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Pollen grain morphogenesis in Triticeae and Avena amphiploids.

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The world of plants offers a wealth of hybrid forms, especially within the grass family with wind pollination expression. The plant breeding system ranges between a recombinational generative reproduction and a replicative vegetative one. At first, variation is created by self-fertilization in autogamy and, secondly, by geitonogamy and cross-fertilization in allogamy (Richards 1988). The balance between auto- and allogamy depends on the floral characteristics, such as cleistogamy or chasmogamy. As early as 1945, Harlan showed in *Bromus carinatus* that such variation may be expressed on in single plant (Harlan 1945). The tribe Triticeae includes species presenting different breeding systems; e.g., from the cleistogamic *Hordeum jubatum* to the allogamic *Secale cereale*. In the genus *Avena*, *A. barbata* is nearly cleistogamic and, in *A. fatua*, 12% outcrossing has been detected (Grant 1981).

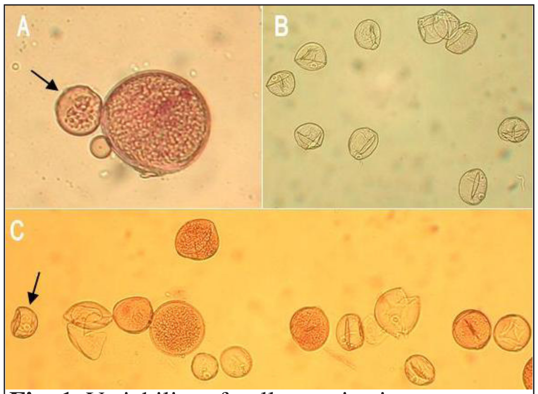

Fig. 1. Variability of pollen grains in a micrograin (arrow) in *Lophopyrum elongatum* (A), dead, empty grains in *L. elongatum* (B), and a large, unreduced, biporate (arrow) and small dead grains (C) in an '*Avena barbata* / *A. sativa* ssp. *nuda*' amphiploid.

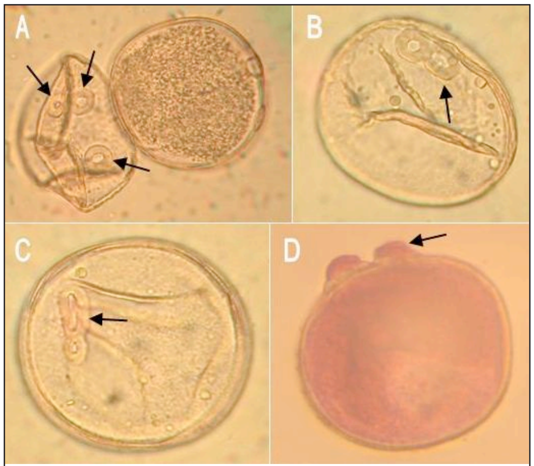

Fig. 2. Multiporate pollen grains in an '*Avena barbata* / *A. sativa* ssp. *nuda*' amphiploid; three pores form separate units (A), two pores develop as one unit (B), a complex structure of three pores (C), and simultaneous germination of a pollen grain through two pores (D).

Pollen quality is usually poor in a hybrid population but can be rapidly improved by natural polyploidization or by plant breeders after the application of colchicine. An example of such pollen variability in a vigorous plant of *Lophopyrum elongatum* produces some amount of micrograins (Fig. 1A) and dead pollen (Fig. 1B). This highly allogamic plant does not show any sign of hybridity and probably has a cryptic hybrid nature, maybe of interspecific origin. A similar pollen grain development was noted in an *Avena* amphiploid (Fig. 1C), in which a biporate grain was also detected. Kihara (1982) presented a pollen grains with two pores in the F_1 a of '*Triticum turgidum* / *Aegilops tauschii*' cross, however he did not mention it. In addition, Kihara also documented partial, unequal, and multipolar cytokineses. Pollen grains from a complex hybrid between *T. aestivum* and *L.* (*Agropyron*) *glaucum* show that multiple cytokineses and elimination of micronuclei occurred during their development (Cicin 1978). Special attention should be given to the morphogenesis of multiporate pollen grains. Such morphs were detected in Triticeae amphiploids between *S. cereale* and some *Aegilops* species (Kalinowski et al. 2001). In a single grain, many pores developed as separate units or as a complex pore formed by two or three joined together. Sometimes the pore is incompletely expressed. Li et al. (2005) presented data on pollen grain morphogenesis in a *T. aestivum–Leymus mollis* partial amphiploid. They found anomalous pollen grains having as many as 10 pores. Ma et al. (2009), in the Panicoideae, discovered that multiporate pollen grains are expressed in apomictic plants. Apomixis is often linked with high level of polyploidy (Grant 1981; Quarin et al. 2001). In the genus *Avena*, multiporate pollen grains also developed in a series of hybrids and amphiploids of high ploidy level (Fig. 2, p. 102). The development of pores is variable, but pollen germination through many pores (Fig. 2D) seems to be most important for pollen tubes competition. This competition creates a genetical status of a hybrid population, wild or cultivated. We concluded that high ploidy level, hybridity, multiporate pollen grains. and apomixis often are linked.

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Cytogenetic events in amphiploids: Triticeae versus Avena

R. Kosina, M. Florek, and K. Markowska.

Cicin (1978) showed that Triticeae amphiploids, derived from hybrids of *Triticum* and *Lophopyrum* through a colchicine treatment, have a stabilized meiosis and improved pollen quality. Young amphiploids of wheat undergo intragenomic alterations occur despite a gross genomic change (Feldman and Levy 2005), including the nonrandom elimination of coding and noncoding DNA sequences, epigenetic changes such as DNA methylation of coding and noncoding sequences, and activation of genes and retroelements. These changes diploidize meiosis and alter gene expression. Han et al. (2003) did not find gross chromosome changes that could be accompanied by the abovementioned means. However, Kosina and Heslop-Harrison (1996) detected structural changes in chromosomes in a young, trigeneric hybrid of the Triticeae. Such changes also are common in stabilized alloploid species. For instance, Leggett et al. (1994) found intergenomic translocations, mainly terminal, in the wild tetraploid oat *Avena maroccana*.

