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"Breeding for stress tolerance in fodder crops and amenity grasses"

Edited by
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Breeding for stress tolerance in fodder crops and amenity grasses

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FORAGE AND PASTURE BREEDING IN PORTUGAL: HISTORICAL SYNTHESIS

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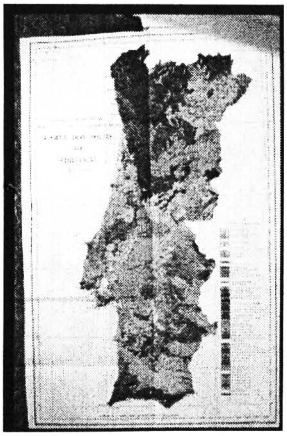
Continental Portugal has a Mediterranean climate, characterised by a dry summer and rainfall is concentrated mainly in Autumn/Winter, becoming frequently excessive and insufficient in spring/summer, rendering difficult for plants when insolation is stronger.

Concerning the agricultural land, we can't say that Portugal is a favored country with very good mesological conditions for agricultural production. About 80 – 85% are acid or moderately acid soils, with a pH between 4 and 6,5. According to Ario de Azevedo, 1970/1971, stony soils, derived of grit stone and sandy soils of dunes occupy 1/6 of the country area.



Loam and clay soils represent only 11% of total surface. Regions with higher rainfall are the hills in the North west, where less developed soils predominate. Most of loam and clay soils are in sub-humid climate areas, in the south, where water logged conditions prevail in winter and summers are characterised by long dry periods.

Orography of Portugal is highly assorted, with two main regions separated by Tejo river. The Northern region is mountainous, whereas the southern region is more or less plane, almost without mountains. More than a half of the area in the Northern region is higher than 400 m; on the other hand, in the Southern region, 98% of the surface is lower than 400 m. The main difference between North and South, as a consequence of the combination of latitude and relief, is the rainfall: 500 mm in the South and 1200-1600 mm in Minho and about 3000 mm in the highest mountain of this province, with a very irregular distribution.

In the south of the country, rain is generally insufficient and irregular, especially after May and stops until September.

As the relationship between heat and moisture is determinant for plant growth, the disorder of these elements limits the season of higher temperatures due to lack of water in the upper lays in the soil. Most of the rainfall occurs during the cold season (Nov.-March) being, sometimes, in surplus during that period of short days and low temperatures, especially in soils poorly drained, which are predominant in our country. Rainfed crops, autumn sowing, mainly cereals, grain legumes, and annual and perennial forages and pasture crops, also suffer of water stress during spring. During May/early June, temperatures are generally too high, which, together with dryness, accelerates maturation of the crops, besides the frequent heat strokes that eventually interfere in seed growth.

Farms area for rainfed cropping is not limiting, either in the North or in the South of the country. Size of the parcels depends on demographic density of occupation and richness of the land. According to INE (1993), the main land agricultural surface used (SAU) is around 3,879,959 ha, where Alentejo and Algarve together represent more than half (> 2 millions). Considering the whole country, farms with 100 ha or more, have 45% of SAU and, in Alentejo, 1% of agricultural enterprises occupy 80% of SAU. In Beira Litoral and Minho the farms with less than 5 ha occupy respectively 40 and 50%.

These are the natural conditions where rainfed crops and, in a lower scale, irrigated crops, are grown. From the 3,9 millions ha of SAU, 2.3 million hectares are occupied by arable land, 789,542 ha with permanent crops and 838,145 ha with permanent pastures.

In Minho and Beira Litoral regions, irrigated crops such as maize, beans and horticulture species are grown during spring/summer, alternating with ryegrass (with or without associated trefoils) during autumn/winter. This is the main region of dairy milk production.

In the inland of the country (Trás-os-Montes, Beira Interior and Alentejo) the most important crops are cereals, that can be used for milk and meat production of cattle, sheep's and goats. In the most flat areas, cattle and lamb production are important. In mountainous and sub-humid areas, sheep production predominates.

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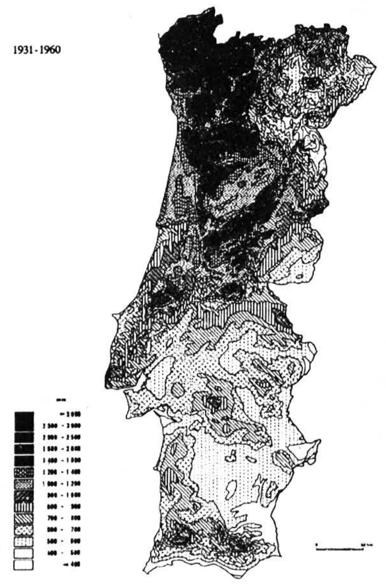

Goats are grown in hills and dry regions (Trás-os-Montes, Beira Interior, Baixas do Guadiana and Serra do Algarve).

Plant breeding of forage and pasture species, as well as technical research to provide its good establishment, to rise the best productivity and proper utilisation, is an under developed sector of agronomical science, when compared with cereals. Thus, evolution and progress verified from beginning of cropping was operated irregularly, leading to contrast positions: in the same farm, in an economical whole, we can see evolved technique to grow cereals side by side with an elementary way to grow pastures. However, with the economical progress of the humanity and the fast increase of populations, one can never expect that natural meadows could produce, by themselves, all products of animal origin needed for food. So, studies on forages and pastures are, nowadays, considered very important tools and, all over the world, we can find specialized Institutions, public or private, dedicating the best of their work to this area of research. However, Portugal is, today, a rare country without a specialised institute for studying, exclusively, pasture and forage crops.

It is also very significant that nations economically more developed, are those which stronger efforts are devoted to reach the best production and use of forage crops, being conscious of the important role carried out in the progress of agriculture and well-being of populations more and more urbanized. In fact, on one hand the grasses influence favorably the fertility of the soil and protection against erosion, and consequently improving its level of productivity; on the other hand they contribute to support a high productivity and quality of cattle at low costs and susceptible to provide the needs of a population in explosive growth. Under the agricultural point of view, we can even say that without the inclusion of herbage production in the agricultural systems, it is not possible to reach profitable and sustainable production, that means an agriculture that tries to reach high levels of productivity but also to preserve the conditions to assure the continuity of those yields.

Among us, these concepts are very important, because one of worst problems affecting the national agriculture is the poorness and degradation of our agricultural soils.

The role of sown pastures is of primordial importance, rendering possible the economical use of inappropriate soils to grow cereals and the establishment of most convenient rotations, helping the protection of the soils against erosion, improving its structure and fertility and the sanitary control of cereal crops.

Long ago it was recognized the need to stimulate the plant breeding work in Portugal, as it was emphasised by legislation from November 22, 1901. There, it was specified that the Agronomic Station of Lisbon and other institutions should make hybridizations of wheat, between national and foreign materials (approving instructions given by General Director of Agriculture, Agronomist Alfredo Carlos LeCoq). Considering the genetic knowledge in that time, this measures must be considered relevant (Victoria Pires, 1939).

It's necessary not to forget that it was precisely after 1900 that Genetics (born in 1866 with studies of Mendel) increases its importance and opens new ways to researchers working on plant breeding. Still according with Victoria Pires (1939), plant researchers in Portugal were not aware of the studies of Hjalmar Nillson in 1881, from Sveriges Ütsädesforening, Svalof which recommended that the wheat selection would be done from descendents of single plants. In 1919 it appeared the first specific institution to obtain new varieties of forages and pasture species: Welsh Plant Breeding Station, created by Sir George Stapledon.

Since 1926, in Portugal, the "Posto Agrário de Elvas" dedicates its main activity to the wheat crop and some preliminary studies on breeding of cereals and forage crops. Meanwhile it is in 1937, after the working up of a plane for breeding cereals and forage crops, by Victoria Pires, at that time head of Plant Breeding Department, at National Agronomic Station (EAN), that the genetic forages breeding begun in our country.

In this document, delivered to the Director of EAN, are defined the orientations of different breeding programs on wheat, barley, oats, rye, maize, pastures and forages crops. Selection objectives are summarised, and methods to be used are described. There were at that time precise ideas about selection and recombination in self-pollinated cereals. Scientific basis of maize breeding were known in detail, throughout simple and double hybrids, from self-pollinated lines. In other species, like rye, bulk selection was considered very important, but other selection methods for those species were not yet specified. Concerning pastures and forages species, nothing was done at that time.

As it is stated in Victori Pires' report, one of the first steps to breed forages is to know the composition of natural pastures in species and separate the different types in the same populations, either in morphological point of view or physiological one, aiming to build the collection of main species which compose the Portuguese grassland and evaluate their potential. Simultaneously, different exotic populations and advanced improved material were introduced in the collections and ecotypes and landraces were collected and joined too.

It was recommended that it would be given special attention to legume species and some grasses, susceptible of good yield under ecological conditions of the country, trying, throughout the same kind of observations suggested for cereals, to select species and cultivars more suitable for each region.

It is noted that a special emphasis was given to selection of plants with low alkaloid content and other active components. A cooperation between the Department of Genetics and the Department of Chemistry and Physiological Chemistry was created, foreseeing the selection of *Lupinus* sp., *Trigonella foenum-graecum*, and *Penissetum* sp. (some of these species are rich in hidrocyanic acid and aromatic products). It was recommended to cooperate closely with laboratories compromised in plant breeding to evaluate the feed quality of each species. Also in order to obtain polyploides it was recommended to make crosses between genus and between species. For this purpose, besides the use of controlled crosses, it was suggested to use other physical means: temperature, X ray, radio, centrifugation, etc.

All this manipulation would be done on species showing high persistence and crosses foreseeing new cultivars having characteristics from several other materials or showing them in highest level. Finally, selection criteria are described, both in pastures and forages, genetic resistance against diseases, preliminary trials, comparative trials and seed multiplication.

As we can see, this workplan for cereals and forages was an advanced document that begun to be implemented on November 1937, in the old "Posto Agrário de Elvas" that joined in this year, as starting material, 399 accessions, being 337 annual species and 61 perennial.

So, to choose the annual species and perennial ones as the most interesting materials to be studied, all the annual legumes species were firstly observed on autumn sowing; sorghum was observed in spring sowing. Perennial grasses and legumes species were observed too.

Besides continuing observations of these sown species, the selection of individual plants, mainly in *Vicias* and *Lathyrus*, started in 1938/39, in which 291 were selected out of 35,913 plants. As these two species were selected for hay production, it was necessary to find a holder plant, and for that some lines of rye and oats were tried.

In spring sowing the best results were found in sorghum and one line was selected in Elvas, hidrocyanic acid free, by Eng° Marques de Almeida, during summer 1940.

Three years later one could conclude that, for dry regions, the most interesting species were *Onobrychis viciifolia, Sanguisorba minor, Hedisarum coronarium, Phalaris tuberosa, Festuca anundracea, some Trifolium repens, Dactylis glomerata, Lolium perenne,* etc. Simultaneously, during the second and third years, together with the selection work, fertilisation trials, mixtures of species and seed multiplication, were initiated.

These studies pursuied and in 1942 it is founded the Plant Breeding Station in Elvas, on the old "Posto Agrário."

Preliminary studies effected during the last years on the original collection of 384 accessions, to determine those ones which would be studied as the most interesting were the main source of knowledge for the new Department of Forages, created with the new Plant Breeding Station (EMP), in1942.

The main objectives of this Department were as follows:

1 - Preserve and improve the collections of plants for forage purpose.

2 - Take care of seed stock avoiding accessions loss.

3 - To carry out breeding of forage species, aiming to create new cultivars of higher economic value and quality, according to methods and techniques usually adopted.

4 - To search new breeding methods more efficient for these purposes.

5 - To study the best technology of cropping to show all the good potentialities achieved by genetic breeding

The responsibility of these works is than of Eng° José Barbas Guerra, Head of Department, who selected several cultivars of annual and perennial species up to January 31, 1966. Some of those cultivars of *Vicia* and *Lathyrus* were widely spread and had an important role to feed animals and in the improvement of soil conditions.

By selection, he obtained the well known cultivars of vetch cv. Caia, from a Portuguese ecotype and the cv. Grão da Gramicha (*Lathyrus cicera*), selected from a population coming from Bari region, Italy.

During the first 17 years of life, the Plant Breeding Station gave to farmers 17 cultivars of pasture and forage species, being 8 annuals and 9 perennials.

In 1966, after 5 months training in plant breeding at Welsh Plant Breeding Station and Grassland Research Institute, Eng° David Crespo becomes the Head of Department at ENMP, extending the previous work selection in some species of annual legumes and perennial grasses, within which we point out *Dactylis glomerata* and *Festuca arundinacea*.

From 1966 to 1985, great evolution arose in the Forages Department of ENMP about utilisation and agronomy research. Several studies were made about mixtures of annual reseeding species with perennial and annual grasses, sowing densities and dates of sowing, fertilisation and its effect on quality and quantity of yielded herbage, etc.

According to Crespo (1992), Portugal, which is one of the Mediterranean countries of EU, is one of the most compromised countries in the use of legumes to improve pastures, due to a program of research and development started in 1965, where EMP was highly involved.

After 1985 up to now, the selection of legumes species of natural reseeding continued and two cultivars of subterranean clover, brachy type were registered in CNV (Variety National Catalog). In 1985 it was developed the selection of some traditional grain legumes for feed (*Cicer arietinum and Vicia faba*) and in 1990 was initiated the selection of peas, for the same purpose. Till now, it was registered in the CNV three cultivars of chickpea, one of faba bean, two *Lathyrus cicera*, and two more cultivars of chickpea and two of peas are ready to be presented to the CNV.

In the area of perennial grasses to associate with annual legumes of natural reseeding, we started again the recurrent selection in *Dactylis glomerata*, summer dormant and with a good development during autumn/winter/spring.

Meanwhile, the Breeding Department of National Agronomic Station in Oeiras, lead by Eng° Marques de Almeida (1936-1978), obtained some cultivars of *Trigonella foenum-graecum, Trifolium resupinatum, T. repens, T. subterraneum and T. alexandrinum*. In 1978 it was appointed Eng° André Mendes Dordio as Head of Genetics and Plant Breeding Department, at EAN and leader of Forages Breeding Section. From that time up to now, some accessions of sweet lupines were evaluated (*L. albus* and *L. luteus*), and some accessions of setaria (*S. splendida, S. sfacelata*) and *Pennisetum purpureum*, to evaluate its potential yield and herbage quality, in our conditions. Also, several plants of grain legumes were evaluated (*Cicer arietinum, Pisum sativum and Vicia faba*). During this period, it listed in the CNV one cv. of sweet lupines (*Lupinus albus* cv. Estoril and *T. resupinatum* cv. Resal).

Final remarks

In 1992, David Crespo wrote that the new conditions imposed to Portuguese agriculture by the integration in EU, would oblige to reformulate the former main objectives of agricultural research, leading to forages and pastures bigger importance than they had before. However, eight years latter, the evolution of cultivated areas didn't lead to a reduction of cereals growing area and the surface of improved pastures didn't increase significantly. Animal products, mainly from cattle origin, have had some problems of commercialisation and farmers haven't increased their production. Agricultural research reduced its activity either for lack of human and material resources or by absence of policy measures.

We hope that, in a short period, with the future extension of EU to east countries, the extensive production of meat and milk may be of more interest to our farmers.

As we just saw, Portugal has large areas where can and must be established permanent or temporary pastures, mainly in rainfed conditions, in more that 2 million hectares.

Being known the most interesting species, to select new cultivars and to produce the needed seed, to develop studies on fertilizations, specificity of rhizobium/host legume association, crops rotation, seed production thecnology, forage evolution quality, resistance and tolerance to drought related to persistence, etc., are subjects to be implemented in a short time.

The species we consider more interesting for rainfed permanents or temporary pastures, are:

Annual medics – *M. polimorpha and M. murex, Trifolium subterraneum, T. resupinatum, T. balansae, T glomeratum, T.incarnatum, Ornithopus compressus, O. sativus, Biserrula pelecinus, Dactylis glomerata, Phalaris aquatica e Lolium rigidum.*

Temporary and permanent irrigated pastures:

Trifolium repens, T. fragiferum, T. pratense, Lotus corniculatus, L. pedunculatus, Medicago sativa, Dactylis glomerata, Festuca arundinacea, Lolium perenne.

Forage crops for forage or for hay:

Vicia sativa, V benghalensis,V. villosa, T. alexandrinum, T. resupinatum sp. suaveolens, M. sativa, Lolium multiflorum, Avena sativa e Triticale.

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Breeding

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BREEDING FOR ABIOTIC AND BIOTIC STRESS IN PERENNIAL TRIFOLIUM

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Abstract

A main objective of most perennial Trifolium breeding and improvement programs is to increase persistence or longevity of the stands. However, both abiotic and biotic stress factors limit progress from selection. Genetic response to these factors in the perennial Trifoliums have, in the most part, been multigenic. Positive responses have most often been achieved through the use of some form of recurrent selection breeding procedures such as mass selection, polycross breeding, or recurrent phenotypic selection. Progeny testing and recurrent selection techniques have been the primary procedures used in the USDA/ARS - University of Wisconsin red clover (*Trifolium pratense* L.) improvement program since the 1960s. Selection for stand longevity and improved pest resistance have reliably extended the persistence of red clover to the third and fourth year in the northern and eastern regions of the U.S. Total four year forage yields of current populations of red clover adapted to these regions are twice those produced by cultivars and germplasm developed in the 1960s. Stand longevity, forage yield, and pest resistance have been improved. Selection for healthy plants in 3- or 4-year-old stands of red clover has simultaneously selected for resistance to root rots caused by Fusarium oxysporum. Continued selection for abiotic stress factors, resistance to soil-borne pathogens, and resistance to root-feeding insects should result in additional improved performance of the species.

Introduction

Red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) are the two perennial Trifolium species most widely used as forage for animal agriculture in the temperate regions of the world. The major limitation in the use of these forage legumes for hay, silage, or pasture is their relative short life span in swards. The stand depletion becomes evident as soon as harvesting, grazing, or cutting commences. The loss of stand continues progressively, depending on the interaction of climatic, pest, edaphic, management, and physiological factors (i.e., abiotic and biotic stress factors), as well as competition among species. The plants' response to these factors and their ability to persist under hay or grazing conditions is dependent upon the degree of stress imposed. In the field, the environment is continually imposing an ever-changing set of stress conditions. Therefore, a main objective of most perennial Trifolium breeding and improvement programs is to develop high yielding germplasm with excellent herbage quality while maintaining or developing a high level of persistence or longevity of stand. However, both abiotic and biotic stress factors tend to act as negative pressures on the species; thus, limiting the progress for improvement. One objective of this paper is to discuss the breeding procedures utilized to improve the tolerance of these species to stress factors. The second objective is to report the progress achieved in the USDA, Agricultural Research Service - University of Wisconsin (USDA/ARS-UW) red clover breeding program over the last four decades in developing germplasm with tolerance to stress.

Breeding Procedures

Breeding procedures utilized for species improvement are generally dictated by the mode of reproduction of the species. Both red clover and white clover are highly self-incompatible (Gibson and Cope, 1985; Smith et al., 1985). The self-incompatibility mechanism can be partially overcome in both species, but selfing leads to severe inbreeding (Bassiri and Smith, 1979; Gibson and Cope, 1985; Goral and Spiss, 1986). Both direct selfing or sibmatting procedures have been attempted in red and white clover with little success (Gibson and Cope, 1985; Smith et al., 1985; Smith, R.R., unpublished data). Genetic male sterility has been isolated in red clover (Smith, 1971), but is not specifically useful in population improvement (Taylor and Smith, 1979). Cytoplasmic male steriles and the respective restoring factors have been isolated (Spiss and Goral, 1990) that may have some potential. These procedures capitalize on both additive and dominant genetic variation that may control the inheritance of stress factors. However, due to the potential for inbreeding, the difficulty in maintaining parent material, and the high economical costs, the reported gains in population improvement have not justified the use of these procedures. The use of the backcross method of breeding to incorporate tolerance to stress factors has been limited. The procedure is time-consuming, laborious, and may result in inbreeding. Taylor et al. (1985) used backcrossing to incorporate bean yellow mosaic virus resistance into the cultivar, Kenstar, but the released germplasm was less vigorous than Kenstar.

In perennial Trifoliums, the most frequently used breeding procedure is some form of mass or recurrent phenotypic selection (RPS). Desirable phenotypes are chosen and intercrossed, generally in a polycross isolation, to produce the subsequent generation. Such polycross seed can be used for progeny testing of the original selections, for testing progress made with initial selections, and/or as a source for further selection. These procedures may be very effective for highly heritable traits. Often some form of progeny (half-sib or polycross) testing is employed in conjunction with the selection process. Propagation of subsequent generations is generally accomplished by using polycross or half-sib family seed because of the difficulty of maintaining parental clones of either red or white clover (Smith et al., 1985; Gibson and Cope, 1985). Excellent reviews on specific responses to selection at the whole plant level on stress factors affecting clovers are presented by Leath (1985); Barnett and Diachun (1985); Manglitz (1985); Caradus and Williams, (1989); and Taylor and Quesenberry, (1996).

The most frequently used phenotypic selection procedures will be described with examples of their use.

Mass selection:

Generally, seed of a large bulk population is seeded either in the field or a controlled environment and exposed to the appropriate stress(es). These may be abiotic stresses (soil type or texture, climate, soil pH, etc.) or biotic (pathogens, insects, nematodes, viruses, etc.). Plants not tolerant to the specific stress(es) are removed or die, and surviving plants are allowed to intercross under isolated natural conditions or controlled pollinations. Seed is bulked from the surviving plants and either used for subsequent cycles of selection or tested for performance and released as new, improved germplasm. Mass selection may be effective for highly heritable traits. One cycle of mass selection was effective in selecting for root rot resistance in red clover (Vanco, 1989). The cultivar AC Charlie was developed using repeated cycles of mass selection for persistence and general plant vigor (Choo et al., 1994).

Polycross selection:

With the polycross method of breeding, desirable, individual clones are identified, asexually propagated, and intercrossed at random in isolation. Seed is bulked by each clone and subsequently progeny are tested. From the results of the progeny tests, the most desirable clones are identified and intercrossed. The polycross seed from the selected clones is bulked to form a new synthetic. This method identifies clones with high general combining ability and may be useful for the improvement of stress factors that are highly complex in inheritance. While effective for complex characters, the procedure is time-consuming and expensive, and maintenance of selected clones over time is quite difficult, primarily due to virus infection. The 10-clone cultivar Kenstar was developed using this method (Taylor and Anderson, 1973). Clones were selected for disease and virus tolerance and for stand longevity. The Russian persistent red clover cultivars, VIK 7 (diploid) and Salynt (tetraploid), and the white clover cultivar, SGP-3, were developed using the polycross method (Novoselova et al., 1983). Parental clones used in this method should be evaluated in several environments where the expected cultivar is to be used to identify inferior parents (Taylor and Quesenberry, 1996).

Recurrent phenotypic selection (RPS):

Recurrent phenotypic selection (referred to as phenotypic recurrent selection in many publications) is probably the most common selection procedure used in breeding perennial Trifoliums. Like mass selection, identification of plants tolerant to stress factors are identified from either natural stands, space plant nurseries, or artificially developed environments. (i.e., laboratory or greenhouse evaluations). Laboratory or greenhouse evaluations may include artificially induced epiphytotics for disease or insect reaction or sophisticated laboratory chemical analyses. Desirable plants are identified and intercrossed in isolation. Seed is maintained from each plant as half-sib family seed rather than bulking all seed as in mass selection. However, equal amounts of seed from each family can be bulked for testing or for parent material for future cycles of selection. Frequently some form of progeny testing is utilized with RPS. Estimates of narrow sense heritablitlty can be obtained from half-sib family means to aid in future selection. Parental selection may be based on half-sib family means or best plants may be selected from the best half-sib families. This latter procedure is often referred to as geno-phenotypic selection (Taylor, 1987).

Taylor et al. (1990) used six cycles of RPS to increase northrn anthracnose (caused by Aureobasidium caulivora (Kirchn.) W.B. Cooke) resistance in 10 polycross populations derived from the 10 parental clones of the cultivar Kenstar. Eighty to 100 plants were selected each year in each population to continue selection. Resistance to the disease was improved in all population, averaging from 9% in Cycle 0 to 52% in Cycle 6. Realized heritability ranged from 9 to 34% per c;ycle, averaging 20% over all populations. Theroretical inbreeding coefficients averaged 2.8% after six cycles of selection. The authors concluded that RPS was an effective method to improve northern anthracnose resistance in these populations. Christie and Martin (1999) increased stand survival (persistence) after one cycle of RPS in the cultivar, AC Charlie. Tofte et al. (1991) reported a substantial increase in resistance to Aphanomyces euteiches in red clover after three cycles of RPS. Narrow sense heritability estimates were high enough (51%) to recommend utilizing family selection for further improvement. A modification of the RPS method, restricted RPS (RRPS), was successfully used by Quesenberry et al. (1989) to select for resistance to the root-knot nematode (Meloidogyne spp.). With RRPS, the source nursery (field or greenhouse) is stratified into a grid system. Plant selection is conducted from each section of the grid in an attempt to control variable effects in the nursery, such as disease distribution, environmental influences, etc.

Breeding for Tolerance to Stress Factors in Red Clover in the USDA/ ARS-UW Program

The long term goal of the USDA/ ARS-UW red clover improvement program has been to improve persistence of the crop. Persistence may best be defined as the survival of plant material against specific stress factors unique to the existing environment. Numerous factors may impose stresses in any one environment, or one factor may impose opposite stresses in different environments. For example, red clover survival in northern USA is a function of tolerance to low temperatures, but in the mid-south and southern USA, tolerance to high temperatures is necessary.

Over the past four decades, the emphasis of the USDA,/ARS-UW red clover breeding program has been to select 70 to 100 surviving, reasonably healthy plants from 3- or 4-year old field tests and to intercross these selected plants and bulking equal amounts of seed from each plant (mass selection) to generate a new parent population for a new cycle of persistent selection. At the same time, the half-sib progeny (recurrent phenotypic selection are evaluated and screened for disease reaction and subjected to further attribute evaluation. Mass selection, RPS, polycross selection, and progeny testing have been employed for plant improvement over this period.

Since the 1950's, this process has been repeated four times with the cultivar Lakeland being released in 1953, Arlington in 1973 (Smith, et al., 1973), and Marathon in 1987 (Smith, 1994). These three cultivars of red clover and the experimental population, WIS EXP, developed in the 1980s, are used to relate the progress from selection for persistence and disease resistance. Seedlings of these populations were evaluated in the greenhouse to determine their reaction to root rot (wilt) caused by Fusarium oxysporum and to northern anthracnose. Procedures described by Venuto et al. (1995) were used to evaluate root rot and those proposed by Smith and Maxwell (1973) for northern anthracnose. Forage yield and other agronomic data were collected on replicated field tests conducted at the Arlington Research Station, Arlington, WI. Data presented on the disease responses are the means of three separate greenhouse evaluations, and agronomic data are the means of two field tests.

Improvement in forage yield (3-year total forage) and resistance to root rot and northern anthracnose in red clover over the four decades of selection are present in Figure 1. Resistance to the two diseases, root rot and northern anthracnose, has steadily increased with the development of new germplasm. Two to three cycles of selection for resistance to northern anthracnose was applied during each cycle of selection for persistence. On the other hand, only one cycle of selection for root rot occurred after the third cycle of selection for persistence. Earlier improvement in root rot resistance was the result of natural selection for resistance with the selection of healthy, persistent plants. Total forage yield increased over the cycles of selection, due in part, to selection for disease resistance and to increased persistence. There was no difference in forage yield (4.0 Mg/ha) in the second year of production among the populations developed over the four decades (Figure 2). The differences among the populations occurred in the third and fourth years of production, with Marathon and WIS EXP producing excellent forage yields in these latter years of production. Selection in exiting 3- and 4-year-old stands of yield trials has been effective in improving persistence, and to some extent, root rot resistance.

Conclusions

Selection for tolerance to stress factors has been successful in the perennial Trifoliums using some form of phenotypic selection or a combination of phenotypic and genotypic selection. However, the identification of plant genotypes tolerant to stress factors is a direct function of the effectiveness of the screening procedure employed (Smith and Kretchmer, 1989). Selection for tolerance to a specific stress trait is dependent upon the effectiveness of identifying tolerant genotypes in the population. As new techniques and procedures become available to identify the genetic composition of the host or causal agent, or we can better define the impact of environmental stress factors, greater progress will be achieved. However, even with new and modern sophisticated techniques and procedures, conventional procedures as described above will still be needed to incorporate the appropriate genetic material into germplasm that will be productive in the target environment.

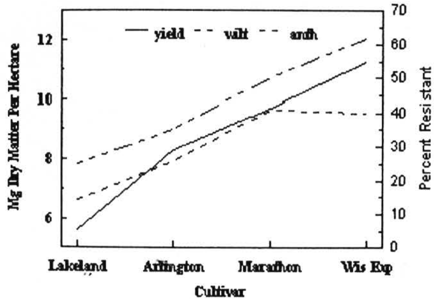

Figure 1. Progressive improvement in the performance of red clover resulting from four decades of breeding for persistence and disease resistance.

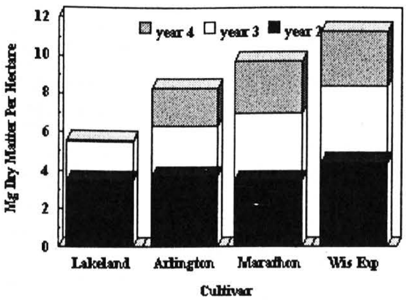

Figure 2. Three year forage production of red clover resulting from four decades of breeding for persistence and disease resistance.

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IMPROVEMENT OF LADINO CLOVER SEED YIELD POTENTIALS IN CLIMATE CONDITIONS OF POLAND

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Abstract

Seed yield capacity of Ladino white clover shall be improved to make it more competitive against to Dutch types. Usually, white clover varieties of Dutch type yielded 10 - 20 % more seed per ha than Ladino type varieties, which are superior for dry matter and protein production in Poland. Since dry matter yield of white clover consisted of heads, leaf blades and petioles, insomuch these characters are positively correlated in between, the single head selection procedure (Staszewski and Staszewski, 1997) has been employed for this study.

Significant correlations have been found between a single head mass and seed weight per head (r = 0.65 - 0.87), a seed weight of 30 heads sample and seed yield per plot (r = 0.45) and between seed weight per head and seed mass per 30 heads sample taken on a plot of next generation.

The breeding procedure included:

1 - Head selection.

2 - Polycross progeny test of best head - families.

3 - Synthesis of new population consisting of the families possessing highest general combining ability . Breeder seed contains Syn . 2 multiplication.

Significant progress in seed yield was received due to head selection procedure.

Introduction

„Trifoglio bianco lodigiano" – Ladino form of white clover (*f. giganteum Lagr. et Fosset*) has started its fruitful spreading all over the world about 140 years ago. It moved from Po Valley (Lombardy, Italy) to the Old and New Worlds. At the end of XIX c. it was being bred by Villmorin in France , as well as by Fruwirth in Germany, and by Sutton in U.K. in the beginning of XXc. (Kostecki, 1928; Brock, Caradus, Hay, 1984; Erith, 1924). Ladino clover was introduced to the western U.S.A. in 1912, but to the much cooler area of Wisconsin in 1916. The unsuccessful trials aimed to introduce Ladino clover to Poland were done by Sempolowski and Sobieszyn (1898-1903), Kaznowski in Pulawy (1921-22) and Mazurkiewicz at Skrzeszewice (1922-23). They were unsuccessful because the imported cultivars were not winterhardy. Staszewski and Starzycki (1959-70) bred winterhardy synthetic population Radi cultivated up to now, and several other ones followed in Poland after 1975 (Staszewski, 1966a; Sempolowski, 1904)

There are several dozens of winterhardy Ladino clovers being grown in Europe and USA teritory in the recent years. The basis for the success of breeders were theoretical studies of Julen , Knoll, Hermerlick , Smith , Wood, Sprague, Roningen, Greenham, Daday, Atwood, Brewbacker, Gibson, Beinhardt, Halpin, Hollowell and others.

Ladino white clover is an autoteraploid mostly self-sterile so that helps to employ heterosis in breeding procedures, (Julen, 1959, Atwood, and Brewbacker, 1953).

Lenoble and Percheron as early as in 1970 in the journal of „Fourrages" pointed out that viruses and poor seed productivity were the main disadvantages of white clover varieties which were intensively bred for the fodder yield capacity. They also underlined a synthetic population breeding to be the most effective way for improvement of cultivars. Numerous winterhardy cultivars giving valuable fodder yield gains were introduced all over the continent of Europe in last decades. But still in Central and Eastern Europe seed yield of Ladino type clover is lower comparatively to Dutch one, since it is a young biotype less adapted to the zone of cooler climate. Hopefully, there are a great seed setting variations within Ladino clover populations, so that, it is possible to bred a synthetic which entirely fit to the growing region to produce high seed yield (Staszewski, 1966b; Lonc, Ramenda, 1982; Gibson et all., 1963; Baker, Williams, 1987; Cebrat, Kobierzynska, Ramenda, 1982; Annicchiaricco, 1993; Jahufer, Cooper, Brien, 1994; Rodes, Webb, 1996).

The micro-plots technique was introduced in Versailles by Demarly (Lenoble, Percheron, 1970a,b), because he found very positive correlations (r=0,86) among the results of micro- versus macro- plots. Micro-plots technique for fodder crops selection was successfully used by Rotili and all in Lodi. Staszewski, 1997, stated great variability 0-0.5g of seed mass per head in the big samples of heads selected in the population grown in the field conditions. Significant correlation (r=0,64-0,87) was found among head mass and seed weight per head. The heaviest heads produced descent families with significantly improved seed yields. Thus the breeding method of synthetic population basing on the selection of heads and micro-plots technique is proposed.

The aim of this work was to study suitability of a new „head selection procedure" for improvement of Ladino clover seed yield capacity.

Material and Methods

Radi cultivar of *Trifolium repens L. f.giganteum Lagr. et Fosset* was taken as initial population to be improved. The variety has been registered in 1970 as a winterhardy synthetic population consisted of clones selected out of English and Belorussian descents. The major disadvantage of Radi cv. is low seed yield comparatively to Dutch clover populations grown in Poland. The program included field experiments to study variations of seed setting within a population and selection of extraordinary seed setters to serve as components for polycross progeny test evaluation.

The main task of our study presented here, was the improvement of seed yield capacity of cultivar Radi. Paralelly, we tried to use new, more simple and cheaper breeding procedure that included the following steps:

Visual selection of 1000-2000 heads among a population grown in the field

Each head mass evaluations – Keep the heaviest heads (25%)

Weight seed mass of each head – keep the best heads (5%)

Poly-cross including 25-35 best heads

Poly-cross progeny test for the both seed and fodder yield and GCA evaluations

Synthesis of syn.1 population consisted of seed reserves of the heads showing the highest GCA

Prebasic seeds are syn.2 or syn.3 multiplications.

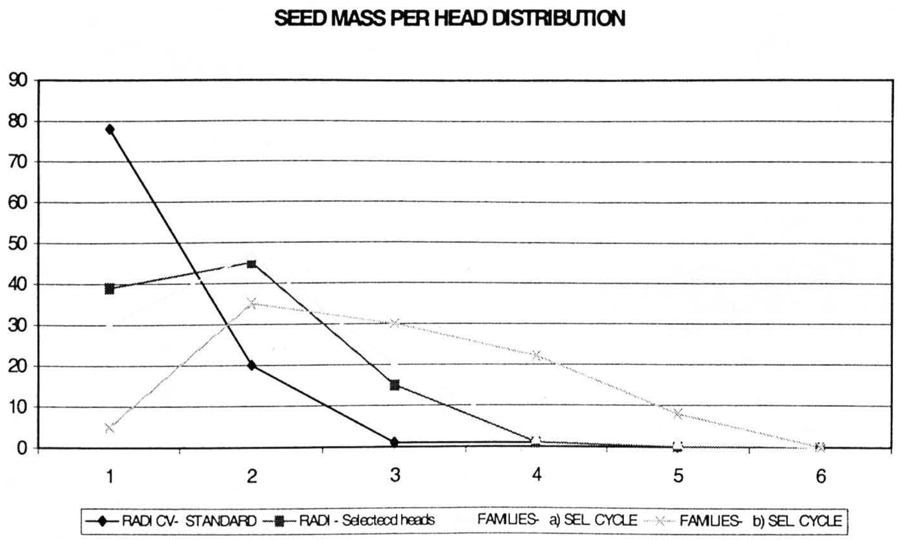
The heads were selected out of the initial population, which were grown in dense sward in the field. The heads being apart 1-2 m were picked up to avoid taking several heads from the same plant. Head and seed mass were weighted in the year of selection.

The plants obtained from seeds received from heads each producing about 0,5g seeds were grown in the poly-cross. The seeds of each poly-cross entry consisting of 20 plants were harvested to sow the trials for GCA evaluations. The poly-cross was installed using small plants grew in greenhouse, so it was possible to receive progeny seeds in the year of planting. Parallelly seeds of heads were used to produce families, to compare their yields with poly-cross progeny in the neighbour trial.

Poly-cross progeny test was carried on the field using microplot technique. The seedlings were grown in pots (no less than 20 plants per pot) in greenhouse. After plants reached 4 weeks age the pot contents were planted in seed-beds spaced 1.5x1.5 m. Each pot produced a microplot. The experiment contained 5 replications of the progeny of each poly-cross entry. Weed control were done using soil rotator 1.25m wide. The area of microplots rested for scoring and harvesting plants covered 0.5x0.5m of soil surface.

Results

The selection of heads was successful because the size of inflorescence is positively correlated with leave blade size and petiole length which are the main yield components (excluding stems) of white clover.



The effects of selection are presented on the figure [the abscissas' axis (y) is given in % of the frequencies of heads in five weight classes, whereas on the ordinate's axis (x) there are shown five weight classes: 1-0,1; 2-0,2, 3-0,3, 4-0,4; 5-0,5g]. The sample of heads taken at random contained 97,5% heads producing 0,1-0,2g of seeds, but only 2% with 0,3-0,4 g seeds per head. The sample of selected biggest, best looking heads contained 16% heads each producing 0,3-0,4g seeds, and the share of heads producing 0,1g of seeds rapidly decreased. The recurrent selection for head size was effective. The families received after the 1st (group „a") cycle of selection were improved slightly. The great progress was observed after the 2nd (group „b") cycle of selection: the heads producing 0,1-0,2 g of seeds shared only 40% and the deal of heads producing 03,-0,4 g of seeds increased up to 52%, and the group of 0,5g seeds per head -which was absent in the random taken sample- dealt 8% of heads.

The comparisons of green mass of poly-cross progenies and families are given in Table 1. The polycross progeny produced higher yields due to better intercrossing of plants comparatively to families multiplicated on separate plots.

Poly-cross progeny test showed high heterosis effects and due to that exceeded significantly standard cultivars. The poly-cross entries in the number of four are valuable to produce a new synthetic due to highest GCA values in green mass.

The seed yield gains caused by heterosis were greater than in green matter (table 2). Ten progenies of poly-cross exceed the best standard cultivar Arta by 26-72%. Seven entries were superior seed yielders but three of them produced unsatisfactory fodder yields.

Table 1. Comparisons of green mass of poly-cross progeny and families.

No of progeny	Poly-cross progeny 1999 / 4 cuts			Families 1999 / 4 cuts		
	kg	GCA	In %	kg	GCA	In %
1	4,9 *	0,2	136,1	3,9 *	0,7	114,7
2	4,9 *	0,2	136,1	3,6	0,4	105,9
3	4,7 *	0,0	130,6	3,2	0,0	94,1
4	4,8 *	0,1	133,3	2,9	-0,3	82,3
5	4,7 *	0,0	130,6	3,2	0,0	94,1
6	4,6 *	-0,1	127,8	3,4	0,2	100,0
7	4,4 *	-0,3	122,2	2,9	-0,3	82,3
8	4,3	-0,4	119,4	2,7	-0,5	79,4
9	5,0 *	0,3	138,9	3,4	0,2	100,0
10	4,6 *	-0,1	127,8	3,6	0,4	109,1
11	4,8 *	0,1	133,3	3,3	0,1	97,1
12	4,8 *	0,1	133,3	3,5	0,3	102,9
13	4,5 *	-0,2	125,0	2,9	-0,3	82,3
14	4,8 *	0,1	133,3	4,6 *	1,4	135,3
15	4,5 *	-0,2	125,0	2,8	-0,4	82,4
16	4,6 *	-0,1	127,8	2,8	-0,4	82,4
17	4,2	-0,5	116,7	3,1	-0,1	91,2
18	5,2 *	0,5	144,4	3,5	0,3	102,9
19	4,1	-0,6	113,9	2,2	-1,0	64,7
20	4,6 *	-0,1	127,8	3,1	-0,2	91,2
Mean	4,7			3,2		
Standars:						
Radi	3,0		83,3	3,3		97,1
Romena	3,2		88,9	3,1		91,2
Arta	3,6		100,0	3,4		100,0
Mean	3,3			3,3		
LSD 0,05	0,71			0,48		

*Significant at 0,05 level

Computing the results of tables 1 and 2 enables to propose the formula of a new synthetic including the entries best in seed and parallelly fodder yields, which displayed highest GCA. There are following entries of the poly-cross: 1,4,9,18.

Since head size in Ladino clover is highly correlated with leaf blade sizes and petiole lengths and parallelly with seed mass per head the selection of biggest heads from a dense sward of clover plants is proved as a good way to improve both seed and forage yields. Progeny testing of the best heads ensured receiving a new population producing significant yield gains. Poly-cross progeny test is very effective to point out heads displaying superior GCA on seed and fodder yields for forming improved synthetic population. It is noteworthly that several poly-cross entries were superior on both: seed and fodder yields. So that receiving a synthetic showing heterosis effect in seed yield parallelly with fodder yield seems realistic.

Open pollinated progeny of selected heads performed worse comparatively to poly-cross progeny of the same set of heads. In spite of that, some of heads being superior in poly-cross progeny test, were producing the best yielding families. Therefore, a selection of superior families among a great number of the descent families of heads cannot be refused.

Table 2. Comparisons of seed yield of poly-cross progeny and families

No of progeny	Poly-cross progenies					Families				
	Head number per plot	Seed weight per 30 heads (g)	Seed yield per plot (g)	In %	GCA	Head number per plot	Seed weight per 30 heads (g)	Seed yield per plot (g)	In %	GCA
1	212	3,15	19,30	135,90	1,70	208	2,76	18,00	122,40	2,10
2	180	2,78	16,40	115,50	-1,20	173	2,21	13,10	89,10	-2,80
3	204	2,21	16,20	114,10	-1,40	162	2,30	13,90	94,60	-2,00
4	216	4,07	24,40	171,80	6,80	188	3,01	18,90	128,60	3,00
5	204	2,24	16,80	118,30	-0,80	234	1,80	14,00	95,20	-1,90
6	184	4,00	20,50	144,40	2,90	200	3,60	18,30	124,50	2,40
7	189	2,54	15,00	105,60	-2,60	207	2,10	14,60	99,30	-1,30
8	190	2,96	18,70	131,70	1,10	160	2,54	15,70	106.80	-0,20
9	184	5,02	26,40	185,90	8,80	151	3,98	20,70	140,80	4,80
10	189	1,85	14,10	99,30	-3,50	196	1,60	14,60	99,30	-1,30
11	188	2,14	14,70	103,50	-2,90	164	1,74	15,30	104,10	-0,60
12	160	1,83	10,90	76,80	-6,70	158	1,68	14,90	101,40	-1,00
13	212	3,18	19,60	138,00	2,00	154	2,60	16,40	111,60	0,50
14	182	2,36	13,10	92,20	-4,50	165	2,01	13,30	90,50	-2,60
15	204	2,61	17,90	126,10	0,30	201	2,56	16,50	112,20	0,60
16	184	2,40	16,70	117,60	-0,90	180	1,78	11,70	75,60	-4,20
17	224	2,14	18,40	129,60	0,80	226	2,56	19,50	132,60	3,60
18	164	3,82	19,00	133,80	1,40	184	2,58	17,50	119,00	1,60
19	185	2,10	14,10	99,30	-3,50	175	1,81	15,30	104,10	-0,60
20	175	2,31	19,90	140,10	2,30	171	1,93	15,80	107,50	-0,10
MEAN	**191,50**	**2,79**	**17,61**	**123,98**		**182,85**	**2,36**	**15,90**	**107,96**	
STANDARDS										
RADI	210,00	1,92	13,10	92,20		189,00	1,81	12,11	82,30	
ROMENA	192,00	1,56	10,60	74,60		190,00	1,63	11,30	76,90	
ARTA	208,00	2,16	14,20	100,00		194,00	2,23	14,70	100,00	
MEAN	**203,33**	**1,88**	**12,63**	**88,93**		**191,00**	**1,89**	**12,70**	**86,40**	
LSD 0.05			1,95					1,84		

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ADAPTATION OF PERENNIAL GRASSES TO A LONG DRY SUMMER

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Abstract

Australian programs for the improvement of perennial ryegrass and tall fescue have employed a range of strategies for improving drought tolerance. Examples are presented to illustrate genetic improvement in perennial ryegrass following utilisation of Australian naturalised germplasm as well as exotic accessions from Southern Europe/North Africa (perennial ryegrass) and, in the case of tall fescue, the Middle East. Extreme drought tolerance found in some exotic grass material is associated with physiological summer dormancy, and is closely linked with early season vigour.

For perennial ryegrass, multi-disease screening has been used to assist selection of parent genotypes. Following progeny evaluation under grazing in several states of Australia, experimental synthetic varieties, selected for persistence and seasonal yield, have been developed and are undergoing field evaluation. Such varieties may extend the area of adaptation and the longevity of plants within the traditional areas. The early season vigour may alleviate feed shortages - either when the variety is used directly, or by blending seed with that of summer-active cultivars.

Grass genotypes exhibiting marked differences in persistence over summer in marginal environments are now being used to examine genetic variability for fructan concentrations and its relationship with drought tolerance. Wide crosses have been carried out in order to develop molecular markers for aspects of drought including fructans, root architecture, summer dormancy and photosynthetic efficiency.

Introduction

The pastoral industries of temperate Australia rely heavily on perennial grasses – particularly perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Schreb.), phalaris (*Phalaris aquatica* L.) and cocksfoot (*Dactylis glomerata* L.). Perennial ryegrass is the most widely sown species with some 7 million ha in use. Other species are used in niche environments, e.g. tall wheatgrass (*Thinopyrum ponticum* (Podp) Liu and Wang) used in saline areas (Smith 1996; Smith 2000).

Whilst these species play important roles in Australian pasture they all suffer from limitations impeding their productivity or persistence. The cool temperate climate of southern Australia is characterised by winter-spring rainfall incidence. At Hamilton in SW Victoria, the mean annual rainfall is 703 mm and long-term rainfall/evaporation for April-November and December-March is 558/588 mm and 148/728 mm respectively. By far the driest inhabited continent, Australia's long dry summers that characterise the majority of grazing environments in the southern temperate zone, present a major challenge to plant breeders seeking to improve the adaptation of productive perennial grasses. Such adaptation has been the major objective of pasture grass breeding programs (Reed 1996). We present three examples from the Australian national perennial ryegrass and tall fescue improvement programs to illustrate progress made by breeders in developing cultivars better adapted to the stress of summer drought.

Progress and Results

Developing winter-active tall fescue

Tall fescue is considered more drought tolerant than perennial ryegrass. However, the summer activity of tall fescue limits its use in regions with long dry summers. A winter-active tall fescue cultivar 'Melik' selected from Israeli germplasm (Rodgers and Beresford 1970) has been used to develop an improved cultivar for regions of approximately 500 mm annual rainfall (Venkatanagappa and Jahufer 1998). In an experiment that ran for six years (mean annual rainfall 575 mm), a winter-active tall fescue selection showed 216% greater herbage production in winter than the standard summer active tall fescue cultivars. It also exhibited greater total herbage production (up to 100%), and better persistence, in an environment where low summer rainfall limited the potential productivity of traditional tall fescue cultivars (Anderson *et al.* 1999). Cultivar 'Fraydo' has recently been released from this program.

Developing drought tolerant perennial ryegrass using Mediterranean germplasm

Germplasm of Mediterranean origin has been widely used in Australian cocksfoot and phalaris breeding programs to develop winter-active, drought tolerant cultivars. Despite early promise (Silsbury 1961) this concept has not been developed with perennial ryegrass where the main ecotypes used have been derived from European seed and the majority of newer cultivars have been developed using New Zealand germplasm (Cunningham *et al.* 1994). As a consequence little viable germplasm of Mediterranean origin was available in Australian collections for use in breeding programs. A large collection was subsequently conducted in North Africa and Italy in the early 1990s (Cunningham *et al.* 1997).

Three thousand perennial ryegrass plants of Mediterranean origin were established at a low rainfall site (610 mm *per annum*) in Victoria and plants were evaluated for herbage yield, persistence and resistance to crown rust, barley yellow dwarf virus and ryegrass mosaic virus. Several experimental synthetics were derived from elite plants selected from this population and were progeny tested at a number of sites in southern Australia. After three years of close rotational grazing, the persistence shown by the best seven families from one of these experimental populations greatly exceeded the mean for the 10 commercially available cultivar comparators (Table 1). A cultivar based on this germplasm is scheduled for commercial release in 2002.

Table 1: Persistence of select families and 10 commercial cultivars of perennial ryegrass after three summers of close rotational grazing (%).

	Balmoral (Vic.)	Harrogate (S. Aust.)	Hamilton (Vic.)
Best 7 families	56.4	68.1	71.8
Cultivars, mean/10	28.2	18.6	46.7
lsd (P=0.05)	16.98	23.33	18.67

Development of cultivars based on naturalised Australian perennial ryegrass

In 1992, 80% of the area sown to perennial ryegrass in Australia was based on the local ecotype cv. Victorian. Victorian perennial ryegrass is noted for its adaptation to Australian conditions. However, it does not have the yield potential of more recently bred cultivars and has low resistance to disease, especially crown rust.

In September 1992, a collection of perennial ryegrass was taken from 56 paddocks across Victoria. Paddocks were required to be productive pasture that had been undisturbed for at least 50 years. The collection of 5600 plants was planted at Hamilton. Seasonal yield and time of flowering were assessed and resistance to crown rust, ryegrass mosaic virus and barley yellow dwarf virus were rated prior to the selection of elite genotypes for crossing prior to progeny evaluation. The first cultivar developed from this population, cv Avalon, was selected for herbage yield and disease resistance (Croft *et al.* 2000).

A comprehensive survey of the incidence of the ryegrass endophyte (*Neotyphodium lolii*) and associated alkaloid production was also conducted on the collection. Each of the 56 populations were found to be infected. The mean frequency of infection within paddocks was 88%. The concentrations of the plant alkaloids, lolitrem B and ergovaline, produced by endophyte infected populations varied significantly (Reed *et al.* 2000).

Avalon perennial ryegrass, together with a range of commercial cultivars, was established in swards in a replicated evaluation trial at Hamilton (Vic.) in the autumn of 1999. The summer was particularly dry and significant tiller death occurred in some cultivars. The number of live tillers present after the first summer was counted in June. Live tiller density for Avalon was less than for G. Impact, a *Lolium x boucheanum* cultivar developed from a New Zealand x Mediterranean cross ($P<0.05$), but greater than for the modern New Zealand cultivars of *L. perenne* (Table 2). Subsequent harvests demonstrated that the cv. Avalon, whilst retaining the persistence of cv. Victorian, has higher second year yield potential ($P<0.001$).

Table 2. Tiller density, and cool season yields of perennial ryegrass cultivars harvested during their second year (2000) at Hamilton, Victoria.

Cultivar	May 18	May 17	June 27	July 28
	Live tillers (no./10 cm row)	Dry matter yield (kg/ha)		
Aries	17.0	174	109	58
Avalon	38.4	490	367	275
G. Impact*	50.2	620	576	386
G. Lincoln	14.5	162	103	75
Vedette	28.9	315	193	158
Victorian	40.2	321	118	63
Yatsyn 1	29.1	212	113	123
LSD (0.05)	8.5	101.7	92.9	92.1

* *Lolium x boucheanum*

Conclusions

Through breeding from appropriate introductions, and by selection within old Australian ecotype material, it has been possible to improve the persistence and productivity of perennial grass in environments subjected to long summer drought. To facilitate further genetic improvement of perennial ryegrass, the current breeding program is now aimed at increasing soluble carbohydrates, particularly the fructan concentration, and developing molecular markers for root architecture and traits associated with drought tolerance (Guthridge *et al.* 1999).

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PROGRESS IN DEVELOPING FACULTATIVE TRITICALE GERMPLASM WITH DUAL PURPOSE FOR THE MEDITERRANEAN REGIONS OF PORTUGAL

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Abstract

Agricultural systems of South Portugal, where Mediterranean Sea strongly influences the environment are unique in many aspects.

Animals (cattle, sheep and pigs) are free-grazing all the year. With this system there are two critical periods for livestock feeding, namely, winter and summer. Suitable varieties of cereals can serve as very important forage resource, with the advantage that large amounts of biomass can be produced under modest nitrogen regimes, even on autumn/winter.

Forage production has not traditionally been a primary selection criterion on the cereals breeding program of ENMP. However during the last 10 years, the changes observed in the national agriculture policy, have motivated new objectives to the breeding programs. Triticale breeding is now centred on dual purposes (forage plus grain) objectives and successful genotypes are now available.

Since triticale for forage has generally to be planted earlier than cereals for grain only, the flowering time is a very important determinant trait of varietal suitability. Using the right vernalisation alleles, very good genotypes have been developed, combining the ability to rapidly establish in autumn with good early forage production and regrowth capacity after winter grazing.

Breeding approach is based on germplasm development, using spring and winter parents, selection and evaluation across distinct regions of Portugal.

This paper is focused on results from the evaluation of early (spring types), intermediate (facultative types) and late (winter types) triticales germplasm. Successful genotypes are those that have facultative growth habit that combine early vigour (winter grazing) with good regrowth capacity (grain production or summer grazing).

Introduction

Cereals are important contributors to animal feeding in Mediterranean conditions, both as forage and as grain. Their drought and cold tolerance make them better adapted than other traditional forage legumes to the long dry summers and cool wet winters, typical of these regions.

Animals (cattle, sheep and pigs), namely land races of these species, are free-grazing all over the year.

With this system there are two critical periods for livestock feeding, winter and summer (Maçãs, *et al.*, 1998). In winter, forage legumes and grasses as ryegrass have problems with lack of resistance to frost. During summer, lack of water and high temperatures makes crop growth impossible.

In South Portugal, cereals are sown in autumn, and the practice of grazing these crops before the jointing stage is one way of complementing feeding on winter. The regrowths may be left for grain production or summer grazing (Royo *et al.*, 1993, Maçãs *et al.*, 1998).

Forage production has not traditionally been a primary selection criterion on the cereals breeding program of Estação Nacional de Melhoramento de Plantas (ENMP) at Elvas.

However, nowadays with the very low prices of small grain cereals, farmers introduced many changes in their farming systems, where the reduction of production costs are the main way to maintain some comparative advantages. Animal production in extensive regime is environmentally friendly and gives high quality products (meat, cheese).

The current research of the cereal breeding program of ENMP includes studies on germplasm development for forage purposes and is mainly, focused on oats and triticale with high early vigor and at the same time regrowth capacity after winter grazing.

Three types of triticale can be distinguished, on what concerns to the development cycle, a) winter triticales, which need vernalization for floral differentiation, b) spring types, with no vernalization requirements and c) facultative types, which varies in the degree of vernalization requirement (Maçãs *et al.*, 1998).

The objective of this study was to compare the suitability of these three types of triticale for forage purpose, based on their winter forage, grain and straw yields.

Materials and Methods

During 3 seasons (1997/98, 1998/99 and 1999/2000), 50 triticale genotypes, 1 bread wheat, 4 oats and 2 barleys were tested to evaluate their forage interest. Trials were sown at Elvas (South Portugal) under rainfed conditions and good soil fertility, to obtain good yield potential. These trials were replicated in 3 other locations under low fertility conditions (data not reported in this paper).

The plots had an area of $12m^2$ containing six rows 20cm apart. The seed rate was 450 viable seeds/m^2 and the sowing date as early as possible after the first rains always before 10 November. Each plot received the following amounts of fertiliser (kg/ha): 110N, $80P_2O_5$, and $30K_2O$. Nitrogen was splited in two applications, at sowing and after winter cutting.

Experimental design consisted of a randomized complete block with 3 replications.

Two harvesting treatments were applied: a) harvesting at crop maturity to evaluate the grain and straw yield and b) cutting the crop for forage in the end of December or mid January (1999/2000) to evaluate green forage, and harvesting the regrowth at maturity. Dry matter content was calculated after drying a sample of 400gr of green forage in an oven at 100° C during 6h. The analytical methods for quality determination were the standard kejdahl procedure (N x 6,25) for protein content and the Weende method for fiber.

The harvest index was calculated from a sample of 1,2 m^2 of each plot.

Results and Discussion

The production of winter forage as well as regrowth capacity was influenced by the growth habit of triticale (Table 1). Climatic variations were very strong among the years imposing high standard deviations for the different variables measured. Early genotypes of triticale, with no vernalisation requirement, developed fast on autumn, showing good dry matter accumulation during the period. However, the winter cutting promoted damage on the growing point and the regrowth capacity was poor when compared with the other types (Table 1). Facultative germplasm revealed better suitability for winter grazing once they produced good amount of green forage during winter and at the end of cycle.

The timing of availability of green forage in winter is very important to feed animals on the field. The regrowth capacity of winter materials do not compensate due to the low forage yield in winter.

Facultative genotypes as well as oats reveals good adaptability for the purposes, with the advantage of triticale that shows complete resistance to powdery mildew that is an important disease on oats. At the same time, triticale shows better adaptation to acid soils, wich are very common in Portugal.

On the other hand, the grain of triticale was significantly higher than oats which is a very important component to feed animals during summer.

The treatment without winter cutting revealed also interesting results (Table 2). Winter germplasm developed slowly during winter, but had advantage for total biomass at the end of the cycle. However, revealed low harvest index, indicating a poor balance between grain and straw when the purpose is silage. Again, facultative types can be recommended to be used as a silage crop, once the harvest index was much higher.

Table 1 – Forage yield and quality for the winter cutting and regrowth, grain and straw yield and harvest index of the materials tested at Elvas during 3 years.

Species / Growth habit	Winter Cutting			Regrowth			
	Biomass (kg/ha DM)	**Protein (%DM)**	**Fiber (%DM)**	**Biomass (kg/ha)**	**Grain yield (kg/ha)**	**Straw (kg/ha)**	**Harv. Index (%)**
Early triticale	4737	17,05	20,53	12581	3787	8794	30
S.D.	2763	0,05	3,74	3383	2331	1052	7
Facultative triticale	4311	17,91	20,82	14761	4198	10563	28
S.D.	2825	0,51	2,84	4714	2296	2419	6
Winter triticale	3678	18,18	20,01	15718	3854	11864	24
S.D.	2362	0,79	1,25	4879	1589	3289	3
Bread Wheat	3613	20,00	19,70	11639	3338	8301	27
S.D.	2830	0,05	1,7	3810	2049	1761	8
Oats	5118	15,70	20,30	14547	2215	12332	15
S.D.	3570	0,2	2,2	4623	1566	3057	4

S.D. – Standard deviation

The timing of availability of green forage in winter is very important to feed animals on the field. The regrowth capacity of winter materials do not compensate due to the low forage yield in winter.

Facultative genotypes as well as oats reveals good adaptability for the purposes, with the advantage of triticale that shows complete resistance to powdery mildew that is an important disease on oats. At the same time, triticale shows better adaptation to acid soils, wich are very common in Portugal.

Table 2 – Days to heading and agronomic performance of materials testing at Elvas during 3 years.

Species / Growth Habit	Days to heading After 1 March	Biomass at maturity without winter cutting	Grain Yield	Straw Yield	Harvest index (5)	Test Weight
Early triticale	21	15275	3931	11344	28	75,97
S.D.	8	1684	1095	639	4	0,88
Facultative triticale	34	16919	4256	11935	28	75,47
S.D.	10	2166	1124	1048	3	1,00
Winter triticale	51	18363	3881	14481	22	74,51
S.D.	14	819	635	717	5	0,94
Bread wheat	40	14259	4148	10111	28	79,20
S.D.	7	514	729	1043	4	0,72
Oats	24	17566	2524	15042	16	44,62
S.D.	7	2659	1328	1823	6	0,72

On the other hand, the grain of triticale was significantly higher than oats which is a very important component to feed animals during summer.

The treatment without winter cutting revealed also interesting results (Table 2). Winter germplasm developed slowly during winter, but had advantage for total biomass at the end of the cycle. However, revealed low harvest index, indicating a poor balance between grain and straw when the purpose is silage. Again, facultative types can be recommended to be used as a silage crop, once the harvest index was much higher.

These results had strong influence on the breeding strategy, revealing that growth habit must deserve special attention.

Research is being conducted in order to identify the right vernalisation alleles to select the better adapted lines.

Conclusions

Triticale is a new crop with good potential for forage. It can compete with other cereals regarding forage purposes. Its early growth and regrowth capacity after cutting makes it suitable for complementing animal feeding during winter and summer. Nevertheless, the differential response of triticale germplasm to the recovery capacity after cutting and green forage production indicate that there is genetic variability among the different growth habits, and selection for forage uses is possible.

Facultative type seems to be better adapted to produce green forage in winter and recovery capacity to assure straw and grain, for feeding during summer. Regarding silage, these genotypes show also good yielding capacity and higher harvest index, than winter types.

On the breeding side, further work is being carried out at present to characterise the vernalisation alleles and to identify the most suitable one(s) for mediterranean conditions.

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CONSTRAINTS IN THE SELECTION OF PARENTS FOR SYNTHETIC CULTIVARS

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Abstract

According to plants breeding textbooks breeding methods can be subdivided into three phases:

i) procurring the initial variation, ii) selection of parents; and iii) testing of the experimental variety. Each of them has its particular constraints and limitations.

The selection of parents for a Synthetic is mostly done on the basis of the testcross performance i.e. gca of the candidates (clones, lines a s o). The reliability of the testcross estimates is crucial.

A balance between high selection intensity (few parents) and the avoidance of too much inbreeding depression (higher numbers) has to be found. Synthetic prediction can help to find the optimum number of parents. The pro cons of additional S1-testing will be discussed on the basis of experimental data.

According to experimental data, the optimum number of parents is variable and depends on the genetic material under consideration.

Introduction

The development of synthetic cultivars is described in various plant breeding textbooks, in the context of the typical breeding scheme (see below) with the Polycrosstest being the central part. According to Schnell (1982), the breeding scheme can be subdivided into 3 distinct phases: i) procuring the initial variation, ii) forming varietal parents (selection) and, iii) testing experimental varieties. The practical pathways which may be followed within each of these phases depend on the species and the breeders preference.

The creation and collecting of the starting material (base population) will not be considered further. However, the availability of genetic variation for the traits of interest is of fundamental importance. The selection of varietal parents (phase 2) depends on the exploitation of the variation available in the base population. To develop the best variety from a given set of parents it would be necessary to know the value of all possible varieties which could be derived from these parents. However, for multi-parent varieties this is only possible with a low number of parents. Prediction formulae at this level are necessary to limit the number of varieties to develop. In the following context and as indicated in the breeding scheme, the selection units are clones, which will be maintained until they are needed for variety synthesis. Other types of parents such as full- or half-sib families are also used in practical breeding as well as the use of remnant seed for synthesis.

Selection of parents - Testcrossing

To obtain genotypic information, i.e. general combining ability (gca) of the parents, testcrossing has to be made and testcross-progenies have to be tested. In most cases the Polycross (PX) test is applied while the Topcross test is not widely used. Unlike in maize, where the tester originates from the opposite pool, this is not (yet) practised in the forages. However, if the tester is the population where the parents derived from, then there will be no genetic difference to the PX.

