Proceedings

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EUCARPIA
Leafy Vegetables 2011

Proceedings

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Preface

In this booklet you find the proceedings of the Eucarpia Leafy Vegetables 2011 meeting, held 24-26 august in Villeneuve d'Ascq, France. It includes a report on the general discussion that took place at the end of the meeting (p81). To illustrate the convivial atmosphere, a compilation of photos taken during different events is available as a separate pdf-file.

The conference was attended by 90 persons, representing 12 different countries, 20 private companies, and 16 public research institutes. About one third (28) of the participants was member of Eucarpia. A complete list of participants with their affiliations and e-mail addresses can be found at the end of this booklet.

During the conference, 22 oral presentations and 14 posters dealing with many different aspects of interest in breeding leafy vegetable crops were organised in sessions covering four themes. As illustrated in the table below, the majority of the presentations and posters concerned lettuce, followed by chicory and spinach.

Leafy vegetable crop	**Lettuce**		**Chicory**		**Spinach**		**Others**		**Total**	
Theme	**op***	**pp**	**op**	**pp**	**op**	**pp**	**op**	**pp**	**op**	**pp**
Culture and management, quality traits	6	-	-	-	-	1	-	1	6	2
Diseases and pests, disease resistance	6	5	-	-	-	-	-	-	6	5
Genetics and biotechnology	3	1	2	4	1	-	-	-	6	5
Germplasms and their diversity	2	1	1	1	-	-	1		4	2
Total	17	7	3	5	1	1	1	1	22	14

*op = oral presentation, pp = poster

The poster entitled 'The development of a breeding strategy for nitrogen efficiency in spinach', by Jose Rafael Chan Navarrete, Pierre-Emmanuel Algoet, Oene Dolstra, Gerard Van Der Linden, Edith Lammerts Van Bueren, was awarded with the best poster price, sponsored by the Société National d'Horticulture de France.

Besides the scientific program, several other activities had been organised, like the cocktail-dinner on the first evening, visit of the experimental station Pôle Légumes Région Nord at Lorgies, a reception at the Villeneuve d'Ascq townhall, and the conference dinner.

We think the program of the Eucarpia Leafy Vegetables 2011 meeting in Lille was diverse and interesting, and we hope that it has offered opportunities to catch up with the latest developments in different aspects of leafy vegetables breeding, as well as to (re)establish contacts with the different persons representing breeding companies, research institutes, and universities, active in this field.

We thank all participants for their contribution to the success of the meeting, and in particular those who presented their work in oral presentations or posters. Their efforts to follow the instructions for submitting abstracts was appreciated since it highly facilitated editing the proceedings. In some instances we made slight adaptations, but only on the form and not on the contents.

For the organisation of the conference, the contributions of the members of the scientific committee, Brigitte Maisonneuve, Ales Lebeda, David Pink, Chris Kik, and Rob van Treuren, has been indispensable. Their experience and advices has made our work much easier. We also acknowledge Ton den Nijs, chairman of the Vegetable Crops section of EUCARPIA, for his continuous interest in the progress of the organisation.

We thank Sophie Lefebvre, Pauline Bachelard, and Anne Bituski of Perspectives & Organisation for their engagement and professionalism in taking care of the more practical sides of the organisation.

Of course, the support of the meeting by sponsors has been very important and we thank Eucarpia, Université de Lille1, Rijk Zwaan, Florimond Desprez, KeyGene, Gautier, Hoquet, Dutcher, and the Société National d'Horticulture de France, for their generosity.

Special thanks we owe to Polytech-Lille for offering us their localities and facilities to host the conference, to Bruno Delbreil for his help in this part of the organisation, and to Daniel Montignies for technical support.

We also thank the city of Villeneuve d'Ascq for their hospitality and the reception at the town hall, as well as Dominique Werbrouck, Maxime Pérus, and Ludovic Vasseur of the Pôle Légumes Région Nord for the opportunity to visit their field trails.

Without the help of all above mentioned persons, institutes, organisations, and companies, the organisation of the Leafy Vegetables 2011 would not have been possible.

The organising committee,

Theo Hendriks, president
Marie-Christine Quillet
Jean-Louis Hilbert

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Program of the meeting

Wednesday, August 24

9h00 – 10h00 Inscription and reception at Polytech Lille
Poster installation

10h00 – 10h15 Welcome and Opening of the conference
Theo Hendriks

10h15 – 10h30 Introduction EUCARPIA
Ton den Nijs

10h30 – 12h00 Theme *'Culture and management, quality traits'*
Chair: David Pink and Rob van Treuren

10h30 – 11h00 Genetics and lettuce seed quality
Steven Groot and Kent J. Bradford

11h00 – 11h20 Developing lettuce with improved quality for processed salads
Ryan J Hayes, Marie-Jose Truco, Leah K McHale, Richard W Michelmore, Rudie Antonise, Paul Hand, Ivan Simko

11h20 – 11h40 Genetic characterisation of post harvest spoilage in lettuce
Laura Atkinson, Paul Hand, Leah McHale, Maria-José Truco, Richard W Michelmore, Johan Schut, David Pink

11h40 – 12h00 Bitter-sweet science: mapping quantitative trait loci for flavour in lettuce
Martin Chadwick, Ascension Martinez Sanchez, Carol Wagstaff

12h00 – 14h00 Lunch

14h00 – 16h00 Theme *'Culture and management, quality traits', suite*
Chair: David Pink and Rob van Treuren

14h00 – 14h20 Detection of QTL associated with rib discoloration and tipburn in crisphead lettuce
Sylvie Jenni, Maria-José Truco, Oswaldo Ochoa, Richard W Michelmore

14h20 – 14h40 A genetic analysis of the introgression process from crops to wild relatives: Mapping Quantitative Trait Loci for plant vigour under abiotic stress conditions
Brigitte Uwimana, Clemens CM van de Wiel, Marinus JM Smulders, Richard GFVisser

14h40 – 15h15 Coffee break

15h15 – 16h45 Bremia discussion meeting

15h15 Co-workers linked to IBEB members
Aad van der Arend**,** Nunhems

15h45 European platform for Leafy vegetables/Lettuce breeding aspects
Michel de Lange, Syngenta

16h15 The American nomination of Bremia isolates
Richard Michelmore, UC Davis

15h15 – 18h45 Poster session

19h00 – 22h00 Cocktail-dinner at the Olivarius Hotel, Villeneuve d'Ascq

Thursday, August 25

9h00 – 12h00 **Theme *'Diseases and pests, disease resistance'***
Chair: Ales Lebeda and Brigitte Maisonneuve

9h00 – 9h30 **Current diseases and pests in lettuce in Western Europe**
Brigitte Maisonneuve and Dominique Blancard

9h30 – 9h50 **Race-specificity in interactions between *Lactuca* spp. and *Golovinomyces cichoracearum***
Aleš Lebeda, Barbora Mieslerová, Pavla Korbelová

9h50 – 10h10 **Plant genotype influences lettuce colonization by the Enterobacteriaceae**
Paul Hunter, Josie Brough, Paul Hand

10h15 – 10h45 **Coffee break**

10h50 – 11h10 **Fine mapping nonhost resistance in lettuce to downy mildew**
Erik den Boer, Ningwen Zhang, Koen Pelgrom, Rients Niks, Richard Visser, Marieke Jeuken

11h10 – 11h30 **Virulence of *Bremia lactucae* populations in Southern France between 2006 and 2011**
Brigitte Maisonneuve, Sandrine Jeuniaux, Emilie Juillard, Marion Lovera

11h30 – 11h50 **Population structure of *Bremia lactucae* isolated from lettuce culture in France**
Romain Valade, Claire Neema, Brigitte Maisonneuve

12h00 – 14h00 **Lunch**

14h30 – 17h30 **Visit of the experimental station Pôle légumes Région Nord in Lorgies**

18h00 – 19h00 **Reception at the Townhall of Villeneuve d'Ascq**

19h30 – 23h00 **Conference dinner at the Hermitage Gantois, Lille**

Friday 26 august

8h30 – 10h15 **Power cut**
Coffee
Best poster price award

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Richard W Michelmore

10h45 – 11h05 Mapping quantitative trait loci for sugar in a RIL population of lettuce
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11h05 – 11h25 Genetic analysis of the genes for dioecism and monoecism in *Spinacia oleracea* L.
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11h30 – 11h50 Identification of alleles conferring delayed bolting in lettuce
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12h30 – 14h00 Lunch

14h00 – 16h00 **Theme *'Germplasms and their diversity'***
Chair: Rob van Treuren and Ales Lebeda

14h00 – 14h30 Genetic resources of leafy vegetables in Europe: conservation and use
Rob van Treuren and Chris Kik

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14h50 – 15h10 Contribution of the French network on chicory genetic resources to a European *ex situ* management of leafy vegetables genetic resources
Pascal Coquin, Valérie Cadot, François Boulineau, Valérie Grimault, Sophie Perrot, M. Benigni

15h10 – 15h30 The diversity of indigenous and traditional leafy vegetable species in South Africa
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15h30 - 16h00 Coffee break

16h00 – 17h00 General discussion, next Leafy Vegetables meeting announcement

17h00 **End of conference**

'Culture and management, quality traits'

Genetics and lettuce seed quality

Steven P.C. Groot[1] and Kent J. Bradford[2]

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Key words: seed quality, seed storage, seed dormancy, lettuce, *Lactuca sativa*

To bring the efforts of plant breeders into effect, farmers have to grow the plants and produce a crop. Seed quality is therefore very important to provide this genetics its full potential in the field. Poor germination or seed borne diseases will result in poor establishment of the crop and consequently loss of productivity. For that reason seed companies put high efforts in seed technology, both in testing the quality of the seeds and in upgrading of the quality. In recent years also genetic factors influencing seed quality were identified, e.g. with lettuce.
Next to seed health the two main issues for lettuce seed quality are dormancy and seed longevity. In general lettuce seeds germinate fast. However, when soil temperatures are too high, germination may be inhibited (high temperature dormancy). In response the seeds may even go into secondary dormancy, resulting in failure of germination even when soil temperatures drop. To overcome this problem, seed companies perform so-called priming treatments with the seeds. With these priming treatments the seeds are pre-germinated and dried before the root protrudes. During the priming treatment seeds several processes are initiated, including the onset of cell cycle activity in the embryonic root tip.
Lactuca serriola is much more tolerant to germination above 30 °C compared to *Lactuca sativa.* Genetic analysis of a recombinant inbred lines (RIL) derived from a cross between these two species, revealed a single QTL associated with high temperature germination (*Htg6.1*) that contained *LsNCED4,* a key gene in biosynthesis of the germination inhibiting plant hormone abscisic acid.
The same RIL population has also been used for QTL analysis of seed storability under a low humidity storage (30% RH and 37 °C) or controlled deterioration (CD) regime (75% RH and 50 °C). Several QTL's were identified, interestingly a deviation was observed in relation to the distinct storage conditions. This distinction can be related to the moisture levels of the seeds during the storage, allowing or not the activity of reactive oxygen scavenging enzyme systems.
Whereas storability of lettuce seeds is already relatively short, shelf life is considerably reduced after seed priming. Seed companies invest in development of priming and drying protocols to maintain storability as much as possible. The damage that is occurring in seeds during storage is a direct or indirect effect of oxidation (membranes, proteins, mRNA and DNA). To improve the analysis of seed storability in relation to seed production, treatments (as priming) and genetic factors, a novel method was developed to analyse seed ageing under various moisture levels, while maintaining the temperature comparable to those during storage conditions in practice. The principle of the method is to increase the rate of oxidation through increasing the oxygen concentration by storing the seeds in steel tanks under high oxygen pressure. The method can also be used to study genetic variation in seed longevity.
Next to awareness during the breeding program, with an increasing importance of seed quality, tests to study genetic effects and generation of molecular markers, it becomes also possible to include seed quality as a trait in breeding programs.

Developing lettuce with improved quality for processed salads

Ryan J. Hayes[1], Maria José Truco[2], Leah K. McHale[3], Richard W. Michelmore[2], Rudie Antonise[4], Paul Hand[5], Ivan Simko[1]

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Key words: *Lactuca sativa* L., quantitative trait loci, genetics, shelf-life, tipburn, breeding

Lettuce is increasingly consumed as minimally processed salads. Cultivars grown for this market may require breeding for improved shelf-life and resistance to physiological defects such as tipburn (TB). Tipburn is a calcium deficiency related defect causing necrosis on the leaf margins, typically on the inner leaves where the damage is not easily observed until processing. Low heritability and correlations between plant morphology and tipburn incidence may impede resistance breeding efforts but make tipburn resistance an excellent candidate for marker-assisted selection. Three recombinant inbred line (RIL) populations (Valmaine x Salinas 88) x Salinas=VS, Salinas 88 x La Brillante=SLa, and Saladin x Iceberg=SI), their parents and the check iceberg type cultivar Calicel were evaluated for TB incidence, head weight, core height, head closure, and head firmness in two replicated Yuma, AZ field experiments. Planting dates were in mid-December and evaluated in early April when high day-time temperatures (27-32°C) may promote tipburn. Tipburn incidence was high in all experiments, occurring in 85% of Calicel heads and 38% of Salinas 88 heads. The variation for TB incidence among RILs was significant (P<0.01). Broad-sense heritability estimates for TB on a per-plot basis were low for all populations (SI = 0.14, VS and SLa = 0.23), indicating that phenotypic selection for reduced tipburn incidence is ineffective. Significant and positive genetic correlations with TB incidence were found with head closure in VS (0.84) and SLa (0.66), core height in VS (0.66), and head weight (0.86) and head maturity (0.67) in SI. The results indicate that specific morphological characters can condition low tipburn incidence and the relationships between tipburn incidence and plant morphology can be population dependent. These associations may hamper successful breeding of new tipburn resistant cultivars.