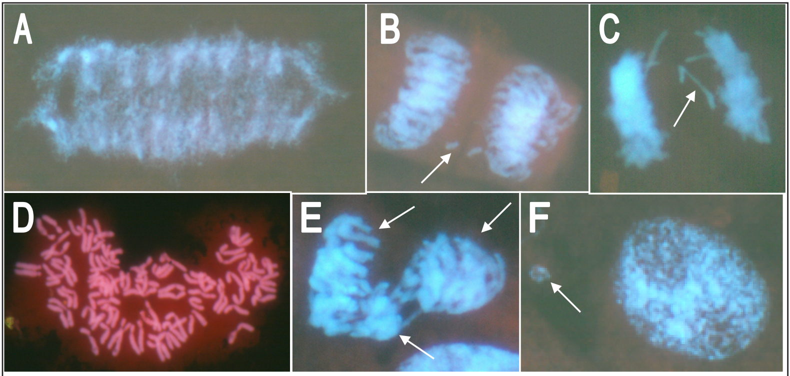

Fig. 3. Mitotic cytogenetic events in a '*Triticum timopheevii* / *Aegilops umbellulata*' amphiploid; an anaphase with highly decondensed chromosomes (A), a telophase with a delayed divided telocentric (B), a delayed dicentric chromosome (C), mitotic nondisjunction (D), tri-polar telophase with bridges (E), and a micronucleus (F).

We exemplify cytogenetic behavior in two, ~50-year-old amphiploids. In a '*Triticum timopheevii* (4x) / *Aegilops umbellulata*' (2x) amphiploid (Fig. 3), an extremely rare high decondensation of anaphase-telophase chromosomes was discovered (Fig. 3A). A delayed division of telocentric chromosome (Fig. 3B), a dicentric chromosome (Fig. 3C), nondisjunction of mitotic sister chromosomes (Fig. 3D), tri- (or multi-) polar telophase (Fig. 3E), and the elimination of micronuclei (Fig. 3F) were noted cytogenetic abnormalities. Studying decondensed chromosomes of *Drosophila*, Steffensen et al. (2001) concluded that their status caused disorder in anaphase, chromosome breakage, and apoptosis. Possibly, multipolar telophases are related to this decondensation. The creation of telocentrics is associated with misdivision of the centromere. In common wheat, Vega and Feldman (1998) discovered that two doses of the *Ph1* gene distinctly increased centromeric misdivision. Both examples (Fig. 3B, C) prove that an interaction between the kinetochore and spindle microtubules is disordered. Sears and Câmara (1952) showed that in *T. aestivum* a dicentric chromosome is normally divided during root mitoses. Such a chromosome moving to the right pole is functionally normal (Fig. 3C). However, Brasileiro-Vidal et al. (2005) reported on dicentric chromatids forming bridges in 'wheat / *Thinopyrum ponticum*' derivatives. They also found that the lack of H3 phosphorylation in a pericentric region causes dysfunction of the centromere and lagging chromosomes (Fig. 3D, p. 103). A similar cytogenetic behavior was found in an octoploid selection of an *Avena* amphiploid, '*A. barbata* (4x) / *A. sativa* ssp. *nuda*' (6x) (Fig. 4), which was manifested in the form of hypoploidy (Fig. 4A), and the presence of telocentrics (Fig. 4B), ring chromosomes (Fig. 4C), micronuclei (Fig. 4D), and bridges (Fig. 4E). In this amphiploid, the breakage-fusion-bridge cycle is active. Tang et al. (2012) showed such cytogenetic instability in somatic tissues of a wheat–rye allopolyploid and attribute the instability to young hybrid offspring. In another complex Triticeae hybrid, rings and telocentrics also were common (Kosina and Heslop-Harrison 1996). Thus, in general, phenomena that are components of cytogenetic instability in hybrids are the same among the grasses or in higher plants.

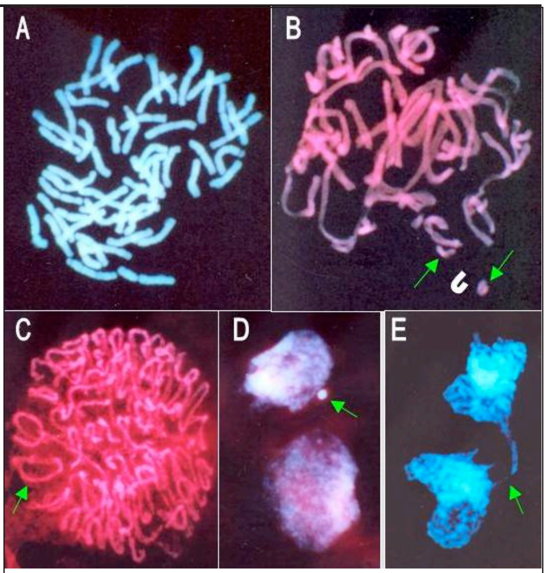

Fig. 4. Mitotic cytogenetic events in an '*Avena barbata* / *A. sativa* ssp. *nuda*' octoploid, a hypometaphase (A), a metaphase with two sister telocentrics (B), a prophase with a large ring (C), a micronucleus (D), and telophase bridges (E).

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The GISH nuclear architecture in Triticeae and Avena amphiploids.

