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VARIATION IN THE LEMMA ABAXIAL EPIDERMIS OF *AVENA STRIGOSA* SCHREB.

Romuald Kosina * & Edyta Franas

Abstract. Microstructure of abaxial epidermis of lemma is presented for 26 accessions of *Avena strigosa* of different geographical origin and for some other oat diploids. Papillae and duplexes of cork and silica cells are main morphogenetic events in the oat lemma. *A. canariensis* and *A. longiglumis*, characterized by a meristemoid activity of the lemma, are situated in an ordination space outside of the *A. strigosa* group, while a cultivated species *A. brevis* is among accessions of *A. strigosa*. The meristemoidal activity of the lemma abaxial epidermis appeared to be a useful taxonomic marker for oat diploids.

Key words: *Avena strigosa*, lemma epidermis, replicas, meristemoids, intra- and inter-specific variation

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Introduction

The microstructures of the abaxial epidermis of lemma are mostly observed in the middle inter-costal region of this bract. In the species of the genus *Triticum* L., such data have been provided by Kosina (1999a). Long cells with thick sinusoid anticlinal walls and specialized short cells, such as cork and silica cells, linked together in the form of a duplex, round papillae with many pits and hairs of various lengths are developed in a wheat lemma epidermis. The microstructure of the epidermis appeared to be an effective taxonomic marker to discriminate wheat species. In the genus *Bromus* L., these characteristics were useful at the sectional level (Kosina 1999b). However, Consaul & Aiken (1993) were not so successful in the establishing the discrimination of *Festuca* L. species when they used the palea characteristics. Qualitative differences in the microstructure of glumellae abaxial epidermis were exemplified for many wild grasses by Parry & Smithson (1964, 1966) and by Kosina (1995) for cereals. Epidermal characteristics of lemma in species taxonomy have been successfully applied such as for the genus *Brachypodium* (Kłyk 2005), a *Bromus secalinus* L. – *B. commutatus* Schrad. – *B. racemosus* L. group (Skowrońska 2005; Kochmański 2008), an *Avena magna* Murphy et Terrell × *A. longiglumis* Dur. amphiploid and its parental species (Świetlikowska 2008), and for perennials and annuals of the genus *Lolium* L. (Kawa 2008).

Material and methods

Seeds of diploid species of the genus *Avena* L. have been obtained from the following collections: Federal Centre for Breeding on Cultivated Plants in Braunschweig, Germany; National Germplasm Resources Laboratory, Aberdeen, USA; the Vavilov's Institute (VIR) in St. Petersburg, Russia; Botanic Gardens in Moscow, Russia; and Rennes, France. As well as they were gathered from the cultivated fields of Podhale region, S Poland. 26 accessions of

A. strigosa Schreb. (As1 to As26 marked as symbols in the diagram) and 4 accessions of other diploids (*A. canariensis* Baum, Rajhathy et Sampson (Ac3), *A. longiglumis* Dur. (Al17), *A. wiestii* Steudel (Aw4), and *A. brevis* Roth (Ab11)) were cultivated on small plots in the same soil and climatic environment in the grass collection maintained by R. Kosina.

The study material was treated as originating from a completely randomised one-way classification design. Varnish replicas prepared according to Hilu & Randall (1984) were microscopically analysed for the microstructure of abaxial epidermis of the first flower lemma in the spikelet. Replicas were taken from the central point of the lemma, a little below the awn attachment. Frequencies of meristemoid cytokinetic events in epidermis observed in the form of papillae, hooks, hairs, cork and silica cells in duplexes, single short cells and anticlinal walls perpendicular to the lemma axis were estimated for random samples $n = 30$. Analyses made for hexaploid oats (Kosina & Warzych 2002) and for bromegrasses (Kosina & Zawerbna 2002) proved that such size of a random sample is sufficient for a quantitative study of the lemma characteristics.

Observations were made under an Amplival microscope and pictures were taken with a Zenith TTL camera and Fuji 400 film. Multivariate data (arithmetic means) for oat accessions treated as Operational Taxonomic Units (OTUs) were numerically elaborated according to Rohlf (1994) with the use of non-metric multidimensional scaling method. The matrix of average taxonomic distances between OTUs was an initial matrix to set OTUs in the form of minimum spanning tree in an ordination space.

Results and discussion

Morphogenesis of the spikelet glumellae is more complex compared to normal grass leaves. The abaxial epidermis of the glumellae contains several differentiated cells; sometimes they express special metabolism, for instance cork and silica cells in duplexes. The awn formed in the middle or lower part of the lemma induces an original morphogenesis in the adjacent parts of epidermis (Warzych 2001).

In the *A. strigosa* and *A. wiestii* accessions, a dead meristemoid field exists just above the awn. No short cells are formed there by anticlinal divisions of the long cells of epidermis. However, a qualitative difference is noted in relation to such a field between both the above species and *A. canariensis*. In the latter, many meristemoids are active in the field (Franas 2003).

Synchronisation of the cell cycle or morphogenetic induction in linear sets of cells in monocotyledonous plants is responsible for the development of series of epidermal short cells (Croxdale 2000).

The middle part of the lemma is the most advanced in morphogenesis. For two accessions of *A. strigosa*, replicas of this part are shown in Fig. 1. The most common types of meristemoids are those that create papillae and cell duplexes (cork cell + silica cell). The latter are less visible in Fig. 1 and a distinct difference is visible between both the replicas. The frequency of meristemoids in the accession As10 is lower and in As13 higher. Both accessions are distant from each other in the minimum spanning tree diagram (Fig. 2). Other oat diploids, *A. canariensis* (Ac3) and *A. longiglumis* (Al17) are distant from *A. strigosa* accessions. In the tree, *A. brevis* is situated among *A. strigosa* units. Accessions of *A. strigosa* situated in extreme positions in the diagram (Fig. 2), As1 and As22 origin from Germany, As13 from Spain, As18 (hidden behind As16) from Portugal and As16 was collected in Podhale, S Poland. In *A. strigosa*, the geographical trend of the accession origin is not documented in the diagram. Similar analyses of glumellae epidermal characteristics made with the use of non-metric multidimensional scaling showed a good discrimination of wild, weedy and cultivated types within a tetraploid complex of *A. barbata* Pott ex Link – *A. abyssinica* Hochst. – *A. vaviloviana* (Maltz.) Mordv. (Kosina & Wach 2002) and separated fatuoids in a complex of *A. sterilis* L. – *A. fatua* L. – *A. sativa* L. (Kosina & Bielewicz-Rzepka 2002). A clear discrimination of *A. longiglumis* and *A. canariensis versus A. strigosa* has also been

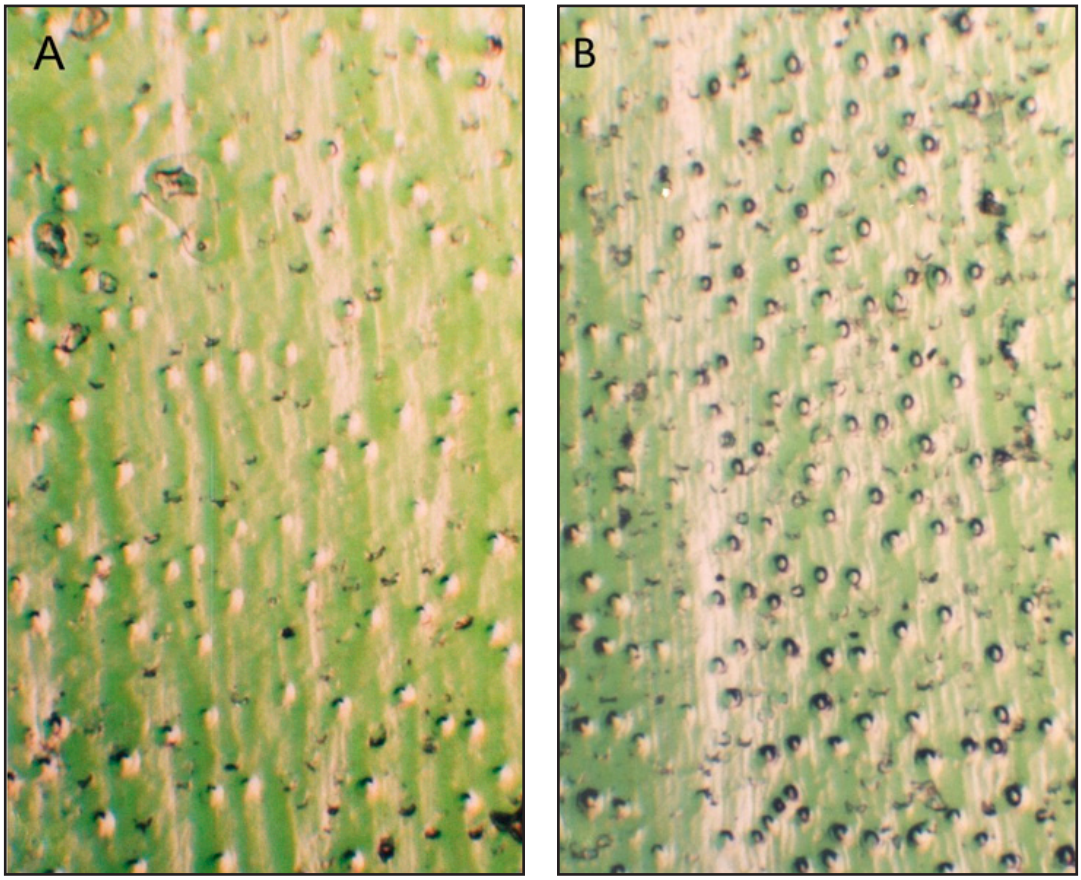

Fig. 1. Varnish replicas of abaxial epidermis of lemma in two *Avena strigosa* accessions. **A** – As10; **B** – As13.

proved on the molecular level for RFLP and RAPD (Alicchio *et al.* 1995; Nocelli *et al.* 1999; Loskutov & Perchuk 2000).

In the *Bromus secalinus* – *B. commutatus* – *B. racemosus* group, a minimum number of meristemoidal events in the lemma epidermis has been detected in the accession of *B. commutatus*, while a maximum number in a putative hybrid *B. racemosus* × *B. commutatus* (Skowrońska 2005). The same approach showed a large taxonomic distance between a perennial *Brachypodium sylvaticum* (Huds.) P. Beauv. and annual *B. distachyon* (L.) P. Beauv. Other species of this genus were intermediate to them (Kłyk 2005). Meristemoidal characteristics of the lemma also appeared to be valuable for the evaluation of taxonomic distances between an amphiploid *Avena magna* × *A. longiglumis* and its parental species (Świetlikowska 2008). A maternal dominance has been detected in the amphiploid.

In the *Lolium* species, papillae are the main morphogenetic event in the lemma epidermis (Kawa 2008). Perennial *L. multiflorum* Lam. described by lemma epidermis characteristics is well distinguished in an ordination space from annuals *L. temulentum* L. and *L. remotum* Schrank. *L. rigidum* Gaud. appeared to be close to *L. multiflorum*. Thus, meristemoid characteristics of abaxial epidermis of lemma describe well inter-specific differences as well distances between the species and their hybrid progeny.