Decay of cut leaf pieces is the limiting shelf-life factor for salads packaged in modified atmospheres. In three spring, summer, or winter replicated field experiments located in Salinas, CA and Yuma, AZ, the SLa population and parents were grown, harvested, processed into salad and packaged into modified atmosphere bags using the method of Hayes and Liu, 2008, J. Am. Soc. Hort. Sci., 133:228–233. Up to nine bags of salad were made for each RIL in each experiment. Each bag was assessed weekly for percent decayed pieces until all bags reached 100% decay. The variation among RILs was significant, and La Brillante had faster decay than Salinas 88 in all experiments. The mean percent decay data was used to detect quantitative trait loci (QTL) on a genetic linkage map comprised of single nucleotide polymorphisms and amplified fragment length polymorphisms. A significant (P<0.01) QTL on linkage group four (LG4) was detected in all field experiments, explaining 27% to 69% of the total phenotypic variation depending on the experiment. Significant (P<0.01) QTL with smaller effects (R_2 <25%) were detected on LG9 in all experiments and LG1 in the Yuma, AZ experiment only. In this population, decay of salad-cut lettuce is a simply inherited trait with limited genotype x environment interaction and suitable for development of molecular markers for use in marker-assisted selection.

Genetic Characterisation of Post Harvest Spoilage in Lettuce

Laura D. Atkinson[1], Paul Hand[2], Leah K. McHale[3], Maria José Truco[4], Richard W. Michelmore[2], Johan Schut[5], David AC. Pink[2].

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Key words: post harvest quality, post harvest discolouration, lettuce, genetic analysis, QTL, linkage map, metabolome, breeding tools

Post harvest discolouration in lettuce is an increasingly important problem due to the shift in the market for prepacked processed salads. However, substantial variations in product quality and consequential losses have been reported. In today's ever-increasing market of 'food perfection', any alteration to the visual characteristics of a product is likely to incite an unfavourable consumer response; with a prime example being prepacked cut salads. It has recently been suggested that almost 50% of salads purchased in the UK are thrown away. Of this 22% is lettuce and 13% being mixed salads (lettuce is a major component of these mixed salads) which are thought to cost £230 million. The main reason for wastage (48%) was that the product had passed its 'sell by date'. Additionally, wastage levels of up to 30% have been recorded for lettuce during processing of raw material due to post harvest discolouration (causing a loss of quality) costing the UK industry an estimated £2.5 million per annum. Pre-packed salads generally have a relatively short shelf life of 3-5 days and there is a need to extend post harvest quality and the resultant shelf life in order to reduce waste and deliver a product of consistently good quality to the consumer. Variation in post harvest discolouration was recorded in a lettuce diversity set of 28 accessions representative of the lettuce primary and secondary genepool. The parents of the Warwick HRI lettuce mapping population, Saladin and Iceberg were included in the diversity set. They showed significantly different responses for discolouration and the difference between them was representative of a major part of the variation seen in the diversity set. F_7 recombinant inbred lines (RILs) derived from a cross between Saladin and Iceberg were therefore suitable for genetic analysis of post harvest discolouration. As a precursor to the genetic analysis, a good quality linkage map based on the F_7 Saladin x Iceberg population was generated. All component linkage groups of this map are in the correct orientation with the correct marker order and can be anchored to the integrated lettuce map by Truco *et al.* (2007) and the MCB19_10NR map under construction at The University of California, Davis. Significant genetic variation in the post harvest response was demonstrated for these RILs. Twenty-one significant QTL were subsequently identified for post harvest discolouration traits, and the markers linked to the QTL can be used for marker assisted selection. Significant but weak correlations were recorded between discolouration and important agronomic traits, however as these were not highly correlated this means that post harvest discolouration and agronomic traits can generally be independently selected for by breeders without having to compromise on other traits. Research was also initiated to understand the metabolic changes underlying the phenotype change. Significant variation in levels of metabolites related to post harvest discolouration including phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and total phenolic content (TPC) was observed in RILs with extreme phenotypes. The differences in metabolite levels were significantly correlated with the discolouration phenotypes. Work was also initiated to identify candidate genes associated with the biosynthetic pathway responsible for discolouration (phenylpropanoid pathway) in an attempt to identify genes underlying QTL. Twenty-three genes have been placed on the Saladin x Iceberg map using comparative genomic approaches. Some of

these co-locate within the region of a discolouration QTL and are therefore candidate genes for the QTL effect. These results indicate that a desired phenotype with reduced levels of post harvest discolouration can be achieved using natural variation, and this study has provided the tools and knowledge to do this.

Detection of QTL associated with rib discoloration and tipburn in crisphead lettuce

Sylvie Jenni[1], Maria-José Truco[2], Oswaldo Ochoa[2], Richard W. Michelmore[2]

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Key words: *Lactuca sativa*, physiological disorder, quantitative trait loci, heat stress

Rib discoloration and tipburn are physiological disorders expressed when crisphead lettuce (*Lactuca sativa* L.) crops are grown in high temperature conditions. The objective of the study was to identify quantitative trait loci (QTL) associated with incidence and severity of these two disorders. Since rib discoloration develops predominantly in crisphead type lettuce, we developed a recombinant inbred line (RIL) population consisting of 152 F_7 families derived by single-seed descent from a cross between two crisphead lettuce cultivars Emperor and El Dorado that are tolerant and susceptible to rib discoloration, respectively. The RIL population segregated for both rib discoloration and tipburn when evaluated at two maturities in one planting and one intermediate maturity in a second planting over two years. Four QTLs for rib discoloration were identified on three linkage groups and accounted individually for 7 to 11 % of the variation in the trait. Decrease in rib discoloration was associated with the Emperor allele at three of the QTLs and with the El Dorado allele at the fourth QTL. A single QTL for tipburn incidence was detected using multi-environment data and described 39 to 73% of the variation in this trait. Tipburn incidence and severity were highly correlated with head type (Empire versus Salinas) and the QTLs for these traits co-localized in the genome. The potential use of this QTL as a potential source of tipburn resistance in other types of lettuce and in other environments needs further investigation.

Bitter-sweet science: mapping quantitative trait loci for flavour in lettuce

Martin Chadwick, Ascension Martinez Sanchez, Carol Wagstaff

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Key words: lettuce, *Lactuca*, quantitative trait loci, nutrition, flavour, metabolite, sugar, sesquiterpenoid lactone

Flavour is one of the major components influencing consumer desire to make repeat purchase of lettuce and there is pressure on plant breeders to develop less bitter and sweeter varieties with a good field holding capacity. Sweetness is influenced by the concentration and balance of sugars in the leaf, with some sugars imparting a sweeter flavour than others. Bitterness in lettuce is primarily regulated by the presence of sesquiterpenoid lactones (SLs) in the leaf and the balance between SLs and sugars. There is a diverse range of SLs in the leaf that function in the plant as defence compounds, but some are also known to have potential health benefits for human consumers. Overall, individual SLs have contrasting levels of bitter taste, raising the possibility that the most bitter compounds could be selected against in a breeding programme if suitable markers were uncovered.

We are using the *L. sativa* cv. Salinas x *L. serriola* lettuce mapping population of 130 recombinant inbred lines in the F10 generation to map quantitative trait loci relating to SL and sugars in the leaves. Sucrose, glucose and fructose were initially assayed by a plate assay and SLs were identified and quantified by LC-MS. Pure standards of SLs were prepared using a fractionating HPLC. The mapping population was initially grown in controlled environment conditions, but the current season's experimental trial used field grown material with contrasting rates of nitrogen application. Mapping was conducted using MapQTL and MQM analysis.

The majority of the SL and sugar compounds examined showed good separation across the population. Significant QTL were mapped for a number of SLs and sugars and a QTL hotspot was revealed where fructose and glucose co-locate, possibly indicating a region of common regulation of these simple sugars. We are currently in the process of mapping a complete metabolite profile as a result of a recent NMR analysis of the entire population. Completion of this multi-trait analysis will enable the development of breeding markers for flavour and nutritional traits in lettuce and provide an insight into commonly synthesised secondary metabolites.

A genetic analysis of the introgression process from crops to wild relatives: Mapping Quantitative Trait Loci for plant vigour under abiotic stress conditions

Brigitte Uwimana, Clemens C.M. van de Wiel, Marinus J.M. Smulders, Richard G.F. Visser

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Key words: Lettuce, *Lactuca serriola* L., *Lactuca sativa* L., GM risk assessment, introgression, crop-wild hybrids, hybrid vigour, abiotic stress, salinity, drought, nutrient deficiency, quantitative trait loci

One of the discussed risks with regard to cultivation of genetically modified crops is the possibility of introgression of transgenes from crops to their wild relatives. After crop-wild hybridization, the persistence in later generations of the hybrids will depend on whether it confers a selective advantage. This will depend on the genetic make-up of the hybrid plant, consisting of a specific combination of wild and crop genomic blocks, on the environmental conditions, and the interaction between the environment and the genotypes.
To understand the contribution of the crop genome and how the crop genome interacts with the wild genome to the performance of the hybrids, we have initiated a study in which we follow the process of introgression from crops to wild relatives using lettuce crop-wild hybrids growing under abiotic stress conditions as a model system. Improved yield under abiotic stress conditions is one of the major objectives of GM crop development at present. We created hybrid generations from *Lactuca serriola* L. (wild prickly lettuce) and *L. sativa* L. (cultivated lettuce). We studied the performance of the hybrids in F_{1S1} and BC_1 hybrid populations under optimum conditions (no stress), drought, salinity and nutrient deficiency conditions. We genotyped the populations using Single Polymorphism (SNP) markers, constructed the genetic linkage maps of the two populations, then combined the phenotypic and genotypic data for Quantitative Trait Locus (QTL) analysis.
In the F_{1S1} and BC_1 populations we obtained QTLs associated with plant vigour under stress conditions as well as under control conditions. More than half of the stress-related QTLs were positively derived from the crop parent, indicating that lettuce crop-wild hybrids could receive traits from the crop that are advantageous for performance under abiotic stress conditions. Although many the QTL regions did not change from one treatment to another, QTL by treatment interaction was significant for biomass and plant height QTLs, suggesting that the mechanisms involved in tolerance to the different treatments were not the same. The QTLs were distributed across all lettuce linkage groups, but for some linkage groups, QTLs were concentrated in specific genomic regions. Such genomic regions are of interest for GM environmental risk assessment. Regions with beneficial QTLs could be avoided as a transgene inserted there would have more chance of persistence through genetic hitchhiking, while regions with non-beneficial or deleterious QTLs for the hybrids would be ideal for containment of a transgene.

NOT PRESENTED

Effect of salinity stress on germination indices in sweet basil (*Ocimum basilicum*) local Iranian cultivars

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Key words: sweet basil, Bojnord local varieties, radical length

In order to study the effects of salinity stress on germination indices in sweet basil local cultivars, an experiment was conducted in factorial form, using a completely randomized design with four replications by NaCl in controlled condition at Islamic Azad University Bojnord Branch in 2011. In this experiment, (four local cultivars) were evaluated at four levels of salinity treatment (distilled water, -3, -6, and -9 bar). The traits were: germination percentage, mean number of days for germination, seed germination, coefficient of germination, radical length, and shoot length considered as components of germination.
Results indicated significant differences among cultivars, salinity stress levels. In all traits, a significant decrease was observed with increase in stress level. Interactions were significant between variety and level of salinity, except germination percentage and shoot length. The lowest germination percentage belongs to Bojnord Rayhan sabz in -9 bar. The highest mean germination times belong to Varamin rahansiah cultivar. The highest coefficient of velocity of germination and speed germination belong to Bojnord local variety, at distilled water and -3 bar. Bojnord rahan siah variety had the lowest radical length, Varamin had longest shoot length in distilled water.

'Diseases and pests, disease resistance'

Current diseases and pests in lettuce in Western Europe

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Key words: *Lactuca*, fungi, bacteria, viruses, nematodes, insects

Lettuce is an important crop for Western Europe vegetables with 2.6 x 10^6 t in 2008. The first production is in Spain (38%) with mainly Iceberg lettuce, the second country is Italy (19%) with different types, and the third production is in France (13%). The first type in France is the Batavia (37%), followed by leaf-lettuce (31%) and butterhead (28%). The French production is over the year with protected crop in winter and open field in summer. The main area is the South-East with 56% of the French production, especially in winter under plastic tunnels. The second area is the North with 11% of the production, mainly in summer between May and October. Three other areas in the West are each producing 8-9 % of French lettuce.
Lettuce is susceptible to many diseases and pests all over the year, in the field as well as in protected culture. The damage for lettuce is not similar for all diseases and pests.
In recent years, the importance of some diseases decreased; for example, damage due to *Lettuce mosaic virus* (LMV) or to *Beet western yellow virus* (BWYV) disappeared from the field. Some fungal diseases, well known by the growers, are still a problem, like *Bremia lactucae* (downy mildew) with the overcoming of the genetic resistance introduced in new varieties, or *Botrytis cinerea* (gray mold) with adaptation to the used fungicides. Bacterial Leaf Spot (*Xanthomonas campestris* pv. *vitians*) is still more or less damageable, depending of the weather in the field.
Recently several soil-based diseases became more important with the break of the methyl bromide and certain pesticides. Among these diseases, there is especially the root knot nematodes (*Meloidogyne* spp.), several viruses transmitted by *Olpidium brassicae*, like the *Lettuce big vein virus* (LBVV), the *Mirafiori lettuce virus* (MiLV), and the *Lettuce ring necrosis agent* (LRNA). Moreover, aphids became a serious problem in some fields in several production areas, with the overcoming of the gene *Nr* conferring resistance to *Nasonovia ribisnigri*,; the damage due to *Pemphigus bursarius* became also more important from 2003, as well as the loss due to *Agriotes* spp., *Sclerotinia* spp., *Rhizoctonia solani*, and *Pythium tracheiphilum*. Other disease were introduced in Southern Europe, like *Fusarium oxysporum* f. sp. *lactucae* in Italy. This could be a threat for other countries close by, especially France where it was reported in the South-Eastern area.
Important for all breeders is the development of more research to provide alternative protection, like organic control with antagonist or Plant Defense Activator. The introduction of new resistance could be also a very efficient method of protection again these diseases and pests.