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Schwarzacher et al. (1989) were the first to successfully use genomic *in situ* hybridizaiton (GISH) to detect parental genomes in plant hybrids specifically a '*Secale africanum* / *Hordeum chilense*' hybrid. This research showed that parental genomes are ordered through a cell cycle, and sets of maternal and paternal chromosomes are positioned side-by-side or concentrically. This spatial arrangement of chromosomes influences gene expression. Linde-Laursen and Jensen (1991) proved that parental genomes also can be identified by a Giemsa method and/or by dimensions of chromosomes. In an '*H. vulgare* / *Psathyrostachys fragilis*' hybrid, chromosomes of *Psathyrostachys* were longer, less stained by Giemsa, and distributed outside of the *Hordeum* chromosomes. A pair of *Hordeum* SAT-chromosomes was nonrandomly arranged within the metaphase plates. In another study, two sets of parental chromosomes could be separated in very distinct sectors in a hybrid nucleus Kosina and Heslop-Harrison (1996).

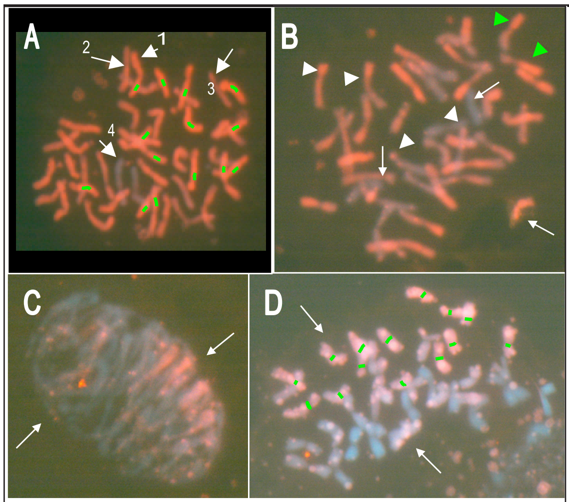

Fig. 5. GISH in root nuclei in a '*Triticum timopheevii* / *Aegilops umbellulata*' amphiploid. Genomic DNA of *Ae. umbellulata* was used as a probe. *Ae. umbellulata* hromosomes are stained green (A). Four types of chromosomes are marked by arrows, translocations by arrowheads, and one reciprocal by green arrowheads (B). A Rabl prophase with red *Ae. umbellulata* chromosomes arranged side-by-side with wheat chromosomes (C). A side-by side chromosomes arrangement with rose chromosomes of *Ae. umbellulata* marked in green.

Here, we present cytogenetic pictures of chromosomes prepared by GISH for two grass amphiploids. DNA in a Triticeae amphiploid, '*Triticum timopheevii* (4x, AAGG) / *Aegilops umbellulata* (2x, UU)' was detected using an *Ae. umbellulata* probe. In an *Avena* amphiploid, '*A. barbata* (4x, AABB) / *A. sativa* ssp. *nuda* (6x, AACCDD)', a probe was prepared from the diploid species *A. nuda*. Baum (1977) reports that *A. nuda* has the AA genomes. Badaeva et al. (2005) considered this species at a subspecific rank within *A. strigosa* with the A^sA^s genomes. In the Triticeae, amphiploid chromosomes of the U genome were discriminated by a light red colour with green marks (Fig. 5A), however, at a 70–75% stringency level, chromosomes of the wheat A and G genomes and the U genome are discriminated in four groups: (1) U genome, (2) a weaker red wheat genome, (3) a other weak, red wheat chromosomes, and (4) a special pair of violet chromosomes most distantly related to the U genome. The relationships between the wheat (AG) and *Ae. umbellulata* genomes need to be studied at an increased level of stringency (80–85%). Better discrimination of *Ae. umbellulata* chromosomes and multiple translocations between U-, A-, and G-genome chromosomes (red, weak red, and violet chromosomes) is shown (Fig. 5). The translocations are mainly terminal. Some translocations are between wheat genomes and some are of the Robertsonian type (green arrows). In a prophase Rabl arrangement (Fig. 5C) as well as at metaphase (Fig. 5D), the side-by-side separation of wheat and *Ae. umbellulata* chromosomes is distinct. Zhang et al. (1998) proved that U-genome chromosomes are homeologous to the A, B, and D genomes of Chinese Spring wheat. The B genome is close to the G genome; chromosomes 2G, 3G, 5G, and 6G are structurally similar to their Chinese Spring equivalents in the B genome (Maestra and Naranjo 1999). In addition, Devos and Gale (2000) highlighted extensive structural rearrangements in the U genome. In a '*T. aestivum* (ABD genomes) / *Ae. biuncialis* (UM genomes)' amphiploid, chromosomes of *Ae. biuncialis* are clearly discriminated (Molnár et al. 2009).

In the *Avena* amphiploid, the A genome of *A. sativa* were discriminated well (light green-yellow) by an A^sA^s-genome probe from *A. nuda*. Many were terminal intergenomic translocations and also Robertsonian type (Fig. 6). Hayasaki et al. (2000) detected many intergenomic translocations in polyploid species of *Avena*. However, Irigoyen et al. (2001) did not find such rearrangements between the genomes of *A. barbata* (AABB). The latter