Breeding Scheme for Synthetics

Base population	Phase 1
Clonal rows	Phase 2
Testcrossing	
Progeny testing	
Syn-0 Synthesis	
Syn-1	Phase 3
Syn-2	

How many testcross candidates? The number of parents which should be testcrossed depends on several factors. To some extent this is due to the phenotypic selection which was practised in the base population and/or among clonal lines. In some situations only a restricted number, due to date of flowering will be available. Since the potential parents will be selected upon their gca the number of candidates will heavily influence selection intensity. Assuming that the synthetic will be based on 10 parents, then with 100 testcross candidates, selection intensity will be much higher as compared to only 40 candidates.

With only a small set of entries in a PX, random mating might not anymore be ascertained. Especially if the range of flowering is too large, non-random mating will occur. In a topcross test with an excess of pollen from the tester these problems will be neglectable. The number of replications in a PX also has an impact on random mating. However, in practise this is mostly seen from a technical point of view, that is the amount of testcross seed needed for plot trials.

Progeny testing

This seems to be the bottle neck in practical breeding. In the forages very little information is available about resource allocation. Fortunately, technical progress (plot harvesting machines) has enabled breeders to run large scale experiments. However, the number of replications and locations are often chosen upon availability and not on rational grounds. To be on the safe side breeders experiments are mostly managed like the official performance trials. Unfortunately, almost no information is available about the interrelationship between testing sites and the average of the official trials.

G x E-Interaction.

Because of the vast range of target environments, genotype x environment interaction are of special interest, since G x E-effects will mask the true value of the candidates.

According to table 1 genotype x year (G x Y) effects seem to be more important as compared to G x L. Especially in stress environments carry over effects take place and enlarge G x Y-effects. The value of any prediction depends strongly on the accuracy of the estimates needed.

Table 1: Variance components from progeny testing in 3 years at 2 locations

Genotype (G)	11.7	49.9
G x Location (GxL)	10.2	4.9
G x Year (GxY)	17.2	29.1
G x L x Y	16.8	23.3

Synthetic prediction

To discuss the various possibilities of selection, the expected yield of a synthetic variety has to be regarded. The expected yield Y of a synthetic in equilibrium was already given by Wright (1922),

(1) $$Y = C - \frac{C - S}{n}$$

where C is the mean of all possible crosses between parents, S is the mean of all intra-parent progenies, and n the number of parents.

S means the performance of the parent itself. If the parent is a heterozygous clone, S is the performance of this clone after one generation of selfing (see Posselt 1984).

Analogous to hybrid prediction, synthetic performance for a selected number of clones can be predicted by a mean value μ plus the sum of gca.

$$Y(gca) = \mu(n) + \frac{2(n-1)}{n^2} x \Sigma gca$$

with: $$\mu(n) = C - \frac{C - S}{n}$$ which is the mean of all (possible) synthetics of size n.

As can be seen from these formulae, the performance of a synthetic variety depends not only on gca but also on the term (C - S)/n which specifies the amount of inbreeding resulting from the limited number of n parents.

If S is not available from experimental data, an estimate value can be used. Though the absolute figures will not give the right yield level, the optimum clone number of the respective breeding material can be determined (see fig. 1).

To determine the number of parents for synthetics two points have to be considered: The first is the inbreeding in the synthetic variety; this effect can be reduced by increasing the number of parents. But a second point to be considered favours small numbers of parents, since with a smaller number of parents a more intensive selection can be applied and only the best parents are included in the synthetic variety. In other words, with increasing number of parents, the mean of all synthetics will increase, but the variance between them will decrease. These considerations lead to the general conclusions that there must be an optimum number of parents.

Table 2: Testcross progeny performance and Synthetic prediction

Clone	Topcross		Prediction			
no.	C	gca	n	μ (n)	Σgca	Y (gca)
1	121.00	12.69				
2	118.50	10.19	2	97.5	22.9	108.9
3	117.50	9.19	3	101.1	32.1	115.3
4	116.40	8.09	4	102.9	40.2	118.0
5	118.90	10.59	5	104.0	50.8	120.2
6	118.20	9.89	6	104.7	60.7	121.5
7	116.40	8.09	7	105.2	68.8	122.1
8	115.80	7.49	8	105.6	76.3	122.3
9	115.20	6.89	9	105.9	83.1	122.3
10	114.30	5.99	10	106.1	89.1	122.2
11	114.10	5.79	11	106.3	94.9	122.0
12	113.80	5.49	12	106.5	100.4	121.8
13	113.80	5.49	13	106.6	105.9	121.7
14	113.10	4.79	14	106.8	110.7	121.4
15	112.30	3.99	15	106.9	114.7	121.1
16	111.30	2.99	16	107.0	117.7	120.7
17	110.30	1.99	17	107.0	119.7	120.3
18	107.20	-1.11	18	107.1	118.6	119.5
19	107.20	-1.11	19	107.2	117.5	118.9
20	106.50	-1.81	20	107.2	115.7	118.2
21	105.90	-2.41	21	107.3	113.3	117.5
22	105.40	-2.91	22	107.3	110.4	116.9
23	104.20	-4.11	23	107.4	106.3	116.2
24	102.80	-5.51	24	107.4	100.8	115.5
25	102.60	-5.71	25	107.4	95.1	114.7
26	101.70	-6.61	26	107.5	88.5	114.0
27	101.30	-7.01	27	107.5	81.4	113.3
28	100.70	-7.61	28	107.5	73.8	112.6
29	100.60	-7.71	29	107.6	66.1	112.0
30	98.90	-9.41	30	107.6	56.7	111.2
31	98.80	-9.51	31	107.6	47.2	110.6
32	97.60	-10.71	32	107.6	36.5	109.8
33	97.50	-10.81	33	107.6	25.7	109.2
34	96.90	-11.41	34	107.7	14.3	108.5
35	94.00	-14.31	35	107.7	0.0	107.7

From fig. 1 two different conclusions can be drawn. An optimum number of parents can be identified. With increasing inbreeding depression 10 % (Syn-90) to 30 % (Syn-70) the optimum shifts from 7 (Syn-90) to 9 (Syn-70) parents. However, as can be seen from the data in table 2, the optimum is rather flat and more or less a plateau. Thus, it depends on the breeder to decide if, according to the gca-estimates, one or two more candidates should be included in the synthetic having the expectation of yielding broader adaptability.

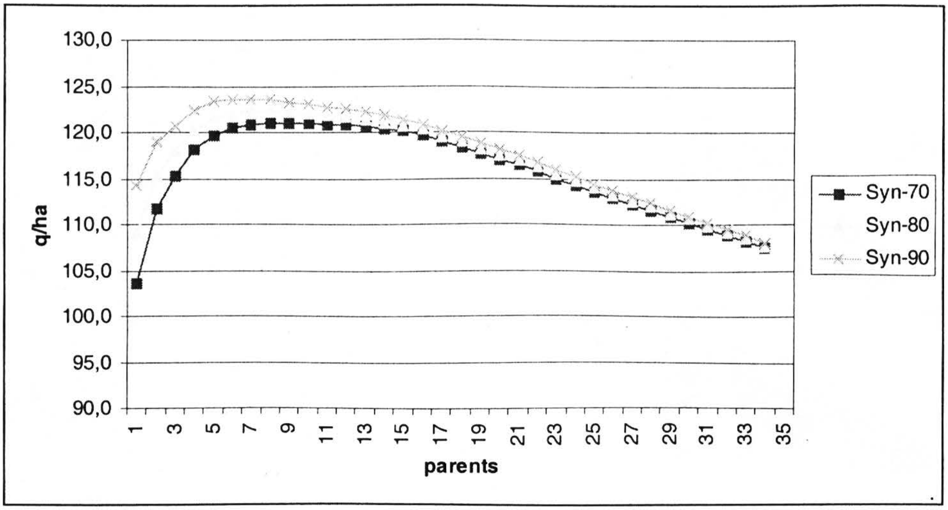

Fig. 1 Synthetic prediction with varying inbreeding depression (see text)

In table 2 an example of experimental and predicted data is given. 35 clones were topcrossed and the total dry matter yield (avg. of 3 years) ranged from 9.4 to 12.1 tons per ha. In the second column the gca estimates are given. Because of positive and negative gca, the sum of gca of all candidates is 0. In the right hand part of the table μ (n), that is the mean of all possible synthetics of size n, is given. According to the diallel formula there are already 595 synthetics with only 2 parents that could be established from these 35 clones. With only the 17 best, there will be still 136 combinations. Because of the increasing mean μ (n) and an increase in the sum of gca, the optimum number of parents will reach a maximum estimate Y(gca). In this case, having chosen 20 % inbreeding depression i.e. Syn-80; the highest estimate will be 122,3 q/ha with 8 parents. However, only small differences to the neighbouring combinations occur. The breeder could either construct several experimental synthetics (n=6 to n=11) for testing, while loosing another few years. He also could take into consideration phenotypic traits with high heritability from the parents themselves. This would then lead to the construction of a selection index. Prediction cannot solve all problems, since it is just a tool. However, since the experimental data is available with very little time prediction could be done as an aid for selection decision of parents for variety synthesis.

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ANDROGENIC AND SOMATIC TISSUE CULTURE IMPROVEMENT IN TIMOTHY BREEDING

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Abstract

Timothy (*Phleum pratense* L.) is the most important grass in northern latitude. It is considered to be allo-hexaploid in nature (2n=6x=42). Biotechnological improvements in timothy breeding by androgenic cell culture, somatic cell culture were achieved in our studies.

Over 400 doubled haploid plants were obtained via anther culture and microspore culture. A series of factors which affect androgenic embryogenesis and green plant regeneration have been evaluated in anther culture and microspore culture. The PG-96 induction media which sharply promote androgenic embryogenesis have been established in this study. Haploid production is extremely attractive as it not only enables selection at the haploid level *in vitro* for desirable agronomic characteristics, but also provides a means for the production of genetically stable homozygous lines, fixed by chromosome doubling. Haploidy offers the advantages of sampling gametes in a random fashion and production of completely homozygous lines from a parental heterozygous cross in one step.

Timothy somatic callus induction was successfully established using immature inflorescences and seedling stems. The capacity of somatic embryogenesis from immature inflorescences was higher than that from seedling stems. The explant size and hormone combination were key factors in callus induction from immature inflorescences. Suspension cultures were initiated from friable nodule-forming callus in modified MS basal medium. The highest green plant regeneration was achieved 5-7 weeks after initiation in suspension culture. More than 100 regenerated green plants were obtained from somatic callus and suspension clumps.

Introduction

Timothy (*Phleum pratense* L.) is an important forage grass, it is valued for its winter hardiness, good palatability, and moderate feeding value. Plant breeding of timothy has been relatively limited in the past due to the low degree of genetic homozygosity traditionally obtainable in this species; hence, selection for desirable traits must be carried out with very heterozygous material. Cell culture techniques for timothy haploid production have been developed but they were recalcitrant (Niizeki and Kati, 1973; Abdullah et al., 1994; Guo et al., 1999; Guo and Pulli, 2000a). Haploid production is extremely attractive as it not only enables selection at the haploid level *in vitro* for desirable agronomic characteristics, but also provides a means for the production of genetically stable homozygous lines, fixed by chromosome doubling.

Somatic tissue culture is a valuable technique in crop improvement programs. Timothy somatic embryogenesis and plant regeneration from mature seed has been reported (Horikawa and Ohi, 1995; Horikawa et al., 1997). However, somatic embryogenesis and plant regeneration in graminaceous grasses were generally difficult (Creemers-Molenaar et al., 1989; Horikawa and Ohi, 1995; Horikawa et al., 1997). Embryogenic cell suspensions provide a key source for regenerative protoplasts of cereal crops. Plant cell suspension cultures offer distinct advantages over stationary cultures. Large amounts of cell materials can be generated rapidly when grown in an agitated liquid medium, because cells are bathed by the culture medium and are evenly exposed to nutrients and plant growth regulators. The suspension cultures were initiated from explant-derived morphogenic callus, or directly from seed-derived mature embryos. Suspension cultures of plants are widely used in biotechnology. However, it is difficult to obtain fast growing, homogeneous and morphogenic suspension cultures (Creemers-Molenaar et al., 1989; Lührs and Lörz, 1988).

Materials and Methods

Timothy donor plant maintenance

For timothy anther culture and microspore culture, seeds were sown in pots in a greenhouse. When the plants had shooted enough, they were transferred into a cool room at +4°C and vernalized for 10-12 weeks. After vernalization the test plants were moved to a greenhouse under the following growth conditions: 18°C/13°C day/night temperature and a 16-h photoperiod at 300 μmol $m^{-2} s^{-1}$ supplemented by fluorescent lamps. Spikes emerged 4-5 weeks thereafter. For timothy somatic callus induction, both immature inflorescences and seedling stems were used.

Anther culture and microspore culture

The optimum developmental stage was between the very late uninucleate and the binucleate stages. The spikes were cut, labelled, bagged and placed into a bottle containing a sufficient amount of water, then they were transferred to cool room at 4 °C in darkness for a cold pretreatment of 2-4 weeks. After cold pretreatment the timothy spikes were surface sterilized, anthers picked and transferred in Petri dishes into a dark incubation chamber at 27°C (Guo et al., 1999). Two different techniques were employed for the mechanical isolation of microspores from timothy spikes, maceration by microblending and maceration with a pestle (Guo and Pulli, 2000a). A series androgenic cell culture media including PG-96 were used in timothy anther and microspore culture.

Somatic callus induction and suspension culture

Somatic callus initiated from immature inflorescence and seedling stem of timothy as discribed by Guo and Pulli (2000b). After several subcultures, two grams of solid, fast growing and small granular embryogenic calli, which were light yellow in colour, were transferred to a 250 ml Erlenmeyer flask for suspension culture as described by Guo and Pulli (2000b). A modified MS medium was used for somatic callus induction (Guo and Pulli, 2000b).

Plant regeneration

Androgenic embryos/calli were transferred onto a solid regeneration medium. Green plant regeneration and chromosome doubling were accomplished as described by Guo et al. (1999). In timothy somatic embryogenesis and suspension culture, green plant regeneration was achieved through immature inflorescence/seedling stem derived calli differentiation directly and from clumps/calli differentiation via suspension cultures as described by Guo and Pulli (2000b).

Results and Discussion

Evaluation of the culture medium effect in timothy anther culture

In this study, a series of anther culture media for cereal and grass crops, including N6, R2M, W14, FHG and Potato II, were tested in both the solid and liquid forms (Figure 1). In addition, a new liquid nutrient medium, PG-96 was developed in our laboratory for timothy androgenic cell culture (Table 1). For many genotypes, the PG-96 liquid medium produced a higher embryo yield, and much better results were obtained by the liquid medium than the solid medium. Compared with all other media, the PG-96 liquid medium sharply increased embryo yield.

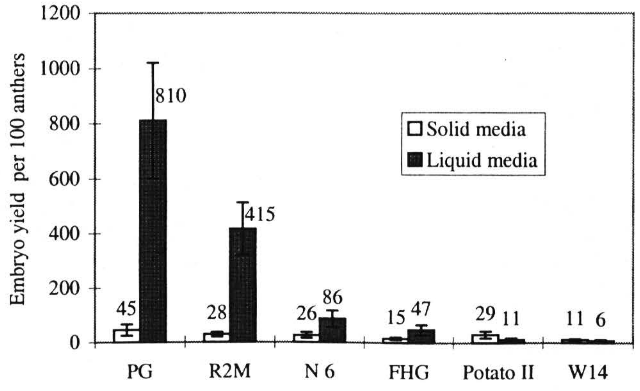

Figure 1. Response of induction medium on embryo yield in anther culture of timothy cv. Adda. Bars indicate the embryos (calli) obtained from solid and liquid induction media. Average values caculated from 5 replicates.

Genotype responses in timothy androgenic cell culture

Genotype is an important factor in androgenic cell cultures. Anther and microspore culture of timothy were genotype-dependent (Table 2).

Evaluation of sugars used in timothy microspore culture

All tissue culture media require the presence of a sugar as a source of carbon and energy. Sugars also act as osmotic regulators in the media. In this study, sucrose, maltose and glucose were tested using filter-sterilized PG-96 liquid medium as the basal medium. Maltose at concentration levels between 9% and 13% gave the best response in timothy anther culture (Guo et al., 1999). PG-96 medium with 6% maltose monohydrate of 0.221 Osm kg^{-1} H_2O was the most suitable for timothy microspore survival (Table 3).

Callus formation in timothy somatic tissue culture

Callus induction from immature inflorescences and seedling stems was achieved in this study. Some parameters such as genotype, explant size and plant growth regulator were evaluated. Genotype is well-established as an important factor influencing the embryogenic response *in vitro*. The choice of explant is perhaps the most important factor in tissue culture. Horikawa et al. (1997) pointed out that there was no differences for callus induction among six timothy cultivars tested for somatic embryogenesis from seeds, but the types of callus such as compact and friable tissue were different.

In this work, the somatic embryogenesis response from immature inflorescence was much better than that from seedling stem. Callus initiation from immature inflorescence was fast and reliable, and the quality of callus was high. There were several types of callus in timothy somatic embryogenesis, compact, friable, very loose and watery. The compact and friable calli were used as initial materials in plant regeneration and suspension culture, respectively. For suspension cultures, friable and nodule-forming callus was needed. The ability to form friable callus among these genotypes was different (Table 4).

Table 1. Composition of PG-96 medium for timothy anther culture.

Components	mgl^{-1}
KNO_3	1500
$(NH_4)_2SO_4$	150
KH_2PO_4	125
$MgSO_4.7H_2O$	200
$Ca(NO_3)_2.4H_2O$	200
KCl	50
$FeSO_47H_2O$	27.8
Na_2 EDTA.$2H_2O$	37.3
$MnSO_4.4H_2O$	10
$ZnSO_4.7H_2O$	3
H_3BO_3	5
KI	0.83
$NaMoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Ascorbic acid (Vitamin C)	10
Proline	20
Aspartic acid	10
Citric acid	10
Biotin	0.05
Glycine	2
Inositol	100
Thiamine-HCl	10
Pyridoxine-HCl	1
L-Glutamine	500
Nicotinic acid	0.5
Glutathione	10
L-Serine	20
Casein hydrolysate	200
L-Alanine	10
2,4-D	1.5
Kinetin	0.5
Maltose	90,000-130,000
pH	5.7

Table 2. Production of embryos (calli) and regenerated plants from anther culture of different timothy genotypes. Data is the average of at least three replicates.

Genotype	Cell division/dish	Embryos/dish (Total)	Green plants (Total)	Albino	Source
Grinstadt	570 ± 108*	277 ± 89	59	180	Norway
Alma	420 ± 69	163 ± 71	73	15	Finland
Kämpe II	290 ± 94	88 ± 30	16	47	Sweden
Iki	280 ± 58	125 ± 22	0	34	Finland
Adda	230 ± 70	46 ± 18	4	60	Iceland
Våti 7701	170 ± 65	97 ± 16	0	57	Norway

*Standard deviation

Table3. Effect of carbohydrate on microspore swollen, division and embryogenesis of timothy microspore culture cv. Grinstadt.

Carbohydrate	Microspore swollen %	Division cells/dish	Embryos /dish
Maltose	65 ± 14 %*	490 ± 66	230 ± 51
Sucrose	31 ± 11 %	160 ± 29	90 ± 18
Glucose	37 ± 9 %	180 ± 35	49 ± 9

* Standard deviation

Table 4. Compact and friable callus induction from immature inflorescences and seedling stems of timothy. (cal.=callus)

Genotypes	Explant number	inflorescence		stem	
		Compact cal.	Friable cal.	Compact cal.	Friable cal.
Adda	45	19 ± 5*	12 ± 4	7 ± 3	6 ± 2
Alma	45	20 ± 4	9 ± 2	11 ± 4	12 ± 3
Tuukka	45	16 ± 2	9 ± 2	4 ± 0	3 ± 1
Grinstadt	45	14 ± 3	6 ± 2	6 ± 1	5 ± 2
Iki	45	11 ± 2	10 ± 4	4 ± 1	0 ± 0
Kampe II	45	6 ± 2	14 ± 2	8 ± 2	5 ± 1

*SD: standard deviation. Means ± standard deviation are from 5 replicates.

Establishment of timothy suspension culture

In many monocotyledonous species, the induction of somatic embryogenesis from the suspension cultures has been difficult. The yellow, nodule-forming and friable callus were easily broken apart and dispersed into small clumps and small cell-aggregate suspension cultures after being moved into MS suspension culture medium. The suspension clumps were finer and the growth of the suspension was fast with 2,4-D 5.0 mg l^{-1} and ABA 0.5 mg l^{-1}.

Plant regeneration from androgenic and somatic cell culture

Albinism is a serious problem in timothy androgenic cell culture. The primary cause of albinism is still unknown. Nuclear gene changes, as well as changes in cytoplasmic genes, are possible reasons for albinism but an additional influence of physiological factors cannot be excluded (Ferrie et al., 1995). Light intensity is very important in green plant regeneration, because the chloroplast formation is sensitive to light. In this study, light intensity played an important role in the formation of green shoots. The green plant regeneration in high light intensity (94.7 µmol m^{-2} s^{-1}) was 1.42% whereas in low light intensity (5.5 µmol m^{-2} s^{-1}) was 4.94%.

For timothy somatic tissue culture and suspension culture, green plant differentiation from suspension culture was more efficient (Guo and Pulli, 2000b). The suspension clumps were very active and had high potential in green plant differentiation.

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EVALUATION OF LOCAL POPULATIONS FOR TIMOTHY BREEDING

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Abstract

Timothy (*Phleum pratense* L) in its cultivated form, is a relatively new species in Northern Norway. In the County of Nordland seeds were grown commonly at farms up to about 1950, and several local strains were in use. Later two varieties 'Bodin' and 'Engmo', developed from these strains and multiplied in South-eastern Norway, have dominated the timothy production in Northern Norway. The South Norwegian variety 'Grindstad' and varieties from Northern Sweden and Finland have also been in use.

In the early 1980 local populations were collected in Northern Norway, multiplied and stored in the Nordic Gene Bank. It would be of interest to study the genetic diversity between the local populations the commercial varieties being used up to now. If they are genetically nearly related, the breeding work should mainly be based on the best varieties for this climatic area. If not, local populations could give valuable new genetic variation to the breeding work.

At Vågønes Research Station, Bodø (67.2°N), 43 of the local population collected in the County of Nordland were tested together with the mentioned Norwegian varieties, the North Swedish variety 'Jonatan' (SW) and the Finish varieties 'Alma' and 'Tuukka' (Boreal). Observations were done in a field test over three years and at controlled freezing tests. Based on phenologic, agronomic and wintering characters cluster analysis were made to assess levels of genetic diversity.

Three main clusters were observed. A smaller main cluster consisted of the Southern variety from Norway and the Swedish and Finish varieties, together with a few local populations. The varieties 'Engmo' and 'Bodin' were each represented in one of the two larger main clusters. The most promising local populations from an agronomical point of view, was found in the main cluster including 'Bodin', but mainly in a sub cluster with some genetic distance from that variety.

The results from these observations, indicated that the local populations still can be of interest when breeding timothy for this northern climatic area. Local populations with high forage yield, good yield distribution, and good winter hardiness were found.

Introduction

Timothy (*Phleum pratense* L.) in its cultivated form is a relatively new species in Northern Norway. In the County of Nordland seeds were grown commonly at some farms up to the1950s, and several local strains were in use. Later two varieties 'Bodin' and 'Engmo', developed from these strains were registered and seed multiplied in South-eastern Norway. These varieties have dominated the timothy production in Northern Norway up to the later years. The South Norwegian variety 'Grindstad' has been used in the best climatic areas, and varieties from Northern Sweden and Finland have also been in some use.

Around 1980 local population were collected in Northern Norway, multiplied and stored in the Nordic Gene Bank. It would be of interest to study the genetic diversity of the local populations and their relationship to the commercial varieties. If the local populations and the varieties were genetically closely related, the breeding work should mainly be based on the best varieties for this climatic area. However, if the local populations possessed new genetic variation compared to the best commercial varieties, they would be of value for the breeding work.

Materials and Methods

43 of the local population collected in the County of Nordland (located from 64° 90' N to 69° 30' N), were tested together with the three Norwegian varieties 'Engmo', 'Bodin' and 'Grindstad', the North Swedish variety 'Jonatan' (SW), and the Finish varieties 'Alma' and 'Tuukka' (Boreal), at Vågønes Research Station, Bodø (67° 17' N, 14° 28' E, 20-43 m a.s.l.). 'Bodin', the most commonly used variety, originated from a neighbour farm of Vågønes Research Station, and 'Engmo', the most northerly adapted variety, from just north of the northern border of Nordland, while 'Grindstad' originated from a farm in South-east Norway. In a field experiment with three complete replicates, several characters were observed over two years after establishment. A two cut system was used with first cut in early July and second cut in early September. All field data were subjected to Nearest Neighbour Analyses and the mean values were corrected using AGROBASE. The plant materials were also studied at controlled freezing tests, where the plants were established in pots in glasshouse and placed outside for natural hardening. Artificial freezing were conducted in mid October and late November.

Results and Discussion

The most northern variety 'Engmo' showed the earliest growth start in spring, while 'Grindstad' started growth latest of the varieties (Table 1). For the local populations growth start ranged from earlier than 'Engmo' to as late as for 'Grindstad'. The heading period was because of the 24 h photoperiod, very short. However, 'Grindstad' started heading earliest, and 'Jonatan' was the latest. Heading time for the local populations were from as early as 'Grindstad' to nearly as late as 'Jonatan'. Proportion of generative tillers at second cut showed great variation. 'Grindstad' had the significantly highest per cent of generative tillers, while 'Engmo' showed a low proportion. In some local populations generative tiller formation was lower than for 'Engmo', while some exceeded the formation in 'Bodin', but the local population with the highest per cent just reached half of the formation in 'Grindstad'.

Table 1. Growth characters of 43 local populations and 6 varieties of timothy.

Pop./Variety	Growth start Day in May	Heading Day in June	Generative tillers at 2nd Cut (%)
Local populations			
Highest	7.0	27.6	18.7
Lowest	4.7	25.6	3.6
'Bodin' *standard*	*6.5*	*26.4*	*11.4*
'Engmo'	5.9	26.5	7.7
'Grindstad'	7.1	25.3	45.9
'Jonatan'	6.7	28.0	13.7
'Alma'	6.2	26.7	15.1
'Tuukka'	6.7	27.5	14.9
C.V.%	13.1	2.53	53.6
L.S.D.0.05	0.75	0.52	5.8

The highest yield at first cut was for the varieties obtained with 'Bodin' (Table 2), while 'Grindstad' showed the lowest yield. The local populations showed a yield range at first cut from below 'Grindstad' to 6 per cent higher than 'Bodin'. At second cut the local populations yielded from 9 units less than 'Bodin' to 17 units more as for 'Tuukka', but far from the second cut yield of 'Grindstad', which significantly exceeded 'Bodin' with 43 per cent units. The result was an almost equal total yield for 'Bodin', 'Tuukka', 'Grindstad' and 'Jonatan', with a range of the local populations from 10 units below to about 6 units above these varieties.

Table 2. Relative yields of 43 local populations and 6 varieties of timothy.

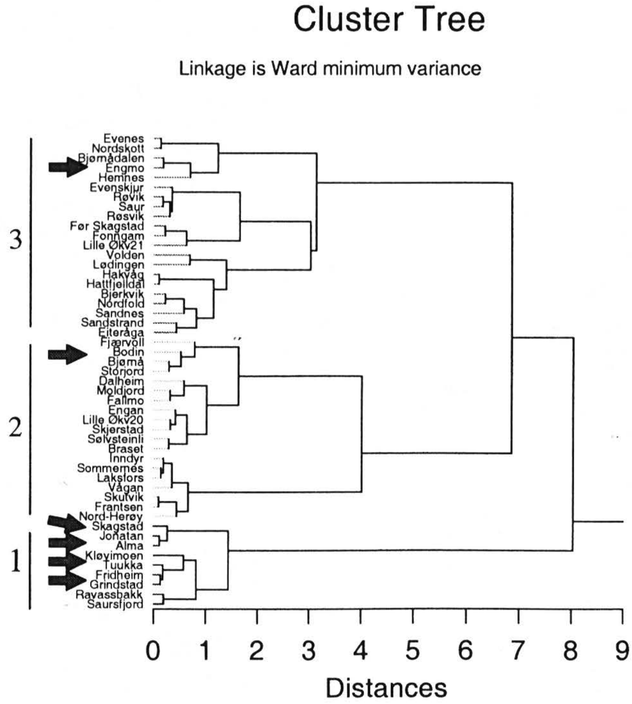
	Relative dry matter yield		
Pop./Variety	1st Cut	2nd Cut	Total
Local populations			
Highest	106	117	106
Lowest	79	91	89
'Bodin' *standard*	*100 (5.66)*	*100 (2.08)*	*100 (7.74)*
'Engmo'	91	99	94
'Grindstad'	83	143	99
'Jonatan'	93	114	98
'Alma'	88	110	94
'Tuukka'	95	117	101
C.V.%	7.22	8.57	5.97
$L.S.D._{0.05}$	0.37	0.18	0.43

Table 3. Persistence characters of 43 local populations and 6 varieties of timothy. Ground cover is presented in per cent, while the scale values for freezing tolerance shows 0 = completely dead plant, and 9 = living without any visible damage.

	Ground cover		Freezing tolerance
Pop./Variety	Spring	Autumn	(0-9)
Local populations			
Highest	90.9	98.0	5.75
Lowest	64.4	70.0	1.56
'Bodin' *standard*	*78.0*	*88.7*	*4.24*
'Engmo'	74.0	74.9	5.16
'Grindstad'	80.5	89.0	2.90
'Jonatan'	78.4	87.8	3.34
'Alma'	72.7	90.4	3.67
'Tuukka'	71.6	87.8	3.18
C.V.%	4.4	10.1	27.7
L.S.D.0.05	5.5	7.7	0.91

Ground cover in spring is mainly related to winter survival of the plants. However, the low spring cover obtained for 'Engnmo' in this experiment was associated with establishment. Nearly the same spring cover was observed for 'Bodin' and the more southern 'Grindstad', indicating low winter stresses during the field test period. However, the late growth start and low first yield of 'Grindstad' might partly have been affected by winter damage. The local populations showed a wide range in spring cover from 64 to 91 per cent. Cover the following autumn showed the ability to most of the entries to recover during growth periods. The artificial freezing test showed the highest tolerance for 'Engmo' and significantly lover tolerance for 'Grindstad'. The freezing tolerance of the local populations ranged from somewhat higher than 'Engmo' to significantly lower than 'Grindstad'. Spring cover corrected for ground cover the previous autumn, was significantly correlated with freezing tolerance, r = 0.6.

Figure 1. Cluster tree based on 10 traits. The selection of traits is based on PCA explaining more than 80 per cent of the variance among the varieties and local accessions.



Following a principal component analyses 10 characters were selected for cluster analysis using Ward minimum variance method, to assess levels of genetic diversity between populations (Figure 1). Three main clusters could be distinguished. Cluster 1 (lowest on the Tree) consisted of the Southern variety from Norway, and the Swedish and Finish varieties, together with a few local populations. The varieties 'Bodin' and 'Engmo' were represented in Cluster 2 (middle) and Cluster 3 (highest), respectively. The most promising local populations from an agronomical point of view, was found in the main cluster including 'Bodin', but mainly in the lowest (on the Tree) sub cluster with some genetic distance from that variety.

Conclusions

The results from these observations indicated that the local populations represent new genetic variation compared to the commercial varieties, and could still be of interest when breeding timothy for northern climatic areas where new cultivars have to be adapted to survive a long period with negative carbon balance and winter stresses, combined with intensive growth during a short summer period with mostly 24 h photoperiod. A number of local populations with high forage yield, good yield distribution, and sufficient winter hardiness were selected for future breeding work.

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A PRELIMINARY COMPARISON OF CLONAL AND PROGENY SWARD SYSTEMS FOR THE EVALUATION OF F_2 GENOTYPES OF PERENNIAL RYEGRASS *(Lolium perenne L.)*

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Abstract

Perennial ryegrass varieties bred in Northern Ireland at the Northern Ireland Horticulture and Plant Breeding Station, Loughgall are used extensively for forage production on farms throughout the UK and Ireland. Primary breeding objectives include herbage yield, persistency, herbage quality and disease resistance. Several breeding methods are being used at the station, based mainly upon the selection of F_2 genotypes, followed by evaluation in progeny swards or clonal swards.

An experiment was conducted to evaluate progeny and clonal swards in their effectiveness in identifying the best genotypes with which to make new synthetics, taking into consideration the labour requirements and costs of both systems. Preliminary results suggest that while both systems can be used effectively to identify superior material, the progeny sward system is less costly.

Introduction

In the British Isles, long-term leys for forage production are based almost entirely on perennial ryegrass because of its high yielding capacity, nutritive value, adaptability to a wide range of environmental conditions and prolific seed production. In order to attempt to produce varieties for the local market, perennial ryegrass breeding commenced in Northern Ireland in 1952 with the main objectives of breeding high yielding, persistent varieties which were well-adapted to local environmental and management conditions. At present varieties such as Moy, Spelga, Glen, Kells and Navan occupy prominent positions on Recommended Lists throughout the UK and Ireland and are extensively used in seeds mixtures.

At Loughgall, most new grass varieties are produced by crossing parental material with complementary characteristics, selecting superior genotypes from the F_2 generation, evaluating this material by means of a performance or progeny test and ultimately polycrossing the top genotypes to produce new synthetics. As commercially successful varieties have been produced at the station by both performance testing in clonal swards (Johnston & McAneney, 1994) and by progeny testing, an investigation was carried out to directly compare the effectiveness of both systems, taking into consideration the labour and other costs relating to the two breeding systems.

Materials and Methods

Crossing and Spaced Plant Selection

The material used for this evaluation arose from a pair-cross carried out in 1993 between Fetione, a high yielding tetraploid perennial ryegrass of intermediate maturity and late-heading F_2 genotypes selected from a cross between Fanal and Tyrone C_1. A total of 15 pair crosses were carried out in crossing bags which were supplied with pollen-free ventilation and seed from each cross was bulk-harvested. Six F_1 seedlings were grown from each cross (total 90 plants) and allowed to freely interpollinate in collective isolation, yielding F_2 seed, which was harvested in bulk.

In October 1994 1000 F_2 seedlings were transplanted into a spaced-plant nursery at 0.5 m x 0.5 m spacing. A compound fertiliser was applied at the time of planting and on three occasions in 1995 d 1996, giving total applications equivalent to 180 kg N, 90 kg P and 90 kg K per hectare per annum. Broad-leaved weeds and indigenous grasses were controlled using selective herbicides, allowing the spaced plants to grow with minimal competition. Throughout the growing season (i.e. March to October) in 1995 and 1996 important characteristics such as plant size, heading date and disease resistance were recorded by visual assessment on a 1 to 9 scale and entered onto a database.

Progeny Sward Preparation

In late May 1996, just prior to anthesis, data collected since March 1995 were used to identify the top 250 plants. Seed heads were subsequently removed from the 750 unselected plants allowing the 250 remaining to freely open-pollinate, yielding 250 lots of progeny (F_3) seed. Further notes taken after harvest and seed yield information were used to reduce the final population to 100 and seed of these was used to sow unreplicated field plots (4.5 m x 1.5 m) in September. Four plots each of the control varieties Fetione and Condesa were also included. In winter 1996/97 the mother plants were moved to a reserve nursery where further notes could be taken on important characteristics.

In 1998 and 1999, plots were cut five times at regularly spaced intervals between April and November using a Haldrup plot harvester. Herbage samples were retained for dry-matter determination and visual assessments were made of sward density and re-heading on several occasions in 1998 and 1999. At Lelystad in the Netherlands where additional plots had been sown in 1997, quite severe attacks of crown rust (*Puccinia coronata*) occurred and visual assessments of resistance were made on five occasions during 1997.

Clonal Sward Preparation

In winter 1996/97, while the 100 mother plants were being transferred to the field reserve nursery, vegetative pieces from each plant were manually propagated into 84 single-tiller ramets. These were allowed to grow in a peat-based compost in the glasshouse until March 1997 when they were transplanted into clonal plots in the field, measuring 0.75m x 1.5 m. with equal spacing between each ramet.

In 1998 and 1999, the plots were cut six times at regularly spaced intervals between April and November at a height of 40mm with a pedestrian operated rotary mower. The fresh yield from each plot was recorded and samples were taken for laboratory dry-matter determination. On several occasions during both harvest years, notes were taken on sward density, crown-rust resistance, mildew resistance and re-heading. Percentage dry matter digestibility was determined on herbage samples retained in September 1998 using the pepsin-cellulase *in vitro* technique (Tilley and Terry, 1963).

Results and Discussion

Spaced Plants

The parental varieties used in this cross were carefully chosen so as to maximise the possibility of developing material which combined the favourable characteristics of high yield with good persistency and rust resistance. Subsequent examination of the F_2 plants displayed wide differences in morphological characteristics: some plants were small or recovered very slowly after cutting while others were quite susceptible to crown rust and other foliar diseases.

Many breeders rely heavily on visual assessment of individual spaced plants, while others are critical of spaced plants as a breeding method because in the absence of competition, poor plants can excel. However, at this station, commercially successful varieties have been bred using spaced plants and this procedure continues to be used. At other breeding stations spaced plants have been used effectively in breeding for other characteristics such as water-soluble carbohydrates (Humphreys, 1989).

Progeny Swards

One of the main practical reasons for establishing progeny tests is the difficulty of obtaining quantitative measurements on individual plants, whereas seed progenies can be sown in small swards. However, criticism can be directed at the method in which seed is produced by open pollination in the spaced plant nursery in that there may not have been an even distribution of pollen, some plants contributing more than others depending upon their location and heading date. The progeny sward system also depends upon being able to keep the other plants alive for a number of years: plants may die if they become infected with viruses.

In 1998 the yield of the progeny swards in this trial ranged from 12.14 to 17.21 $t.DM.ha^{-1}$ while in 1999 the highest yielding was 12.91 tonnes, recorded in progeny 136 which was also highest yielding in 1998. Notes taken throughout the trial period indicated that most plots had an acceptable level of sward density while a good correlation was observed between re-heading notes taken in 1998 and 1999. Notes taken in the Netherlands indicated that, in general, most of the progenies had quite acceptable rust resistance although there was a range in rust scores between 4.4 and 8.6.

Three new synthetics, differentiated by heading date and rust resistance, were produced from this material in 2000 and will be evaluated on several sites in the UK and Ireland prior to NL application.

Clonal Swards

The use of clonal swards is a feature of the work at Loughgall and has been used successfully in the breeding of successful varieties such as Corbet, which holds a prominent position on the current NIAB Recommended List for England and Wales. One of the reasons for adopting this technique was to be able to evaluate the performance of superior F_2 material in mini-swards, without the drawbacks associated with producing progeny seed. A further advantage is the ability to be able to evaluate vegetative material over a long period of time, possibly giving an accurate assessment of persistency. However, the clonal sward system gives no indication of the Specific or General Combining Ability of the material.

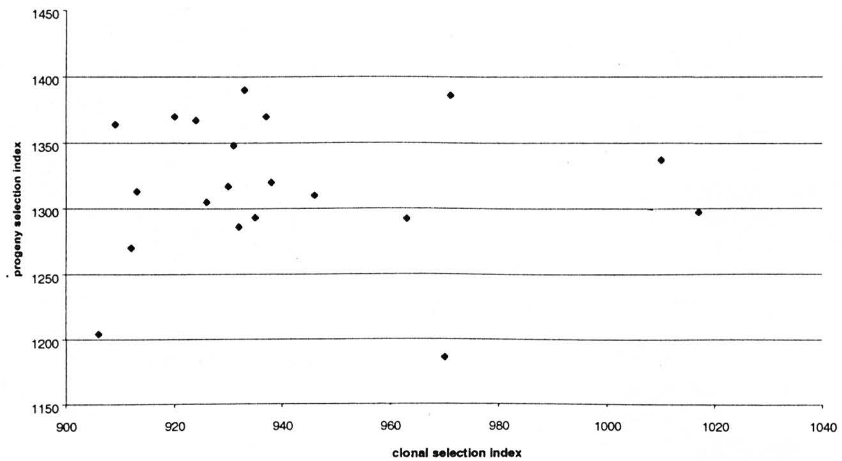
In this experiment two new synthetics, differentiated by heading date, were made and will be fully tested in the same trials as the synthetics made from the progeny swards.

Comparison between both systems

In autumn 1999, when the material had been tested for two complete growing seasons in both clonal and progeny swards, new synthetics were made from the top genotypes. Additional data from the clonal field reserve and the small plot trial in the Netherlands was fully utilised by means of a selection index, applying the heaviest weightings to yield and sward density.

When this exercise was completed it was found that 7 genotypes were placed in the top 20 of both clonal and progeny sward rankings. However, the correlation coefficient in Figure 1 was not significant at -0.03. This and the wide scatter of the other points suggests that there was little relationship between the two systems in terms of the order of the genotypes which were favoured. This lack of relationship was demonstrated by top yielding progeny 136 which was ranked 83rd in the clonal swards in terms of 1999 total yield.

Figure 1: Relation between selection indices for clonal and progeny plots



Labour Requirements and Costs

A comparison of the labour requirements and other costs associated with establishing and maintaining progeny and clonal swards for two complete harvest years was made and showed that clonal swards were approximately twice as costly as progeny swards. This was due mainly to the high labour requirement for manual tiller preparation and planting.

Conclusion

The final conclusion of the comparison cannot be made until new synthetics made from both breeding systems have been thoroughly tested in sward trials on several sites. However, several preliminary conclusions can be drawn:-

1. Clonal swards are a good alternative to progeny swards if specialised equipment, such as a plot drill or harvester, or adequate land is not available.

2. Clonal swards have a much higher labour requirement than progeny swards, resulting in higher costs. However, the labour intensive task of vegetative propagation may be carried out during the winter months.

3. The availability of progeny seed provides the opportunity of evaluating material on several sites where environmental conditions are different, thereby increasing the chances of breeding varieties with a low genotype x environment interaction. However, on occasions some spaced plants produce insufficient seed with which to sow a plot.

As outlined earlier, commercially successful varieties have been developed at Loughgall by both the clonal and progeny sward system. Other systems, such as the inbred full-sib method and recurrent selection have also been used. While some breeding systems may be more effective or less costly in developing superior varieties, it is our opinion that the quality of the germplasm and the location of the breeding station are both more important than the breeding system.

Acknowledgements

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HETEROSIS EFFECT IN SYNTHETIC POPULATIONS OF HYBRID ALFALFA (*Medicago media* PERS.)

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Abstract

Genetic and statistical analysis of hybrid vigour carried out for 11 traits in 9 synthetic populations in SYN – 1 and SYN – 2 generations of alfalfa indicated high differentiation with respect to the analysed traits between the population.

Narrow variability range of the value of hybrid vigour was found for: the number of shoots, protein content and the number of flowers per inflorescence (V% = 9). The highest value of the value of hybrid vigour was noted for: the yield of fresh mass, the yield of dry mass. The yield of protein, the number of pods per fructification, setting of pods and the yield of seeds (V% = 42.3). The calculated high coefficients of determination and repeatability (p = 0.834) for the analysed traits indicate the possibility of carrying out effective selection.

Hybrid vigour for the analysed traits and populations was, generally, by 18% Lower in SYN-2 generation than in SYN-1 one.

Introduction

Breeding of classic hybrid cultivars of alfalfa is difficult and unprofitable for many reasons. The yield increase of hybrid cultivars (F_1) is not always high enough to make development and maintenance of inbred lines or clones profitable. Also the biology of alfalfa flowering including pollination by insects and often considerable extent of self-pollination, is an obstacle. It prevents making precise crossings between selected genotypes. The increase in green mass and seeds brought about by heterosis in case of alfalfa can be successfully used in breeding synthetic cultivars. They are created as a result of panmictic over-crossing of selected genotypes with proven high combination capacity. The aim of this work was presentation of the heterosis effect in synthetic alfalfa populations Syn-1 and Syn-2 with respect to several chosen traits.

Plant Material and Methodology

From the developed initial material 56 inbred lines were selected. They were evaluated in the following generations: S_1, S_2, S_3, S_4 and S_5. From the inbred lines in the S_5 generation with the highest general and specific combination ability (G.C.A. and S.C.A.), expressed by the increase of green mass yield by 20-40% higher as compared to control cultivars, 9 synthetic populations were bred. The inbred lines (S_5) were combined in such a way that in a given synthetic population there were no lines related to each other. An exception were populations N° 5 and 6 made from the related lines in order to determine the influence of affinity on the heterosis effect expressed in the yield of green mass and seeds. Composition of the analysed synthetic populations is given in Table 1. Experiments with the synthetic populations Syn-1 and Syn-2 were made in a system of completely randomised blocks with four replications. The control cultivars were Radius and Boja. On the plots of 10 m^2 12 kg/ha of seeds were sown in rows 40 cm apart. The synthetic populations were evaluated during three years of full utilisation.

The plants in each synthetic population in the Syn-1 and Syn-2 generations and in the control cultivars Radius and Boja were assessed basing on observations and measurements of developmental phases and the traits related to the yield of green mass and seeds. The hybrid vigour was calculated for 12 traits of 9 synthetic alfalfa populations in the Syn-1 and Syn-2 generations. It was calculated as a value of this trait with respect to its mean value in parent plants. For these traits also the coefficients of repeatability and determination were calculated. The results are given in Tables.

Results

The mean yield of green mass for the parental populations was 555.2 dt/ha (Table 2). The hybrid vigour in the populations Syn-1 varied from 20.9 to 136.3%. In the populations of the Syn-2 generation the hybrid vigour fell on average by 15%. An exception was the population N° 5 which in the Syn-2 generation had higher vigour than in Syn-1, i.e. 55.1 and 88.3%, respectively (Table 2). The populations N° 4, 8 and 9 indicated slight fall of the hybrid vigour in the generation Syn-2, whereas the green mass yield for the populations N° 3, 6 and 7 had negative value (Table 2).

The dry mass yield for the parental populations ranged from 112.8 to 180.6 dt/ha (Table 2), while the hybrid vigour in the populations Syn-1 varied from 27.1 to 40.8%. In the Syn-2 populations the hybrid vigour dropped. The value of the fall for different populations varied – from slight for the populations N° 1, 4 and 9 to considerable for the populations N° 3 and 8 (Table 2). An exception were the plants of the population N° 5 whose dry mass yield in Syn-2 was higher than in Syn-1.

The mean seed yield from 1 ha for the parental populations was 1.45 dt, while the percentage of the hybrid vigour for the yield of seeds from synthetic populations in the generation Syn-1 varied from 133.3 to 461.0% (Table 2a) and for the populations N° 2, 4, 5, 8 and 9 was over 250%. In the Syn-2 generation there was a fall in the heterosis effect. An exception were the plants from the population N° 5 whose percentage of the hybrid vigour in the Syn-2 generation was higher than in Syn-1 by 57.0%, reaching the value of 484.6% (Table 2).

The results of the analysed traits of the synthetic populations in the generations Syn-1 and Syn-2 constituted the basis for calculating repeatability coefficients for the values of these traits in subsequent generation of the population and in subsequent years of the experiment. Calculated repeatability coefficients of some traits are given in Table 3. The highest repeatability was found for the number of shoots (0.837), green mass yield (0.814), dry mass yield (0.834, protein yield (0.825), number of flowers per inflorescence (0.915), number of inflorescences per shoot (0.894), number of pods per fructification (0.856), pod setting (0.924) seed yield (0.837) and number of seeds per pod (0.880). The calculated coefficients of repeatability indicate small changes in the "behaviour" of the population in the Syn-2 generation with respect to the values of the traits obtained in the generation Syn-1.

Table 3 presents also the coefficients of determination in per cent. They define the degree of match between the values of different traits estimated for each population in the Syn-2 and their expected values. The higher determination coefficient the lower is the dispersion of the obtained results with respect to the expected ones (Table 3). The determination coefficients were high for plant height (95.8%), green mass yield (91.3%), dry mass yield (89.7%), number of plants per inflorescence (92.1%), pod setting (95.8%) and number of seeds per pod (93.2%).

Conclusions

1. Wide range of variability with respect to hybrid vigour observed for green mass yield, dry mass yield, protein yield, number of pods per fructification, pod setting and seed yield can constitute the basis for successful and effective selection of the best synthetic populations.
2. In the Syn-2 generation, for most populations and analysed traits, a 15% fall in the hybrid vigour was noted. An exception was the population N° 5 whose genotype reaction was different. Its value of the hybrid vigour for most features was higher in the Syn-2 generation than in the Syn-1 one.
3. The highest value of the hybrid vigour resulting from the heterosis effect was found, for most analysed traits, in the synthetic populations N° 4, 5, 8 and 9. They can be an object for the next stage of genetic and breeding studies.

Table 1. Composition of the synthetic populations in the Syn-1 and Syn-2 generations

Synthetic population N°	Inbred lines included in the population	Degree of inbreeding
1	A_3, B_{10}, F_1, $G_{1/1}$	S_5
2	A_3, B_{10}, F_1, $G_{1/1}$	S_5
3	A_3, $E_{1/2}$, F_1, $G_{5/1}$	S_5
4	B_{10}, F_1, $G_{1/1}$	S_5
5	D_3, D_5, $G_{1/1}$	S_5
6	A_3, D_5, $G_{1/1}$, $G_{5/1}$	S_5
7	D_5, E_{12}, $G_{1/1}$	S_5
8	D_5, $E_{1/2}$, $G_{5/1}$	S_5
9	$E_{1/2}$, F_1, $G_{5/1}$	S_5

Table 2. Characteristics of the heterosis effect in the synthetic populations Syn-1 and Syn-2 of hybrid alfalfa (*Medicago media* Pers.)

Syntheses	Plant height (cm)			Number of shoots (item)			Green mass yield			Dry mass yield			Protein content (%)			Protein yield		
	P̄*	Syn-1 (%)	Syn-2 (%)	P̄*	Syn-1 (%)	Syn-2 (%)	P̄* (dt/ha)	Syn-1 (%)	Syn-2 (%)	P̄* (dt/ha)	Syn-1 (%)	Syn-2 (%)	P̄*	Syn-1 (%)	Syn-2 (%)	P̄* (dt/ha)	Syn-1 (%)	Syn-2 (%)
Syn -1	57,9	44,6	22,8	64,8	10,8	3,4	586,9	136,3	110,2	114,6	40,8	39,2	22,0	4,9	4,0	25,2	14,2	17,4
Syn – 2	59,0	12,5	-2,2	65,5	26,0	11,9	526,4	31,1	15,7	119,8	35,4	26,9	21,8	10,0	8,7	26,1	-3,8	-7,0
Syn – 3	60,2	4,0	-12,1	63,5	-17,6	-15,9	551,3	-11,1	-20,3	124,3	28,4	7,2	21,6	-0,8	1,9	26,8	-9,0	-5,0
Syn – 4	54,4	49,1	43,6	65,2	15,3	4,3	525,4	50,2	48,9	112,8	35,0	33,8	22,1	8,9	7,2	24,9	20,8	23,6
Syn – 5	55,4	65,4	55,2	62,0	30,8	31,0	620,4	55,1	88,3	131,8	39,2	47,0	21,9	12,2	15,3	28,9	19,3	24,2
Syn – 6	60,0	9,5	-2,2	62,6	-9,3	-10,4	525,6	6,8	-3,3	118,1	-14,6	-20,8	22,1	-15,4	-13,1	26,1	-11,3	-18,8
Syn – 7	55,0	9,8	-2,9	64,9	-25,1	-22,7	511,9	-20,9	-26,0	114,3	-27,1	-35,4	22,6	-9,7	-7,1	25,8	-24,3	-14,2
Syn – 8	57,5	80,6	78,1	63,4	8,9	5,6	517,9	41,9	37,4	120,6	40,3	18,9	21,8	19,2	10,1	26,3	16,4	16,3
Syn – 9	59,4	71,9	69,1	63,5	17,8	22,5	631,2	59,2	53,2	180,6	38,2	37,4	21,5	4,1	3,4	38,9	18,6	10,6
Radius (check)	58,3			62,8			580,2			109,5			21,4			25,2		
Boja (check)	57,0			59,2			574,7			106,6			22,0			26,9		

* - mean value of a trait for parental forms

Table 2a. *Characteristics* of the hetereosis effect in the synthetic populations Syn-1 and Syn-2 of hybrid alfalfa (*Medicago media* Pers.)

Syntheses	Number of flowers per inflorescence (item)			Number of inflorescences per shoot (item)			Number of pods per fructification (item)			Pod setting (%)			Seed yield		
	- P*	Syn-1 (%)	Syn-2 (%)	- P*	Syn-1 (%)	Syn-2 (%)	- P*	Syn-1 (%)	Syn-2 (%)	- P*	Syn-1 (%)	Syn-2 (%)	- P* (dt/ha)	Syn-1 (%)	Syn-2 (%)
Syn -1	17,2	10,6	24,8	35,4	24,3	20,2	6,5	172,3	140,0	33,4	131,7	93,4	1,5	173,3	126,7
Syn – 2	16,3	24,3	20,5	26,2	20,5	14,5	6,3	88,9	65,1	34,7	61,4	38,6	1,7	247,1	170,6
Syn – 3	16,0	30,2	36,4	27,4	31,4	27,4	6,6	36,4	1,5	32,2	46,9	9,9	1,5	133,3	133,0
Syn – 4	18,8	14,1	12,4	39,4	17,2	10,3	5,9	184,7	193,2	33,1	128,4	118,7	1,2	461,0	322,0
Syn – 5	17,4	35,4	54,3	36,2	45,2	58,2	6,7	88,1	49,3	40,5	109,2	166,4	1,7	308,6	484,6
Syn – 6	15,8	14,3	10,8	28,2	31,4	34,2	6,6	59,1	33,3	21,9	35,7	12,9	1,3	140,0	126,6
Syn – 7	17,2	15,0	10,2	20,4	26,2	31,2	6,7	64,2	17,9	32,3	86,9	53,3	1,5	173,3	93,4
Syn – 8	15,3	68,5	49,2	32,8	68,4	72,3	6,4	179,7	123,4	31,9	96,2	71,5	1,5	357,5	285,6
Syn – 9	18,4	54,3	46,4	40,3	61,4	43,2	6,0	173,3	101,7	41,5	118,4	82,5	1,2	330,9	314,6
Radius (check)	15,4			28,4			9,9			34,5			1,8		
Boja (check)	17,9			31,2			5,4			27,0			1,1		

*** - mean value of a trait for parental forms**

Table 3. Repeatability coefficients and determination coefficients between the populations of the Syn-1 and Syn-2 generations for selected traits

N°	Trait	Repeatability coefficient	Determination coefficient
1	Number of shoots	0,837**	81,39
2	Plant height	0,791**	95,87
3	Green mass yield	0,814**	91,36
4	Dry mass yield	0,834**	89,78
5	Protein content	0,737**	73,24
6	Protein yield	0,825**	85,34
7	Number of flowers per inflorescence	0,915**	92,10
8	Number of flowers per shoot	0,894**	77,36
9	Number of pods per fructification	0,856**	83,80
10	Pod setting	0,924**	95,89
11	SEED YIELD	0,837**	76,72
12	Number of seeds per pod	0,880**	93,24

** - significance level = 0.01

P. Monjardino, A. da Câmara Machado, V. Carnide (eds.): Breeding for stress tolerance in fodder crops and amenity grasses; Department of Agricultural Sciences, University of Azores, Terceira, 2001

EVALUATION OF INBRED LINES IN ITALIAN RYEGRASS

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Abstract

Italian Reygrass (*Lolium multiflorum* Lam.) is a fodder grass for a one to two years cropping system. It is self incompatible with two incompatibility loci S and Z (Fearon et al. 1983).

Therefore selfings of L. *multiflorum* do not occur naturally. However, self progenies can be obtained by temperature treatment of flowering plants in a growth chamber (Eickmeyer 1994).

With this method several hundered inbred lines (I1) were obtained. 170 of them, derived from different populations were tested as spaced plants (20 plants per line) in 1999. Simultaneously the inbred lines were pollinated in a topcross design with two good pollinator varieties.

The results of the 1999 trial can be summarized as follows: Significant differences between population were obtained for tillering, sward density, growth habit, ear length, number of fertile tillers, tiller diameter, flag leaf length and 1000-Kernel-weight, but not for regrowth, plant heith, plants girth, flag leaf width and see yield. Significant differences between lines with populations were found for all traits to be measured on single plants, that means all traits beside seed yield and 1000-Kernel-weight.

Introduction

Italian Ryegrass (*Lolium multiflorum* L.) is a fodder grass for a one to two years cropping system. It is self incompatible with two incompatibility loci S and Z (Fearon et al. 1983). That is why selfings of *L. multiflorum* do not occur naturally. In breeding programmes lines are tested for general combining ability (GCA) in topcross or polycross tests. Productivity is measured on clones. Clones are heterozygous and many weak recessive genes are covered. Therefore after crossings the heterosis of the clones cannot be used in its full extent. Self progenies of *L. multiflorum* can be obtained by temperature treatment of flowering plants in a growth chamber (Eickmeyer 1994). On inbred lines the genetic potential can be obtained at an earlier stage of the breeding programme since the level of homozygosity is increased, offering the possibility to reduce the number of expensive tests for productivity including yield components.

Materials and Methods

170 inbred lines of *L. multiflorum* derived from 15 populations were tested as spaced plants during spring and summer 1999. Simultaneously the inbred lines were pollinated in a topcross design with two good pollinators. Data were obtained from 20 plants per line, 10 of them were cut in June, the remainig 10 plants were used for seed production.

Results

Variances of traits are given in Table 1. Some traits differed between populations: tillering, sward density, growth habit, ear length, number of fertile tillers, tiller diameter, flag leaf length and 1000-kernel-weight. Variances of traits between lines within populations demonstrate that they were all usable for selection. Correlation coefficients between traits are shown on Table 2.

Discussion

To make use of results from inbred lines showing large differences one has to check if these results are correlated with the GCA from topcross progenies.

Synthetic populations still have a large genetic potential for improvement. This is not exhausted yet, as the results of the analysis of variance between inbred lines within populations show. Therefore it is worth to select extensively between inbred lines of a population.

Correlations between traits exist although they are not very close. Therefore it should be possible to combine desired traits in one variety.

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Acknowledgements

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Table 1: Relative values of variances between populations (n=15) to between inbred lines within populations (n=170) and variances between inbred lines to plants within inbred lines

traits	between populations: between lines	between: within lines
tillering[2]	1.19 *	7.36 **
sward density[2]	1.92 *	7.10 **
growth habit[2]	1.84 *	10.86 **
regrowth[1]	1.76	4.09 **
ear length[1]	1.78 *	9.81 **
number of fertile tillers[2]	1.93 *	8.61 **
plant height[2]	1.00	7.84 **
plant girth[1]	0.90	6.05 **
fresh matter[1]	0.75	5.31 **
tiller diameter[1]	3.07 **	6.42 **
flag leaf width[1]	0.78	6.72 **
flag leaf length[1]	2.32 **	5.03 **
seed yield[1]	0.89	.
1000-kernel-weight[1]	2.19 **	.

*,** significant at P = 0.05 and P = 0.01, respectively
[1] measured on 10 plants
[2] measured on 20 plants

Table 2: Some selected correlation coefficients between single plant traits

trait	number of fertile tillers	tillering	fresh matter	sward density	plant girth	plant height	1000-kernel-weight	seed yield
ear length	0.15	0.23	0.42	0.10	0.39	0.47	0.22	0.26
number of fertile tillers		0.62	0.59	0.46	0.60	0.22	0.05	0.32
tillering			0.58	0.78	0.53	0.40	0.04	0.43
fresh matter				0.43	0.85	0.51	0.21	0.42
sward density					0.38	0.33	-0.01	0.42
plant girth						0.39	0.11	0.35
plant height							0.15	0.34
1000-kernel-weight								0.16

All correlation coeffitients >0.20 are significant at P=0.05

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RELATIONSHIP BETWEEN THE AGRONOMIC CHARACTERS AND SEED HARDNESS IN ALFALFA (*Medicago sativa* L.)

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Abstract

In natural populations seed hardiness is an adaptation to extreme climatic conditions. For the alfalfa production technology the seed hardiness is a disadvantage. To compensate for seed hardness, large quantities of seed are usually sown, but due to the protracted germination a control is difficult to make however it should take place at a particular phenophase of alfalfa, since plants emerging later may be damaged or exhibit retarded development. Further more, an excess of 40 % seed hardness is a disadvantage for the western seed markets, since only seed germinating within 10 days are considered to be able to germinate.

If small lots are to be marketable as „bio-alfalfa" the ratio of hard-coated seeds must not exceed 5%.

Four genetically different populations were divided each in a normal and a hard seed fraction, on which green yield and yield components (plant height and shoot number per plant) were studied in field.

Our experimental data suggested that before beginning selection the varieties or lines capable of highest yield under the given ecological conditions should be chosen. Preliminary experiments will then show whether in any of these the plants emerging from normal seeds have more favourable characteristics than those arising from hard seeds. This will then be followed by mass selection (positive or negative) of initial population(s).

Introduction

The seedcoats of alfalfa seeds may not be permeable to water in the soil. The permeability of alfalfa seedcoats can change over time. For the alfalfa production technology the hard seedcoat is a disadvantage. Lute (1928) and Leggatt (1927) found that environment of soils decreased hard-seededness and that fewer hard seeds remained in soil tests than in blotter tests.

The use of germinated lucerne seeds in salads is a special use of the crop. If small lots are to be marketable as „bio-alfalfa" the ratio of hard-seeds must not exceed 5%. However it is important to study whether selection against the hard seedcoats will not have a negative impact in other economically important characters.

Materials and Methods

Normal and hard-coated seeds were selected from four genetically different populations. The germs were rubbed out between two rubber surfaces in order to preserve the original state of the seed coat and to avoid mechanical injuries which would promote water inhibition. Seeds were pre-germinated of 10 days. Those that did not germinate were regarded as the hard seed fraction and were then established in the greenhouse. 60 genotypes per entry were planted out in the field in four replications. The green yield and its main yield components (plant height and shoot number) were investigated for each genotype.

Results

Differences up to 44,6 % were recorded between the yield potentials of the four selected breeding populations. Compared to the 1st population, the 2nd yielded +2,9 %, the third +27,4% and the fourth +44,6 % more (Tab. 1.). Over all four populations, the hard-coated fractions had a +7,7 % higher four-year green yield compared to the normal seed populations. A comparison within each population showed that the hard-coated seeds fractions were more productive for populations 1 and 4, while in the other two populations (2, 3) they yielded 9,9-21,6 % less than the normal seed fractions (Fig. 1)

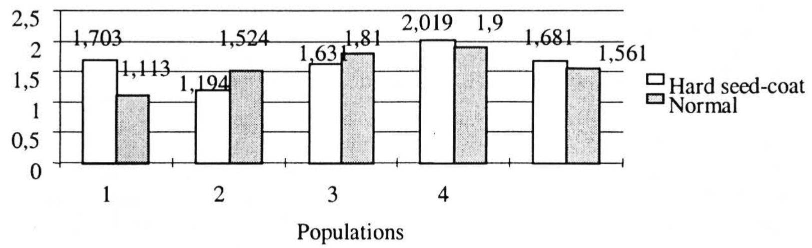

Fig. 1 Four-year green yild of normal and hard seed-coated alfalfa genotypes of four populations (1-4) from 1993-1996.

Table 1. Green yield averages of alfalfa genotypes with normal and hard-coated seeds

Combination	Green yield average	
	kg/genotype	Rel.(%)
1. population	1,356	100,0
2. population	1,395	102,9
3. population	1,727	127,4
4. population	1,960	144,6
Experimental mean	1,618	119,3

An analysis of the annual green yield showed that the yield dynamics of the normal and hard-coated fractions was similar: in the second year highest yields were observed, as expected, followed by a decline in the third year. In the fourth year, however, there was a increase in the average yield per plant (Fig. 2)

The persistence was analysed on the basis of annual plant number. For the first two populations only 50 % of the plants produced a yield in the fourth year (64 plants in population 1 and 60 in population 2) while this ration was 84 % and 85 % for populations 3 and 4, respectively. Much greater differences among populations were observed over the four years between the normal fractions (41,2 %) than between the hard-coated fractions (30,6 %) in plant height. Genotypes from hard-coated seeds of the 1st and 4th populations were taller in all the years than those of the normal fractions. Within the individual populations a clear difference between the fractions was only observed for the 1st population: the plants in the hard-coated group were 45 % taller than those in the normal group. In the other populations this difference ranged from 4,2 % to 16,5 %. The annual dynamics of plant height was similar to that of the green yield.