Race-specificity in interactions between *Lactuca* spp. and *Golovinomyces cichoracearum*

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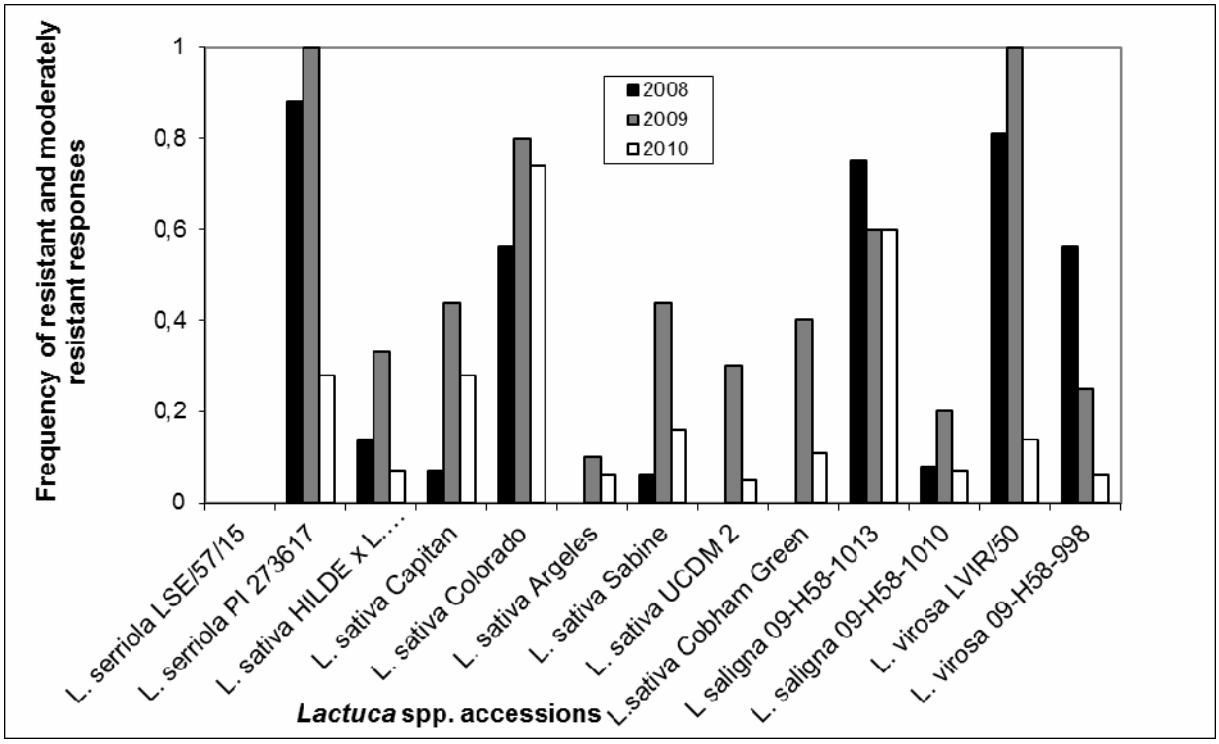
Key words: lettuce, powdery mildew, resistance, reaction patterns, races

Golovinomyces cichoracearum s. str. (DC) VP Gelyuta is a worldwide spread obligate biotrophic fungus, which infects predominantly plant species of family Asteraceae, on cultivated crops can be dangerous on lettuce and chicory (Braun, 1995; Davis et al., 1997). Representatives of *Lactuca* spp. are one of the main hosts of this pathogen; in Europe the following *Lactuca* spp. are affected by powdery mildew: *L. muralis, L. perennis, L. quercina, L. serriola, L. saligna, L. sibirica, L. viminea* and *L. virosa* (Lebeda, 1994, 1999). The development of infections occurs in dry and warm weather from early summer to early autumn. *G. cichoracearum* infects leaves and stems at mature stage primarily (Lebeda and Mieslerová, 2010). The aim of this work was to characterize pathogenicity variation of *G. cichoracearum* isolates collected in wild populations of prickly lettuce (*L. serriola*) in the Czech Republic.

The several expeditions were undertaken during summer 2008-2010 as a part of complex research of interactions between prickly lettuce (*L. serriola*) and powdery mildew (*G. cichoracearum*). Observations were mainly focused on recording of natural infection of powdery mildew on prickly lettuce in localities in East Bohemia and Moravia. The powdery mildew isolates were obtained from single pustules of *G. cichoracearum* occurring on *L. serriola* leaves and maintained on plants of highly susceptible *L. serriola* (LSE/57/15). For study of pathogenic variability of *G. cichoracearum* s. str. was used the differential set of 13 *Lactuca* genotypes (2 *L. serriola*, 6 *L. sativa*, 1 hybrid *L. sativa* × *L. serriola*, 2 *L. saligna* and 2 *L. virosa*) (Mieslerová et al., 2009). During 2008-2010 the number of collected and studied *G. cichoracearum* isolates varied (16 in 2008, 13 in 2009, and 19 in 2010). The leaf discs bioassay was used. The leaf discs were cut from the leaves of *Lactuca* spp. plants (8-week-old) and the upper side of each leaf disc was inoculated by surface contact with fresh conidia of *G. cichoracearum*. For assessment of degree of infection 0-3 scale was used. The reactions were divided to the three categories: R – resistant (% max DI < 30); MR – moderately resistant/susceptible (% max DI 30-60); S – susceptible (% max DI > 60).

The recent and more-less stable differential set of *Lactuca* spp. accessions was developed according to the results from previous study (2005-2007) and consists of accessions which in previous years showed the most differential response patterns to infection of *G. cichoracearum* isolates (Mieslerová et al., 2007). *G. cichoracearum* isolates collected in the Czech Republic during 2008-2010 differed in pathogenicity towards *Lactuca* spp. The incompatible interactions were represented by highly resistant responses (without sporulation and mycelia development); moderately resistant responses were characterized with limited sporulation. There was recorded slight variation in frequency of resistant and moderately resistant responses during 2008-2010; in most of studied accessions in 2009 the frequency of resistant responses was higher, while in 2008 was lower (with some exceptions). The highest percentage of resistant responses showed *L. sativa* (cv. Colorado), *L. saligna* (09-H58-1013), *L. virosa* (LVIR/50) and *L. serriola* (PI 273617) (Fig. 1). None of the tested accessions were found to be resistant to all studied isolates of *G. cichoracearum*. The obtained results confirmed the existence of race-specific interactions in natural populations of *G. cichoracearum* on prickly lettuce and also temporal changes of pathogenicity during the studied period. We have no information about genetic basis of these interactions. The results could be used as a background for lettuce resistance breeding against powdery mildew.

Fig. 1. Frequency of resistant and moderately resistant responses in the interactions of *Lactuca* spp. accessions and *G. cichoracearum* isolates



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Plant genotype influences lettuce colonization by the Enterobacteriaceae

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Key words: Lettuce, phyllosphere, bacterial populations, Enterobacteriaceae, plant genotype

The aerial part of the plant (phyllosphere) is known to support large and diverse naturally occurring bacterial communities which have been shown to vary between plant species. Numerous environmental and plant factors have been associated with this variation, however, much less is known about the drivers of variation in such communities within plant species. The Enterobacteriaceae are of particular interest as this taxonomic group contains some of the most important bacterial genera associated with food poisoning outbreaks, particularly *Salmonella* and *E. coli*. Both of these genera have been implicated in food poisoning outbreaks traced back to minimally processed salad crops.
In an attempt to identify some of these drivers, the naturally occurring bacterial phyllosphere populations from twenty six lettuce accessions were compared using terminal restriction fragment length polymorphism (T-RFLP) profiling (a culture-independent profiling technique). Results were compared with morphological and physiochemical data collected for each of the lines at two growth stages; 4-weeks post-emergence (baby leaf) and 10 weeks post-emergence (mature). The bacterial populations showed significant diversity between the lettuce accessions. Statistical analyses revealed that plant traits with complex underlying genetic controls such as those influencing plant architecture and leaf surface topology, leaf surface waxiness, tissue water content and levels of calcium, soluble carbohydrate, phenolic compounds in the plant tissue were associated with these differences. A more detailed investigation into the composition of the populations using 16S rRNA gene clone library analysis of bacterial samples from three lines (cv. Iceberg, cv. Saladin and the wild lettuce relative, *L. serriola*) which are parents of two genetic mapping populations, showed that variation in the Enterobacteriaceae was a major component of these differences (Hunter et al (2010) Applied and Environmental Microbiology 76(4): 8117-8125). Further experiments showed significant differences in the ability of the parental lines to support both epiphytic (external) and endophytic (internal) colonization by a non-pathogenic strain of *E. coli* which has been used successfully to mimic pathogenic strains in attachment studies. Cultivar Saladin showed the highest level of epiphytic colonization followed by cv. Iceberg, with *L. serriola* showing the lowest level. In terms of endophytic colonization, cv Iceberg and *L. serriola* showed similar levels and were both more heavily colonized than cv. Saladin. The availability of well characterised genetic mapping populations should allow for the genetic mechanisms underlying these differences to be better identified and potentially lead to genetic markers for factors which may influence the risk of pathogen contamination.

Fine mapping nonhost resistance in lettuce to downy mildew

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Key words: Lettuce, downy mildew, nonhost quantitative resistance, fine mapping

Bremia lactucae causes downy mildew in lettuce (*Lactuca sativa*) and leads to high yield losses in commercial lettuce cultivation. Breeding for resistance by single dominant resistance (R) genes is effective; however those genes are easily broken by the pathogen. Nonhost resistance from related *L. saligna* might be a good durable alternative. In an earlier phase we tested F2 populations and a set of 29 backcross inbred lines (BILs), each with a single introgressed chromosomal segment from *L. saligna* in the *L. sativa* genetic background; this set of BILs represented 96% of the *L. saligna* genome [1,2]. These disease tests revealed that the nonhost resistance of *L. saligna* is not explained by R genes but by multiple resistance QTLs [3,4]. The resistance of most of the resistant BILs was plant stage dependent. One of the BILs (BIL8.2), with an introgression of 26 cM long, was effective in both the young and adult plant stage with an infection reduction of 70% and 30%. In order to fine map the resistance within the *L. saligna* introgression of this BIL and to separate this resistance from undesired traits, we developed near isogenic lines (NILs) with smaller *L. saligna* introgressions. Disease evaluation of those NILs both at young and adult plant stage revealed four QTLs. From those four QTLs; QTL1 reduces the infection level in both young and adult plants; QTL2 at the young plant stage only; QTL3 at the adult plant stage only; and QTL4 neutralize the effect of QTL1 at the adult plant stage. NILs with introgressions that covered QTL1 and QTL2 spanning 6 cM, could explain the infection reduction of 70% at young plant stage. Only one NIL that contained two introgression regions (5 and 8 cM) covering QTL1 and QTL3 and lacking QTL4 could explain the 30% infection reduction of the original resistant BIL8.2 at adult plant stage.

Virulence of *Bremia lactucae* populations in Southern France between 2006 and 2011

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Key words: downy mildew, *Lactuca*

Bremia isolates collected in the Avignon area between 2006 and 2011 were studied for virulence spectrum and compared to some isolates from other French areas. The collection processed from different origins: (1) isolates collected in protected culture of 4 growers on lettuce cultivars which were rather new varieties with large resistance, and also in air with "trap boxes"; (2) isolates from other growers near Avignon; (3) many isolates collected in INRA lettuce cultures on accessions of old and new varieties in plastic tunnels in winter or in open field in May and (4) several isolates harvested on *Lactuca serriola* often neighbour to lettuce cultures. The virulence was studied in artificial inoculation on 19 to 25 differential hosts.

In Avignon, 70 isolates were collected on lettuce cultivars, 25 were collected in "trap boxes" and 8 came from *L. serriola*; in other place of Vaucluse, 16 isolates were collected on cultivars, 4 on *L. serriola* and 2 in "trap boxes". All these 125 isolates could be classified in 2 groups present from fall 2006 to spring 2011. One group was collected only on *L. serriola* and cv Kigalie. These isolates were not virulent on Cobham green, neither on *Dm1* to *Dm 6*, neither *Dm10* to *Dm13*; but they were virulent on *Dm7*, *Dm15*, *Dm16*, *R17*, *R18*, *R38*. The other group was collected on many cultivars with different resistances. These isolates were similar to BI: 22, BI: 24 or BI: 25, not virulent on Dandie (*Dm3*) and LS102 (*R17*); some were virulent on Discovery (*Dm7*, *Rsal*), some others were not.

Only in March 2010 and in 2011, few collected isolates were virulent on Dandie (*Dm3*); these isolates were collected on 2 cultivars, on *L. serriola* or in "trap boxes". These 7 isolates were also virulent on LS102 (*R17*), but were not virulent on LSE/18 (*Dm16*), Colorado (*R18*) and Discovery (*Dm7*, *Rsal*). These types of isolates were collected also near Lyon in March 2009 on 2 other lettuce cultivars.

The isolates collected on the farm of one grower showed a global stability of virulence for 4 years on 5 cultivars with different resistances and in "trap boxes". Isolates collected on *L. serriola* and on "trap-plants" in INRA culture, or in "trap-boxes" placed in grower culture, show the presence of *Bremia* strains that were not collected in cultivated varieties. The *Bremia* isolates collected in one open field on old varieties were similar with those harvested on new cultivars.

These results will be discussed in relation with the virulence of European BI strains.

Population structure of *Bremia lactucae* isolated from lettuce culture in France

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Key words: *Bremia lactucae*, population genetics, clonality, selection pressure

Bremia lactucae, the causal agent of lettuce downy mildew is an important economic problem for the lettuce crops. To limit the damages caused by this pathogen, breeders used race-specific monogenic resistances. However, under selection pressure, *B. lactucae* populations showed a rapid adaptation to host resistances. Characterizing of the population genetic structure could be an important step toward the identification of the evolutionary strategy used by *Bremia* to overcome host resistance. Thus, in collaboration with Gautier Semences and Rijk Zwaan, we compared the genetic diversity of *Bremia* isolates collected in France since 2006 with the diversity of European isolates and official races (Bl: races). We also studied the population structure of French *Bremia* isolates.