The arrangement of OTUs in the ordination space shows (Fig. 2) that the statistics of a curvilinear regression between a line connecting the points of maximal values of x and y axes (coefficient of correlation $r = -1$)

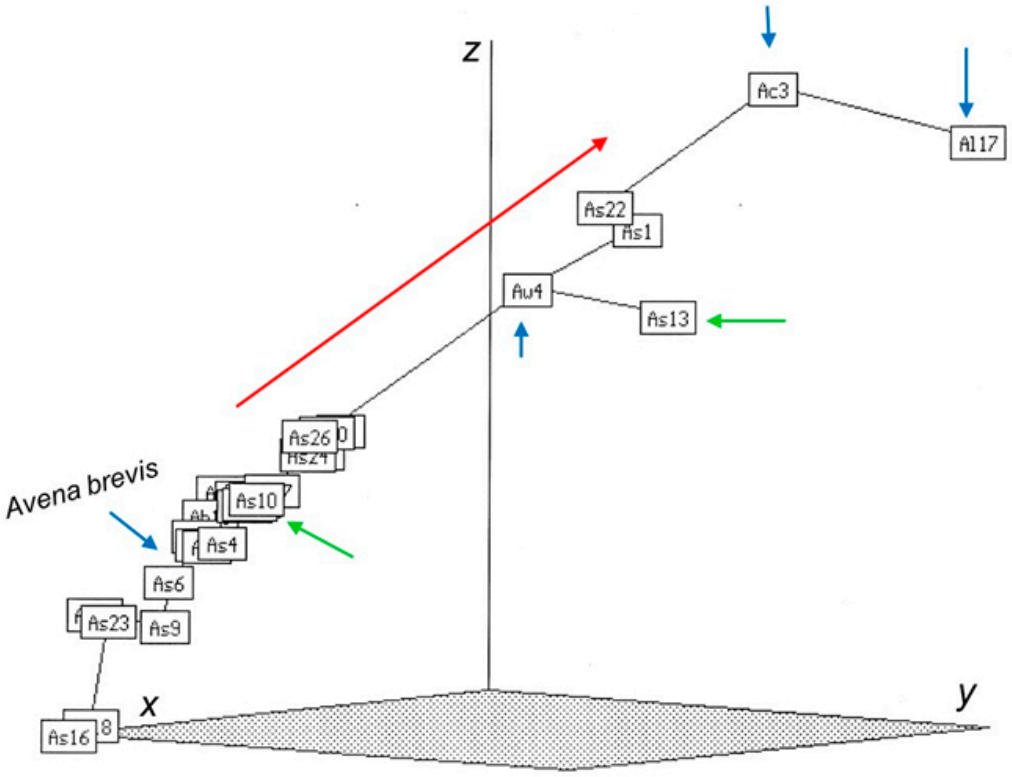

Fig. 2. A set of *Avena strigosa* (**As**) accessions and other oat diploids *A. canariensis* (**Ac**), *A. brevis* (**Ab**), *A. longiglumis* (**Al**) and *A. wiestii* (**Aw**) (see **blue arrows**) distributed in an ordination space in the form of minimum spanning tree. **Green arrows** show the As accessions presented in Fig. 1. A diploid cultivated species *A. brevis* is hidden within the *A. strigosa* set. The **red arrow** shows a directional trend of increased meristemoid frequency in the set of accessions.

and values of the *z* axis can be new taxonomic characteristics, and such type of approach has already been pointed for other grasses by KOSINA (2004).

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VARIATION OF STARCH GRANULES IN DIPLOID SPECIES OF THE GENUS *AVENA* L.

Romuald Kosina * & Anna Grabińska

Abstract. Composite starch granules, the main product of assimilation in oat endosperm, were analyzed in the accessions of both wild and cultivated diploid species of the genus *Avena*. Simple starch granules are mostly synthesized in the outer parts of the endosperm tissue. The size of sub-grains in a composite granule does not depend on a wild or cultivated status of the species. Inter-specific variation in the size differences of composite granules is large. Also, a broad variation has been detected for granules analyzed in a Lugol's solution or polarizing light. This analysis revealed a difference between the synthesis of amylopectin *versus* amylose in a granule. Examples of occurrences of low levels of amylopectin synthesis are provided.

Key words: *Avena* diploids, starch granules, variation

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Introduction

Many studies have shown that the development of endosperm tissue in grass caryopsis occurs according to a clonal pattern. The nature of the tissue in a free nuclear stage has been described as a body composed of sub-syncytial units, that is, groups of nuclei of different origin (Kosina 2009). For instance, in *Avena strigosa* Schreb., two adjacent cells of endosperm can synthesize starch granules of different size due to their genetic difference. On the other hand, the interior of the cell can differ in starch synthesis in its two regions, central *versus* external, and such a type of cells can create a single clone, *e.g.* in *A. brevis* Roth (Kosina 2009).

In wheat, starch granules of three different sizes, A, B and C are synthesized (Wilson *et al.* 2006; Kosina & Tomaszewska 2011b). The A- and B-granules dominate in the genus *Hordeum* L. (Baum & Bailey 1987). A bimodal size of starch granules (A and B) has been noted for rye, but in millet, rice or triticale (× *Triticosecale* Wittm. ex A. Camus) it is unimodal (Tester *et al.* 2004). Some data on the inter-specific variability of the size of subunits in the *Avena* L. composite starch granules have already been provided by Kosina (2007).

Changes in the size of starch granules were described by Klemsdal *et al.* (1986) in the *Risø* high lysine barley mutants. Granules were small and caryopses poorly filled by endosperm tissue. Other starch mutations such as *Risø 17* and *Notch-2* were studied by Burton *et al.* (2002). These mutants synthesize phytoglycogen and in their plastids several starch granules develop. In ripe caryopses, atypical starch granules are composite.

Other characteristics of starch granule can be detected by a Lugol's reaction or by imaging a granule in a polarized light. The amylopectin-poor starch granule appears as a Lugol's light

body or is lightly colored when observed under a polarizing microscope. Amylopectin-poor mutations of single starch granules were found in amphiploids such as *Elymus canadensis* L. × *Pseudoroegneria libanotica* (Hack.) D.R. Dewey and *Triticum dicoccum* Schrank ex Schübl. × *Aegilops squarrosa* L. (*A. tauschii* Coss.) or *Leymus racemosus* (Lam.) Tzvelev (Kosina *et al.* 2015). Patron *et al.* (2002) described cultivars in *waxy* barley with a low level or with no amylose starch granules. The level of amylose depends on the activity of granule-bound starch synthase I (GBSSI). Types with low- or free-amylose contents are probably of Chinese origin. *Waxy* mutants expressing lowered activity of GBSSI have also been obtained in *Avena strigosa* (Verhoeven *et al.* 2004). In potato, starch granules with low activity of GBSSI are not stained by Lugol's iodine (Edwards *et al.* 2002) and such types have also been documented in some members of Triticeae (Kosina *et al.* 2015).

Material and methods

Accessions of the *Avena* diploids were cultivated on small plots under the same soil and climatic conditions in R. Kosina's grass collection. Thus, the study material was treated as originating from a completely randomised one-way classification design. Starch granules of the following species were studied (in brackets, the numbers of accessions are provided): *A. brevis* Roth (4), *A. canariensis* Baum, Rajhathy et Sampson (1), *A. hirtula* Lag. (2), *A. longiglumis* Dur. (5), *A. nuda* L. (1), *A. pilosa* (Roem. et Schult.; syn. *A. eriantha* Dur.) (1), *A. strigosa* Schreb. (34) and *A. wiestii* Steudel (4). From broken caryopses, starch granules were isolated, mounted on slides in glycerin and stained by a Lugol's solution according to Broda (1971). Non-stained starch granules were observed under a polarizing Amplival microscope and documented by a Zenith TTL camera with Fuji 400 film. Granules with high amounts of amylopectin stained with Lugol's iodine were dark brown in color, and when observed under a polarizing microscope a distinct, red and blue coloration was noted.