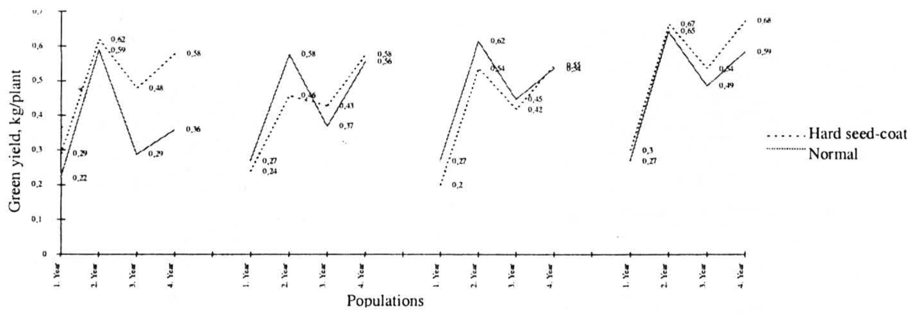

Fig. 2 Yield dynamics of normal and hard seed-coat alfalfa genotypes of four populations (1-4) in 1993-1996

The difference between the three-year averages of shoot number of the two fractions (5,4 %) increased still in the fourth year, with a total of 11,6 % more shoots of plants grown from had-coated seeds than for those emerging from permeable seeds. A particularly large difference was recorded for the 1st population, where the hard seed fraction produced 57,5 % more shoots than the normal group. The trend was similar to that observed for green yield and plant height: the hard-coated seeds produced plants with 7,6-18 % more shoots in populations 1 and 4, and an opposite trend in the other populations.

Over the years great differences between the two fractions were constantly observed in the 1st population, though with a decreasing tendency (2nd year: 57,6 %, 3rd year: 47 %, 4th year: 38,7 %)

Table 2. Correlation between yield and yield components and spring development in the second year.

Year	Green yield		Plant height		Tiller number	
	Normal	Hard-coated	Normal	Hard-coated	Normal	Hard-coated
1993	0,70	0,41	0,93	0,65	0,94	0,21
1994	0,45	0,71	0,86	0,87	0,87	0,20
1995	0,33	0,75	0,71	0,77	0,49	0,12
Three-year total	0,46	0,66	0,74	0,75	0,43	0,27

Table 3. Correlation between green yield (x) and its components (y) in normal and hadcoated alfalafa populations

Character	Plant height		Tiller number	
	Normal	Hard-coated	Normal	Hard-coated
Green yield (three-year)	0,93	0,91	0,60	0,90
Plant height (three-year)			0,29	0,71
Green yield (four-year)	0,96	0,91	0,83	0,90
Plant height (four-year)			0,68	0,70

Conclusions

Cocozza and Pacucci (1965) described seed hardness as a variety-specific, hereditary trait. This was confirmed by the analysis of various Hungarian varieties (Gimesi, 1958). Hard seeds not only had higher biological value from a survival point of view, but also had greater green yields (Czimber, 1967). Therefore the size of the yield surplus and the cost of overcoming seed hardness would decide whether it was worth increasing the proportion of hard seeds in alfalfa varieties. In the present experiments a better yield potential of 7,7 % of the hard seeds was recorded for the average over the four lines. When the difference between the two groups (hard and normal seeds) was studied for each line separately, superiority of hard-coated seeds was not a general phenomenon. The majority of producers prefer lots with less than 10 % hard-coated seeds (Fedorov, 1968). This is particularly evident if alfalfa seed is required for human consumption (germinated seed for salads), where it is desirable for all the seeds to germinate at the same time. It is characteristic of the annual yield dynamics of the four populations that the yield increased dramatically in the second year compared with the first, but dropped by 25-45 % again, in the third year. Irrespective of whether the normal or hard seeds gave the highest annual yield, the yield dynamics exhibited a similar pattern in both seed fractions of alfalfa. With respect to regrowth ability, the annual changes showed the same trend in both fractions of populations 2 and 3. However, in population 1 the hard-coated fraction appeared to be more valuable, not only due to the higher shoot number observed each year, but also because there was hardly any decline in shooting ability in the third and fourth year compared with the excellent value measured in the second year, while in the normal group there was a drop of 18 % (29 shoots per plant). If seed hardness is to be reduced it would be advisable to use a population such as population 3 as starting material, where an increase in shooting ability was observed in all four years.

The experimental data do not demonstrate whether hard-coated or permeable seeds are the more valuable in alfalfa. Before beginning selection the varieties or lines capable of the highest yield under the given ecological conditions should be chosen. Preliminary experiments will then show whether in any of these plants emerging from normal seeds have more favourable characteristics than those arising from hard seeds. This will then be followed by negative or positive mass selection on the selected variety.

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VARIOUS APPROACHES TO GENERATE BASIC MATERIAL FOR BREEDING OF FORAGE GRASSES BY WIDE HYBRIDIZATION

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Abstract

Wide hybridization offers a great potential for crop improvement by broadening the intraspecific genetic variability. Four strategies were used to introduce novel basic material in grass breeding: generation of novel alloploids with inhomologous genomes (1), generation of chromosome addition or substitution lines (2), development of stable and highly fertile alloploids from parental species with homoeologous genomes (3), introgression of desired qualitative characters from one species into another species (4).

1) Artificial autotetraploids from *Lolium* and *Festuca* were crossed as females with natural autotetraploids like *Dactylis glomerata* and *Arrhenatherum elatius* as male parents to generate meiotically stable amphidiploids. Additionally, *L. multiflorum* was crossed with *Phleum pratense* (2n = 14 and 42) and *Cynosurus cristatus* (2n = 14). Overall more than 30 F_1 hybrids were produced.

2) The generation of chromosome addition lines from *L. multiflorum* and *F. pratensis* having distinct chromosome pairs from *D. glomerata* is in progress. One BC_2 plant with 21 *L. multiflorum* and 1 *D. glomerata* chromosome(s) was detected.

3) For overcoming the insufficient fertility and the chromosomal instability of *Lolium-Festuca* hybrids (*F. pratensis* × *L. multiflorum*, *L. perenne* or *L. hybridum*), attempts are in progress to reduce the chromosome number of F_x hybrids via anther culture from the tetraploid to the haploid level and then to select symmetrical intergeneric chromosome translocations (centromeric region from *Lolium* and telomeric from *Festuca* or vice versa) by GISH among doubled haploids.

4) *L. multiflorum* with high crown rust (*Puccinia coronata*) resistance was produced by three successive back crosses of *F. arundinacea* × *L. multiflorum* and *L. multiflorum* × *F. pratensis* hybrids. Resistant BC_3 plants were selfed and crossed between each other, and several breeding companies introduced the BC_3S_1 and BC_3F_1 into their breeding programmes.

Introduction

The aim of wide hybridization is the combination of desired features from different species. Two important strategies should be distinguished: the generation of novel hybrids (alloploids) and the enlarging of genetic variability within one species (introgression). The combination of high yield with high quality, persistence, winter hardiness and different resistances is significant particularly for forage grasses.

On the one hand, parental species with significantly increased phylogenetical distances were used for intergeneric crosses during the last decade. In vitro and in vivo techniques were optimized to overcome the barriers of generative hybridization. Even crosses between Pooideae and Panicoideae species are successful now (reviewed by Matzk et al., 1997). The potential for combination of remotely related genomes by generation of novel alloploids or by addition of alien chromosomes seems by far not exhausted.

On the other hand, intergeneric hybrids with closely related or homoeologous genomes are intensely studied already during a long period. Despite of the efforts made in controlled crosses, cytological studies and prebreeding, the fertility and chromosomal stability of these hybrids especially for *Lolium/Festuca* allopolyploids are still insufficient for a broad acceptance in plant breeding. Even examples of a successful introgression of single features from *Festuca* into *Lolium* species or vice versa are rare up to date. Four new approaches were followed to overcome the barriers or difficulties and to exploit wide hybridization for grass breeding.

Materials and Methods

Crosses between parents with inhomologous genomes were carried out as described by Oertel et al. (1996). Plants of *Lolium multiflorum* (*L. m.*), *L. perenne* (*L. p.*), *Festuca pratensis* (*F. p.*) and *Lolium-Festuca* hybrids as female parents were crossed with *Dactylis glomerata* (*D. g.*), *Phleum pratense* (6x), *Phleum pratense ssp. nodosum* (2x), *Arrhenatherum elatius* and *Cynosurus cristatus* as male parents (Table 1). Additionally, *Lolium* species were crossed with *F. rubra.*

Anther culture was used to develope highly fertile alloploids with stable chromosome number and symmetrical translocations from F_x hybrids of *F. pratensis* × *L. multiflorum*, *L. perenne* and *L. hybridum*. Anthers with microspores at about late uninuclear stage were placed onto solid medium containing auxin for induction of cell divisions. After 1 to 2 months calli were transferred to a medium without auxin for plant regeneration. Green plants were planted in soil after the 3-leaf-stage. The ploidy of the regenerated plants was determined by flow-cytometry and in some cases by chromosome counting in mitotic metaphase squashes. The viability of pollen was estimated by staining with acetocarmine.

The way and the methods for introgression of crown rust (*Puccinia coronata* Corda) resistance from *Festuca* species into *L. multiflorum* were described by Oertel and Matzk (1999). The introgression should be confirmed by genomic in situ hybridization (GISH) in rust resistant BC_3S_1 plants, the procedure was as described by Jiang et al. (1995). The GISH was also used to analyse the chromosome constitution of various intergeneric F_1 hybrids as well as backcross progenies (procedure as described by Oertel et al., 1996).

Results and Discussion

(1) Remotely related autotetraploid species with inhomologous genomes were crossed in the expectation to obtain allotetraploids showing regular meiosis as a prerequisite of fertility. 11 allotetraploid hybrids of *F. pratensis* × *D. glomerata* and *L. multiflorum* × *D. glomerata* were produced (Table 1). Regular meiosis was observed in the *L. multiflorum* × *D. glomerata* hybrid, but nevertheless the plants were sterile. Probably the course of gametogenesis was interrupted as a consequence of genomic disharmonies (OERTEL et al. 1996). Five *F. pratensis* × *D. glomerata* hybrids interrupted their development at different stages (e. g. tillering, differentiation of anthers and ovarys, before meiosis) and spores were never formed. Three three-species hybrids were produced by crossing *Lolium-Festuca* hybrids with *D. glomerata*, but they showed low vigour and no fertility.

Moreover, crosses were carried out between other remotely related species with various ploidy levels. Embryo development occured in all cross combinations due to efficient auxin application in vivo after pollination. For the first time one hybrid between *L. multiflorum* and *Cynosurus cristatus* was produced. Therefore, the potential of wide hybridization may not be exploited yet.

(2) The combination of desired features of *Lolium* or *Festuca* species with *D. glomerata* may be achieved by addition of only 1 or 2 pairs of *D. glomerata* chromosomes to the *L. multiflorum* chromosome complement, too. One BC_1 plant and 37 BC_2 plants were generated by backcrossing with *L. multiflorum*. BC_1 and BC_2 plants with 1 to 7 chromosomes

from *D. glomerata* were identified (Table 2). The fertility was increased from F_1 to BC_2. Therefore, chromosome addition lines may be more promising than allotetraploids between *Lolium/Festuca* and *D. glomerata*.

Table 1. Results of crosses of parental species with inhomologous genomes

Parental species (ploidy); [shaded]: autotetraploid female × male	Number of florets pollinated	% of grains with embryos	Number of hybrids obtained
F. pratensis (2x) × D. glomerata (4x)	731	1,91	1
F. pratensis (4x) × D. glomerata (4x)	5567	1,78	4
L. multiflorum (2x) × D. glomerata (4x)	1984	0,96	2
L.multiflorum (4x) × D.glomerata (4x)	20142	1,21	7
L. perenne (2x) × D. glomerata (4x)	497	1,41	0
L. perenne (4x) × D. glomerata (4x)	1325	1,66	0
[L. m./L. p. × F. p.] (4x) × D. glomerata (4x)	1386	0,29	3
F. rubra (6x) × L. perenne (4x)	345	2,90	2
L. multiflorum (4x) × F. rubra (6x)	168	8,93	4
L. multiflorum (4x) × F. rubra (8x)	301	10,96	14
L. multiflorum (4x) × Arrhenatherum elatius (4x)	1164	1,55	0
L. multiflorum (4x) × Cynosurus cristatus (2x)	617	0,49	1
F. pratensis (4x) × Phleum pratense (2x/6x)	290	2,07	0
L. multiflorum (4x) × Phleum pratense (2x)	454	1,32	0
L. multiflorum (4x) × Phleum pratense (6x)	2976	1,61	0
L. perenne (4x) × Phleum pratense (6x)	351	1,99	0
[L. m./L. p. × F. p.] (4x) × Phleum pratense (2x/6x)	3438	0,47	0

Table 2. Characterization of *Lolium-Dactylis* hybrids after backcrossing with *L. multiflorum*

Backcross generation	Number of plants	Chromosome constitution*	Seed set and pollen vitality
BC_1	1	21 Lm + 7 Dg	very low
BC_2	1	33 Lm + 7 Dg	low
	1	28 Lm + 7 Dg	
	1	34 Lm + 4 Dg	low
	1	? Lm + 0-7 Dg	
	1	21 Lm + 1 Dg	moderate
	6	14-28 Lm + 0 Dg	
	26	analyses in progress	

* number of chromosomes from the parental species; Lm: *L. multiflorum*, Dg: *D. glomerata*

(3) Translocations between the parental chromosomes occur frequently in *Lolium-Festuca* hybrids (Zwierzykowsky et al., 1999). The homoeologous genomes cause meiotic abnormalities and therefore, the allotetraploids are chromosomal instable and insufficient fertile. These disadvantages should be overcome by a novel approach using anther culture and GISH as efficient tools. The chromosome number should be reduced from the tetraploid to the haploid level via two cycles of anther culture. After chromosome doubling , doubled haploid plants (DH) arise which have identical pairs of translocated chromosomes. Karyotypes with a complete set of symmetrical intergeneric chromosome translocations

(centromer region from *Lolium* and telomer region from *Festuca* or vice versa) can be selected by GISH among the haploid or DH-plants. These plants should show regular meiosis.

For the first cycle of anther culture, 47 allotetraploid donor plants were selected. Anthers of 24 plants developed calli and 103 green plants arose from 14 donor plants. The capability to produce calli, regenerants and green plants varied significantly between the donor plants. The green plants were either diploid (71 %), tetraploid (12 %) or aneuploid (17 %). All regenerants showed a low male and female fertility. The analyses by GISH confirmed high frequency of intergeneric translocations and are now focused on the selection of the desired karyotypes.

(4) Introgression is a powerful tool to transfer desired features from *Lolium* to *Festuca* or vice versa. Humphreys and Pasakinskiene (1996) reported successful transfer of drought resistance from *F. arundinacea* into *L. multiflorum*. We introduced crown rust resistance from *F. arundinacea* and *F. pratensis* into *L. multiflorum* (Oertel and Matzk, 1999). Prerequisites were: various resistant hybrids, high translocation frequency between the homoeologous chromosomes, efficient screening method (detached leaf test) and exclusive selection of high resistance. The resistance is stable already over several succeded generations contrary to results from Wilkins et al. (1974). 120 highly resistant BC_3F_1 progenies were incorporated into the breeding programmes of several plant breeding companies. All plants possess high fertility and phenotypic identity to *Lolium*. As an evidence for a real introgression of the crown rust resistance from *Festuca* into *L. multiflorum* two small terminal translocations originating from *Festuca* were detected by GISH in one resistant BC_3S_1 plant along the complete *Lolium* background.

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COLLECTING AND EVALUATION OF GRASSES FOR PRESERVATION OF THE GENETIC DIVERSITY

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Abstract

In the following explanation it will be shown – as an example of grasses collection – how to get, to expand and to maintain the genetic diversity through collecting missions. Further how by the evaluation trials the value of plant genetic resources will be assessed.

The collecting missions will be carried out with specific aims and to complete the special collections. The main goal is thereby to close gaps – geographic origin and in the traits/characteristics – in the collection and to prevent the further gene erosion.

During the last three years, the Genebank has organised and carried out collecting missions in Croatia, Bulgaria and Spain. These trips followed in co-operation with other national and international institutions under exact arrangements (Material Transfer Agreement).

Only particular qualitative material (seed) was collected from which we could get essential passport data. The value of collected material was estimated in evaluation trials (2-3 years). These led to an expansion of intraspecific diversity of grasses.

Finally, the users of Plant Genetic Resources can feed back on high quality seed and informative data about the accessions.

The results of the evaluation show that still more accessions are missing in special fields of variability of traits (e.g. crown rust resistance, maturity group: very early and very late).

Conclusions

→ Collecting missions are carried out to close gaps – geographic and in the traits.

→ From collecting to availability of seed and data 3...10 years will pass by; it depends on mode of evaluation (primary or/and secondary evaluation).

→ Analysing of evaluation data is a continuous process. The results have to be added into the database.

→ Further studies are necessary to compare the variability of culture and wild material (molecular marker), in order to arrange the mode of maintenance strategy (ex-situ or in-situ).

Introduction

IPK collecting missions were undertaken to fill gaps for important economic traits and to expand eco-geographic coverage (Lellbach et al., 1997; Willner et al., 1998; Willner, 1999) in our grasses collection.

During the last three years, in co-operation with national and international institutions, the IPK Genebank has organised and carried out collecting missions to Croatia, Bulgaria and Spain (Table 1).

All missions were organised in co-operation with partners in the respective countries.

Preceding each mission we negotiated an agreement to exactly determine all the conditions of the collecting mission for both partners.

Both partners participated in collecting. Tasks were divided. The collected material and information were shared at the end of each mission.

We are interested in developing closer relationships with the donor countries of the germplasm. Ideally, joint work with the new accessions on multiplication, characterization and evaluation is developed after the collecting mission. Until now there are no arrangements and concrete examples of such continued co-operation after joint collecting missions. Currently there is successful co-operation between the IPK genebank station at Malchow and the Deutsche Saatveredelung (DSV).

Materials and Methods

Seed and detailed passport data were collected. The data were recorded on prepared collection forms (Table 2). These forms are routinely used by CLIMA/ICARDA missions and were kindly provided by Dr. Clive Francis, CLIMA, Perth, Australia.

At first the data of collection site were recorded: geographic/physical, site habitat, soil, climate, botanical structure. Furthermore data of donor (address, region) and notes about cultivation /utilization are important. The second list consist of data on collected material such as botanical name, seed or living material (how many?), local use etc.

Table 3 shows the process of a collecting mission and follow-up work with the collected material.

After collecting the new material has to be grown in order to determine the botanical identity and the description of some morphological and traits relevant for breeding.

Afterwards, seed multiplication is being carried out in isolation plots in a rye field. The seed harvested of the most valuable material is transferred to storage in the following year, one part as base collection and the other part as active collection. A small seed sample is stored in our Genebank at Gatersleben as safety collection.

The evaluation of collected material is done for some species in extra trials with partner institutions like breeder firms and/or research institutions.

Results and Discussion

As mentioned above a total of 998 accessions were collected (Table 1). Evaluation of this new material and seed production for long term storage is an ongoing process. 29% of the collected accessions have already been integrated into the collection.

As example *Lolium perenne* accessions from Bulgaria 1998 is presented some collecting and cultivating data (Table 4) for intraspecific variability.

The value of collected material is evaluated in field trials (2-3 years).

Our results show that there is continued need to find further sources for traits such as maturity at the extremes of early and late (Fig. 1) and crown rust resistance (Fig. 2).

All data of collection, characterization, multiplication, evaluation are recorded into the internal data base. The first and most important informations are to find in the external data base in internet (http://mws.ipk-gatersleben.de/malchow/index.html). But the evaluation data are not yet available in internet.

Conclusions

→ Collecting missions are carried out to close gaps for eco-geographic coverage and economically useful traits.

→ Evaluation and seed bulk up is time consuming. It takes 3 to 10 years from collecting to the availability of seed and data, depending on the mode of evaluation (primary or/and secondary evaluation).

→ Analysis of evaluation data is a continuous process. The results have to be added into the database.

→ The collected material provides an opportunity to compare the genetic variability of the wild material to the cultivated gene pool (molecular markers). Such information is needed to devise appropriate in-situ and ex-situ conservation strategies.

→ Further collecting missions are needed

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Abbreviations:
CLIMA: Centre for Legumes in Mediterranean Agriculture, University of Western Australia
ICARDA: International Center for Agricultural Research in the Dry Areas, Aleppo, Syria

Table 1 Collecting missions 1997-1999

Species	Croatia 1997 collected / integrated	Bulgaria 1998 collected / integrated*	Spain 1999 collected / integrated*
Lolium perenne L.	34 / 24	73 / 62	83 / 0
Dactylis glomerata L.	16 / 9	29 / 1	37 / 0
Festuca pratensis Huds.	13 / 12	20 / 0	0
Poa pratensis L.	24 / 20	13 / 10	0
Phleum pratense L.	14 / 3	27 / 6	0
Others	55 / 40	335 / 100	225 / 0
TOTAL	156 / 108	497 / 179	345 / 0

* multiplication not yet finished

Figure 1 Inflorescence emergence of collected *Lolium perenne* accessions in comparison to cv. Fennema

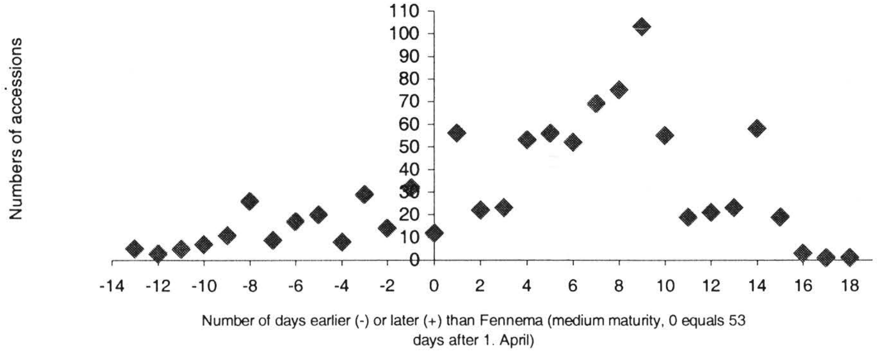

Figure 2 Rust susceptibility of collected *Lolium perenne* accessions in comparison to cv. Fennema

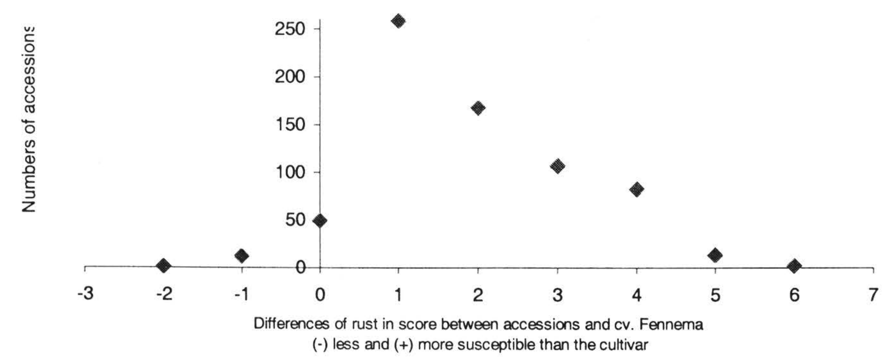

Table 2 Collection data sheet Spain 1999

2.1. Informations of collection site

Site No.: ________ Date: ___/___/___ Collectors: ________________

Latitude: ________ Longitude: ________ Altitude: ______ m Rainfall: ______ mm

Country: ________________ Province: ________________

Village: ________________ Location: ________________

Donor: ________________________________

Photo No: ________ Aspect: ______ deg. Soil pH: ____ Soil colour: ________

Site Habitat

- ☐ 1= Sown pasture
- ☐ 2= Grassland
- ☐ 3=Field margin
- ☐ 4=Fallow
- ☐ 5=Garden/Orchard
- ☐ 6=Agricultural crop
- ☐ 7=Disturbed land-road/pathside
- ☐ 8=Woodland
- ☐ 9=Seaside
- ☐ 10=River/ streamside
- ☐ 11=Market
- ☐ Other ________________

Slope

- ☐ 1=level 0 - 3 %
- ☐ 2=undulating 3 - 8 %
- ☐ 3=gently rolling 8 - 16 %
- ☐ 4=sloping 16 - 30 %
- ☐ 5=steep > 30 %
- ☐ 6=mountainous

Grazing pressure

- ☐ 1=nil
- ☐ 2=light
- ☐ 3=moderate
- ☐ 4=heavy
- ☐ 5=mowed/ cut

Area sampled

- ☐ 1= 1m²
- ☐ 2= 1 - 10 m²
- ☐ 3= 10 - 100 m²
- ☐ 4= 100 - 1000 m²
- ☐ 5= >1000m²

Parent rock

- ☐ 1=Granite
- ☐ 2=Basalt
- ☐ 3=Schist
- ☐ 4=Calcareous
- ☐ 5=Limestone
- ☐ 6=Allnvial
- ☐ 7=Sandstone
- ☐ 8=Dunes
- ☐ Others: ________________

Soil type/texture

- ☐ 1=Stones/Rocky
- ☐ 2=Gravel
- ☐ 3=Sandy
- ☐ 4=Sandy loam
- ☐ 5=loam
- ☐ 6=clay loam
- ☐ 7=clay
- ☐ 8=Highly organic
- ☐ 9=Others: ________________

Soil depth

- ☐ 1= 0 - 10 cm
- ☐ 2= 10 - 20 cm
- ☐ 3= 20 - 40 cm
- ☐ 4= > 40cm

Water relation

- ☐ 1=Freely drained
- ☐ 2=Moderately drained
- ☐ 3=Water table
- ☐ 4=Swamp

Salinity

- ☐ 1= None ☐ 2=Low
- ☐ 3=Medium ☐ 3=High

Associated Species:

Dominant: ________________________________

Others: ________________________________

____

2.2. Informations of collection source

Site No.: ____ Collection No.: ____ Photo No.: ____
Genus: ____ Species: ____ Spp./Var.:
Common name: Herbarium Sp: Y/N Rhizobium Sp: Y/N
Flowering: Y/N Fruiting: Y/N Irrigated: Y/N Threshed Y/N
Flower colour: [ ] Pop.type: [ ] Sample status: [ ] Density: [ ]
No. plants/seeds collected: ____
Local use.: ____
Remarks: ____

Site No.: ____ Collection No.: ____ Photo No.: ____
Genus: ____ Species: ____ Spp./Var.:
Common name: Herbarium Sp: Y/N Rhizobium Sp: Y/N
Flowering: Y/N Fruiting: Y/N Irrigated: Y/N Threshed Y/N
Flower colour: [ ] Pop.type: [ ] Sample status: [ ] Density: [ ]
No. plants/seeds collected: ____
Local use.: ____
Remarks: ____

Site No.: ____ Collection No.: ____ Photo No.: ____
Genus: ____ Species: ____ Spp./Var.:
Common name: Herbarium Sp: Y/N Rhizobium Sp: Y/N
Flowering: Y/N Fruiting: Y/N Irrigated: Y/N Threshed Y/N
Flower colour: [ ] Pop.type: [ ] Sample status: [ ] Density: [ ]
No. plants/seeds collected: ____
Local use.: ____
Remarks: ____

Site No.: ____ Collection No.: ____ Photo No.: ____
Genus: ____ Species: ____ Spp./Var.:
Common name: Herbarium Sp: Y/N Rhizobium Sp: Y/N
Flowering: Y/N Fruiting: Y/N Irrigated: Y/N Threshed Y/N
Flower colour: [ ] Pop.type: [ ] Sample status: [ ] Density: [ ]
No. plants/seeds collected: ____
Local use.: ____
Remarks: ____

Site No.: ____ Collection No.: ____ Photo No.: ____
Genus: ____ Species: ____ Spp./Var.:
Common name: Herbarium Sp: Y/N Rhizobium Sp: Y/N
Flowering: Y/N Fruiting: Y/N Irrigated: Y/N Threshed Y/N
Flower colour: [ ] Pop.type: [ ] Sample status: [ ] Density: [ ]
No. plants/seeds collected: ____
Local use.: ____
Remarks: ____

Table 3 Overview of working from collecting to access of sources in the gene bank

COLLECTING

WHERE?
On natural places
On cultivated sites

WHAT?
1. seed samples (minimum 20 inflorescences)
2. living material (minimum 10 clones parts per place)
3. data

a) collection site: geographic/physical data
site habitat/soil data
climat
botanical structure

b) donor: name
address
region

c) cultivation: land use/management
intensity

d) utilization: meadow
pasture
mowing pasture
livestock numbers

CHARACTERIZATION

In comparison cultivation

Description of morphological traits and traits relevant to breeding, for example:

- leaf colour
- leaf width
- leaf length
- characteristic of inflorescence
- habitus
- bulk to different stages
- persistence
- inflorescence emergence
- flowering date
- disease susceptibility

MULTIPLICATION in field cultivation (isolation plots)
in greenhouse

recording of valuable plant genetic resources in base collection

EVALUATION primary (in field trials)
secondary (in field or laboratory experiments with cooperation partners)

DOCUMENTATION internal data base
External data base in internet
(http://mws.ipkgatersleben.de/malchow/index.html)

- Recording of collecting data (passport data)
- Recording of seed (in basis and active collection)
- Recording of characterization and evaluation data

Table 4 *Lolium perenne* L. from Bulgaria 1998

4.1. Collecting data

Photo	Collect_nr.	Origin Region/site	Latitude	Longitude	Elevation In m.a.s.l.	Habitat
6	271	Pirinmountains/Bansko	4149N	2336E	640	pasture/sheep grazing dry, valley slope, extensive utilization
7	247	Pirinmountains, Banja	4146N	2326E	775	Meadow/after cut grazing, sheep, cows Valley bottom, intensive util.
8	790	Northbulgaria, Ruse,	4346N	2557E	50	Natural place near the river Beli Lom, dry, stony, sandy-loamy
9	647	Black Sea, Eminska Mountains, northern Burgas	4247N	2735E	85	Valley slope, NW-Aspect, sheep grazing, extensive, very dry, sandy stony
10	595	Southern Black Sea, near Primorsko	4215N	2743E	5	Pasture, sheep, cows grazing, extreme dry

4.2. Cultivating data

Photo	Collect nr.	Habitat before winter	Habitat after winter	Growth in springtime	Emerg. date	Flowering date	Harvest	Remarks
6	271	6	7	7	25.05.	07.06.	17.07.	Prostrate-half erect, high rust suscept.
7	247	6	7	7	10.05.	30.05.	03.07.	Dark green and wide leaf, rust suscept.
8	790	8	8	9	18.05.	10.06.	27.07.	Medium rust suscept.
9	647	8	8	9	16.05.	09.06.	24.07.	Medium Rust suscept.
10	595	9	9	9!	16.05.	12.06.	17.07.	Low rust suscept.

CHARACTERISATION OF GERMPLASM OF ANNUAL MEDICS (*Medicago polymorpha* L.) IN THE "BAIRRO RIBATEJANO" REGION

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Abstract

This work corresponds to the first stage of a project whose aim is the further selection of cultivars of annual medics for the limestone massif of Central Portugal, and it reports on the characterisation and preliminary evaluation of 45 populations of annual medics (Medicago polymorph L.) collected in the " Região Agrária do Ribatejo e Oeste".

Thirty six traits, decided after the descriptors for annual medics of the International Board for Plant Genetic Resources (IBPGR), were observed throughout the vegetative and reproductive stages. Data were analysed by means of the NTSYS-pe (Numerical Taxonomy and Multivariate System) Program.

Traits that showed greater variation were peduncle hairiness, left surface hairiness at mean flowering, leaf area one month after transplantation, seed yield per plant, single pod weight and basal internode length.

Relatively long life cycles (140 to 181 days from sowing to flowering) were observed in all populations. Forage and seed yield were high in most populations. Hard-seededness, evaluated in the months of August, September and October following seed ripening, was always very high, though it decreased with time.

Introduction

The aim of this research is the characterisation and preliminary evaluation of 45 populations of annual medics (*Medicago polymorpha* L.) collected in the "Região Agrária do Ribatejo e Oeste", which stands in the limestone massif of Central Portugal, in order to identify suitable germplasm for breeding. As commercial varieties are not well adapted to the region, new varieties are needed, based upon naturally well adapted germplasm.

Material and Methods

During the summer of 1995, 45 accessions of *M. polymorpha* were collected in the Agrarian Region of "Ribatejo e Oeste" (Fortunato, 1997). On October 4 th of the same year, the 45 populations were sown in jiffy seven pots, and on November 14th the seedlings were planted in the field; by that time seedlings averaged the 2nd-3rd true leaf stage.

The field trial was conducted in the "Bairro Ribatejano", in an homogeneous clay lime soil, alkaline [pH (H_2O) = 8.3], with high levels of phosphorous (149 ppm) and very high levels of potassium (352 ppm). The growing season happened to be unusually rainy (1234.8 mm against the thirty year average of 710 mm), particularly in the late season, with 157 mm in May. The field layout followed an experimental design of two completely randomised blocks, with spaced plants (the distances within and between rows were 0.5 and 1.0 m, respectively).

A total of 36 traits (Table 1), decided after the IBPGR list of descriptors for annual medics, were observed along the growing season and after harvest (IBPGR, 1991).

Numerical taxonomy techniques were used for the analysis of data, performed with the NTSYS-pc (Numerical Taxonomy and Multivariate System), version 1.8. Data were first standardised. A cluster analysis by UPGMA (Unweighted Pair Group Method using Arithmetic Averages) and a principal components analysis were performed (Rohlf, 1992).

Results and Discussion

The mean, the limits of variation and the coefficient of variation (CV) observed for each of the traits under observation are shown in Table 1. Average and maximum seed yield per plant and averages and maximums of life cycle length traits are quite high if we take into account the data available in literature for the Australian commercial varieties.

The largest coefficients of variation were found in peduncle hairiness, leaf surface hairiness at mean flowering, leaf area one month after planting, seed yield per plant, pod weight and internode length (at 4^{th}-5^{th} internode stage from the base).

Table 1- Range of variation, means and coefficients of variation (CV) for 36 traits

Trait	Range	Mean	CV (%)
1 – Growth habit (1-4)	1 - 2	1.07	23.7
2 - Internode length, at 4th-5th internode stage from the base (cm)	1.48 - 4.49	2.86	26.3
3 - Number of primary branches	3.50 - 12.75	7.57	24.8
4 - Early vigour, one month after planting (1-10)	4 - 10	6.69	20.3
5 - Leaf area, one month after planting (cm^2)	1.43 - 7.58	3.89	32.0
6 - Leaf area, at mean flowering (cm^2)	5.98 - 15.58	11.31	22.5
7 - Winter hardiness (1 - 10)	3.50 - 9.50	6.23	18.5
8 - Herbage yield (dry matter/plant) at mean flowering (g)	210.75 - 567.25	376.11	21.2
9 - Herbage yield (dry matter/plant) at pod maturation (g)	272.50 - 580.00	433.58	17.0
10 - Leaf surface hairiness, one month after planting (1 - 9)	1 - 3	1.82	24.2
11 - Leaf surface hairiness, at mean flowering (1 - 9)	1 - 2	1.40	35.4
12 - Petiole hairiness, one month after planting (1 - 3)	1 - 1	1.00	- -
13 - Petiole hairiness, at mean flowering (1 - 3)	1 - 1	1.00	- -
14 - Peduncle hairiness, at mean flowering (1 - 3)	1 - 3	1.49	39.5
15 - Days to the first flower bud primordium	134 - 173	156.29	7.9
16 - Days to mean flowering	140 - 181	163.24	7.7
17 - Days to last flowering	245 - 254	246.93	1.1
18 - Days to first mature pod	210 - 230	219.36	3.4
19 - Days to last mature pod	247 - 268	255.49	1.7
20 - Maturation period (number of days)	37 - 77	56.11	17.3
21 - Days to death plant	251 - 275	263.96	1.9
22 - Node number at first flowering	6.75 - 17.25	11.28	17.6
23 - Mean number of florets per inflorescence	2.80 - 5.50	4.40	12.9
24 - Mean number of pods per inflorescence	2.60 - 5.00	4.08	14.5
25 - Pod whorl number range	2.13 - 5.63	4.60	14.5
26 - Pod hairiness (1 - 3)	1 - 2	1.04	20.0
27 - Pod spininess (1 - 4)	3 - 4	3.69	12.7
28 - Amount of pod spininess (1 - 10)	5 - 9	6.84	12.0
29 - Pod weight (mg)	26.05 - 161.70	84.20	27.7
30 - Seed yield per plant (g)	20.85 - 118.86	76.64	28.6
31 - Pod yield per plant (g)	127.34 - 295.00	217.09	18.7
32 - Weight of 1000 seeds (mg)	2529.60 - 5277.90	4053.40	15.2
33 - Number of seeds per pod	3.60 - 10.30	8.08	15.4
34 - Seedcoat permeability, the 2^{nd} of August 1996 (%)	89.00 - 100.00	96.28	3.0
35 - Seedcoat permeability, the 12^{th} of September 1996 (%)	79.00 - 100.00	95.29	4.5
36 - Seedcoat permeability, the 17^{th} of October 1996 (%)	61.00 - 100.00	85.69	10.9

The phenogram after the UPGMA method is presented in Figure 1. Population 42, which is well separated from the others, seems to belong to *Medicago polymorpha* var. *vulgaris*; the remaining populations, which apparently belong to *M. polymorpha* var.

polymorpha, do not separate into clear-cut clusters, but some populations show a tendency to cluster together. Such is the case of the group formed by populations 1, 41, 45, 46, 63 and 48, as well as that formed by populations 23, 38, 33, 37 and 24. The populations of the first group showed low early vigour, below average winter hardiness, early flowering, long maturation period, below average herbage, pod and seed yield per plant and a low percentage of hard seeds in October. The populations of the second group showed long basal internodes, high early vigour and winter hardiness, and above average herbage, pod and seed yield per plant; as in the first group, the populations of the second group are early flowering and present a slightly below average percentage of hard seeds in October.

A principal component plot is given in figure 2. The first two principal components together account for 36.5% of the total variation. The first principal component mostly consisted of winter hardiness, seed yield per plant, early vigour, leaf area and internode length. Population 19 is well distinguished from the others as it shows high winter hardiness, very high early vigour, large leaf area (mainly at flowering) and the highest seed yield per plant (118.86 g). Inversely, populations 42, 64 and 72 showed low early vigour, low winter hardiness, small leaf area and low seed yield. Population 37 and population 2 are clearly distinguishable from the others as they presented the longest and shortest internode length, respectively. The second principal component was strongly associated with seed coat permeability in September and, particularly, in October, the number of days to the first flower bud primordium, number of days to flowering, number of days to the first mature pod and herbage yield at pod maturation. Populations 11 and 23 showed the highest percentage of hard seeds in September and October, while the populations 1, 33, 46 and 48 had the lowest percentages. Populations 2 and 9 were the most late flowering and populations 6, 16, 33, 37 and 41 the most early flowering.

Conclusions

The morphological characterisation indicates that, for many traits, there is a significant variation between populations. Leaf surface and peduncle hairiness at mean flowering, leaf area one month after planting, seed yield per plant, pod weight and internode length at 4^{th} - 5^{th} internode stage from the base were the traits that exhibited the largest variations.

Numerical taxonomy techniques allowed the identification of several differentiating traits, in particular, winter hardiness, early vigour, seed yield per plant, leaf area, internode length, hardseededness in September and October and some life cycle length traits. However, these results must be taken with care, as they have come out of a single year study.

Some populations clearly exceeded the Australian commercial varieties for traits such as early vigour, winter hardiness, herbage and seed yield. Besides, most populations showed late flowering (140 to 181 days from sowing to flowering) when compared with standard commercial varieties, which is much convenient for many regions of Portugal. Such performances clearly recommend the use of some of this germplasm for breeding.

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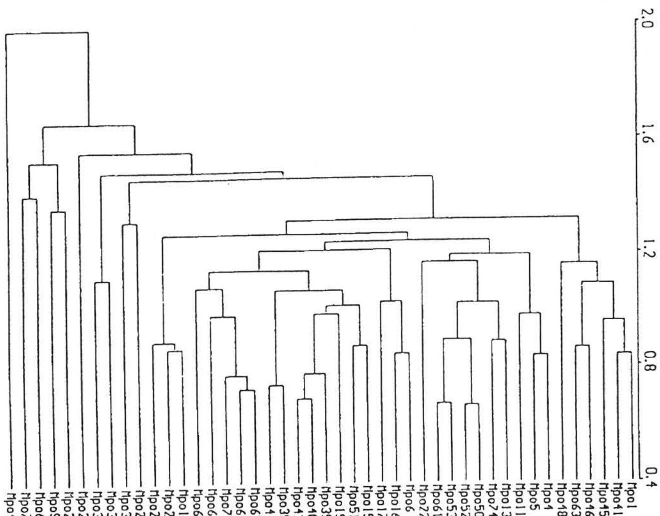

Figure 1 - Phenogram of 45 populations obtained by UPGMA applied to the matrix of distances (r = 0,770) Mpo - *M. polymorpha*.

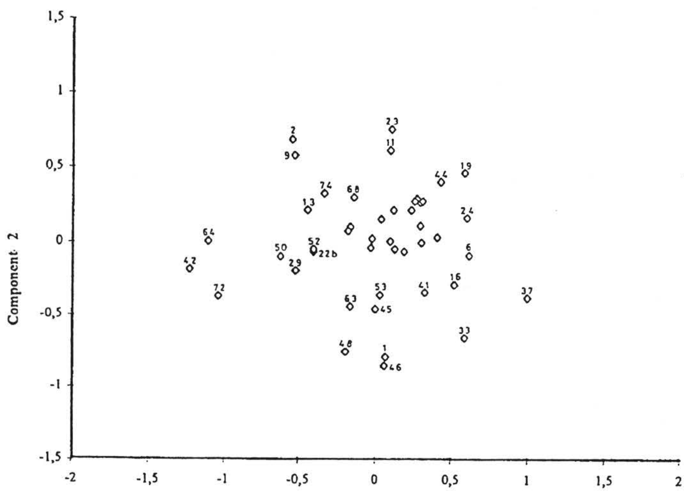

Figure 2 - Plot of 45 populations on the plan defined by the two first principal components.

GERMPLASM EVALUATION OF ANNUAL MEDICS COLLECTED IN PORTUGAL

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Abstract

The capacity of annual *Medicago* species to grow in drought conditions is well documented, which makes it quite interesting to use them in large areas of Southern Portugal. Another environmental constraint is the adaptability to acid soils, which are frequent in such regions.

We've done, on a germplasm collection of ecotypes from the Center and South of Portugal, a preliminary evaluation and we've selected the best populations, 73 entries, 65 of them coming from portuguese natural vegetation, classified in seven species of *Medicago* genus. The 73 entries were agronomicaly evaluated on five sites. The soils of four sites are acid (Cambisol and Luvisol) and one of them is neutral (Cambisol). We've completed the screening with trials in pots with the same soils.

It was found that the most common species in Portugal is *Medicago polymorpha*, which includes some *accessions* with great adaptability. Some ecotypes of this species have the ability to answer very well, in terms of dry matter accumulation, when environmental index increases. As ecotypes with specific adaptation to the acid soils checked we found that, all accessions of *M. murex* tested showed good adaptation level. A large group of *M. polymorpha* species were tolerant to those conditions, as well as *M. murex.*

Trials in pots, using the same soils and ecotytpes, and different fertilisers (lime and P), showed a positive effect of P application on dry matter production and the lime improved the performance of all species except *M. murex*.

Introduction

Annual medics are more resistant to water stress than subclover because of fast root penetration of the soil and a deep rooting habit. *Medicago laciniata* can even be found in areas with an annual rainfall of 25-50 mm. In Western Mediterranean regions the most representative species of annual medics are *M. polymorpha*, *M. truncatula*, *M. doliata*, *M. orbicularis*, *M. minima* and *M. arabica* (Francis, 1979; Carneiro *et al.*, 1993/94; Carneiro e Serrão, 1996; Fortunato, 1997).

Soil acidity and high levels of some trace elements limit the growth of annual medics. The survival of *Rhizobium* sp. in acid soils also influences the adaptation of these plants to acidic conditions. There is different tolerance between and within species of *Medicago* to these soil factors, especially in *M. polymorpha* and *M. murex*. Some authors consider that *M. truncatula* is a cosmopolitan species adapted to a very large diversity of soil conditions. Limited to clay soils, *M. rugosa* is also present in Portugal.

Germplasm collections can be used to obtain genetic variability and to select new cultivars of annual medics to use as forage.

Material and Methods

Germplasm Collection

We collected pods from plants belonging to 10 species of annual medics from Central and Southern regions of Portugal. We also collected soil samples from the same sites. A preliminary evaluation of this collection was carried out, based on some agronomic traits. We selected those that produced more than 20.0 g of dry matter per plant in 1991/92, and more than 10.0 g of dry matter per plant in 1992/93.

The collection we used for further studies was composed of 65 Portuguese ecotypes belonging to 7 species of *Medicago*, and 8 commercial cultivars.

Field Trials

The 73 members of the collection were evaluated on three sites (4 environments since in one site they were grown in two years). The soils of two sites were acid (Luvisol derived from schistic rocks - '*Quinta do Valongo*' at Mirandela and '*Revilheira*' at Reguengos de Monsaraz). The third soil was neutral (Cambisol from granite rocks associated with calcareous rocks- '*ENMP*' at Elvas). Plants were harvested at flowering from 0.75 m^2 plots, dried at 65 °C for 48 h and weighed.

Pot Trials

Two acid soils were used in the pot trials, '*Revilheira*' and a Cambisol, from granite rocks - '*Couto da Várzea*'. Ecotypes 66, 172 and 179 of *M. polymorpha*, and 148 of *M. murex* were used. Pots were kept in a glasshouse, and were watered weekly to 70 % of the water holding capacity. Two factors were studied: i) liming - without liming (C_0), and limed to $pH_{(H2O)}$ 6,8 (C_1); ii) fertilisation with phosphate - without application (P_0), with 0,05 g P_2O_5 kg^{-1} of soil (P_1), and with 0,15 g P_2O_5 kg^{-1} of soil (P_2), applied as Ca $(H_2PO_4)_2.H_2O$. Plants were harvested at flowering, 116 days after sowing, and their dry matter evaluated as before.

Results

Species Collected

Annual medics were more frequent in soils with pH $_{(H2O)}$ between 7,6 and 8,5 (Figure 1) (Carneiro, 1999). The species represented in larger numbers was *Medicago polymorpha.*

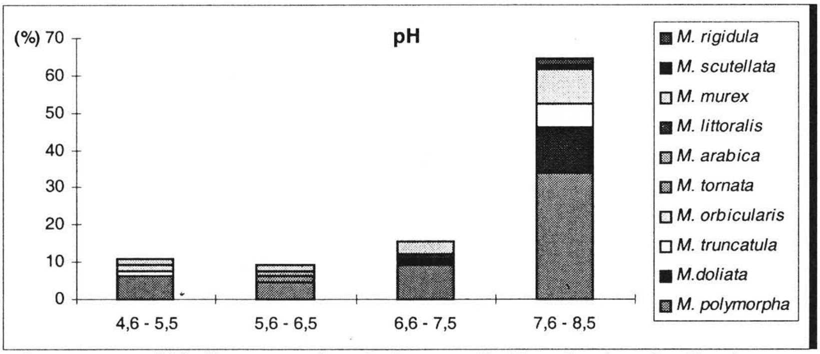

Fig. 1 - Occurrence of *Medicago* spp. in relation to soil pH at the site of collection.

Field Trials

The results obtained in the four environments, (one year at '*Quinta do Valongo*', one year at '*Revilheira*' and two years at '*ENMP*'), showed that both the 'Environment' and the 'Ecotype' influence dry matter accumulation (table 1).

Using the 'Method of Determination of Stability' described by Eberhard and Russel (1966) to the parameters measured lead to the evaluation of ecotypes 22, 29, 5, 11, 111, 19, 179, 8, 174, 171, 159 and 101 of *M. polymorpha*, 149 of *M. scutellata*, 36 of *M. doliata* 87, 95, 182 of *M. truncatula* and 88 of *M. tornata*, as those with greatest stability (large yield in diverse environments).

Ecotypes 14, 66 and 48 of *M. polymorpha*, 2, 153 e 18 of *M. doliata* and 186 of *M. tornata* had the greatest increases in yield in relation to the increase of the environmental index, which represent the potential of one environment. It's measured by the average of all ecotypes in that environment.

Using the 'Method of the Consistency of Performances' (Ketata *et al*.. 1989), ecotypes 66, 76, 7, 11, 49, 19, 50, 71, 6, 8, 22, 39, 114, 179, and 5 of *M. polymorpha*. 18 and 36 of *M. doliata*, 12 of *M. scutellata,* and 64 of *M. truncatula* were consistently superior (Figure 2). Some ecotypes grew well only in one environment. Ecotypes 2, 153, 187, 163, 102, and 90 of *M. doliata,* 48 of *M. polymorpha,* 186 of *M. tornata,* 94 of *M. littoralis,* 93, and 194 of *M. scutellata* seemed well adaptated to the environmental conditions of '*Quinta do Valongo*'. Ecotypes 14, 57, 63, and 177 of *M. polymorpha,* 72, and 10 of *M. doliata* were adapted to the conditions of '*ENMP*'. Ecotype 194 of *M. murex,* 11, 76, and 66 of *M. polymorpha* seemed adapted to the conditions of '*Revilheira*'.

Pot Trials

The results showed a significantly effect of 'Soil'. 'Lime', 'Phosphorus' and 'Ecotypes', of simple interactions and of the triple interaction 'Soil × Lime × Ecotype' (table 2).

Plants grown on the soil of '*Revilheira*' had greater biomass than those from the soil of '*Couto da Várzea*'. Liming the soil lead to a 100% increase in biomass. Plants from pots with the highest level of P grew more than the others. Ecotype 66 have the greatest biomass accumulation and ecotype 172 the smallest (Table 2).

The effect of phosphorus was similar in both soils, but the effect of lime was not. Liming lead to increased biomass accumulation in pots with soil from '*Revilheira*' in all ecotypes but 148. This is a *M. murex*. a species very often referred to as well adapted to soil acidity. Liming lead to increased yield in all ecotypes grown in the soil from '*Couto da Várzea*'. However. ecotype 148 responded less to liming than the others.

Conclusions

The evaluation in the field of annual medics lead to the identification of ecotypes with a great capacity to adapt to different environments and other ones that were specific to one environment.

The pot trial showed that the application of phosphorus had a positive effect on yield in two acid soils. Liming was also beneficial to ecotypes 66, 172, and 179 of *M. polymorpha,* but not for ecotype 148 of *M. murex*.

Table 1 - Biomass accumulation (g per 0.75m^2) of 73 annual medics in four environments and some statistical parameters.

Ecotype	Species	Quinta do Valongo 1993/94	ENMP 1993/94	ENMP 1994/95	Revilheira 1995/96	Average	sd	b_i	s^2_{di}	W_i	Order Number	sd
66	*M. polymorpha*	881	840	236	221	545	316	1.88 *	11499	106255	7	7.9
76	*M. polymorpha*	790	615	231	223	465	246	1.47	6521	36161	10	7.9
14	*M. polymorpha*	768	860	119	51	449	367	2.15 *	23394	187696	28	28.4
12	*M. scutellata*	689	654	287	123	438	241	1.46	1406	26077	12	7.6
2	*M. doliata*	1057	356	187	98	424	377	1.95 *	82017	259899	26	17.8
48	*M. polymorpha*	910	470	158	150	422	310	1.73 *	31861	121681	22	18.4
186	*M. tornata*	761	628	247	44	420	288	1.76 *	99	62473	19	17.7
18	*M. doliata*	761	593	262	62	419	273	1.67 *	709	49632	19	13.1
11	*M. polymorpha*	570	625	215	246	414	185	1.04	10040	20326	17	13.8
49	*M. polymorpha*	763	487	192	202	411	235	1.35	13569	40375	18	13.8
7	*M. polymorpha*	733	504	275	115	406	233	1.40	4120	25748	15	7.0
57	*M. polymorpha*	678	681	97	165	405	275	1.58	18616	73415	24	28.6
64	*M. truncatula*	657	619	213	111	400	241	1.46	2018	27214	21	7.6
50	*M. polymorpha*	682	602	164	141	397	246	1.48	4833	34416	23	14.4
153	*M. doliata*	998	266	266	17	386	367	1.82 *	93451	258689	33	31.3
94	*M. littoralis*	714	493	313	19	384	255	1.51	8320	44499	25	24.4
71	*M. polymorpha*	627	576	182	135	380	223	1.34	2952	18746	24	10.7
102	*M. doliata*	665	549	261	34	377	247	1.50	1324	29974	26	17.2
19	*M. polymorpha*	612	454	229	177	368	175	1.05	2452	5193	20	9.6
72	*M. doliata*	525	636	155	146	365	219	1.23	14759	35365	26	18.5
10	*M. doliata*	580	624	223	26	363	249	1.49	6409	38440	29	18.7
63	*M. polymorpha*	533	664	125	103	356	247	1.39	18561	53592	31	22.7
6	*M. polymorpha*	564	557	216	83	355	211	1.28	1978	12252	27	7.8
93	*M. scutellata*	678	325	336	55	348	221	1.16	25889	54565	27	21.0
39	*M. polymorpha*	673	497	173	48	348	249	1.52	1306	31529	31	12.2
187	*M. doliata*	792	340	219	36	347	279	1.54	29723	90453	31	20.3
8	*M. polymorpha*	450	603	238	62	338	205	1.14	15027	32219	27	14.7
114	*M. polymorpha*	618	503	153	59	333	233	1.43	792	20988	33	12.7
22	*M. polymorpha*	507	441	195	165	327	149	0.91	1094	3139	28	11.5
177	*M. polymorpha*	576	528	94	103	325	228	1.35	6710	26483	35	21.7
90	*M. doliata*	626	375	264	35	325	213	1.24	9536	24997	30	16.9
163	*M. doliata*	695	322	237	25	320	242	1.34	21053	54874	34	21.0
80	*M. doliata*	741	372	144	15	318	275	1.60	16357	70587	41	22.0
29	*M. polymorpha*	424	543	111	162	310	179	0.96	15755	31718	35	24.1
179	*M. polymorpha*	578	372	148	126	306	184	1.08	6058	12789	35	14.0
104	*M. truncatula*	597	410	167	10	296	225	1.36	1975	18136	42	17.0
5	*M. polymorpha*	468	453	175	87	295	168	1.02	1056	2159	35	6.2
87	*M. truncatula*	495	417	234	10	289	187	1.12	3023	7617	39	19.6
36	*M. doliata*	520	375	227	32	288	181	1.08	2983	6732	37	11.9
149	*M. scutellata*	462	321	301	47	283	150	0.82	9472	22573	35	20.2
111	*M. polymorpha*	568	289	133	93	270	186	1.04	11481	23187	42	13.6
174	*M. polymorpha*	521	229	183	106	260	157	0.82	13769	30996	41	17.9
171	*M. polymorpha*	436	386	115	95	258	154	0.93	1690	3904	43	15.0
141	*M. truncatula*	348	329	333	14	256	140	0.65	16788	46553	41	26.8
4	*M. polymorpha*	272	394	208	142	254	93	0.46	6260	43744	34	16.7
195ª	*M. truncatula*	406	231	308	70	254	123	0.56	13624	47746	38	25.8
159	*M. polymorpha*	469	406	46	94	253	186	1.08	6948	14629	43	18.9
95	*M. truncatula*	416	415	160	8	250	174	1.05	1921	4134	48	14.9
182	*M. truncatula*	424	347	189	14	243	157	0.95	1563	3388	47	11.7
139	*M. scutellata*	274	452	205	15	236	157	0.78	16856	38883	44	16.7
108	*M. doliata*	256	352	262	64	233	105	0.48	9865	48340	37	18.4
195	*M. murex*	281	240	207	169	224	42	0.25 *	177	60337	39	25.2
109	*M. polymorpha*	339	225	151	167	220	74	0.40	2598	44035	44	24.8
194	*M. murex*	202	325	91	247	216	85	0.17 *	12950	100700	45	30.4
180	*M. polymorpha*	113	487	129	125	213	158	0.39	42065	124541	42	23.9
88	*M. tornata*	387	239	172	44	210	124	0.82	3155	14611	50	9.6
101	*M. polymorpha*	409	286	26	86	202	154	0.88	6726	15164	52	17.1
118	*M. truncatula*	227	311	202	40	195	98	0.49	6698	41493	48	11.7
140	*M. truncatula*	114	355	270	37	194	125	0.27 *	27499	111534	41	24.6
128	*M. truncatula*	168	293	267	23	188	106	0.33 *	16805	81436	46	24.8
162	*M. rigidula*	244	286	183	8	180	106	0.57	5112	29684	55	12.8
92	*M. truncatula*	304	263	125	9	175	117	0.71	502	9974	60	5.7
157	*M. truncatula*	242	288	99	64	173	94	0.55	2038	26203	53	13.0
142	*M. truncatula*	200	165	242	24	158	82	0.26 *	9698	76931	51	24.2
172	*M. tornata*	308	154	128	6	149	107	0.60	4162	25514	63	9.8
100	*M. polymorpha*	160	257	151	22	147	84	0.40	5903	51165	59	7.0
147	*M. polymorpha*	200	269	43	47	140	98	0.53	4040	31276	59	11.6
10ª	*M. truncatula*	138	281	132	5	139	98	0.43	9317	53220	63	8.0
106	*M. truncatula*	79	270	135	26	127	91	0.27 *	82976	82976	60	9.3
148	*M. murex*	94	185	63	158	125	49	0.01 *	4744	114065	56	28.4
47	*M. murex*	96	118	118	168	125	26	-0.13 *	372	138253	53	30.3
89	*M. rugosa*	111	183	144	8	111	65	0.25 *	5096	69929	65	8.7
144	*M. tornata*	89	79	93	8	67	35	0.14 *	1292	80344	71	2.2

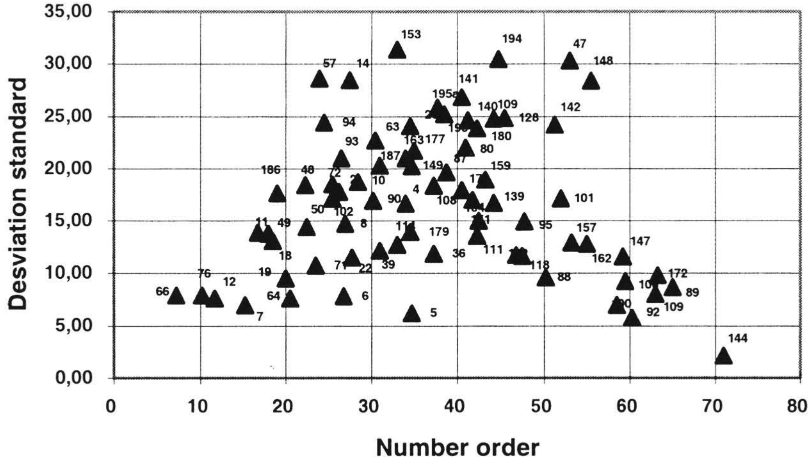

Fig. 2 - Distribution of the ecotypes in relation to order number and standard deviation.

Table 2 – Biomass accumulation by annual medics grown in two acid soils. limed or unlimed. and with three levels of added phosphorus.

Treatments	Biomass (g plant^{-1})		Triple interaction	Biomass (g plant^{-1})	
Revilheira	1.39	a	Revilheira C_0 66	1.55	cd
C. da Várzea	0.83	b	Revilheira C_0 148	1.14	e
n = 72			Revilheira C_0 172	0.46	fg
C_0	0.72	b	Revilheira C_0 179	1.28	de
C_1	1.50	a	Revilheira C_1 66	2.44	a
n = 72			Revilheira C_1 148	1.36	de
P_0	1.02	b	Revilheira C_1 172	1.26	de
P_1	1.09	b	Revilheira C_1 179	1.65	c
P_2	1.22	a	C. Várzea C_0 66	0.39	fg
n = 48			C. Várzea C_0 148	0.43	fg
66 (*M. pol.*)	1.61	a	C. Várzea C_0 172	0.16	g
148(*M. mur.*)	1.05	b	C. Várzea C_0 179	0.37	fg
172(*M. pol.*)	0.64	c	C. Várzea C_1 66	2.04	b
179 (*M. pol.*)	1.15	b	C. Várzea C_1 148	1.26	de
n = 36			C. Várzea C_1 172	0.67	f
			C. Várzea C_1 179	1.31	de
			n = 36		

Means were compared with the 'LSD' Test.

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Breeding for biotic stresses resistance

GRASS/*NEOTYPHODIUM* INTERACTIONS AND THEIR ROLE IN THE IMPROVEMENT OF STRESS TOLERANCE

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Keywords: Endophytes; *Neotyphodium lolii; Lolium perenne*; Grass/endophyte associations; peramine; lolitrem; ergovaline; stress tolerance

Abstract

The infection of perennial ryegrass and tall fescue by *Neotyphodium* spp. endophytes establishes a mutualistic symbiosis between grass and fungus. Populations of perennial ryegrass infected with *N. lolii*, collected in natural pastures of different grassland areas, are being searched in a attempt to select endophyte strains free of lolitrem B and ergovaline, which are toxic to grazing animals, but producing the non-toxic peramine which is responsible for the deterrence of insect attacks.

Research conducted on infected populations collected in Portuguese natural pastures allowed to select a small number of ryegrass genotypes free of ergovaline in which only traces of lolitrem B were detected but with peramine. Work in New Zealand that led to the release of the novel *Neotyphodium* strain - AR1 is also reported.

Reference is made to selection of non-toxic strains of N. *coenophialum* in naturally infected populations of all fescue. Finally in the breeding of amenity grass cultivars the importance of grass/endophyte associations is enhanced.

Introduction

In the last two decades extensive research has been devoted to the mutualistic symbiosis (Clay, 1988) of grass/endophyte interactions in the major temperate grasses perennial ryegrass (*Lolium perenne* L) and tall fescue (*Festuca arundinacea* Schreb.) but the benefits are currently limited because of the production of alkaloids toxic to livestock.

The benefits which are conferred to these pasture grasses by their association with Neotyphodium endophytes are too valuable to be under-estimated and set aside. Therefore inter-disciplinary work is being conducted to develop grass/endophyte associations in which the protection against insect atacks, discouragement from over-grazing by herbivores and enhanced persistence conferred on the grass symbiont will be combined with endophyte strains that are harmless to grazing livestock (Siegel, Latch and Johnson, 1987; Easton, 1999; Tapper and Latch, 1999).

In perennial ryegrass infected with *Neotyphodium lolii* endophyte the compound produced which confers resistance to some insect pests is the pyrrolopyrazine alkaloid peramine, yet this compound has no apparent detrimental effects on grazing animals (Prestidge, Popay and Ball, 1994). While in tall fescue infected with *N. coenophialum* endophyte the compounds associated with endophyte presence which confer resistance to some insect pests are the saturated 1-aminopyrrolizidine alkaloids N-formyl loline and N-acetyl loline, yet these compounds have little effect on livestock. Similarly meadow fescue (*Festuca pratensis* L.) infected with *N. uncinatum* also produces these compounds (Bush, Wilkinson and Schardl, 1997).

The known hazards to livestock are caused by two groups of endophyte alkaloids, indole diterpenes and ergopeptines. The major indole diterpene alkaloid is the tremorgenic neurotoxin lolitrem B, causes the livestock malady known as "ryegrass staggers", with symptoms ranging from slight trembling of the neck and shoulder muscles to staggering during movement, resulting in collapse (Siegel, Latch and Johnson, 1985; Van Heeswijck and McDonald, 1992; Bush, Wilkinson and Schardl, 1997). The ergopeptine alkaloid ergovaline causes elevated body temperature, vasoconstriction, perturbed reproduction, depressed milk production and reduced liveweight gain (Bush, Wilkinson and Schardl, 1997; Easton, 1999).