Twelve microsatellites were specifically developed for *B. lactucae* in order to analyze the genetic diversity of the lettuce downy mildew. Isolates were recovered from different varieties of lettuce, carrying different resistance genes, from the most important production areas of lettuce in France. More than 400 isolates were genotyped using these twelve markers.

Eighteen different multilocus genotypes (MLG) were observed for 20 different official Bl: races, 17 MLG were observed for 30 European isolates, whereas only twelve different MLG were identified for 340 isolates collected in France. Two out of these twelve MLG were found the most frequently at French scale and were observed in almost all the lettuce production areas that had been sampled. This low genetic diversity in France indicated high clonality in all populations and important gene flow between populations. However, different indexes suggested that rare events of sexual recombination occurred, especially in populations of Western France, where genotypic diversity was slightly higher. Moreover, analyzing the population genetic structure suggested the presence of different clonal lineages, possibly resulting from selection pressure of resistance genes.

These results will be discussed considering the impact of host resistance gene on the population structure of *B. lactucae.*

'Genetics and biotechnology'

Genetics and genomics of disease resistance in lettuce

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Key words: *Lactuca sativa*, Disease resistance, Genome sequencing, Plant-pathogen interactions.

The advent of inexpensive DNA sequencing opens up several opportunities for characterization of plant germplasm, the identification of genes for disease resistance, and the development of more durable strategies for disease control. Sequencing of the lettuce genome is underway in collaboration with the BGI and a consortium of ten breeding companies. Whole genome shotgun data are being integrated with gene space and transcriptome assemblies as well as with an ultra-dense transcript-based genetic map of over 13,000 unigenes. Syntenic comparisons to sequenced plant genomes are providing insights into the evolution of the lettuce genome as well as assisting in map-based cloning of disease resistance genes. The genomic architecture of disease resistance in lettuce is being determined by genetic analysis of resistance to over ten diseases resulting in the identification of more than 50 phenotypic disease resistance loci. Resistance to most but not all diseases maps to NBS-LRR encoding genes. Genetic associations between resistance phenotypes and candidate genes are being validated by RNAi. Genes for resistance to diverse diseases are clustered in the genome. Knowledge of the genetic architecture of resistance allows pre-emptive breeding to prevent breeding for resistance to one disease inadvertently introducing susceptibility to another. The genome sequences of several bacterial non-pathogens are available and have provided insights into the basis of non-host resistance. The genome of the number one pathogen of lettuce, the oomycete *Bremia lactucae*, is also being sequenced. This sequence is providing genes encoding candidate virulence effector proteins. These are being analyzed using yeast two-hybrid protein-protein interaction assays to determine whether they target the same points of vulnerability in the plant as bacterial effector proteins. In addition, high-throughput sequencing provides the opportunity for high-resolution analysis of variation in pathogen populations. Combined with the establishment of a pipeline for the introgression of disease resistance genes, this will allow the strategic deployment of resistance genes that should fragment selection pressures on pathogen populations, consequently slowing the evolution of virulent isolates and resulting in more durable disease control.

Mapping quantitative trait loci for sugar in a RIL population of Lettuce

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Keywords: lettuce, RILs, QTLs, sugars, glucose, fructose, sucrose, organoleptic quality

It is well known that carbohydrates play important roles in plant growth and development, in stress responses, and as signalling molecules to contribute to organoleptic quality of fruit and vegetables. Improving organoleptic quality is an important but complex goal in fresh-cut lettuce. The organoleptic quality of fresh-cut lettuces involved parameters as flavour, texture and odour. The principal compounds that influence flavour in lettuce are organic acids, phenolic and sesquiterpenoids compounds as well as sugars. Sugars are one of the compounds that provide an appealing sweet flavour in lettuce. On the other hand, offodours are detected sometimes during the storage in fresh-cut lettuce, especially in fresh-cut iceberg, which have been associated with fermentation process, and tentatively related to sugar content. We have studied quantitative trait loci (QTLs) associated with the sugar (glucose, fructose and sucrose) content using a population of 97 F10 recombinant inbred lines (RILs) from a cross between *Lactuca sativa* and *Lactuca serriola*. The two parent lines have different sugar content. *L. sativa* showed the highest content in glucose, fructose and sucrose. RILs showed a large range of variation for all the traits. A genetic map with 1,335 markers was used, and several QTLs were detected by simple interval mapping and composite quantitative mapping. These analyses identified two significant QTLs for glucose explaining a range of 12.6 – 16.4% of the phenotypic variation, and in the case of fructose identified five significant QTLs explaining a range of 10.0-33.1% of the phenotypic variation. These QTLs are distributed in different chromosomes. However, one of the QTLs for fructose is co-located with one of QTLs for glucose. This result could be due to the fact that both are monosaccharides and could be relative to their common biosynthetic of signalling pathway. Our results are an important step toward marker-assisted selection for sugarrelated traits and help to maintain the quality of fresh-cut lettuce.

Genetic analysis of the genes for dioecism and monoecism in *Spinacia oleracea* L.

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Key words: AFLP, F1 hybrid, Sex chromosome, Sex determination, Spinach

Spinach (*Spinacia oleracea* L.) is one of the most nourishing leafy vegetables being grown worldwide, mostly in countries with a temperate climate. The crop is generally considered as a dioecious species with an even ratio of female and male individuals. However, certain lines and crosses will produce monoecious individuals, among which the ratio of female to male (or hermaphrodite) flowers varies widely.

In developed countries, most of the current spinach cultivars are F1 hybrids. The hybrid seeds production in its infancy was carried out with roguing of male individuals in dioecious lines designated as seed parents. But, now most of hybrids are produced from seed parent lines breeding true for highly female monoecious character. Furthermore, present breeding programs aim at using highly male monoecious inbred lines as pollen parents.

Sexual dimorphism in dioecious lines has been shown to be controlled by an allelic pair termed *X* and *Y*. Monoecism was proposed to be controlled by a single major gene (*Xm*), which is allelic to the *X*/*Y* (Janick and Stevenson 1954). However, the single major gene alone is insufficient to completely explain the wide range of monoecious characters actually observed. Several lines of evidence indicate an involvement of (an) additional allele(s) and/or modifier(s) in monoecism. However, nothing further is known about monoecism in spinach, and the molecular basis of sex determination has not yet to be described.

Ten AFLP markers, closely linked to the *X/Y* locus, were identified using bulked segregant analysis, four of which were revealed to cosegregate with *Y* in the present mapping population. We mapped the AFLP markers and two known male-specific DNAs to a 13.4 cM region encompassing the locus. These markers will be the basis for positional cloning of the sex determination gene.

We also showed that a single, incompletely dominant gene is responsible for the highly male monoecious character. The gene was found to be located at a distance of 4.3 cM from microsatellite marker SO4, which mapped 1.6 cM from the *X/Y* locus. This indicates that the monoecious gene is allelic to or closely linked to the *X/Y* gene pair. SO4 will enable breeders to efficiently select highly male monoecious plants for preferential use as the pollen parent for hybrid seed production. We are currently investigating the allelism between the male-determining gene (*Y*) and the monoecious gene. Moreover, we also have constructed a spinach genomic BAC library, not only toward development of more accurate diagnostic markers for sex determination but also toward isolation of the sex determination gene(s).

Identification of alleles conferring delayed bolting in lettuce

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Key words: EMS-mutagenesis, polymorphism, flowering time genes, delayed bolting

Cultivated lettuce, *Lactuca sativa*, is a major horticultural crop, being one of the world's most important leafy salad vegetables. UK production of lettuce in 2009 covered an area of 5592 hectares producing 117,300 tonnes of crop, with a home production value of £84.7 million.
The timing of bolting/flowering is a significant factor in lettuce production since it affects crop quality, yield and scheduling of production. Associated with the initiation of bolting, which occurs before any visible signs of the bolt appears, is the biosynthesis of secondary metabolites. Such compounds include the sesqiuterpene lactones, that may be produced to protect the floral buds from insect predators, and these give the leaves a bitter taste. This is an undesired trait for human consumers and bolting thus renders the crop unsaleable. Bolting is promoted by higher temperatures and with the increase in temperature predicted to continue with climate change bolting in the field will become an increasing issue.
Significant advances in the understanding of the regulation of flowering time have been gained from studies in *Arabidopsis*. The precise timing of floral initiation is governed by complex interactions between several endogenous and environmental factors and the key genetic pathways underpinning the flowering process have been elucidated. Studies in several species reveal conservation between these genetic components, although studies in lettuce have been limited.
Cultivated lettuce lines carrying EMS-induced mutations have been identified that have altered bolting characteristics in relation to wild type plants. Late bolting lines have been back-crossed to reduce background mutations and homozygous plants identified. Growth of late bolting lines in the glasshouse under natural light and artificially lit long day conditions, under controlled environment conditions at a range of specific temperatures and under different field conditions demonstrated the robustness of the late bolting phenotypes in the lines generated.
Homologues of genes involved in the photoperiodic, autonomous and vernalisation floral induction pathways, as well as the floral pathway integrator *FLOWERING LOCUS T (FT*) have been identified in lettuce. Genomic sequence, including upstream regulatory regions, and cDNA sequence have been isolated for each of these target genes. Functional complementation studies have been carried out in *Arabidopsis* for a subset of the genes, which demonstrate that they act as regulators of flowering time. PCR-based and high throughput sequencing technologies are being used to detect polymorphisms in these target genes, and also within unknown genes between wild type and late bolting lines. Any polymorphisms detected are screened in segregating populations to determine whether they co-segregate with, and are the cause of, the late bolting phenotype.
New alleles that are identified in the late bolting lines can be used in breeding programs aimed at delaying bolting and improving the holding ability of commercial lettuce crops.

Genetic and genomic resources in *Cichorium intybus* L. (Asteraceae) and their applications in fundamental and applied research on reproduction

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Key words: chicory (*Cichorim intybus* L., Asteraceae), reproduction (vegetative, sexual), genetics, genomic resources, somatic embryogenesis, self incompatibility, male sterility (nuclear, cytoplasmic)

Resolving the molecular mechanisms underlying traits controlling sexual and vegetative reproduction is a key factor in the improvement of chicory breeding but will also provide new insights into these traits which so far are poorly understood at the molecular level in chicory and other Asteraceae. An overview of the genetic and genomic resources for chicory, as well as the molecular tools to explore and exploit them, applied in our studies on the reproduction traits somatic embryogenesis (SE), sporophytic self incompatibility (SSI), and male sterility (ms), will be presented.

To identify genes underlying traits controlling reproduction in chicory, we use a combination of gene and/or QTL mapping, and map-based cloning. A candidate gene approach is often applied in parallel, by mapping genes identified in chicory or other (model) plants. The genetic analyses are performed on different mapping populations showing segregation for one, but mostly a combination, of the traits under study. A consensus genetic map based on three mapping populations was established containing 472 transferable codominant SSR and SNP markers covering the 9 chromosomes of the *C. intybus* haploid genome (Cadalen *et al* 2010). For one mapping population the genetic map was complemented with AFLP markers. Furthermore, two BAC libraries of chicory were constructed for map-based cloning from two genotypes that differed in traits related to sexual and vegetative reproduction. The two libraries together contain 170,500 clones and cover 12.3 haploid genome equivalents (Gonthier et al 2011) The candidate gene approach is facilitated by over 80,000 *Cichorium* (chicory and endive) EST sequences that are publicly available on the sites NCBI dbEST and Compositae Genome Project.

The induction of somatic embryogenesis (SE) in chicory has been studied for over 20 years in our laboratory using the interspecific hybrid '474' (*C. intybus* × *C. endivia*). Genetic variability with respect to SE was found present in the Hungarian landrace 'Koospol' from which the *C. intybus* parent of the hybrid '474' originated. Among plants from this landrace, embryogenic (E, K59) and non-embryogenic (NE, K28) genotypes were identified, and crosses among them were realized to identify genes implicated in the SE process. Interestingly, the K28xK59 progenies also showed segregation for traits related to sexual reproduction, in particular SSI and nms, and thus allowed us to initiate molecular genetic studies on these traits as well.

The identification of E and NE genotypes in a similar genetic background allowed the creation of E and NE cDNA libraries by suppression subtractive hybridisation, using mRNAs isolated from E and NE explants. Transcriptomic analyses of genes represented by cDNAs in either library allowed the identification of several genes differentially expressed during SE, and thus represent candidate genes possibly implicated in this process (Legrand 2006, Legrand *et al* 2007, Lucau-Danila *et al* 2010).

QTL analysis in progenies obtained from crossing K28 (NE, male sterile) x K59 (E, hermaphrodite) was performed by determining the number of plantlets formed from root explants after SE induction, and resulted in finding several chromosomal regions that

showed co-localization of QTL with one or more candidate genes identified previously. These results should allow the identification of genes of which the expression is causally implicated in direct SE in chicory, and may help the introgression of this trait into commercially interesting chicory varieties that lack SE competence. In addition, genotypes with high SE or regeneration capacities are being used to develop a transformation protocol for the functional validation of cloned genes.

Chicory is naturally allogamous, with a self incompatibility sytem that is sporophytically determined by a single S-locus. Complete diallele analysis in the K28xK59 progeny allowed the identification of 4 S-haplotypes, and the S-locus was mapped on linkage group 2 of the consensus map of chicory. A screen of 2500 descendants from the K28xK59 cross resulted in identifying molecular markers closely linked to the S-locus that are being used to screen the BAC libraries to obtain clones containing the S-locus. A detailed report on the progress of the map-based cloning of the S-locus will be presented separately (Gonthier 2011)

Recently, we learned that self pollen germinate on immature stigmas, indicating that the female determinant of the SSI system is installed relatively late during flower development. This result formed the basis for a transcriptomic strategy to identify the gene encoding the female SSI determinant, and that was initiated by the extraction mRNAs from immature (self compatible) and mature (self incompatible) stigmas from two groups of plants with different S-haplotypes.