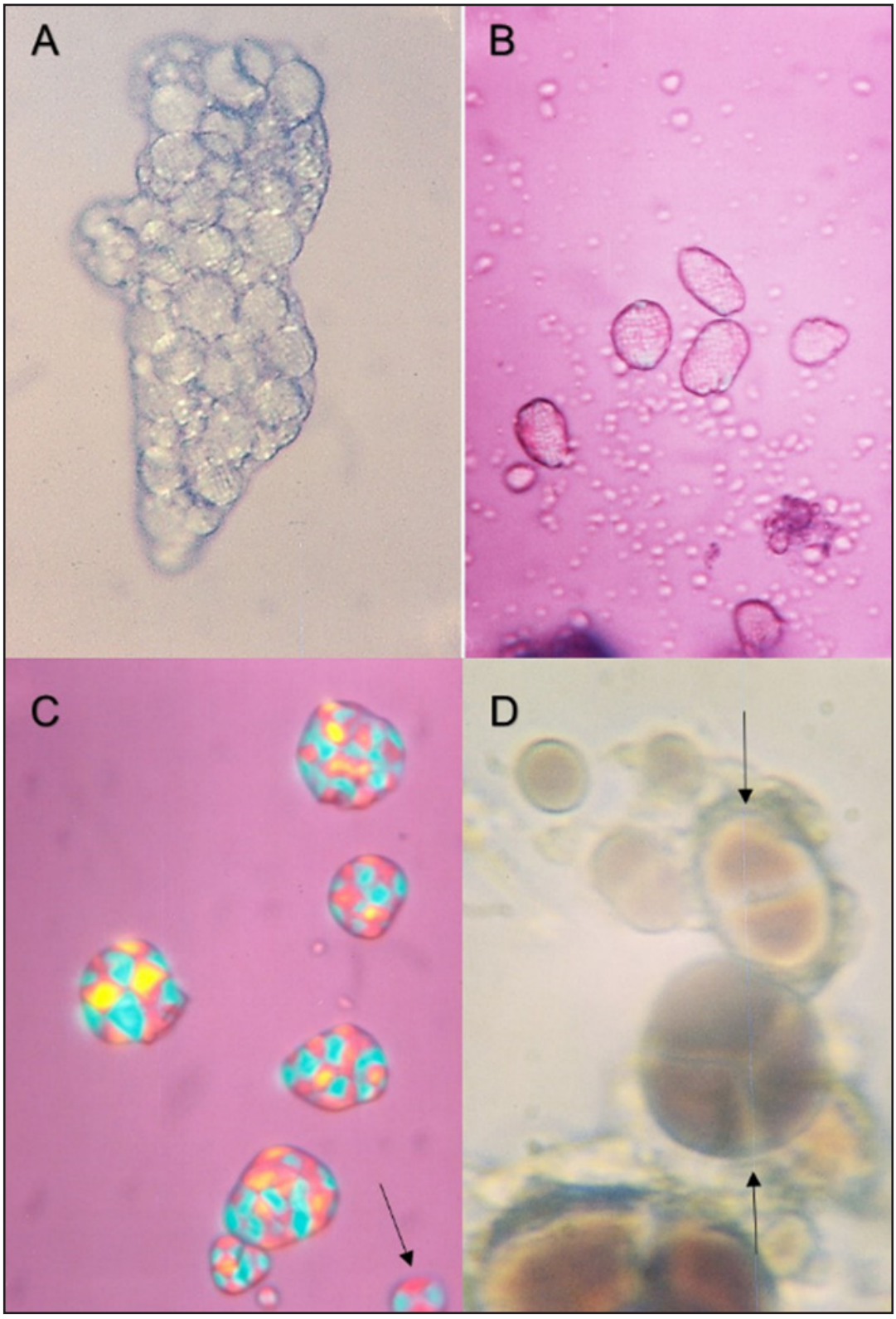
Results and discussion

In general, composite starch granules are synthesized in the endosperm of oats. However, the synthesis of simple granules adds them to a total starch pool in the tissue. Such dual morphogenesis especially occurs in the outer cells of endosperm which are adjacent to a high-protein (HP) subaleurone layer, *e.g.* in *A. hirtula* (Kosina 2009). The simple granules are also synthesized and embedded in a protein mass in the HP layer. The size of composite granules appeared highly variable among diploid oat species. This variation is exemplified in Fig. 1. In *A. pilosa* and *A. eriantha*, the granules are very fine-grained and composed of many grains. In *A. pilosa*, fine-grained granules, mainly circular, are embedded in a fatty mass (Fig. 1 A); *A. eriantha* has granules that are elongated and ellipsoidal in shape (Fig. 1 B). Granules in *A. wiestii* are larger and composed of only several starchy units (Fig. 1 C, D). Under polarizing light, they presents strong coloration (Fig. 1 C). This proves that the synthesis of amylopectin in these granules is very effective. *A. canariensis* has granules similar to those in *A. wiestii*. However, granules from another sample in *A. wiestii* differ with respect to amylopectin synthesis (Fig. 1 D). Lighter granules synthesize less amylopectin.

In a cultivated species, *A. strigosa*, fine-grained granules are several times larger than those in *A. eriantha*. A wild species, *A. longiglumis*, expresses a similar Lugol's staining diversity as *A. wiestii* (Grabińska 2008).

The above examples show that oat diploid species vary with respect to size of sub-grains present in the composite starch granules. Two types of granules are distinguished, coarse-

Fig. 1. Composite starch granules: **A** – *Avena pilosa*; **B** – *A. eriantha*; **C, D** – *A. wiestii*. **A-D** are taken under a light microscope; **A-C** under a polarised light; **D** – shows the granules stained in a Lugol's solution. Microscopic magnification: **A-C** – ×320; **D** – ×800. ►


A
B
C
D

grained in *A. wiestii* and *A. canariensis versus* fine-grained in *A. strigosa*, *A. pilosa* and *A. eriantha*. Each pool of granules is composed of composite and single ones, and proportion between both probably depends on the age of the tissue and its location in the caryopsis. Granules between each other also differ in the level of amylose *versus* amylopectin synthesis.

Morphogenesis and metabolic potential of starch granules can be inter- and intra-cellular and intra-granule variable (Kosina 2009). Such types of variation have also been noted in the endosperm aleurone layer (Kosina & Tomaszewska 2011a), and create mosaic patterns of the endosperm development (Kosina 2007). These variations are of mutational origin. Amyloplasts expressing amylose synthesis mutations were detected in barley (Patron *et al.* 2002), oat (Verhoeven *et al.* 2004) and pea (Edwards *et al.* 2002).

A. pilosa has been considered to be a heterotypic synonym of *A. eriantha* (Baum 1977). And the differences between both species in endosperm microstructure support such status of *A. pilosa*.

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ANTIPODAL SYMMETRY AND ASYMMETRY IN THE EMBRYO SAC OF *AVENA SATIVA* L.

Romuald Kosina * & Joanna Krawczyk

Abstract. Inter- and intra-antipodal variation is presented for a free-nucleolar developmental stadium in the *Avena sativa* endosperm. Antipodals of common oat are uni- or multinuclear. Multinuclear antipodals, in a single cell, show nuclei of the same mitotic stage, but this is different for various antipodals. Examples of DNA amplification and anomalies occurring during mitosis are provided.

Key words: *Avena sativa*, antipodals, DNA amplification, karyokineses, variation

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Introduction

In various grasses, antipodals are polyploidised up to different level and can be active in the form of an antipodal tissue even in the cellular endosperm (Batygina 1987). In a common wheat × triticale cross, antipodal nuclei can reach 512*n* within three days after pollination (Wędzony 1992-1993). Some data points that nuclei in a multinuclear antipodal cell are mitotically synchronous, *e.g.* in *Triticum durum* Desf. (Ivanovskaya 1983). However, Chaban *et al.* (2011) observed synchronous and asynchronous karyo- and cytokinesis in antipodals of common wheat. As a rule, giant chromosomes are formed in the uninuclear antipodals, and following this, the number of rDNA loci does not increase, *e.g.* in a *Triticum durum* × *Aegilops tauschii* Coss. amphiploid (Kosina 1994). Less is known on the behaviour of antipodals in the genus *Avena* L. Some results have already been presented by Krawczyk (2008).

Material and methods

Young fruits of *Avena sativa* var. *grisea* Körn. were dissected from spikelets in a stadium of free-nuclear endosperm. Fruits were fixed in a Carnoy's solution and stored in a freezer at -20 °C. Embryo sacs were isolated from the fruits and washed in distilled water and then three times for 5 min in a 0.01 M citrate buffer. Material was enzymatically digested in a mixture of pectinase and cellulase in a hybridisation oven at 37 °C. The digested material was centrifuged three times for 3 min at 800 g, each time in fresh citrate buffer. After centrifugation, the supernatant was discarded. The material was prepared by squash or dropping methods according to Schwarzacher *et al.* (1980) and Ambros *et al.* (1986). For chromosome staining, 100 µl/slide of 0.5 µg/ml DAPI and 0.025 µg/ml propidium iodide were used, respectively. Slides were washed in PBS buffer and mounted in a medium that prevented the fading of fluorescence. Slides were stored in a refrigerator at 5 °C. Cytogenetic material

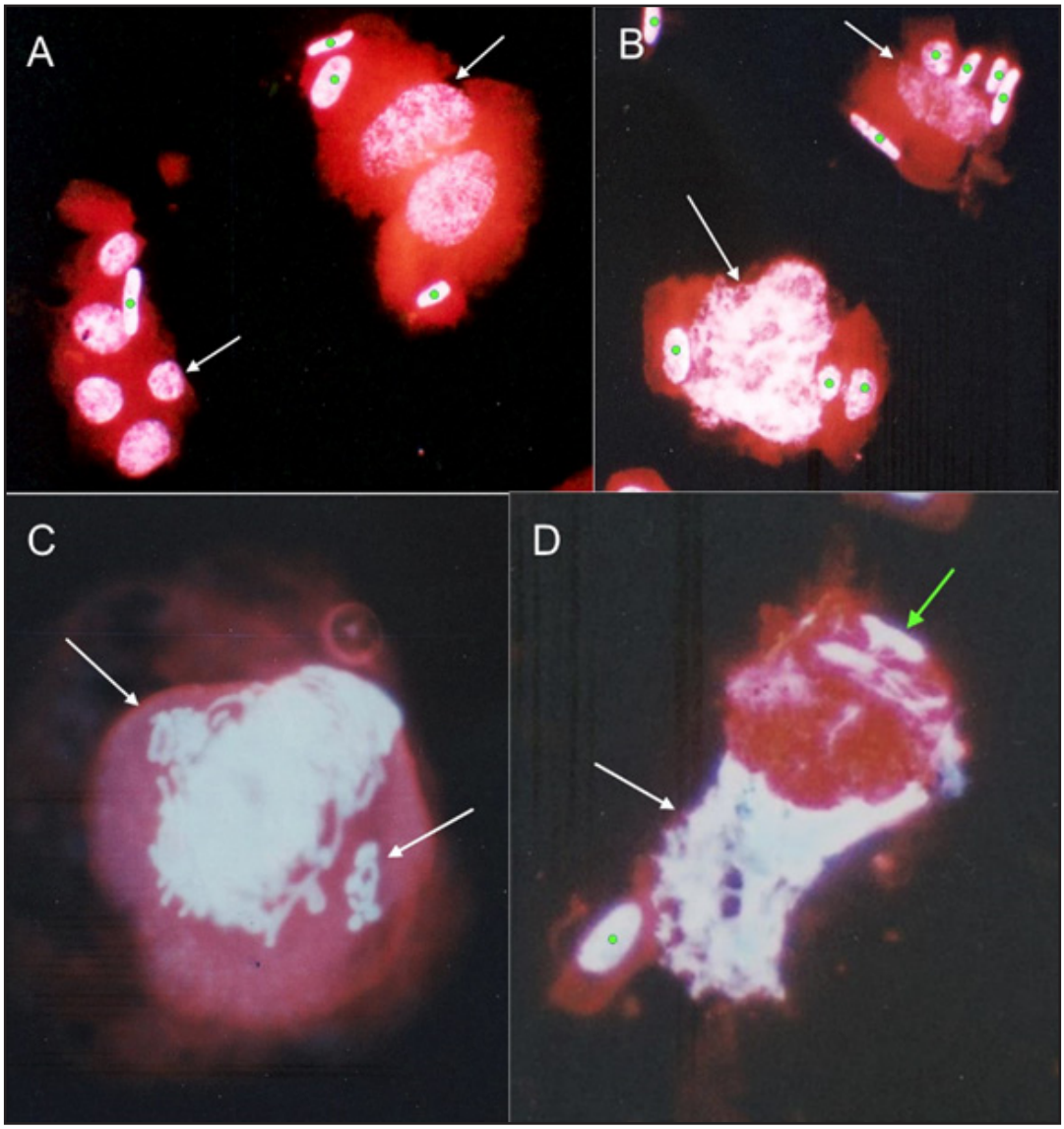

Fig. 1. Nuclear antipodal morphology at the free-nuclear endosperm stage in *Avena sativa*: **A** – two multinuclear (2 and 5 nuclei) antipodals (**arrows**) showing the different levels of ploidy and phase of the cell cycle; **B** – two uninuclear antipodals (**arrows**) with various DNA amplification and, probably, at a different stage of prophase; **C** – a highly polyploidised antipodal cell at prophase stage with two groups of laggard chromosomes (**arrows**); **D** – a partly apoptotised antipodal nucleus (**white arrow**) and semi-telophase of a small nucleus (remnant DNA) perhaps was formed from the laggard chromosomes (see **green arrow**). Other nuclei, not from antipodals, are marked by **green dots. A-D** – DAPI and propidium iodide sequential staining.

was documented under an Olympus BX60 epifluorescence microscope with a triple filter (DAPI, TRITC, FITC) and pictures were taken with Zenith TTL camera and Fuji 400 film.