Although not much work in this field of research has so far been carried out in Europe, this does not signify that the grass-endophyte association should not be considered as a way to improve pasture performance, particularly those grown in the southern areas with great biotic and abiotic stresses. This paper reports the results of research on infected perennial ryegrass ecotypes collected in natural pastures of North-east Portugal that seldom receive any rain in the three hot summer months.

Materials and Methods

Ecotypes of perennial ryegrass collected in natural pastures through the North-east of Portugal were assessed for the presence of *N. lolii.* At least ten tillers were taken from each ecotype and tested for endophyte infection using the microscopic staining method described by do Valle Ribeiro, Gurney and Bush (1996). Ecotypes in which the fungal mycelium was detected in at least one of the tillers were classified as infected.

Twenty-two infected genotypes, chosen on the basis of seed availability, from five different ecotypes were analysed for alkaloid content. The assessment of ergovaline and peramine alkaloids was carried out in infected seeds and the presence and concentration of the neurotoxin lolitrem B was detected in samples of plant pseudostems. These samples were collected from infected plants, at full ear-emergence, which were grown in Vila Real, Portugal.

The technique used to quantify lolitrem B was based on the method adopted by Tapper and Latch (1999). Ergovaline was extracted with 80% methanol from powdered samples and quantified by HPLC (Siegel et al., 1990). Peramine was also extracted with 80% methanol and concentration determined by TLC (Fannin et al., 1990).

Two infected genotypes, UT 13/11/15 and UT 15/6/11 respectively, which were free of ergovaline and have only traces of lolitrem B, were crossed with a new PGG Seeds selection in New Zealand. The above endophyte containing genotypes were used as maternal parents and hybrid plants infected with UT 13/11/15 endophyte strain were backcrossed to the new selection.

Results and Discussion

The use of perennial ryegrass/endophyte associations is known to offer benefits in environments where pest problems or drought stress are common. In pastures of Northern Europe where pest problems or drought stress are not severe their use is not justified, but in Southern Europe where summers are very hot and dry they may offer advantages provided the associated animal health problems can be minimised. In these experiments perennial ryegrass ecotypes, collected from natural pastures in an area of North-east Portugal with extremely hot and dry summers, were commonly infected with *N. lolii.*

No lolitrem B accumulated in 18 (81.8%) of the 22 genotypes whose herbages were analysed for the presence of this alkaloid (Table 1) but the insect deterrent peramine was found in all of them. Three genotypes only had low lolitrem B and the fourth had 5.6 µg/g dry matter in a seed sample. The eighteen chemotypes (Tapper and Latch, 1999) with no lolitrem B had a high level of ergovaline and in most cases a content of peramine sufficient to deter the feeding by adults of Argentine stem weevil. These associations were similar to those firstly selected by Latch and Tapper (1988) with the aim to obtain strains of *N. lolii* that did not cause ryegrass staggers and avoided the herbivory of the grass by Argentine stem weevil. An initial release of an endophyte strain free of lolitrem B lead to the discovery of the importance of ergovaline in infected perennial ryegrass (Latch, 1994). Since their findings infected plants have been screened for the absence of both mammalian toxins in perennial ryegrass/*N. lolii* symbiota and six novel endophyte strains were obtained. Strain AR1, which is harmless to livestock and has the deterrence of insect feeding conferred by peramine, has been released by AgResearch for being inoculated in cultivars of perennial ryegrass free of fungal infection. All six AR strains, including AR1, were inserted into cultivar Grasslands Nui endophyte-free and six seed lines were propagated and sown in three sward trials in 1996 in which their performances were compared with those of the same cultivar infected with the wild type and endophyte-free respectively (Popay *et al.*, 1999). Table 1 results confirm the relationship of high ergovaline content with low lolitrem B content.

The data in Table 1 clearly indicate that among the 22 ryegrass/endophyte associations, there are only two, UT13/11/15 and UT15/6/11, worthy of being incorporated into endophyte-free plants of cultivars or lines of perennial ryegrass. The *N. lolii* strains present in those associations yield desirable alkaloid profiles free of ergovaline, only low content of lolitrem B, and peramine contents sufficient to assure insect feeding deterrence.

Alkaloid contents in hybrid plants obtained from crossing maternal plants containing the selected endophytes with a new ryegrass selection are presented in Table 2. It also shows a comparison with the same selection inoculated with AR1 strain. Further backcrosses of the new selection to these hybrids will create populations of similar genetic background with different endophytes. This would then allow a reasonable comparison of animal performance of endophytes containing all three alkaloids with those containing only peramine (AR1) and with those containing only peramine and lolitrem B (UT13/11/15). Such work will be important to help understand the animal health problems associated with endophytes. The development of an endophyte strain such as UT13/11/15 with small amounts of lolitrem B may be of interest to give greater insect resistance than endophytes lacking lolitrem B, as Argentine stem weevil larval growth is reduced by lolitrem B (Prestidge, Popay and Ball, 1994). There is some possibility that grasses with endophytes lacking ergovaline and lolitrem B may be subject to greater grazing pressure in drought periods than wild type endophytes, a feature which in itself may place the plant at risk of being overgrazed leading to plant death. The presence of small amounts of lolitrem B may provide some animal feeding deterrent value under these conditions to prevent pasture overgrazing.

As ergovaline has some insecticidal activity (Bush, Wilkinson and Shardl, 1997) some associations in Table 1 with high contents of ergovaline and sufficient peramine may possibly be used in the improvement of perennial ryegrass cultivars for amenity. In turf grasses, where livestock toxicosis is not a problem, endophytes can clearly add value by protecting the plants against biotic and abiotic stresses (Shardl and Phillips, 1997).

Bouton (1999) in the U.S.A. inserted a non-toxic AgResearch strain of *N. coenophialum* into cultivars "Jesup" and "Georgia 5" of tall fescue which were tested against "Jesup" with wild endophyte and endophyte-free respectively. On the basis of these tests he concluded that the insertion of non-toxic endophyte strains into elite cultivars is a good strategy to provide better animal performance and survival in tall fescue pastures.

The results reported above give a great incitement to pursue the research on perennial ryegrass infected with *N. lolii* collected in Portugal. Alkaloid profiles of wild type associations emphasise the possibility of selecting endophyte strains that do not synthesise toxins but confer biological protection against insect attacks and drought tolerance to the ryegrass symbiont. Such novel strains will have great value as they would enhance the performance of adapted cultivars under the unfavourable conditions prevalent in some areas of Portugal and other Southern European countries.

Table 1: Alkaloid contents, µg/g dry matter, of 22 genotypes of perennial ryegrass

Genotype	**Lolitrem B**	**Ergovaline**	**Peramine**
UT 1/3/8	ND	13.8	4.2
UT 2/3/10	ND	15.5	4.5
UT 3/4/2	ND	17.2	3.6
UT 4/7/5	ND	28.5	5.7
UT 5/7/12	ND	13.6	3.6
UT 6/10	ND	16.2	4.6
UT 7/10/9	ND	19.5	5.2
UT 8/10/10	ND	20.7	4.4
UT 9/10/12	ND	22.0	5.0
UT 10/6/3	ND	38.6	4.2
UT 11/6/6	ND	65.0	6.0
UT 12/6/7	ND	16.2	4.6
UT 13/11/15	0.1	ND	4.3
UT 14/11/1	ND	34.2	4.9
UT 15/6/11	0.2	ND	5.3
UT 16/11/7	ND	38.6	4.2
UT 17/4/5	ND	13.4	4.6
UT 18/7/9	ND	16.5	4.7
UT 19/3/11	ND	2.0	1.1
UT 20/10/2	ND	15.1	4.7
UT 21/6/9	0.1	0.2	4.4
UT 22/6/12/8	5.6	ND	4.6

Table 2: Alkaloid content (µg/g dry matter) of hybrids containing selected strains of endophytes

Seed line	Endophyte strain	Lolitrem B	Ergovaline	Peramine
New selection	Wild type	2.1	1.8	15
PS4 x New selection	UT 13/11/15	0.4	0.0	2
PS5 x New selection	UT 15/6/11	0.2	0.0	0.1
New selection - 1	AR 1	0.0	0.0	5

PS4 = UT 13/11/5; PS5 = UT 15/6/11

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RESISTANCE TO INFECTIOUS DISEASES IN AMENITY GRASSES GROWN IN CENTRAL ITALY

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Abstract

A survey carried out in Perugia, central Italy, concerning the resistance/susceptibility of different cool season grasses (*Lolium perenne*, *Festuca arundinacea*, *Festuca rubra* subsp. *rubra*, *Festuca rubra* subsp. *tricophylla*, *Festuca rubra* subsp. *commutata* and *Poa pratensis*) to biotic agents of diseases made it possible to obtain the following information:

1) during the period 1999/2000 the most dangerous diseases were represented by rusts in *Poa pratensis*, particularly *Puccinia striiformis* and *P. graminis*. Differences in the level of resistance/susceptibility among varieties of Kentucky bluegrass have been recorded so that, for this species, a proper varietal selection could have a positive effect in reducing chemicals in rust control;

2) in late Spring and early Summer of 1999 and 2000, minor diseases caused by *Fusarium* spp. and *Rhizoctonia* spp. have been detected in *P. pratensis* and *F. rubra*. In few cases *Drechslera siccans* was also found. During September 1999 traces of *P. coronata* have been recorded in *L. perenne*. *F. arundinacea* appeared as the most resistant species.

Introduction

In Italy turfgrass diseases have not been extensively monitored in the past. To date *Puccinia graminis* Pers., *Puccinia coronata* Corda, *Puccinia striiformis* West., *Curvularia lunata* (Wakker) Boedijn, *Colletotrichum graminicola* (Ces.) Wilson and *Drechslera siccans* (Drech.) Shoemaker have been recorded in plants (Govi *et al.*, 1974; Cappelli *et al.*, 1993), while *Drechslera siccans* (Drech.) Schoemaker and *Drechslera andersenii* Lam. have been isolated from seed samples (Cappelli, 1991). Using an isolate *of P. coronata* to screen perennial ryegrass varieties in the greenhouse and in the field evidenced a reasonably good level of resistance (Cappelli *et al.*, 1993). However, the monitoring of turfgrass diseases across many Italian environments (ranging from continental to true Mediterranean climate) is nowadays improving, with the establishment of a national turfgrass research network which involves also a series of research institutions dealing with plant pathology. Information on resistance/susceptibility of turf varieties present in the market and belonging to the cool season grasses most utilised in Italy are essential for the operators.

Materials and Methods

Data reported in this paper are referred to the monitoring carried out in Perugia, central Italy (43°03' N 12°23' E), a site characterised by a sub-Mediterranean climate, with a mean annual rainfall of 794 mm and a mean temperature of 13.5 °C. The evaluation was conducted on 40 varieties of perennial ryegrass (*Lolium perenne* L.), 20 of tall fescue (*Festuca arundinacea* Schreb.), 20 of Kentucky bluegrass (*Poa pratensis* L.) and 30 of red fescue: 10 of *Festuca rubra* (L.) subsp. *rubra*, 10 of *Festuca rubra* (L.) subsp. *tricophylla* (Gaud.) Richter and 10 of *Festuca rubra* (L.) subsp. *commutata* Gaud., respectively. The sample of entries includes European and U.S.A. varieties whose list is reported in Annichiarico *et al.* (in press).

The turf entries were established in the Autumn of 1998 in plots of 2x3 m, arranged in a Group Balanced Block Design with three replications.

Observations on the diseases presence were carried out both in 1999 and 2000. Plots showing symptoms of diseases were sampled for laboratory diagnosis. The degree of infection was scored for each plot according to the percentage of area attacked by the pathogen.

Rust fungi in leaf samples were identified on the basis of the morphological criteria proposed by Cummins (1971) while the identification of other micro-organisms was performed through *in vitro* isolation from infected tissues. Infected part of plants were surface-disinfected with 1% sodium hypochlorite for 2 min., followed by three rinses in sterile distilled water on Potato Destrose Agar (PDA) pH 6.5. After 6 to 8 days of incubation at 20-25 °C, the developing colonies were examined visually and microscopically for morphological characteristics.

Results

Major diseases

During the period 1999/2000 the most dangerous diseases were represented by the rusts in *Poa pratensis,* particularly *Puccinia striiformis* and *Puccinia graminis.*

The first attacks of *P. striiformis* were observed during April 1999 and the heaviest incidence of the pathogen was recorded at the end of May 1999. *P. graminis* was observed first during June 1999 and the maximum disease severity was observed during September of the same year.

Differences in the level of susceptibility among varieties of Kentucky bluegrass have been recorded for both the rusts (Tab. 1); as a consequence, for this species the varietal choice has to be considered important in efforts aiming at decreasing the use of chemical treatments.

Tab.1 Behaviour of varieties of *P. pratensis* against rusts.

Causal agent	Resistant varieties (No)	Low susceptible varieties (No)	Susceptible varieties (No)	Highly susceptible varieties (No.)
Puccinia striiformis	9 *	6	4	1
Puccinia graminis	5**	7	6	2

* Names of resistant varieties: Barcelona, Bartitia, Cocktail, Compact, Miracle, Moonlight, Optigreen, Princeton, Unique.

** Names of resistant varieties: Compact, Conni, Haga, Princeton, Saskia.

Minor diseases

In late Spring and early Summer of 1999 and 2000, rings patches of blighted plants with browning symptoms at the collar and at the base of the leaves were recorded and sampled in plots of *P. pratensis* and *F. rubra*. Laboratory assessments ended up with the isolation of *Fusarium* spp. and *Rhizoctonia* spp. In few cases *D. siccans* was also found. During September 1999 traces of *Puccinia coronata* have been recorded in some perennial ryegrass varieties.

Conclusions

The results of our investigation indicate that in central Italy the species most prone to fungi attacks is *Poa pratensis*, while *Festuca arundinacea* is the most resistant. The main turf diseases are caused by *Puccinia* spp. However, the varieties tested showed different amounts of resistance/susceptibility to *P. striiformis* and *P. graminis*. As a consequence, in Kentucky bluegrass a proper choice of variety could have a positive effect in reducing chemicals to control rust diseases. This could be of great importance in low input lawns, such as those in public gardens and private houses. The higher intensity of damages recorded in 1999 could be the consequence of different environmental conditions in respect to year 2000. Further analysis are currently in progress to get more information in this area.

During the period of investigation perennial ryegrass, tall fescue and red fescues varieties have been characterised by low levels of disease damages. Nevertheless, previous investigations in central Italy carried out in perennial ryegrass showed that some of these diseases (rusts in particular) can develop with high level of infection (Cappelli *et al.*, 1993; Cappelli and Polverari, 1994). As a consequence, results obtained in the present research needs to be validated in time.

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SELECTING MEADOW FESCUE ECOTYPES FOR REDUCED SUSCEPTIBILITY TO BACTERIAL WILT

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Abstract

Bacterial wilt caused by *Xanthomonas campestris* pv. *graminis* is an important stress factor for meadow fescue (Festuca pratensis Huds.). We investigated the susceptibility of meadow fescue ecotypes by means of artificial infection trials. Seedlings were raised from seed collected in 9 permanent meadows and pastures in eastern Switzerland. They were inoculated by clipping with scissors contaminated with culture of the standard Xanthomonas isolate used in the breeding programmes at the FAL Reckenholz. Disease scores and survival rates were recorded during three weeks of regrowth in a controlled environment chamber (23/19 ° C day/nigth temperature).

All ecotypes were significantly more susceptible to bacterial wilt than the moderately resistant standard variety PREVAL. However, There were significant differences in disease scores and survival rates among the ecotypes. Those from species-rich, very extensively managed meadows and pastures were the most susceptible. There was a positive correlation between the fertilizer status of the collection site, as estimated from the botanical composition, and resistance to *Xanthomonas*

The differences in susceptibility among the ecotypes suggest that they contain some sources of genetically baseb resistance. At the RAC Changins-Nyon, breeding material based on ecotypes was subjected to several cycles of selection after artificial infection with *Xanthomonas*. Although the initial material was hitghly susceptible, a marked progress in resistance was observed.

Introduction

Meadow fescue (*Festuca pratensis* Huds.) is known to be affected by bacterial wilt caused by *Xanthomonas campestris* pv. *graminis (X.g.)* since the disease was first discribed by Egli *et al.* (1975). Schmidt (1990) ranks the susceptibility of meadow fescue at the same level as perennial ryegrass, next lower to Italian ryegrass, the most susceptible of forage grasses. Therefore, improving resistance to baterial wilt was an important objective of the meadow fescue selection programmes conducted at the Swiss Federal Research Station for Plant Production at Changins-Nyon (RAC). Because Swiss Federal Research Stations were restructured, these activities were recently transferred to the FAL Reckenholz. With the aim to rejuvenate the breeding material, we collected ecotypes from different sites in eastern Switzerland. The objective of the present study was to assess the level of susceptibility of the collected material to bacterial wilt. The results were compared with the botanical composition of the collecting sites to define criteria when searching for more promising ecotypes. We also assessed the progress in resistance obtained formerly by the colleagues of the RAC using successive cycles of selection aided by artificial *Xanthomonas* inoculations.

Materials and Methods

Comparison of ecotypes. Flowering stems of about 50 meadow fescue plants were collected in 9 natural meadows and pastures in May 1999. Seeds were allowed to ripen by placing the stems in tap water containers in isolated greenhouse cabins. 208 seedlings (8 reps. of 26) of each ecotype and of cv. PREVAL were grown in boxes for one month and then inoculated with a highly pathogenic isolate of *X.g.* The base of leaf blades was cut with scissors contaminated with a bacterial suspension (10^8 to 10^9 cells ml^{-1}). Incubation occurred at day/night temperatures of 23/19 °C and with supplemental light for 16 hours. Ten days after the inoculation, disease of individual plants was assessed on a 1 to 9 scale (with 1 = no disease, 5 = whole plant wilting and 9 = dead). Scores were averaged per replication before applying standard analysis of variance procedures.

Comparison of successive cycles of selection. Seed of the initial plant material and of the bulk of selected plants after most of up to 10 successive cycles of selection carried out at the RAC was available. The material had been split after the first cycle, giving rise to two separate breeding lines, each with several more cycles of recurrent selection. For each of the breeding generations and for cv. PREVAL, 168 seedlings (4 reps. of 42) were grown. The same procedure as for the comparison of ecotypes was applied to assess *X.g.* susceptibility.

Results

All ecotypes were significantly more susceptible to bacterial wilt than the standard cultivar PREVAL (Table 1.) However, there were significant differences in disease scores among the ecotypes. These differences were not related to the type of utilization of the collecting sites: When neighbouring sites of different utilization history were compared, in one case the pasture (Birmensdorf) and in one case the meadow (Albis) produced ecotypes of significantly lesser susceptibility. In a third case (Hüttlingen), there was no difference.

Table 1. Susceptibility of 9 meadow fescue ecotypes to *Xanthomonas campestris* pv. *graminis (X.g.)* as compared to cv. PREVAL.

Cultivar/ecotype	Utilization	*X.g.* disease score, 10 d after inoc.
PREVAL	Cultivar	3.04 a
Albis M	Mowing	4.06 b
Gibswil	Pasture	4.47 bc
Birmensdorf P	Pasture	4.66 c
Hüttlingen P	Pasture	4.75 c
Hüttlingen M	Mowing	4.83 cd
Spreitenbach	Pasture	4.93 cde
Birmensdorf M	Mowing	5.28 de
Albis P	Pasture	5.32 de
Rheinau	Pasture	5.36 e

Scores not followed by the same letter are significantly ($p<0.05$) different.

The number of surviving plants decreased dramatically with increasing disease scores. The relationship between the two figures was very close: a curvilinear (exponential) regression on disease score after 10 days explained over 95 % of the variation in percentage surviving plants after 50 days (Figure 1).

Resistance was improved progressively by recurrent selection of superior individuals among inoculated plants with a susceptible breeding population (P), which had been derived mainly from Swiss ecotypes. (Table 2). The most marked relative progress was made in the first selection cycle. In both sub-populations investigated, the degree of resistance levelled off after 4 to 6 cycles of recurrent selection.

Figure 1. Relationship between *X.g.* disease score and survival rate for 9 meadow fescue ecotypes and cv. PREVAL.

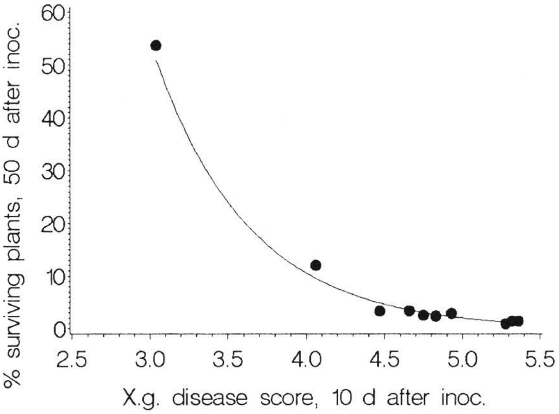

Table 2. Progress in resistance against *X.g.* in breeding populations subjected to recurrent selection after artificial inoculation. Lines A and B were derived starting from the F1 of the common origin (P). PREVAL obtained a score of 5.3.

Generation	P	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
				X.g. disease scores, 10 d after inoculation							
Line A			-	4.25 b	4.53 b	3.93 b	2.43 a	2.28 a	2.68 a	2.05 a	2.38 a
Common	6.28 c	4.55 b									
Line B			-	4.10 b	2.38 a	2.48 a	-	2.18 a	1.88 a	1.75 a	-

Scores not followed by the same letter are significantly ($p<0.05$) different.

Discussion

Although ecotypes of meadow fescue are highly susceptible to bacterial wilt, the significant differences among accessions (Table 1) suggest that they will potentially respond to selection. Indeed, within breeding populations based on ecotypes, it was possible to increase the level of resistance markedly by recurrent selection of resistant plants after artificial inoculation (Table 2). We analyzed the botanical composition of the collecting sites (Table 3) and found some significant correlations between disease scores of meadow fescue ecotypes and sward characteristics of their site of origin (Table 4).

The ecotypes originating from sites with a high proportion of meadow fescue were more susceptible to bacterial wilt. Probably, at these sites there was little natural disease pressure: susceptible meadow fescue genotypes were able to survive, leading to higher proportions of the species in the sward. Susceptibility of meadow fescue also increased with poorer nutrient status, less water supply and higher proportions of leguminosae (other than white clover) in the sward. Such swards are often interesting from the point of view of species diversity but apparently, in terms of serving as new sources of genetic diversity for resistance breeding, they are less valuable than more intensively managed grasslands.

Table 3. Botanical composition of the sites of origin of 9 meadow fescue ecotypes

			Collecting sites sorted by ascending susceptibility for X.g.								
Utilization (M=mowing, P=pasture)			M	P	P	P	M	P	M	P	P
Species sorted by descending nutrient index within functional groups	*Humidity index (Landolt 1977[1])*	*Nutrient index (Landolt 1977[1])*	**Albis M**	**Gibswil**	**Birmensdorf P**	**Hüttlingen P**	**Hüttlingen M**	**Spreitenbach**	**Birmensdorf M**	**Albis P**	**Rheinau**
			Sward percentages per species or functional group								
Grasses			**60**	**75**	**70**	**80**	**65**	**65**	**45**	**60**	**55**
Festuca arundinacea Schreber	4	4	5		3		1		2	5	5
Alopecurus pratensis L.	4	4			8		1			2	
Lolium perenne L.	3	4	10	25	20	18	13	10	5	10	4
Festuca pratensis Hudson	3	4	3	4	5	6	6	10	6	8	10
Poa trivialis L.	3	4	8		8	15	5	10	2	4	4
Dactylis glomerata L.	3	4	5	1	2	6	15	5	2	3	4
Bromus mollis L.	3	4	10	5	5		5	5	2		3
Trisetum flavescens L.	3	4	8	3	1	4	1		5		4
Arrhenatherum elatius L.	3	4	2				2		10	5	
Lolium multiflorum Lam.	3	4	5				13				
Poa annua L.	3	4		3	2	10		2			
Phleum pratense L.	3	4	1	1							4
Agrostis stolonifera L.	4	3		3	4						
Anthoxanthum odoratum L.	3	3	2	6	2			3	3	10	4
Poa pratensis L.	3	3	1	3	5	15		2		2	1
Holcus lanatus L.	3	3		5		1	1	10	5	5	
Cynosurus cristatus L.	3	3		8	5			8	3	2	
Festuca rubra L.	3	3		7		5	1				
Helictotrichon punescens (Hudson) Pilger	3	3					1			4	2
Agrostis tenuis Sibth.	3	2		1							
Bromus erectus Hudson	2	2									10
Forbs			**25**	**15**	**15**	**13**	**23**	**19**	**30**	**25**	**25**
Ranunculus repens L.	4	4	5		5	1	3	7		3	
Cardamine pratensis L.	4	4		1				1			
Taraxacum officinale Weber	3	4		1	1	1	6		2	3	1
Crepis biennis L.	3	4							6		
Galium Mollugo L.	3	4	1				1		2		1
Rumex obtusifolius L.	3	4				3		2			
Veronica Chamaedrys L.	3	4	1			3				1	
Bellis perennis L.	3	4	1	1			1				
Plantago major L.	3	4									3
Plantago media L.	3	4									3
Heracleum Sphondylium L.	3	4					2				
Anthriscus sylvestris L.	3	4	1								
Prunella vulgaris L.	3	4							1		
Capsella Bursa-pastoris (L.) Med.	2	4					1				
Alchemilla vulgaris auct. pl.	4	3		2							
Ranunculus acer L.	3	3	5	1	4	3	3	8	3	2	1
Ajuga reptans L.	3	3	1	1					4	1	
Cerastium caespitosum Gilib.	3	3	1	1	1		1	1	2		
Rumex acetosa L.	3	3	2				1		2	2	
Leontodon hispidus L.	3	3	2						1		2
Ranunculus Ficaria L.	3	3				2					
Chrysanthemum Leucanthemum L.	3	3	2								
Knautia arvensis (L.) Coulter	3	3									1
Veronica filiformis Smith	3	3					1				
Plantago lanceolata L.	2	3	2	7	4		3		5	5	4
Achillea millefolium L.	2	3							2	8	5
Geranium molle L.	2	3									2
Potentilla erecta (L:) Räuschel	2	3									1
Equisetum palustre L.	4	2	1								
Daucus Carota L.	2	2									1
Legumes			**15**	**10**	**15**	**5**	**10**	**15**	**25**	**15**	**20**
Trifolium repens L.	3	4	5	8	10	4	4	7	5	3	4
Trifolium pratense L.	3	3	9		5		4	4	10	8	7
Vicia sepium L.	3	3		1		1	2	2	5	4	1
Lathyrus pratensis L.	3	3		1				2	5		
Vicia Cracca L.	3	3									1
Lotus corniculatus L.	2	3									5
Medicago lupulina L.	2	3	1								2

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Unfortunately, in intensively managed permanent grasslands in Switzerland, meadow fescue often has completely disappeared already.

Table 4. Correlations (r) between *X.g.* disease score of meadow fescue ecotypes and parameters of sward description of their site of origin. r values followed by an asterisk are significantly (p<0.05) different from 0.

Description of sward at collecting site	coefficient of correlation (r)
% meadow fescue	0.781 *
nutrient (nitrogen) index	-0.626 *
humidity index	-0.626 *
% leguminosae other than white clover	0.607 *
% grasses	-0.504

The results suggest that when searching for ecotypes with a reduced susceptibility to bacterial wilt, one should rather avoid sites of permanent grassland where meadow fescue is too abundant. Chances to find sources of resistance should be best where meadow fescue is able to maintain a low proportion in the sward in the presence of an important natural disease pressure.

Conclusions

Selection programmes based on ecotypes of meadow fescue must consider their often extremely high susceptibility to bacterial wilt. However, selection aided by artificial inoculation can be successful. Choice of appropriate collection sites can help obtaining base material with a reduced susceptibility.

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GENETIC ANALYSIS OF CROWN RUST RESISTANCE IN *Lolium perenne*

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Abstract

For the genetic analysis of the resistance reactions towards crown rust an in situ resistance test was established. Under standardised test conditions three types of resistance reactions could be distinguished on leaf segments inoculated with uredospores of crown rust. Plants without any symptoms or with clorotic patches only (type 1) or with single small pustules (type 2) were considered as resistant, whereas those of type 3 (large pustules and sporulation) as susceptible. Homozygous inbred lines in L. *perenne* were selected through evaluation of their resistance. Resistance is expressed dominantly in the F1 progenies. Using these lines F2 and BC1 progenies were produced and tested for their resistance to P. *coronata.*

Segregation analysis reveals that resistance is controlled by two dominantly acting genes. These genes named *Crl* and *Cr2*. In addition, genetic analysis were carried out to identify modifying resistance genes in L. perenne. Results point out, that genes have to exist, which modify the expression of genes *Crl* and *Cr2.*

Introduction

Crown rust, caused by *Puccinia coronata* is one of the most serious diseases of perennial ryegrass (*Lolium perenne*). It occurs worldwide on perennial ryegrass which is used for forage and turfgrass. In previous studies on perennial and Italian ryegrass (*L. multiflorum*) resistance to crown rust was reported to be polygenically inherited in some instances whereas in others it was found to be controlled by few major genes (Kopec et al., 1983, Wilkins, 1975a and b). To render resistance toward regional crown rust pathogen more amenable to efficient selection procedures, we established a detached-leaf test for crown rust resistance. In addition, $I_{0,1}$ lines and their progeny were subjected to segregation analysis to obtain insight into the mode of inheritance of crown rust resistance.

Materials and Methods

A homozygously resistant line was selected out of seven $I_{0,1}$ lines of *Lolium perenne* with resistance to crown rust. Plants of this line were crossed with homozygously susceptible plants of *Lolium perenne* to produce F1 generation. All individuals of this generation were found to be completely resistant to crown rust. F2 and BC1 progeny were produced by intercrosses among resistant F1 individuals or by crossing resistant F1 plants to susceptible individuals, respectively. The youngest fully developed leaves of 12-week old plants were used for detached-leaf tests. Leaf segments of about 3-4 cm long were placed on agar containing 30 ppm benzimidazol. Sample sizes were 70-80 individuals per progeny with two replications per individual. Uredospores of a standard crown rust mixture were suspended in water and sprayed onto the leaf pieces with 300-400 spores/cm² leaf area. About 12 days after inoculation, leaves were evaluated visually for three reaction types to crown rust infection as follows:
Type 1= No pustules and chloroses (resistant)
Type 2= Chloroses with little pustules and sporulation (resistant)

Type 3= Pustules with strong sporulation (susceptible)

Results and Discussion

Segregation of resistant and susceptible plants in F_2 and BC_1 progeny is given in Tables 1 and 2, respectively. Segregation analysis supports the hypothesis that resistance is controlled by two independently acting, dominant genes which we have designated *Cr1* and *Cr2*. There are two resistance types with different reaction to crown rust, i.e., infection 'Type 1' and 'Type 2'. In F1 generation no plants of 'Type 2' were observed. In F2 and BC1 generations resistant 'Type 2' individuals appeared.

To further investigate 'Type 2' phenotypes in their genetic constitution and to test whether they arise from *Cr1cr1Cr2cr2* genotypes, seven 'Type 2' plants from an $I_{0,1}$ progeny were pseudocompatibly selfed at 30°C. Without additional assumptions, segregation data for the seven selfed families does not fit well with the 11:4:1 expectation for the ratio of types 1, 2, and 3, respectively (Table 3). Excess or lack of susceptible individuals is evident within the first and the remaining six selfed progeny, respectively, which indicates gametic selection. It is known from previous studies in rye and ryegrass (Wehling and Uphoff, 1990; Eickmeyer, 1994) that even under high-temperature treatment to induce pseudo-compatibility, male gametic selection may occur upon selfing due to marked differences in the rate at which different *S* or *Z* alleles are knocked out by high temperature. It is also known from other grasses and from Triticeae that a number of rust resistance genes reside on homoeologous group 1 chromosomes. In rye, for instance, leaf rust resistance genes were located on chromosome 1R and one of them found to be closely linked to the self-incompatibility locus *S* (Roux et al., 2000). Considering existing synteny relationships among grasses and Triticeae, thus, location of crown rust resistance genes in *Lolium* sp. and their linkage to a self-incompatibility gene appears as a realistic scenario.

To conclude, a preliminary explanation for segregation data in Table 3 is as follows:

(1) 'Type 2' phenotypes may arise from double-heterozygous resistance genotypes, *Cr1cr1Cr2cr2*.
(2) One of the two segregating *Cr* loci, say *Cr1* may be linked to a segregating self-incompatibility locus, say *S*.
(3) One of the two segregating *S* alleles, say S_1 remains completely functional at high temperature, i.e., the fraction of S_1 pollen grains which can fertilize the egg cell is zero (f_s= 0, with $0 \leq f_s \leq 0.5$).

Fitness of segregation data to expectation for this model is given in Table 3, under the assumption that recombination fraction between *Cr1* and *S* is r= 0.3 and that F2 family #2843 differs from the remaining six F2 families in respect to linkage phase.

Although the model presented has to be verified by more extended progeny testing and the mode of gene action in 'Type 2' individual deserves more thorough investigation, the F2 and BC1 segregation data indicate that resistance of *Lolium perenne* to our crown rust inoculum may be traced back to the action of not more than two major dominant genes. Whether this type of resistance is also effective under field conditions is currently tested. In addition, molecular markers shall be applied for further characterisation of *Cr1* and *Cr2* resistance genes.

Table 1: Segregation of crown rust resistance in F2 generation

F2 Family	n	Segregation Resistant	Susceptible	$\chi^2_{(15:1)}$
2749	75	66	9	4.26
2750	77	73	4	0.14
2751	74	69	5	0.04
2752	74	67	7	1.33
2753	76	71	5	0.01

Table 2: Segregation of crown rust resistance in BC1 generation

BC1 Family	n	Segregation Resistant	Susceptible	$\chi^2_{(3:1)}$
2745	53	40	13	0.01
2746	77	64	13	2.71
2747	74	58	16	0.45
2748	76	59	17	0.28

Table 3: Segregation of crown rust resistance in selfed progenies of type 2 resistant plants

FAMILY	n	Resistant		Susceptible	$\chi^2_{(11:4:1)}$ Linkage *Cr1-S* & gametic selection [a]	
		Type 1	Type 2	Type 3	no	yes
2843	77	42	28	7	7.34*	5.58 [b]
2844	77	56	21	0	5.38	3.08 [c]
2845	77	47	27	3	4.84	4.25 [c]
2846	66	49	16	1	2.61	1.00 [c]
2847	77	62	15	0	7.37*	4.77 [c]
2848	76	58	17	1	4.10	1.69 [c]
2849	77	58	19	0	5.47	3.08 [c]

* Significance, $\alpha = 0.05$

[a] for $r = 0{,}3$; $f_s = 0$
[b] for linkage phase CrS_1/crS_2
[c] for linkage phase CrS_2/crS_1

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RESISTANCE OF RED CLOVER TO *FUSARIUM* FUNGI

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Abstract

In the last decade, red clover disease research has centered on crown and root-rot resistance. Most investigators implicate *Fusarium* spp. as the main causes of root-rot. Species most often mentioned are *F. oxysporum*, *F. solani*, *F.avenaceum* or *F. culmorum*. Control of root rot is difficult. Because root-rotting organisms tend to build up in soils when red clover is grown continually, an obvious control measure is to use crop rotation or to change to a crop that is not susceptible to the same root-rot organisms as is red clover. Resistance breeding is an dominantly method for control of root rot. Various artificial techniques have been used to access and screen for resistance. *In vitro* inoculation procedures, indirect methods based on biological activity of toxic fungal metabolites, *in vivo* screening methods were used. Part of selection must be tests of field resistance because results obtained under model conditions do not always correlate with resistance level. In paper will be summarized results with breeding for resistance of red clover to *Fusarium* fungi not only in Czech republic.

Forage crops occupy a specific position in agricultural primery production. Although they rank high from the viewpoint of the total area devoted to these crops, they are not cash crops in the true sense of the word. They are mostly a tool essential for making profit in plant and animal production. This economic aspects is often taken into account in assessing the importance of forage crops and it also plays a significant role in forage protection against harmful agents. The data collected over the past years have reported nearly no fungicide-treated areas under major leguminous forage crops on the crop land. This fact does not, however, suggest that these crops are free from damage caused by pathogenic agents. Of course, it does not mean that large-scale fungicide application is the only and the best method of protection. But on the other hand, it is an integral part of modern protection systems as well as resistance breeding, the importance of which is increasing in the context of what has been said about forage crops. As suggested by the present literature , the problems of increasing resistance of major forage legumes including red clover to biotic agents have received increased attention worldwide.

The chief object of research workers and plant breeders is to produce plant materials with combined resistance capable of eliminating genetically the negative effects of several pathogens all at once. Plant materials modelled in this way might exhibit not only increased resistance to biotic agents but also increased general compatibility of a cultivated crop with the remainder of the agrosystem and increased competitivenes. An important factor which correlates with resistance is also increased persistence. Last but not least, this might prevent losses in green matter or seed quality.

The success of selection work is dependent on a number of factors which must be taken into account. Among the most significant are a suitable selection technique, understanding of the bionomics of a pathogenic organisms, information about the genetic basis of resistance, knowledge of correlation between resistance and other characteristics, etc. In pathosystems of red clover some of the theoretical questions can only hardly be precisely answered.

In the last decade, red clover disease research has centered on crown and root-rot resistance. Most investigators implicate *Fusarium* spp. as the main causes of root-rot. Species most often mentioned are *Fusarium oxysporum*, *F. solani*, *F. avenaceum* and *F. culmorum*.

Root rot implicated aslo other genera as *Cylindrocarpon* or *Pseudomonas,* but their significance are not so important as *Fusarium spp.*

Control of root rot is difficult. Because causal organisms tend to build up in soils when red clover is grown continually, an obvious control measure is to use crop rotations in which red clover is less frequently sown, or to change to a crop that is not susceptible to the same root-rot organisms. Pesticide control is other possible of control, but level of efficiency and relatively great financial expenses are limiting factors. From these reasons resistance breeding is a dominantly method for control of root rot.

Various artificial techniques have been used to access and screen for resistance in red clover. *In vitro* inoculation procedures include inoculation plants in the cotyledon stage in sterile test tubes for example with a mixture of *Fusarium* species. Cultivars were differentiated according to their resistance. A similar test can be used to measure the frequency and intensity of seed infection on plants grown on a nutrient medium in test tubes. Intensity of infection was highest in seeds of moderately to highly susceptible cultivars. High correlation of laboratory and field assessments of the resistance of 16 cultivar of red clover indicated that laboratory tests could be used for preliminary screening of breeding materials before test start under natural conditions.

Indirect methods of assessing root-rot resistance *in vitro* have been attempted by a number of investigators. Biological activity of toxic fungal metabolites of *Fusarium* spp. has been shown to be correlated with virulence of the isolates. Culture filtrates of *F. roseum* also have been tested on red clover.

In vivo methods for evaluation and/or screening for resistance to root rots of red clover have been used. Some authors dipped rooted cuttings in a suspension of *Fusarium* spp. spores and was able to differentiate resistance in diploid and tetraploid cultivars. A similar method is placed severed roots in pots in contact with *Fusarium* inoculum. After two cycles of selection, progenies had less root rot than their parents. Many authors indicate that the material must be tested also under field conditions to determine if populations have been improved.

Many investigators report the evaluation and screening for root-rot resistance under greenhouse and/or field conditions, either under natural or artificially induced epiphytotics. It is interesting to note that, under field conditions, factors of general adaptability were often involved with resistance to root rot. The best results of selection are usually found in local cultivars.

Unfortunately we have recently a little evidence of heritability of root rot resistance. Some papers suggests that the similarity of progeny reaction to *Fusarium* spp. to that of the mother strain indicated that the degree of resistance might be heritable, and that transfer of resistance was possible.

RECURRENT SELECTION FOR CROWN RUST RESISTANCE AND ESTIMATION OF ITS HERITABILITY IN MEADOW FESCUE

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Abstract

We have run a recurrent selection programme since the early 1990´s to improve the resistance to crown rust in meadow fescue.

A comparative study of the effect of recurrent selection for crown rust resistance in meadow fescue was recently undertaken. Such a study require a simultaneous comparison among the different generations of selection including the base generation. We carried out such a comparison in four different varieties.

The results indicated a significant improvement of the resistance against crown rust in each of the four varieties worked with. On average over the 4 varieties ,with the disease rating of the base population set to 100, two generations of selection brought the disease rating down to 28 % of that of the base population. That is a reduction ,difference to the base population, of 72 % with the least significant difference at the 5 per cent probability point ($LSD_{0.05}$) = 10%.

Keywords:
Meadow fescue (*Festuca pratensis*), Resistance, Crown rust (*Puccinia coronata*), Heritability, Recurrent selection.

Introduction

Crown rust (*Puccinia coronata*) is the most important rust disease of grasses in Sweden. Although more important on perennial ryegrass (Jönsson &Engqvist 1998), meadow fescue may suffer significant yield losses from rust attacks some years in the late season growth. Such losses justifies plant breeding efforts in material intended for use in southern Scandinavia and further south.

We have run a recurrent selection programme since the early 1990´s to improve the resistance to crown rust in meadow fescue. A comparative study of the effect of recurrent selection for crown rust resistance in meadow fescue was recently undertaken. Such a study require a simultaneous comparison among the different generations of selection including the base generation. We carried out such a comparison in four different varieties, and also estimated the heritability of the resistance to crown rust.

Material and Methods

Plants for laboratory assessment were raised in the greenhouse at ca 20°C with 18 hours of light. They were inoculated with uredospores at the 3-4 leaf stage. Inoculum was produced on plants of susceptible varieties. These susceptible varieties varied during the years. The races of the fungus also varied since new inoculum was repeatedly collected from naturally infected plants in the field and added to the bulk of inoculum. The aim was to use a composite inoculum in order not to select for major gene resistance.

After inoculation the plants were covered with a plastic sheet for two days to ensure a high relative humidity and kept at 20°C. After another 10-12 days the plants generally were ready for rust assessment. The scoring was done on a 1-4 scale, where 1 is completely resistant, and 4 is susceptible. The disease index (DI) was calculated in the traditional way (Engqvist and Jönsson 1997). Repeated selections for crown rust resistance have been made in Sigmund and 3 other populations after inoculation with the rust fungus. The selected plants in each cycle of selection were planted out in isolated multiplication for recombination within the populations.

For inheritance studies 50 plant progenies in two of the populations were inoculated and assessed for rust in order to make an estimation of the heritability.

Results

The effect of the selections was studied in a comparative test of the seed obtained in the various cycles of selection. A significant improvement in the degree of resistance against crown rust was obtained in the material worked with. On average over the 4 varieties ,with the disease rating of the base population set to 100, two generations of selection brought the disease rating down to 28 % of that of the base population (Table 1).

On an average the heritability of rust resistance was 0.68 when estimated through variance components (Table 2). Estimations through regression analysis of disease indices were lower and especially so in the population with a superior resistance in the K0 generation.

Table 1. Meadow fescue, *Festuca pratensis* Hudson. Comparative test of the effect of 2 cycles of selection against crown rust, *Puccinia coronata* in the 4 different populations studied. DI = Disease Index 0-100, where 0 = no attack. % = Susceptibility relative to base population, K0.

Generation of selection	Variety								Average	
	Sigmund		**SW ÄS 85 D1**		**IL95/4337**		**IL97/4515**			
	DI	%	DI	%	DI	%	DI	%	DI	%
K0	64	100	13	100	49	100	32	100	40	100
K1	34	53	10	77	26	53	23	72	23	58
K2	16	25	7	54	8	16	12	38	11	28
$LSD_{0.05}$	12	19	5	38	6	12	8	25	4	10

Table 2. Meadow fescue, *Festuca pratensis* Hudson. Heritability estimates for resistance to crown rust, *Puccinia coronata*.

Mode of calculation	Sigmund	SW ÄS 85 D1	Average
Variance components	0.81	0.55	0.68
Regression	0.63	0.30	0.47

Conclusions

Recurrent selection appears to be effective in improving the resistance to crown rust in meadow fescue, which is also indicated by the heritability estimates.

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Breeding for abiotic stresses resistance

BREEDING PERENNIAL GRASSES FOR ABIOTIC STRESS TOLERANCE

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Abstract

Perennial forage and turf grasses are subjected to numerous stresses throughout the lifetime of a pasture, ley, lawn, or athletic field. Abiotic stresses can be manifested as reductions in vigor or quality of a grass sod up to complete stand mortality, including all intermediate possibilities. Throughout the lifetime of a stand, abiotic stresses can increase in intensity and severity (e.g. soil toxicities, air pollution), they can be constant (e.g. cultural practices, soil physical properties), or they can fluctuate regularly (e.g. cold, freezing) or irregularly (e.g. draught, anoxia). Breeding for stress tolerances is complicated largely by the need for a screening procedure thats is constante, repeatable, and meaningful. Screening procedures are complicated by issues such as duration and intensity of the stress, growth stage of selection units, cultural practices, and interactions with other stresses. Long – term natural selection in existing grass stands is very effective at creating useful levels of stress tolerances, but requires too many years to be effective beyond the first generation of cultivars. Introduction of foreign genes by plant transformation will eventually allow development of new cultivars from stress-tolerant transgenic plants. However, breeders must exercise caution in the development of transgenic cultivars, ensuring that the benefits of the transgenic product to its consumers exceed the costs of its development. The need to share royalty payments among numerous contributing organizations may lead to marking difficulties for transgenic forage and turf grasses that already have low profit margins

Introduction

Genetic polymorphisms in perennial grasses are due to the interaction of plants with their environment, ie. the occurrence of stresses. As new genes arise due to mutation, they increase in frequency if they confer a fitness advantage in a particular environment, or disappear if they do not. In a constant environment, the single genotype with the greatest accumulation of genes conferring fitness to individuals will always be favored. In reality, environments fluctuate in both space and time and genotypes are plastic, ie. they have genes that regulate their phenotype depending on the current or local environmental conditions (Via, 1994). Individual genes may confer increased fitness in one environment, decreased fitness in another environment, and be neutral in still another, resulting in genotype x environment interaction.

Environmental conditions are determined by numerous interacting and often interdependent climatic and soil factors. Most perennial grasses have evolved adaptation to a wide range of climates and habitats, suggesting that they possess intraspecific genetic variability for numerous stress tolerances. Synthetic cultivars, which comprise the majority of the forage and turf market, can be designed with a multitude of genotypes that have various stress tolerances, conferring greater phenotypic plasticity to the cultivar than would be possible with a single genotype. This creates a challenge for the plant breeder: to define the target and selection environments with sufficient clarity and purpose to ensure that the most favorable genotypes are selected.

Recent reviews have focused on breeding for stress tolerances in forage (Casler et al., 1996) and turf grasses (Duncan and Carrow, 1999). These reviews focus largely on individual stresses, citing a large body of literature on a range of abiotic stresses. This review will emphasize selection and breeding methods, documenting their strengths and weaknesses, using individual stresses largely as examples.

Natural Selection

Genetic variation in tolerance to low temperatures has evolved in numerous perennial grasses. When evaluated under extremely low temperatures, germplasm collections of perennial grasses from widely different climates generally demonstrate considerable variability in survival or persistence. Much of this variation is associated with local conditions, such as mean temperature of the coldest month of the year, altitude, or duration of snow cover (Badoux, 1979; Humphreys and Eagles, 1988; Klebesadel and Helm, 1986). In festulolium [*Festulolium loliaceum* (Hudson) P.V. Fournier], selection of surviving plants from 3-year-old hay fields or a 5-year-old pasture resulted in a 10-fold increase in plant survival and a 40% increase in tiller survival of surviving plants following 3 days exposure to -11°C (Casler et al., 1997). This increase in freezing tolerance resulted in a 12.4% increase in ground cover of living crown tissue following two or three winters and a 1.2% increase in 2-year-total forage yield at 14 field sites (Casler et al., 2000, unpublished data).

Natural selection for increased tolerance to toxic elements such as salinity, numerous heavy metals, and atmospheric SO_2 and NO_2 is well documented (Casler et al., 1996). As exposure to these toxins increases, intolerant plants are removed from populations of perennial grasses, leaving tolerant plants to take their place by vegetative or sexual reproduction. In many cases, acute exposure can result in rapid increases in the frequency of tolerant plants, as rapidly as 3 years in the case of SO_2 (Wilson and Bell, 1985). The rapidity and/or the degree to which natural selection increases a population's tolerance to a toxin is typically proportional to the degree or duration of exposure (Antonovics et al., 1971; Horsman et al., 1979; Venables and Wilkins, 1978). In some cases tolerance to toxins carries a physiological cost, leading to reductions in the frequency of tolerant plants when exposure is ceased (Hickey and McNeilly, 1975; Wilson and Bell, 1986). Generally, tolerance to toxins is inherited independently, with little evidence of cross-tolerances between different toxins.

Natural variability for drought tolerance within many species suggests that natural selection may also be useful in identifying more drought tolerance genotypes. Perennial ryegrass (*Lolium perenne* L.) accessions from relatively dry collection sites were more likely to be tolerant of drought stress (Reed et al., 1987; Wedderburn et al., 1990). However, in both studies, relatively drought-tolerant accessions generally had lower vigor and forage yield upon rewatering, indicating the presence of a negative genetic correlation between performance in drought-stress vs. non-stress environments. The negative genetic correlation suggests that it is highly unlikely that genotypes with both drought tolerance and high yield under both stress and non-stress environments can be identified (Rosielle and Hamblin, 1981). Indeed, research on barley (*Hordeum vulgare* L.) in drought-stress vs. non-stress environments confirms Rosielle and Hamblin's theoretical predictions (Ceccarelli, 1996).

In summary, natural selection can be very useful for developing stress tolerances in forage and turf grasses. However, lack of environmental control leads to variation in selection pressure, which increases the time required to achieve meaningful progress. Furthermore, because most leys, pastures, lawns, and athletic fields are prevented from undergoing sexual reproduction, natural selection is essentially a non-recurrent process. It has been useful in developing numerous cultivars, but may have reduced utility in the future of perennial grass breeding.

Methodical and/or Recurrent Selection

Direct Selection for Stress Tolerance

Definition of the proper selection environment is one of the most important factors influencing the success of a recurrent selection program. Identification of the proper degree and duration of exposure to any stress will determine the genetic variation observed under stress, the heritability of measured stress tolerance, and the rate of progress. Identification of optimal conditions may require preliminary experimentation. In some cases, plants can be exposed to multiple stress factors if multiple stress tolerances are required. Multiple-stress screening experiments should be planned carefully, because additional selection criteria may decrease selection differentials (and the rate of progress) for each individual stress tolerance.

While screening for disease resistances has worked remarkably well on young seedlings, due to high correlations with adult-plant resistance (Casler and Pederson, 1996), this generalization is not always true for abiotic stress tolerances of the perennial grasses. Selection for cold tolerance (percentage tiller survival at low temperature) in Italian ryegrass (*L. multiflorum* Lam.) seedlings was highly successful, averaging a 19% reduction in tiller mortality (Hides, 1979). However, when exposed to field conditions, selected populations averaged 8% less plant survival than the original populations. Similarly, lines of two wheatgrass species selected for germination in high-salt solution culture were inconsistent in subsequent adult-plant evaluations of vigor and/or forage yield (Dewey, 1962; Pearen et al., 1997). An exception to this is Zn tolerance in colonial bentgrass (*Agrostis tenuis* Sibth.), in which two cycles of phenotypic recurrent selection for seedling growth achieved the same results as 21-31 years of natural selection (with minimal to no recombination) under field conditions (Al-Hiyaly et al., 1993).

Perhaps because of the difficulties inherent with defining and sustaining proper selection environments and the potential problems associated with selection on seedling traits, there are few reports of direct selection for abiotic stress tolerances in forage and turf grasses. High-stress environments tend to reduce genetic variability and heritability (Allen et al., 1978; Ceccarelli, 1989), making the plant breeder's job more difficult by demanding increased precision of forage yield/vigor measurements, a more highly-trained eye for turf quality ratings, and higher selection intensities to offset reductions in heritability (thereby increasing population sizes). These problems have led many plant breeders and physiologists to collaborate in the identification of stress tolerance mechanisms, with the goal of developing simpler and more heritable selection criteria.

Indirect Selection for Component Traits

Drought has received the most attention to identify mechanisms of stress tolerance and develop better selection criteria. Numerous experiments have identified several physiological factors that were heritable and positively correlated to measures of drought tolerance. High stomatal resistance (Wilson, 1975a), deep ridging of the adaxial leaf epidermis (Wilson, 1975b), low leaf water conductance (Gay, 1989), low osmotic potential (Thomas and Evans, 1989), and low carbon-isotope ($^{13}C/^{12}C$) discrimination ratio (Read et al., 1993) were all heritable traits that were found, subsequent to successful selection, to confer some degree of increased drought tolerance or water-use efficiency. Despite the positive and encouraging results from the majority of these experiments, there is little evidence that other laboratories or breeding programs have incorporated these traits into their plant improvement programs. Reasons for this may include the need for specialized equipment and training, the potentially daunting task of making laborious and expensive measurements on thousands of plants, and a perception that drought tolerance is not a necessary selection criterion.

Deep planting (5 cm) is a drought-avoidance mechanism, allowing seedling roots to reach soil water more rapidly, but potentially compromising seedling emergence and survival. Four cycles of mass selection for emergence from deep planting in Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski] resulted in a 175-254% increase in deep-planting emergence, and a 70% increase in field emergence from deep planting with a 26% increase in forage yield at dryland sites (Lawrence, 1979). Although no root measurements were made on these populations, the results suggest that higher root growth rates may be partly responsible for the observed gains.

Selection of seedlings with the longest roots in high-salt solution culture resulted in 67-100% increases in adult-plant dry weight for seven temperate grass species exposed to three levels of salt stress (Ashraf et al., 1986). The selection protocol also increased adult plant dry weight in a no-salt environment for three of the seven species, suggesting that salt tolerance may not carry a physiological cost. Alternatively, the selection criterion (increased root growth in high-salt solution) may be a composite trait, resulting from both increased tolerance to high salt concentrations and increased root growth rate per se.

Root growth and development is largely ignored in forage and turf grass breeding programs. Duncan and Carrow (1999) call the root system the "first line of defence in adaptation to abiotic and edaphic stresses." Perennial grasses have highly plastic root systems with individual roots possibly as short-lived as individual leaves (Eason and Newman, 1990; Langer, 1963). Root systems are constantly adjusting and responding to stress factors both below and above the soil surface (Grime, 1988). Indeed, a highly plastic, dynamic, and fast-growing root system may be the most important determinant of a plant's ability to compete against its neighbours (Donald, 1958; Hofman and Ennik, 1980). In tall fescue (*Festuca arundinacea* Schreb.), genotypes with larger primary root diameters, but fewer primary roots, are better able to penetrate hardpans and can extract water from twice the depth as genotypes with a larger number of smaller roots (Torbert et al., 1990).

Interspecific Hybridization

Breeders have long sought to combine favourable traits of different species into a single genotype, such as the drought tolerance of tall fescue with the forage quality and seasonal growth characteristics of Italian ryegrass. A rain shelter was used to screen *L. multiflorum* x *F. arundinacea* hybrid derivatives, followed by one cycle of phenotypic selection (Humphreys and Thomas, 1993). Drought tolerance scores for the introgression lines averaged 72% of the *L. multiflorum* parent prior to selection and 131% following selection, 43% of the *F. arundinacea* parent prior to selection and 76% following selection. Genomic in situ hybridization identified a single *F. arundinacea* chromosome segment on the long arm of *L. multiflorum* chromosome 2, which DNA probes identified as deriving from the *F. pratensis* subgenome of *F. arundinacea* (Humphreys and Pasakinskiene, 1996). A further evaluation of these introgression lines identified individual lines with both drought and freezing tolerance exceeding that of the superior parent, *F. arundinacea* (Humphreys et al., 1997).

Phalaris (*Phalaris aquatica* L.) is highly intolerant of acid soils, but its close relative reed canarygrass (*P. arundinacea* L.) is relatively tolerant. *P. aquatica* x *P. arundinacea* hybrid derivatives that were morphologically similar to *P. aquatica* were tested on limed and unlimed acid soils (Oram et al., 1993). Introgression lines had average vigour ratios (unlimed:limed) of 0.99 vs. 0.35 for *P. aquatica* cultivars.

Several cultivars of various wheatgrass species have documented improvements in tolerance to saline or dryland soils (Asay and Jensen, 1996), but it is impossible to know how much of the improvements are due to interspecific hybridization per se or to selection among amphiploid progeny. Tall wheatgrass [*Thinopyrum ponticum* (Podp.) Liu and Wang] has served as a valuable donor of genes for salinity and drought tolerance in wheat (*Triticum aestivum* L.) breeding programs (Sharma and Gill, 1983).

Plant Transformation

Over 100 potential transgenes have been identified as genetic factors affecting abiotic stress tolerances (Duncan and Carrow, 1999). Transgene expression has been confirmed for genes controlling drought tolerance, salt tolerance, and potential shade tolerance (modification of the red:far red ratio to increase light interception) (Duncan and Carrow, 1999). Transgene expression for increased stress tolerance has not been reported in forage or turf grasses, although there are efforts at Rutgers University to transform creeping bentgrass (*Agrostis palustris* L.) with the mannitol-1-phosphate dehydrogenase gene to improve drought tolerance (Duncan and Carrow, 1999).

Despite the promise and potential advantages of transgenic approaches for improving stress tolerance, this technology may not be well suited to many forage and turf grasses. The problems usually encountered with plant transformation will exist for perennial grasses: technological development costs, limited germplasm sources capable of regeneration, the need for considerably backcrossing, and transgene instability. Additional disadvantages will include transgene introduction to wild relatives by outcrossing, potential loss of genetic variability and stability due to narrow transgenic germplasm bases, and the need for additional selection pressure within transgenic populations which will lengthen the breeding cycle. Finally, royalty payments or licensing fees must be paid to patent holders at each step of the transformation process, further reducing the already marginal profit margins of most perennial grass cultivars. History has shown in the USA that excessive royalty demands, which necessitate excessive retail seed prices, are the death knell of perennial grass cultivars. Transgenic cultivars must have an obvious economic benefit to consumers if they are to survive in commercial markets.

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GENETIC AND PHYSIOLOGICAL ASPECTS OF COLD TOLERANCE OF FODDER CROPS

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Abstract

The accumulation of free proline in the overground plant part is used to estimate frost resistance of ryegrass growing in artificial climatic conditions. Varieties and species of reygrass were found to differ in frost hardiness and in their capacity to accumulate proline. The proline accumulation coefficient (PAC) allows to differentiate varieties in terms of their frost hardiness after the first phase the hardening.

Frost resistance can be increased by selecting the plants that survived freezing and by concentrating the genes controling this character in the population or using intergeneric hybridization.

Key words: free proline, frost resistance, proline accumulation coefficient, reyrgass varieties, freezing tolerance.

Introduction

The crop grows successfully, develops, produces grass and seed yields under favourable conditions. However, they are often unfavourable, therefore plants are affected by stress environmental factors. Most often it is temperature. It has the greatest impact on genetic diversity and geografical distribution of plants whereas variety and population diversity proves their genetic adaptation in different temperature regimes (Perez de la Vega, 1997).

The degree of stress resistance is genetically controlled and inheritable, under the optimum growth condition it is latent potential character revealing itself in extreme conditions (Udavenko, 1988).

Cold accumulation in plants is associated with the expression of cold regulated genes (Nancy N., *et.al.* 1996). Under our country climatic conditions the impact of low temperatures on perennial grasses, i.e. their frost resistance and overwinter survival is extremely important.

Frost, like any other stress, causes physiological changes in every all of the living organism. These changes are reflected by changes in the content of proline and sugars (Mantyla, 1997; Windt, 1999) and by the activity of some other enzymes (Bredemeijer *et.at.* 1995). Variation in the content of free proline and carbohydrates under the impact of stress depends on plant genotype (Thomas *et.at.* 1993). Biologic role of proline in plants is very important (Britikov ,1975) and is the subject for heterogenous research of its functional meaning. Variuos researchers explain the accumulation of free proline differently. Pagoun (1984) suggests using it as "metabolic marker of hardening", however. Chu (Chu *et.at.*,1978) did not find any connection between proline accumulation and frost resistance in barley and wheat. The objective of this study was to examine the changes in free proline during low temperature and frost resistance of perennial grasses and their intergeneric hybrids under controlled and natural conditions.

Materials and Methods

The content of free proline was determined in every freezing, temperature and control plants growing at 20 ^{0}C using Bates (Bates et.at., 1973) method. Accumulation of proline in plants was estimated using proline accumulation coefficient (PAC): ratio amound of free proline of freezing plants to control plants.

The plants were grown in a greenhouse until the 2^{nd}-3^{nd} leaf stages. Then they were hardened cunder controlled conditions at a temperature of +2 ^{0}C for two weeks the first phase of hardening) and at a temperature of -3 ^{0}C in the dark for 3 days (the second phase of hardening). The resistance to low temperatures was estimated by using the standard freezing regime in a low temperature freezing chamber KHT-1. The plants that survived after the freezing grown in a greenhouse, vernalized and then were planted in the field to obtain seeds of F_1 hybrids. After freezing the plants were defrosted at a temperature 2 ^{0}C and grown in a greenhouse for two weeks and then counted.

Results and Discussion

Ryegrass species and varieties differ by their tolerance to low temperature. The most cold resistant was perennial ryegrass variety 'Pašavy' which survived at the temperature –12 ^{0}C 40,6 % plants (Table 1). F1 hybrids selected from cold tolerant individuals by the method of direct freezing were significantly more resistant than their parental forms (Table 1). Frost resistance is determined genetically. Therefore, frost resistance can be increased by selecting the plants that survived freezing and by concentrating the genes controlling this character in the population.

As a rule tetraploid forms are more resistant to low temperature, but it depends on the species. In our investigations winter-hardiness of tetraploid variety *Doctylis glomerata* was higher then in diploid forms (Fig.1). On the contrary *Festuca pratensis* diploid variety was more resistant then tetraploid populations (Fig.1) The number of genes has a different effect for different species. The metabolitic processes occurring in a plant cell define the plant tolerance to unfavourable environmental conditions. The role of aminoacid proline is very important in these processes. Under normal conditions the proline content in plants is very low. It rises under the influence of cold and depends on grass species and variety (Fig.2). Various cold-tolerant varieties accumulate proline differently (Fig.2). Change of proline content can be used as an indirect indicator of frost hardiness in perennial grasses. The proline accumulation coefficient (PAC) in the overground part of plants differentiates perennial species and varieties by frost hardiness after the phase of hardening (Fig.2). The proline amount in plants that survived after freezing and were regrown to 2-3 leaves was 1,77 times higher in var. 'Veja' and 1,44 times higher in var. 'Varpe' in comparison with the proline amount before hardening.

Exogenic proline increases surviving of *Lolium perenne* seedlings after freezing at –7^{0}C and -10 ^{0}C temperatures (Fig.3). These results confirm the protective role of proline to cold stress.

Another way to develop cold tolerance population is intergeneric hybridization between fescue and ryegrass. The main goal of these hybrids is to concentrate a good forage value of ryegrass, winterhardiness and durability of fescue. The intergeneric hybrid variety 'Punia' was bred by the hybridization of the tetraploid forms *Festuca pratensis* Huds variety 'Dotnuva I' and *Lolium multiflorum*. Lam variety 'Muljam'. The new variety and two standards were sown in the variety trials. The variety of perennial ryegrass 'Veja' and the variety of meadow fescue 'Dotnuva I', both of which were registrated in Lithuania.

According to the date variety trials, the yield of forage of the variety 'Punia' was 58,2 and dry matter yield 15,4 t/h every year.(Table 2) Winter-hardiness of 'Punia' was 4,5 points, 'Veja'-3,7 and 'Dotnuva I'4,8 points. 'Punia' was infected by crown rust only by 0,4 points, 'Veja' 2,4,'Dotnuva I' by 3,0 points.

In 'Punia' the leaf spot was evaluated by 1,0 point, in 'Veja'3,5 points,'Dotnuva I' 4,0 points. This means that the new variety had a four times higher resistance to this disease (Table2).

Since 1997 variety 'Punia' is registrated in Lithuania.