Though naturally self incompatible, some seeds may be obtained upon selfing in chicory, and in witloof chicory some varieties have been selected for a high seed set upon selfing. Thus, in chicory ms is an important tool for F1 hybrid production in circumventing the time-consuming emasculation of flowers of the seed producing plants. In chicory, a single nuclear ms source is known under the name 'Edith' (Desprez *et al* 1994), whereas cytoplasmic ms was introduced by fusion of chicory protoplasts with protoplasts of CMS sunflower (Rambaud *et al* 1993).

Segregation of ms 'Edith' in the K28xK59 progeny allowed us to map the *nms1* locus to linkage group 5 of the consensus map, and a detailed cytological analysis learned that in *nms1* microspores were degenerated shortly after meiosis. By comparing the events and their timing with those published for ms mutants in Arabidopsis and rice, we selected 2 candidate genes, both encoding bHLH transcription factors. Subsequent genetic analysis of the polymorphisms displayed by each of the two candidate genes learned that one of them co-localized with the *nms1* locus. The complete sequences of the alleles *NMS1* and *nms1* was determined from BAC clones and showed that the *nms1* allele contained a CACTA-type transposon. That this was the causal mutation conferring ms was confirmed upon the identification of male fertile revertants in which the transposon had left the *nms1* locus.

As for CMS, we focus on the identification of CMS restorer genes. Soon after the introduction of CMS in chicory by protoplast fusion, crosses with a certain genotype of chicory revealed that the artificially introduced CMS could be restored. To further explore this intriguing phenomenon, we try to identify the gene(s) responsible for the restoration by a similar strategy as outlined above.

Cadalen *et al* (2010) Mol Breeding 25:699-722
Desprez *et al* (1994) CR Acad Agric Fr 80 (7): 47-62
Gonthier *et al* (2010) BMC Research Notes, 3:225-234
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Rambaud *et al* (1993) Theor Appl Genet 87, 347-352;

Towards the map-based cloning of the S-locus in *Cichorium intybus* L., a self incompatible Asteraceae species

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Key words: chicory (*Cichorium intybus* L., Asteraceae), self incompatibility, S-locus, map-based cloning

Self incompatibility (SI) is one of the most important mechanisms to prevent selfing in hermaphrodite plants. This mechanism is generally under the control of a single multiallelic locus, the S-locus. Pollen and pistil determinants have been identified in some plant families (Brassicaceae, Solanaceae, Convolvulaceae) and are different in each family, suggesting that different molecular mechanisms of SI have evolved independently. Chicory (*Cichorium intybus* L.) is a sporophytic self incompatible species belonging to the Asteraceae family for which these determinants are different and still unknown.
Our goal is the positional cloning of male and female determinants of SI in chicory. We assigned the S-locus to one end of one of the 9 linkage groups of the chicory consensus map (Cadalen et al, 2010). In order to obtain a high density high resolution map we have developed a marker-assisted BSA strategy and have genotyped more than 2500 individuals to increase the number of specific markers in the S-locus region. In parallel, two 6X BAC libraries were constructed from two genotypes with different S-alleles (Gonthier et al, 2010). The most tightly linked markers to the S-locus are being used to screen both libraries to obtain a physical map. The results obtained until now (Gonthier 2011) will be presented.

Cadalen et al (2010) Mol Breeding 25:699–722
Gonthier et al (2010) BMC Research Notes, 3:225-234
Gonthier (2011) PhD thesis, Université Lille Nord de France, USTL1, Lille, France

‘Germplasms and their diversity’

Genetic resources of leafy vegetables in Europe: conservation and use

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Key words: European cooperation, Germplasm utilization, PGR management

Leafy vegetables constitute a highly variable group of crop plants. Chicory, lettuce and spinach are generally regarded as the main leafy vegetables. A fourth group, denoted by 'minor leafy vegetables' includes, amongst others, artichoke, asparagus, lamb's lettuce, rhubarb and rocket salad. In 2003, the leafy vegetables working group of the European Cooperative Programme for Plant Genetic Resources (ECPGR) was established in order to create a cooperative basis for the conservation and utilization of genetic resources of leafy vegetables in Europe. Priority areas identified by the working group included documentation, characterization and evaluation, regeneration, safety duplication, redundancies within and among collections and crop-related wild relatives. Outputs of the working group included the International *Lactuca* Database (ILDB) and minimum descriptor lists for the characterization of various leafy vegetable crops (http://www.ecpgr.cgiar.org/Workgroups/Leafy_Vegetables/Leafy_Vegetables.htm). Support to the activities of the working group was provided by the EU funded project 'Leafy vegetable germplasm, stimulating use', carried out from 2007 until the end of 2010 by 12 participating organizations from 10 European countries (http://documents.plant.wur.nl/cgn/pgr/leafyveg). Within the framework of this project the ILDB was updated, while new databases were developed for spinach, chicory and minor leafy vegetables, respectively. Gaps within the newly established databases were identified and priorities for future acquisition were recommended. Safety duplication of accessions was realized for project members that previously lacked such an arrangement. Experimental work in the project was carried out for lettuce, spinach, chicory, rocket salad, lamb's lettuce and orache, and included removal of backlogs in regeneration, morphological characterization and evaluation of accessions for resistance to pests and diseases, quality and abiotic characters. In addition, accessions were evaluated for utilization and marketing purposes in order to identify germplasm with potential for the development of varieties for local markets. All experimental project data were linked to the databases and were made publically available as downloadable files (http://documents.plant.wur.nl/cgn/pgr/LVintro). Currently, joint activities concerning leafy vegetables in Europe are carried out in the framework of the ECPGR project 'A European Genebank Integrated System' (AEGIS) aiming at the establishment of a virtual European collection (http://www.aegis.cgiar.org/). For this purpose, activities are focused on the nomination of the 'most appropriate accessions' in national collections and on the development of minimum standards for gene bank operations involving leafy vegetables.

Distribution and variation of wild *Lactuca* species in North America – a challenge for future lettuce breeding

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Key words: *Lactuca serriola,* USA, Canada, geography, ecology, distribution, variation, morphology, DNA content, genetic polymorphism

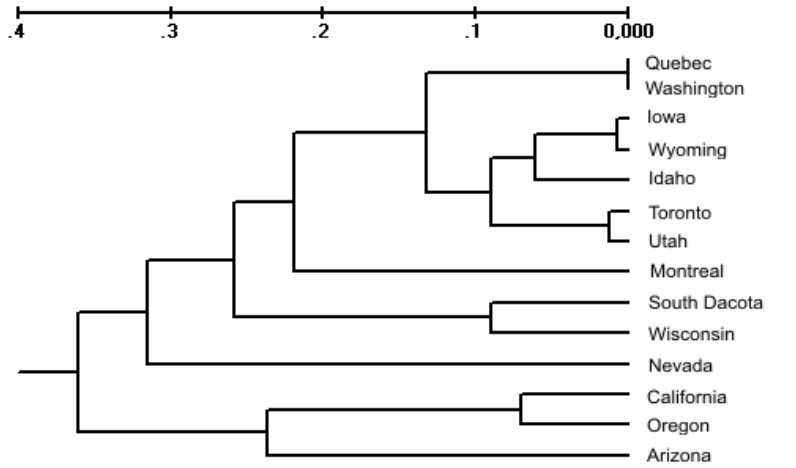
The wild relatives of *Lactuca* spp. constitute a broad genetic base that can provide essential materials for lettuce breeding (Lebeda et al., 2007, 2009). During the last 30 years, there has been considerable progress in the collection, characterization, and practical application of *Lactuca* L. germplasm collections, as well as in filling in gaps in our knowledge about the biology and ecology of many *Lactuca* species (Lebeda et al., 2009). However, current information about the geographic distribution of wild and weedy *Lactuca* species in North America is still incomplete (Lebeda et al., 2004b), and readily available genetic resources of *Lactuca* from this geographic area are limited (Lebeda et al., 2004a). For these reasons, exploration missions to the United States and Canada were conducted (Lebeda et al., 2011). The aim of the research described herein is to summarize information on the ecogeography of *Lactuca* spp. in North America, to show the preliminary results on phenotypic and genotypic variation of accessions collected, with a strong emphasis on *L. serriola* L.

To study the distribution and ecogeography of wild and weedy *Lactuca* in North America, extensive parts of the United States and Canada were explored in the years 2002, 2004, 2006 and 2008. In the United States, 16 states representing the Northeast (New York), Midwest (Wisconsin, Iowa, Minnesota, South Dakota), West (Wyoming, Montana, Idaho, Utah, Colorado, Arizona, Nevada, California, Oregon, Washington) and South (North Carolina) were surveyed. In Canada, expeditions were made only in 2 provinces (Quebec, Ontario). Morphological assessments of 231 *L. serriola* seed samples were performed during the course of their regeneration (during the vegetative period in 2009) in a greenhouse under controlled conditions. Assessments included 12 quantitative and qualitative characters of stems, rosette and cauline leaves, inflorescences and flowers, following published descriptor lists for wild *Lactuca* species (Doležalová et al., 2002). Variation of absolute content of nuclear DNA was measured in ten genotypes of *L. serriola* from USA and Canada. A set of 165 *L. serriola* accessions were analysed using allozyme analysis (6-PGDH, PGI, PGM, GOT, LAP, ME, and NADH-DH) in order to to reveal the level of genetic variability. A subset of 92 accessions covering a broad transect of territory between the southwest (California, USA) and northeast (Quebec, Canada) was used for preliminary evaluation of genetic differentiation of *L. serriola* accessions using AFLP analysis (Lebeda et al., 2011).

In total, seven wild and weedy *Lactuca* species (*L. serriola*, *L. saligna* L., *L. virosa* L., *L. canadensis* L., *L. biennis* (Moench) Fernald, *L. floridana* (L.) Gaertn., *L. ludoviciana* (Nutt.) Riddell), an interspecific hybrid (*L. canadensis* × *L. ludoviciana*), and an undetermined *Lactuca* species were observed and collected in 200 North American locations (Lebeda et al., 2011). Field observations and detailed surveys of naturally occurring populations provided new information on geographic distribution, ecology, and inter- and intrapopulation structure of *Lactuca* species in North America (Lebeda et al., 2011). Based on the morphology of rosette and cauline leaves, 80% of plants representing 231 North American accessions could be assigned to *L. serriola* f. *serriola.* Formation of basal rosettes was recorded in 77% of the plants, while the remaining ones quickly bolted. The mean value of absolute 2C DNA content of *L. serriola* as determined was 5.86 pg and ranged from 5.79 to 5.89 pg. The lowest absolute 2C DNA value was found in a sample originating from Toronto, Ontario, the highest was in a sample from Dodgeville, Wisconsin. Differences within individual samples were low, ranging from 1.7% in a sample from Buffalo, Wyoming to 2.89%

in a sample from Toronto. During allozyme analysis seven enzymatic systems (6-PGDH, PGI, PGM, GOT, LAP, ME, and NADH-DH) were resolved and 15 loci were interpreted (66.67% polymorphic at 95% criterion). A low level of genetic variability indices were observed (H_o = 0.000, H_e = 0.204) and indication of grouping of samples according to its geographical origin on an UPGMA (Coancestry coefficient) dendrogram (Fig. 1). However, this grouping in sensu of geographical distribution is not strict and there is a weak bootstrap values supporting this distribution.

Fig. 1. UPGMA (Coancestry coefficient) dendrogram based on interpretation of 15 allozyme loci of 14 geographical areas on North American continent.



Nevertheless, results of isozyme analysis supportst our findings based on AFLP data (Lebeda et al., 2011). In total we detected 324 AFLP fragments with 268 (82.7%) polymorphic among 47 accessions analyzed. Bootstrapped UPGMA clustering distinguished accessions into four major groups with significant bootstrap values. Evidently, the Sierra Nevada Mountains and other geographic barriers in the west form a natural boundary between *L. serriola* populations collected along the Pacific coast and in the southwest and those populations sampled northward and eastward (Rocky Mts., Great Plains and Southeastern Canada). We intend to continue in detailed morphological, phenological, resistance and molecular polymorphism studies to elucidate relationships among *L. serriola* populations in North America.

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Contribution of the French network on chicory genetic resources to a European *ex situ* management of leafy vegetables genetic resources

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Key words : chicory, network, disease resistance evaluation, utilization, genetic resources

The *Cichorium* genus consists of two main cultivated species but many production methods and different consumption uses: cut and plain endives (*C. endivia* L.), witloof and Italian leaf chicory (*C. intybus* var *foliosum*) are consumed as raw or cooked salads and the root chicory is used either for roasting or inuline production (*C. intybus* var *sativum*). The European varieties Catalogue contains approximately 500 chicory varieties divided in 5 sub-lists: 140 in the cut endive type, 120 in the plain endive type, 60 in the witloof type, 130 in the leaf chicory and 40 in the root chicory type. Due to this diversity, breeding objectives vary depending on the species and uses: adaptation to novel growing habits for cut and plain endives and Italian leaf chicory, and productivity and improvement of nutritional and taste components for witloof and root chicory. Nevertheless, disease resistance represents one common breeding aim especially for witloof and cut and plain endives and mainly in order to respect the EU and national legislations on MRL (Maximum Residus Level) on vegetables.

Disease resistance is usually tested by the breeding companies involved in endives and leaf chicory (witloof and Italian types). Compared to the European lettuce market (95 000 in the EU), the EU chicory market is relatively small and divided in 6-8 countries with their own production methods and consumption uses: 20 000 ha for endives (60 % of the EU area in Italy, 20 % in France, …) and 35 000 ha for witloof and Italian leaf chicory (40 % of the EU area in Italy, 38 % in France, …). By another way, the wide range of diversity in the chicory genetic resources has not been still fully characterized and evaluated. Operators involved in chicory breeding have to evaluate the available genetic resources of the whole *Cichorium* genus for this disease resistance breeding aim.