Results and discussion

Amplification of DNA in an antipodal cell can occur by karyokineses or by the replication of DNA, without the separation of chromosomes. Karyokineses give cells

with the different numbers of nuclei and such a picture is presented in Fig. 1 A. In the red cytoplasm of two cells, five smaller nuclei in one cell *versus* two larger nuclei in another are compared. In both the cases, the nuclei are in prophase stage, but the larger nuclei are at a higher level of ploidy. This emphasizes the asynchrony between the two antipodals and these differences relate to the number of nuclei and their level of ploidy. However, synchrony exists among nuclei within each of cells. In Fig. 1 B, asynchrony is evident at the level of DNA amplification in the two uninuclear cells. No information is presented regarding the laggards in antipodals. Fig. 1 C shows that a highly polyploid prophase nucleus can be formed by an earlier non-disjunction of chromosomes. Some chromosomes are not included in the nucleus, providing micronuclei and creating intracellular nuclear asynchrony. The morphology of a nucleus presented in Fig. 1 D is not easy to interpret. A large light mass of highly condensed DNA is separated from some DNA remnants by a large fine-grained RNA nucleolar body stained by propidium iodide. These DNA remnants seem to be a micronucleus during a condensed telophase. Amplification of rDNA signals on polytene chromosomes, but not their number, has been detected by Kosina (1994) in a *Triticum* L. × *Aegilops* L. amphiploid.

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CARYOPSIS TRANSFER SYSTEM IN AN *AVENA MAGNA* MURPHY ET TERRELL × *A. LONGIGLUMIS* DUR. AMPHIPLOID

Romuald Kosina * & Maria Świetlikowska

Abstract. Evaluation of the structure of the caryopsis transfer system is presented for an *Avena magna* × *A. longiglumis* amphiploid. Each component of the system such as vascular bundle, pigment strand, nucellar projection and ventral aleurone layer varies between the amphiploid and its parental species. The number of xylem vessels present in the caryopsis bundle expressed heterosis-like inheritance. The position of the caryopsis xylem bundle in *A. longiglumis* shows a lower efficiency in assimilate transport. Some anomalies in the development of the ventral aleurone layer and parenchyma, adjacent to the transfer system, are presented.

Key words: *Avena amphiploid*, caryopsis, transfer tissues, xylem

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Introduction

The caryopsis transfer complex is composed of various tissues like vascular bundle, pigment strand, nucellar projection, and ventral aleurone layer. These tissues are in the crease area. In the *Hordeum* mutants, this complex decides on successful assimilate storage in the endosperm (Felker *et al.* 1985). In many grasses, transfer cell walls have been detected in these tissues, especially in nucellar projection. A distinct correlation has been discovered in wheat tetraploids between the number of xylem vessels presented in the vascular bundle of the caryopsis and the morphogenesis of starchy-protein endosperm tissue (Kosina 1988). Bundles composed of many vessels were positively correlated with the development of a thick high-protein subaleurone layer. Demethylation of genomes in a *Triticum timopheevii* Zhuk. × *Aegilops umbellulata* Zhuk. amphiploid led to the development of a poor nucellar projection, acellular pigment strand and sclerification of the tissue adjacent to pigment strand (Kosina *et al.* 2013; Koźlik 2013). Two wheats, *Triticum kiharae* Dorof. & Migush. and *T. fungicidum* Zhuk. differ from each other in size of the transfer ability in the xylem bundle, pigment strand and nucellar projection (Kosina & Bureś 2011). Structural differences detected in the nucellar projection of *Brachypodium distachyon* (L.) P. Beauv. appeared to be almost qualitative, where three distinct types have been distinguished (Kosina & Kamińska 2013a).

Material and methods

Accessions of the parental species, *Avena magna* Murphy et Terrell and *A. longiglumis* Dur., and an *A. magna* × *A. longiglumis* amphiploid were cultivated on small plots under the same soil and climatic conditions in R. Kosina's grass collection. The studied material was treated as originating from a completely randomised one-way classification design. Samples of size $n = 30$ were elaborated.

Caryopses were fixed in FAA, in the following proportions: formaldehyde (40%) : ethanol (50%) : glacial acetic acid (10%). After fixation, caryopses were rinsed three times in tap water and cut in the central part, in a plane perpendicular to the caryopsis axis. For cutting, a freezing microtome K TS-II (USSR) was used. Cross-sections of caryopses about 40 μm in thickness were mounted in glycerin as semi-permanent slides. The slides were documented in a polarizing Amplival microscope (Carl Zeiss, Jena, Germany). A natural fluorescence of tissues has been observed in an Olympus BX60 epifluorescence microscope with triple filter (DAPI, TRITC, FITC). Images were taken with an Olympus E-520 camera (Olympus Imaging Europa GMBH, Hamburg, Germany).

Results and discussion

The organization of the caryopsis transfer system in oats is similar to that of other grasses. In the maternal species, *A. magna*, the xylem bundle is composed of nearly 19 vessels. Some of them are clearly visible in Fig. 1 A. In the paternal species, *A. longiglumis*, the xylem bundle is distinctly smaller, and consists from about 9 vessels (Fig. 1 C). In *A. longiglumis*, the distance between the xylem and the pigment strand is larger compared to that in *A. magna* (Fig. 1 C *vs* 1 A), and this distance can be several times larger (Fig. 1 D, see a dotted line between pigment strand and vascular bundle). Thus, the efficient transport of assimilate provided by vascular bundle into endosperm is distinctly lower in *A. longiglumis*. On the other hand, in the amphiploid, the vascular bundle and adjacent parenchyma are isolated from other parts of pericarp by a thick-walled structure (Fig. 1 E, see white arrows). Also, a pericarp epidermis with thick internal and external tangential walls additionally isolates this area. Under polarizing light, these walls show a strong reaction, because of the presence of high cellulose content. Sclerification of the caryopsis transfer system has also been noted in other oat species such as *A. brevis* Roth and *A. strigosa* Schreb. (Grabińska 2008). The xylem bundle in the amphiploid is large and composed of up to 30 vessels (Fig. 1 B). Such a significant transgression of parental characteristics in the amphiploid can be a result of heterosis. After colchicine treatment of F1 plants, genomes of each parent occur as homologous sets in an amphiploid; however, interactions between the genomes of both parents and multiple translocations observed in the oat hybrid progeny create a heterozygous complex with expression of heterosis. This phenomenon of inheritance observed in successive generations in plant hybrids can be considered as a multiple heterosis (Palilov 1976). In the amphiploid, the morphology of the xylem bundle, but also multivariate characteristics of spikelet and abaxial epidermis of lemma show that the uniparental dominance is expressed, showing a distinct shift towards the maternal species, *A. magna* (Świetlikowska 2008). In another cereal amphiploid, *Triticum timopheevii* × *Aegilops umbellulata*, the number of placental xylem vessels is highly positively correlated with the width and thickness of caryopsis and height of nucellar projection (Kosina 2014).

As a rule, the aleurone layer develops between the nucellar projection and the starchy endosperm. In this area, the aleurone cells have transfer walls that are not present in others parts of the caryopsis. Some anomalies in the development of transfer system are detected in this area in *A. longiglumis* (Fig. 1 D, green arrow) and to a greater extent in the amphiploid. The aleurone layer is not developed there and the cells of the starchy endosperm adjoin nucellar projection. Such a pattern of development has been observed in other grasses such as *Brachypodium sylvaticum* (Huds.) P. Beauv. (Kłyk 2005), *B. distachyon* (Kosina *et al.* 2012; Kosina & Kamińska 2013b), *Avena wiestii* Steudel (Grabińska 2008) and *Bromus secalinus* L. (Kochmański 2008), but it seems to be more frequent in plants of hybrid origin (Bureś 2008; Tomaszewska 2009; Zając 2009; Koźlik 2013).

The development of the amphiploid caryopses appeared to be less stable than that in parental species (Świetlikowska 2008). In amphiploid plants, the most frequent

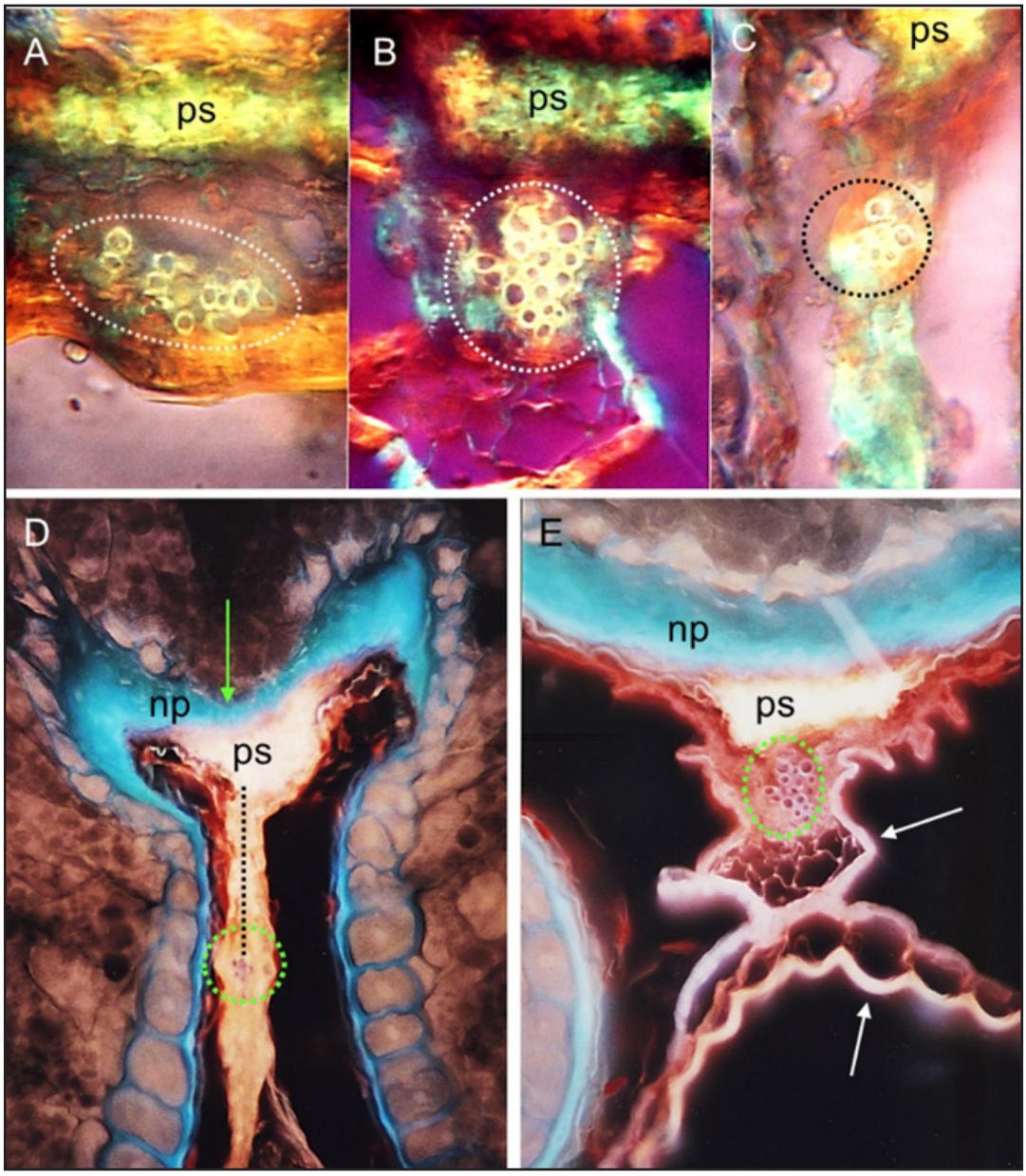

Fig. 1. Caryopsis xylem bundles in parental species *Avena magna* (**A**) and *A. longiglumis* (**C**), and in the amphiploid (**B**). Caryopsis transfer system in *A. longiglumis* (**D**) and in the amphiploid (**E**). **np** – nucellar projection; **ps** – pigment strand. Xylem bundles are encircled by **green dotted lines**. Thick cell walls isolating the vascular bundle are indicated by **white arrows** in **E**. Absence of aleurone layer at the border between the nucellar projection and starchy endosperm is indicated by a **green arrow** in **D**. **A-C** – as observed under polarising light; **D, E** – epifluorescence images. Microscope magnification for **A-C** – ×200; for **D, E** – ×80.

chromosome number is 41. Cytogenetic behaviour, with bridges, laggards and telocentrics, can lead to this hipohexaploid level.