Conclusions

Frost resistance is determined genetically, and resistance genes can be concentrated in plants in various ways. It can be obtained by selecting hybrid generations after several freezing cycles or by intergeneric hybridization, therefore genetic diversity is the main factor determining plant adaptibility to stress. The proline accumulation coefficient enables to differentiate among the ryegrass varieties according to their cold tolerance after the first stage of harding. Increase number of genomes has a specific effect for different species.

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Table 1. The survival of ryegrass species and their hybrids

Species, varieties	The survival, %					
	-5 ^{0}C		-10 ^{0}C		-12 ^{0}C	
	parental	F_1	parental	F_1	parental	F_1
L.p. Pašavy	85,0	93,3	37,8	56,7	40,6	31,6
L.p. Veja	82,5	98,3	24,0	41,6	7,6	10,8
L.m. S_{22}	49,2	74,0	10,8	17,3	5,1	12,5
L.w. Varpė	21,8	43,4	1,6	2,5	0,0	0,0
LSD $_{05}$	13,5	6,2	7,5	13,5	7,5	4,6

Table 2. Evaluation of variety 'Punia' and registrated varieties *L.perenne* 'Veja' and *F.pratensis* 'Dotnuva I'

Name of variety	Productivity, t/ha				Winter-hardiness (scale 0-5)	Disease resistance (scale 0-5)	
	dry matter	%	seeds	%		crown rust	leaf spot
Veja	10,7	100,0	0,65	100,0	3,9	2,4	3,4
Punia	15,4	143,3	0,76	125,9	4,5	0,4	1,0
Dotnuva I	13,1	122,4	0,94	144,6	4,8	3,2	4,2
LSD_{05}	1,7						

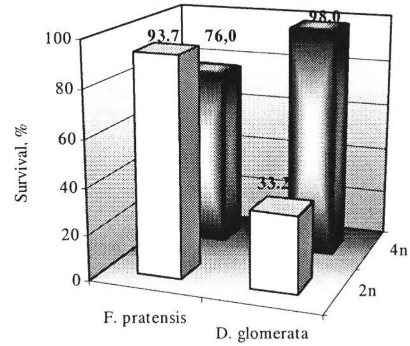

Fig. 1. Winter- hardiness diploid and tetraploid forms *F.pratensis* and *Dactylis* glomerata (3rd year of growing)

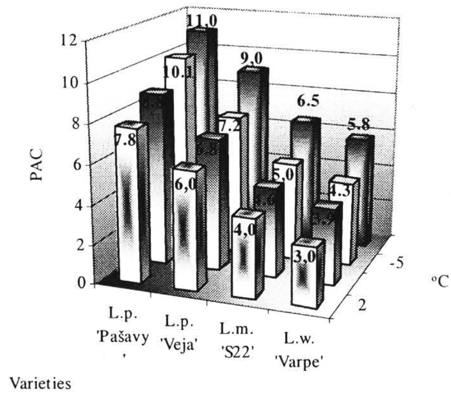

Fig. 2. Proline accumulation in different species *Lolium*

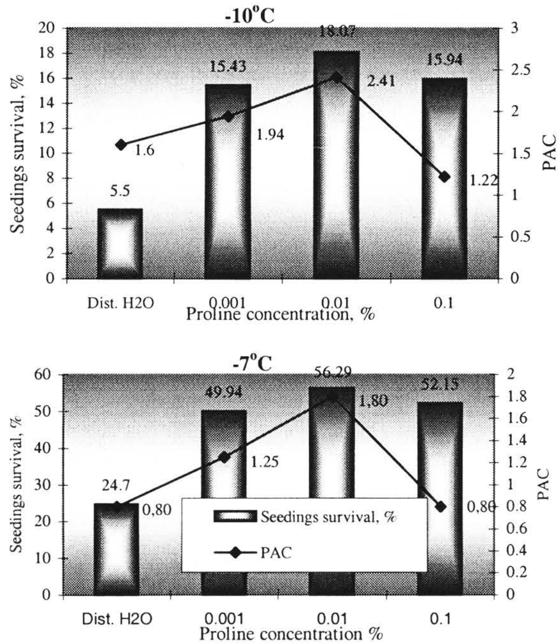

Fig. 3 Effect of exogenic prolins on the *Lolium perenne* L. frost hardiness

GROWTH RATES OF A *Dactylis glomerata* POPULATION UNDER RELATIVE LOW TEMPERATURES

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Abstract

Cool-season grasses do not become completely dormant during winter, thus whenever ambient conditions are favorable they grow. Growth rates of plants depend on environmental conditions, mainly air temperatures and genotypes as well. The aim of the present study was to evaluate the Relative Growth Rate (RGR) within a local broad-based population of *Dactylis glomerata* in relation to plants' productivity capacity and air temperature, during winter. The study was conducted in Northern Greece at 500 m elevation. The mean maximum temperature range from 5 to 22 ^{0}C and mean minimum temperature range from 0.7 to 8 ^{0}C. Tillers' height of sixty plants was measured weekly from October to March. Dry matter production of the plants was evaluated at the end of November, January and March. The RGR of low and high yielding plants did not differ significantly under favourable growth conditions (mean maximum air temperature 10-20 ^{0}C) from October to November, while it was higher for the high yielding plants, under cold stress conditions (5-10 ^{0}C), from February to March. During this period the high yielding plants were growing 2.5 times faster than the low yielding plants. The RGR was zero for the both groups, when temperature dropped below to 5 ^{0}C (December-January). It seems, that dry matter production of plants during winter depends more on genes controlling tolerance to cold stress, rather than on genes controlling yield potential of plants. Consequently, the development of productive cultivars with wide adaptation presupposes efficient incorporation of genes controlling yield per plant and genes controlling tolerance to stress into a single genotype.

Introduction

Herbaceous plants (Boudet et al., 1993) are gradually adapted to the low winter temperatures, by changes in membrane properties, accumulation of solutes with cryoprotective properties and synthesis of new proteins as result of new genes expression. The above physiological changes partly restrict plants' photosynthetic capacity (Oquist, 1983). However, some herbaceous species such as *Secale cereale* retain a high photosynthetic capacity even during the winter (Huner et al., 1986). Dry matter production is in function not only of photosynthetic production but of photosynthates' partioning between growth and storage as well and is highly influenced by environment and mainly by air temperatures (Griffith and McIntyre, 1993). This depends on genotype and show intervarietal differences within many species (Pollack an Eagles, 1988). The aim of the present study was to evaluate the growth rates within a local broad-based population of *Dactylis glomerata* in relation to the productive capacity of plants and the air temperature, during winter.

Materials and Methods

The study was conducted in Northern Greece at 500m elevation, with a mean annual precipitation of 600mm. The mean maximum temperature range from 5 to 22 ^{0}C and mean minimum temperature range from 0.7 to 8 ^{0}C (Fig. 1). A broad-based population of *Dactylis glomerata* was evaluated in a R-21 honeycomb design (Fasoulas and Fasoula, 1995), for dry matter production during winter, for two consecutive years. During the third year thirty high and thirty low yielding plants from the above population were selected. The selected plants were clipped at 5cm above ground on October 7^{th}. Three shoots of each plant were marked and their height were measured at weekly intervals until March 24^{th}. The experimental period (October-March) was divided in to three parts, according the mean maximum air temperature. The first part (1^{st} period) was from October to November (22-12 ^{0}C), the intermediate period from December to January (< 4^{0}C) and the third part (2^{nd} period) from February to March (10-4 ^{0}C). The intermediate period was not analysed, as the growth rates were zero. Dry matter production was measured at the beginning and the end of each test period. Weekly dry matter production was evaluated from regression equations developed by correlation dry weight and height. The Relative Height growth Rate (RHR)=1/h*Dh/Dt and the Relative weight Growth Rate (RGR)=1/W*DW/Dt was calculated for each period. Growth Performance Coefficient (GPC=W/t) was calculated as an indicator of plants' productivity potential for each period. Data were subjected to analysis of variance and significant differences between means were evaluated by the LSD test (Steel and Torrie, 1980) at a probability level of p=0.05.

Results and Discussion

No significant differences in mean RHR (Table 1) of the 1^{st} period, were observed between low and high yielding plants under favourable growth conditions, while there was significantly higher RGR for the high yielding plants. Both RHR and RGR decreased drastically (Fig.2), as air temperature dropped from 22 to 12^{0}C. Although, the high yielding plants had higher mean RGR of, the GPC (Table 1) of the two groups did not differ significantly. It seems that there are differences among low and high yielding plants in partitioning of photosynthates between organs. The high yielding plants allocate higher proportion of photosynthates in developing new leaves, while low yielding plants in shoot expansion.

In contrast, under relative low temperatures, there were significant differences in both mean RHR and RGR of the 2^{nd} period (Table 1), between low and high yielding plants. The high yielding plants were growing 2.5 times faster than the low yielding (Table 1). In fact, high yielding plants were initiating growth even at 5 ^{0}C, whereas the RHR and RGR of low yielding were zero (Fig. 3). Due to such differences in height and dry matter growth rates, the GPC of high yielding were 79% higher (Table 1) compared to low yielding plants. It is obvious, that high yielding plants exceeded in dry matter production during winter (October-March), because their faster growth rates under stress conditions. It seems, that dry matter production under relatively low temperatures depends more on genes controlling tolerance to cold stress, rather than on genes controlling yield potential of plants (Dragavtsev, 1997). The role of genes on photosynthetic rates, partitioning of photosynthates between organs, under low temperatures, requires further investigation.

These results indicate that selection probably would be more effective when based on seasonal rather than total annual forage production. Similar results were reported by McClain (1981) studying the general and specific combining ability for forage yield in D.gl. The disadvantage of this practice is that cultivars selected under stress condition have narrow adaptation range. This was obvious in the present experiment. The high yielding plants exceeded in dry matter production only under stress condition, while no significant differences was detected under favorable condition. The development of productive cultivars with wide adaptation range pressupposes efficient incorporation of genes controlling yield per plant and genes controlling tolerance to stress into a single genotype. Probably, a selection technique, which combine productivity and adaptation (Fasoula and Fasoula, 2000) is an efficient selection practice.

Table 1. Relative Height Rate (RHR), Relative Growth Rate (RGR) and Growth Performance Coefficient (GPC) during the 1st (October-November) and 2nd period (February- March).

	1st period			2nd period		
	RHR	RGR	GPC	RHR	RGR	GPC
Low yielding	0.09a	0.37a	0.45a	0.07a	0.007a	0.06a
High yielding	0.10a	0.54b	0.65a	0.17b	0.020b	0.29b

1 Means followed by the same letter in the same column are not significantly (P <0.05) different.

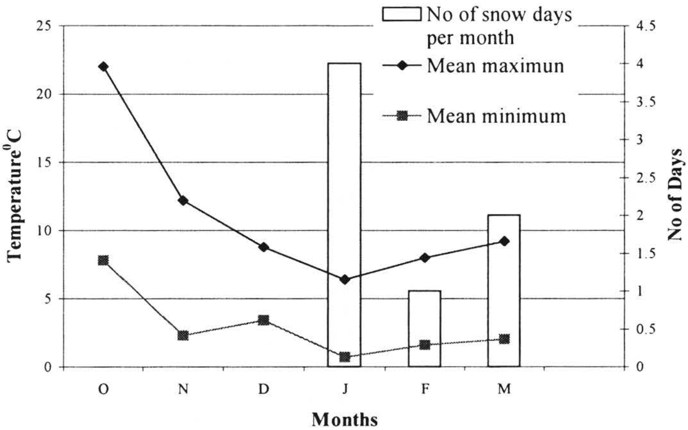


Fig.1. Climatological data of the experimental area

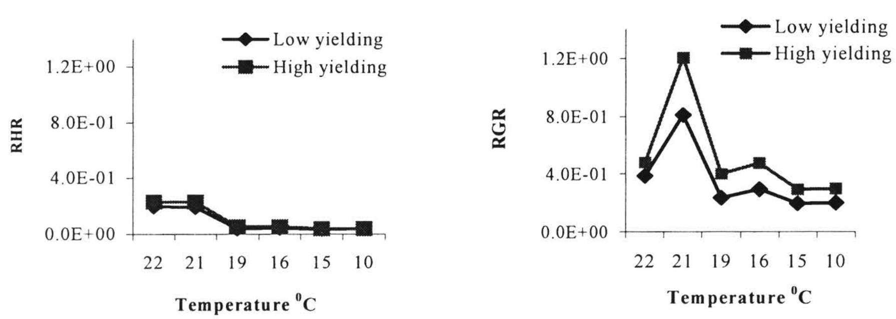
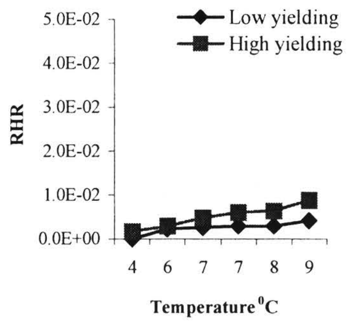


Fig. 2. RHR and RGR of high and low yielding plants in relation to weekly mean maximum air temperature during the 1st period of the experiment.



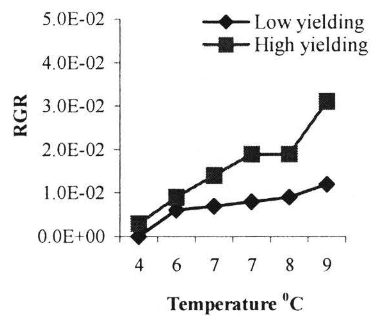


Fig 3. RHR and RGR of high and low yielding plants in relation to weekly mean maximum air temperature during the 2nd period of the experiment.

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SCREENING FOR SHADE TOLERANT AMENITY GRASSES: USE OF PHOTOSYNTHESIS MEASUREMENTS

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Abstract

In modern sporting arenas and in parks or ornamental lawns shade can cause serious problems for a good establishment and development of turf grass. The selection of shade tolerant grass cultivars forms therefor one of the future challenges for the breeders. Ideal photosynthetic characteristics for shade tolerance are a combination of a low light compensation point (I_c) with a high quantum efficiency (α_c). In order to have an idea about the natural variability for these parameters in *Festuca rubra* L. spp *commutata* and *Lolium perenne* L., respectively 100 and 200 individual plants were screened using an infrared gas analysis system. Results showed that for both species sufficient genetic variation was available for selection and breeding purposes. For the chewing red fescues average I_c was 18.5 μmol m^{-2} s^{-1} with maximum and minimum levels measured of 28.8 and 12.8 μmol m^{-2} s^{-1} respectively. For α_c the average value was 0.83 mmol m² mol^{-1} g^{-1} with extremes of 1.26 and 0.45 mmol m² mol^{-1} g^{-1}. For the tested perennial ryegrass plants I_c varied between 57.8 and 15.5 μmol m^{-2} s^{-1}, with an average of 26.2 μmol m^{-2} $s^{-}1$, while α_c reached maximal 1.58 mmol m² mol^{-1} g^{-1} with a minimum of 0.38 mmol m² mol^{-1} g^{-1} (average 0.88 mmol m² mol^{-1} g^{-1}). A measuring technique was also developed for screening of individual seedlings in an early stage. Pre-screening of seedlings on physiological traits in combination with the evaluation of field performance could offer possibilities to enhance breeding programmes for shade-tolerant turf grass cultivars.

Introduction

In modern sport arenas and in parks or ornamental lawns shade can cause serious problems for a good establishment and development of turf grass. In a low light regime, grass plants tend to have thinner and more elongated leaves and reduced root growth and shoot density (Allard *et al.*, 1991a, 1991b; Budryte-Aleksandraviciene and Schulz, 1999; Kephart *et al.*, 1992). Weakened grass is also more susceptible to diseases. Under trees shading is also combined with competition for water and water stress. On the other hand in shade, grass surfaces dry out more slowly, which is also associated with a higher risk for fungal disease infections.

Good management of the turf such as adjusting the mowing height and mowing frequency and reducing nitrogen supply, can tackle partly the negative aspects of shade. Apart from management practices, also a great difference between grass species is observed (Budryte-Aleksandraviciene and Schulz, 1999). The right choice of the grass species and mixture can therefor overcome some problems (Newell *et al.*, 1999). More recently a new turf grass species (*Deschampsia cespitosa*) was introduced. This species seems to have a higher shade resistance (Schnotz, 2000). Besides differences between species also within cultivars of one species significant variation in shade tolerance is observed (Budryte-Aleksandraviciene and Schulz, 1999).

Differences in the photosynthetic characteristics and the physiology of the species or the cultivar, as was shown earlier (Van Huylenbroeck *et al.*, 1999), can explain the different behaviour under shade conditions. In *Festuca arundinaceae* similar variations were found and it was concluded that the heritability of these photosynthetic characteristics were large enough to facilitate progress by recurrent selection (Asay *et al.*, 1974; Wilhelm and Nelson, 1985). However instead of screening at canopy level, the variation of individual plants is more important in case we would use these parameters in breeding and selection programs for shade tolerance. Therefor the aim of this work was to get an insight in the natural variation of the photosynthetic characteristics at individual plant level in perennial ryegrass and chewings fescue.

Materials and methods

Plant material

Individual plants of perennial ryegrass (*Lolium perenne* L.) and chewings fescue (*Festuca rubra* spp. *commutata*) were sawn in april in a peat mixture and transplanted in the field in the beginning of June. Plants were cutted regularly at 4 cm height.

Photosynthesis measurements

Photosynthesis was measured in a closed system. Each cuvette had a volume of 50ml. The CO_2 concentration was measured by an infra red gas analyser (IRGA, MK3, Analytical Development Co., Hoddesdon, England). Signals from the IRGA, thermocouples and quantum sensor (Li-Cor, Licoln, USA) were fed to a datalogger (DL-2, Delta-T, Cambridge, England) connected to an IBM compatible computer, which enabled the program controlled logging of these parameters. Before the cuvettes were closed, they were flushed with air containing a constant CO_2 concentration of 380 $\mu l\ l^{-1}$. During the experiments air temperature was controlled at 20°C and relative humidity at 65%.

For the measurements, per plant five fully developed leaves were placed in the cuvette. Dark respiration (R_d) and photosynthesis at 65 $\mu mol\ m^{-2}\ s^{-1}$ were determined. Light compensation point (I_c) and quantum efficiency (α_c) were then calculated. In the experiment, 200 individual perennial rygrass and 100 chewings fescue plants were measured separately.

Results and discussion

In figure 1 an overview of the obtained results are presented for both perennial ryegrass (Fig. 1 A, B, C) and chewings fescue (Figs. D, E, F). The results show that significant differences occur between both species. Chewings fescue plants had on average a higher dark respiration, a lower light compensation point and a lower quantum efficiency compared to perennial ryegrass plants. Similar results were also obtained when comparing at canopy level different cultivars from both species (Van Huylenbroeck *et al.*, 1999).

Within each species also significant differences in the measured parameters were found at individual plant level (Fig. 1). For the chewing red fescues the average I_c was 18.5 $\mu mol\ m^{-2}\ s^{-1}$ while the extreme values varied between 28.8 and 12.8 $\mu mol\ m^{-2}\ s^{-1}$. For α_c the average value was 0.83 $mmol\ m^2\ mol^{-1}\ g^{-1}$ with maximal and minimal values of 1.26 and 0.45 $mmol\ m^2\ mol^{-1}\ g^{-1}$, while R_d varied between 8.5 and 27.6 $nmol\ CO_2\ g^{-1}\ DW\ s^{-1}$. For the tested perennial ryegrass plants I_c varied between 57.8 and 15.5 $\mu mol\ m^{-2}\ s^{-1}$, with an average of 26.2 $\mu mol\ m^{-2}\ s^{-}1$, while α_c reached maximal 1.58 $mmol\ m^2\ mol^{-1}\ g^{-1}$ with a minimum of 0.38 $mmol\ m^2\ mol^{-1}\ g^{-1}$ (average 0.88 $mmol\ m^2\ mol^{-1}\ g^{-1}$). Here R_d varied between 14.8 and 32.6 $nmol\ CO_2\ g^{-1}\ DW\ s^{-1}$.

These results show that actually in both species plants can be found with improved photosynthetic characteristics for shade tolerance. Pre-screening of seedlings on physiological traits can therefore, in combination with the evaluation of field performance, offer possibilities to enhance breeding programmes towards a significant progress in the development of shade-tolerant turf grass cultivars. Optimisation of measuring techniques should allow us to screen at an acceptable speed the starting populations.

Acknowledgements

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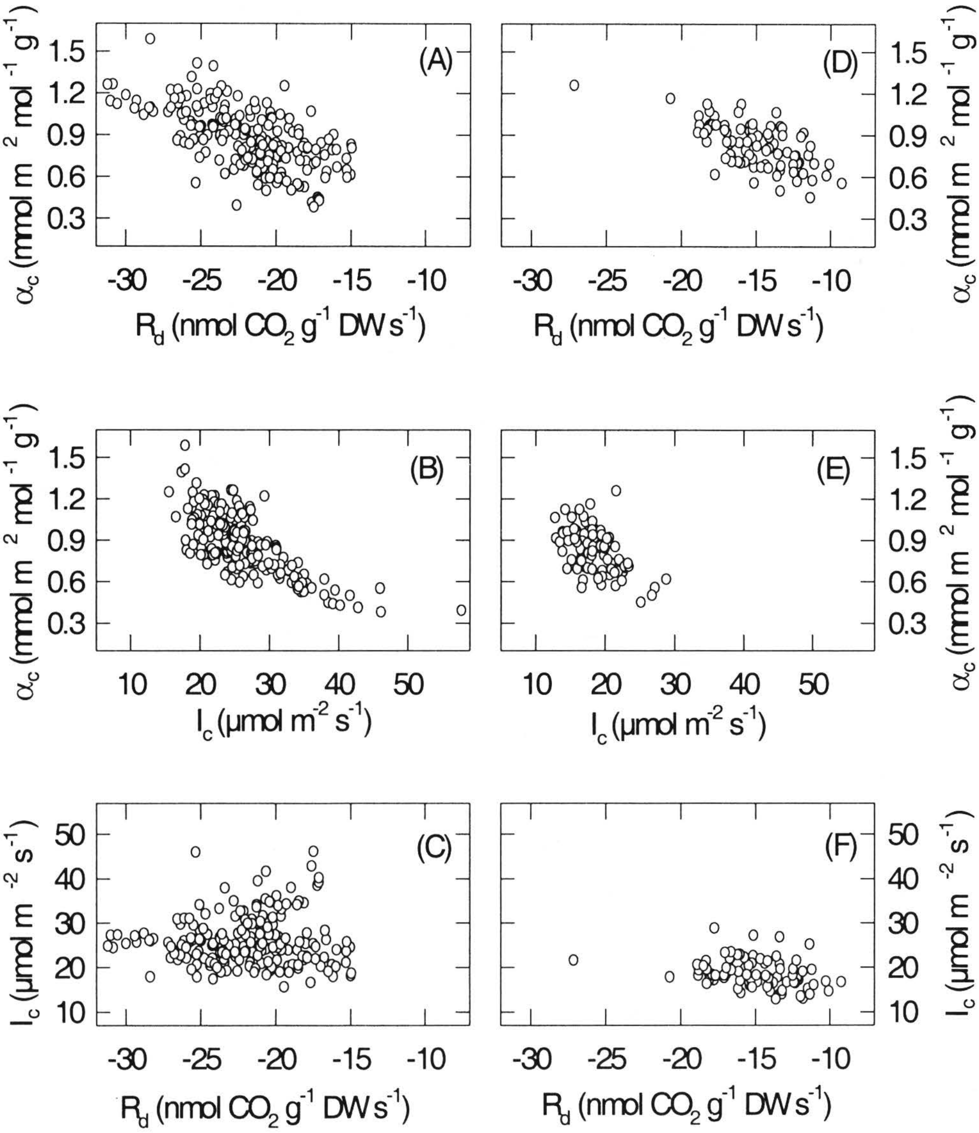

Figure 1. Variation in the photosynthetic characteristics [dark respiration (R_d), light compensation point (I_c) and quantum efficiency (α_c)] for *Lolium perenne* (A, B, C) and *Festuca rubra* ssp. *commutata* (D, E, F) individual plants

A PAN-EUROPEAN APPROACH TO "DISSECTING" STRESS RESISTANCE TRAITS IN THE FORAGE GRASSES

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Abstract

A new consortium (called SAGES) of European geneticists, plant physiologists, plant breeders, and seed merchants has been formed with EU funding. Perennial grassland represents 40% of the total agricultural land utilised by the EU. SAGES uses conventional breeding and the natural biodiversity of Europe's grasslands to develop high quality forage grasses adapted to diverse abiotic stresses encountered throughout Europe. SAGES uses new technology and unique opportunities provided within *Lolium* and *Festuca* species to "dissect" the complex traits associated with stress resistance. It will help develop understanding of the physiological mechanisms involved, and will provide genetic markers in order to select gene combinations governing desirable traits for future use in variety production. The new varieties will contain functioning stable gene combinations designed to improve productivity and persistency and reduce costs of fertiliser, irrigation, and resowing. Increased productivity will benefit incomes and help maintain fragile rural communities and keep people on the land.

Background:

Perennial grassland comprise more agricultural land (40%) in Europe than any other crop and help support vulnerable rural communities that rely on livestock farming for their livelihood. Some perennial grasslands have simply developed *in situ* from the indigenous flora. Others have been established *de novo* by sowing local, or improved, varieties. Existing high-quality forage cultivars (*Lolium*) have been bred for intensive systems in benign environments, but have proved to be not sufficiently robust to meet many of the environmental challenges that face extensive agriculture in less favoured areas. The relative importance of different environmental stresses varies widely over Europe, and each region requires cultivars combining specialised combinations of stress resistance. The stresses are complex – in the field, drought tolerance requires more than just tolerance of dry soil, winter hardiness more than tolerance of freezing. The ways in which plants respond to stresses are even more various, and their genetic control complex.

The EU have agreed under their Framework V "Quality of Life" "Sustainable Agriculture" programme to fund a project to evaluate for genotype-environment interactions, the performance of new gene combinations for stress resistance in different genetic backgrounds and under contrasting stress conditions. The results will be robust new forage grasses designed to achieve productive, more persistent, and sustainable grasslands, for diverse regions of Europe. This will be achieved through the application of biotechnology allied with new advances in conventional breeding. *Lolium* varieties will be developed (subsequent to the project) which are adapted to harsh climates (drought and cold stress). The results from greater adaptation of *Lolium* to stress will: **(i)** extend their geographic range, **(ii)** lengthen their growing season, **(iii)** require less intensive production systems, **(iv)** reduce dependence on imported protein concentrates for animal feed, **(v)** cause less habitat disturbance to wildlife and **(vi)** obviate the need for using transgenics in these outbreeding species. Greater persistence and reliability will reduce need for reseeding, and hence reduce land degradation caused by ploughing. By growing high quality ryegrass in certain less favourable areas farmers will produce their own high-quality feed and thus should be less dependent on 'bought-in' cereals and concentrates.

Genes for persistency, and for tolerance of cold, drought and poor soils, can be found in currently under-exploited native *Festuca* ecotypes that are adapted to stresses across Europe. These *Festuca* ecotypes cannot, however, compare with *Lolium* cultivars for productivity or quality under favourable conditions (See Table 1). Prior to the start of the project, *Festuca* genotypes from a wide range of habitats across Europe have been used in introgression breeding programmes with *Lolium* cultivars to generate diploid *Lolium*-like plants with stress-tolerance in excess of that expected in *Lolium*. Progeny from these plants will provide the basis for future development of new cultivars.

Over recent years, the Institute of Grassland and Environmental Research (IGER), the Institut National de la Recherche Agronomique (INRA), the Institute of Plant Genetics, Polish Academy of Sciences (IPG) in association with the Agricultural University of Craców (AUC) and the Norwegian Crop Research Institute (NCRI), in association with the Agricultural University of Norway (AUN) individually, have developed research programmes designed to enhance adaptation of *Lolium* specifically for their respective growing conditions (Humphreys *et al.*, 1998). This project will realise the synergy and efficiency achievable by formally combining available European expertise in a single project.

By combining the expertise and research activities of the partners of the consortium will have:

- access to all major collections of native European *Festuca* and *Lolium* (Zwierzykowski, 1996); http://www.igergru.bbsrc.ac.uk).
- access to all current major grass genetic mapping populations in Europe (Bert *et al.*, 1999); Hayward *et al.*, (1998); King *et al.*, (1998).
- enhanced ability to map traits associated with stress resistance (Humphreys and Pašakinskiene, 1996); Humphreys *et al.* (1998); Pašakinskiene *et al.*, (2000)
- the ability to design breeding programmes to encourage precision gene transfer in order to assemble the optimum gene combinations (Humphreys and Thorogood, 1993); Humphreys and Ghesquière, (1994).
- the necessary physiological screens to select grasses capable of withstanding expected environmental stresses (Durand *et al.*, 1997); Thomas *et al.* (1999); Rapacz (1999).

The species to be used are *Lolium multiflorum* (2n= 2x=14), *L. perenne* (2n=2x=14), *Festuca pratensis* (2n=2x=14), *Festuca glaucescens* (2n=4x=28), and *Festuca arundinacea* (2n=6x=42) (See Table 1). All species are inter-fertile, and, when crossed, produce hybrid plants that are themselves fertile. The hybrids:

1. allow very high levels of intergeneric chromosome pairing, recombination, and gene exchange (Humphreys *et al.* 1998).
2. allow genes from any region of the *Festuca* genome to be transferred to homologous sites on *Lolium* chromosomes, where they are stable and can function normally and effectively (e.g. Humphreys and Ghesquière, 1994)
3. allow detection of intergeneric recombinant chromosome segments using genomic *in situ* hybridisation (GISH). This which we have termed introgression mapping (King *et al.*, 1998), enables the physical location of genes for stress resistance transferred from *Festuca* into *Lolium* (Humphreys and Pašakinskiene, 1996).

4. allow androgenesis (recovery of haploid or polyhaploid plants from anther culture) to isolate unique gene combinations where recessive genes are expressed and contrasting extremes of stress tolerance generated from the same hybrid gene pool - a unique system for genetic linkage mapping (Humphreys *et al.*, 2000). This will be explained further below.

Lolium x *Festuca* hybrids have been known and used in agriculture since at least the 1970s, and some are currently available on the market. However, compared with *Lolium* (especially *L. multiflorum*) they have poor productivity, and have frequently been found to be unstable (Thomas and Humphreys, 1991); Canter *et al.* (1999). Recent advances in the fundamental understanding of genetics and the development of efficient new introgression approaches enable:

1. the physical location on the chromosome of quantitative traits associated with stress resistance (e.g. Humphreys and Pašakinskiene, 1996).
2. the association of molecular markers with desirable agronomic traits to enable selection for desirable gene combinations (e.g. Hayward *et al.* (1998).
3. functioning stable natural gene combinations that are ready for direct use in breeding programmes (e.g Humphreys and Thomas, (1993).
4. the incorporation of detailed physiological studies to improve understanding and enable exploitation of the complex mechanisms involved in stress resistance by associating pinpoint changes in the *Lolium* genotype by the introgression of different combinations of *Festuca* genes with changes in plant phenotype and gene expression (e.g. Thomas *et al.*, 1999).
5. the studying of complex interactions between genes governing high quality forage production and good stress resistance, to generate good agricultural grasses with improved persistency (e.g. Ghesquière *et al.*, 1996).

F. pratensis will be used primarily as a source of genes for improved winter-hardiness in *Lolium*. *F. arundinacea* will be used primarily as a source of enhanced drought and heat resistance in *Lolium*. *F. glaucescens* will also be used as a source of genes for drought resistance but has a different strategy of drought-survival and is an ideal source of genes for *Lolium* survival in southern Europe. Furthermore, ecotypes of the three *Festuca* species are adapted to mountainous conditions, and genotypes that have been used in breeding programmes as a source of genes for improved *Lolium* production in upland and mountainous regions of Europe will be used in this project.

The relevance of *Festuca* genes in *Lolium* as adaptation against a range of abiotic stresses will be assessed at different European sites. Each genotype will comprise mainly *Lolium* DNA, thereby retaining most, if not all the good growth characters of *Lolium*. The introgression lines are each expected to contain one or more independently transferred *Festuca* sequence, a part of which comprise genes sufficient to significantly improve the stress resistance of *Lolium*. The SAGES project utilises a very efficient strategy for mapping stress resistance traits. It will allow identification of the *Festuca* chromosome introgressions with GISH, and will subsequently generate *Festuca*-specific genetic markers for genes located within those introgressed chromosome segments. The project thereby eliminates time-wastage and costs incurred by mapping the remainder (expected to be >95%) of the *Festuca* genome. By introgressing into *Lolium, Festuca* genes from different regions of the *Festuca* genome, the complex components of stress resistance will be "dissected" into their constituent parts. The outcomes will be improved understanding of the mechanisms of stress resistance and their genetic control. Procedures will facilitate breeding for targeted gene sequences without linkage drag of any putative deleterious *Festuca-Lolium* gene combinations and will lead to the production of more robust and persistent high quality *Lolium* cultivars ideally suited to improve sustainability of grassland systems.

The stresses:

So far as the plant is concerned, drought is a complex phenomenon. It involves not just a lack of available water but also high transpiration rates, supra-optimal temperatures, photo-oxidation, mineral deficiency, and hard soil. Their relative importance varies with location and year. Definitions of drought resistance must ultimately take into account the climate and prevailing agriculture. An over-riding requirement is that the crop should survive and re-grow rapidly when autumn rains set in. A secondary requirement is that yields should not be greatly reduced during mild drought (water deficit less than 50 – 100 mm, say), a response which occurs in many Mediterranean grasses which rapidly become "quiescent" at first indication of drought.

Winter hardiness is similarly complex, involving tolerance not only of sub-zero temperatures, but also of ice-encasement, anoxia, low light inputs (especially near the Arctic Circle), and resistance to disease attack. Winter hardiness may be lost in Spring more rapidly than it is acquired, and accordingly persistence over winter may be worse in relatively mild climates if they induce dehardening, because this leads to more frost damage than may occur in colder climates in which hardiness is maintained. Winter dormancy can help prevent potentially dangerous dehardening. Breeders face the compromise that there is often a negative correlation between growth in early spring growth or late autumn, and winter hardiness.

Advantages of using androgenesis from *Lolium* x *Festuca* hybrids

Androgenesis involves regeneration of novel plants from pollen mother cells by anther culture. The genome of each androgenic plant is unique and represents the products of meiosis of the parent plant. From *Lolium* x *Festuca* hybrids it is possible to generate large populations from a parent plant and thereby isolate high numbers of alternative combinations of genes from the same parental genome. IGER and IPG have demonstrated (e.g Humphreys *et al.*, (2000) that certain gene combinations are expressed in androgenic plants that otherwise remain "hidden" in the parent plant or in hybrid derivatives. This "hidden variation" may prove a valuable resource for future crop improvement. Furthermore, androgenesis can avoid the potential problem of low male transmission of putative desirable gene combinations that may arise due to pollen competition or pollen-stigma interactions during conventional breeding programmes. In the absence of such selection pressures, useful gene combinations may well be recovered amongst androgenic lines. Procedures are available subsequently to fix desirable gene combinations as homozygotes reducing many years of backcrossing normally required in plant breeding to achieve the same objectives. Pioneer research carried out at IGER and IPG and more recently NCRI has indicated that amphiploid cultivars of *F. pratensis* × *L. multiflorum* ($2n = 4x = 28$) cultivars such as Sulino and Felopa provide the best starting point for anther culture programmes in the *Lolium/Festuca* complex. The majority of androgenic lines derived from such cultivars have 14 chromosomes. These we define to be dihaploids since they were developed from a tetraploid form and retain half the tetraploid chromosome number. A proportion (c10%) of the dihaploid lines are fertile, and it is possible to use these to generate new populations for plant breeding. The new lines will be functionally equivalent to natural diploid *Lolium* x *Festuca* hybrids although such hybrids are highly sterile and as a consequence cannot be incorporated into breeding programmes. Preliminary research carried out at IGER has identified dihaploid lines with high stress resistance that can be maintained in subsequent generations in *Lolium*-like new populations. These new lines are expected to improve the persistency of *L. multiflorum*.

The project:

There are 3 principal workprogrammes:

Workprogramme 1 - Technologies for dissecting and manipulating traits

The objective of this workpackage is to exploit and evaluate in a co-ordinated way the experimental introgression lines developed independently by each research partner. There are 4 main objectives:

- Locate using GISH *Festuca* genes for stress resistance to *Lolium* chromosome arms.
- Associate AFLP markers with these *Festuca* genes and use anchor markers to integrate information from mapping populations.
- Seek festucoid stress-resistance traits in elite genotypes.
- Associate markers with these traits to design "breeders' toolkits". These we define as a combination of: 1) one (or ideally 2) genotypes of a *Lolium* cultivar, containing a single *Festuca* chromosome segment that contains a gene (or genes) that together with *Lolium*, combine to generate a significant improvement in the stress resistance of that *Lolium* cultivar, and 2) two or more genetic markers that flank the *Festuca* introgression on either side, and enable its selection in future breeding programmes.

Workprogramme 2 - Exploitation of androgenesis

The SAGES project will use androgenic plants derived from *F. pratensis* x *L. multiflorum* (4x) cultivars. There are four main objectives:

- The identification of novel gene combinations governing complementary and desirable traits from *Lolium* and *Festuca*.
- The production of homozygous lines of desirable gene combinations.
- The allocation of molecular markers to stress-tolerance traits.
- The comparison of the recovery of *Festuca* derived mechanisms for winter hardiness in *Lolium/Festuca* introgression lines in workprogramme 1 with those only recovered in the androgenic populations developed in workprogramme 2.

Workprogramme 3 - Technology transfer and the commercial development of improved novel germplasm

The scientific and commercial partners will:

1) construct and promulgate a strategy to exploit new breeding technology to help develop improved and more sustainable grasslands for the whole of Europe. The outcomes are to:

- encourage and improve collaboration between commercial grass breeders, seeds merchants and public sector research institutes.
- promote a common and logical approach to the classification and testing of *Lolium* x *Festuca* hybrids and hybrid derivatives in Europe for "National Lists".
- promote the concept of sustainability, the exploitation of native ecotypes and the use of novel germplasm.

2) construct a consortium of major European scientific and commercial partners with expertise in grass breeding and marketing, to exploit commercially the outcomes of workprogrammes 1 and 2. This involves:

- Pan-European field-trials of *Lolium-Festuca* introgression lines.
- assembling a suite of novel, stress-tolerant pre-breeding germlines and associated markers to provide the breeders with "toolkits".
- converting model systems for marker assisted selection into commercial enterprises for improved grass variety production.

Table 1. Complementary traits in the *Festuca–Lolium* complex

Trait	*F.pratensis*	*F.arundinacea*	*F.glaucescens*	*L.multiflorum*	*L. perenne*
Quality					
Speed of establishment	++	++	+	++++	+++
Early spring growth rate	+++	++++	++	++++	+++
Summer growth potential	+++	+++	+	++++	+++
Herbage digestibility	+++	++	+	++++	+++
Stress resistance					
Winter hardiness	++++	+++	+++	+	++
Drought resistance	++	++++	++++	+	++
Persistency	+++	+++	+++	+	+++

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EARLY CRITERIA USED TO SCREEN SOME FODDER CROPS FOR WATER FLOODING RESISTANCE

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Abstract:

The objective of this study was to analyse the influence of water excess in roots of selected grass species. Morphological and anatomical behaviour were recorded. Among these species studied, some genotypes did not show any symptom of water-excess (tall fescue), the others showed typical evidence of hypoxy effects (barley) while some others were intermediate (oat).

Prepared histological sections from main roots of tolerant species showed formation of aerenchyma, which feed the roots with oxygen from the aerial part. However, moderately tolerant species showed aerenchyma only in adventitious root system when sensitive species were not able to modify their root structure to withstand water logging. This process could be used to identify, at early stage of development, the most tolerant species to water excess.

Key words: water logging, fodder crop, abiotic stress, screening criterion

Introduction

Hydromorphy resulted from the presence of permanently or temporarily water table, which its out flows was prevented by waterproof zone. It may be due to the soil topography that make difficult voiding of water excess obtained after plentiful rain or irrigation.

In the word there are many hydromorphic land. Even in the arid and semiarid zone of the Mediterranean region we can find many hydromorphic land. For example, in Tunisia the fourth (1/4) of agricultural area used was affected by hydromorphy and salinity (Ben Naceur, 1994).

Among these zones, some are exploitable during dry years or during summer period. Some other are unfortunately wasted land. In these regions a hypoxia around the root reduce hydrous and mineral absorption of submerged plant (Levitt, 1980; Fitter and Hay, 1987; Ben Naceur and Paul, 1993). As a result we can note a slowing up of growth, a foliar yellowing (Waters et al., 1991), a loss of apex dominance and an appearance of partial or total necrosis in the root.

To the water excess effects were added the soil microorganism toxin effects. The response of plants to these stress conditions was variable. Some plants unable to tolerate this constraint showed various adaptations forms (Atwell et al., 1988; Thomson and Greenway, 1991; Ben Naceur, 1994).

In this paper we propose to study the behaviour of some fodder genotypes of selected species, under water excess conditions and to highlight the early adaptation criteria that can be used to prevent oxygen deficit around roots.

Materials and Methods

The study was carried out on two barley genotypes (*Hordeum vulgare* L., cv Aurore-Esperance and Roho), two oat genotypes (*Avena sativa* L., cv «Avon and «Av. 86) and two tall fescue genotypes (*Festuca arendinacea* Shreb., cv Grombalia and Mornag).

The seeds were sown in a pot with 17.5 cm high and 23 cm diameter on the basis of 6 seeds per pot.

The experiment was conducted on pot shelter used as controls. Water logging was performed by subjecting 3 pots of each genotype to water excess. The 3 others pots were maintained at field capacity (2/3 of retention capacity of the pot) by water supplying the quantity that lost during each two days.

Morphological and anatomical behaviour were studied during water excess of different species. The parameters recorded were scoring of yellowing, dry matter production, adventitious roots and the ability to modify cortical parenchyma to have aerenchyma formation and to prevent oxygen deficit at the root level.

Results and Discussion

Morphologic modification observed under water excess

The observations scored during our experimentation allowed us to characterise the behaviour of the studied species.

Figure 1: Yellowing and growth slowing down of barley (left) and normal growth and colour of tall fescue plants under water excess (right).

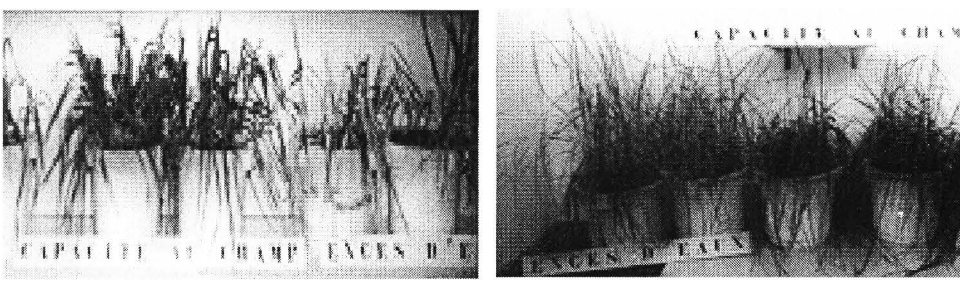
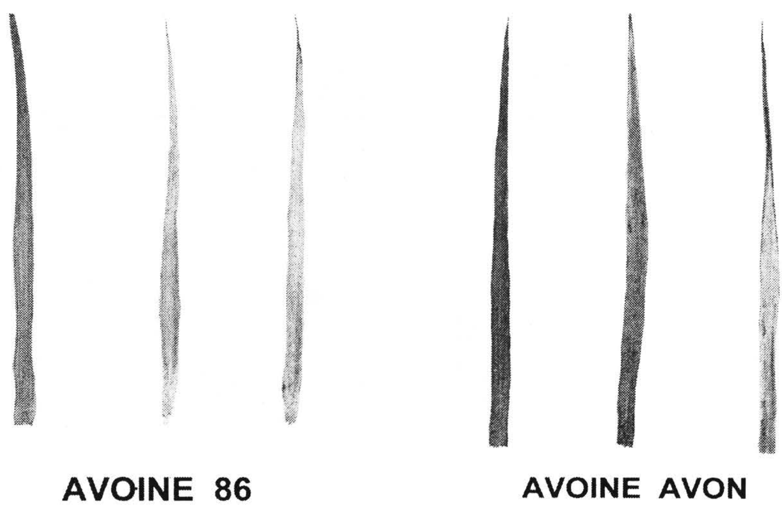


Figure 2: Partially and then totally foliar yellowing of oat.



For barley, plants exposed to water excess showed complete yellowing, while the control plants grew normally (Figure 1).

Differences among genotypes were observed for oats: genotypes «Av. 86» was completely susceptible to water logging, showing symptoms of complete yellowing while genotype «Avon» showed moderate tolerance to water excess (Figure 2). In the last case yellowing was located only in leaf tip, however, it may cover the whole plant leaf when submersion was prolonged.

Partially root necrosis was also observed at barley and oat. These roots were replaced by adventitious root system more vigorous at barley than at tall fescue (figure 3) or by both vigorous adventitious root system and floating root system in oat (figure 4).

Anatomic modifications observed under water excess

Figure 3: The adventitious root was more vigorous for barley (right) than for tall fescue (left). Nevertheless it was more vigorous for oat comparatively for the two other species (Figure 4).

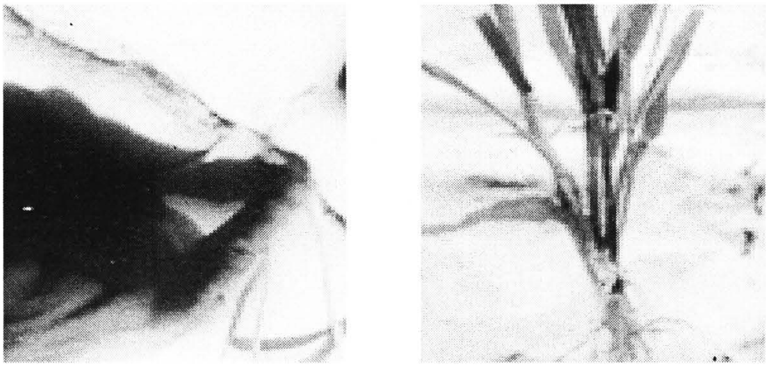
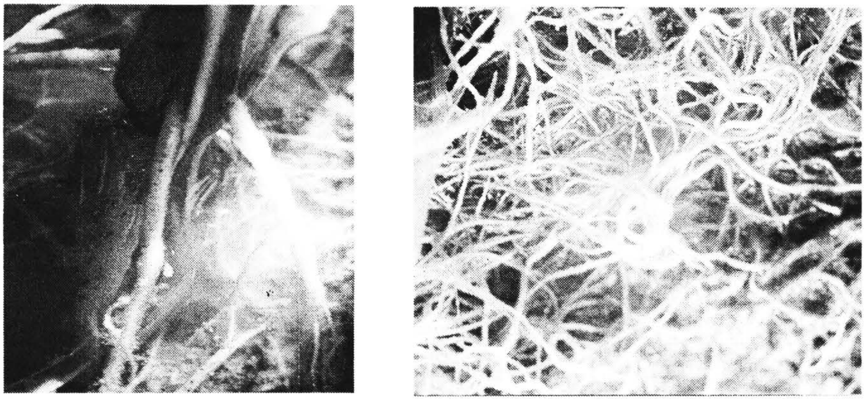


Figure 4: Appearance of vigorous adventitious root system (left) and a floating root system (right) for oat.



These observations showed that barley genotypes were sensitive to water excess. In the opposite tall fescue genotypes were able to grow under these stress conditions. In the other hand, oat behaviour varied according to the genotype. It was intermediate.

Dry matter production

Differences were observed for dry matter production (table 1). Variations were observed for the barley and oat genotypes. On the other hand, the response of tall fescue genotype «Grombalia» was similar to that of «Mornag».

Table 1: Dry matter production under the two water regimes (Results were expressed on g/plant).

Species	**Genotypes**	treatment	control	% reduce
Hordeum vulgare	Aurore-Espérance	0.1992	1.469	86.44%
	Roho	0.42857	0.94543	54.67%
Avena sativa	AvoineAvon	0.382	0.740	48.38%
	Avoine86	0.16	0.636	74.84%
Festuca arendinacea	Grombalia	0.7165	0.7702	7%
	Mornag	0.172	0.4379	6.07%

Table 1 showed similar behaviour of the two tall fescue genotypes. It did not show a significant reduction of their dry matter under excess water. These results agree with our previous work (Ben Naceur and Paul, 1993), and suggests that these species received oxygen from aerial part and circumvents the asphyxia around the root.

The oat genotype «Avon» was more tolerant than «Av 86». The barley genotype «Roho» was more tolerant than «Aurore-Esperance».

The comparison of barley and oat (all genotypes included) showed a superiority of oat to barley. The dry matter reduction for these two species varied from 48 to 86%. Nevertheless, the oat genotype «Avon» was closely related to the barley genotype «Roho».

Prepared histological sections from roots of various species allowed us to confirm and to distinguish among tolerant and sensitive species to water excess. In fact, the tall fescue species were able to modify their cortical parenchyma to form aerenchyma which feed the roots with oxygen from the aerial part. This ability was observed for both tall fescue genotypes «Grombalia and Mornag» (Figure 5a, b and c). This may be explained by the fact that there were no differences between the treatment and control for dry matter production.

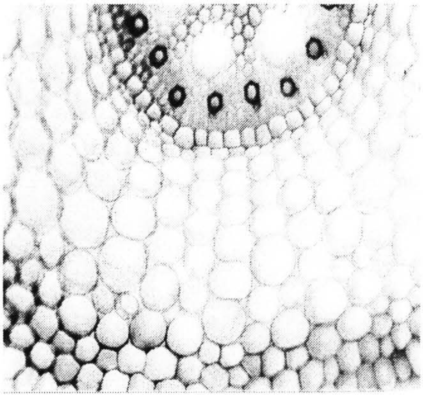
For the oat genotypes, the sections prepared at the normal root system did not show any parenchyma modification (Figure 6). However, we observed these parenchyma when section were made at the adventitious root system (Figure 7).

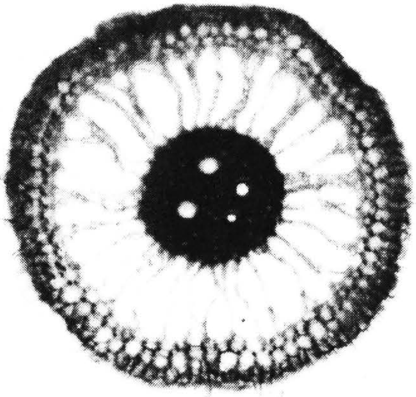
We observed that adventitious root were more numerous and more vigorous for oat than tall fescue or barley. They may partially feed plant by Arial oxygen as it was shown for tall fescue normal root system. Based on these observations, oat may be considered as moderately tolerant to water excess.

Figure 5: Histological section prepared at tall fescue root for control plant (left) or treatment (right).

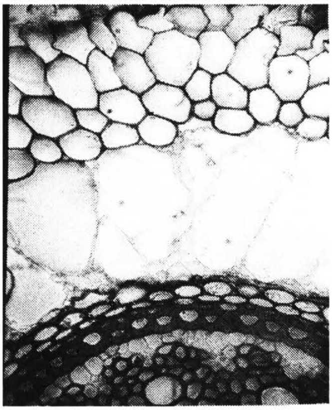
a) Control

b) Treatment





c) Extending of tall fescue root section part under water excess.



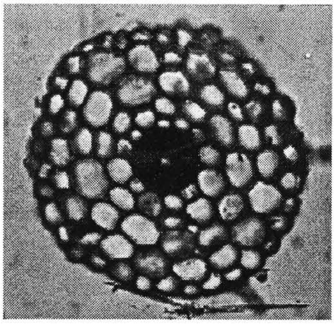
For the barley genotypes, no modifications were recorded both at normal and adventitious root system (Figure 8). This signifys that barley was a sensitive species to water excess.

Conclusion

This study allowed us to notify various stress symptoms at barley species. We can mention foliar yellowing, purple coloration of stem bottom, dry matter reduction and the unability to form aerenchyma in its both root system, under water excess conditions.

Contrarily to barley, oat species showed a variable foliar yellowing according to the genotype. This means than some genotypes were more tolerant than others to water excess.

Figure 6: Inability of oat genotypes to form aerenchyma in normally root system.
Oat genotype «Avoine 86» «Avoine Avon» oat genotype



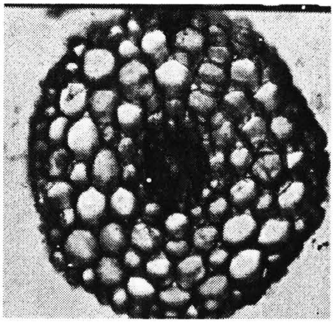


Figure 7: Appearance of some aerenchyma at adventitious root system of oat.

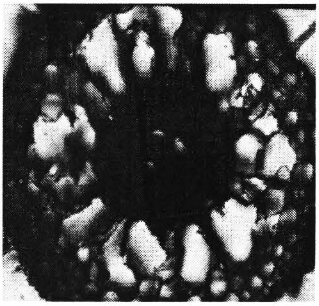
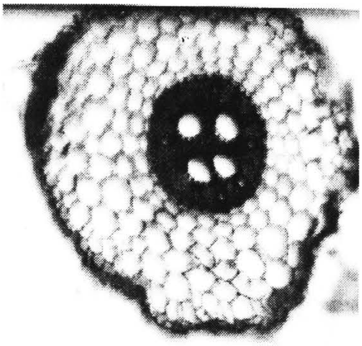
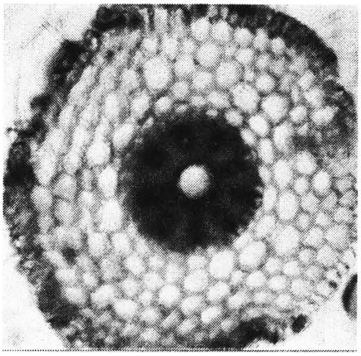


Figure 8: Barley genotypes were unable to form aerenchyma at both root systems (the main root system or the adventitious root system).

Barley «Roho» genotype Barley «Aurore-espérance» genotype





Histological section showed aerenchyma formation only at adventitious root system of oat. This result confirm their intermediate position between barley (sensitive) and tall fescue (tolerant).

The adventitious root developed was more numerous and more vigorous than barley and tall fescue root. This leads to have a dry matter production little affected under stress especially in «Avon» genotype comparatively to barley

For the tall fescue species, all observations recorded and concerned foliar coloration, growth and parenchyma formation converge to the conclusion that tall fescue is the most tolerant to water excess and it may valorise frequently flooded zone.

As a result of this study we can say that aerenchyma formation at normally root system constitute the best criterion of selecting tolerant species to water logging.

Numerous and vigorous adventitious root bearing aerenchyma is the second criterion for the moderately tolerant species to water excess. Further more, completely and quickly foliar yellowing of submerged plants could be a good indicator of sensitive species to water excess.

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THE BIOLOGY OF LICORICE AND IT'S INTRODUCTION TO CULTURE IN RUSSIAN SEMIARID ZONE

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Abstract

Involving of the salted lands in agricultural turnover and their effective use in agriculture is an important scientific-technical and economic task. This task can be solved with helping of the ecologically specialized species of natural flora. Among the perspective species, capable to grow on the secondary salinity soils is licorice (*Glycyrrhiza glabra* L.). It is a perennial legume plant with a versatile use. Since the ancient times *Glycyrrhiza glabra* was cultivated for the purposes of growing licorice root — a very important pharmaceutical and technical row material. Licorice was included in pharmacopoeia of more than 33 countries of the world. The technological components of licorice are used in wide scale in food, chemical and metallurgy industries.

Licorice has not only medical and fodder value, but also still large meliorative value. Due to effective occultation of a surface by above-ground mass ramified and a bully developed rooted system, pump and the biological drainage functions, licorice plantation provide sharp lowering of physical evaporation, lowering of a level of ground waters, thereof provide desalinization.

High salt-tolerance of licorice in a combination to major economic conditions predetermine necessity for introduction in culture of this plant on second salted lands, which occupy more than 1 mln.ha in arid areas of the Russia.

With the purposes of comparative ecology-biological study and selection of the salt resisting forms with valuable medical and fodder qualities, an expedition was conducted in republics of Central Asia (Uzbekistan, Turkmenia, Kazakhstan), Northern Caucasus (Dagestan, Azerbaijan), in arid areas of Russia (Astrakhan, Volgograd, Novosibirsk provinces, Stavropol and Krasnodar Territories). 85 samples of five licorice species were collected. The field experiences are included in an experimental-industrial farm "Leninskoe" of Caspian Research Institute of Arid Agriculture (Chernyi Yar district of Astrakhan province).

Introduction

Genera *Glycyrrhiza* consist of 13 species, among them *G. glabra* is the most important as fodder, medicine and biomeliorant culture.

Licorice, is a legume plant and a particularly very useful forage crop in Central Asia and south-east regions of Russia. Protein content in dry matter reached 18-20%, oil 5.9-9.0% and has a high content of water-soluble carbohydrates. Because of its estrogen content, licorice is used for the stimulation meat and milk production in different kinds of animals (Larin et al., 1951; Burtzeva, 1969; Lysov et al., 1969; Goryachev et al., 1969; Shimanov, 1972).

The second direction in licorice use is medicine. From ancient times the licorice roots are used as pharmaceutics and technical feedstock. Licorice was included in pharmacopoeia in more than 33 countries of the world. From roots extracted such chemical components as triterpens, saponins and flavonoids, which have antialergic, antiphlogistic, anaplerotic and other kind activity. Licorice roots can be used in food, chemical, metallurgical industries and in other spheres of human life.

Biological and synecological specialities of licorice gave possibilities for using it as environmental forming factor, because it has ability for growing in saline conditions. Because of the large above-ground green mass and well developed root system, licorice has very important biological drainage functions, decreasing level of underground water table, and resolving salinity of soils.

So licorice value determine not only as a forage and medicine crop, but also as an environmental factor for the degraded lands restoration.

In connection with splitting of USSR, Russia was separated from the main licorice growing zones. So it is appeared the scientific and practical necessity to investigate licorice biology in new North-west Caspian region of Russia.

Materials and Methods

In this study of selection for salt-tolerant forms of licorice with high forage and medicine value, populations from 5 licorice species were collected. The samples for initial nurseries were collected in expeditions in arid Russian zones (Astrakhan, Volgograd, Saratov, Novosibirsk, Stavropol, Krasnodar provinces, Dagestan and Kalmyk republics), and in arid countries of Central Asia (Turkmenistan, Uzbekistan, Kazachstan). As a result more than 85 samples were collected to form a gene pool. Field experiments were conducted in 1996-2000 years in scientific experimental farm "Leninskoye" of Caspian Research Institute of Arid Agriculture (Cherny Yar district, Astrakhan province).

The territory belong to part of semi-desert zone, which is characterised with continental climate (hot dry summer, cold and insufficient low snow cover winter, insufficient number of precipitation, with high air temperatures and a lot of windy days with dust storms). Mean yearly temperature is about 7.8°C. The coldest month is January (-9.2°C) and the hottest is July (24.3°C). Mean yearly precipitation is 256 mm. Yearly sum of temperatures is 3300-3500°C. The number of days without frost is 175-186.

Light chestnut soils are sandy loam with low salinity. Concentration of salts is 0.735-0.764 g/l. Water table level is seasonal (in winter 3.0-4.0 m, in summer — 1.5-2.0m). Mineralization reached 2.08-2.56 g/l.

Sampling of initial material, forming of gene pool, and multiple ecology-biological study of licorice were made by instruction of All-Russian Fodder Research Institute.

Breeding of new varieties was made by mass selection and screening individual plants in several generations. The competitive testing were made by the instruction of Russian States Variety Committee.

Results and Discussion

In order to understand the biology of licorice it is necessary to know its geographical distribution, in particular its climatic and edaphic conditions.

G. glabra is an ecological plastic culture and can grow in wide ecological gradients environmental conditions, from dry steppe to salting flood plains. In the same time licorice grows well in flooding plains and river valleys in Central Asia. In such conditions licorice dominated and formed dense tangled vegetation.

On the plant life form licorice is semicryptofyte, vegetable mobile polycarpic. Stems are simple, well leafed, 70-200 cm tall. Licorice is long root-stalked plant. Roots penetrate in to the soil at the fist year of life till 153 cm, and on the third-fourth years 180-200 cm. Deep penetrated roots principal function is water uptake, whereas the roots from upper horizons primary function is mineral nutrition. Root stalks of licorice are 1-4 m long and are distributed in all directions of the upper soil horizons (10-40 cm).

Licorice prefers light texture soils, sand, sandy loam, sandy clays with 10-30% clay content. But licorice can also grow on heavy soils, if underground horizons have a high proportion of light substrates. Optimal edaphic conditions for growing and forming above-ground green masse are alluvial-meadow, alluvial meadow-tugay, meadow-marshy, meadow saline marsh.

A fact of high importance in biology of licorice is forming hard seeds, that determine low laboratory and field germination. In connection with this phenomena we carried out the cycle of investigations dealing with the characters of seed germination.

It was stated, that the optimal temperature to licorice seed germination is +40°C, the minima ranges from 6 to 8°C. The optimal water content for germination the main part seeds is 60% of soil water holding capacity.

Mechanical and chemical scarification licorice seeds render positive on seed germination. The most effective is chemical treating with sulfuric acid, which gave a possibility to increase seed germination up to 88% in a South-Turkmenian population and up to 90% in Astrakhan population, whereas the non-treated seeds only germinated 4-16%. Field germination for treated seeds and non-treated seeds were 38-40% and 6-10%, respectively.

In relation to salinity *G. glabra* is halomesophit. At the period of seed germination and juvenile stage salt tolerance is not very large. On the future stages of plant development salt tolerance is raised.

In connection with the domestication, there is a need to organise breeding, seed growing technologies, and investigation of flowering process of licorice.

G. glabra is a polymorphyc species, which included different geographical, edaphic and coenosis ecotypes. The ecotypes are different on their characteristics: yield of above-ground fodder phytomass and the content of nutritional and medicine substances. Such interspecies ecological and economic different characteristic dictate the necessity of creating collections and selecting salt tolerant plants which combine high yield potential and contain a significant amount of biologically active substances.

We created a collection on the base of this gene pool. As a result we managed to select licorice forms with tall plants (124-160 sm) and with high ecological adaptability. The most salt tolerant and productive plants were selected from Astrakhan and South-Turkmenian populations, and they formed 18.2-23.3 t/ha above-ground dry fodder mass with 7.8-9.5% concentration of glycyrrehizic acid in roots.

Selected forms of *G. glabra* are promising fodder crops, especially in saline soils, because of their phytomass productivity and content of glycyrrehizic acid in roots, which enable future commercial plantations in semi-desert regions of Russia. On the second year of life they formed dense vegetation and decreased physical water evaporation 5 fold. As a result soil salinity was stopped and then began soil resolving. The yield of dry above-ground fodder reached on the second year of life 6-8 t/ha.

On another farm (collective farm "Rodina", Narimanovsky district, Astrakhan province) in Volga low lands, 300 ha of non-cultivated lands, formerly used for vegetable crops production, were planted by licorice. The yield of licorice reached 7-8 t/ha above-ground dry matter and 8-10 t/ha roots as a valuable medicine material.

On the base of knowing biology and ecology of licorice two technologies of licorice plantation were created on the irrigated lands of North-west Caspian region. These technologies are the planting with root-stalked 10-15 cm long and 10-20 cm deep and cultivation by seed sowing.

Conclusion

On the base of our investigation, the following conclusions were made:

1. *G. glabra*, being on the life plant strategy as violent species, belong to C-species, distinguished with high salt tolerance, which gave a possibility for growing on saline soils with water-table 0.5-3.0 m. or under irrigation by saline water. It is characterised with intraspecific diversity, morphological and ecological traits. In North-west Caspian region, perspective licorice populations from Astrakhan province and South Turkmenia were selected.
2. *G. glabra* — is a valuable fodder and medical species for the effective exploitation of secondary saline soils for producing high energy, for its high protein content and for the roots as a valuable medical raw material.
3. Licorice, for the sake of environmental forming capacity carried out function of degraded soils biological restoration. After 5-7 growing years on the saline irrigated soils, the process of desalinization started and appeared a possibility to grow another traditional agricultural crop of multiple common value.