In France, for 25 years, management of genetic resources has been organized through specialized networks by species or group of species (carrots, cereals, fruit trees, …): they bring together all actors involved in this topic around a pilot structure. Besides the preservation of patrimonial material, this organization has identified and characterized accessions through work carried out by the different partners of each network. Most of these networks are coordinated by INRA. Comprising 14 private and public partners (breeding companies, local organizations, technical institute) around GEVES (Group for Testing and control of Varieties and Seeds) as the coordinator of this network, the *Cichorium* network was created in 1996 and contains today 1800 accessions of which 650 belong to the National collection. Through contributions of its partners and collaborations generated with public research structures, this chicory network has carried out general operations as well as:

- regenerations (more than 500 accessions),
- morphological characterizations (1000 accessions),
- evaluations for genetic resistance against 5 diseases (180 accessions) and bitterness (100 accessions)
- distribution (1000 accessions) for different uses such as diversity presentation, breeding programs, official registration, ...

Accessions included in this collection maintained in the Brion GEVES station have different origins : old varieties maintained by breeding companies, deleted varieties from the French

and EU Catalogue and material from INRA – Versailles researchers when the chicory breeding program ended. Consequently, duplicates have occurred. In order to develop a core collection approach, field characterization have been realized, and use of ISSR markers has been developed to enhance the core collection and markers for each cultigroup have been identified.

Nevertheless, at European level, before 2007, the European gene bank community did not have a good overview of their stored leafy vegetable germplasm due to the absence of global crop databases for spinach, chicory and minor leafy vegetables whereas the existing international lettuce database needed significant updating. There was also limited access to trait data in databases which made it difficult for the user to select appropriate accessions. During the 2007-2010 period, a consortium of twelve partners from ten European countries, including three universities, six public research institutes and three non-governmental organizations conducted a project entitled 'Leafy Vegetables, stimulating use' in the framework of the European GENRES program. Coordinated by the Centre for Genetic Resources, the Netherlands (CGN) and resulted in an unique leafy vegetable crops portal on the web (http://documents.plant.wur.nl/cgn/pgr/LVintro/), this program concentrated most of the partners efforts on regeneration (1250 accessions of which 200 chicory accessions), characterization (1300 accessions of which 180 chicory accessions) and evaluation (800 accessions of 270 chicory accessions) for the genetic resources management and workshops to general public for the use stimulation. All these accessions on which trials and characterizations have been conducted and other chicory accessions are gathered in a unique accession database which contains today more than 1700 chicory accessions. Results of all future characterizations and evaluations will be included in this EU chicory database.

Among evaluation activities done within this program, work on pest and disease resistance was undertaken by a number of specialists and evaluations were focused one side on chemical contents and other side on genetic resistance against lettuce, spinach and chicory diseases. Especially on witloof cultivated mainly in France, Belgium and the Netherlands (total area of 15 000 ha of which 9000 ha are produced in France), evaluation against root and foliage disease was conducted: *Alternaria cichorii*, *Sclerotinia sclerotiorum* and *Thielaviopsis basicola*. For *S. sclerotiorum* and *T. basicola*, recent years have shown great impacts on the root and witloof production for both conventional and organic producers. A specific method has been developed by SNES, the central laboratory of GEVES, for an early resistance test against *T. basicola* and accessions have been identified for new breeding programs. Even if *A. cichorii* is more of a problem for the Italian leaf chicory, interesting accessions have been identified. For *S. sclerotiorum*, other screenings need be done on other accessions: resistance shown in some accessions is only partial. Nevertheless, the developed method by F.N.P.E. (French Federation of Witloof Producers) is currently used for commercial varieties providing useful information for the producers. On endives (cut and plain chicory), sclerotinia has been largely developed and its impact is growing. Besides that, development of organic productions and reduction of MRL will give new opportunities to screen our gene bank for genetic resistance.

Tomorrow, the French chicory network managed by GEVES has to characterize remaining accessions and to evaluate accessions for genetic resistance against pests for which no genetic resistance has been found (sclerotinia) or for which no work have been apparently conducted (marsonia).

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The diversity of indigenous and traditional leafy vegetable species in South Africa

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Key words: Indigenous vegetables, traditional vegetables, Amaranth, Cleome, Corchorus, pumpkin, cowpea, morogo, imifino

A wide diversity of plant species are traditionally used as vegetables in South Africa. Some of these species are indigenous but many of them are indigenised species that became part of the culinary tradition of the local people. These traditional vegetables are seldom cultivated and most of the species are collected in the wild or in fallow land where they are commonly regarded as weeds. Some of these species prove to be very nutritious. Despite these characteristics, these crops have been regarded as backward, and have been neglected by modern science for a long time.

Baseline information on the use of indigenous and traditional vegetables was gathered using questionnaires and other participatory tools in seven rural villages in South Africa. These villages differ in terms of ethnicity, geography and climate.

The species utilised and the preferences vary between agro-ecological zones as well as between ethnic groups. The knowledge of different crops was based with different groups within the community. Leafy vegetables tend to be the domain of the woman, while cash crops, fruit grains and cereals tend to be the domain of the men. The knowledge tends to be rudimentary in the youth

The most popular wild harvested species are amaranth (*Amaranthus* spp.), spider flower (*Cleome gynandra*) and jutes mallow (*Corchorus* spp) amongst others. Cultivated species are local landraces of cowpea (*Vigna unguiculata*) and pumpkin (*Cucurbita* spp). Many species are used as leafy vegetables in localised areas, for instance Lambsquaters (*Chenopodium album*), Blackjack (*Bidens pilosa*), balsam pear (*Momordica baslemima*) and many others.

African vegetables are still used extensively in rural and even urban and peri-urban South African households. It is a major contributory factor towards food security and balanced diets of households. Some of these species, like amaranth, contribute considerably to the daily intake of iron and vitamin A of rural households. However, over the years the use of African vegetables has diminished and in certain parts there is a real danger of losing the knowledge associated with African vegetables.

'Culture and management, quality traits'

AWARDED BEST POSTER PRICE

The development of a breeding strategy for nitrogen efficiency in spinach

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Key words: spinach, nitrogen use efficiency (NUE), hydroponics

Spinach is recognized as a food crop with an increasing consumption and The Netherlands is the leader in seed production worldwide. Spinach is a crop that has a high demand for nitrogen in order to rapidly produce harvestable leaves with a dark green colour as required by the market, but it has low nitrogen use efficiency (NUE). This implies that its cultivation requires a high input of nitrogen, which may have a negative impact on the environment. Cultivars adapted to low-input growing conditions are needed for both conventional and organic agriculture to enable a more sustainable crop production system. This breeding challenge has not yet been addressed by the breeding sector.

Therefore, the aim of this project is to develop knowledge, tools and genetic material that facilitate the development of varieties that perform well (good yield stability and quality) under low nitrogen conditions. The following aspects will be addressed: i) development and use of a rapid screening method for nitrogen use efficiency (NUE) under hydroponic conditions, ii) the analysis of Genotype x Environment (GxE) interaction for NUE in different field conditions and iii) development of molecular tools for the analysis of genetic variation of NUE using a dedicated mapping population.

In a first pilot two spinach cultivars were evaluated at different N-levels hydroponics and data on root and shoot growth, canopy development and chlorophyll content were collected. The cultivars responded differently to low N availability, and displayed GxE interaction: the best-performing cultivar under N-limiting conditions outperformed under low-input conditions the other cultivar.

It indicates that hydroponics is a suitable system for evaluation of N-use efficiency in spinach. In a next step, genetic diversity for N-use efficiency in a wider set of spinach cultivars will be assessed both on hydroponics and in field trials. The research started in September 2011 and will be performed until 2014, and is funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

The project will be conducted in close cooperation with the spinach breeders of Enza Zaden/Vitalis, Nunhems Seeds, Popvriend, and Rijk Zwaan.

Breeding of multiple-crop dill varieties

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Key words: dill, breeding, multiple harvesting

During cultivation of dill under the long-day conditions, two periods can be distinguished:
- basic growing period – from emergence to the marketable size of plants (12-15 cm), duration of the period is 40-45 days;
- marketable conformance period – from reaching marketable maturity of plants to bolting, the period can last for 7-40 days.

Depending on duration of marketable conformance period 2 methods of dill cultivation can be applied. If this period is short (7-12 days) the conventional culture cultivation technology with single-crop complete harvesting of marketable plants is applied. In extended period of marketable conformance (20-40 days) the multiple (2-6 times) selective leaf harvesting (length of lamina is 18-20 cm) or mass cutting of all leaves except the apex and youngest leaves for further extended growth is possible. In this respect single-crop and multiple-crop dill variety breeding is essential.

Single-crop dill varieties for fresh salad production should include plants with elevated or semi-elevated rosette of dark-green or green leaves. Leaves should have dense arrangement of plane medium-length segments in the lamina. The period from emergence to the beginning of harvesting should be as short as possible (30-35 days). Seeds should be large, with high germinating ability. Plants should have resistance to fungus pathogens. Dill varieties with active growth and elevated rosette of leaves are necessary for low-density growing and multiple-crop cultivation. Lamina should be intensively green with dense arrangement of applanate medium-length segments. More extended market conformance period, fast leaf growth after cutting, resistance to fungus pathogens are important characteristics for varieties of this group.

Multiple-crop dill varieties are suitable for conveyor green production without additional sowings, but modifications of the conventional technology is modified for their cultivation. To increase productivity it is necessary to decrease the sowing rate to 1-3 kg/ha. Low-density growing (distance between rows is 30-45 cm, between plants in a row – 15-25 cm) provide better nutrition and better conditions for growth and development of plants.

Use of multiple-crop dill varieties allows:
- to economize seeds due to sowing rate decrease and reduction of sowings;
- to get the higher yield of green leaves – up to 50 tones/ha, moreover, the yield is of higher quality, because the leave fragrance increases during plant aging;
- to increase the sales at higher prices during rather long period of time, without fear that the plants will overgrow and lose their marketable quality.

For the multi-crop dill variety breeding selections from hybrids between varieties Ducat, Gribovskiy, Salute and others were used as the parent material. As the result of breeding, the following multiple-crop dill varieties with various duration of the period of marketable conformance were produced: Alligator, Amazon, Iney, Almaz.

NOT PRESENTED

Effect of salinity stress on germination indices in *Allium ampeloprasum* ssp *persicum* local Iranian (Bojnourd cultivars)

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Key words: *Allium ampeloprasum* ssp *persicum*, bojnord local variety, radical length

In order to study the effects of salinity stress on germination indices in *Allium ampeloprasum* cultivar, an experiment was conducted in factorial form, using a completely randomized design with four replications by NaCl in controlled condition at Islamic Azad University Bojnord Branch in 2011. In this experiment, two Bojnord local cultivars were evaluated at five levels of salinity treatment (distilled water, -3, -6, -9, and -12 bar). The traits were: germination percentage, mean of day germination, seed germination, coefficient of germination, radical length, and shoot length considered as components of germination.
In all traits, a significant decrease was observed with increase in stress level. Interaction were non-significant between variety and level salinity expect germination percentage and shoot length. The lowest germination percentage belongs to bojnord 1 in -12 bar. The highest mean germination times belong to bojnourd 1 local variety. The highest coefficient of velocity of germination and speed germination belong to bojnord 2 local variety, at distilled water and -3 bar. Bojnord 1variety had the lowest radical length, bojnourd 1 had longest shoot length in distilled water.

'Diseases and pests, disease resistance'

Improvement of the differential lettuce set for *Bremia* virulence evaluation: new *sativa* monogenic lines

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Key words: *Lactuca*, downy mildew, differential set, resistance

Several accessions of the actual set of host differentials are difficult to use to determine the virulence of *Bremia* isolates; some are *Lactuca serriola* with reflex capitula and slow germination, some are not monogenic, like Ninja (*Dm3*, *Dm4*, *Dm11*, and a resistance from *L. saligna* called here *Rsal-1*) or Discovery (*Dm7*, *Rsal-1*). Besides, some *Bremia* isolated recently on some cultivars do not sporulate on Cobham green, the accession used as universal susceptible. In collaboration with 5 breeding societies (Enza Zaden, Gautier Semences, Rijk Zwaan, Seminis, Syngenta) and the Geves-Snes, and with some financial support from French Agricultural Ministry, a project was developed to create some new differential lines: a susceptible line without the resistance from Cobham green (called *Rcg* here), a *sativa* monogenic line with *Dm16* to replace *L. serriola* LSE/18, and the *sativa* line CGDM16 (*Dm16*, *Rcg*), a monogenic line with *Rsal-1* to replace Ninja and Discovery.

To produce a universal susceptible line and to eliminate *Rcg* from the differential line CGDM16, the cross [CGDM16 x F_1 (Cobham green x LSE/18)] was produced. Progeny of that cross was tested with Serr84/99, a *Bremia* strain isolated by A. Lebeda in the French Alps which was not virulent on Cobham green. Only 18 I_1 progenies of 100 hybrid plants [CGDM16 x F_1 (Cobham green x LSE/18)] were in segregation for the resistance to Serr84/99; 8 out of these 18 I_1 families were homogeneous resistant and 10 showed segregation for *Dm16* resistance. In the progenies from these 18 selected hybrid plants, I_2 families were produced on 71 I_1 plants tested as susceptible to Serr84/99. The capitulum of these 71 plants was observed and the I_2 progenies were tested with Serr84/99; therefore 14 lines I_2 susceptible to Serr84/99 and with an erect capitulum were selected. A ring test was realized with Serr84/99 and BI:22 in 6 laboratories: one line without resistance (susceptible to BI:22 or NL2) and 8 lines with *Dm16* (resistant to BI:22 or NL2) were selected. These 9 lines were tested with the 16 UPOV strains of *Bremia* used for cultivar inscription in Europe, and 2 I_2 were selected: one line (*Rcg*$^+$, *Dm16*$^+$) and one line (*Rcg*$^+$, *Dm16*). These two lines are good candidates as a susceptible genotype and as a *Dm16* line in the set of lettuce differentials for *Bremia* evaluation.

To create a differential line with only *Rsal-1*, a screening in the progenies from (Discovery x Angie) was realized with BI:17 to eliminate *Dm6* from Angie (a cultivar *Dm6*, *Rsal-1*) and the strain FR30/99 (sextet 63-62-16-01) to eliminate *Dm7* from Discovery. In a first step, 22 out of 287 F_3 families, issued from 287 harvested F_2 (Discovery x Angie) plants, were selected as homozygous for *Dm6*$^+$ and *Dm7*$^+$ (susceptible to BI:17 and to FR30/99). After a ring test in 6 laboratories, 5 F_3 were selected and tested with the 16 UPOV strains of *Bremia*; the results were up to expectation except with BI:5.