Concluding remarks

The *4x/2x Avena* amphiploid expressed some cytogenetic and developmental instability

(Świetlikowska 2008). Also, such a status was detected for the ventral aleurone layer development. In the amphiploid plants, heterosis and maternal dominance were noted. A positive transgression in the number of caryopsis xylem vessels creates some assimilate storage advantage of the amphiploid over its parental species. Perhaps, this phenomenon can be more enhanced in cytogenetically stable hexaploid plants.

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INTRODUCTORY DATA ON MEIOTIC STRUCTURES IN *ALLIUM SENESCENS* L. SUBSP. *MONTANUM* (POHL) HOLUB

Romuald Kosina * & Agnieszka Małecka

Abstract. Examples of zygotene chromosomal configurations in *Allium senescens* subsp. *montanum* are presented. Bivalents do not dominate in a zygotene stadium. Some bivalent segments show deletions. Also, tri- and tetravalents segments are observed. The conjugation of SAT-chromosome shows that a translocation has occurred in the SAT segment. In addition, univalents indicate that meiotic behaviour of the species is unstable.

Key words: *Allium senescens*, meiosis, conjugation, translocations

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Introduction

Allium senescens L. subsp. *montanum* (Pohl) Holub is a perennial plant distributed mainly in Central Europe, with extreme points in Northern Germany, Central Ukraine, Northern Spain and Southern Italy (Friesen & Herrmann 1998). Zhang *et al.* (2012) have provided data on its vegetation also in China. Its synonyms are *A. montanum* Lam. and *A. lusitanicum* F.W. Schmit. The species has been recognized as allotetraploid (Gillies 1989) with chromosome numbers ranged from 16 to 32 (Pastor 1982; Speta 1984). Breeding strategy is the factor that decides the survival of the species in the environment, and hence research of meiotic behaviour is important in assessing the evolutionary fate of the species. A detailed study by Małecka (2008) showed that the process of microsporogenesis in the species is highly unstable. Laggards, bridges, micronuclei of different size and extrusion of nucleolar RNA into cytoplasm are common. Zhang *et al.* (2012) observed similar anomalies in plants of *A. senescens* growing in China. Therefore, presence of B chromosomes has to be considered when meiotic behaviour is evaluated in alliums. One B chromosome was noted in *A. senescens* (Shopova 1966). However, Loidl (1988) did not find too many anomalies during pachytene synapsis in allotetraploid *A. montanum*. In this polyploid species one can at least expect some meiotic configurations related to multivalents formation.

Material and methods

Stamens of *A. senescens* subsp. *montanum* were collected during two years, 1993 and 2006, from plants cultivated in the Botanic Garden, University of Wroclaw, Wroclaw, SW Poland. The stamens were fixed in a Carnoy's solution and stored in a freezer at -20 °C. Before slide preparation, stamens were washed in distilled water and further three times for 5 min in a 0.01 M citrate buffer. Material was enzymatically digested in a mixture of pectinase and cellulase in a hybridization oven at 37 °C. The digested material was centrifuged three times for 3 min at 800 g, each time in a fresh

citrate buffer and the supernatant was discarded. The material was prepared by squash or dropping methods according to Schwarzacher *et al.* (1980) and Ambros *et al.* (1986). For better dispersion of chromosomes, a hot plate method according to Henegariu *et al.* (2001) was applied. For chromosome staining, 100 µl/slide of 0.5 µg/ml DAPI and that of 0.025 µg/ml propidium iodide were used, respectively. Slides were washed in PBS buffer and mounted in a medium that prevented the fading of fluorescence. Slides were stored in a refrigerator at 5 °C. Slides were documented under an epifluorescence microscope Olympus BX60 with a DAPI filter and pictures were taken with Zenith TTL camera and Fuji 400 film.

Results and discussion

The size of an *Allium* L. chromosome can be observed in a specimen shown in the left upper part of Fig. 1 A. This is a univalent chromosome, not conjugated. A univalent segment is also shown in the lower part of the picture. Another chromosome, in which chromatids are indicated by black arrows (Fig. 1 A), shows probably two crossing-overs with two other chromosomes not distinguished in a chromosomal mass. These chromosomes form a trivalent segment. Trivalent association is also indicated by green arrows in the lower right part of the zygotene group. The arrows point to the conjugation of heteromorphic arms of different length. This trivalent group is associated by a short terminal segment with another chromosome, and a tetravalent group is formed as a result of several translocations. In Fig. 1 B, a univalent segment (see white arrow in the upper left part) proves that in this site some deletion is present. Several long bivalent segments are shown (red arrows); however, in the lower right part, two bivalent segments are involved to form a tetravalent segment. A short translocation forms a trivalent segment between the bivalent and SAT-chromosome. A NOR-constriction of the SAT-chromosome is well documented. Both zygotene pictures show that some uni-, bi- and multivalents are formed during meiosis. Translocations are responsible for such configurations. SAT-chromosomes also undergo these changes.

Małecka (2008) showed that the studied *Allium* is an allotetraploid, therefore it could expect a high percent of bivalents. In fact, meiosis in this accession was highly irregular. Multiple translocations, multivalents, rings, heteromorphic bivalents, laggards, bridges, dicentric chromosomes and a spectrum of micronuclei were noted. The frequency of these chromosomal aberrations reached 15%.

B chromosomes which present in alliums during meiosis are mostly univalents and are preferentially transmitted. In polyploids, like the allotetraploid here, they are lost. In *A. senescens* one B chromosome has been detected (Shopova 1966). No multivalents have been observed from diplotene to metaphase stage in allotetraploid *A. montanum* (Loidl 1988). But Fig. 1 shows that some partial multivalent associations occur. Also, multivalents were noted in diakinesis (Małecka 2008). In addition, the presence of heteromorphic bivalents and some deletions contradict the possibility of pure bivalents occurrence. Here, chromosomal configurations cannot be 'true multivalents' but multivalent association caused by translocations. Configurations presented in the upper part (Fig. 1 A, black arrows) and in the lower part (Fig. 1 B, blue and red lines) can be interpreted as tetravalent formation, and such figures have been documented in *A. porrum* L. by Khazanehdari *et al.* (1995).

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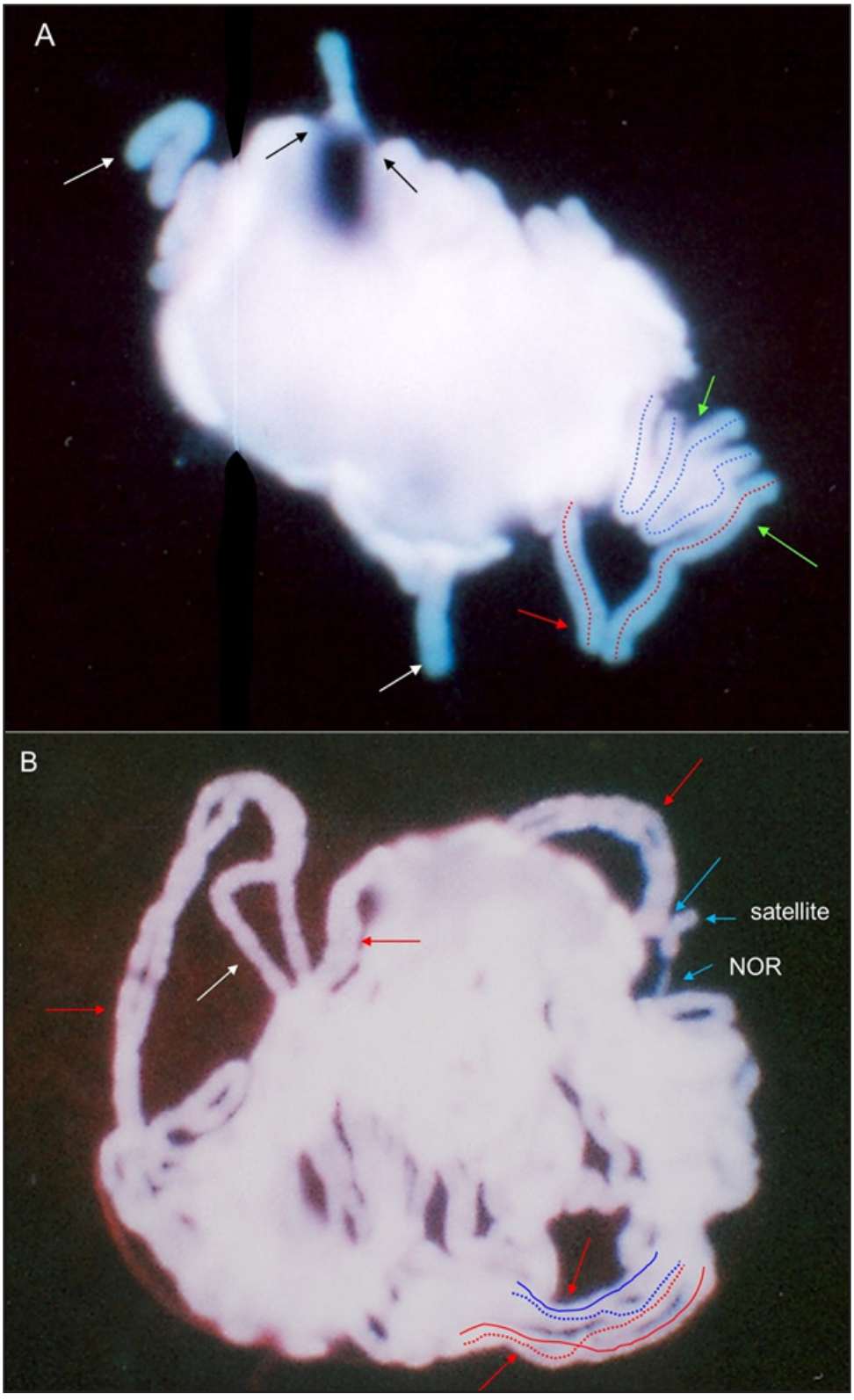
Fig. 1. Zygotene chromosomal configurations in *Allium senescens*. Univalents (**white arrows**), bivalents (**red arrows**), ▸ and trivalent formation (**black** and **green arrows**), SAT-chromosome (**blue arrows**).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RANUNCULUS POLYRHIZOS AS A NEW RECORD FOR IRAN, WITH ECOLOGICAL AND MICROMORPHOLOGICAL EVIDENCE

Mahmoud Bidarlord [1*], Farrokh Gahremaninejad [1], Maneezheh Pakravan [2]

Abstract. *Ranunculus polyrhizos* is reported as a new noteworthy record for the flora of Iran. This species was collected from alpine dry gravelly slope in Talesh Mountains. Taxonomic remarks and notes geographical distribution and habitat for this species are provided. Moreover nectar scale, pollen and achene micromorphological characters of the species are added and compared with related species.