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CHICKPEA BREEDING FOR DROUGHT TOLERANCE

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Abstract

At the National Plant Breeding Station (ENMP) in Elvas – Portugal, we have done some studies about drought tolerance in chickpea plants. In this area, the crop is very important for food and feed.

In the Mediterranean basin usually rainfall is during the winter season; chickpea is traditionally sown in spring because the local landraces are susceptible both to ascochyta blight and cold. Chickpea crop sown during spring, therefore experiences considerable drought stress and produces low yield.

The principal research of chickpea at ENMP is to improve this crop and its adaptation to our mediterranean conditions. Screening for biotic and abiotic stresses was given high priority. Tolerant materials have been identified for two diseases: *Ascochyta rabiei* and fusarium wilt. Efforts are underway to transfer genes for resistance using conventional crosses between resistant material coming from ICARDA and others origins and local germplasm.

The low rainfall registered during the last years provided an excellent opportunity to select chickpea-lines against drought tolerance.

Twenty genotypes of chickpea with different origin, phenology, seed size, growth habit, height and yield were tested in winter (W) and spring (SP) sowing and under normal rainfall and irrigated conditions during several years. These genotypes were submitted to morphological and physiological characterisation.

The number of days from sowing to different phenological stages varied among genotypes. In the driest treatment, there were less variations in time to maturity among the genotypes. Irrigation delayed maturity date. These genotypes presented low water potentials and high RWC (Relative Water Contents).

The yields in the driest treatment were lower but varied significantly among the cultivars. Seed yield ranging from 5211 kg/ha (genotype ChK 3226 – irrigated treatment in Winter 98 with wet spring) and 207 kg/ha (genotype ChK 3099 – non irrigated treatment in Spring 96, with driest spring) was noted.

All variables evaluated were correlated with seed yield. In the rainfall treatment, early phenology appeared to be the most important attribute for high seed yield. The results also indicate that cultivars that gave high seed yield in the drier treatment are also responsive to increased moisture supply.

Two tolerant genotypes were selected and are in inscription process in CNV (Catálogo Nacional de Variedades – National Catalogue of Varieties).

Introduction

The Chickpea

Chickpea is a traditional low-input crop in the farming systems of the semi-arid tropics (350mm - 600mm annual rainfall).

In Mediterranean countries is an important raw material of human diet and at the same time an interesting way to provide vegetable protein. In Portugal, the chickpea production is used for food. However, in the past, some part of the harvest was used for feeding, pigs and mules mainly in very favourable seasons where surplus of grain were registered.

Until the middle of this century the chickpea cultivation area was quite considerable (Fig 1). On the second half of the century chickpea acreage clearly declined but at the same time grain yield persists very low.

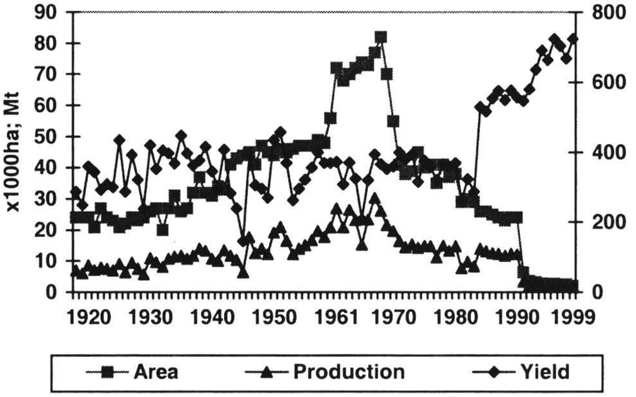


Fig. 1 – Surface, production and grain yield evolution

During the nineties this problem occurred mainly to lack or low CAP subsidies to the crop and also lower prices of imported raw material. There were also some problems on the agronomy and varieties extremely susceptible to the main diseases and drought; plant structure was not adapted to mechanical harvesting and insufficient market organisations as concerning the farmer's side.

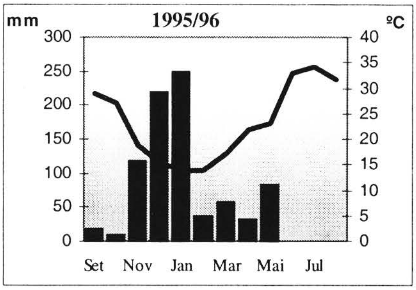
The production of chickpea normally is low because this crop is currently spring-sown.

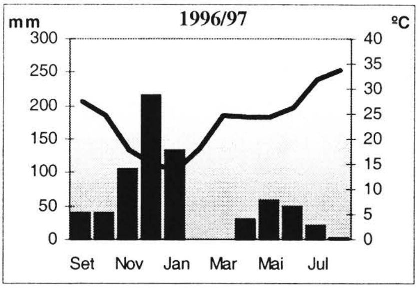
There are two main types, the Desi type with small seeds, coloured and angular shape, and Kabuli type, with large and beige colour seeds and ram-head shape. More than 80% of the world production of chickpea is Desi type, predominantly grown in subsistence agriculture.

Growth Cycle

Chickpea is traditionally sown in Spring. Diseases and drought are the most important limiting factors of production. Ascochyta blight and Fusarium wilt, are two major diseases of chickpea. Leaf miner in the Mediterranean region and pod borer in other regions are the major pests. Drought is the major abiotic stress throughout the chickpea growing areas.Date of sowing has an extreme influence on the crop performance once it determines the environmental conditions to which the various phenological stages of the crop will be exposed. Low shoot biomass in the Mediterranean basin is an important reason among others for poor yield. Yield can be increased substantially by advancing sowing date from spring to Autumn, where chickpea plants have favourable atmospheric conditions; the plants develop more biomass, flowers and pods. However these plants have some problems during the late spring without rain, when higher biomass means more transpiration leading to genotypes that cannot avoid terminal stress.

On Spring-sowings the low soil moisture level in the seed-bed, promotes poor stand establishment due to an inadequate germination or emergence and high seedling mortality and plants develop all the flowering and pod setting stages under water stress conditions. On the other hand, on Autumn/Winter-sowings lack of water associated with high diurnal temperature fasten plant development and increases flower and pod abortion, even if late rains could occurr (Fig 2 - year 1996/97).





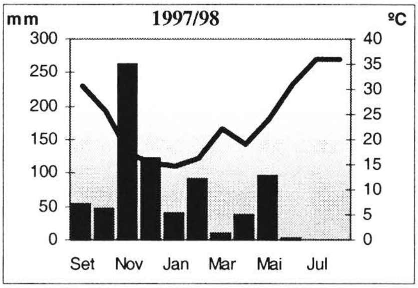


Fig. 2 – Rainfall and maximal daily temperature during 3 years at Elvas

Breeding

Chickpea breeding in the National Plant Breeding Station started in 1986 with some material received from ICARDA, ICRISAT and Portuguese populations, that were tested in two seeding periods: Autumn and Spring.

The major objectives of the breeding programme in chickpea are:

a) To develop cultivars and genetic stocks with high and stable yield.

b) To promote strategic research to complement objective a). Specific objectives in the development of improved germplasm for our climate are:

1) Winter sowing: resistance to Ascochyta blight, tolerance to cold, tolerance to drought in final stages of the cycle, suitability for machine harvesting, medium to large seed size;

2) Spring sowing: cold tolerance at seedling stage, resistance to Ascochyta blight and Fusarium wilt, tolerance to drought, early maturity, medium to large seed size.

Material and Methods

Experiments were conducted during 1995/96, 1996/97 and 1997/98, with 20 genotypes of chickpea (ChK 2983, ChK 508, ChK 309, ChK 3254, ChK 3203, ChK 3213, ChK 3226, ChK 3221, ChK 504, ChK 301, ChK 1150, ChK 1140, ChK 933, ChK 2909, ChK 2381, ChK 2844, ChK 3087, ChK 3099, ChK 1695, ILC 3279), previously selected for their response to drought in winter (W) and Spring (SP) sowing. All genotypes are adapted to winter sowing and *Ascochyta rabiei* tolerants. They were planted under irrigation (I) and non irrigated (NI) conditions. The interaction between treatments, years and genotypes was calculated by factorial analysis and correlations. They allow us to select the best genotype. Yield data were plotted against the best yield on WI (winter irrigated) treatment, during the season 1996/97, to access the relationship between yield potential and yield under all treatments.

Results

Table 1 - Factorial ANOVA analysis for the grain yield, harvest index (HI) and seeds/m^2, during 3 years.

Genotype	*YIELD*				*HI*				SEEDS/m2			
	WINTER		SPRING		WINTER		SPRING		WINTER		SPRING	
	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI
ChK 2983	2496	1347	1971	979	40.7	51.5	44.1	43.6	695	361	530	272
ChK 508	2954	1465	2146	1199	35.7	39.2	33.6	32.6	615	304	453	263
ChK 309	3098	1869	2103	1253	38.1	51.3	40.0	37.9	1001	580	669	462
ChK 3254	3054	1738	2198	1030	38.8	43.9	42.4	35.9	674	395	451	230
ChK 3203	3172	1686	2124	1395	44.8	47.8	38.6	44.6	894	458	592	391
ChK 3213	3693	1858	2827	1683	46.1	50.4	47.4	39.1	885	452	677	425
ChK 3226	3775	1707	2520	1457	45.1	43.7	39.8	37.3	1221	534	796	490
ChK 3221	3793	1645	2844	1600	44.8	42.2	39.4	36.8	1080	434	785	484
ChK 504	2713	1646	1719	1158	41.4	48.7	40.3	37.2	613	359	383	330
ChK 301	2566	1138	1915	1224	38.6	40.2	34.8	34.3	895	369	697	418
ChK 1150	2679	1205	2112	1462	39.3	37.9	39.1	41.5	937	382	703	482
ChK 1140	2876	1242	1726	694	39.1	33.8	33.8	38.4	966	404	618	245
ChK 933	2669	1517	1913	879	34.0	38.2	31.8	29.1	1013	528	719	329
ChK 2909	3579	1834	2610	1013	40.8	38.4	32.6	35.1	1054	529	746	289
ChK 2381	3684	1562	2165	1228	41.2	39.8	36.7	34.4	1063	421	601	368
ChK 2844	2966	1591	2229	846	39.9	40.3	32.7	35.2	895	473	669	266
ChK 3087	2765	1487	1972	634	37.5	36.6	32.1	27.7	1009	520	707	247
ChK 3099	3019	1190	2174	998	42.4	47.3	40.7	33.0	880	368	652	308
ChK 1695	2666	1216	1870	1215	40.3	45.8	38.8	42.4	673	318	523	347
ILC 3279	2959	1335	2036	863	36.2	34.9	36.0	34.0	946	427	599	296
T	***		***		***		*		***		***	
Y	***		***		**		***		***		***	
G	***		***		***		***		***		***	
TxY	***		***		**		***		***		***	
TxG	*		ns		***		ns		***		**	
YxG	***		***		ns		ns		***		***	
TxYxG	ns		*		ns		ns		**		*	
CV (%)	20.6		30.8		12.4		19.2		18.5		31.1	

T - treatment; Y - year; G – genotype; *P>0.05; **P>0.01; ***P>0.001; n.s. not significant

Conclusion

Chickpea is a well-adapted crop to the Mediterranean conditions, where drought occurs during end of grain filling period. However good results can be obtained with moderate irrigation during and grain growth phases.In the future the aim of breeding programme will be the selection of adapted materials to drought and heat stress, during the beginning of flowering. Plants with high recovery capacity after stress are also an objective of selection.

A screening technique will involve:

1) Physiological and morphological evaluation of resistances in comparison with susceptible materials from ICARDA and from other local germplasm.

2) Adaptation trials during Autumn/Winter under irrigation, and select the best genotypes, once our data shows good genotype performance under more unfavourable conditions for the highest yield potential lines.
3) To select lines for irrigation and tolerant to main diseases, especially Ascochyta blight.

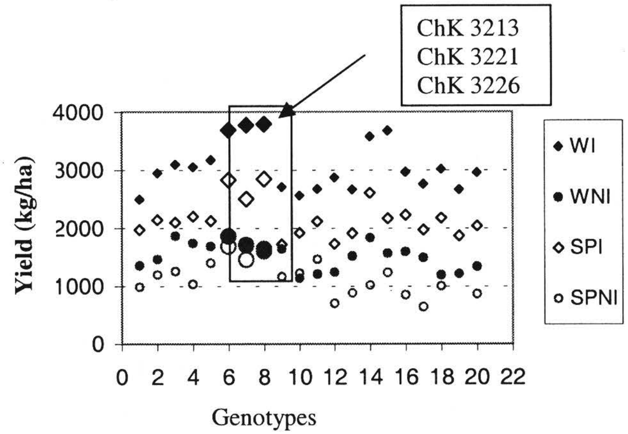


Fig. 3 – Factorial analysis: grain yield

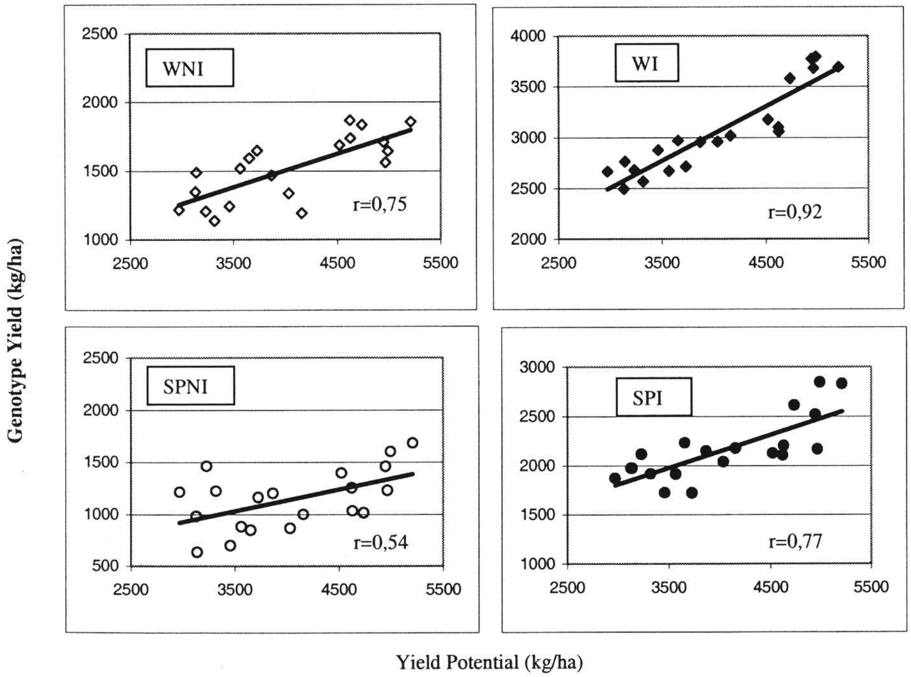


Fig. 4 – Factorial analysis: yield potential vs. treatments

ASSESSMENT OF VARIATION IN SALT TOLERANCE AMONG ALFALFA VARIETIES

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Abstract

Alfalfa (*Medicago sativa* L.) is considered to be moderately sensitive to salinity. Exploitation of genetic variation to improve salt tolerance offers an effective mean to sustain production in areas affected with salinity. Fourteen alfalfa varieties with diverse origins and growth behaviour were assessed for salt tolerance under greenhouse conditions. The varieties included four Egyptian (Giza-1, Ismailia-1, Ismailia-94 and Sewa); five Polish (Boja, Kama, Kometa, Radius and Tula); four Dutch (Marina, Medina, Midi and Oro) and one American (WL-605). The experiment was a randomised complete block with three replications. Treatments' design was a split plot, with levels of salt as the main plot and varieties as the sub plot. Plants were grown in pots filled with soil mixture (2 : 3 : 3 : 4, peat, sand, perlit and plotting mix), irrigated with two salt levels, control and 90 mM NaCl. Three cuts were taken at 10 - 20% bloom to investigate the effects of salinity on plant performances. In addition, heritabilities of the traits on a plot basis were also determined.

The results showed that, all the measured traits were influenced significantly by salt levels and varieties except for shoots number/ plant and protein %. Although the reduction in plant performances as a result of stress was pronounced for all traits, the ratio of root : shoot and percent of protein were increased in the stressed plants compared with control. Generally, the Egyptian varieties tolerated more than other tested materials since, recorded the highest fresh and dry weights of shoots, root dry weight and tallest shoots. Coefficients of broad sense heritability estimated at 90 mM revealed that, Root : Shoot ratio and root dry weight were moderately heritable (h^2 = 50-60%). While, plant height, fresh and dry forage yield were highly heritable (h^2 = 80-92.5%). The results concluded that, the genetic variation for salt tolerance exists and it is possible to select tolerant genotypes within varieties. The nutritional value of plants was not affected by NaCl up to 90 mM. Additionally, the above mentioned traits are promising to improve and select for salt tolerance in alfalfa.

Key words: Alfalfa, *Medicago sativa* L., Heritability, Salt tolerance, Variation.

Introduction

Salinity in soil or water presents a stress condition for crop plants that is of increasing importance in agriculture. It is estimated that a third of the world's irrigated area is already affected by excess salinity (Staples and Toenniessen, 1984). Reclamation, drainage might reduce the severity of salinization, but the costs are prohibitive. However, using of salt-tolerant crops is one of the solutions to problems associated with salt affected soils or with the utilization of brackish water for irrigation (Pasternak and De Malach, 1993). Alfalfa (*Medicago sativa* L.) is the world's most important forage crop (Barnes *et al.*, 1988), grown extensively under both rainfed and irrigated conditions where both salt and drought are prevalence problems.

Smith *et al.*, (1994) emphasised that, utilising genetic variation from germplasm collections could facilitate improvement of tolerance trait. McKimmie and Dobrenz (1991) also reported that, selection within a population or cultivar for salt tolerance is possible only if variability exists. The same researchers mentioned that variability in salt tolerance within a progeny of 54 clones from alfalfa population grown under conditions of -0.7 MPa salinity was manifested in term of shoot length. Depending upon morphological and agronomic characters as the main criteria, it could be possible to identify salt-resistant genotypes. Khan *et al.*, (1994) found that, increasing salinity level from 10-100 mM NaCl substantially reduced dry weight of roots. Johnson *et al.*, (1992) found a 7.3% reduction in alfalfa forage yield for each dSm^{-1} (≈11mM NaCl) increase above a threshold of 2.0 dSm^{-1} (≈22 mM NaCl). Recurrent selection at the adult stage increased salt tolerance in alfalfa and breeding showed that this trait was highly heritable (Noble *et al.*, 1984). It is evidenced that, genetic diversity is a prerequisite for any breeding program (Salam *et al.*, 1999) and by selection from within cultivars it should be possible to achieve improvement in salt tolerance. This research is a preliminary study to investigate the differential responses of different alfalfa cultivars at adult stage to irrigate with NaCl. In addition, heritabilities in broad sense were determined for all the measurements to quantify the extent of genetic variation controlling salt tolerance and superior genotypes could be identified within cultivars for further breeding studies.

Materials and Methods

I-Plant materials and procedures

A total of 14 alfalfa cultivars, representing a wide range of origin and growth type were included in this study: four Egyptian (Giza-1, Ismailia-1, Ismailia-94 and Sewa); five Polish (Boja, Kama, Kometa, Tula and Radius); four Dutch (Marina, Medina, Midi and Oro) and one American (WL-605). Seeds were germinated in petri dishes for 5 days, further, transplanted to plastic pots containing soil mixture of 2:3:3:4 of peat, perlite, sand and potting mix covered with sand to a depth of 1 cm. Seedlings were irrigated with non-saline water for 14 days and then with 0.25-strength Hoagland solution plus 0 or salinized to 90 mM NaCl. Three harvests were undertaken when the plants were at 10 - 20 % bloom. For each cut the following traits were recorded on the basis of individual plant: Plant height, forage fresh weight (clipped 5 cm above the crown), No. of shoots, forage dry weight (oven dried for 48 h at 70 °C). After the third harvest, five plants from each treatment in each replicate were selected randomly, separated into shoots and roots. Root samples were washed in tap water to remove adhering soil particles, dried at 70 °C and weighed. Root : shoot ratio was also determined by dividing root dry weight at the third harvest by dry shoot weight (mean of 3 cuts). Dry forage was ground and total N % was analyzed by semi-micro Kieldahl method using Kyelfec Auto 1030 Analyzer. To calculate protein %, N % was multiplied by 6.25.

II-Experimental design and statistical analysis :

A split-plot design with 3 replicates was used with salt levels (0 and 90 mM NaCl) as main plots and varieties as sub-plots. Each sub-plot (variety) was represented by 10 pots each with one plant, placed randomly within each main plot (salt level).

Data were analysed statistically using COSTAT program and tested for its significance by F-test. Heritability in broad sense was expressed as the ratio of the genetic variance component to the phenotypic variance:

$Ms_{(G)} - Ms_{(GE)}] / [Ms_{(G)}]$ (Link *et al.* 1999).

$Ms_{(G)}$ and $Ms_{(GE)}$ being the ANOVA mean squares due to varieties and to interactions of varieties with salt levels, respectively.

Results and Discussions

The analysis of data indicated that, all the studied characters were affected significantly by both varieties and salt levels except for protein % and shoots/plant (Table 1). Exposing alfalfa plants to salt stress cause a reduction in plant growth parameters. In contrast, R : S ratio and protein % were increased with increased root zone salinity. The reduction in growth in terms of plant height, dry weight of shoots and root dry weight recorded 59.40 %, 60.90 % and 77.50 %, respectively. Whereas, increasing in R : S ratio and protein % recorded 128.30 % and 124.30 %, respectively. Although, growth of all cultivars decreased in response to NaCl stress, the Egyptian cultivars maintained the highest growth values compared with other investigated cultivars. Broad-sense heritability estimates are presented in Table 1.

Table 1. Performances and broad sense heritability estimates of traits associated with yield for fourteen alfalfa cultivars grown under control and salt-stressed conditions.

Varieties	Salt levels (mM)	Plant height (cm)	Shoots $plant^{-1}$	Fresh yield (g/plt)	Dry yield (g/plt)	Root dry weight (g/Plt)	R:S	Protein %
Giza-1	0	49.00	2.80	9.08	2.21	1.06	0.48	14.52
	90	28.20	2.68	5.69	1.37	0.84	0.61	19.67
Ismailia-1	0	4620	3.08	8.99	2.18	1.06	0.48	15.62
	90	2680	2.80	5.72	1.36	0.92	0.68	19.92
Ismailia-94	0	44.80	2.85	8.84	2.10	0.91	0.44	15.59
	90	28.30	2.88	5.70	1.39	0.80	0.57	19.40
Sewa	0	47.00	3.00	9.26	2.26	1.12	0.50	14.94
	90	29.40	2.68	5.63	1.35	1.00	0.73	18.88
Boja	0	39.10	2.99	8.06	1.97	0.95	0.48	16.92
	90	21.30	2.98	5.08	1.18	0.79	0.67	19.87
Kama	0	37.20	3.07	8.35	2.09	1.04	0.49	17.47
	90	22.30	2.75	4.72	1.15	0.67	0.58	20.88
Kometa	0	38.00	3.08	8.54	1.99	0.97	0.48	17.17
	90	22.80	2.90	4.90	1.22	0.74	0.60	20.53
Radius	0	36.00	3.17	8.18	1.97	1.01	0.52	16.51
	90	21.10	2.74	5.14	1.24	0.76	0.61	21.08
Tula	0	36.90	3.19	8.20	1.97	0.91	0.46	16.24
	90	21.40	2.83	5.25	1.23	0.76	0.62	20.75
Marina	0	42.10	2.98	8.58	2.12	0.91	0.43	15.77
	90	25.90	2.55	5.64	1.30	0.68	0.52	19.52
Medina	0	43.30	3.06	9.46	2.32	0.98	0.42	16.12
	90	25.80	2.76	5.27	1.31	0.71	0.55	18.58
Midi	0	43.00	2.84	8.11	1.99	0.89	0.45	16.00
	90	25.80	2.50	5.22	1.31	0.74	0.56	20.14
Oro	0	41.90	2.92	7.24	2.06	0.97	0.47	15.93
	90	25.70	2.58	5.06	1.23	0.61	0.49	20.74
WL-605	0	45.30	3.15	9.03	2.22	0.88	0.40	15.61
	90	25.90	2.76	5.56	1.32	0.69	0.52	18.93
LSD								
Var.		0.84	ns	1.04	0.13	0.17	0.10	ns
salt levels		1.66	ns	0.50	0.09	0.12	0.07	ns
var. x salt		2.35	ns	ns	0.13	ns	ns	ns
h^2bs (%)	0	95.34	30	71.43	76.92	50	30	40.54
	90	92.52	15	80	83.33	60	50	0.68

ns: not significant.

Most of traits recorded high estimates of heritability under stress than control condition, supporting the existence of genetic variation for these traits within and between the genetic materials. In contrast, shoots / plant and protein % recorded low values of heritability at 90 mM and moderate value at 0 mM NaCl suggesting that, both traits were more influenced by environmental conditions, therefore, genetic variability is less importance. In this regard, Ray *et al.* (1999) on their work on alfalfa grown under water-stressed field condition, recorded moderate heritability estimate for forage yield (41%) and low estimate for shoot height (23%). Also, Ashraf *et al.* (1987) obtained moderate value of heritability for shoot length (31%) of alfalfa grown in salinized solution. On the other hand, Al-Khatib *et al.* (1994) mentioned that, salinity tolerance in term of shoot length is heritable with broad sense heritability estimated at 74 %.

From the previous results it can be concluded that, using the salt level 90 mM NaCl caused reduction in most plant growth parameters. High protein alfalfa forage can be obtained with saline water up to 90 mM. Depending upon morphological criteria such as, dry weight of shoots and roots, plant height and R:S is effective to select tolerant genotypes. The Egyptian varieties tolerated NaCl stress and surpassed the other tested materials. Selection for salt tolerance and further hybridization between the selected genotypes is possible within the tested varieties hence, most of characters recorded moderate to high heritability estimates.

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WINTERHARDINESS OF NORTH AMERICAN AND EUROPEAN *Medicago sativa* L. VARIETIES

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Abstract

The study on winter hardiness of lucerne European (7 entries) and North American (21 entries) varieties was carried in the field experiment at Radzików (Central Poland). Dry matter yield was evaluated in the field experiments in 4 research stations. Spread all over the country of Poland. There were no winterhardiness difference between the North American and the European groups of varieties. In both groups existed great variability of winterhardiness and many varieties are suitable for cultivation in Poland. Because of relatively week winters in 1997 – 99 the winterhardiness of varieties were not correlated with dry matter yields in the field conditions.

Material and Methods

The material for study consisted of seed samples of 21 North American and European varieties. Their names and origin are given in Table 1.

Field experiment for evaluation of winterhardiness were carried out in the experiment where plants were grown in space 20 x 100 cm, 25 plants per plot in 5 replicated. The plants were sown in early June 1997, and clipped in 3 leaf stage to remain 25 plants per 1 row plot. CUF – non dormand variety has been used to check if winter condition were severe enough.

Plant number on each plot were counted in the autumn and the spring (Table 1). In 1998 frequent cuttings were employed to make plants weaker. In April 1999 the plants remained on plots were scored in the five rates : 1 = no injury, 5 = dead plant (Table 2). The observations were completed according to the procedure of University of Wisconsin - Study 93-06. The experiments for evaluation dry matter yields were carried on according to randomized block design in 5 replications and 4 experimental stations : Bartazek (Northern PL), Borowo (Western PL), Radzików (Central PL), Sandomierz (South-East PL). Nineteen entries were included into this experiment: 16 USA, 4 European (Table 3).

2 cuts were harvested in 1997 (the year of sowing), 4 cuts in 1998 as well as in 1999. The plants were cut in early bloom stage. The standard technology of lucerne growing were applied during the vegetation of plant.

Results

Near to 30 % of plants were lost during first winter, due to early frosts in November and December 1997, and also February 1998 was unusually frost. The snow cover smaller then usually unfavoured plant survival too. There were no significant differences among varieties excluding CUF 10, which plants were totally damaged.

Due to frequent cutting employed in the vegetation period of 1998 (six cuts taken off) another 10 % of plants were dead, but still 61 % of plants remained on plots, and Radius, Kometa, Alpine II were the best in this respect.

There was a very deep depression of plant number per plot in April 15 1999, followed frequent cutting stress in spite of that winter 1998/99 was mild. The plants of CUF 101 were totally damaged by frost and only about 23 % of plants remained on plots (the experiment mean) and also great variability of the plant survival was observed among the entries (Table 1).

In general many USA entries displayed good plant survival after the stress and also European ones, excluding local varieties (unregistered populations). Several new bred American cultivars performed very well.

The vigor of plants remained on plots in the spring 1999. After one month many plants died, 17 % were very injured, 30,8 % partially injured, and only 20 % were good and very good. But great variability among entries existed and one may have chosen many of them with satisfactory winter hardiness (Table 2).

The comparison of winterhardiness and dry matter yields of cultivars displayed that in the given climatical conditions in 1997 – 99 on Poland's territory the winterhardiness did not influence the dry matter yield. All varieties produced a satisfactory amount of dry matter, anymore, some of them were very good exceeding by several per cent the local standard Kometa. The ratios given in Table 4 did not show correlation among winter hardiness and dry matter yields. But the results would have been quite opposite if they were exposed to real severe winter conditions.

Conclusions

1. Both North American and European varieties varied in winterhardiness when were evaluated in stress conditions.
2. New bred American cultivars possessed very good winter hardiness : ABT 205, F-G 97-2, Treasure, Alpine II and Magnum 1V.
3. In spite of that then the list of European varieties was limited some of them were winterhardy : Kometa, Radius and RAH 100.
4. If the weather conditions are similar to the long term mean the winterhardiness of varieties do not influence their yield performances.
5. Many of North American recent varieties exhibit good winterhardiness and dry matter yield to be cultivated in Poland.

Table 1 Comparisons of winterhardiness of various alfalfa varieties under stressed conditions in the field.

Object	Origin	Plant survivals in %			
		Oct. 23. 1997	Apr. 15. 1998	Oct. 23. 1998	Apr. 15. 1999
USA :					
Magnum 111	Dairyland Seed	100,0	72,8	68,8	33,6 *
Magnum 1V	Dairyland Seed	100,0	80,8	58,4	35,2 *
Magnum 111 Wet	Dairyland Seed	100,0	79,2	64,8	22,4
Magna Graze	Dairyland Seed	100,0	81,6	60,8	23,2
Haygrazer	Dairyland Seed	100,0	80,0	69,6	34,4 *
Haygrazer Dual	Dairyland Seed	100,0	74,4	50,4 *	16,0 *
Alpine II	Peterson Seed	100,0	78,4	75,2 *	35,2 *
Stampede	Peterson Seed	100,0	79,2	64,8	24,0
ABT 205	Clark Seed	100,0	76,0	55,2	43,2 *
ABT 405	Clark Seed	100,0	75,2	57,6	29,6 *
Treasure	Clark Seed	100,0	75,2	63,2	37,6 *
F-G 97 - 1	Forage Genetics WI.	100,0	79,2	66,4	32,0 *
F-G 97 - 2	Forage Genetics WI.	100,0	72,8	65,6	40,0 *
F-G 97 - 7	Forage Genetics WI.	100,0	72,8	64,0	32,0 *
ACW PO3	Cal West	100,0	76,0	58,4	18,4 *
W-L 92 - 28	W - L Research	100,0	73,6	62,4	21,6
W-L 92 - 132	W - L Research	100,0	83,2	61,6	25,6
W-L 93 - 39	W - L Research	100,0	80,0	56,8	27,2
W-L 93 - 110	W - L Research	100,0	80,8	64,8	18,4 *
Vernal	Public Variety	100,0	76,8	62,4	24,8
Europe :					
Bar MS 63424	Barenbrug (Fr)	100,0	80,4	61,6	11,2 *
RAH 100 **	IHAR (PL)	100,0	79,2	66,4	24,0
Radius	Public Variety (PL)	100,0	77,6	71,2 *	24,8
Kometa	Public Variety (PL)	100,0	83,2	76,0 *	30,4 *
Trwała Czyżów	Local Variety (PL)	100,0	71,2	58,4	16,8 *
Miechowska	Public Variety (PL)	100,0	72,8	56,0	18,4 *
Iłowska	Local Variety (PL)	100,0	73,6	56,8	9,6 *
CUF 101	Non dormant standard	100,0	0,0 *	0,0 *	0,0 *
CUF 101 ***	Non dormant standard		100,0 *	84,0 *	0,0 *
mean			75,4	61,4	24,5
LSD $_{0,05}$			10,31	9,48	4,26

* Significant at α = 0,05 level compared to the mean

** The first variety with long raceme peduncle governed by *lp* gene

*** CUF 101 plants were planted again in 1998 y

Table 2 Evaluations of plant vigour in the spring after frequent cutting stress (third year of vegetation)

Object	Origin	Rate (May 18. 1999) : 1 = no injury, 5 = dead				
		1	2	3	4	5
<u>USA :</u>						
Magnum 111	Dairyland Seed	6,2	19,1	59,7 *	12,1 *	2,9 *
Magnum 1V	Dairyland Seed	4,8 *	20,1	37,6	29,5 *	8,0
Magnum 111 Wet	Dairyland Seed	0,0 *	21,7	36,0	39,0 *	3,3 *
Magna Graze	Dairyland Seed	3,6 *	39,1 *	14,0 *	43,3 *	0,0 *
Haygrazer	Dairyland Seed	6,2 *	25,0 *	56,2 *	12,6 *	0,0 *
Haygrazer Dual	Dairyland Seed	0,0 *	4,0 *	40,4	40,4 *	15,2 *
Alpine II	Peterson Seed	11,4 *	26,0 *	29,1 *	33,5 *	0,0 *
Stampede	Peterson Seed	15,5 *	13,6 *	52,1 *	12,1 *	6,7
ABT 205	Clark Seeds	22,0 *	18,8	29,4 *	11,8 *	18,0 *
ABT 405	Clark Seeds	4,0 *	24,5 *	42,0	24,2	5,3
Treasure	Clark Seeds	10,9 *	24,6 *	39,6	16,9	8,0
F-G 97 - 1	Forage Genetics WI.	6,2	19,5	41,3	23,0	10,0 *
F-G 97 - 2	Forage Genetics WI.	15,5 *	28,9 *	42,1	5,6 *	7,9
F-G 97 - 7	Forage Genetics WI.	5,0 *	19,8	50,5 *	18,6	6,1
ACW PO3	Cal West	15,5 *	12,2 *	31,3 *	31,0 *	10,0 *
W-L 92 - 28	W - L Research	11,7 *	31,6 *	35,0	16,7	5,0
W-L 92 - 132	W - L Research	4,3 *	27,6 *	53,3 *	8,1 *	6,7
W-L 93 - 39	W - L Research	9,1	27,7 *	37,3	25,9	0,0 *
W-l 93 - 110	W - L Research	11,7 *	5,0 *	35,0	43,3 *	5,0
Vernal	Public Variety	6,8	20,5	53,9 *	18,8	0,0 *
<u>Europe :</u>						
Bar MS 63424	Barenbrug (Fr)	0,0 *	0,0 *	49,0 *	44,3 *	6,7
RAH 100 **	IHAR (PL)	11,4 *	9,7 *	48,9 *	20,7	9,3 *
Radius	Public Variety (PL)	2,5 *	17,5	63,4 *	13,7 *	2,9 *
Kometa	Public Variety (PL)	14,7 *	14,3 *	50,5 *	12,9 *	7,6
Trwała Czyżów	Local Variety (PL)	6,3	28,1 *	25,0 *	9,4 *	31,2 *
Miechowska	Public Variety (PL)	3,3 *	13,8 *	38,7	40,8 *	3,4 *
Iłowska	Local Variety (PL)	18,7 *	37,5 *	37,5	0,0 *	6,3
CUF 101	Non dormant standard	0,0 *	0,0 *	0,0 *	0,0 *	0,0 *
CUF 101 ***	Non dormant standard	0,0 *	0,0 *	0,0 *	0,0 *	0,0 *
mean		7,8	19,0	38,9	21,0	6,6
$LSD_{0,05}$		2,14	4,51	6,22	4,92	1,69

* Significant at $\alpha = 0,05$ level compared to the mean

** The first variety with long raceme peduncle governed by *lp* gene

*** CUF 101 plants were planted again in 1998 y

Table 3 Dry matter yield of North American and European varieties

Object	Origin	Mean of 4 locations					
		First cut, May 1998		First cut, May 1999		Ten cuts 1997-99	
		t/ ha	% of Kometa	t/ ha	% of Kometa	t/ ha	% of Kometa
USA :							
Magnum 111	Dairyland Seed	5.9	96.4	5.8	112.1	37.35	101.9
Magnum 1V	Dairyland Seed	6.0	98.7	5.8	112.9	37.94	103.5
Magnum 111 Wet	Dairyland Seed	5.7	93.6	5.6	109.3	37.30	101.8
Alpine I I	Peterson Seed	6.0	98.3	5.7	111.5	37.93	103.5
Stampede	Peterson Seed	6.2	101.8	6.0	116.7	39.43	107.6
Treasure	Clark Seeds	5.8	95.6	6.0	116.0	37.60	102.6
F-G 97 - 1	Forage Genetics WI.	6.0	97.4	5.3	103.8	38.27	104.4
F-G 97 - 2	Forage Genetics WI.	6.0	98.8	5.7	111.6	38.10	104.0
F-G 97 - 7	Forage Genetics WI.	6.0	98.2	5.7	110.6	38.21	104.3
ACW PO3	Cal West	6.1	99.2	5.3	102.4	36.89	100.7
W-L 92 - 28	W - L Research	6.1	100.5	5.7	111.5	39.43	107.6
W-L 92 - 132	W - L Research	6.0	98.8	5.5	107.6	38.22	104.3
W-L 93 - 39	W - L Research	5.8	95.4	5.7	110.8	37.93	103.5
W-I 93 - 110	W - L Research	6.1	99.3	5.8	112.5	37.82	103.2
Vernal	Public Variety	5.7	93.3	4.5	87.5	33.56	91.6
Europe :							
Bar MS 63424	Barenbrug (Fr)	5.8	95.0	5.6	109.7	37.47	102.2
RAH 100 *	IHAR (PL)	6.5	107.1	5.9	114.6	38.56	105.2
Radius	Public Variety (PL)	6.2	101.0	5.8	113.6	37.87	103.3
Kometa	Public Variety (PL)	6.1	100.0	5.1	100.0	36.65	100.0
mean		6.0		5.6		37.71	
$LSD_{0,05}$		0.62		0.72		1.58	

* The first variety with long raceme peduncle governed by *lp* gene

Table 4 Correlation coefficients between plant survival and the yield

Variable	Plant survival in % Apr. 15. 1998	Plant survival in % Apr. 15. 1999
Dry matter yield of first cut (May 1998)	0,66	-
Dry matter yield of first cut (May 1999)	-	0,15
Total dry matter yield of 10 cuts (1997-99)	-0,04	0,07

Table 5 Climatical conditions at Radzików

Month	Temperature (°C)				Precipitation (mm)			
	1997	1998	1999	Mean of 1975-95	1997	1998	1999	Mean of 1975-95
January	-4,4	**1,1**	**0,9**	-2,0	0,0	**6,6**	**0,5**	18,5
February	2,2	**3,6**	**-0,5**	-1,2	10,0	**9,2**	**2,3**	18,1
March	4,9	2,6	5,8	2,6	12,8	22,3	7,5	24,4
April	6,1	10,7	12,6	7,5	19,4	36,7	94,9	31,8
May	14,5	16,9	15,5	13,7	56,0	30,1	39,3	45,1
June	17,2	19,6	19,9	16,1	84,3	91,5	155,1	63,2
July	18,3	19,4	23,1	17,9	227,7	83,9	30,7	68,8
August	19,9	18,5	21,0	18,1	31,9	24,6	5,0	48,1
September	13,9	14,0	19,1	13,1	18,6	7,4	5,4	42,5
October	6,8	8,7	8,8	8,2	26,6	32,4	18,8	28,4
November	-1,5	3,2	2,2	2,8	43,7	22,1	7,5	28,7
December	**-2,4**	**0,4**	1,4	-0,2	**18,9**	**0,0**	13,0	24,9

GROWTH EVALUATIONS SPLIT WITH DROUGHT TOLERANCE OF ALFALFA UNDER WATER STRESS AND FREQUENT CUTTING

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Abstract

Alfalfa (*Medicago sativa* L.) is a major legume crop grown in arid and semiarid regions for forage where it requires abundant watering for high production under frequent cutting. In places where the drought is an important determinant factor of alfalfa growth, the improvement of alfalfa water-use efficiency could be effective to achieve varieties for profitable production in these zones. Therefore, eighteen alfalfa varieties were studied in order to evaluate their water stress tolerance. Following varieties were included: five Egyptian (Giza-1, Ismailia-1, Ismailia-94, New Valley and Sewa), eight of USA (CUF-101, Haygraze Dual, Legend, Magna Graze, Magnum IV, Vernal, WL-605 and WL-610), one French (Luzelle) and four Dutch (Marina, Medina, Midi and Oro). The plants were subjected to five water capacity variations (75%, 60%, 45%, 30% and 15%) and harvested for ten cuts at 10 to 20 % bloom. The experiment was carried out in greenhouse in 8 kg capacity pots which were filled with a mixture (3 : 1 : 1 of soil, sand and peat, respectively) and mean daily maximum / minimum temperature was 35 / 20 °C. Each variety was grown in 4 pots and 4 plants per pot. The experiment design was a randomised complete block with a two-factorial set of treatments. The statistical analysis revealed significant influence of water capacity on investigated varieties and their interactions for most measured characters. The varieties from arid centers such as Egyptian varieties exceeded the rest varieties in most traits such as plant height, shoots per plant, fresh and dry weights, leaf: stem ratio and root dry weight. Generally, the drought stress reduced alfalfa growth and had increased root : shoot ratio and decreased shoot number per plant, shoot, root and total plant weights comparatively to non-stressed plants. Genetic and phenotypic correlations among studied characters which accompanied with significant estimates of heritability and genetic variability concluded that, the selection criteria based on these characters will be effective for improvement of drought tolerance in alfalfa.

Key words : Alfalfa, *Medicago sativa*, water stress, water capacity, drought, heritability correlations

Introduction

In the arid and semiarid regions, alfalfa production may benefit from improvements in water-use efficiency (WUE). Whereas, high average daily temperatures exceeding 35 °C during the summer also drastically reduce water use efficiency in alfalfa (Kipnis *et al.*,1989 and Robinson and Teuber, 1992). Wissuwa and Smith (1997) reported that, the combination of these factors makes summer the least economical time for alfalfa production in long-season desert environments. Although, drought reduce the forage yield of all legumes, alfalfa has been reported to be drought tolerant because of its deep rooting system and has the greatest yield potential in drought (Peterson *et al.*, 1992). They added that, alfalfa herbage yield, leaf : stem weight ratio (LSWR) and herbage quality were often less affected by drought and produce more nutrients per hectare than the alternative legumes (birdsfoot trefoil, cicer milkvetch and red clover). Melton *et al.* (1989) reported that, attempts to improve water-use efficiency (WUE) in alfalfa have been limited, and when successful, have required multiple years of field evaluation to complete. Also, limited success from selection under drought stress has led several researchers (Turner, 1981; Blum, 1983; Austin, 1989 and Ludlow and Muchow, 1990) to propose conducting indirect selection for plant characteristics associated with performance under stress. In addition, evaluating physiological and agronomic traits under irrigation may also reduce the error variance relative to drought conditions where the influence of soil heterogeneity is magnified (Ray *et al.*, 1998). Currier and Melton (1995) found that, coefficients of variation for alfalfa forage yield in New Mexico have averaged three to four fold greater under drought conditions (Unpublished data). The objective of this study was to determine the influence of drought stress imposed to five water capacity levels on different alfalfa varieties. Heritability and genetic and phenotypic correlations among traits under investigation were also estimated.

Materials and Methods

Eighteen alfalfa varieties were studied in order to evaluate their water stress tolerance. Following varieties were included: five Egyptian (Giza-1, Ismailia-1, Ismailia-94, New Valley and Sewa), four Dutch (Marina, Medina, Midi and Oro), one French (Luzelle) and eight of USA (CUF-101, Haygraze Dual, Legend, Magna Graze, Magnum IV, Vernal, WL-605 and WL-610). Alfalfa seeds were germinated in petri dishes in a growth chamber at 26 ± 1 °C and seedling were transplanted to 8 kg capacity pots containing 3 : 1 : 1 of Soil : Sand : Peat mixture after one week in the greenhouse. Mean daily, maximum / minimum greenhouse temperature during the experiment was 35 / 20 °C. Ten seedlings were planted per pot and irrigated with Hoagland solution for two week, then thinned to four plants per pot. The plants were subjected to five water capacity variations (75, 60, 45, 30 and 15 %) and harvested for ten cuts at 10 to 20% bloom. The experiment was a factorial arrangement of a randomised complete block design with four replications. Whole plots contained the five water capacity variations and within whole plots were the eighteen of alfalfa varieties. The pots were weighted twice daily, watered if necessary and re-randomised in the greenhouse. These water capacity were maintained within ± 2 %. Because of the big numbers and weight of the pots, the weight of the growing plants was not considered when maintaining water capacity.

Significant treatment effects at $P < 0.05$ and $P < 0.01$ were identified by ANOVA procedures. When significant treatment effects occurred, means were separated using Fisher's L.S.D (Steel and Torrie, 1980). Genetic ($\delta^2 g$) and phenotypic ($\delta^2 ph$) variances were estimated from the obtained data of the measured traits averaged over all means the five water capacity variations and eighteen alfalfa varieties, hence, broad sense heritability (h^2_{BS}) was calculated according to Wricke and Weber (1986). Genetic (r_g) and phenotypic (r_{ph}) correlation coefficients among traits under investigation were also estimated (Miller *et al.*,1958).

Results and Discussion

The analyses of variances revealed highly significant influence of water capacity on the investigated varieties and their interactions for most traits such as plant height, shoots per plant and forage fresh and dry weights (Table,1 and 2). Otherwise, the interaction effects did not differ significantly for rest traits such as root dry weight and leaf : stem as well as root : shoot ratio. In addition, the varieties from arid centers such as Egyptian varieties exceeded the rest varieties in most characters, with exception of New Valley variety which represented lowest mean root : shoot ratio (1.68). Among USA varieties, Legend was given the highest mean of leaf : stem ratio (1.92) and Vernal in root : shoot ratio (2.73). On the other hand, the

Dutch variety Oro was markedly affected by drought and exhibited the lowest means of plant height (21.50), shoots per plant (2.14),leaf : stem ratio (1.44), root dry weight (0.95) and forage fresh as well as dry weights (Table, 1 and 2).

Drought has been shown to reduce plant height, shoots / plant, forage fresh and dry weights, root as well as total dry weight and increases leaf : stem and root : shoot ratios. The high ratio of root : shoot in the varieties possessing low dry forage production under water stress revealed that, root : shoot ratio could not be well correlated to drought tolerance. This result was in agreement with Chapin (1980) who mentioned that, high R : S ratio are characteristic of plants having low absorption capacity and low relative growth rates. Whilst, Blaha and Vancura (1990) reported that, varieties with a high ratio of root dry matter : shoot dry matter were characterized by greater nutrient uptake, yield stability and drought resistance.

The magnitude of the heritability estimates as well as the significant and positive genetic correlations among most studied traits suggest rapid initial responses and effectiveness of selection for superior genotype within and between investigated varieties as shown in Table (4). The relationships between growth characters under investigation are influenced by genetic and environmental variations as well as the interaction between them. In addition, genetic correlations were in general slightly higher than phenotypic correlations

Conclusion

Genetic and phenotypic correlations among growth characters, together with significant estimates of heritability and genetic variability, leads us to conclude that the selection criteria based on these characters will be effective for improvement of drought tolerance in alfalfa.

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Table (1) : Mean of plant height , number of shoots / plant and forage fresh weight for eighteen alfalfa varieties under five water capacity.

Varieties	Plant height (cm)						No. of shoots / plant						Forage fresh weight (g) / plant					
	75 %	60 %	45 %	30 %	15 %	Mean	75 %	60 %	45 %	30 %	15 %	Mean	75 %	60 %	45 %	30 %	15 %	Mean
Giza -1	68.70	63.50	37.70	15.70	11.50	39.40	7.00	4.92	2.80	1.50	1.12	3.47	19.30	12.50	7.10	1.80	1.00	8.30
Ismailia -1	70.20	65.20	39.50	16.00	11.70	40.50	6.52	4.52	2.82	1.32	1.10	3.26	18.30	11.80	7.00	1.40	0.90	7.90
Ismailia -94	67.70	62.20	38.20	10.70	11.50	38.10	6.50	4.50	2.70	1.17	1.17	3.21	17.50	11.20	6.60	1.00	1.00	7.50
New Valley	72.00	67.00	40.20	15.70	11.20	41.20	6.97	5.07	2.95	1.37	1.10	3.49	20.00	13.60	8.00	1.80	1.00	8.90
Sewa	69.00	63.70	37.00	15.20	10.20	39.00	7.67	5.67	3.17	1.37	1.12	3.80	21.20	14.40	7.30	1.40	1.00	9.10
CUF - 101	60.00	53.20	36.70	15.20	10.20	35.10	6.12	4.00	2.17	1.17	1.10	2.91	15.10	8.90	5.70	1.20	0.90	6.40
Haygraze Dual	46.20	37.50	19.20	12.00	9.20	24.80	5.45	3.57	2.15	1.12	1.02	2.66	10.10	5.30	3.00	0.90	0.60	4.00
Legend	51.50	43.50	35.00	13.50	9.50	30.60	6.42	4.25	2.32	1.17	1.05	3.04	13.20	7.50	4.70	1.00	0.70	5.40
Magna Graze	43.50	34.20	19.20	13.00	9.20	23.80	5.67	3.62	2.00	1.05	1.05	2.68	9.90	5.00	2.70	0.90	0.60	3.80
Magnum IV	46.20	39.50	20.70	12.50	8.50	25.50	5.45	3.40	1.90	1.12	1.07	2.59	10.20	5.30	3.00	1.00	0.80	4.10
Vernal	42.70	35.50	17.00	10.70	8.20	22.80	5.22	3.30	1.85	1.05	1.00	2.48	9.00	4.70	2.20	0.80	0.50	3.40
WL-605	53.20	43.20	19.50	13.50	9.50	27.80	6.07	4.07	2.22	1.35	1.10	2.96	13.00	7.10	3.00	1.20	0.80	5.00
WL-610	47.70	40.70	18.20	13.50	9.70	26.00	5.25	3.25	1.75	1.07	1.05	2.47	10.10	5.50	2.60	0.90	0.60	3.90
Luzelle	46.70	38.70	18.50	12.70	8.70	25.10	6.65	4.52	2.77	1.27	1.12	3.27	12.50	6.90	3.20	1.00	0.70	4.90
Marina	40.00	35.70	17.00	12.00	8.50	22.60	4.95	2.95	1.62	1.00	1.00	2.30	8.10	4.40	1.90	0.80	0.60	3.20
Medina	42.00	33.70	17.50	12.00	8.20	22.70	4.87	2.87	1.47	1.12	1.00	2.27	8.20	3.80	1.90	0.80	0.60	3.10
Midi	44.20	34.50	16.70	11.70	8.00	23.00	4.75	2.75	1.50	1.07	1.00	2.21	8.30	4.00	1.80	0.80	0.60	3.10
Oro	40.50	31.70	15.50	11.50	8.20	21.50	4.67	2.67	1.30	1.05	1.02	2.14	7.50	3.60	1.60	0.80	0.60	2.80
Mean	52.90	45.80	25.70	13.20	9.60	29.40	5.90	3.88	2.19	1.19	1.07	2.85	12.90	7.50	4.10	1.10	0.80	5.30
L.S.D (Water) 0.05	0.95						0.20						0.46					
L.S.D (Water) 0.01	1.25						0.27						0.61					
L.S.D (Varieties) 0.05	1.81						0.39						0.88					
L.S.D (Varieties) 0.01	2.38						0.51						1.16					
L.S.D Wat.x Var. 0.05	4.04						0.86						1.97					
L.S.D Wat.x Var. 0.01	5.32						1.14						2.59					

Table (2) : Dry weights of shoots , root and total plant for eighteen alfalfa varieties under five water capacity.

Varieties	Forage dry weight (g) / plant						Root dry weight (g) / plant						Total dry weight (g) / plant					
	75 %	60 %	45 %	30 %	15 %	Mean	75 %	60 %	45 %	30 %	15 %	Mean	75 %	60 %	45 %	30 %	15 %	Mean
Giza -1	3.50	2.60	1.60	0.50	0.30	1.70	1.69	1.56	1.47	1.31	1.10	1.43	5.19	4.16	3.07	1.81	1.40	3.13
Ismailia -1	3.30	2.40	1.60	0.40	0.30	1.60	1.71	1.54	1.40	1.27	1.12	1.41	5.01	3.94	3.00	1.67	1.42	3.01
Ismailia -94	3.10	2.30	1.50	0.30	0.20	1.48	1.67	1.55	1.45	1.33	1.12	1.42	4.77	3.85	2.95	1.63	1.32	2.90
New Valley	3.60	2.80	1.80	0.50	0.30	1.80	1.76	1.62	1.48	1.38	1.12	1.47	5.36	4.42	3.28	1.88	1.42	3.27
Sewa	3.70	2.90	1.70	0.40	0.20	1.78	1.77	1.63	1.50	1.36	1.23	1.50	5.47	4.53	3.20	1.76	1.43	3.28
CUF - 101	2.70	1.80	1.30	0.40	0.20	1.28	1.48	1.36	1.25	1.17	1.04	1.26	4.18	3.16	2.55	1.57	1.24	2.54
Haygraze Dual	1.70	1.10	0.70	0.20	0.20	0.78	1.19	1.08	0.99	0.90	0.81	0.99	2.89	2.18	1.69	1.10	1.01	1.77
Legend	2.30	1.60	1.10	0.20	0.20	1.08	1.48	1.37	1.26	1.14	1.02	1.25	3.78	2.97	2.36	1.34	1.22	2.33
Magna Graze	1.70	1.10	0.60	0.20	0.10	0.74	1.23	1.13	0.99	0.88	0.72	0.99	2.93	2.23	1.59	1.08	0.82	1.73
Magnum IV	1.80	1.20	0.70	0.20	0.20	0.82	1.18	1.10	1.01	0.90	0.66	0.97	2.98	2.30	1.71	1.10	0.86	1.79
Vernal	1.60	1.10	0.50	0.20	0.10	0.70	1.19	1.08	0.99	0.87	0.67	0.96	2.79	2.18	1.49	1.07	0.77	1.66
WL-605	2.30	1.50	0.70	0.30	0.20	1.00	1.46	1.36	1.25	1.07	0.79	1.19	3.76	2.86	1.95	1.37	0.99	2.19
WL-610	1.80	1.15	0.60	0.20	0.20	0.79	1.34	1.24	1.14	1.02	0.81	1.11	3.14	2.39	1.74	1.22	1.01	1.90
Luzelle	2.20	1.40	0.70	0.30	0.20	0.96	1.46	1.36	1.25	1.09	0.90	1.21	3.66	2.76	1.95	1.39	1.10	2.17
Marina	1.40	0.90	0.40	0.20	0.10	0.60	1.23	1.10	0.98	0.86	0.75	0.98	2.63	2.00	1.38	1.06	0.85	1.58
Medina	1.40	0.80	0.40	0.20	0.10	0.58	1.18	1.09	0.96	0.80	0.69	0.94	2.58	1.89	1.36	1.00	0.79	1.52
Midi	1.50	0.80	0.40	0.20	0.10	0.60	1.18	1.05	0.94	0.81	0.71	0.94	2.68	1.85	1.34	1.01	0.81	1.54
Oro	1.30	0.70	0.40	0.20	0.10	0.54	1.20	1.09	0.96	0.78	0.70	0.95	2.50	1.79	1.36	0.98	0.80	1.49
Mean	2.30	1.60	0.90	0.30	0.20	1.10	1.41	1.29	1.18	1.05	0.89	1.16	3.68	2.86	2.11	1.33	1.07	2.21
L.S.D (Water) 0.05	0.09						0.03						0.10					
L.S.D (Water) 0.01	0.12						0.04						0.14					
L.S.D (Varieties) 0.05	0.17						0.06						0.20					
L.S.D (Varieties) 0.01	0.23						0.08						0.26					
L.S.D Wat.x Var. 0.05	0.39						N.S						0.44					
L.S.D Wat.x Var. 0.01	0.52						N.S						0.58					

N.S : Non significant

Table (3) : Leaf : stem and root : shoot ratios for eighteen alfalfa varieties under five water capacity.

Varieties	Leaf : Stem ratio						Root : Shoot ratio					
	75 %	60 %	45 %	30 %	15 %	Mean	75 %	60 %	45 %	30 %	15 %	Mean
Giza -1	1.55	1.80	1.95	2.00	2.05	1.87	0.49	0.61	1.11	2.99	4.14	1.87
Ismailia -1	1.50	1.77	1.95	2.02	2.02	1.85	0.52	0.64	0.92	3.43	4.23	1.95
Ismailia -94	1.55	1.82	1.85	2.02	2.07	1.86	0.53	0.69	1.07	4.17	5.07	2.31
New Valley	1.55	1.77	1.85	2.00	2.12	1.86	0.50	0.59	1.00	2.83	3.50	1.68
Sewa	1.42	1.65	1.72	1.82	2.02	1.73	0.48	0.58	0.93	3.85	5.17	2.20
CUF - 101	1.50	1.80	2.00	2.07	2.12	1.90	0.55	0.75	0.99	3.25	4.29	1.97
Haygraze Dual	1.37	1.65	1.75	1.77	1.95	1.70	0.68	1.00	1.45	3.77	4.66	2.31
Legend	1.62	1.90	1.95	2.02	2.10	1.92	0.64	0.90	1.19	4.72	5.09	2.51
Magna Graze	1.40	1.62	1.72	1.85	1.92	1.70	0.74	1.05	1.57	3.65	5.42	2.49
Magnum IV	1.45	1.70	1.75	1.82	1.87	1.72	0.68	0.95	1.53	3.82	4.14	2.22
Vernal	1.32	1.55	1.67	1.80	1.90	1.65	0.78	1.03	2.02	3.99	5.81	2.73
WL-605	1.65	1.90	1.97	2.00	2.05	1.91	0.63	0.98	1.95	3.37	3.66	2.12
WL-610	1.37	1.52	1.62	1.75	1.90	1.63	0.77	1.12	2.26	4.24	5.20	2.72
Luzelle	1.57	1.65	1.70	1.85	1.90	1.73	0.66	0.96	1.81	3.87	4.19	2.30
Marina	1.20	1.35	1.42	1.47	1.57	1.40	0.91	1.29	2.43	3.95	5.65	2.85
Medina	1.35	1.55	1.62	1.72	1.85	1.62	0.84	1.41	2.47	3.71	5.21	2.73
Midi	1.25	1.45	1.52	1.70	1.72	1.53	0.81	1.35	2.44	4.04	5.35	2.80
Oro	1.20	1.37	1.42	1.57	1.65	1.44	0.89	1.51	2.67	3.57	5.24	2.78
Mean	1.44	1.66	1.75	1.85	1.93	1.73	0.67	0.97	1.66	3.73	4.78	2.36
L.S.D 0.05	0.06						0.29					
(Water) 0.01	0.09						0.38					
L.S.D 0.05	0.12						0.54					
(Varieties) 0.01	0.16						0.72					
L.S.D 0.05	N.S						N.S					
(Wat.xVar.) 0.01	N.S						N.S					

N.S : Non significant

Table (4): Broad sense heritability(h^2_{BS}) and genetic (above diagonal) and phenotypic (below diagonal) correlations among eight characters for eighteen alfalfa varieties under five water capacity.

Character	h^2_{BS}	Correlation							
		Plant height	shoots / plant	Forage fresh weight	Forage dry weight	Root dry weight	Total dry weight	Leaf : Stem ratio	Root : Shoot ratio
Plant height	0.98	---	- 1.00**Φ	1.00**Φ	0.63**	0.61**	0.81**	0.53**	0.39**
No. of shoots / plant	0.71	- 1.00**Φ	---	0.53**	0.45**	0.38**	0.58**	0.37**	- 0.55**
Forage fresh weight	0.94	1.00**Φ	0.50**	---	0.61**	0.66**	0.58**	0.50**	0.86**
Forage dry weight	0.93	0.61**	0.41**	0.43**	---	0.33**	0.59**	0.48**	0.41**
Root dry weight	0.95	0.58**	0.35**	0.58**	0.31**	---	0.63**	0.39**	0.88**
Total dry weight	0.97	0.76**	0.52**	0.60**	0.54**	0.60**	---	0.38**	0.63**
Leaf : Stem ratio	0.91	0.47**	0.39**	0.48**	0.45**	0.35**	0.37**	---	- 0.50**
Root : Shoot ratio	0.42	0.42**	0.61**	0.56**	0.37**	0.85**	0.83**	0.47**	---

** Significant at 0.01 level of probability.

Φ Calculated values exceeded 1.

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GROWTH OF PERENNIAL PLANTS ON THE FLOTATION TILLINGS

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Abstract

Exploitation of sulphur resources in Poland with underground ore smelting method caused devastation of arable land. Those soils become very acid with pH 4 and in some cases to pH 2,5.

Our research was focused on reconstruction of soil conditions and flora on waste tip of flotation tilling (resulting from hydraulic purification of sulphur extracted with open pit method in south-east of Poland) used to cover devastated arable land. Waste tip of flotation tiling was 50 ha large with average depth of 150 cm.

In 1995 those waste tip were irrigated with thickened municipal sludge at the amount of 250; 500 and 750 m^3/ha and well mixed to the 25 cm depth. Than tubers of *Heliantus tuberosum* as well as seed of *Festuca arundinacea Scherb.*, were planted. Besides that, on plots of 20 m^2, not irrigated with thickened municipal sludge, following species were planted: *Colamagrastis epigeios (L.) Roth., Artragalus cicer L., Artragalus glycyphyllos L., Coronika varia L., Galega officinalis L., Lupinus polyphyllus L. dl., Spontina pectinata Linle and Sylphium perfoliatum L.*.

Result have shown that among tested species only grasses with strong root system (*Festuca arunilimacea and Calamagrostis epigeios*) grew quite well on flotation tillings. *Heliantus tuberosum* grew well of flotation tilling irrigated with thickened municipal sludge. Organic matter of roots and green parts of perennial plants recovered biological live in substrate speeding up the process of soil formation.

Record of soil pH was among 7,0 to 7,5 and were not dependent on the amount of thickened sludge , however amount of N, P and K in soil were low and increased with the amount of thickened municipal sludge with almost no influence of species used for land reclamation.

Introduction

Exploitation of sulphur resources in Poland caused large devastation of arable land. Those soils became toxically acid with pH below 4 . In some cases, devastated land were oust of flora because of high soil acidity to pH 2,5 or because of covering fertile soil with layer of waste with no biological life.

The present research was focused on reconstruction of soil conditions and flora on waste tip of flotation tilling resulting from hydraulic purification of sulphur extracted with open pit method in south-east of Poland, used to cover devastated arable land.

Material and Methods

Waste tip of flotation tilling was 50 ha large with average depth of 150 cm. In 1995 those waste tip were irrigated with thickened municipal sludge at the amount of 250, 500, and 750 m^3/ha and well mixed to the 25 cm depth. Additionally mineral fertilisers at the rate of 68; 39 and 39 kg/ha of N, P, K, respectively, were applied. Than tubers of *Heliantus tuberosus* (0.25 ha*)* as well as seed of *Festuca arundinacea Scherb.* (ca 1 ha), were planted.

Besides that, on plots of 20 m^2 that were not irrigated with thickened municipal sludge, the following perennial species were planted:

1. *Calamagrostis epigejos (L.) Roth.*,
2. *Astragalus cicer L.*,
3. *Astragalus glicyphyllos L.*,
4. *Coronilla varia L.*,
5. *Galega officinalis L.*,
6. *Lupinus polyphyllus Lindl.*,
7. *Sportina pectinata Link,*
8. *Sylphium perfoliatum L.*,
9. *Reynantia japonica Houtt.*,
10. *Miscanthus sacchariflorus (Maxim) Hack*

Results

Three years of observation have shown that *Heliantus tuberosus* grew well on recultivated soil and its plants have reached 2 m height (Table 1). In the following years plants showed good regrowth, and setting of tubers, giving compact overgrowth. Every year early spring cut and ploughed of *Heliantus* plants enriched ground with organic matter and speed up soil formation process.