In conclusion, interesting candidates for *Bremia* differential lines were obtained to replace (1) Cobham green by a line susceptible to all know *Bremia* strains, called FrDm0, (2) CGDM16 by a monogenic *Dm16 sativa* line, called FrDm16, and (3) Ninja and Discovery by a monogenic *Rsal-1* line, called FrRsal-1. The partners of that project decided to improve the homogeneity of these F_3 lines with one extra generation of selfing and observations on the morphology of the lines. An extra control of the conformity of the susceptibility/resistance especially with Serr84/99 and with BI:5 will be also realized before proposing the material to the IBEB group.

Identification and denomination of "new" races of *Bremia lactucae* in Europe by IBEB until 2011.

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Key words: *Bremia lactucae, Lactuca sativa*, Lettuce downy mildew, virulence, variability, sextet coding, IBEB, lettuce varieties, resistance characteristics, Dm genes.

The International Bremia Evaluation Board, IBEB, a joint effort of European lettuce breeders and authorities since 1998, has identified and named new races of *Bremia lactucae* (Bl:) in the last 12 years. Since 2006, the races Bl:26, 27 and 28 have been nominated.

2011	GreenTower	Lednicky	UC DM2	Dandie	R4T57D	Valmaine	Sabine	LSE 57/15(Blam)	UC DM10	Capitan	Hilde II	Pennlake	UC DM14	NunDm15	CG Dm 16	NunDm17	Colorado	Ninja	Discovery	Argeles	RYZ-2164	RYZ-910457	Bedford	Balesta	Bellissimo	EU-B
DM nr/R nr	0	1	2	3	4	5/8	6	7	10	11	12	13	14	15	16	17	18	36	37	38						
Sextet nr		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Sextet value		1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32	Sextet code
Bl:1	+	+	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	(m)	11-58-00-00
Bl:2	+	+	+	+	+	+	+	-	+	(-)	+	+	+	-	-	-	(-)	-	-	+	-	-	-	+	+	63-58-00-49
Bl:4	+	+	+	-	+	+	(-)	+	+	(-)	+	+	+	-	(-)	-	(-)	-	-	-	-	-	(-)	-	-	27-59-00-00
Bl:5	+	+	-	+	-	-	-	+	+	-	+	+	-	+	(-)	-	-	-	(-)	-	-	(-)	-	-	-	05-27-01-00
Bl:6	+	+	+	-	+	+	(-)	-	+	+	+	+	+	-	(-)	-	-	-	-	-	-	(-)	-	(-)	(-)	27-62-00-00
Bl:7	+	+	+	+	+	-	+	+	+	-	+	+	+	(-)	-	-	-	-	-	-	-	-	-	-	-	47-59-00-00
Bl:10	+	+	+	+	+	+	+	+	+	(-)	+	+	(+)	(-)	-	-	-	-	-	-	-	(-)	(-)	-	-	63-59-00-00
Bl:12	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	57-63-03-00
Bl:13	+	+	-	+	-	+	(-)	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	21-63-00-00
Bl:14	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	63-62-00-00
Bl:15	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	31-31-00-00
Bl:16	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	63-31-02-00
Bl:17	+	-	+	+	-	+	-	+	+	-	+	+	+	+	-	-	+	-	+	-	-	-	(m)	-	-	22-59-41-08
Bl:18	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	59-31-10-00
Bl:20	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	(-)	63-31-10-00
Bl:21	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	(-)	(-)	-	-	63-31-51-00
Bl:22	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	(-)	+	-	59-63-09-16
Bl:23	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	63-31-02-01
Bl:24	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-	-	-	-	-	59-31-10-01
Bl:25	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-	(-)	59-31-42-00
Bl:26	+	+	+	+	+	+	(+)	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	-	63-31-58-01
Bl:27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	+	-	63-63-13-19
Bl:28	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-	+	-	-	(-)	59-31-10-05

Reactions: - resistant, (-) resistant with necrosis may interfere with result, (m) moderate/ mixed, (+) susceptible with necrosis, + susceptible Bl:1 till Bl:16 are aliases for NL1 -NL16. Bl:3, 11 and 19 are not available and found anymore and therefore skipped from the list.

Resistance to *Bremia lactucae* in natural populations of *Lactuca saligna* from some Middle Eastern countries

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Key words: willow-leaf lettuce, lettuce downy mildew, non-host resistance, race-specific resistance, seedling stage, adult-plant stage

Lactuca saligna (willow-leaf lettuce) has a European-Asian distribution area, and it also occurs in North Africa and North America. In Israel it is commonly distributed all over the country except for desert areas (Beharav et al., 2008). *L. saligna* has never been recorded as a natural host of *Bremia lactucae* (lettuce downy mildew), however it can be infected artificially (Beharav et al., 2006). Lettuce breeding strategies have been focused on searching for and utilizing novel and potentially durable sources of resistance from wild *Lactuca* and closely related species, especially *L. serriola* L., *L. saligna* and *L. virosa* (Lebeda et al., 2007). In *L. saligna*, resistance to *B. lactucae* has been explained as having mostly quantitative and race non-specific character (Lebeda, 1986), involving quantitative trait loci (QTLs) (Jeuken and Lindhout, 2002). The aim of our present study was to screen accessions of *L. saligna* (N=146) originating from the natural populations (Israel: N=136, France: N=8, Jordan: N=1) for their resistance to several isolates (races) of *B. lactucae* (N=10) differing in virulence. Experiments were carried out at a seedling stage and for a few selected accessions also at an adult-plant stage.
Results showed generally high level of resistance to all isolates used, both for seedlings and adult plants, which is in accordance with previously postulated thesis that *L. saligna* is a non-host for *B. lactucae* (Lebeda, 1986). Nevertheless, at the seedling stage for a low number (N=20) of the screened accessions a bit higher degree of infection by either one or a few of the races was recorded. These accessions originated mainly from non-Israeli populations. Sporulation intensity in those cases was however not so high as usual for a susceptible response of other *Lactuca* spp. Some previous studies (e.g. Lebeda, 1986; Beharav et al., 2006) refer to the race-specific character of *L. saligna* responses, as well. These 20 accessions were used for adult-plants screening to verify race-specificity observed at the seedling stage. The obtained results did not correspond with those obtained for seedlings and all accessions showed race-nonspecific resistance.

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Comparison of controlled environments for the screening of resistance to *Xanthomonas campestris* pv. *vitians* in lettuce

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Key words: *Lactuca sativa*, bacterial leaf spot, disease resistance, vegetable breeding

Bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians* (Xcv) is a worldwide problem of economic importance in many lettuce-producing areas. Small angular leaf spots first appear as water-soaked lesions and then become papery, finally coalescing to form large necrotic regions on the leaves. The disease reduces quality and yield and increases the potential for postharvest losses. The lack of effective control measures to prevent pathogen proliferation and dispersion makes the use of less-susceptible cultivars the most promising way to reduce disease. The identification of resistant germplasm is the first step in selecting parents for breeding purposes. However, inconsistency among trials in the United States and Canada for the ranking of lettuce cultivars for bacterial leaf spot symptoms was reported, possibly because of genotype-by-environment interaction, different evaluation methods, or genetic diversity in the Xcv strains. The objective of the present work was to develop a screening test that provides a reliable and consistent evaluation of bacterial leaf spot resistance. A total of 24 lettuce varieties, namely 15 romaines, six crispheads, two Latins, and one Batavia, were grown seven times in a growth chamber and three times in a greenhouse equipped with a mist system. The lettuce plants were artificially inoculated with three isolates of Xcv originating from Quebec. The plants were evaluated using a standardized key with a 0-to-7 scale corresponding to specific amounts of surface area with damage. The greenhouse and growth-chamber environments were correlated (r = 0.78) and showed consistent ranking of genotypes in relation to bacterial leaf spot resistance. Disease severity rankings remain to be validated under field conditions.

Fine mapping nonhost resistance in lettuce to downy mildew

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For abstract see P31

'Genetics and biotechnology'

QTL analysis of bitter compounds in Lactuca species

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Key words: Lettuce, Lactuca, terpenoid sesquiterpene lactone, bitterness, Quantitative Trait Loci

One of the most critical sensory aspects of lettuce (Lactuca spp.) is the perceived bitterness, especially in certain varieties and those which have suffered from environmental stresses during growth. The compounds primarily responsible for the bitterness in lettuce are a group of terpenoids highly typical of Asteraceous plants; the sesquiterpene lactones (SLs). These compounds comprise a range of constitutive and induced phytoalexins in lettuce, and are therefore important to successful growth of the plant. However, we hypothesise that the SL profile could be manipulated to bring a less bitter variety to the market.

We have assessed the presence of these compounds in a recombinant inbred line (RIL) population in order to identify QTL (quantitative trait loci) for some of the bitter compounds found in our population. LC-MS using a fluorescence detector was used for identification and quantification of the compounds. Significant QTL were found for three of the sesquiterpene lactones analysed. A strong QTL was identified for lactucin-15-oxalate on chromosome 2 and which accounted for 27.1% of the variance associated with the compound. Multiple QTL were found for 15-p-hydroxyphenylacetyllactucin-8-sulphate which, in addition to a pair of separate QTL on chromosome 3 accounting for 27.2% and 23.6% variance, also had a single QTL on chromosome 4 accounting for 29.1% variance. There were three closely occuring QTL on chromosome 3 for 8-deoxylactucin which accounted for 30.1%, 23% and 26.4% of the variance respectively. We hope to refine our findings with NMR data on a wider population in the hopes of finding QTL for the other compounds, as well as refining the QTL already identified.

We hope that this information can be fed into a breeding program in order to remove the perceived bitterness of lettuce for some consumers, preferably in a manner what has least impact on the plant's ability to grow and function efficiently and deal with environmental stresses such as pathogenesis.

Mapping QTL and candidate genes for hydroxycinnamate metabolism in chicory (*Cichorium intybus* L.)

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Key words: chicory (*Cichorium intybus* L.), hydroxycinnamates, caftaric acid, chlorogenic acid, chicoric acid, genetic control, quantitative trait loci (QTL), candidate gene, antioxidants

Secondary metabolism corresponds to the production of compounds allowing plants to survive in their environment. Among these molecules, polyphenols display widely studied antioxidant properties that may have health benefits. The aim of this work was to study the genetic control of the biosynthesis of hydroxycinnamates detected in chicory (*Cichorium intybus* L.) leaf tissue, using a combination of a quantitative trait locus (QTL) and candidate gene approach.
A high-throughput protocol was set up to analyse and quantify the polyphenolic content of chicory leaves. The presence of 3 major phenolic acids was detected by HPLC and confirmed by mass spectrometry: caftaric acid, chlorogenic acid, and chicoric acid. The protocol was combined with a radical scavenging ability test (DPPH assay).
From 201 genotypes of the F1' progeny K28xK59, each represented by 5 clones, were phenotyped for 6 traits that showed significant variability: caftaric, chlorogenic and chicoric acid contents (ACAFT, ACHLO, and ACHIC, respectively), the total contents of these three molecules (ATOT), the ratio caftaric acid/total phenolic acids (PCAFT), the ratio chlorogenic acid/total phenolic acids (PCHLO), and the radical scavenging ability (AAR). For QTL mapping a genetic map was used with 126 markers covering the nine linkage groups of the chicory consensus map (Cadalen et al, 2010). Furthermore, 16 polymorphic candidate genes encoding transcription factors and enzymes potentially implicated in the biosynthesis of phenolic acids were mapped.
In total 20 QTL were detected: 1 for ACAFT (R^2 = 16.4%), 3 for ACHLO (R^2 = 50%), 2 for ACHIC (R^2 = 13.9%), 5 for PACFT (R^2 = 44%) and PCHLO (R^2 = 61%), and 4 for AAR (R^2 = 31%). Amongst the mapped candidate genes, 7 genes encoding enzymes involved in the phenylpropanoid pathway co-localised with QTL.
This study (Bahri 2010) is a first step toward understanding the genetic control of the biosynthesis of the main phenolic acids in chicory leaves, particularly caftaric and chicoric acid, only synthesised in a few other plant species. For breeders, the identification of molecular markers and specific genes associated with loci controlling antioxidant activity and phenolic acids amounts in chicory leaves opens the possibility to apply this knowledge to commercially interesting chicory cultigroups.

Cadalen *et al* (2010) Mol Breeding 25:699–722
Bahri (2010) PhD thesis, Université Lille Nord de France, USTL1, Lille, France

Mapping QTL and candidate genes for somatic embryogenesis in chicory (*Cichorium intybus* L.)

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Key words: chicory (*Cichorium intybus* L.), somatic embryogenesis, genetic control, quantitative trait loci (QTL), candidate genes

Somatic embryogenesis (SE) is an asexual reproduction pathway in which somatic cells form embryos in a process that resembles zygotic embryogenesis. In chicory (*Cichorium intybus* L.) a genetic variability in the capacity of SE was found in plants originating from a Hungarian chicory landrace called 'Kospool', and two contrasting genotypes were selected: K59, highly embryogenic, and K28, hardly embryogenic. From the cross K28xK59 two progenies were obtained in 2001 and 2004, F1'a (n=123) and F1'b (n=104), respectively. Variability for SE capacity in both progenies was exploited to identify chromosomal regions (QTL) and candidate genes implicated in this process.

After 7 days of culture of root explants from *in vitro* plantlets under SE inducing conditions and 30 days of development, the number of plantlets (PL) and shoot-like structures (SH) formed were counted. Inspection of the phenotypic data showed that in contrast to the total number of events (PL + SH), the progenies F1'a and F1'b differed in the capacity of plantlet formation, F1'a clearly possessing a higher PL than F1'b. Nonetheless, in both progenies the traits PL and SH showed continuous and normal distributions after log10 transformation, and heritabilities superior to 0.63.