Key words: *Ranunculus polyrhizos*, Talesh Mountains, new record, flora, autoecology

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Introduction

Genus *Ranunculus* L. (Ranunculaceae Juss.) with about 60 taxa is one of the largest genera in the flora of Iran (Iranshahr *et al.* 1992; Naqinezhad *et al.* 2016). This genus is distributed in different habitats including forests, dry and wet meadows, flood plains, lakes, rivers, and in alpine regions (Johansson 1998). Recently, studies have been carried out in taxonomy (Pakravan 2010, 2012), palynology (Pakravan *et al.* 2010, 2014), phylogeny (Rastipishe *et al.* 2011) and morphology of nectaries (Emady *et al.* 2010; Nemati *et al.* 2009) of some Iranian *Ranunculus* species.

Pollen morphology of Ranunculaceae was investigated by various authors (Wodehouse 1936; Kumazawa 1936; Santisuk 1979; Hamilton 1976; Petrov *et al.* 1981). Clarke *et al.* (1991) described three different pollen types of *Ranunculus* viz *R. acris, R. arvensis* and *R. parviflorus* types. In particular, *R. acris* type he divided onto nine groups which are commonly characterized by their tricolpate (rarely pentacolpate) apertures.

Babington (1856) described different shape of the nectaries of *Ranunculus*. Cook (1966) introduced three main nectary types: lunate, circular and pyriform. Subsequently, Dahlgren (1992) described eight types of nectaries for subgen. *Batrachium*.

The main classifications (De Candolle 1818; Tamura 1995; Davis & Cook 1965; Iranshahr *et al.* 1992) showed that characters of achenes are suitable in separation of taxa in *Ranunculus*. *Ranunculus* achenes are usually with a persistent glabrous beak, without distinct longitudinal wrinkles, rarely faintly wrinkled on lateral faces, pericarp with sclerenchymatous layer, have important role in infrageneric classification.

The aim of the current paper is to introduce Ranunculus polyrhizos as a new record from Iran. Additionally, some ecological and micromorphological characters of the species are represented.

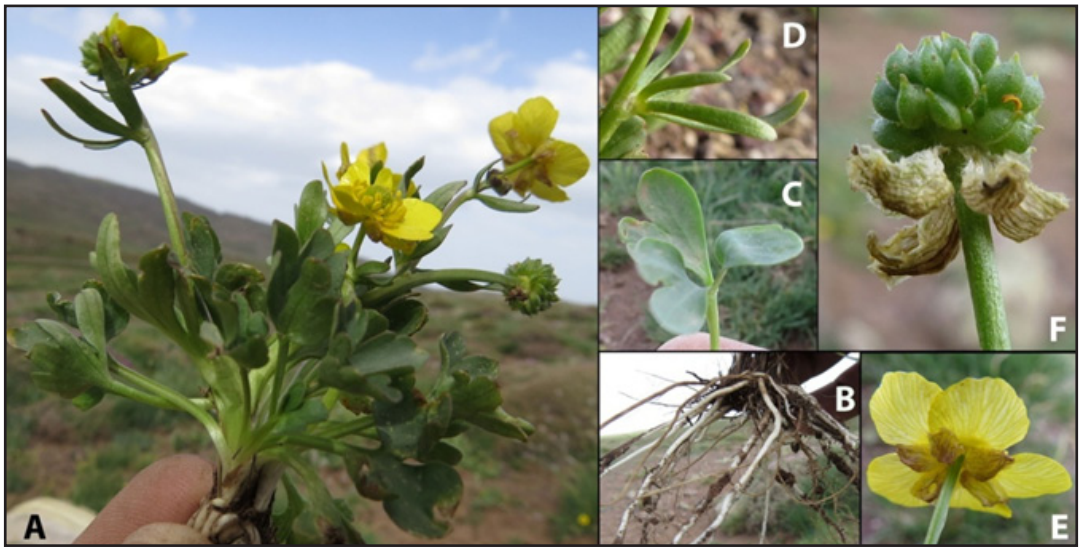

Fig. 1. *Ranunculus polyrhizos*: **A** – habit; **B** – root; **C** – basal leaf lamina; **D** – stem leaf lamina; **E** – flower (sepals and petals); **F** – friut.

Material and methods

During the field work in Talesh mountains some interesting *Ranunculus* specimens were collected. Later these specimens were crosschecked with Floras (Boissier 1867; Ovchinnikov 1937; Davis & Cook 1965; Iranshahr *et al.* 1992; Wang & Gilbert 2001) and then, on the base of inspection of herbarium collections at T, FAR, TARI and W, the specimens have been identified as *R. polyrhizos* Stephan ex Willd. Recorded material was deposited at FAR, T and W herbaria.

Some morphological characters were measured in the field on living plants, while others were analyzed on herbarium specimens by using stereomicroscope Zeiss Stemi SV 6. Micromorphological analyses were carried out on scanning electron microscope KYKY-EM 3200. Soil samples were taken from the center of the population to 30 cm depth. Measured soil variables include physical and chemical properties. Soil texture was determined by the hydrometric method (Bouyoucus 1951). Soil pH and soil electrical conductivity (EC) were determined by pH-meter with glass electrode and EC-meter respectively. Organic matter (OM) was estimated by Walkley and Black method (Nelson & Sommers 1996).

Results and discussion

Ranunculus polyrhizos Stephan ex Willd., Sp. Pl., ed. 4 [von Willdenow.] **2 (2)**: 1324, 1799; Ovchinnikov, Flora of USSR **7**: 301, 1937; Davis, Flora of Turkey **1**: 170, 1965; Wang, Flora of China **6**: 282, 2001 (Fig. 1).

Specimens examined. IRAN: Ardabil province, 43 km on the road of Ardabil to Khalkhal, Neor, Lissar protected area, Bacrodagh mountain, 2800-2900 m a.s.l., 37° 58′ N, 48° 36′ E, 03 May 2014, *Bidarlord 15887* (FAR, T, W).

Additional specimens examined. CHINA: Manchuria, Tigrowe Prope Schi-touhodse. 5.6.1928, N. Kozlow, *W 12438*, 1940. **RUSSIA:** *W 9910*, 1964; *W 21992*, 1974; *W 12892*, 1992; *W 09052*, 1991. **TURKEY:** B8 Erzurum, Palandoken Dag Gebirgs steppe, 2900 m, 1978, *W 12892*.

Morphological remarks. Perennial glabrous plants, 5-17 cm high. Roots fasciculate, slender. Collar fibrous. Stems 1-3, usually ascending or erect, distally branched, mostly 2-5-flowered. Basal leaves petiolate, petioles 1.5-5 cm, glabrous; blade 0.7-1.8×1-3.2 cm, more or less reniform or rounded-reniform in outline, deeply tripartite or trisected, the segments tapering to a petiole like base, the middle segment oblong

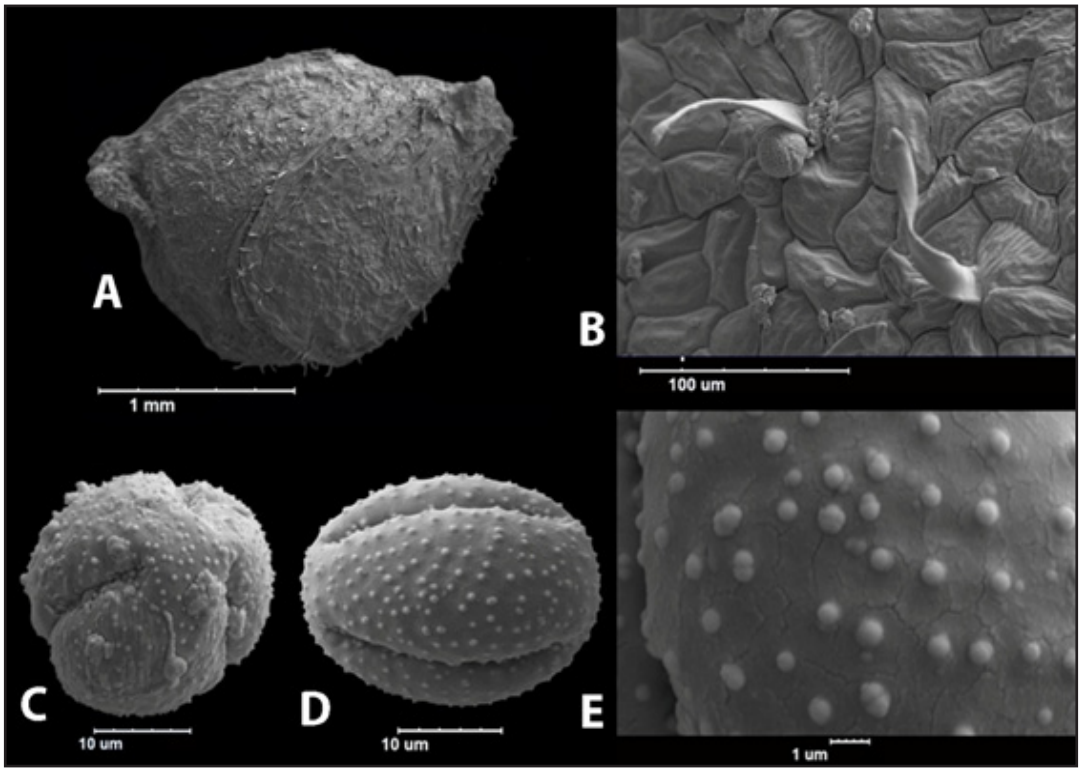

Fig. 2. SEM micrographs of *Ranunculus polyrhizos*: **A** – achene; **B** – achene surface; **C** – polar view of pollen grain; **D** – equatorial view of pollen grain; E – exin surface.

obovate, cuneate, with 3 rounded apical teeth or small lobes, the lateral segments broader than the middle one, bifid, with entire obtuse margin. Lower stem leaves similar to basal leaves. Upper stem leaves sessile, trisected, segments linear, bracts sessile, 2-3-partite, with linear lobes. Peduncles finely sulcate, often divaricate, in groups of 2 or 3, glabrous or minutely hairy distally. Flowers solitary, terminal, 1-2 cm in diameter. Receptacles puberulent. Sepals 5, as long as half of petals, more or less broad ovate, convex, with numerous prominent rather dark longitudinal partly branching veins, abaxially sparsely yellowish and puberulent; the edges whitish, scarious, hairy. Petals 5-7, 5-10×5-8 mm obovate, with prominent veins on both surfaces, the margin rounded, with a few hairs near nectary, nectar scale sacate. Stamens numerous; anthers narrowly oblong. Achenes 2-2.5 mm long, *ca.* 1.5-2 mm wide, more or less obovate or oblong-obovate, turgid, covered with a short whitish-scarious hairs, without transverse wrinkles, but pericarp often in lower seed-bearing part with two longitudinal spongy wings; the beak to 0.3 mm, glabrous, uncinate. Scanning electron microscope analysis of achene morphology showed that epidermal cells are imbricate, with strip-like hairs. Seeds light brown with dark brown longitudinal ribs, ellipsoid or triangular, 1-1.5 mm long and 0.8-1 mm wide, outline on the hilum face triangular (Figs 2 & 3).