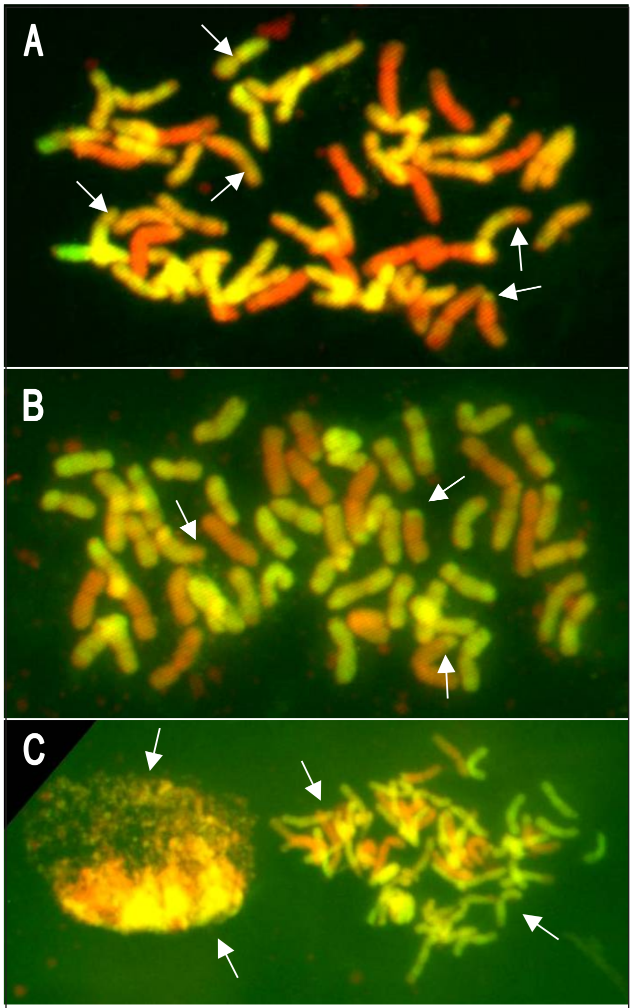

Fig. 6. GISH in the root nuclei in an '*Avena barbata* / *A. sativa* ssp. *nuda*' octoploid amphiploid. Genomic DNA from the diploid species *Avena nuda* (A^sA^s) was used as a probe to detect metaphase yellow and green chromosomes (A and B) and terminal and Robertsonian translocations (arrows). A side-by-side arrangement of chromosomes in prophase and metaphase (C).

study successfully hybridized the As120a sequence from *A. strigosa* (A^sA^s) to the A genome of *A. barbata*, but not to B genome. The selection of this octoploid from a decaploid resulted in the rejection of two genomes. Six A^s genomes in the octoploid metaphase proves high homology between the A and D genomes (Fig. 6B, p. 105). Linares et al. (1998) discovered that the sequence As120a from *A. strigosa* is absent in C genome. In our material, 14 red chromosomes were assigned to the B or C genomes. Because no translocations between the A and B genomes were found by Irigoyen et al. (2001), the red–green translocations (Fig. 6B) probably are between chromosomes of the A and C genomes. Such A–C translocations have been discovered by Leggett et al. (1994) in *A. maroccana*. Our conclusion is that chromosomes of B genome are not present in the octoploid *Avena* amphiploid. In addition, the B genome of *Avena* is inactivated earlier and does not control apoptosis (Kosina and Tomaszewska 2013). The arrangement of the parental genomes (green versus red) in the *Avena* amphiploid is side-by-side, in both prophase and metaphase nuclei (Fig. 6C).

A common pattern of intermediate inheritance in plant hybrids is probably correlated with such an arrangement of parental genomes. Both sets of parental genomes can be expressed without epigenetic restrictions. However, the spatial arrangement of parental genomes can be changed when a nucleus approaches to apoptosis, in the root as well as in the endosperm (Kosina and Tomaszewska 2013).

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Nucleolar variability in grass antipodals.

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The clonal and mosaic nature of grass endosperm has been studied by Ivanovskaya (1983), Kosina (1992, 2007, 2009, and 2012), and Kosina and Tomaszewska (2012). Mosaics are frequent and more variable in grass hybrids and amphiploids (Kosina and Tomaszewska 2010; Kosina and Zając 2010). In the endosperm tissue, antipodals are very special cells with respect to their origin, function, and death. In some grasses, for instance in rye, antipodals are uninuclear and uninucleolar (Poddubnaja-Arnoldi and Dzhalilova 1976). Antipodal chromosomes can by polytenized in Triticale (Kaltsikes 1973), and the number of rDNA loci is not increased, as in the '*Triticum* (4x) / *Aegilops tauschii*' amphiploid (Kosina 1995). At the final stage of their activity, antipodals are apoptized, and this process in wheat varies from that in the synergids (An and You 2004). Parental genomes are differentiated in their roles during apoptosis of endospermal nuclei in the Avena amphiploid (Kosina and Tomaszewska 2013). In an antipodal cell, many nuclei and nucleoli can appear similar (Fig. 7), but between antipodal cells, the nuclei can vary in the number and stage of the cell cycle (Fig. 7B). Nucleolar and rDNA variation in the grass endosperm has been described (Kosina 1995, 1996, 1997, and 2011a, b). The number of nucleoli is less variable in the diploid Triticeae species and more variable in polyploids. Dendrograms presenting the variation of endosperm nucleoli can be properly constructed with the use of the Canberra metric (Kosina 1997). The number of rDNA loci and nucleoli can be changed due to multipolar anaphases, which are frequent in the endosperm, and/or to loss of loci located on bridges or laggards (Kosina 1995; Kosina and Kłyk 2011). In our study, free-nuclear endosperm tissue was stained *in vivo* by acridine orange (DNA green and RNA red) and on squashed slides by complex staining (DAPI+PI) or by means of a FISH method with using rDNA probe pTa71, stained Cy3. Two types of antipodal nuclei were discovered:

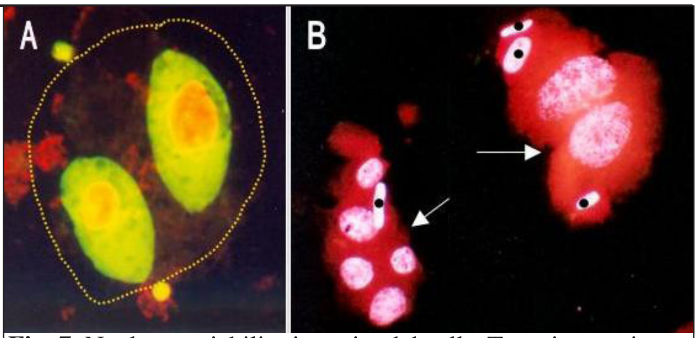

Fig. 7. Nuclear variability in antipodal cells. Two sister uni-nucleolar nuclei in one antipode of *Elymus caninus* (nucleolar RNA in orange, nuclear DNA in green) (A) and two antipodal protoplasts (red) with prophase nuclei (light blue) of different ploidy level in each, in *Avena sativa* (foreign nuclei are marked by black dots) (B).