Festuca arundinacea in first year of its vegetation have formed canopy of a good compactness and could be twice cut. In following years growth of this grass was quite good, however stronger reaction to summer drought comparing to *Festuca arundinacea* grown on arable soils were observed.

Among wild species plants, the *Calamagrostis epigejos* showed the best growth on flotation tilling, giving tight canopy. *Spontina pectinata* also grew quite well but did not express the vitality typical of this species. Similarly, during three years, *Sylphium perfoliatum* behaved the same way.

Species of legumes were poorly adapted to grow on flotation tilling. The highest stability was observed for *Astragalus cicer*. However, plants of *Galega officinalis* disappeared in first year of vegetation (Tab.2). Plants of *Lupinus polyphyllus* were not able to form tap root but form fibrous-like root system.

Conclusions

- Grasses with strong rooting system *Festuca arundinacea* and *Calamagrostis epigejos* grew quite well on the devastated soils covered with layer of calcium carbonate flotation tilling from sulphur mine.

- *Heliantus tuberosum,* because of water and assimilates deposit in tubers, is growing well on calcium carbonate flotation tilling with thickened municipal sludge.

- Organic matter of roots and green parts of perennial plants, after three years of its incorporation into the ground, recovered its biological life and speeded up the process of soil formation.

Tab1. Plant heigth of chosen perennial species grown on calcium carbonate flotation tilling from sulphur mine.

Years	Perennial species				
	Calamagrostis epigejos (L.) Roth.	Reynantia japonica Houtt.	Spartina pectinata Link.	Helianthus tuberosus L.	Miscanthus sacchariflorus (Maxim.) Hack.
1996	76.6	115.3	-	213.3	-
1997	111.0	127.3	71.0	205.2	77.0
1998	112.0	215.0	105.0	180.0	85.0
1999	110.0	195.0	155.0	155.0	88.0

Tab.2. Survival of plants of chosen species used for recultivation of calcium carbonate flotation tilling.

Years	Perennial species							
	Calamagustis epigejos (L.) Roth.	Astragalus cicer L.	Astragalus glicyphyllos.L .	Coronilla varia L.	Galega officinalis L.	Lupinus polyphyllus Lindl.	Spartina pectinata Link.	Sylphium perfoliatum L.
Spring 1995	-	81	83	78	91	-	-	-
Fall	-	76	69	73	50	-	-	-
Spring 1996	173	71	57	69	0	-	-	-
Fall	173	49	29	39	0	-	-	-
Spring 1997	173	19	12	1	0	256	23	25
Fall	173	19	10	0	0	200	23	25
Spring 1998	173	19	7	0	0	35	23	25
Fall	173	19	6	0	0	30	23	25
Spring 1999	173	19	4	0	0	20	23	25
Fall	173	19	3	0	0	5	23	25

INTRODUCTION OF TROPICAL FORAGE SPECIES IN TEMPERATE PASTURES TO FACE WATER AND TEMPERATURE STRESSES

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Abstract

With the enlargement of total irrigated area in southern Portugal, forage and pasture production might be an interesting hypothesis for the economic exploitation of such facilities. For this purpose, the C_4 grasses can offer a great potential of growth during Spring and Summer. Some species/varieties which belong to this group of plants, were experimented in Portugal, by various research workers, being possible now to joint up the information available. Even though these species have the maximum return, in terms of dry matter production, for a great amount of irrigation supply, we can document yields as high as 13 or 14 t ha^{-1} per year, for water provision such as 3000 m^3 ha^{-1}. They denote a good behaviour under high temperatures, which are very frequent in our conditions, during July and August, while other genera show a great depression in productivity.

These genetic materials are of great interest and we want to identify the main constraints of its use - frost susceptibility, low crude protein and sometimes also low digestibility - in order to know which characteristics must be chosen to define the most defensible lines for genetic improvement. Relatively to species that we have studied, *Paspalum dilatatum* has higher tolerance to low temperatures than *Paspalum urvillei* and both larger than *Setaria sphacelata* var. *sericea* × *S. sphacelata* var. *splendida* (cultivar Splenda) and *Pennisetum clandestinum.* The crude protein content, different from species to species, is generally low but affected by date of cutting. For the mentioned species, in the second and third years of utilisation, the values varied from 55 g kg^{-1} to 100 g kg^{-1}.

In Mediterranean conditions these species can be used for pasture in mixtures with temperate perennial grasses and legumes or as sole crops for fodder production. So it should be very important to continue the study of these species in Portugal building up a germplasm collection and evaluating these genetic resources on the most representative environments of irrigated areas.

Introduction

Conventional agriculture intensification conducted to environmental problems. For this reason, nowadays, agricultural activity has to conciliate food production, to meet the nutritional needs of an increasing world population, with environmental protection and improvement. Considering these targets, and aiming for economic viability, it is essential searching for solutions that can contribute for better utilisation of non-renewable energy sources and water.

Concerning to forage production under irrigation, in Mediterranean conditions, the introduction of tropical forage species in mixtures with temperate perennial grasses and legumes or as sole crops, might contribute to rationalise the utilisation of water in the poorest soils since they grow well under high temperatures prevailing in the summer.

With that purpose, research work has been carried out in Portugal with several perennial species for agronomic evaluation under limited water supply.

Brief description of undertaken research

There is a long list of species that might have interest. Some of them have already been tested, and results for dry matter yield are presented in Table 1.

Table1.Results for dry matter yield of tropical forage species obtained under irrigation (2950 to 3067 $m^3 ha^{-1}$)

Species	Dry matter yield ($kg\ ha^{-1}$ by year)	Reference
Setaria splendida	18640	Rebelo, 1990
Paspalum dilatatum	11189	Lourenço *et al.*, 1998
Pennisetum clandestinum	9324	Lourenço *et al.*, 1998
Pennisetum purpureum	12067	Lourenço *et al.*, 1998
Setaria sphacelata var. *sericea* × var. *splendida* (Splenda)	11230	Lourenço *et al.*, 1998
Setaria splendida	4307	Lourenço *et al.*, 1998
Setaria sphacelata var. *sericea* × var. *splendida*	8529	Nolan *et al.*, 1997
Paspalum dilatatum	7592	Nolan *et al.*, 1997
Paspalum urvillei	8222	Nolan *et al.*, 1997
Cenchrus ciliaris	2337	Nolan *et al.*, 1997

From Nolan *et al.* (1997) work, it can be concluded the following:

- *Pennisetum clandestinum*, *Paspalum dilatatum* and *Setaria sphacelata* var. *sericea* × var. *splendida* were easy to establish, but the same did not happen with *Setaria splendida*
- *Paspalum dilatatum* showed the highest tolerance to low temperatures
- *Setaria sphacelata* var. *sericea* × var. *spendida* seemed to be the most advantageous with respect to dry matter digestibility and crude protein yield.

Other species with possible interest and already evaluated in Portugal were the following:

- *Cenchrus setigerus*
- *Chloris gayana*
- *Chrysopogon fuiurus*
- *Diacantum annulatum*
- *Panicum maximum*
- *Sehima nervosum*
- *Pennisetum purpureum*
- *Paspalum urvillei*.

Constraints to overcome

The main limitation of these species with respect to the utilisation in animal nutrition are the low values of dry matter digestibility that rarely reach the content of 600 g kg^{-1}, and also the low concentration of crude protein that usually varies between 65 and 125 g kg^{-1}. In this way, when considering germplasm selection, a great attention should be given to the quality traits in order to find the species with the highest potential of reaching the best performances in our conditions.

The establishment by seeding was found quite difficult for most of the species already studied. So the easiness of this way of propagation should be taken into account when considering germplasm selection.

Also, winter survival might be a problem for some species.

Research topics to be pursued

The results already available show that there are research topics with interest to follow in order to evaluate and select ecotypes of tropical species to be used under irrigation in Mediterranean conditions.

There is a need to constitute germplasm collections to be studied in Portugal in order to supply information and selected material to use with rentability in irrigated areas with limited water supply. Agronomic studies allowed for the identification of several aspects that should be considered in the screening of ecotypes of different species such as:

- Ease establishment
- Early beginning of growth
- Stability and duration of growth
- Evolution of growth along the year
- Nitrogen and water use efficiency. Rebelo (1990) found that dry matter yield increased significantly up to the application of 250 kg ha^{-1} of nitrogen.
- Other nutrients use efficiency
- Low temperature resistance, important for the species survival during the winter
- High temperature resistance
- Plant height
- Persistence under grazing
- Dry matter digestibility
- Crude protein content.

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YIELD STABILITY OF CHICKPEA IN TERMINAL DROUGHT ENVIRONMENTS

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Abstract

Seed production stability is important in sustainable agriculture, allowing farmers to regularise their income. This is very important in forage plants, like chickpea, for animal food production, because industry buys grain at minimum price. This way it is necessary to produce at low costs in a regular way.

With the objective of selecting stable and productive varieties in different water conditions, eight genotypes of chickpea (with different flowering date) growing on eight different water availability conditions, have been studied, in a three-year period (1997 through 1999). The study was carried out in the Plant Breeding Station at Elvas. Seed production (whose results will be presented), seed number, weight of a seed, harvest index and reproductive development where observed. Using several stability evaluation methods, variability was observed between genotypes, allowing to explore this feature in plant breeding. Not always bigger seed production matching greater water availability was observed. This may indicate low water use efficiency occurrence.

From the eight genotypes tested in eight environments we choose the genotype G6 that better reconciles stability and yield. This genotype is stable and productive when tested with 24 genotypes in two environments. This genotype, with its early flowering and high harvest index, should be the most appropriate for low rainfall conditions. In spite of genotype G7' relative instability, this genotype has shown a better adaptation to high rainfall conditions.

Introduction

Effects of weather and management on yield variation depend in part on the species and cultivar employed. Consequently, plant breeders are concerned about the stability characteristics of cultivars.

Obtaining stable cultivars is very importance for grain legumes of indeterminate growth habit, like chickpea, because they are less domesticated crops, usually cultivated in marginal environments and the cultural techniques aren't always the more adequate, the result is that the incomes are low and unstable for the farmers.

The grain legumes have the physiological ability to prolong their vegetative phase after the onset of flowering, so they have a potentially long cycle and late ripening. In consequence they are subject to water limitations, very frequents at the end of cycle in Mediterranean conditions.

After Becker (1981) there are two concept of stability: biological and agronomic. In the first concept, the genotypes are stable when the behaviour in different environments have a small variation. In the second concept, a genotype is stable when the interaction value is minimal. The plant breeders and farmers are interested in stable and greater yielding varieties. So, the biological concept has a small interest, because the new varieties must respond with a higher production in more favourable conditions.

In this work we will study the stability of seed production of eight genotypes of chickpea, tested in eight environments which differ mainly in their water availability at the end of the cycle. In two of these environments, the stability of each genotype will be tested in a germoplasm collection of twenty-four genotypes.

Materials and Methods

Eight genotypes of chickpea with different flowering dates were tested in a total of eight environments (which were assigned names from E1 through to E8) whose main difference was their water availability during the reproductive phase. The study was carried out in the National Plant Breeding Station (ENMP) at Elvas in a three year period: 1997 (E1, E2 and E3), 1998 (E4, E5 and E6) and 1999 (E7 and E8). Three different water conditions were tested: without stress – irrigated when necessary (environments E1, E4 and E7); with late stress – irrigated until 500 degree-day after flowering of the earliest genotype (E2 and E5); with early stress – natural precipitation conditions (E3, E6 and E8).

Sowing was done at the end of January or during February. These genotypes belong to ENMP's germoplasm collection and are named ChD322, ChK510, ChK2909, ChK2983, ChK3095, ICCV92024, ICCV93219 and Sombrero. They will be referred to as G1, G2, G3, G4, G5, G6, G7 and G8, respectively. In 1999 the genotypes were integrated in a collection of twenty-four genotypes. The experiment was delineated in Split Block Design, with three replications. A fixed effect model was used for the analysis of variance.

The following approaches were used to study genotypes' stability:

i) regression analysis (Eberhart and Russel, 1966) - the mean yield of each environment (Ei) is arrayed on a linear scale along the abscissa and yield of genotype Gi is plotted on the ordinate. The slope (bi, dY/dE) is taken as the stability coefficient. A genotype is unstable if the bi is significantly higher than 1, or S^2di (sum of square deviation) significantly different from zero.

ii) coefficient of variation (CV) of Francis and Kannenberg (1978): CVi=(standard deviation/mean)x100;

iii) ecovalence of Wricke (1962) cited from Cubero et al. (1997): $Wi=\Sigma j(Yij - \bar{Y}i. - \bar{Y}.j + \bar{Y}..)^2$. Ecovalence measures the contribution of a genotype i for an interaction G*E. The genotype with the lowest Wi is the most stable. Kangs and Miller (1984) proposed the ecovalence's mean squares method (QM-Wi) assuming this function behaves like an F of Snedecor. The instability of a genotype is significant if $F_{calculated}>F_{table}$;

iv) stability variance ($\alpha^2 i$) of Shukla (1972):

$$\alpha^2_i=((g(g-1))\Sigma j(Yij- \bar{Y}i.- \bar{Y}.j+ \bar{Y}i..)^2-\Sigma i\Sigma j(Yij- \bar{Y}i.- \bar{Y}.j+ \bar{Y}i..)^2)/((g-1)(g-2)(a-1)),$$

where: Yij = average value for genotype i in environment j; g = number of genotypes; a = number of environments. $F = \sigma^2_i/\sigma^2_o$ is calculated (σ^2_o being the variance of the error within the environments, with (a-1) and ag(r-1) degrees of freedom) and checked for its significance. If significant, the genotype is unstable

Results and Discussion

The analysis of variance for yield (table 1) indicates that the effects of main factors (Genotype and Environment) and their interaction are both significant, but the environment is the major contributor to the total variation observed (65,5%), indicating high variation in the environments studied.

According to Bidinger et al. (1996), the variation due to the genotype represents, in multilocal trials, a small percentage of total variation in the yield. The information from the genotype (G) effect is consequently very limited. In this case we must exploit the information from the environment (E) and the interaction (G*E). Our results show that the contribution of the genotype sum of squares (SS) for the total SS is only 5.8% and the contribution of interaction is 13.3%. Once this interaction has been detected, its study was carried out using the above mentioned stability analysis. Its values are presented in table 2.

Table 1 - Analysis of variance for seed production of eight genotypes in eight environments

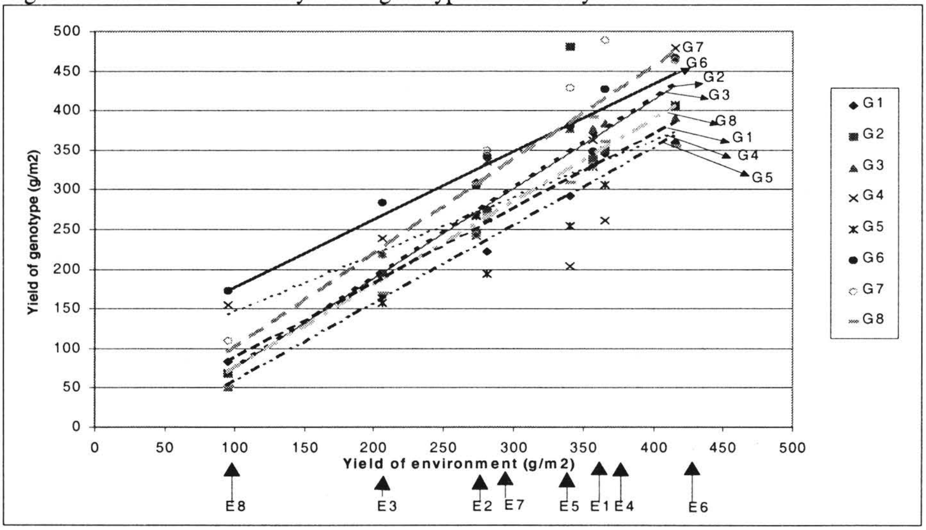
source of variation	df	sum of squares (SS)	F	Contribution SS
Evironment (E)	7	1773818	40.71 ***	65.5%
Rep/Evironment	16	99605	2.18**	3.6%
Genotype (G)	7	156524	7.82***	5.8%
E*G	49	359495	2.57***	13.3%
Error	112	320321		11.8%
Total	191	2709763		

** P<0.01; *** P<0.001

Figure 1 represents one of the stability analysis methods (Eberhart & Russel, 1966), relating each genotype's average seed production with the average seed production on each environment. In this way we can have a global perception of the average seed production and of the genotypes' behaviour for each environment.

Extreme average seed production values were attained for E8 (Spring of 1999, with low water levels retained in the soil and no irrigation) and E6 (Spring of 1998, with high water levels retained in the soil and rainfall during the reproductive phase). In E3 (the second least productive environment) rainfall was prevented, with an anti-rain protection, after flowering, although there was a high quantity of water retained in the soil. In all other environments there was irrigation after the first genotype had flowered.

Figure 1 – Relation between yield of genotype and mean yield of environment



Genotype G6 is the most productive in the least productive environments (E8 and E3), revealing its adaptation to low water availability. Even in the most productive environments this genotype is still one of the most productive with only genotype G7 being more productive. Genotype G6's bi isn't significantly different from 1 and its S^2di isn't significantly different from 0, which means it can be considered stable.

Figure 2a was built upon Francis & Kannenberg's (1978) coefficient of variation (CV) method. Genotypes G8 and G5 are on the south-eastern quadrant with low production levels and low stability. Genotypes G4 and G1 are on the south-western quadrant, meaning they are stable but nonetheless low productive. Genotype G7, on the north-eastern quadrant, is very productive but is also very unstable. On the north-western quadrant, genotype G6 is the most interesting one with its high production and stability levels. Genotype G6's behaviour was ascertained when tested in environments E7 and E8 (figure 2b), along with 23 other genotypes. Some more stable genotypes were identified, although none so productive.

Figure 2a – Yield and coefficient of variability (CV) for eight genotypes in eight environments

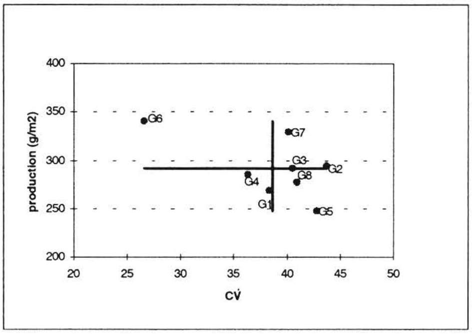
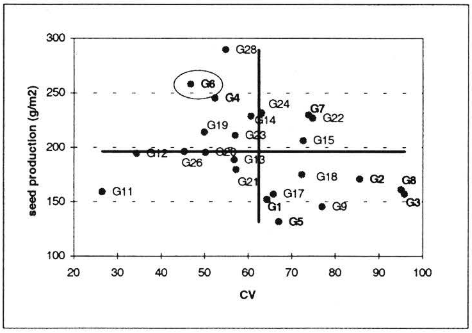


Figure 2b – Yield and coefficient of variability (CV) for twenty four genotypes in two environments



As shown in table 2, similar results are obtained when joint regression, ecovalence and its average square and Shukla's stability variance methods are applied: genotypes G3 and G6 are still the most stable. Between these two, genotype G6 should be the elected since not only its average production is higher but also it is the most productive genotype in the worst, least productive, environment (E8). This is a Desi type genotype with early flowering and high harvest index.

Table 2 – Mean yield from each genotype and statistic parameters of yield stability

Genotype	Yield (g/m2) (a)	bi	S^2di	CV	Wi	MS-Wi	α^2_i–Shukla variance
ChD 322 (G1)	268.8 bc	0.95	1385.9	38.4	8532	1422	1217.5
ChK 510 (G2)	294.4 b	1.14	*3406.4***	43.7	21817	*3636***	3748.1**
ChK 2909 (G3)	291.2 b	1.12	**666.4**	40.5	**5147**	**858**	**572.8**
ChK2983 (G4)	284.7 b	0.72	*6055.6****	36.3	*42064*	*7010****	*7604.6****
ChK 3095 (G5)	247.8 c	0.98	1208.3	42.8	7268	1211	976.8
ICCV 92024 (G6)	340.8 a	0.85	**662.6**	**26.6**	**5625**	**938**	**663.9**
ICCV 93219 (G7)	328.9 a	1.19	*3043.5***	40.2	20792	3465**	3552.8**
Sombrero (G8)	276.9 bc	1.05	1399.3	41.0	8588	1431	1228.2

(a) means with the same letter aren't significantly different
* values significant (Fcalc. > Ftab.), means that the genotype is unstable; at bold are the values more favourable

Conclusions

Comparison of genotypes in different environments cannot be accomplished only through the analysis of the genotype's effect, because the effect of the environment and of the interaction G*A are relatively more important. In such cases, stability analysis is more relevant.

From our analysis we can conclude genotypes G3 and G6 have the most stable seed productions, with genotype G6 being generally more productive, specially in environment E8 with high water stress during reproductive phase. Genotype G6's stability and high production levels were confirmed in a 24 genotype group, tested in two different environments. This genotype, with its early flowering and high harvest index, should be the most appropriate for low rainfall conditions.

In spite of genotype G7's relative instability, this genotype has shown a better adaptation to high rainfall conditions.

On future analysis we should study the characteristics of each genotype and their influence on seed production, and thus determine selection criteria for further more stable genotype's research.

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ALUMINUM TOLERANCE OF RYE POPULATIONS WITH FORAGE APTITUDE

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Abstract

Soil acidity is responsible for a high content of free Al^{3+} ions in the soil which affect the plants development. One way to overcome this abiotic factor is to select plants more tolerant to aluminum toxicity.

Rye is an important cereal in North Interior Portugal where the soils are acid. This cereal is exploited either for grain production or for green forage.

Two rye populations (Vila Pouca and Montalegre) were evaluated for aluminum tolerance in nutritive solution at 10, 20, 30 and 60ppm of aluminum concentration. The dry matter yield at booting and milk/dough stages was also evaluated. Montalegre population registered, on average, the highest root growth and dry matter yield.

These two populations had a similar growth but are more precocious than the cultivars from Central Europe, especially during the vegetative period. In general, the Portuguese rye populations revealed to be more tolerant to aluminum than the European cultivars. The higher growth of the regional rye populations during winter allows it profit as a forage crop when the green forage availability is very low.

Introduction

Rye is an important crop in North Interior Portugal, in particular in the Trás-os-Montes region. This region provides more than 60% of the total national production. Rye is grown for grain production or for animal feed. In Trás-os-Montes, when rye is explored for forage crop it can be profitable when managed with a single cut, usually in May, or with multiple cuts. In this specific case it is sown early, during September.

Because of the semi-erect habit of rye populations from Trás-os-Montes, in comparison with the prostate habit of the cultivars from central Europe, and their good development during October and November it is possible to do a cut, or to use them as pasture in November/December. The later regrowths are usually cut in May allowing for the introduction of a second crop, maize or potatoes.

The soils of the Trás-os-Montes region are acid (pH 5.0 and 5.5). Soil acidity is responsible for the high content of free Al^{3+} ions in the soil, which affect the root development of plants. One way to overcome this abiotic factor is to select plants that are more tolerant to aluminum toxicity.

The aim of this study was to evaluate the aluminum tolerance of two rye populations from the Trás-os-Montes region and their forage aptitude.

Material and Methods

Two rye populations (Vila Pouca and Montalegre) from farmers of Trás-os-Montes and the cultivar Dankowskie Zlote (a tolerant tester from Poland) were studied at 10, 20, 30 and 60 ppm aluminum concentrations. The kernels were sterilised with 0.1% of Hg_2Cl_2 aqueous solution for 10 min, rinsed with tap water and germinated on filter paper in petri dishes. After germination, when the roots had reached about 5 mm in length they were placed in the following nutrient solution: 0.4 mM $CaCl_2$, 0.65 mM KNO_3, 0.25 mM $MgCl_2.6HO$, 0.01 mM

$(NH_4)_2 SO_4$ and 0.04 mM NH_4NO_3. Forty eight hours later, the seedlings were transferred to another nutrient solution containing Al in the form of $AlCl_3.6H_2O$ at 10, 20, 30 and 60 ppm aluminum concentrations. After this treatment (48 hours) the roots were stained with Eriochrome cyanine R for 10 min and washed with tap water. All the solutions were aerated continuously and adjusted to pH 4.0.

After treatment the plantlets were transferred to a new nutrient solution without aluminum, for 48 hours, in order to observe the root regrowth which was measured in three roots per plant. The data presented are the average of three replications (about 150 plants per cultivar/population).

Dry matter yield of the two rye populations was evaluated at the booting and at the milk/dough stages for three years at Vila Real. The trials were sown at the end of October and the seeding rate was 350 viable seeds/m^2.

Forage samples were collected and dried in a forced air dryer at 65°C and yields calculated on an oven-dry basis. Sub-samples of forage were ground and used for *in vitro* organic matter digestibility (IVOMD) and crude protein content (CP), which were evaluated according to Marten and Barnes (1980) and AOAC (1975), respectively.

Results

Root regrowth of the rye populations at different aluminum concentrations is presented in Table 1.

Table 1 – Mean of root regrowth (mm) of rye populations at 10, 20, 30 and 60 ppm aluminum concentrations.

Populations/ Cultivar	10 ppm	20 ppm	30 ppm	60 ppm	Mean
Vila Pouca	1.685	1.063	0.535	0.073	0.839
Montalegre	1.620	1.163	0.488	0.120	0.848
Dank. Zlote (tolerant tester)	1.520	0.691	0.270	0.020	0.625
Mean	1.608	1.152	0.431	0.071	

When the aluminum concentration was increased the average values of root regrowth decreased. The values obtained on the average of the cultivar/populations were 1.61, 1.15, 0.43 and 0.07 at 10, 20, 30 and 60 ppm, respectively.

Both populations registered higher values of root regrowth at all aluminum concentrations in comparison with the tester Dankowskie Zlote, considered to be a tolerant cultivar. Vila Pouca and Montalegre populations were characterised by similar behaviour at all concentrations tested.

With regard to dry matter yield, the Montalegre rye population produced the highest dry matter yield at both stages (1741 Kg/ha at booting stage and 7471 Kg/ha at milk/dough stage) (Table 2).

Although the two rye populations showed a similar forage quality (IVOMD), the Montalegre population had, at both stages, a higher *in vitro* organic dry matter and crude protein content (CP) than the Vila Pouca rye population (Table 3). At the booting stage, the *in vitro* organic dry matter of the Montalegre rye population was 74.88% and crude protein content was 18.0%. The values for these parameters at the milk/dough stage decreased to 48.88% and 5.98% for *in vitro* organic dry matter and crude protein content, respectively.

Table 2 – Dry matter yield (kg/ha) of two rye populations at the booting and at the milk/dough stages (average of three years).

Population	Booting Stage	Milk/dough Stage
Vila Pouca	1338	5350
Montalegre	1741	7471

The good response of the two rye populations shows that they are an important source of genes for aluminum tolerance and that it is important to collect and preserve these and other threatened rye populations. These populations and others from the Trás-os-Montes region must be included in future breeding programmes in order to obtain new cultivars.

Table 3 – *In vitro* organic matter digestibility (IVOMD) (%) and crude protein content (CP) (%) at the booting and at the milk/dough stages (average of three years).

Population	Booting stage		Milk/dough stage	
	IVOMD (%)	CP (%)	IVOMD (%)	CP (%)
Vila Pouca	72.24	16.51	47.30	5.69
Montalegre	74.88	18.01	47.88	5.98

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Agronomy and Crop Physiology

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MAN-MADE STRESSES IN THE GRAZING RESOURCES OF THE MEDITERRANEAN REGION

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Abstract

The diversified grazing resources of the Mediterranean Region are the result of a multimilenary influence of humans and their grazing animals, primarily on the Mediterranean forest, which through fire, cutting, grazing and cultivation has given place to the various forms of natural and cultivated grazed feed resources existing today. This influence of man with their grazing animals can be either positive or negative. In a meeting subordinated to the theme "Breeding for Stress Tolerance in Fodder Crops and Amenity Grasses" dealing mainly with biotic and abiotic stresses, the author calls the attention to other type of stresses, those resulting from errors committed by humans when exercising their planning and managerial capacities to exploit grazing resources, either natural or sown.

After briefly characterising the Mediterranean Region and its grazing resources, the most common man-made stresses affecting their sustainable productivity are identified and some remedial solutions presented. Among them, those derived from: 1) an inadequate choice of plant material to establish sown pastures or plant fodder shrubs; 2) failing to satisfy plant nutrient requirements or to deal with nutrient unbalances in sown pastures; 3) grazing mismanagement, pointing out that in Mediterranean conditions, undergrazing may lead to a so severe degradation as overgrazing, and that extremely destructive effects of overgrazing only occur when the grazing animals receive extra feed energy from outside the grazed area; the anarchic use of grazing resources in collective grazing lands, the subsidies to feed grains, and the mechanization of the transport of the grazing animals, together with feed grains and water resources; the sedentarization and excessive pressure of population on collective grazed land in certain areas, and the use of plough as a form of land appropriation; the abandon of land and grazing activities by the rural populations in some areas of private property and the return to shrub/forest land with increasing risk of fire .

1.Introduction

In this 23rd EUCARPIA Fodder Crops and Amenity Grasses Section Meeting, subordinated to the general theme "Breeding for Stress Tolerance in Fodder Crops and Amenity Grasses", plant breeders are reporting on the results of their efforts to minimise the negative effects of various natural biotic (mostly diseases and pests) and abiotic (cold, heat, shade, drought, waterlogging, salinity, etc.) stresses, which are responsible for losses of productivity in fodder crops or for defective performance in amenity grasses.

When the author (a pasture agronomist) received an invitation from the Organising Committee to give a lecture in the section "Agronomy and Crop Physiology", he decided to identify and discuss other type of stresses, those resulting from errors committed by humans when exercising their planning and managerial capacities to exploit grazing resources, either natural or sown. Indeed, any sown pasture, even if based on top cultivars resistant to biotic and abiotic stresses, may have its potential productivity significantly reduced as a result of inadequate planning and management. A similar result may occur in natural grasslands, which can be easily degraded through mismanagement.

To analyse problems of degradation of grazing resources as a consequence of man-made stresses, the Mediterranean Region is particularly suitable for the following reasons:

-a) The Region was the first to have grazing animals domesticated (Blondel and Aronson, 1999), being known that all the existing grazing resources are the result of a multimilenary evolution caused by the action of man on the primitive forests, through fire, cutting, uprooting, grazing and, in the case of arable land, also cultivation, with eventual introduction of plants through sowing and planting.

-b) The grazing resources of the Region are of paramount economic and social importance. They are produced in a large percentage of the land surface, and in many areas are the main (not to say the only) source of income for the rural populations, who, through the grazing animals, convert them in meat, milk, drought power, or fibber, and in certain areas also in leisure activities, such as hunting.

-c) In the Region we can find a mosaic of edapho-climatic conditions, which together with the existence of different management systems (sedentary, transhumant and nomadic grazing; set-stocking, rotational, and deferred/intermittent grazing), the diversity of grazing animals (sheep, goats, cattle, pigs, horses, donkeys, camels and wild herbivorous), and other various forms of direct intervention of man (cultivation, fertilising, seeding/planting, irrigation, drainage, etc.) have created not only a wide range of types of grazing resources, but also a great variability in their productivity and biological diversity.

-d) There are in the Region two types of legal property for the grazing resources, with a profound influence on their use and productivity: private grazing lands, which predominate in most of the European countries; collective or public grazing lands, predominating in countries of North Africa and West Asia, where, apart certain areas of private grazing land and a few cases of organised common grazing, it rules the principle "first to arrive, first to be served".

It is within this context, involving a great variability of physical and human influences that the Mediterranean grazing resources and the most common man-made stresses affecting the sustainability of their productivity are going to be briefly analysed.

2.The Mediterranean Region and its Grazing Resources

2.1.The Mediterranean Region extends over a vast area in 3 continents, including totally or partially several countries in South Europe (Portugal, Spain, France, Italy, ex-Yugoslavia, Albania and Greece), West Asia (Turkey, Cyprus, Syria, Lebanon, Israel, Jordan, Iraq, and Iran) and North Africa (Morocco, Algeria, Tunisia, Libya and Egypt).

2.2.The climate of the Region is characterised by dry and hot summers, and mild/cool wet winters, although an accentuated variation occurs between different places according to their respective geographic position, thus originating a range of micro-climates, with different rainfall and temperature patterns.

Average annual rainfall may range, among different localities, from as little as 100 mm to more than 2000 mm, but even for the same locality, rainfall and its distribution may suffer great variations from year to year. For instance, a zone receiving 600 mm of average annual rainfall may have extreme dry years of less than 300 mm, and very wet years of more than 900 mm. Even the distribution of the rain during the wet season is very irregular and impossible to predict. Therefore, irregularity and unpredictability of rainfall during the wet season are other important characteristics of Mediterranean climate.

Mean annual temperature also varies from place to place (but not so much as rain for the same place), within a range going from 2-3°C. (in high mountain areas) to more than 20°C. (in some coastal areas of North Africa).

2.3.The soils are also greatly variable, and in certain areas they suffer frequent changes, being not rare to find 2 or 3 soil types of different geological origin, in a transection of just 1 km. There are vast areas of marginal lands (thin, steep, stony, sandy, etc., sometimes with acidity or alkalinity in excess, or with drainage or salinity problems), alternating with zones of deep soils. However, most of the Mediterranean soils are poor in organic matter, and many are nutrient deficient, particularly in phosphorous, although deficiencies in potassium and in other macro or micronutrients may occur.

2.4. *The grazing resources* are very diversified and found almost everywhere, in valleys, plains, slopes, mountains, etc., either in the form of natural or sown/planted vegetation. Most of these grazing resources can be used all the year around, in the green stage during the wet season, and as dry pasture during the summer, but in certain areas, the existence of fodder trees/shrubs, or irrigated pastures, may provide green feed also during the dry season.

The great majority are produced in semiarid and sub-humid grasslands, as well as in arid steppic grasslands dotted by shrubs, or even in evergreen shrublands and open forests, but a significant amount is also produced in arable land, under the form of stubbles, fallows, and annual or multiannual sown pastures. Some grain crops, such as cereals, may also provide green grazing, particularly during the autumn/early winter period, and straw as a feed reserve.

Although the large majority of the areas producing grazing resources are still occupied by natural pastures, the increasing demand of animal products and the higher pressure on agricultural land, are determining a constant increase of improved pastures through sowing and fertilising, and in some cases also through irrigation. These improved pastures are more frequent in countries where the grazing resources are private, particularly in the semi-arid, sub-humid and humid areas of Portugal, Spain, France, and Italy, but many other countries in the Region have progressed in this matter. Even in the more arid zones of the South and East, where collective grazing lands are dominant, national and international development programmes have also stimulated the plantation of fodder shrubs or trees, to increase the availability of feed resources, particularly during certain periods of scarce herbaceous pasture.

2.4.1. *Range of productivity.* Due to the wide range of natural and man-made conditions, the yield and composition of the Mediterranean grazing resources exhibit a huge variation. As an indication, grazing lands may produce from as little as 20-50 feed units (FU)/ha in very degraded collective grazing lands of the arid zone, to as much as 1500-5000 FU/ha in well managed sown rainfed pastures in semi-arid, sub-humid or humid zones, and up to 5000-15000 FU/ha in irrigated pastures.

2.4.2. *Biodiversity and its role.* The wealthy in plant species of a pasture is generally related to its productivity. Indeed, a high degree of plant biodiversity functions as a stabilizer factor against the great variability of the edapho-climatic conditions prevailing in the region, and may also attenuate the damage caused by grazing mismanagement. If not degraded, Mediterranean pastures are very rich in species, being not rare to find 50-60 or more species in a good natural pasture. A drastic reduction of this number may well be the result of a severe degradation under the action of man. In sown pastures, the use of rational mixtures, highly diversified in species and cultivars, also favours productivity and stability.

2.4.3. *Plant composition.* Apart from various legumes (*Fabaceae*) and grasses (*Poaceae*) which are present in most environments, many other herbaceous species of different families (*Asteraceae, Cruciferae, Plantaginaceae, Umbeliferae, Borraginaceae, Liliaceae, Ranunculaceae, Geraniaceae,* etc.,etc.), and also various trees and shrubs (*Fagaceae, Coniferae, Chenopodiaceae, Cistaceae, Fabaceae, Rosaceae,* etc.,etc.) may be found.

There are different plant associations according to edapho-climatic and management conditions, and each plant association may suffer drastic changes as a result of management. In certain areas, associations of herbaceous species, shrubs and trees may occur, all contributing with diverse feed resources to the grazing animals, as in the case of the "montado/dehesa", an interesting Mediterranean agro-forestry system existing in the Southwest of the Iberian Peninsula. Apart from the herbaceous pasture and eventual cereal stubbles, the "montado" provides also leaves of shrubs, and particularly fruits (acorns) of trees (*Quercus rotundifolia, Q. suber*) which mature and fall down during the most critical period of herbage feed availability (autumn/early winter).

A few naturally occurring plants (or part of plants) may be poisonous for the grazing animals, but often these plants possess mechanisms of repulse in order to prevent their ingestion by the animals. Other plants have spines and thorns, which function as a defence to overgrazing. Some others are refused in the green stage, but animals may safely graze them in summer after drying off.

2.4.4. The role of legumes and grasses. Herbaceous legume species have a special place in the Mediterranean grazing resources (Crespo, 1987). They enjoy the excellent light and temperature conditions of the Mediterranean climate, and are very important components in natural and sown pastures, as they act as a motor to raise pasture productivity and building soil fertility, through their ability to fix symbiotic nitrogen (up to 200 kg N/ha/year in legume rich rainfed pastures, and up to 600 kg in legume based irrigated pastures). However, grasses play also an important role in the productivity and persistence of pastures, as they contribute to balance the energy/protein ratio and, above all, remove the eventual excess of nitrogen fixed by the legume/Rhizobium symbiosis.

2.4.5. Legume and grass herbage species. Practically, all the grass and legume species providing an important contribution to Mediterranean herbaceous pastures, have originated in the Region, and a good number of them have been domesticated. Among these, we find annuals (little or no hard seed content), self-reseeding annuals (medium to high content of hard seeds), and perennials (with physiological summer dormancy or very deep root systems), many of them with specific and varietal adaptation to different edapho-climatic conditions.

The domestication of annual legumes (*Trifolium alexandrinum, T. incarnatum, T. resupinatum ssp. suaveolens, T. squarrosum, Ornithopus sativus, Vicia spp., Lathyrus spp., Pisum spp.*.etc.) as well as some perennials (*Medicago sativa, Onobrychis viciifolia, Hedysarum coronarium, Trifolium repens* and *T. pratense*) and a few annual grasses (*Avena sativa, Hordeum vulgare, Secale cereale, Lolium multiflorum*), since long used in the Region as fodder/pasture crops (for cutting or grazing), was mostly a result of local breeding and selecting efforts. However, for the self reseeding annual grasses (*Lolium rigidum*) and legumes (*Trifolium subterraneum sensu latu, T. michelianum, T. resupinatum ssp. resupinatum, T. vesiculosum, T, cherleri, T, hirtum, T. glanduliferum, Medicago polymorpha, M. sphaerocarpos, M. murex, M. truncatula, M, scutellatta, M. rugosa, M.littoralis, M. rotata, M. tornata, Ornithopus compressus, O. pinatus, Biserrula pelecinus*, etc.), and for a few summer dormant types of perennial grasses (*Dactylys glomerata, Lolium perenne, Festuca arundinacea,* and *Phalaris aquatica*) and deep rooted legumes (*Trifolium fragiferum*) the process of domestication is more recent, being mainly the result of the efforts of Australian scientists, who, during this last century, have collected, evaluated and developed a range of species and cultivars, from plant material originated in the Region. The example of Australia has been followed in many countries in the Region during the last 30-40 years and a few more cultivars have been released by national or international institutes, although the availability of commercial seed is so far entirely dependent of the Australian seed industry.

The breeding and selection activities involving pasture legumes have been accompanied by the selection of specific-effective strains of *Rhizobium* spp. which are used in the production of inoculants at a commercial scale, in order to enhance symbiotic N fixation.

Today, there are seeds available of a very wide range of herbaceous legume and grass species and cultivars, differing in the length of the vegetative/reproductive cycles and in adaptation to edapho-climatic conditions, that can be used in the composition of adequate diversified mixtures, which if properly established, fertilised and managed can lead to persistent, low cost and productive pastures in practically all the Mediterranean environments, with the exception of zones where annual rainfall is less than 200-250 mm, where seeds of adequate herbaceous plant material are still missing.

2.4.6. Trees and shrubs. For the arid zones, a few exotic shrub/tree species (*Acacia cyanophylla, A. salicina, Atriplex nummularia, A. canescens, Opuntia ficus-indica* var. *inerme,* etc.), have been extensively planted through high cost operations carried out in countries of North Africa and West Asia. Other species of local origin are now calling the attention of scientists, due to their better adaptation and therefore longer persistence. Among these are the local trees/shrubs *Acacia tortilis, Atriplex leucoclada, A. halimus, Rhus tripartitum, Periploca laevigata, Caligonum comosum, Lysium arabicum* as well as the small shrubs *Salsola vermiculata, Artemisia herba-alba, Rhantherium suaveolens*, etc..

For the semi-arid and sub-humid zones, there are valuable indigenous trees and shrubs (e.g. *Quercus spp., Ceratonia siliqua, Fraxinus angustifolius, Arbutus spp.* etc.) naturally available in many ecosystems. However, for the zones where these components have been eliminated, other local or introduced fodder shrub/tree species are also receiving attention, such as *Medicago arborea, Chamaecytisus proliferus, Colutea arborescens, Morus albus, Gleditchia triacanthos, Robinia pseudoacacia*, etc..

3. Man-Made Stresses on Grazing Resources

By man-made stresses on grazing resources we mean any direct or indirect impact derive from the decision capacity and action of man, leading to a loss of productivity or/and persistence. The following cases are the most common and will be briefly presented:

3.1. Stresses derived from a wrong choice of plant material in sowings or plantations. Apart from assuring a timely and adequate soil preparation and sowing/planting operation, the establishment of pastures or fodder shrub/trees must involve plants well adapted to the prevailing edapho-climatic conditions. In the case of permanent sown pastures or shrub/tree plantations the plants have to be able to reproduce and persist under the existing conditions. Otherwise their persistence and productivity will be ephemerous. This has been the case of extensive plantations of *Acacia cyanophylla* or *Atriplex nummularia* in certain excessively arid or frosty areas of North Africa and West Asia, which, apart from being costly in their establishment, had a relative short life, due to their inadaptation to climate. A similar situation may occur with sown permanent pastures, and particularly in those based on self-reseeding annuals, when the species and cultivars used are unadapted to soil or climate conditions, or when they do not produce a sufficient amount of hard seeds to assure a good persistence, or even when the seed mixture used does not contain enough biodiversity to sustain productivity under eventual edapho-climatic variations, or under other abiotic or biotic stresses. Therefore, a good knowledge of the characteristics of the species and cultivars available is required to avoid this type of stress. Particularly, in relation to edaphic adaptation, it should be kept in mind that is much wiser to use plants adapted to soil conditions than to adapt the soil conditions to the plant requirements. However, sometimes the use of pH soil amendments or the improvement of drainage conditions may be required to allow maximum economic returns from certain species, particularly legumes.

3.2.Stresses derived from insufficient plant diversity in sown pastures. The climatic and edaphic variations mentioned before, require the use of complex seed mixtures, which shall adopt the concept of "maximum rational diversity of plant species and cultivars", i.e., an association of all available plants well adapted to the average soil and climate conditions, which includes those able to survive under stresses induced by eventual niches of soil (drainage, depth, pH, etc.), or by inter annual rainfall variations. Therefore, an appropriate seed mixture should include not only plants varying in vegetative/reproductive cycles, but also plants adapted to eventual soil variations which may occur in the area If a seed mixture to establish a permanent or temporary pasture does not contain enough plant biodiversity, there will be a tendency for a progressive loss of productivity and persistence.

3.3. Stresses derived from nutrient deficiencies or unbalance. To establish and/or maintain a productive and persistent pasture, the plant components must find sufficient and balanced nutrients to fill up their requirements. It is worth noting that in a natural pasture the existing plants are generally adapted to the level of nutrients and other soil conditions, and therefore a high response to applied nutrients should not be expected. However, when dealing with sown pastures, based on selected cultivars with higher nutrient requirements, attention shall be given to the satisfaction of those requirements, both at the establishment and maintenance phases. When dealing with legume based pastures, the first step to assure satisfactory nitrogen nutrition should consist in inoculating the seeds with specific Rhizobium strains, in order to achieve an effective symbiotic nitrogen fixation. A second step is to assure a convenient level of phosphorous or other missing nutrients, through adequate fertilisation. Unfortunately, and in spite of a severe phosphorous deficiency in many Mediterranean soils, some graziers fail to regularly topdress their high phosphate demanding sown legume/grass pastures, and therefore they risk seeing them suffering a progressive degradation in productivity to the level of a natural pasture.

Another type of stress related with unbalanced nutrients, may be found in legume dominant pastures, where excessive amounts of symbiotic nitrogen accumulate in the soil, due to insufficient grasses to use it, thus giving place to an invasion of unpalatable nitrophilous plants (mainly thistles), able to ruin the pasture and eventually having negative impacts on soil acidification and on nitrate/nitrite environmental pollution. The introduction of well adapted grasses corrects this situation.

Other similar situation may arrive in sheep/goat camping sites inside the paddocks, where the excessive accumulation of feces may cause nutrient unbalance, able to eliminate valuable legume species, even to kill trees, and leading to the invasion of other non palatable species (e.g.*Urtica spp., Malva spp., Erodium spp.*, thistles, etc.). The removal and spreading out of this manure, at the end of summer, solves this problem.

Another stress of the same nature is induced by excessive applications of fertiliser N in grass/legume pastures (Crespo, 1983), leading to the reduction or total elimination of legumes, and therefore to a higher cost of production and a loss of quality of the pasture. Indeed, in Mediterranean legume rich pastures, the regular use of fertiliser N is not required to achieve maximum economic returns, and only in exceptional circumstances, such as late autumn sowings (when the temperature is already too low for adequate Rhizobium nodulation) or in winter dominant grass pastures, it is recommended to fertilise with moderate quantities of nitrogen.

3.4.Stresses caused by grazing mismanagement.

Grazing mismanagement, particularly overgrazing, is accused of being the most important factor of degradation of Mediterranean grazed feed resources, responsible for the severe desertification of large areas in North Africa and West Asia. However this concept requires some clarifications. Although opportunistic spring and summer overgrazing of self reseeding sown pastures may negatively affect the seed bank, it should be mentioned that most of the vegetation involved in Mediterranean pastures is particularly well adapted to grazing, being very resilient even after a long period of intensive use, providing the animals are leaving exclusively on that vegetation during the entire period. Indeed, in a situation of overstocking, the grazing animals are much more vulnerable to death than the plants they graze, and severe consequences of overgrazing are evident only when man brings from outside other forms of energy-feed to keep the grazing animals alive. Therefore, the main factor of desertification is not the grazing animal, but the man himself, who through wrong policies (collectivisation of previous tribal land, sedentarization of nomads or transumants, and particularly feed grain subsidies) creates the conditions for an anarchic destruction of valuable grazing resources, many of which have survived in a reasonable condition until the second half of the twenty century. Indeed, the great period of ruin started some 40-50 years ago, when the policy makers of North Africa and West Asia countries accepted to promote the use of feed grains, through subsidies, thinking that this was the best way to increase animal production and simultaneously protect the grazing resources of the steppe. If the first objective was fully achieved (there has been a 3 to 5 fold increase in the number of animals during that period), the second failed completely and produced catastrophic results, as the rise of animal numbers kept alive and productive on the steppe has been accompanied by a continuous and severe degradation of the grazing resources, due to overgrazing. An example taken from Algeria, where sheep population increased from 3.8 to 16.1 million (Aidoud and Nedjraoui, 1992, cit. by Blondel and Aronson, 1999), refers to a steppe of alfa (*Stipa tenacissima*) which, just in a period of 13 years (1976 to1989), suffered a considerable degradation, represented by a change from 16 to 83% of bare ground, from 2100 to 750 kg dry matter/ha, and from 130 to 60 FU/ha/year, with a tremendous loss in biodiversity, particularly in the perennial species. More recently, this tragic problem has been aggravated by the mechanised transport of the animals, feed grains and drinking water, which introduced a great mobility to the livestock in the search for the rare but essential fibrous element, provided at no cost by the steppic vegetation. In many of these areas the fibrous vegetation is already so scarce that the price of 1 kg of straw (0.3 FU), coming from arable land, may reach 2-3 times the cost of 1 kg of barley grain (1 FU)! After these last 40-50 years of ruin, some policy makers have decided to restrict the subsidies to feed grains but the results seem not to be very encouraging, as pastoralists have adopted other strategies to keep their animals alive, such as feeding subsidised bread to their flocks (as it was observed by the author in 1993 in Iran) or, much worse, appropriating collective land through ploughing (a common form of land appropriation in these countries) to cultivate barley in areas in which the average rainfall does not allow an economic yield of grain. To justify this tremendously destructive practice pastoralists argue that "if the barley does not produce any grain, it will produce at least some green feed or straw"! The result of such policies and attitudes is no doubt the continuous advance of the desert, as it can be already seen in many areas of these countries. If some plants are still found in abundance on those desertified areas, certainly they are unpalatable to the grazing animals!

A different type of man-made stress associated to mismanagement is found in southern European countries, where private property is the rule. Here, the stress is induced through undergrazing and abandonment of grazing lands, generally associated to the rural exodus. However this type of stress can be as destructive of the grazing resources as overgrazing. It affects particularly the herbaceous vegetation, particularly the annual species, which if not properly grazed do not regenerate well, giving way to the invasion of woody shrubs and trees, therefore returning to matorrals and forests, highly susceptible to fire. In some regions, like in certain areas bordering the French "Côte d`Azur", where the abandon of grazing areas has been more accentuated, policy makers are now aware of the important role of grazing animals in the prevention of wild fires, contradicting the old accusation of being responsible for the destruction of forests and landscapes. As a consequence, there are programs for the development of sown pastures associated to well managed forest and eventually to fire breaks (Etienne, 1996), with the shepherds being stimulated to keep their grazing animals.

The stress induced through undergrazing may affect also pastures usually well managed. Indeed, in years of prolonged growth in the Spring, and particularly when dealing with sown pastures based on self-reseeding annual legumes and grasses, it may become difficult to graze all the dry pasture before the opening of the Autumn rains. Under these circumstances, the excessive cover of dry pasture until the beginning of the rainy season, not only reduces the rate of hard seed breakdown (the insulation effect of the dry pasture diminishes the daily termic amplitude, responsible for the breakdown of hard seeds) but also prevents the germinated seedlings of coming out through the accumulated pasture. The insufficient removal of the dry pasture is also associated to a deficient trampling and grazing effect, required for a convenient spreading and positing of the seed bank in the soil. This affects pasture regeneration, which if continued year after year will lead to an exhaustion of the seed bank and therefore to a severe degradation of the pasture. To solve this problem two alternative attitudes may be adopted: adjust the stocking rate to the availability of pasture through buying or selling animals; keep a fixed reasonable stocking rate, and in years of higher production cut hay in alternate paddocks to be kept as a feed reserve for the poorer production years.

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GRASS BLACK BOX.
A NEURAL NETWORK APPROACH TO A DAIRY FARM PRODUCTION SYSTEM

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Abstract

The usual conceptualisation of a dairy farmer production systems involves two interrelated production systems: a feed production function which inputs are fertiliser, land, weather, machinery and labour; and a milk production function based cows, feed - bought or produced - machinery and labour. The aim of this paper is to conceptualise the dairy farm production system in only one system using a neural network's mechanism with the whole set of inputs (fertiliser, land, weather, feed, cows, machinery and labour) and with only one output, milk.

Introduction

The usual conceptualisation of a dairy farmer production system involves three interrelated production systems: a feed production function which inputs are fertiliser, land, weather, machinery and labour; a cattle production function based cows, feed - bought or produced - machinery and labour; and a conversion production system that generates milk and beef.

The aim of this paper is to conceptualise the dairy farm production system in only one system using a neural network's mechanism with the whole set of inputs (fertiliser, land, weather, feed, cows, machinery and labour) and with only one output, milk.

In point 2 we review the concept of agricultural production systems. Along point 3 we systematise some models of agricultural systems. Point 4 explains the fundamental ideas related to neural networks. Point 5 calibrates a neural network model to the milk production in Terceira Island.

Agricultural Systems

System analysis reflects and holistic view of the reality through which the complexity of the system stems from the interaction between the various components. According to Caldwell (1994), an agricultural system represents a reasonably stable standard behaviour of farm and non - farm activities. It is managed taking into account available resources and pre defined aims, through a set of well defined cultural practices and restricted either by the internal behaviour of the system or by the biophysical, sociological, cultural, economic and institutional surrounding environment. Being so the agricultural activity involves different knowledge fields, and its whole behaviour is not properly understood whenever each subsystem is analysed detached from the others (Braga et al., 1997).

A cattle system can be represented by three interrelated sub systems (Hodgson, 1990): Vegetal growth (which result from resources such as soil, weather, and crops); animal consumption; and conversion into animal products (beef, milk, wood). Useful indicators are the productivity, stability and sustainability of the system (Pearson e Ison, 1987).

Agricultural Simulation Models

A model is a useful personal interpretation of a perception about the reality (Wilson, 1984, cited by Pearson e Ison,1987). Simulation models are simplifications of the real interactions between the various components of a system which, nevertheless, can give useful views and practical information (Braga et al., 1997) for farmers, extension services and researchers (Pearson e Ison, 1987). They can be systematised according to their uses: management or research models (Jørgensen, 1994); and taking into account the scale of the approach (crop, farm, region, global) Braga et al. (1997).

Neural Networks Models

Neural networks are like different arrangements of simple artificial neurones, which try to reproduce - at least to some extent - the structure and the adaptative learning of human beings (Herbert Simon, 1945).

The usual model neuron (i) (Santin Alves e Pasquareli, 1997) computes, at time t, a logistic function $[g(h_i)]$ - with a parameter (β) - of a weight (${}^tw_{ij}$) sum of its inputs (${}^tV_j$) from other units $[h_i=\Sigma_j{}^tw_{ij}{}^tV_j]$, and outputs the resulting signal of that function (${}^{t+1}V_j$) , for time (t+1).

(1) $${}^{t+1}V_j = g(h_i)$$ where,

(2) $$g(h_i) = 1 / [1 + \exp(-2\ \beta\ h_i)$$ and,

(3) $$h_i=\Sigma_j{}^tw_{ij}{}^tV_j$$

The learning process is done by updating the weights (${}^tw_{ij}$)[1] according to some rule that could induce final outputs to converge with predefined targets (O_j)

The use of these neural networks has spread through many fields (signal prediction, optimisation, image recognition, fitting functions and curves).

The statistical question (Jørgensen, 1994) we are interested to solve is to disguise a suitable mechanism to reproduce the quantity of milk as a function of different variables (fertiliser, feed, cows, land,...).

In a experience conducted in Kentucky (USA) Shearer et al. (2000) concluded that neural networks are a promising tool to predict the spatial variability of agricultural productions.

Model Calibration

Data used on this model came form (21 farms x 3 years) observations obtained from accountancy farm records from Terceira Island. The data used in the calibrations are synthesised in Table 1. The results are presented in Figures 1, 2 and 3.

The minimum error is obtained when we considered the Farm, the Year and the Farmer and active explanatory variables. Other tests and data should be done in order to improve the quality of this first attempt to create a management regional model for Terceira Island dairy production.

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[1] In the case of multi-layer feed-forward networks - used in this subsection - the algoritm that can be applied to update the weights is called back propapation procedure. It is described in (Hertz, Krogh & Palmer, 1991, pp.120) and it includes the following steps:

a) Compute a measure of distance ($\delta^o{}_j$) between the predefined target (O_j) and the actual final output (${}^tV^f{}_j$):

(1) $$\delta^f{}_j = g'(h_i)[O_i-{}^tV^o{}_j]$$

where $[g'(h_i)]$ is the derivative of function g(h) which, in this case, is equal to $[2\beta g(1-g)]$;

b) Compute the measures of distance ($\delta^{m-1}{}_j$) for all layers (m-1) and for every unit, using the formula:

(2) $$\delta^{m-1}{}_j = g'(h^{m-1}{}_i)\Sigma_j{}^tw^m{}_{ij}{}^t\delta^m$$

c) Update the weights (${}^tw^m{}_{ij}$) using the expression:

(3) $${}^{t+1}w^m{}_{ij}= {}^tw^m{}_{ij}+ \eta\delta^m{}_j{}^tV^{m-1}{}_j$$

Table 1: Synthesis of the Data Used in the Model Calibration

	Year	Area	Cattle	Fertil.	Feed	Vets	Fuel	Milk	Farm
		Hectare	Number	1000 PTE	1000 PTE	1000 PTE	1000 PTE	1000 PTE	
Average		28	60	833	3319	472	586	9757	
STD		12	27	602	2935	555	312	6202	
MIN	1996	12	24	91	77	38	194	2463	1
MAX	1999	51	123	3721	16143	2914	1724	32063	21

Figure 1

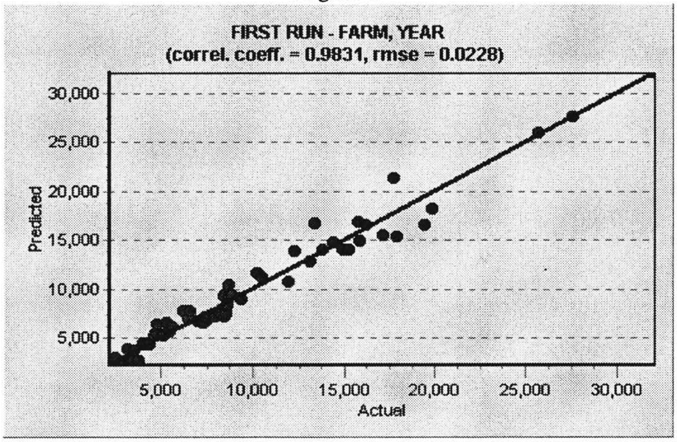


Figure 2

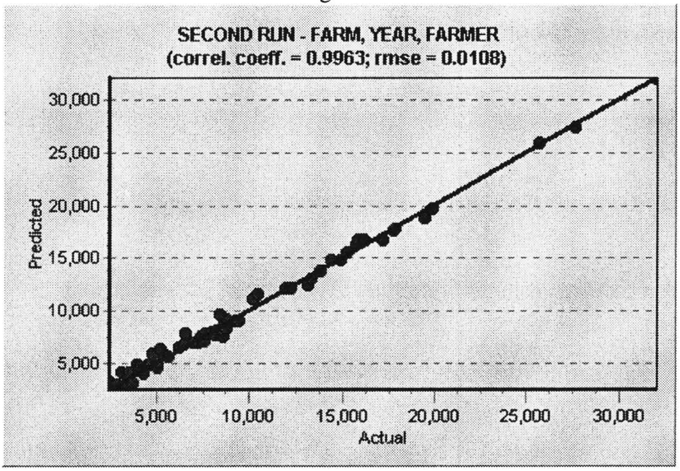
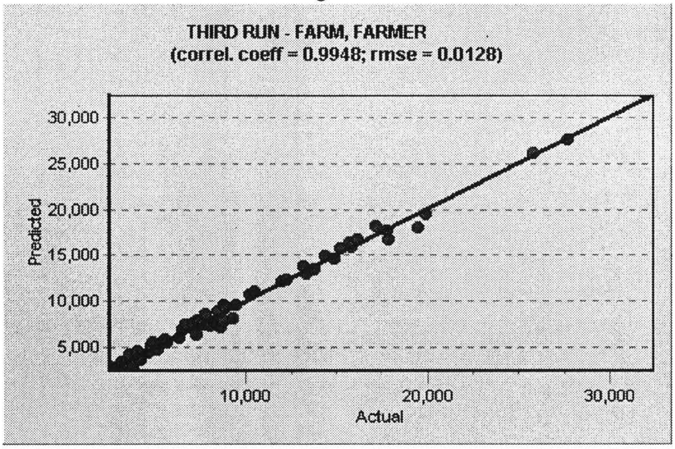


Figure 3



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THE EFFECT OF NITROGEN RATE AND CUTTING FREQUENCY ON DRY MATTER YIELD IN PERENNIAL RYEGRASS F2 POPULATIONS

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Abstract

Because of risk of soil water pollution by nitrogen leakage nitrogen fertilisation on grassland in Belgium should be reduced. Therefore we need grass varieties that still have a high dry matter production at lower levels of N application. When breeding grasses we usually test our material for dry matter production at low cutting frequency although its final goal is to be cut frequently by grazing. Have breeders to adapt their methods to create the highest yielding variety for the different management conditions.

To answer this question we installed a split plot trial including 24 unrelated F2 populations of perennial ryegrass at 2 cutting frequencies (4 and 6-8 cuts a year) and 2 nitrogen application levels (450 and 270 kg N/ha.year).

The ranking of the dry matter yield of the F2 populations and the genotype/cutting frequency/nitrogen level interactions are discussed.

Introduction

Because of risk of soil water pollution by nitrogen leakage, nitrogen (N) fertilization on grassland in Belgium should be reduced. Therefore we need grass varieties that still produce a lot of dry matter at lower levels of N application. When breeding grasses we usually test our material for dry matter yield at low cutting frequency although its final goal is to be cut frequently by grazing. What is the effect of this range of management on the plot performance when breeding perennial ryegrass?

Materials and methods

A strip-plot trial in 3 replicates was sown in 1998 with N levels and cutting frequency as main plots within which populations were randomised. We applied 2 N levels: 370 and 260 kg N/ha.year and 2 cutting frequencies: 4 and 6 cuts a year. Fertiliser was applied as split-dressings after each cut in the proportion 5:2:6 N:P:K. 30 populations were involved: 24 diploid F2 populations and 6 control varieties (3 diploids and 3 tetraploids) spread over 8 early, 11 intermediate and 11 late types. The plots of 9 m² were harvested with a Haldrup plot harvester. We determined dry matter (DM) yield for each cut in 1999 and 2000.

Results

The significancy of the treatment effects and their interactions is shown in table1.

Average yield figures are presented in fig. 2a. Low nitrogen reduced yield by 2.4 t DM/ha.year (18 %). Frequent cutting reduced yield by 3.0 t (22 %). Largest difference in mean yield between varieties was 1.6 t (12 %).

Figure 1 shows the relationship between the high and the low N level for the 2 cutting frequencies and the 30 populations. As a general trend populations performing well under high N level perform well under low nitrogen level for both cutting frequencies. Significant interactions for both N level x population and for cutting frequency x population are illustrated in figures 2b and 2c.

Table1. Significancy of treatment effects
(** p<0.01; *** p<0.001; NS not significant)

treatment factor	significance
N level	**
cutting frequency	**
N level x cutting frequency	NS
population	***
N level x population	**
cutting frequency x population	***
N level x cutting frequency x population	NS

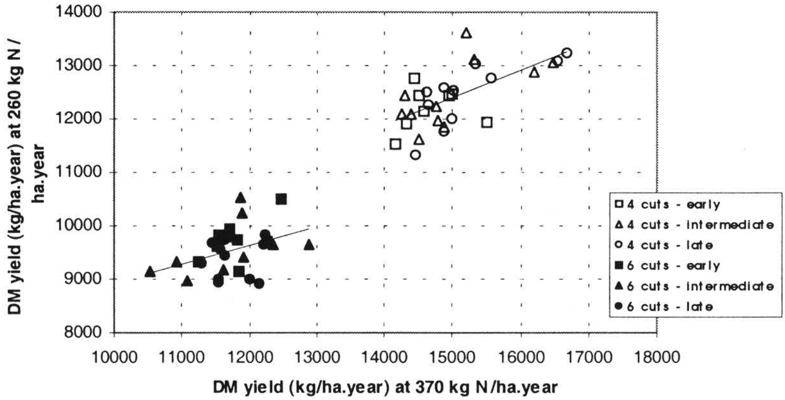

Fig. 1. Relationship between DM yield at low and high N level for 2 cutting frequencies and 30 perennial ryegrass populations.