Phenology. Flowering in April – May, fruiting in May – June.

Distribution and habitat. *R. polyrhizos* has been collected from alpine dry gravelly slope of the Backrodagh mountain in the Talesh mountains, ranging from altitudes of 2800-2900 m a.s.l. Accordingly to conducted analysis, this species grows on the loamy soils (sand 48, clay 20, silt 32) with pH 7, soil EC – 670 μSiemens/cm, and organic matter near 4.5%. This species was accompanied by such alpine species as *Allium derderianum* Regel,

Alopecurus aucheri Boiss., *Artemisia melanolepis* Boiss., *Astragalus aureus* Willd., *Campanula stevenii* M. Bieb., *Colchicum raddeanum* (Regel) K. Perss., Festuca rupicola Heuff., *Ficaria kochii* (Ledeb.) Iranshahr & Rech. f, *Jurinea monocephala* Aitch. & Hemsl., *Minuartia recurva* (All.) Schinz & Thell., *Onobrychis cornuta* (L.) Desv., *Poa bulbosa* var. *vivipara* Koch, *Scutellaria pinnatifida* A. Ham., *Tanacetum chiliophyllum* (Fisch. & E. Mey. ex DC.) Sch. Bip, *Thymus kotschyanus* Boiss. & Hohen., *Valeriana leucophaea* DC., and *Veronica kurdica* Benth.

R. polyrhizos is an Euro-Siberian element. It was firstly described from Siberia (Wildenow 1799). It is distributed from Turkey, Transcaucasia, Central and South Russia, Siberia, Kazakhstan to China (Xinjiang). This species grows in alpine screes, steppes, meadows, among scrubs, sometimes on abandoned fields, dry gravelly slopes in altitude from 1200 to 3000 m a.s.l. (Ovchinnikov 1937; Davis & Cook 1965; Wang & Gilbert 2001).

According to molecular results (Emadzade *et al.* 2010), the Central Asian specimens of *R. polyrhizus* were nested within North American clade. However, some previous investigations (Nemati *et al.* 2009; Emady *et al.* 2010) showed that characters of nectar scale have taxonomical value and separate these *Ranunculus* species.

In *R. polyrhizos* nectar is excreted by nectar scale at the base of petal on the yellow claw. Nectar scale is about 1×0.8 mm. It arises directly from the petal to which it is laterally attaching in the whole of its length and forming a sack at the petal bottom. At the top it is hairy and sometime dentate (Fig. 3). Basing on Nemati *et al.* (2009) *R. polyrhizos* nectar scale shape is similar to such in *R. asiaticus*, but in color it is golden-yellow instead of red-purple.

The pollen grains of *R. polyrhizos* are triporate, radically symmetrical, and heteropolar. The length of polar axis (P) is 29.5 μm and equatorial length (E) is 22.01 μm, ±P/E = 29.5/22.1. Pollen shape is prolate. Ornamentation is verrucate. Pollen characters *R. polyrhizos* is similar to *R. glacialis* (Halbritter *et al.* 2011). These pollen characters occurred in the *R. acris* type in accordance to Clarke *et al.* (1991).

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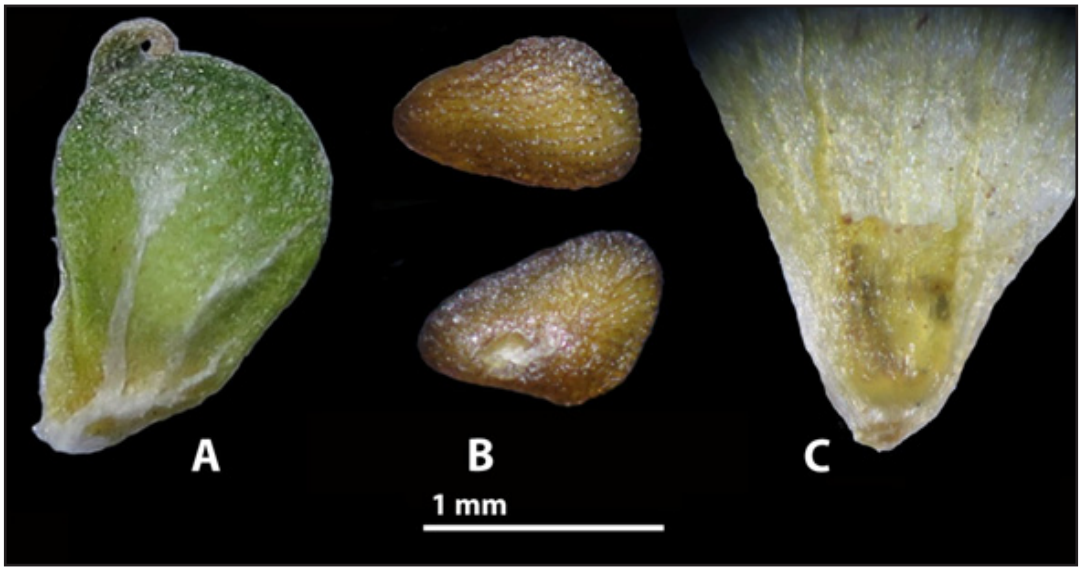

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CHENOPODIUM BADACHSCHANICUM (AMARANTHACEAE), A NEW RECORD FOR IRAN

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Abstract. *Chenopodium badachschanicum* Tzvelev is recorded for the first time for the flora of Iran. The species has been collected from Hamoon lake basin in South East of Iran. Characteristics, exact localities, habitat and geographical distribution are explained.

Key words: Amaranthaceae, *Chenopodium badachschanicum*, new record, Iran

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Introduction

Chenopodium L. comprises about 150 annuals and perennials in the world (Fuentes-Bazan *et al.* 2012), out of which at least 15 species occur in Iran (Uotila 1997; Assadi 2001).

Due to phenotypic plasticity, polyploidy and hybridization, taxonomy of *Chenopodium* always was a problematic task (Cole 1961; Rahiminejad & Gornall 2004; Kurashige & Agrawal 2005; Zhou *et al.* 2005). Among this genus, two controversial taxa, *i.e. C. album* L. aggregate and *C. hybridum* L. aggregate, comprise different species, subspecies, varieties and forms (Jüttersonke & Arlt 1989; Dvořák 1990, 1992; Zhu *et al.* 2003).

C. hybridum agg. is described in Flora of China as two species or subspecies (Zhu *et al.* 2003). Although *C. badachschanicum* Tzvelev was reported for Central Asia by Tzvelev (1960), in Eastern Asia it was not known before this time (Zhu *et al.* 2003).

Several new species of genus *Chenopodium* have been recorded in recent years from Iran (Rahiminejad *et al.* 2004; Rahiminejad & Ghaemmaghami 2005), confirming that this genus here is represented by more species than it was suggested before (Uotila 1997). During field investigation in Sistan and Baluchestan provinces at Hamoon Lake district (south east of Iran), an interesting *Chenopodium* specimen was collected. Identification of the specimen was done in the herbarium of Alzahra University (ALUH), and as a result *C. badachschanicum* is reported for the first time for the flora of Iran.

Results

Chenopodium badachschanicum Tzvelev, *Notul. Syst. Herb. Inst. Bot. Nomine V.L. Komarovii Acad. Scient. URSS* **20**: 434,

Fig. 1. Map showing locations of *Chenopodium badachschanicum* in Iran (★) and adjacent countries (●).

1960; Ovtšinnikov, Fl. Tadž. SSR **3**: 328, 1968; Pratov in Bondarenko & Nabiev, Consp. Fl. As. Med. **3**: 42, 1972; Uotila in Rech. f., Fl. Iran. **172**: 40, 1997.

Description. Sparsely farinose to subglabrous annual, stem to 30 cm, yellowish-green, erect, angular, branched, lower branches sub-opposite. Petiole usually *c.* 1/3 of the length of leaf blade, blade thin, 3-8(-15) cm, lanceolate, with outward-projecting acute basal lobes and 0-2 lobe-like acute teeth on both sides, otherwise entire, apex acute to acuminate, base sub-truncate to slightly cordate, bracts narrowly triangular, hastate, entire, uppermost lanceolate. Inflorescence narrow, lax, mostly leafless, terminal and axillary, cymose -dichasial, branches divaricate, solitary or several loosely together. Perianth segments 5, connate to below the middle, partly spreading in fruit, with a strong midrib visible especially inside, back apically keeled. Stamens 5. Stigmas 2-3. Part of fruits falling with perianth. Pericarp persisting. Seeds horizontal, black, (1.2-)1.4-1.6(-2.0) mm in diameter, round in outline, margin somewhat acute, testa with large, irregular but mostly radially elongated pits, radial furrows and other rugosities, sometimes almost smooth (Figs 1-3).

Flowering and fruiting. June – September.

Studied population. IRAN: Sistan & Baluchestan, 7 km south of Hirmand, Barahoii village (*ALH – ha105*).

General distribution. C Asia, NE Afghanistan, N Pakistan, N India, China, Nepal.