1. A highly polyploidized antipodal nucleus has one or a few large nucleoli, for instance in *T. aestivum* and *Lophopyrum elongatum* (Fig. 8). A similar nucleolar status has been expressed in many other member of Triticeae. However, a different nucleolar and rDNA pattern was detected in Triticeae amphiploids, the antipodal nuclei have many nucleoli and many rDNA sites (Fig. 9).
2. A huge polyploidized nucleus has many, often more than 100, small nucleoli. Examples are *Avenula planiculmis*, *Lolium temulentum*, and *Briza maxima* (Fig. 10). Other members of the Avenae tribe express a similar nucleolar pattern, but also the same was noted in some species of Triticeae such as *Hordeum bulbosum* and *Elytrigia repens*.

Both kinds of antipodal cells are good models to study developmental, functional, and apoptotic variation.

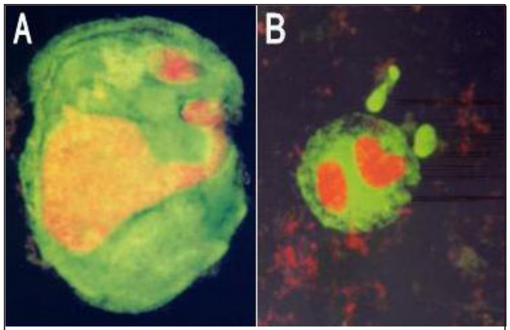

Fig. 8. 'Poor' nucleolar antipodal nuclei in *Triticum aestivum* (A) and *Lophopyrum elongatum* (B) (DNA in green, RNA in red).

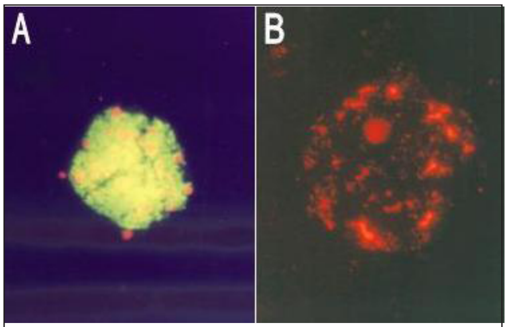

Fig. 9. Nucleolar variability in amphiploid antipodal nuclei. A multinucleolar nucleus from the cross '*Triticum turgidum* subsp. *carthlicum* / *Aegilops tauschii*' (DNA in green, RNA in red). Multiple red loci of rDNA (a pTa71 probe) in the nucleus of a '*T. turgidum* supsp. *turanicum* / *Ae. tauschii*' hybrid (B).

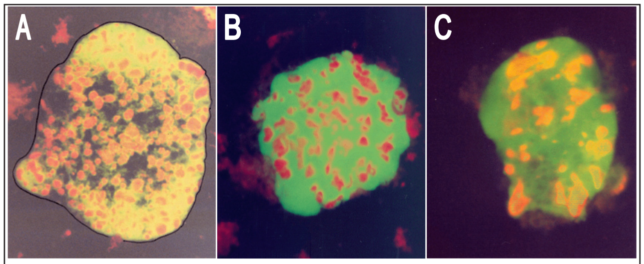

Fig. 10. Multinucleolar highly polyploidized antipodal nuclei (DNA green or yellow, RNA red or orange) in *Avenula planiculmis* (A), *Lolium temulentum* (B), and *Briza maxima* (C).

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Variability of vegetative propagation in Brachypodium distachyon.

R. Kosina and P. Tomaszewska.

Natural phenotypic variation is still of great interest, to relate it to DNA characteristics. Many studies on *Brachypodium distachyon* provide new data in this area. For instance, Tyler et al. (2014) presented variability of habit in *B. distachyon*, including a prostrate one with elongated and flexible shoots, whereas in the Kosina collection of *B. distachyon*, the longest shoots were found in an accession from Italy (ITA1, Fig. 11A). Some plants from this accession exhibited a winter behavior, not flowering in the first year of cultivation. Schwartz et al. (2010) also noted a winter habit in the species. Under moist weather conditions, these plants formed vegetative tufts (Fig. 11A), whereas blooming individuals developed several tillers with spikes. Vegetative tufted plants were cultivated through the autumn and winter in a greenhouse. They developed very long shoots (~1 m), with internodes reaching 12 cm, and sporadically

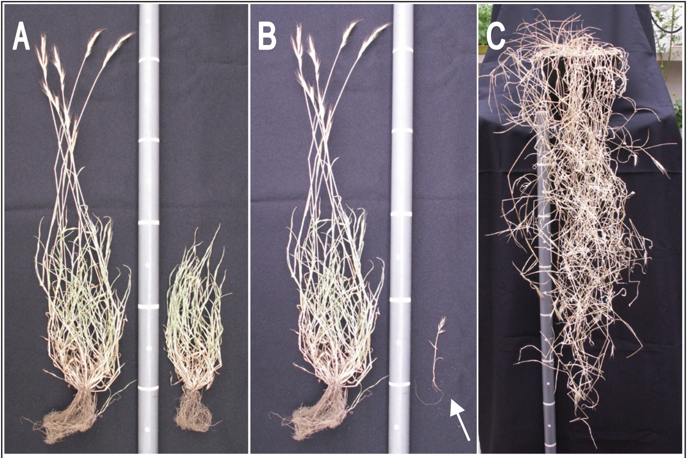

Fig. 11. Plant morphology in different accessions in *Brachypodium distachyon*; generative and vegetative habit in ITA1 (Italy) (A), two plants of various vigor and height (ITA1 on the left and PAK1 (Pakistan) on the right (B)), and plants of ITA1 presenting an excessive elongation growth in the greenhouse (C).