The seasonal distribution of dry matter for the 2 populations involved in the N level x population interaction showed a very good spring growth (fist cut) for the population with the best performance at the low N level. This pattern for the 2 populations involved in the cutting frequency x population interaction showed that the highest yielding population at the low cutting frequency had a much higher yield in the second cut (beginning of June) i.e. in the reproductive growth stage. The population performing best at the more frequent cutting also had a better sward density.

Table 2 shows the top 5 populations for each treatment. One population is in the top 5 ranking of each management. 3 of the 5 best performing populations at 370 N and 4 cuts are control varieties that have been selected under this management. 4 of the top 5 populations at 260 N and 6 cuts are new F2 populations.

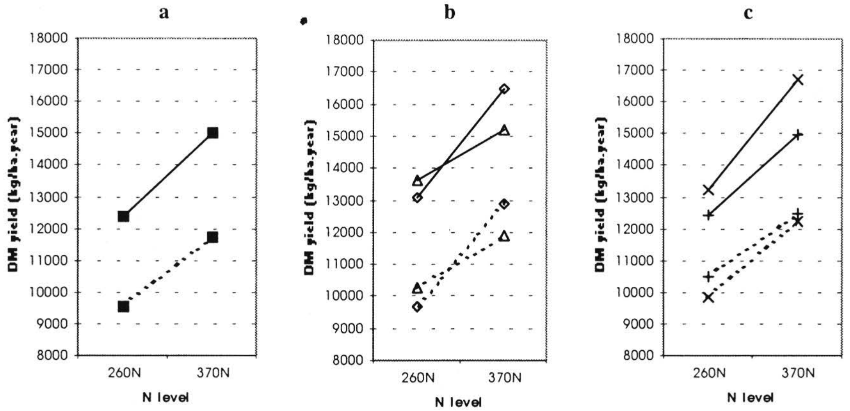

Fig. 2. DM yield at low and high N level and 2 cutting frequencies (4 cuts __ ; 6 cuts ...) showing (a) average effect, (b) interaction N level x population and (c) interaction cutting frequency x population.

Table 2. Top 5 populations for each treatment
(italic: best at 370 N and 4 cuts; underligned: best at 260 N and 6cuts)

ranking	370 N		260 N	
	4 cuts	6 cuts	4 cuts	6 cuts
1	***1511***	*roy*	2164	2167
2	*pomerol*	2171	***1511***	2171
3	*roy*	2182	2167	2164
4	*ritz*	rebecca	*pomerol*	2160
5	*1488*	***1511***	*roy*	***1511***

Discussion

In former trials with perennial and italian ryegrass varieties we found that their ranking for DM yield was almost the same at the high and the reduced level of nitrogen fertilisation (Baert et al., 2000). Schapendonk et al. (1989) and Wilkins et al. (1997) came to the same conclusion in trials in flowing solution culture. Although the actual trial confirms this general trend at the 2 cutting frequencies, some significant N level x population interactions were observed. Such interaction was found when comparing perennial ryegrass with timothy cultivars, the latter better performing at low N level (Baert et al., 2000). A good spring growth seems to be in favour of a good yield at a reduced N level.

More frequent cutting caused an important yield reduction. The cutting frequency x population interaction could be explained by differences in reproductive growth. Wilkins et al. (1989) showed already that poor reproductive growth had a greater effect on reducing total annual yields under infrequent harvesting.

Populations with a well spread seasonal distribution of high dry matter yield may be adapted to a wide range of managements. Some of the cultivars bred at high N level and low cutting frequency performed also vary well at low N level or high cutting frequency. They are however outyielded by populations that perform especially well under these managements.

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CORRELATIONS BETWEEN SEED WEIGHT AND INITIAL GROWTH STAGES IN LUCERNE

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Abstract

Seed weight is a very important agronomic characteristic in many forage species. A study was carried out to evaluate the influence of seed weight on the initial growth stages of four distinct populations of *Medicago sativa* L.. Significant differences in seed weight existed among the populations and significant correlations were found between this trait and various morphological and physiological characters for the varieties and ecotypes tested.

Introduction

Medicago sativa L. is the most commonly cultivated forage species in the world; it is grown on about 32 million hectares (Michoud *et al.* 1988). Compared with other fodder crops lucerne has several advantages in terms of productivity, nutritional value and soil fertility restoration. Although lucerne is the most investigated fodder crop, studies on the relationship between seed weight and initial growth stages are lacking. Research on other forage species has revealed that seed weight is positively correlated with young plant vigour (Thomas 1966, Whalley *et al.* 1966, Falcinelli and Negri 1980).

Several aspects in relation to the environment are involved in the establishment of forage species (Panella 1975, Lorenzetti 1977, Talamucci and Falcinelli 1977). If plantlets grow quickly, they avoid most of the disadvantages, excluding the environmental stresses that take place in the period of sowing (Falcinelli and Veronesi 1987).

The aim of this work was to evaluate 4 alfalfa populations in the initial growth stages for physiological and morphological traits in relation to seed weight.

Materials and methods

The experiment was carried out in Perugia (Italy) in 2000. Four distinct populations were used: the local ecotype "Casalina"; the Italian varieties "Classe" and "Equipe" and the Pioneer variety "Capital". Sixty seeds from each population were taken at random, weighed and randomly divided into 5 groups of 12 seeds each, in order to produce 5 replications per population. The seeds were put in germination boxes (12 seeds per box) fitted with filter paper arranged vertically, so that the roots could grow downwards and the leaves upwards.

The boxes were put in a germination chamber at a constant temperature of 20°C with a photoperiod of 10 hours (2,500 lux). Data were recorded on: length of seminal root (LSR, mm, 7, 9 and 12 days after sowing), emission of cotyledon leaves (EC, days from sowing), emission of the unifoliate leaf (EUFL, days from sowing), emission of the first trifoliate leaf (EFTL, days from sowing), emission of the second trifoliate leaf (ESTL, days from sowing), development score (DS, from 1=minimum to 9=maximum, 60 days from sowing), plantlet height (PH, cm, at 60 days from sowing) and plantlet weight (PW, mg, at 60 days from sowing).

The data collected were subjected to analysis of variance and the linear coefficient of correlation was calculated between seed weight and the other characteristics for all the populations studied. Statistical analyses were performed with the SAS program (SAS Institute, Cary, NC.1985).

Results

Analysis of variance

Results of ANOVA are summarised in table 1. There was a significant difference in seed weight (SW) among the populations examined. The varieties "Capital" and "Classe" scored average seed weights of 2.45 mg and 2.26 mg, respectively, while the variety "Equipe" and the ecotype "Casalina" both scored 1.99 mg. There were also significant differences among the four populations for other traits such as LSR taken at 7-9-12 days, EC and ESTL. No significant differences were found for the rest of the traits investigated, i.e. EUFL, EFTL, DS, PH and PW.

Table 1. Seed weight (SW), length of seminal root (LSR), emission of cotyledon leaves (EC), emission of unifoliate leaf (EUFL), emission of first trifoliate leaf (EFTL), emission of second trifoliate leaf (ESTL), development score (DS), plantlet height (PH) and plantlet weight (PW) of 3 varieties and 1 ecotype tested in the experiment.

Varieties and ecotype	SW	LSR (7)	LSR (9)	LSR (12)	EC	EUFL	EFTL	ESTL	DS	PH	PW
Classe	2.26 b	2.09 a	3.39 a	5.09 a	3.15 b	12.71	25.85	33.64 a	5.69	28.25	33.28
Capital	2.45 a	1.70 bc	2.93 a	4.64 a	3.38 ab	12.55	24.81	31.74 b	5.38	29.11	31.66
Equipe	1.99 c	1.47 c	2.26 b	3.61 b	3.71 a	12.72	25.13	33.07 a	5.36	26.98	29.57
Casalina	1.99 c	1.87 ab	2.98 a	4.58 a	3.37 ab	13.36	24.94	34.17 a	7.67	26.09	31.05

Means followed by the same letter are not significantly different at $P \leq 0.05$.

Correlation analysis

Table 2 reports the coefficients of correlation between seed weight (SW) and the other traits investigated in each population. Seed weight was positively correlated with the plant height (PH) (0.28, 0.44, 0.52) and development score (DS) (0.32, 055, 0.39) in "Classe", "Capital" and "Equipe", respectively, while there was no correlation with these traits in the "Casalina" ecotype, in which there was a positive correlation between SW and EC (0.53). In the Capital variety SW was correlated positively with LSR 9 (0.30), LSR12 (0.32) and negatively with ESTL (-0.47); in the "Classe" variety SW was negatively correlated with ESTL(-0.32). In the "Equipe" variety SW was positively correlated with PH (0.31). On the whole seed weight was highly positively correlated with plantlet weight (0.37) and negatively correlated with ESTL (-0.37), EFTL (-0.20). Seed weight was also positively correlated with LSR 12 (0.17).

Table 2. Coefficients of correlation between seed weight (SW) and the other traits investigated.

	SW				
Traits	**Classe**	**Capital**	**Equipe**	**Casalina**	**All data**
LSR 7	-0.12	0.19	0.05	-0.02	0.04
LSR 9	-0.13	0.30*	0.08	-0.03	0.11
LSR 12	-0.10	0.32*	0.19	0.05	0.17*
PW	0.28*	0.44**	0.52**	0.25	0.37**
EC	0.01	-0.07	0.02	0.53**	0.01
EUFL	-0.09	-0.26	-0.13	-0.08	-0.14
EFTL	-0.15	-0.46**	-0.30*	-0.21	-0.20**
ESTL	-0.32*	-0.47**	-0.18	-026	-0.37**
DS	0.32**	0.55**	0.39**	-016	0.10
PH	0.23	-0.27	0.31*	-0.01	-0.02

*, ** Significant at 0.05 and 0.01, respectively.

Conclusions

The results obtained from the analysis of variance indicate that there were significant differences in seed weight among the populations of *M. sativa* analysed. Moreover, significant correlations between this trait and the various morpho-physiologocal characters were found for the varieties and ecotype tested. In the population "Casalina" the only significant correlation was with the emission of the cotyledon leaves (EC), which are emitted later in genotypes with larger seed. In the varieties, instead, the physiological characters related to the first stages of development were correlated with seed weight. The correlation of seed weight (SW) with plantlet weight (PW) and development score (DS) in all varieties is very interesting, with the "Capital" variety being the most evident. An experiment in the open field will be carried out to further investigate these preliminary results.

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LIGHT INTERCEPTION AND GREEN MATTER YIELD IN A GRASS TRIAL

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Abstract

Ligth interception and green matter yield was studied in 1992 in a third year trial with 10 varieties of timothy (Phleum pratense L.). The radiation measurements were made with a Sunfleck Ceptometer type CEP from Delta-T Devices Ltd during the early vegetation and the regrowth periods. The variation in light interception among varieties was significant, which resulted in a correlated response in green matter yield. On an average the correlation co-efficient amounted to 0.32 in the first vegetation period and 0.60 in the regrowth. To illustrate the variation between the studied entries, the light interception of two varieties, Kampe II and SW T128 was chosen. Kampe II capture more light during the first period but less during the regrowth compared with SW T128. Plant growth habit was thought to be the main reason for their different light interception. Kampe II has a more heterogeneous leaf orientation compared to SW T128' s more upright leaves. Under South Swedish conditions erect leaves seemed to absorb less light than a more horizontal or heterogeneous leaf orientation. The order among varieties in light interception was completely different in the first growth and in the regrowth periods.

Keywords: Light interception, radiation, green matter yield, timothy, *Phleum pratense*

Introduction

Quite a few papers have been published on light interception in agricultural crops. But few have dealt with the variation among varieties of a single species in this character and still fewer have covered the light interception of different forage grass varieties. In the present work light interception and green matter yield of timothy (*Phleum pratense L*) varieties were studied in a trial sown in 1989.

Material and methods

The trial was sown with 10 varieties and four replications. The varieties were Kämpe II, Carola, Vanadis, and several numbered varieties.

The radiation measurements were made in the third harvest year, in 1992. They were made with a Sunfleck Ceptometer type CEP from Delta-T Devices Ltd. The first recording was made on May 7th, soon after commencement of growth, and the measurements were made every 2-3 days till the first green matter harvest was made on June 9th. Measurements were again made during the regrowth period from August 3rd to September 7th. Because of varying sunshine during the measurement, some recording days had to be disregarded and in the regrowth period only the measurements on August 10th and September7th are reported here.

The solar radiation was measured horizontally, alternating above and below vegetation. In each plot, measurements were made between each of the 10 rows, i e 9 recordings were made above and 9 below vegetation. The intercepted or absorbed light was defined as the incident radiation minus the radiation transmitted through vegetation ($PAR_a = PAR_i - PAR_t$, according to the designations of Gosse et al, 1986). Light reflection from the ground (PAR_{tr}) or from the vegetation itself (PAR_r) has not been considered, as they are of minor importance.

The intercepted light in two of the varieties, Kämpe II and SW T128, is shown in diagrams 1 and 2. Light interception and green matter yield of the two harvest cuts, as well as earliness (heading date) of the tested varieties are listed in table 1.

Results

The solar radiation increases considerably from measurement No.1 to 2 and 3 but is then fairly constant over the first growth period. The measured radiation below the vegetation increases also from the first to the second recording but decreases then as a consequence of a gradually denser leaf mass. Only during the last measurements before the first cut the radiation below vegetation again increases when the leaves slowly start to wilt. The harvest was made some days after heading of the latest timothy.

The correlation between intercepted light and green matter yield varied of course between measurements, starting low at the first day, increasing up to measurement No.6 when the correlation was significant, and then decreasing again to a low level at the last recording. On an average the correlation amounted to 0.32 during the first vegetation period. In the regrowth period the correlation between intercepted light and green matter yield was quite high, although not significant. On the two days which were considered here the correlation coefficient was 0.61 and 0.56 with an average of 0.60 which is not quite significant.

The analysis of variance of light interception (not reported) showed that there were highly significant differences both between measurements and between varieties in the first growth as well as in the regrowth period. The variation in green matter yield was also significant among varieties both in the first and in the regrowth cut. To illustrate the variation between varieties, light interception of Kämpe II and SW T128 is shown in diagrams 1 and 2. Kämpe II captured more light during the first period but less during the regrowth compared with SW T128.

Discussion

Kämpe II was superior to SW T128 in light interception during the first growth period, perhaps because of being one day earlier in heading, but more likely because of its heterogeneous leaf orientation compared to SW T128's more upright leaves. Kämpe II was also superior to SW T128 in the first cut green matter yield. In the regrowth the order of light interception, as well as yield, was the opposite. SW T128 had a quicker start of growth after the first harvest, and it kept this superiority during the whole period. A superior regrowth capacity is often typical of late varieties, and even if the difference between these two entries was only one day, the regrowth yield difference could be due to this. Bélanger & Richards (1995) found very small differences in intercepted PAR among 7 timothy varieties and concluded that the variation in radiation use efficiency is greater than the variation in light interception. In the present study, significant differences between varieties in light interception resulted in correlated yield differences. Madakadze et al (1998) compared the light transmission in a number of switchgrass *(Panicum virgatum)* varieties and found that early varieties had a higher PAR_t value in a late development stage. This is in agreement with the results of this trial where the PAR_t increased slightly during the two last measurements of the first growth period.

Diagram 1. Light interception in two timothy varieties before first harvest

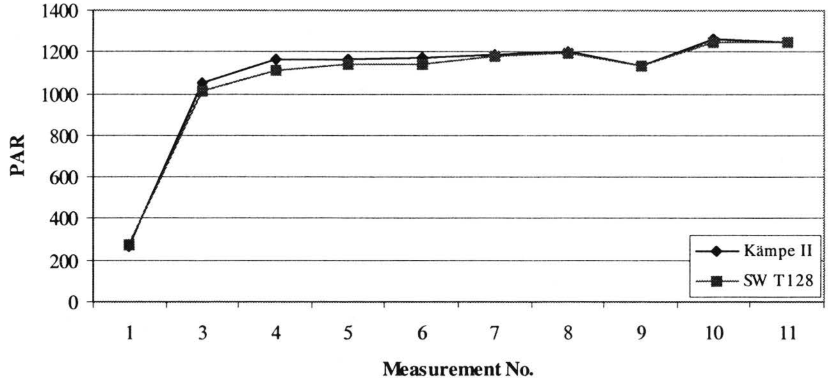

Diagram 2. Light interception in two timothy vrieties before second harvest

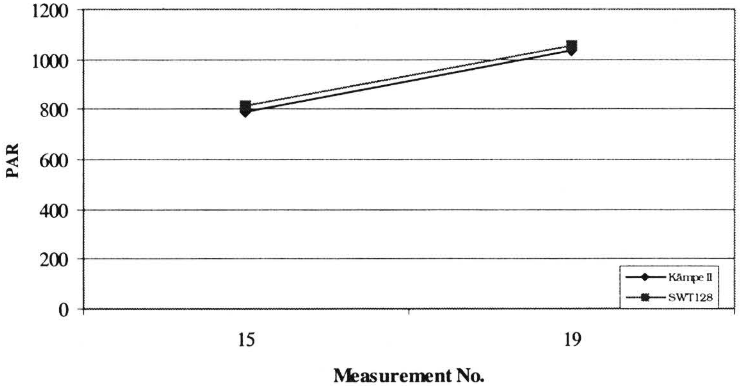

Table 1. Heading date, light interception and and green matter yield in timothy varieties.

Variety	Heading date	First harvest cut Light interception, PAR	First harvest cut Green matter yield, Kg/ha	Second harvest cut Light interception PAR	Second harvest cut Green matter yield, Kg/ha
Kämpe II	June 2	1086 c §	11600 a	915 b	4730 ab
Carola	June 2	1086 c	12480 abc	919 b	4750 ab
SW T118	June 1	1087 c	13900 c	918 b	5420 b
SW T118 U1	June 2	1082 bc	12370 ab	880 a	5400 b
SW T119	June 1	1070 a	12330 ab	921 b	5940 b
SW T120	June 1	1072 ab	13120 bc	886 a	4670 ab
SW T122	June 1	1082 bc	11540 a	910 b	4810 ab
SW T128	June 3	1068 a	11060 a	933 c	5480 b
SW T129	June 3	1074 ab	11980 ab	920 b	5900 b
Vanadis	June 2	1089 c	12520 abc	877 a	3480 a
LSD 0.05		11	1470	12	1380

§ Means within a coloumn followed by the same letter are not significantly different at the 0.05 level.

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SELECTION OF *Dactylis glomerata* L. PORTUGUESE ECOTYPES BY ANALYSIS OF QUALITY WITH NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

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Abstract

Dactylis glomerata L. (cocksfoot, orchardgrass) is one of the main perennial grasses of the natural meadows used for grazing or hay, widespread in our country from the sea level to 1500-1600 m.

In breeding programs for improved yield and quality, it is very important the knowledge of both, as well as their relation. The conventional methods to evaluate the feeding quality take long time and have high costs so, we need a methodology which allows the determination of feeding quality of forages rapidly and accurately. NIRS technology has the potential for rapid, non-destructive, simultaneous prediction of many plant constituents associated with forage quality.

This study was conducted in the National Plant Breeding Station, Elvas, and the main objectives are: i) to investigate whether NIRS technology can reliably assess feeding quality of de *D. glomerata*; ii) evaluate the plant production, evaluate the feeding quality and relation between both, in forty Portuguese ecotypes and five checks of cocksfoot, to select them for improved yield and quality.

The evaluation of yield/plant was done with four cuttings (June/99, February, April and June/00).

For NIRS calibration, the samples were assayed by reference methods for moisture, crude protein, crude fibber, ash and *in vitro* digestibility, in 114 samples that had been selected among a total of 328. The calibration equations were obtained by multiple linear regression. Equations were validated with 20 samples.

Selected samples had a wide range of values, and for the calibration parameters, were chosen five wavelengths to fit the best equations. The squared coefficients of determination (r^2) of these equations ranged from 0,97 for crude protein, 0,90 for crude fibber, 0,86 for ash, 0,7327 for moisture and 0,40 for *in vitro* digestibility.

We concluded, by ANOVA, that protein was significantly different between ecotypes and cuttings. Moisture, fibber and ash were significantly different between cuttings and we didn't obtain significant differences for *in vitro* digestibility.

Even that the accuracy obtained with NIRS technology is not so high as wished, it allows us to do a previous screening and to obtain a few number of samples to be assayed by conventional laboratory techniques, giving us the possibility to analyse more samples and identify with efficiency and low costs the best samples.

Introduction

Dactylis glomerata L. (cocksfoot or orchardgrass) is a perennial grass widespread in Portugal. Cocksfoot is often present in natural pastures, being used for hay and grazing and it is a major forage grass consumed by livestock. Knowledge of both yield and quality traits as well as their interrelationships provides a sound basis for a breeding program to improve this species.

Conventional procedures to determine feeding quality are slow and have high costs so we need a methodology that allows the determination of feeding quality of forages rapidly and accurately. The technology of near infrared reflectance spectroscopy (NIRS) has the potential for rapid, non-destructive, simultaneous prediction of many plant constituents associated with forage quality (Norris *et al.*, 1976).

This study was conducted in Estação Nacional de Melhoramento de Plantas, and the main objectives were: i) to investigate whether NIRS technology can reliably assess feeding quality of *D. glomerata*; ii) evaluate plants yield, evaluate the feeding quality and relation between both, in Portuguese ecotypes of cocksfoot, to select them for improved yield and quality.

Materials and Methods

We've installed in 1997 a trial with forty-one Portuguese ecotypes and four checks (cultivars Currie, K2M, Porto and Vila Viçosa) of *D. glomerata*. The ecotypes were complete randomised and each one was represented by a plot of two lines with ten isolated plants (50 cm apart) each line, in a total of 20 plants X 2 replications.

Management consisted in a cutting in vegetative growth, to estimate Winter yield done in first two weeks of February of 2000, and two cuttings to estimate Spring yield, at the beginning of medium blooming (first two weeks of April of 2000) and at medium earing (first two weeks of June of 2000).

To estimate yield per plant, after each cutting, we weighed total green matter yielded in each plot and replication. To evaluate dry matter, samples were dried at 80°C for 24 hours and were ground through a 0.8 mm hammer mill for feeding quality determinations.

The NIRS equipment used in this study was Inframatic 8120 with 20 filters and wavelengths from 1400 to 2400 nm and software Percon version 4.84, 1986 from Perten Instruments.

For NIRS calibration, samples were assayed by reference methods for moisture, crude protein, crude fibre, ash and *in vitro* digestibility in 114 samples that had been selected by the computer software among a total of 328. To select the best combinations of four wavelengths we used the following criteria: least calibration constants, value of F test higher than 100, least standard error of calibration, highest squared coefficients of multiple determination, existence of a specific and/or a reference filter in the combination. The calibration equations were obtained by multiple linear regressions. Equations were validated with 20 samples. Feeding quality of cocksfoot ecotypes was evaluated by NIRS after the calibration.

Results and Discussion

In table 1 we present the quality components studied, it's range of variation, average and calibration equations for *D. glomerata*.

Table 1 – Quality components studied in samples, range of variation, average and calibration equations for *D. glomerata*. Log values are correspondent to a specific wavelength: 2=2336 nm; 3=2310 nm; 5=2230 nm; 6=2208 nm; 8=2180 nm; 9= 2139 nm; 10=2100 nm; 11=2050 nm; 13=1940 nm; 14=1818 nm; 16= 1759 nm; 19=1680 nm

QUALITY COMPONENT	MAXIMUM (%)	MINIMUM (%)	AVERAGE (%)	CALIBRATION EQUATIONS
Moisture	6.78	2.19	4.59	4.8-42.2log5+27.3log13+211.33log14-178.1log16
Crude Protein	20.82	5.96	14.86	1.3+792.7log8-629.8log9-584.6log14+398.4log19
Crude Fibre	36.70	19.09	17.61	31.6+1193.6log2-932.7log3-251.7log8-75.7log11
Ash	17.50	5.70	11.80	19.9-100.7log3-1011.7log5+2632.7log6-1486.6log8
In vitro Dig.	72.10	36.20	35.90	38.7-685.0log10+1232.8log11+1512.8log14-2082.7log16

Table 2 – NIRS calibration and validation statistics R^2= coefficient of determination; F Test = value of F test; SEC=standard error of calibration, MAX=maximum difference between laboratory values and predicted values by NIRS; LAB AVE=average of laboratory values; NIRS AVE=average of values predicted by NIRS; SD=standard deviation of LAB and NIRS quality values ; r= correlation coefficient

QUALITY COMPONENT	CALIBRATION			VALIDATION					
	R2	F Test	SEC	MAX	LAB AVE	NIRS AVE	SD		r
							LAB	NIRS	
Moisture	0.73	75	0.55	1.85	4.83	4.25	1.70	1.19	0.94
Crude Protein	0.97	966	0.63	2.74	11.59	11.20	5.10	5.07	0.97
Crude Fibre	0.90	258	1.21	4.21	32.04	31.27	5.96	4.78	0.97
Ash	0.86	169	1.06	3.48	9.07	8.37	2.99	2.47	0.92
In vitro Dig.	0.40	18	5.60	5.78	47.09	63.21	2.64	12.41	0.02

The squared coefficients of multiple determination (R^2) of known forage quality values on NIRS values were 0.73 for moisture, 0.97 for crude protein, 0.90 for crude fibre, 0.86 for ash and 0.40 for *in vitro* digestibility (table 2). The R^2 and standard errors of calibration (SEC) for NIRS-predicted assays were both lower for moisture (Silva, 1998), essentially equivalent (Marten *et al.*, 1983; 1984) or higher (Winch and Major, 1981) for crude protein, higher for crude fibre (Silva, 1988), higher for ash (Silva, 1998) and R^2 was lower but SEC was higher (Marten *et al.*, 1983; 1984) or equivalent (Winch and Major, 1981) for *in vitro* digestibility than those previously reported for NIRS analysis.

The correlation coefficients between predicted and actual quality values (table 2) were higher for moisture (Silva, 1988), equivalent for crude protein (Marten *et al.*, 1983; 1984; Silva, 1988) and higher for crude fibre (Silva, 1988) than those previously reported. The correlation coefficient for *in vitro* digestibility was almost zero. We expected these correlations to be in order that they occurred, because NIRS has repeatedly been shown to be very applicable to the prediction of plant proteins and because *in vitro* digestibility is a bioassay that commonly has less precision than chemical assays.

The results found for moisture show that probably NIRS is not able to do accurate predictions of this trait. Looking at our results we can confirm that crude protein can be accurately predicted by NIRS. For crude fibre the results don't give the guarantee that NIRS can predict accurately this component but that could have happened because we have a small range of variation of crude fibre content and to it's complex nature. We think that there is a possibility of NIRS to predict accurately crude fibre in face of others (Burdick *et al.*, 1981; Marten *et al.*, 1983; Marten *et al.*, 1984; de Ruiter *et al.*, 1988) and our results. We expected these results for ash because it's very difficult to analyse this component once the reference method doesn't analyse a chemical entity well defined but some components which means that two samples with the same ash content can have different mineral combinations that absorb in a different way the energy emitted by NIRS (Shenk and Westerhaus, 1991). Once *in vitro* digestibility is the component that's more complex chemically of the five components studied we might have to use more wavelengths to calibration equations. It will be needed more studies to demonstrate if NIRS can be used to predict accurately *in vitro* digestibility of cocksfoot. Thought the results weren't completely acceptable, except for protein, we think that values of crude fibre and ash could be used for certain applications, mainly because we can rapidly obtain these data.

The most interesting ecotypes will be the ones that have better Winter yields in order to go with legumes growth when in consociation. Ecotype 237, with the best yield/plant in first cutting had the lowest content in crude protein. In second and third cuttings had good yields/plant too, but of the lowest crude protein content. Ecotype 5042 with a good yield/plant in first cutting had a low crude protein content and a high crude fibre content. In second cutting had averaged values for three parameters. In third cutting had a high yield/plant and crude protein and fibre contents near average. Ecotype 227 has a good Winter yield with one of the highest crude protein content and a low crude fibre content. Its behaviour in the other two cuttings was similar. With a high yield per plant in first cutting, ecotype 234 has an average content in crude protein and average to high of crude fibre. In second cutting the yield per plant was good but crude protein content was low and crude fibre content average. In third cutting 234 had of the lowest yields but the best crude protein content and the lowest crude fibre content. A low crude protein content, high crude fibre content and high yield per plant were the results of ecotype 5056 in first cutting. In the next cuttings, yields/plant weren't so high but it had the same behaviour for crude protein and fibre. Ecotype 225 had good yields in first cutting and averaged in second and third. Crude protein content was average in first cutting but lower in the other two cuttings. Crude fibre content was average in Winter cutting but high in the other two cuttings. Yield/plant of 3 A ecotype was high in first cutting and average in second and third, but crude protein content was high in first two cuttings, but low in third. Herdade dos Tomazes was an ecotype that had a good yield/plant in first and second cuttings and had high crude protein and low crude fibre contents. In third cutting had less yield per plant but a reasonable content of crude protein and high content of crude fibre. At last, ecotype 5046 had a good behaviour in two first cuttings for yield per plant but low crude protein and high crude fibre contents. In third cutting had a low yield per plant, average crude protein and high crude fibre contents (table 4).

The ideal ecotypes for selection in what concerns to yield and quality should have high yields in Winter associated with high crude protein content, as well as a good behaviour in Spring. Ecotype 227 had behaviour similar to this one. Ecotype 234, thought with some limitations, had a good behaviour, mainly in the end of cycle in which it has a good crude protein content. Herdade dos Tomazes although had a low yield in last cutting had a good crude protein content and had a good behaviour in first cutting.

These results showed a negative correlation between crude protein and fibre contents in three cuttings. In third cutting we also found a positive correlation between crude protein content and ash and a negative one between crude fibre content and ash (table 3).

Conclusions

In a breeding program we can accept a lower level of precision if that allows us to analyse hundreds of forage samples and obtain quality components data rapidly and at a low cost than with reference methods. On other side, in cases that precision is lower than we wished, this technique can be used like a rapid method to do material screening that is analysing rapidly a high number of lines or populations. After pre-selection with NIRS, reference methods can be used in a less number of samples allowing with more efficiency and at a low cost the identification of the best samples.

We can conclude that there is a negative correlation between crude protein and crude fibre contents.

Selection of ecotypes 227, 234 and Herdade dos Tomazes can't result in best yield or best feeding quality but it points to the existence of Portuguese germplasm with good relations yield/quality allowing its selection in breeding programs to future attainment of cultivars.

Table 3 –Correlations between studied quality parameters and yield per plant, in three cuttings: yield/plant, crude protein, crude fibre and ash

	Crude Protein			Crude Fibre			Ash		
	1 cut.	2 cut.	3 cut.	1 cut.	2 cut.	3 cut.	1 cut.	2 cut.	3 cut.
Crude Fibre	-0.826***	-0.721***	-0.344*	-	-	-			
Ash	0.147	-0.060	0.538***	-0.128	-0.081	-0.316*	-	-	-
Yield/Plant	-0.144	-0.131	-0.022	0.104	0.197	0.286	-0.120	-0.223	0.046

*, *** significant at the 0.05 and 0.001 probability levels, respectively

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Table 4 – Dry matter yield per plant (g), content (%) of crude protein (CP), crude fibre (CF) and ash (A) obtained for ecotypes/cultivars of *D. glomerata*, in each cutting

Ecotype/Cultivar	First Cutting				Second Cutting				Third Cutting			
	Yield/Pl	CP	CF	A	Yield/Pl	CP	CF	A	Yield/Pl	CP	CF	A
V. Viçosa	16.45	14.85	25.68	13.56	21.95	19.10	24.41	9.77	110.85	7.72	35.58	5.26
Porto	14.00	17.24	23.91	13.15	27.60	17.08	24.89	10.31	121.25	7.56	35.43	6.11
Currie	39.20	11.93	27.29	13.11	33.30	14.29	26.25	10.97	107.70	7.15	35.70	5.56
K2M	11.90	13.17	25.33	13.46	19.00	13.43	26.27	9.79	55.60	8.77	33.43	7.36
H. dos Tomazes	29.45	16.17	25.38	12.60	56.90	16.95	25.27	10.89	87.70	8.43	34.77	7.38
1 A	11.35	17.48	24.56	13.55	39.75	18.79	25.43	9.63	91.45	8.58	35.84	5.80
2 A	13.80	13.52	26.87	13.76	21.55	18.74	25.31	10.10	99.55	8.11	34.78	6.43
3 A	29.55	13.18	27.53	13.38	33.20	15.21	26.71	10.42	119.05	8.23	34.85	6.80
221	13.65	12.72	27.81	13.03	23.65	15.40	26.79	10.22	89.75	7.81	34.87	6.94
222	19.00	15.75	25.82	13.63	28.10	17.91	26.07	10.33	99.80	7.90	35.33	6.30
223	13.40	14.63	26.43	13.35	28.60	17.31	26.64	9.92	97.25	7.96	35.70	6.48
224	23.30	16.94	25.06	13.31	41.75	17.90	26.10	9.53	108.25	8.37	34.77	6.65
225	31.40	14.88	26.24	13.09	36.40	15.71	26.53	10.64	106.10	7.69	35.73	6.86
226	18.45	13.93	26.57	13.19	26.55	16.03	26.24	11.43	83.55	7.77	36.52	6.56
227	32.25	16.59	25.11	13.18	39.50	18.34	24.48	9.54	138.60	8.42	35.17	7.93
228	27.65	13.71	27.17	14.17	42.15	16.41	25.97	10.50	107.90	7.90	35.27	6.83
229	23.35	16.24	25.75	14.22	50.95	16.39	25.21	10.34	103.25	8.60	35.45	6.29
230	19.75	16.35	25.72	13.10	35.60	16.18	26.69	9.66	100.70	8.81	36.03	5.88
231	14.50	15.59	25.75	14.46	17.50	16.96	27.20	11.87	89.00	7.95	36.52	6.76
232	21.70	13.87	26.29	14.73	25.25	17.08	24.95	11.26	90.65	8.29	34.66	6.65
233	21.70	14.90	25.54	14.52	31.45	15.99	26.38	10.25	119.05	9.40	33.89	7.50
234	32.00	14.74	25.57	13.14	44.65	15.38	25.99	9.87	77.30	9.42	33.21	8.10
235	17.90	12.50	27.27	13.32	11.05	16.75	26.05	9.69	71.90	8.11	35.38	6.39
236	20.75	12.38	26.97	13.82	20.85	16.35	24.23	10.74	46.80	8.25	33.62	6.57
237	40.85	11.50	26.83	13.35	42.75	14.67	26.92	9.55	134.25	7.83	34.81	6.65
238	18.55	14.57	25.17	12.44	36.55	17.73	25.69	9.65	109.00	8.16	35.10	6.69
239	13.05	14.44	26.57	14.24	12.95	18.67	24.60	9.43	128.75	8.14	36.24	5.88
5035	22.00	15.98	24.86	13.27	36.55	18.00	24.72	9.27	126.05	9.08	35.32	6.78
5036	16.70	14.33	26.01	13.47	28.40	14.99	27.24	10.82	101.65	7.50	35.55	6.18
5037	28.25	13.59	27.60	12.48	29.75	16.52	25.34	11.00	110.40	7.77	35.32	6.58
5038	23.95	17.26	25.19	13.47	44.85	19.09	25.59	10.33	121.20	9.13	35.33	6.84
5039	36.05	17.77	24.73	13.71	43.20	16.79	26.80	9.14	109.70	8.22	35.31	7.30
5040	19.35	16.08	24.97	13.11	30.60	17.19	26.37	10.19	117.25	8.55	35.55	7.61
5041	15.55	15.12	25.27	13.46	34.45	15.47	26.79	11.42	104.85	7.55	35.16	5.47
5042	38.40	12.67	27.44	13.18	32.80	16.84	25.96	9.99	139.80	8.17	36.05	6.65
5043	27.25	13.52	27.33	14.78	37.80	15.84	26.19	10.73	129.80	7.94	36.04	6.13
5044	28.20	17.15	25.54	13.47	41.90	18.43	25.61	9.94	122.20	8.78	34.93	6.95
5045	25.05	15.50	26.02	13.29	31.75	19.63	24.99	10.31	144.05	7.67	35.85	6.32
5046	28.75	13.28	27.23	12.69	61.95	13.55	28.93	10.20	58.65	8.09	36.04	6.55
5056	31.70	12.02	26.72	12.37	30.35	15.08	26.33	9.89	126.00	7.26	35.01	6.60
5057	28.30	14.83	26.02	14.04	37.25	17.69	25.23	10.18	145.15	8.27	35.40	6.42
5058	20.40	13.81	26.79	13.42	28.60	15.34	25.88	11.06	89.20	7.79	35.68	6.04
5119	21.60	15.63	25.91	13.57	34.50	16.74	25.91	10.58	109.60	8.38	35.24	6.63
5120	18.05	15.95	25.64	12.86	29.20	18.04	25.47	9.24	109.80	7.88	35.38	6.34
5121	20.85	14.15	26.78	13.23	28.90	18.04	25.40	10.71	131.50	7.15	36.04	5.82
Average	23.10	14.72	26.09	13.44	33.16	16.76	25.91	10.25	106.49	8.14	35.29	6.57
LSD (P=0.05)	16.83	2.57	1.97	-	23.57	3.16	2.03	-	53.74	1.24	1.53	1.20

DIFFERENCES IN NITROGEN CONTENT AND NITROGEN EXPORT BY DIFFERENT VARIETIES OF *Lolium perenne* L

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Abstract

Lolium perenne L is the dominating grass species in belgian cultivated grassland. Differences in N export between species or between varieties could be of importance in minimising the risk of N-leaching. N-efficiency is calculated as N_{output} x 100 x (N $fertilizer_{input})^{-1}$

The purpose of this study was the examination of differences in relative ADM yield, N-content and N- export in a wide range of 40 varieties of *Lolium perenne* at two locations in Belgium: a sandy loam and a sandy soil. Diploid and tetraploid varieties as well as early and late varieties are represented in this comparison. The trials were cut 4-6 times a year during 3 years after the sowing year.

Keywords: nitrogen, exportation, varieties, *Lolium perenne*

Introduction

It is well known that all the grass varieties do not react in the same way to nitrogen in terms of dry matter yield, nitrogen content and nitrogen export. *Lolium perenne* is the dominating grass species in Belgian cultivated grassland. Maybe differences between varieties of this species in N content and N exportation should receive more attention in perspective of efficient N use for grass production and the restriction of the amount of leachable N in the soil at the end of the growing season.

Baert et al (1999) and De Vliegher et al (1999) published results of genotype variation in nitrogen use efficiency at two levels of nitrogen fertilisation .The purpose of this study was to examine the differences in relative DM-yield, N-content, N-export in a range of 40 listed or candidate varieties of *Lolium perenne* at two locations in Belgium.

Material and methods

In 1995, 40 varieties of *Lolium perenne* were established in a complete block design with 4 replicates in Merelbeke (sandy loam) and in Geel (sand). Diploid (28), tetraploid (12), early (9), intermediate (15) and late (16) varieties were involved. In the 3 following years, the level of N-input by mineral fertilisers was 425 $kg.ha^{-1}.year^{-1}$. This experiment was executed during 3 growing seasons in a 5-6 cutting regime.

Results

A very large quantity of the applied nitrogen is exported by harvesting the grass under mowing conditions: 88% in Merelbeke and 104% in Geel. In Geel the N-output exceeded the N-input by mineral fertiliser, especially in the first harvest year, because there was a supplementary uptake of mineralised soil nitrogen (table 1).

The range in nitrogen export within the 40 varieties was on average 40-46 kg N ha^{-1} $.year^{-1}$ and corresponds with 10% of the mineral N-input. Within a year the differences could increase to 79 kg N.ha^{-1}. Although there is no difference in the level of N-export between the early, intermediate and late group, the range for the early and intermediate types varies between 11 and 30 kg N.ha^{-1}.year^{-1} and the variation between the late varieties is much higher: there are candidate varieties with a very low and with a very high N-output (table 2).

There is not a good correlation between N-output and N-content but the range in N-content in Geel is very large: 5.6 g N.kg^{-1}DM.

The correlation between N-export and DM yield is good (Merelbeke R^2= 0.54, Geel R^2= 0.55) (fig1 and 2). In general, high yielding varieties export large quantities of nitrogen. As a result, breeding for high nitrogen efficiency (or exportation) can be achieved by breeding for high DM yield.

The correlation between the N-export in the 2 locations is not promising (fig 3) in perspective that breeders select mostly in one location. However, some varieties combine in both locations a high DM yield with a substantial or high nitrogen content and can be integrated in a specific selection program for nitrogen efficiency.

Conclusion

In general, breeding for dry matter yield is breeding for nitrogen efficiency. The correlation between the N export in the 2 locations is not promising. However, some varieties have potential in this perspective because they combine a high DM yield with an substantial or high N content.

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Table 1: DM yield, N content and N export of 40 varieties of Lolium perenne in 1996-1998 in two locations in Belgium

character-ristic		Merelbeke (sandy loam)				Geel (sand)			
		1996	1997	1998	cycle	1996	1997	1998	cycle
DM yield kg/ha	average	14531	13253	15320	**14368**	17212	14686	12597	**14832**
	stand.dev.	369	485	565	**396**	498	863	666	**516**
	range	1416	1998	2705	**1867**	2250	3596	2788	**2142**
N content g/kg	average	24.7	23.9	28.6	**25.7**	32.4	25.4	31.9	**29.9**
	stand.dev.	0.5	0.7	0.9	**0.5**	0.9	0.7	1.0	**0.7**
	range	2.2	2.8	4.7	**2.4**	3.1	3.5	5.6	**3.8**
N export kg/ha	average	360	316	437	**371**	557	373	402	**444**
	stand.dev.	9	10	13	**8**	18	17	21	**11**
	range	34	44	76	**46**	66	59	79	**40**

Table 2: DM yield, N content and N export of the different types of Lolium perenne in 1996-1998 in two locations in Belgium

characte-ristic		Merelbeke (sandy loam) type			Geel (sand) type		
		early(9)	interm.(15)	late(16)	early(9)	interm.(15)	late(16)
DM yield kg/ha/year	average	14443	14284	14404	14908	14828	14793
	stand.dev.	191	306	537	404	535	578
	range	526	1068	1867	1117	1707	2142
N content g/kg	average	25.6	25.8	25.7	29.8	30.0	29.9
	stand.dev.	0.4	0.4	0.5	0.5	0.8	0.7
	range	1.2	1.3	2.3	1.4	2.7	3.0
N export kg/ha/year	average	371	370	372	443	445	444
	stand.dev.	4	7	11	11	10	11
	range	11	20	46	30	27	40

Workshops

WORKSHOP 1: BREEDING FOR DISEASE RESISTANCE

Chairman: U. K. Posselt,

Several general topics were discussed and the following decisions made or conclusions drawn.

1. The 3rd Conference on Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf was held in September 26th at Soest, Germany. It was concluded, that there were too many papers about endophytes which are not considered being that important to European conditions. It was suggested not to continue this working group with separate meetings. It was decided to add a session of resistance breeding every 2nd or 3rd year to the section meeting. This should help to enable more researchers to attend the section meetings, and to attract specialists from the field of phytopathology to our main meetings.

2. Virus diseases

After a brief discussion it was concluded that the occurrence of viruses is mainly in the breeding nurseries, i.e. under spaced plant conditions and that research work should not have priority.

3 Rust resistance

Speakers from almost all countries considered the rusts being the most important diseases. There is some concern of rust dispersal further to the north of Europe and also some indication of changes in the rust populations and/or changes in the level of resistance in current cultivars. To study this in a more detailed way it was suggested to have a joint experiment across Europe to yield information about cultivar x rust/ country - interaction. Beat Boller from Switzerland agreed to coordinate this project.

4 Disease assessment techniques

It was suggested to establish a collection of infection and assessment techniques and to standardise them. A final decision about this topic could not be made yet, but will be on the agenda of the next section meeting.

WORKSHOP 2: BREEDING FOR DROUGHT TOLERANT GRASSES

Chairman: M. W. Humphreys, Institute of Grassland and Environmental Research, UK

Introduction: *Questions for consideration*

1. What is drought?
2. What is drought resistance?
3. Can we measure it?
4. Is drought important in performance of forage and amenity grasses
5. Do we want to do anything about it? Is it commercially feasible?
6. If it is, how do we do it? Empirical or mechanistic? What species? C3 or C4?

What is drought?

So far as the plant is concerned, drought is a complex phenomenon. It involves not just a lack of available water but also high transpiration rates, supra-optimal temperatures, photo-oxidation, mineral deficiency, and hard soil. Their relative importance varies with location and year. Definitions of drought resistance must ultimately take into account the climate and prevailing agriculture. An over-riding requirement is that the crop should survive and re-grow rapidly when autumn rains set in. A secondary requirement is that yields should not be greatly reduced during mild drought (water deficit less than 50 – 100 mm, say), a response which occurs in many Mediterranean grasses which rapidly become "quiescent" at first indication of drought.

What is drought resistance (DR)?

Operational definition: Drought in agriculture is defined as a dry spell that results in a loss of yield below that expected under optimal water supply. For grasses, DR is the ability to produce the desired product (herbage, green lawn) during slight, temporary or early drought; survive severe drought (quiescence, growth rhythm, toughness); having survived, respond rapidly to renewed water supply.

Definition of product: From a plant breeding perspective, DR in grasses is assessed based on their: capability to produce herbage mass; herbage quality (WSC, protein, palatability, digestibility), seasonal distribution; regrowth potential; visual quality and uniformity; resistance to poaching, trampling, or root-pulling.

Environment specificity: Breeders must decide whether they wish to target DR to maritime, Mediterranean, or continental environments?

Environment characterisation criteria: Environmental factors for consideration are:

1) **climate**: the rainfall distribution (reliability of summer rainfall, probability of thunderstorms); evaporative stress; high temperatures;
2) **soil**: depth, water-holding capacity, mineral status (nutrients, metal toxicity, winter waterlogging).

Can we measure drought resistance?

What is suitable for one environment is not necessarily suitable for another. With increasing degree of control, but decreasing degree of reality we can rely on the following tools:

(a) Accumulated experience from general trials over a range of environments over many years.

Advantages: realistic, minimal technology; can take into account age of sward (DR may vary according to age).
Disadvantages: almost impossible to interpret except in the most general terms; deconstructing effects of environment, locality, year, management, expensive; time-consuming; difficult to standardise; lack of uniformity within sites; only suitable for populations and not for individual genotypes.
(b) Specific trials in areas of known drought susceptibility.
Advantages: similar to (a).
Disadvantages: similar but not as great as (a).
(c) Trials under rain-out shelters.
Advantages: One year's trials may be adequate; simpler to organise; drought is the principal effect making analysis simpler.
Disadvantages: Dry soil in a wet year not necessarily the same as a true drought year; limited area available; expense of shelter; affect of the rain-out shelter on other environmental factors.
(d) Trials in deep containers in glasshouse, or controlled environment room.
Advantages: Convenient; enables comparisons between individual genotypes without neighbour effects; allows analyses of the roots.
Disadvantages: Unreal high transpiration rates (oasis effect); plants often young (<1 yr); roots grow unrealistically fast, especially if the soil is well-fed throughout.
(e) Trials in normal flower-pots.
Advantages: Allows evualuation of constitutive traits.
Disadvantages: Totally unrealistic; drought usually extremely rapid.
(f) Trials of plants growing in nutrient solution with added osmoticum (e.g. PEG).
Advantages: Convenient and inexpensive; allows control of water potential at roots.
Disadvantages: See (d); possible toxicity of the osmoticum; very short term.

Is drought important?

Even in the wet maritime climate of the UK, shortage of available water during the growing season is the main cause of year-to-year variability in yield of herbage.
Do we want to do anything about it?
Improved C3 grasses for temperate regions would:

(a) displace C4 grasses which are generally of lower quality (forage & turf) than C3 grasses;
(b) reduce costs of re-sowing and waiting for the new crop;
(c) improve sustainability and reduce environmental damage.

How do we go about improving grass drought resistance?

A Framework for determining priorities.

Level of characterisation and targetting of traits	Approach to selection and breeding Evaluate existing lines or ecotypes	Select out of existing lines or ecotypes	Produce synthetic hybrids of elite parents	Marker-assisted selection
General vigour, establishment rate, competitivity				
Performance under a wide range of environments (yield stability)				
Performance under drought in particular				
Expression of DR strategies (avoid, delay, tolerate)				
Expression of putative traits (rooting, osmotic adjustment, WSC, etc)				
Expression of proteins (enzymes, antioxidants, osmo-protectants, etc.)				

General discussions of the meeting

It was thought likely that the basis for genetic variation in drought resistance within the *Lolium/Festuca* complex was dependent more on allelic variation than the presence of any unique gene combinations. Furthermore, it was widely accepted that root development was of key importance for future research on drought resistance and had not over the years received sufficient attention. Genotype variation in mineral uptake and mineral use-efficiency was further reason for research on rooting characters. It was accepted that research on root development is laborious and complicated, but nevertheless essential for further progress. Decisions were necessary on the optimum conditions for studying root development. Should research concentrate on root growth under non-stress, under stress environments, or ideally both? Research on the production, location, and translocation of storage carbohydrates should be combined with that on root development, and will have direct relevance to a plant's ability to recover following drought. Whilst root development was of prime importance, it was noted that there are also known differences between drought resistant and drought sensitive grass species in leaf and shoot characters. For example, *Festuca arundinacea* (drought resistant) has more stomata on its abaxial leaf surface than *L. multiflorum* or *L. perenne* (generally drought susceptible) and has a greater tendency to roll its leaves under drought. Whilst a number of grass species are potential sources of genes for drought resistance, only species with deep-rooting systems are effective in clovers.

Field trials for drought resistance should be undertaken at different sites throughout the world to optimise selection procedures. The requirements for drought resistance alter for different climatic conditions. For Mediterranean conditions, summer quiescence is essential for plant survival. As parent plants, use should be made of species adapted to the selected growth conditions as a source of genes for drought resistance. Considerations should also be made of the positive effect of endophytes on drought resistance and water-use-efficiency and the advantages and disadvantages of their inclusion in new varieties.

It was accepted that a better understanding of the mechanisms of drought resistance was urgently required. There is a definite role for plant physiologists to work closely with geneticists, and plant breeders to develop new more robust grass varieties.

Breeding for complex traits such as drought resistance presents opportunities for marker-assisted selection (MAS). At present, AFLPs are being used to construct dense maps on alien introgressed chromosome segments containing genes for drought resistance. However, microsatellites will be used increasingly as the marker technology becomes available to the plant breeder. Use should be made of syntenic relationships, known to exist between different crop species, to maximise efficiency of current MAS plant breeding strategies. It is becoming increasingly obvious that within the *Gramineae*, species retain many gene sequences from a common ancestor in the same order and within the same linkage groups. The consequence of this is that information from an extensively studied crop species may be applied directly to genetic studies of genomes of other less understood crop species, such as *Lolium*. Any QTL for drought resistance previously mapped in say rice or wheat may be conserved in *Lolium* and may be targetted directly using the relevant markers.

Questions were asked whether transgenic technology was necessary to improve drought resistance in forage and amenity grasses. It was widely accepted that the transfer of quantitative traits was impractical by transgenic methods and that conventional breeding offered far more effective procedures. Gene transfer between drought resistant *Festuca* and *Lolium* species in hybrids is high. Sites of intergeneric recombination have been detected along the entire chromosome-arm with the highest frequency at the median positions. Using conventional breeding technology an EU project co-ordinated by researchers at IGER Aberystwyth, would provide *Lolium* lines with *Festuca* introgressions for drought resistance. Such plant materials and markers for drought resistance traits would be commercially available within five years.

WORKSHOP 3: BREEDING METHODS

Chairman: B. Boller
Reporter: D. Reheul

Prior to the meeting, a number of grass breeders had been asked to present breeding schemes they use to develop commercial varieties of allogamous grass species. There was a very encouraging response to this request. Nine breeders from seven different countries presented one or several posters with often highly detailed information about the different steps of their preferred way of developing varieties:

Joost Baert of DvP, Belgium
Bernard Bayle of Limagrain-DLF, France
Beat Boller of FAL Reckenholz, Switzerland
Jan Dijkstra of Barenbrug, The Netherlands
Fred Eickmeyer of Saatzucht Steinach, Germany
Marc Ghesquière (Poster presented by Michael Humphries) of INRA, France
Ulf Feuerstein of DSV, Germany
David Johnston of DARDNI, Northern Ireland
Hans-Arne Jönsson of Svalöf, Sweden.

Routine breeding schemes for *Lolium* species

Seven breeders presented breeding schemes they use most in their current (and partly past) routine variety development programmes of *Lolium* species (mainly *Lolium perenne*). It was agreed that they remain anonymous in the present workshop summary. We therefore designate them as Breeder A to Breeder G.

The great majority of the schemes presented involve the use of clones selected phenotypically from spaced plant nurseries. Their progenies are tested individually, typically at a single location. Based on these tests, a number of superior clones are combined to synthetic varieties. These more "conventional" schemes differ mainly in the method used to obtain the seed from the selected clones. Open pollination among non-repeated clones, polycross and topcross methods are used. In all cases, the half-sib offspring of the clones ("F1") is used to assess their breeding value in terms of general combining ability. Further details of the particular schemes are described below.

A more fundamentally different breeding scheme ("Full-sib F2") was presented by breeder G as his current mainstream method, and by breeder D as an alternative to the "conventional" scheme. Here, a large number of selected individuals are arranged in isolated pairs to produce full-sib F1 seed. The F1 offspring of each cross is grown in a separate isolation to produce a larger amount of F2 seed, and in parallel in row trials used to assess disease (rust) resistance. The F2 seed is used to establish a plot trial with yield determination and observation (row) trials in several additional locations. Superior pair cross progenies are finally combined to synthetic varieties. Because inbreeding occurs to an important extent when F2 seed is produced, the results of the progeny tests give a direct estimate of "varietal ability" of the genotypes (including their tolerance to inbreeding), rather than of just combining ability. Another obvious advantage of this system is that unlike with clones, the components of the synthetics can be kept over a long period without any labour and very little risk. The discussion revealed that this breeding scheme is used rather extensively by at least one additional breeder not present with a poster.

Table 1. Routine breeding schemes for Lolium species: Estimates of volume and selection intensity of breeding steps

step		Breeder code						
		A	B	C	D	E	F	G
greenhouse screening (disease inoculation	no. sown/planted yr^{-1}	15000	7500	none	15000	none	none	none
	% retained	**50**	**50**	**-**	**33**	-	-	-
spaced plant nursery	no. sown/planted yr-1	7500	4000	20000	5000	15000	n.a.	n.a.
	% retained	**8**	**5**	**5**	**10**	**4**	**10**	n.a.
observation of clones	no. planted yr^{-1}	600	200	1000	500	600	n.a.	n.a.
	% retained	**40**	**38**	**50**	**20**	**33 to 50**	**10**	n.a.
type of testcross (PC=Polycross, TC=Top cross, O=open pollination, X=Pair Cross)		**PC**	**O**	**TC**	**PC (+ X)**	**O**	**none / O**	**X**
genotypes (clones) in test cross	no. harvested yr^{-1}	240	75	500	100	200 to 300	n.a.	600
tests of genotype progenies	no. locs. yield	none	1	2	1	2	none / 1	1
	no. locs. observation	1	none	1	none	none	none / 1	3
	% retained	**65**	**10**	**33**	n.a.	**20 to 40**	n.a.	**5 to 10**
variety synthesis	no. of components	15	3 to 10	4 to 10	5	10	10 to 15	at least 6
	no. of synthetics yr^{-1}	16	10 to 15	75	n.a.	few	n.a.	6
	% retained/applied	**25**	n.a.	**25**	n.a.	n.a.	n.a.	n.a.
plot trials with yield determination, series 1	seed generation used	syn-2	syn-3	syn-1	n.a.	syn-1	n.a.	"syn-3"
	no. of locations	3	2	4	n.a.	2	several	5
	no. of rep. per loc.	3	4	3	n.a.	3	n.a.	3
observation trials, parallel with plot trials	no. of locations	1	none	4	n.a.	none	n.a.	n.a.
plot trials with yield determination, series 2	seed generation used	syn-2	no trials	no trials	no trials	syn-2	no trials	no trials
	no. of locations	3	-	-	-	6	-	-
	no. of rep. per loc.	3	-	-	-	3	-	-

"n.a." (not available) indicates insufficient information on volume of that particular step.

Details: (see Table 1)

Base material. Few details were given on the development of the base material. Pair crosses between selected individuals from superior cultivars were often mentioned as the predominant source of genetic materials. Ecotypes, or more generally collected material from permanent or semi-permanent grassland, are also still used extensively. Some form of recurrent selection and recombination with more advanced breeding material is used to create the "starting population" for the "conventional" schemes.

Greenhouse screening: Three breeders (A, B and D) indicated that they use artificial inoculation in the greenhouse to screen for disease resistance. Typically, about half the seedlings are discarded based on this first screening. Rusts, snow mould and bacterial wilt were mentioned as pathogens involved in artificial inoculation.

Spaced plant nursery: Most breeders implant spaced plant nurseries with several thousand individuals each year. All of them apply a rather strong selection intensity at this step. Only 4 to 10 % of the individuals are retained and pass on to observation of clones. Disease resistance was often mentioned as the most important selection criterion. Some breeders find it useless to try and select for yield on the basis of spaced plant observation. However, breeder F mentioned that in his breeding programme, having a strong focus on yield, successful varieties have been selected based uniquely on spaced plant selection, without any progeny testing.

Observation of clones: Clones are most often observed as clonal rows with 5 to 6 ramets per clone. Breeder F sometimes uses clonal swards, i.e. small plots allowing for yield and quality determination. Generally, selection intensity at the level of clones is much lower than in the spaced plant nurseries. Up to 50 % of the clones are retained for test crosses.

Test cross: Surprisingly often, the simplest form of test cross is used: open pollination among non-repeated, selected clones (Breeders B, E, and F). In this case, the potentially important influence of the predominant pollinator (a neighbouring plant) is disregarded altogether. Breeders A and D most often plant polycrosses for the purpose of collecting clonal seed for progeny tests. However, they do not usually reach the number of replications necessary in theory for completely random mating. Only Breeder C uses a method which is entirely conform to breeding text books: the top cross method with alternating (planted) clonal and (sown) tester rows. The pair crosses performed by Breeder G can also be regarded as a form of test cross. However, at the same time, they create the experimental units (combination of two genotypes) to be tested.

Apart from it's function to produce seed for the progeny tests, the test cross can also be used to get an estimate of seed yielding ability. This was particularly mentioned by Breeder E who discards about 50 % of the clones based on insufficient seed yield.

Test of genotype progenies: Most breeders systematically conduct yield trials (some at more than one location) to evaluate the genotype progenies. These plot trials are often complemented with "observation" trials in rows, mainly to evaluate disease resistance. At this step, estimates of selection intensity varied the most among the breeders. They indicated to retain between 5 and 65 % of the tested genotypes. The average selection intensity is similar to that applied after observation of clones.

Variety synthesis: In breeding schemes involving clones, these are generally maintained vegetatively during the progeny tests, so as to use the original genotypes for variety synthesis. Two deviations from this "text-book" method were described: Breeder A uses the Fl offspring of the clones grown in the progeny tests (isolated for each polycross) to harvest the original seed of the final variety. Breeder B grows the offspring of the selected clones of a synthetic as individual plants for one or two cycles of mass selection.

The majority of the breeders aim at creating synthetic varieties with less than 10 components. Some indicate that they sometimes create synthetics with even less than 6 clones, a number which is often considered minimal to avoid excessive inbreeding depression. Conversely, Breeders A, E and F tend to use larger numbers of components.

Trials to evaluate synthetics. All breeders conduct plot trials, generally at two or more sites to evaluate the synthetics. Two breeders (A and E) systematically sow each synthetic in two consecutive years (series) of evaluation trials. Breeder E uses syn-1 seed for the first and syn-2 seed for the second, more extensive series of trials. The other breeders, with the exception of Breeder C, use syn-2 or syn-3 seed for their evaluation trials. Breeder C uses syn-1 seed for extensive yield and additional observation trials. In the "Full-Sib" scheme of Breeder G, the seed used for evaluation of the synthetics corresponds with syn-3 of a synthetic based on clones.

Only Breeders A and C gave an estimate of the selection intensity applied after the evaluation of the synthetics. Both indicated to retain 25 % of the synthetics for application to official trials.

Experimental breeding schemes

Two breeders presented results they obtained with breeding schemes outside a regular variety development scheme:

Hans-Arne Jönsson presented a scheme he used in timothy breeding. It involved three cycles of recurrent selection within separate breeding populations. After each cycle, equal numbers of superior genotypes from each breeding population were combined to a synthetic. So far, the results were rather disappointing. The author suspected that an important degree of inbreeding depression took place during recurrent selection where a high selection intensity had been applied: in each cycle, only 12 genotypes per population were retained.

Marc Ghesquière developed a breeding strategy to use marker assisted selection in *Lolium* material introgressed with *Festuca*. QTL markers were effectively detected by analyzing a tetraploid "BC2" population obtained by backcrossing hybrids between tetraploid (*4x*) Lolium *multiflorum* and *Festuca glaucescens* to the *4x Lolium* parent. Further development of the *4x* hybrids involved crosses and backcrosses with *2x Lolium*. This lead to major loss of *Festuca* chromosomes and resulted in an introgressed *2x Lolium* population. The results suggested that within this population, for certain traits of low heritability, marker assisted selection could be an efficient alternative to conventional phenotypic selection.

Discussion

Although the general outline of the different breeding schemes has a lot in common, every breeding program has its own particular characteristics. The breeding strategies vary from very simple to quite complicated and yet all the breeders succeed in breeding good (local) varieties. In order to breed *widely adapted* varieties, the ability to manage the interaction genotype × environment is often considered just as important as the breeding method. This is why the early testing of new varieties is conducted in different environments and this is why the location of the main breeding station never can be the ideal place to assess all the demands of a modern variety bred to be used in different environments.

An ever lasting question is this : "Where should the selection of superior genotypes be concentrated: before or after the progeny testing?" It is a general idea that one should not rely too much on phenotypic selection to indicate polygenic characteristics. The absence of a close correlation between the performance of single plants and their progenies, pushes breeders to put much emphasis on the progeny testing. Hence all breeders start in their plant nursery with thousands of individual plants and select quite *sharply* for characteristics with a presumed simple inheritage. All breeders eliminate *mildly* in their clonal fields (on average two thirds of the clones are discarded) and produce progenies of as many genotypes as possible. A sharp early selection may narrow too much the genetic base of the population with disappointing results (see Hans-Arne Jönsson).

Breeders are searching intensively for the ideal genotype(s) or combinations of and between genotypes witch complement each other. In this respect the specific combining ability becomes more important and since we need several generations between the initial cross and the final product, the performance of inbreds or consanguinous plants might be very essential. The more the genetic range between components gets smaller, the more important this principle becomes. Maybe this is why the concept of general combining ability is getting devaluated in many breeding schemes: an unbiased pollination is not a prerequisite for several breeders while producing offspring of selected clones. "Cross the best with the best and hope for the best" is an important driver in several programmes. The evolution towards quick and less complicated schemes, as those working with pair crosses, is also a consequence of this concept.
Not completely in line with this idea, is the careful constitution of the synthetic varieties with quite a lot of components (on average nine components) or, if pair crosses are on the basis of a selection cycle, to mix or to polycross several "F2's" to finalize the new variety.

At the entrance of yield trials, there is no uniformity in the seed generation under test. Some breeders test the syn-1 generation, but breeder B uses syn-3. However if more than 1 series of testing is used, the final series usually is conducted with syn-2. The seed generation supplied to the official trials might influence the performance in the official trials.

The discussion was regarded as very useful owing to the open minded exchange of ideas that might inspire breeders to reflect on their own schemes, resulting in smaller or major adaptations to the proper breeding strategies.
The discussion was closed with the wish to continue the exchange of ideas and reflections on breeding methods in future meetings.

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