Notes. *C. badachschanicum* is an element of the *C. hybridum* aggregate. This is a tiny annual species. Type specimen was not available; there

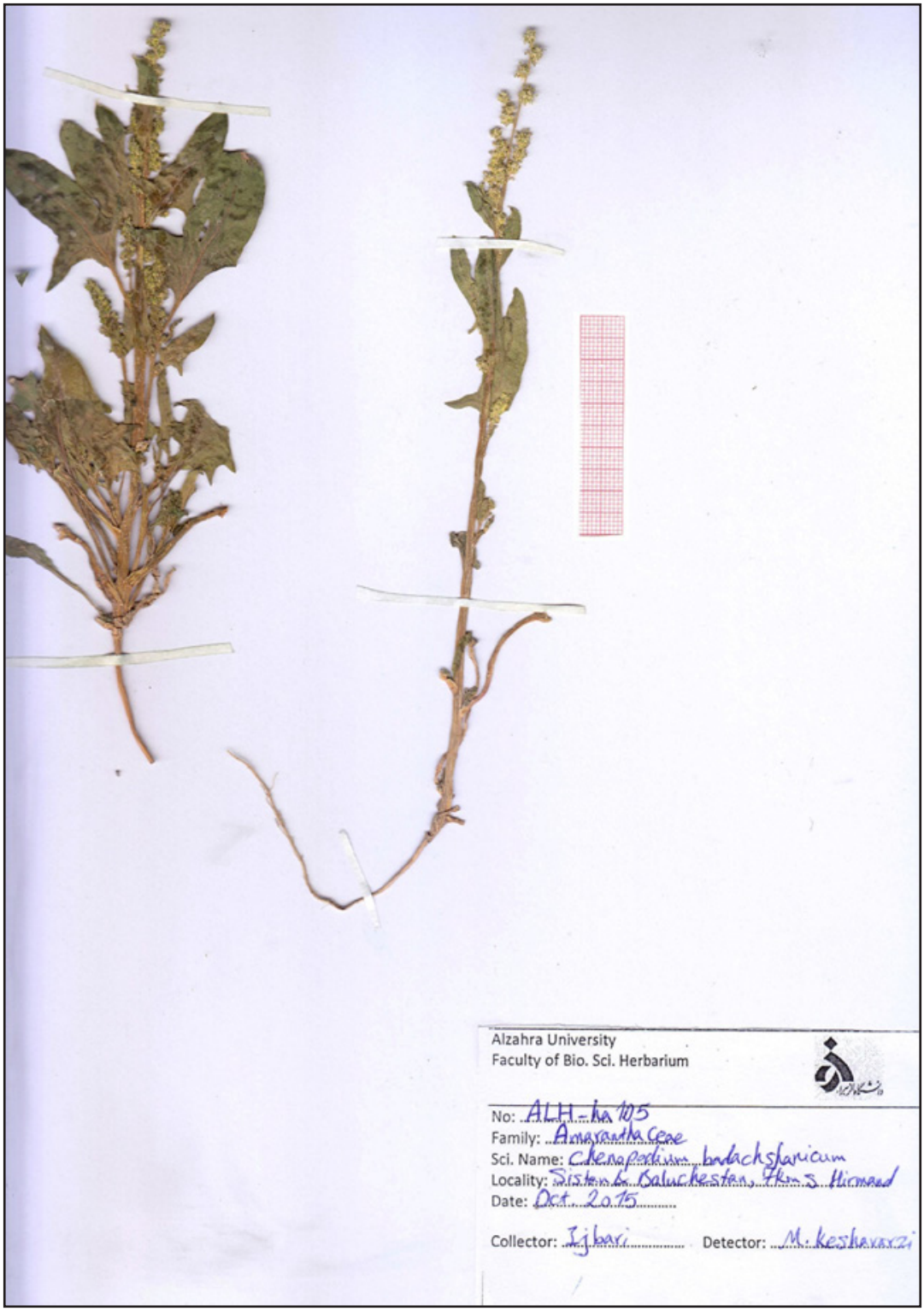

Fig.2. General view of collected *Chenopodium badachschanicum*.

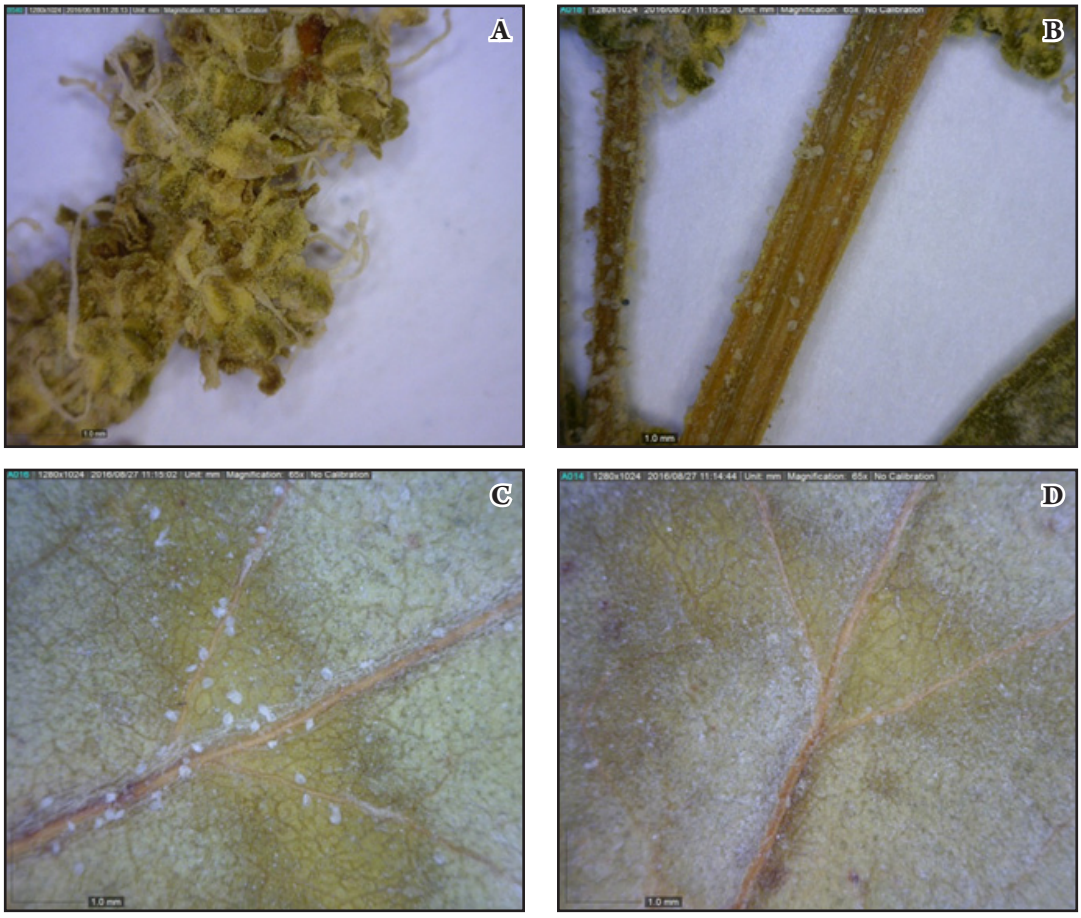

Fig.3. Different parts of collected *Chenopodium badachschanicum*: **A** – flowers; **B** – stem; **C** – abaxial surface of the leaf; **D** – adaxial surface of the leaf.

was only description of the species from the Flora of Pakistan (Perveen & Qaiser 2012). Details of its description were compared with available references (Freitag *et al.* 2001). According to the descriptions given in Flora of Pakistan, the specimen was identified as *C. badachschanicum*. To be sure about the species identification, micro-morphology of the pollen grains was studied (Fig. 4).

This species is sympatric with *C. murale* L. which shows great morphological variability in different localities of Iran, so their pollen grains were compared. In particular, exine thickness in studied *C. badachschanicum* (2.52 μm) was in concordant with Perveen & Qaiser (2012) measurements for Pakistan.

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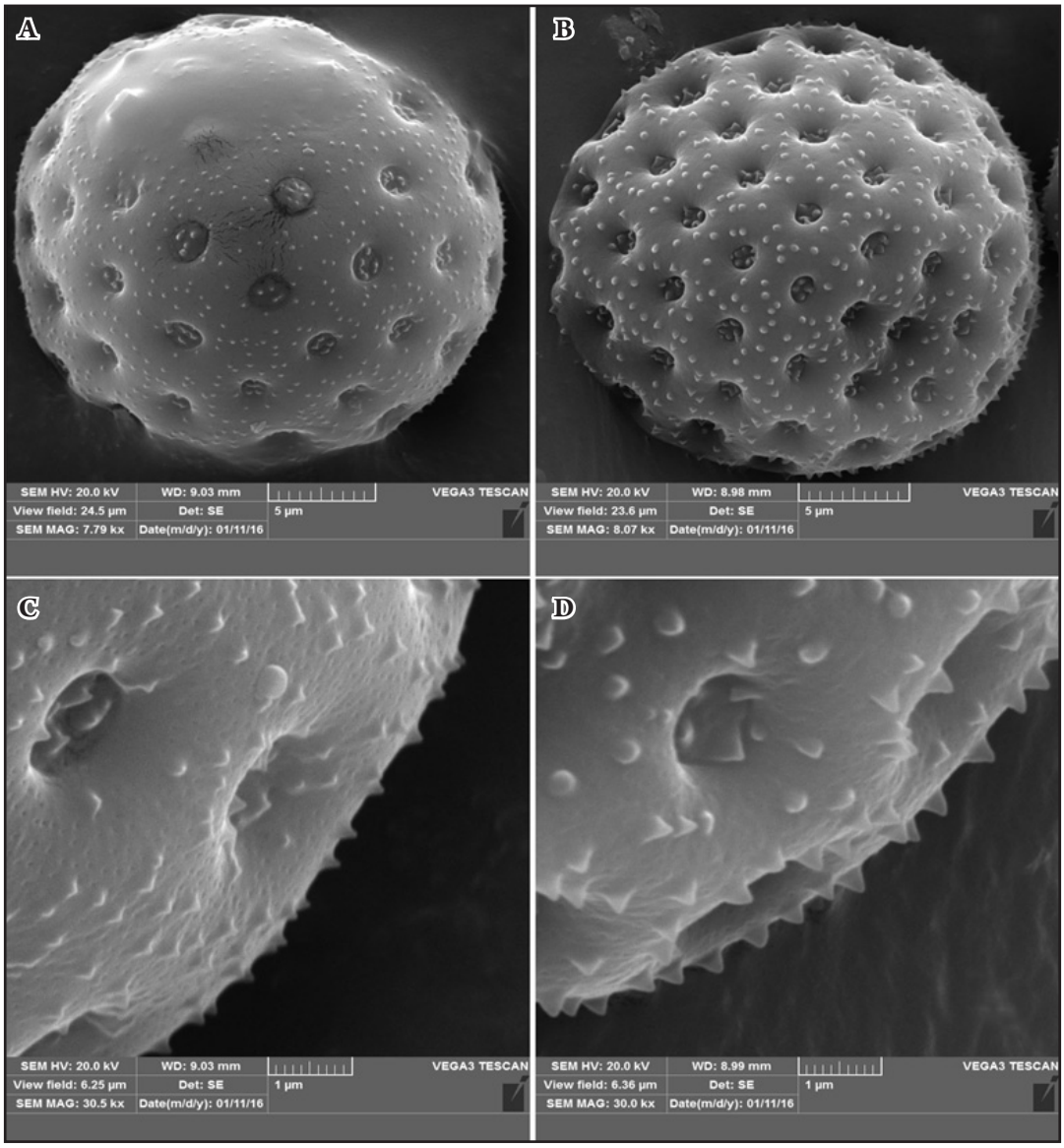

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INFORMATION FOR AUTHORS

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