terminated by inflorescences. Opposite variation was noted, especially in accessions from Spain (ESP2) or Pakistan (PAK1), plants with one short (less than 10 cm) tiller terminated by a spike (Fig. 11B, p. 108). Thus, in *B. distachyon*, the variability related to vegetative versus generative potential is large. In late autumn in accession ITA1 and several others, prostrate shoots rooted and from axillary buds new tillers were produced (Fig. 12). These plants resembled perennials. Such a rooting behavior is a new characteristic in annual *Brachypodium* and can change its invasive potential in areas adjacent to cultivated fields or in pastures. Interaccessional (-populational) differences in reproductive versus vegetative effort could be studied more effectively under water stress (Aronson et al. 1993).

Fig. 12. An autumn rooting of nodes in accession ITA1 of *Brachypodium distachyon* from Italy.

In the perennial *B. pinnatum*, a vegetative dispersal is prevalent over generative, but Schläpfer and Fischer (1998) discovered a high clonal diversity within the population of this grass. The vegetative propagation allows *B. pinnatum* to invade effectively new disturbed areas (Buckland et al. 2001). The number of tillers created by a grass plants is under genetic control (Kebrom et al. 2013), and a gene (*tin3*) inhibiting the tiller development was discovered in *Triticum monococcum* (Kuraparthy et al. 2006). Possibly, the same genes were expressed in the uniculm bread wheat Gigas (Atsmon and Jacobs 1977) and in the dwarf form of *B. distachyon* (Fig. 11B).

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Questions about the nature of an anomalous plant in Brachypodium distachyon.

R. Kosina and P. Tomaszewska.

New types of plants can be mutational or hybrid origin. Any trait of an individual can be changed by mutation and a new phenotype is, *de facto*, only a small component of the total variation of a given taxon. In plant breeding, induced mutants are widely used to create new cultivars. Most often, the new characters are easily identified, such as size and shape of the plant or its separate organs (Allard 1960). New phenotypes created by hybridization of two parental plants are represented by the F_1 or next generations. The progeny of distant parents is often completely sterile due to many meiotic anomalies (Cicin 1978).

In the collection of *Brachypodium* maintained by R. Kosina, an extraordinary plant has been identified (Fig. 13B, p. 110). This plant exhibits extremely long and multiflowered spikelets and was found within a selection from an accession AFG1 from Afghanistan (Fig. 13A). Possible donors of such a trait could be two accessions from Morocco, MAR1 and MAR2 (Fig. 13D and E). All the mentioned accessions are within *B. distachyon*. Comparative morphological data show that traits of the new plant are closest to the characteristics of MAR1, especially those related to the spikelet (Table 1, p. 110); however, some characteristics of the plant are remarkable. In the very long spikelets composed of more than 40 flowers, no caryopsis developed. The analysis of pollen grains showed that this plant is completely sterile (Fig. 13C). A spontaneous cross-pollination with MAR1 as a pollen donor is possible in nature, because all accessions in the collection express chasmogamic behaviour (Kosina and Tomaszewska 2012). Such a case of pollen sterility is rather impossible after an intra-specific cross-pollination. Another pollen donor that expresses long and multi-flowered spikes is *B. phoenicoides*. This species is also cultivated in the above-mentioned collection and represents strong chasmogamy and produces a lot of pollen grains. Khan and Stace (1999) crossed *B. distachyon* with *B. pinnatum*, but no seeds were obtained from the F_1 hybrid and its plants were sterile as well.

Fig. 13. Spikes of various accessions in *Brachypodium distachyon*: AFG1s, a selection from an accession AFG1 (Afghanistan) (A), a sterile hybrid found in AFG1s (B) and its sterile pollen grain (C), MAR1 from Morocco with short and long spikelets (D), and MAR2 from Morocco with long spikelets (E).

Table 1. Data on plant morphology of some accessions in *Brachypodium distachyon* presented in Fig. 13.

Character	AFG1s	AFG1s (a sterile plant)	MAR1	MAR2
Plant height (cm)	27.0	39.0	23.0	34.5
Number of tillers	3	6	4	10
Number of main tillers	2	1	4	1
Number of secondary tillers	1	5	0	9
Spikelet length (cm)	3.5	8.0	7.0	5.5
Number of spikelets/spike	1–2	1–2	1–2	1–3
Number of flowers/spikelet	16	44	27	26

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Variability of coleorhizal hairs in the Triticeae and Brachypodium.