A genetic map for the K28xK59 progenies (Cadalen *et al* 2010) was used to map 47 polymorphic candidate genes previously identified to be differentially expressed during SE (Legrand 2006, Legrand *et al* 2007, Lucau-Danila *et al* 2010) as well as to map QTL for PL and SH. For each of the two traits, 6 QTL were detected that together explained 23% and 44% of their phenotypic variation, respectively. All QTL for PL and SH seemed to map to the same chromosomal regions, suggesting that the formation of plantlets and shoot-like structures was under common genetic control, and that the latter represented malformed embryos.

Among the mapped candidate genes, 16 co-localised with QTL for PL and SH. In view of their role in the maintenance of undifferentiated stem cells in shoot apical meristems, the co-localisation of genes homologous to *SHOOT MERISTEMLESS* (*STM*) and *ARGONAUT* (*AGO*) in Arabidopsis with QTL is particularly interesting.

With the detection of QTL for SE, the results of this study (Clabaut 2009) have for the first time revealed elements in the genetic control of SE in chicory, and open the possibility of their introgression by marker assisted selection of QTL in commercial chicory cultigroups which are generally recalcitrant for SE. Furthermore, the co-localisation of certain candidate genes with QTL provides a basis for future research on their implication in SE.

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A first characterization of the genomic regions flanking the S-locus in *Cichorium intybus* L. (Asteraceae)

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Key words: chicory (*Cichorium intybus* L., Asteraceae), self incompatibility, S-locus, positional cloning

Self incompatibility (SI) is one of the most important mechanisms to prevent selfing in hermaphrodite plants. This mechanism is generally under the control of a single multi-allelic locus, the S-locus. Pollen and pistil determinants have been identified in some plant families (Brassicaceae, Solanaceae, Convolvulaceae, Papaveraceae) and are different in each family, suggesting that different molecular mechanisms of SI have evolved independently. Chicory (*Cichorium intybus* L.) is a sporophytic self incompatible (SSI) species belonging to the Asteraceae family for which these determinants are unknown, but are again different from those identified so far.
Our goal is the positional cloning of male and female determinants of SSI in chicory. A high-density map around the S-locus has been created to perform physical mapping of this region, based on the availability of two 6X BAC libraries (Gonthier 2010). Chromosome walking toward the S-locus of chicory has begun, and BAC clones sequences have permitted us to obtain the first information about the chicory genome in the S-locus region. Comparative sequence analyses have allowed us to investigate new approaches to accelerate chromosome walking. Indeed, we found a very high level of co-linearity between genes of some sequenced Dicotyledonous species and chicory. These results, and a strategy to exploit them in our map-based cloning approach of the S-locus, will be presented (Gonthier 2011).

Gonthier *et al* (2010) BMC Research Notes 3, 225-234
Gonthier (2011) PhD thesis, Université Lille Nord de France, USTL1, Lille, France

Cloning and characterization of nuclear male sterility 1 (*nms1*) in chicory (*Cichorium intybus* L., Asteraceae)

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Key words: chicory (*Cichorium intybus* L., Asteraceae), nuclear male sterility *(nms1)*, transcription factor (bHLH), dysfunctional tapetum-like, transposon (CACTA)

Chicory (*Cichorium intybus* L.) is naturally self incompatible but some seeds may be obtained upon selfing, and in witloof chicory some varieties have been selected for a high seed set upon selfing. Thus, in chicory male sterility is an important tool for F1 hybrid production in circumventing the time-consuming emasculation of flowers of the seed producing plants. So far, in chicory only a single nuclear ms source is known under the name 'Edith' [2].
Segregation of ms 'Edith' in the K28xK59 progeny allowed us to map the *nms1* locus to linkage group 5 of the consensus map [1], and a detailed cytological analysis learned that in *nms1* microspores were degenerated shortly after meiosis. By comparing the events and their timing with those published for ms mutants in Arabidopsis and rice [4-7], we selected 2 candidate genes, *ABORTED MCROSPORES* (*AMS*) and *DYSFUNCTIONAL TAPETUM* (*DYT1*). These genes, as well as their homologues in rice, encode basis Helix_Loop-Helix (bHLH) transcription factors implicated in the functioning of the tapetum. Subsequent genetic analysis of polymorphisms displayed by each of the candidate genes learned that one of them co-localized with the *nms1* locus. The complete sequences of the alleles *NMS1* and *nms1* was obtained after screening BAC libraries [3] and showed that the *nms1* allele contained a CACTA-type transposon in a *DYT1*-like gene. Identification of male fertile revertants in which the transposon had left the *nms1* locus indicated that the presence of the transposon was the causal mutation conferring ms. Further confirmation was provided by analysis of the expression of Ci*DYT1* in wt and *nms1* capitular buds, showing the lack of mRNA of this gene in *nms1* plants.
The results suggest that the role of certain bHLH transcription factors in pollen formation, as identified in Arabidopsis and rice, is conserved in chicory. To obtain support for this hypothesis, we will transform the Arabidopsis *dyt1* mutant [7] with Ci*DYT* to see whether this will restore its male fertility.
The identification of the mutation responsible for *nms1* (ms Edith) will allow chicory breeders to screen progenies rapidly for ms plants, i.e. at the seedling stage, with a single PCR reaction using 3 primers.

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'Germplasms and their diversity'

Characterization of developmental stages in *Lactuca saligna* germplasm from Europe and USA

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Key words: *Lactuca saligna*, *Lactuca serriola*, willow-leaf lettuce, bolting, flowering, geographic origin

Lactuca saligna L. (willow-leaf lettuce, fam. Asteraceae), highly polymorphic and widely over the world distributed species meets raising importance in lettuce (*L. sativa*) breeding programmes as an important donor of valuable traits (resistance) since last quarter of 20th century (Lebeda et al., 2007). Large variability among its populations were observed for morphological characters in their natural habitats in Europe, USA and Near East (Lebeda et al., 2001; Beharav et al., 2008), during cultivation of plants under greenhouse conditions (Křístková et al., 2009), and also on the level of molecular markers (Kitner et al., 2008). The purpose of this study was to describe the variation in developmental stages in *L. saligna* germplasm from Europe and USA during their cultivation in greenhouse, and to compare to corresponding data on *L. serriola* samples from similar regions. The set of 30 samples of *L. saligna* were collected in Italy (Po river lowland, Riviera di Ponente), France (Rhône Alpes), South and Eastern Slovakia and in the USA (Salinas). The beginning of bolting varied within the whole set from 92 to 133 days after sowing (DAS), the beginning of flowering was recorded 133 to 183 DAS, the period between both developmental stages was 21 – 62 days. Beginnings of bolting and flowering of *L. serriola* samples were recorded more early (20 days for bolting, 40 days for flowering). Plants *L. saligna* originating from the most southern regions (Riviera di Ponente and Po river lowlands) entered to the stage of bolting very early (97 and 119 DAS) but the period between bolting and flowering of 47/45 days (Riviera di Ponente/Po river lowland) was longer than for plants from remaining three regions (from higher elevations in the South Europe - Rhône Alpes, Central Europe - Slovakia and the USA). Plants from these regions bolted later (124, 126, 124 DAS), but the period to beginning of flowering (32, 36, 25 days) was significantly shorter. This phenomenon was observed also for samples of *L. serriola* from similar regions. Highest levels of heterogeneity in both developmental stages between samples from one region, and between individual plants within one sample were observed for samples from Po river lowland. Plants of all samples from Riviera di Ponente were highly uniform in both developmental stages.

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Contribution of the French network on chicory genetic resources to a European *ex situ* management of leafy vegetables genetic resources

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For abstract, see P50

‘General discussion’

General discussion

During the general discussion at the end of the meeting, the following items were dealt with:
- The next EUCARPIA Leafy Vegetables meeting in 2015
- How to improve the organisation and participation of the Leafy Vegetables meetings?
- Is lettuce dominating the Leafy Vegetable meetings?

The next EUCARPIA Leafy Vegetables meeting in 2015

At several occasions during the meeting, the participants were encouraged to think about possibilities for the organisation of the next Leafy Vegetables meeting. This resulted in a proposal by Carol Wagstaff, that Dr Maria Isabel Gil, from CEBAS-CSIC, based in Murcia, Spain, is willing to host the 2015 leafy vegetables meeting (http://www.cebas.csic.es/dep_ingles/aliment/group_quality.html). This proposal was highly appreciated by all participants as an opportunity to enlarge the number of organizing countries.

How to improve the organisation and participation of the Leafy Vegetables meetings?

Following the announcement of the next meeting, a discussion was started on the possibilities to improve the organisation and participation. This was also instigated by the slightly disappointing number of participants at the present meeting. It was suggested that one reason for this may be the absence of spinach breeders, which decided to attend the ASP 2011 International Spinach Conference, 3-4 October in Amsterdam, The Netherlands.
As a critique with respect to the organisation of the present meeting was particularly mentioned the omission of the affiliations of the participants on the badges.
To improve participation, the following suggestions were made:
- information on the next meeting should be communicated as soon as possible via internet, both on a site dedicated to the conference as well as via the EUCARPIA site. With respect to the latter, it was also mentioned that membership of EUCARPIA is the best way to be informed on this kind of meetings.
- lists of e-mail addresses of participants of previous Leafy Vegetables should be exploited to stimulate participation, for instance by asking those persons to add a link to the conference site to their e-mail signature and to suggest other people to be contacted. The generation of a cleaned and updated version of the e-mail addresses would be very helpful. Similarly, sponsors should be asked to present the link to the conference site.
- to stimulate in particular the participation and integration of PhD students by offering them the possibility to present their thesis' results. The organisation of a dedicated session at the beginning of the meeting for these presentations was suggested (though it should be prevented that this part of the meeting is abstained as 'less important').

Is lettuce dominating the Leafy Vegetables meetings?

As a large part of the oral presentations and posters were dedicated to lettuce (17 of the 22 oral presentations, and 7 of the14 posters), the question was raised whether lettuce started to become too dominating in comparison to other leafy vegetable crops. Apart from the fact that lettuce does represent a (the?) major leafy vegetable crop, it was argued that the number of presentations on chicory had increased (though mainly by contributions of the organizing laboratory), and that the relative low number of contributions on spinach during the present meeting was influenced by a reduced participation of the spinach breeders mentioned before. Moreover, it was put forward that in view of the many collaborations in lettuce research projects, and the results this has generated, this species merits a status as model to stimulate similar collaborative research networks for other leafy vegetables.

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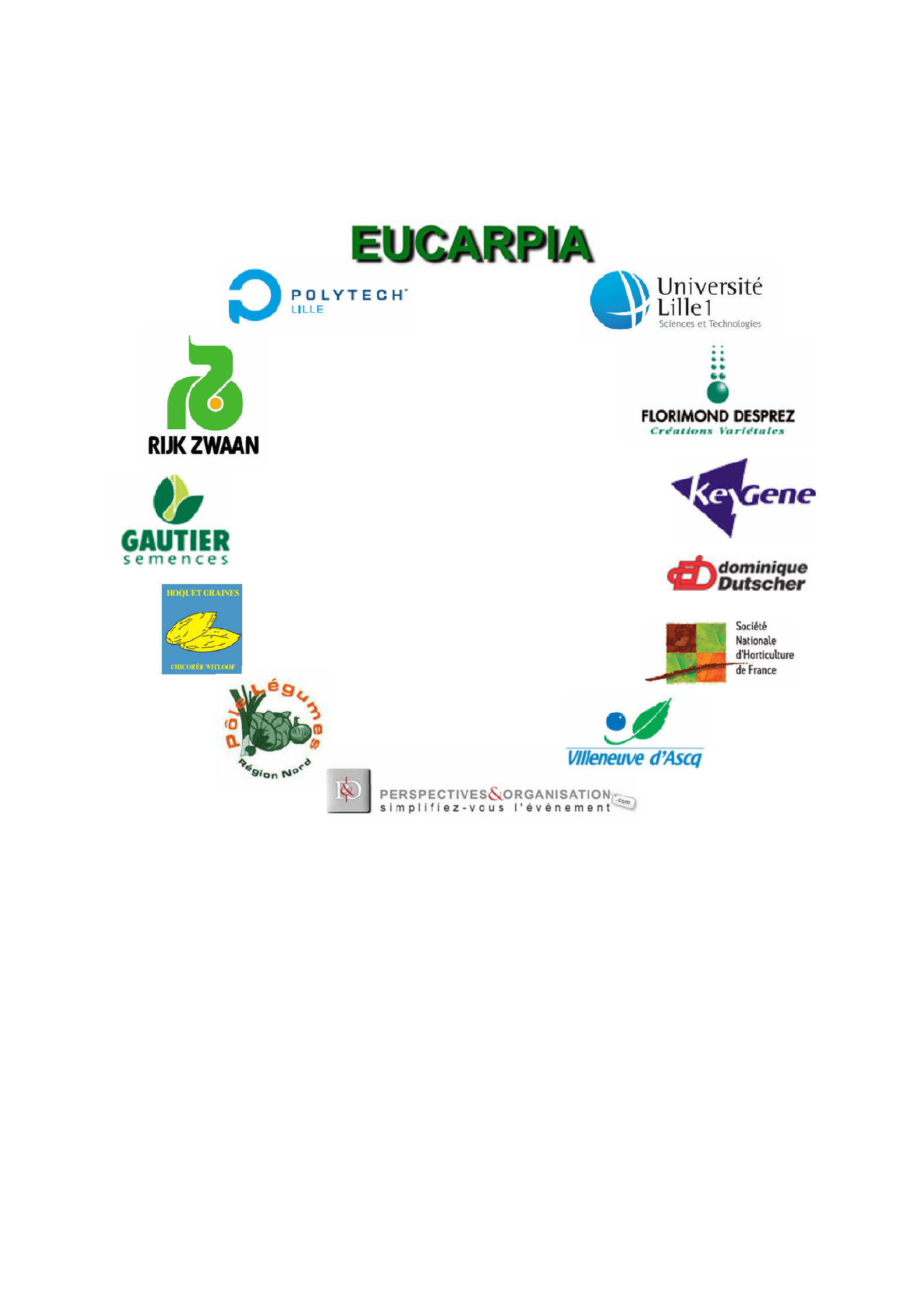
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