R. Kosina and P. Tomaszewska.

The efficient growth of a seedling and its competitiveness in a population depend on successful germination of its diaspore. Many factors, including the morphology of diaspore, affect germination. Coleorhizal hairs are one of these factors. Coleorhizal hairs are components of a coleorhiza epidermis, and they are able to absorb water from capillary channels in the soil (Fig. 14B, a seed on the left having abundant hairs). The capillary potential of hairs is increased in specimens with many

Fig. 14. Germination of naked caryopses in various accessions in *Brachypodium distachyon* 48 h after imbibition. Coleorizal hair spheres and the tips of coleoptile are outlined in white. An early heading, spring accession from Iran (IRN1, A), a biennial accession from Iraq (IRQ, B), and a form with smooth glumellae (C), a facultatively biennial accession from Iraq, a form with hairy glumellae (D). All pictures were taken at the same magnification.

long hairs. When soil humidity is high, water highly condenses on them. Previously, Rost (1975) assigned the role of water absorption to the coleorhizal hairs in *Setaria lutescens*. Northam et al. (1996) documented that in *Taeniatherum caput-medusae* (Triticeae), development of coleorhizal hairs strongly depends on the germination temperature. At the end of the first 24h of germination, at 18°C, 15% to 74% of caryopses had these hairs. Bureś (2008) discovered that in a '*Triticum turgidum* subsp. *dicoccum* / *Aegilops tauschii*' amphiploid the coleorhizal hairs are expressed poorly (Fig. 15A), a trait similar to that found in a '*T. timopheevii* subsp. *timopheevii* / *Ae. umbellulata*' amphiploid (Fig. 15B). Another amphiploid, *T. kiharae* with the genomic formula AAGGDD, develops a rich sphere of the coleorhizal hairs (Fig. 15C). Germination tests showed that hairs also are variable in *T. aestivum*, from a sphere with many hairs to almost none (Fig. 16A), and this variation seems to be continuous. In *Secale cereale* (Fig. 16B), the development of the coleorhizal hairs can be described rather by a bimodal distribution with states of hairs, present versus absent. The coleorhizal hairs do not develop on the coleorhizal papilla, especially on its attachment point. The 0–1 character state for hair is also typical for *Ae. umbellulata* (Figs. 17A and 17B), and a winter form of *Brachypodium distachyon* (Fig. 17C). The 0–1 state, in fact, is rather an intermediate state towards a continuous one. The coleorhizal hairs develop quickly in accessions of the annual *B. distachyon* (Fig. 14A, p. 110); however, there is intrasample variable. In the facultatively biennial form (Fig. 14B and 14C), for smooth and hairy diaspores of accession IRQ from Iraq, coleorhizal hairs are shorter on average and in some caryopses absent. Kosina and Jaroszewicz (2007) presented a large difference between hairs in *B. distachyon* and *B. sylvaticum*; being long and abundant in the former species. The development of the coleorhizal hairs appears to be very variable. In species from the tribe Triticeae, this development is more variable. The coleorhizal hairs are shorter, less dense, and often the tip of the coleorhiza is naked. We can assume that their role in the quickly germinated seeds of cereals is not as significant as in the seeds of wild species, such as in *Brachypodium*, with slower germination and a slower seedling growth rate. The coleorhizal hairs function over many days of the seedling development.

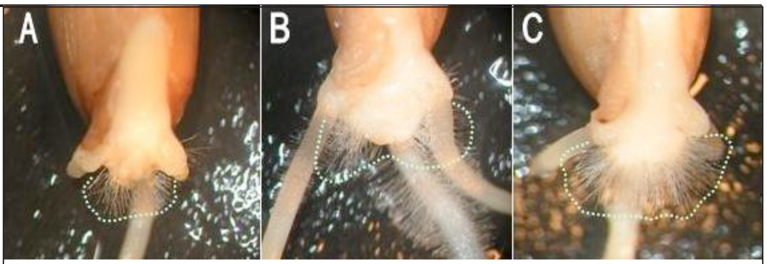

Fig. 15. Coleorhizal hairs spheres (outlined) 24 h after imbibition in the amphiploid K221-13 (*Triticum turgidum* subsp. *dicoccum* / *Aegilops tauschii*) (A); the amphiploid K217-1 (*T. timopheevii* subsp. *timopheevii* / *Ae. umbellulata*) (B), and *T. kiharae* (C). All specimens are at the same magnification.

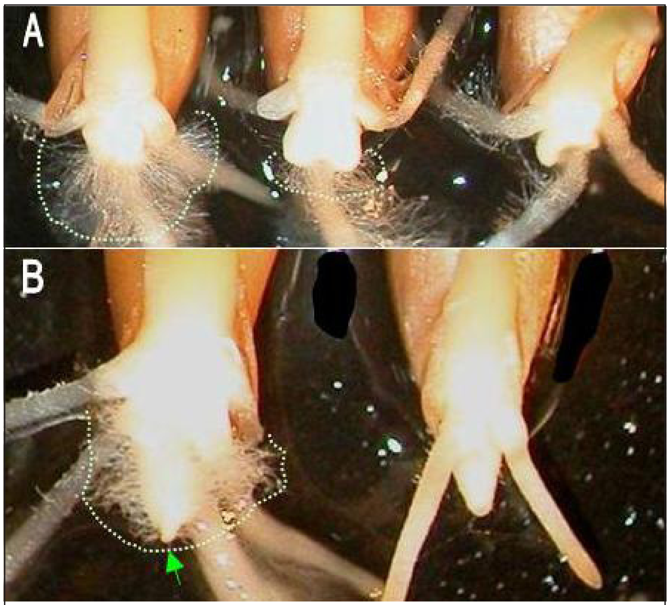

Fig. 16. Variability of the coleorhizal hair development in *Triticum aestivum* (A) and *Secale cereale* (B). The coleorhizal hair sphere is outlined. Pictures were taken 48 h after imbibition at the same magnification.

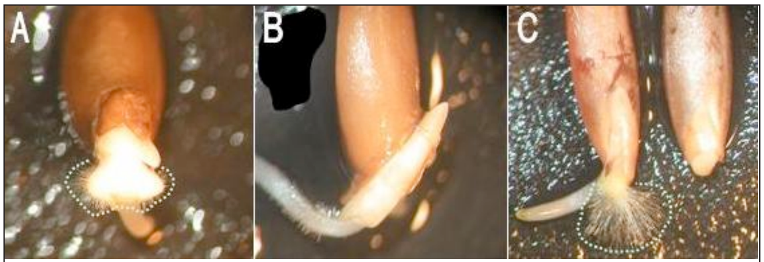

Fig. 17. Variability of the coleorhizal hair development 24 h after imbibition in *Aegilops umbellulata*, with a small coleorhizal hair sphere (A) and without hair (B); and *Brachypodium distachyon* accession ITA1 (from Italy) with a rich sphere on the left and without hair on the right (C). A, B, and C are not at the same magnification.

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Intrapopulational variation of germination in Triticeae and Brachypodium.

R. Kosina and P. Tomaszewska.

Bromus tectorum (cheatgrass) is an annual grass which is very similar with respect to its autogamic breeding system to common wheat and *Brachypodium distachyon*. Beckstaed et al. (1996) identified germination differences in cheatgrass depending on temperature. This variation appeared to be habitat dependent (interhabitat variation). A cheatgrass population growing in a more favorable habitat expressed larger phenotypic plasticity. The great variation in seed dormancy between individual plants in populations (intrapopulational variation) of four weeds was detected by Andersson and Milberg (1998); however, we do not know anything about their breeding systems.

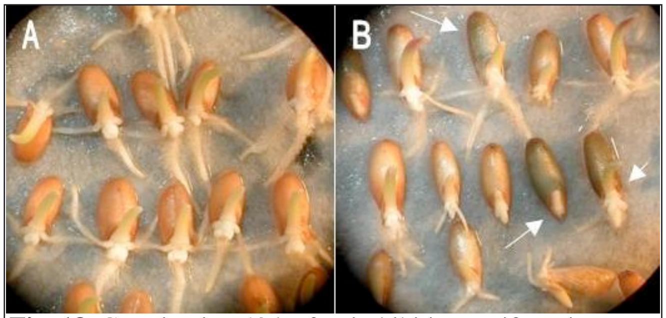

Fig. 18. Germination 48 h after imbibition, uniform in *Triticum aestivum* (A) and uneven in *Secale cereale* (B).

In our research material, germination tests show that within random samples of diaspores, a distinct difference between individuals is noted. Caryopses of common wheat germinate very quickly and uniformly (Fig. 18A). The germination behavior of *Secale cereale* is different (Fig. 18B), and at least three classes can be distinguished 48h after imbibition: 1. good germination and growth, 2. later germination and slower growth, and 3. germination only beginning. Perennial species of *Brachypodium* are situated at the other end of the germination spectrum. We present examples of *B. rupestre* and *B. pinnatum*. Their germination is very variable within a given population (Figs. 19A and 19B). Dormant diaspores, stored for 1 year, germinate later (9 days after imbibition). Growth of seedlings ranges from those with long coleoptiles and roots to those with only a little marked growth of coleoptile under the lemma (Fig. 19B, green arrow). In an annual *B. distachyon*, germination 48h after imbibition is quick in accession IRN1 (Fig. 20A). Three classes of germination, as in rye, can be distinguished. Another type of germination was observed in a facultatively biennial form of *B. distachyon* (accession IRQ from Iraq). Regardless of the type of glumellae surface, smooth or hairy, diaspore germination was delayed. A distinct imbibition of coleoptile was noted several hours after maintaining the diaspore in a watered Petri dish, however, no further development was seen during the 24h after imbibition. The diaspores of the biennial

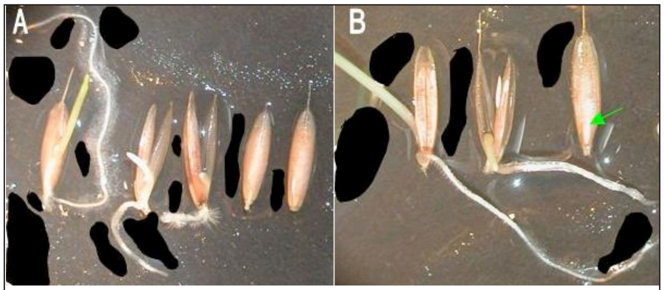

Fig. 19. Intrapopulational germination variability, 9 days after imbibition, in *Brachypodium rupestre* (A) and *B. pinnatum* (B). Coleoptile growth under the lemma is indicated by a green arrow.

Fig. 20. Intrapopulational germination variability of *Brachypodium distachyon* diaspores 48 h after imbibition. Coleorizal hair spheres and tips of coleoptile are outlined in white. Different stages of germination are shown by arrows. Early heading, spring accession IRN1 (Iran, A), biennial accession IRQ (Iraq, B), a form with smooth glumellae, and facultatively biennial accession IRQ (Iraq), C, a form with hairy glumellae. Pictures are not at the same magnification.

form are a little more dormant than those of the spring type. Forty-eight hours after imbibition, coleorhizal hairs were visible, growth of root ceased, and growth of coleoptile progresed under the lemma (Figs. 20B and 20C, see marked coleorhizal spheres and coleoptiles, p. 112).

Variation in germination is created by two main patterns of grass variability. One is associated with the grass breeding system, namely autogamy versus allogamy. The proportion of homozygotes and heterozygotes is determined by the breeding system. Heterozygous populations express obviously larger intra-populational variation in germination. The second pattern relates to annual versus perennial grasses. Our results prove that perennials express larger variation in germination. Interactions between the four above-mentioned syndromes can form the background for studying germination differences.

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Interpopulational and interspecific variation of germination in Brachypodium.

R. Kosina and P. Tomaszewska.

A germination analysis was performed for several perennial species of *Brachypodium*, *B. pinnatum* (Bp), *B. phoenicoides* (Bph), *B. rupestre* (Br), and *B. sylvaticum* (Bs), and for annual *B. distachyon* accessions ITA (Italy), TUR (Turkey), IRQ (Iraq), IRN (Iran), and AUS (Australia). The various accessions in the species were numbered (Fig. 21) and the percent germination and root growth rate was observed and recorded for successive days. In *B. distachyon*, spring, winter, and biennial biotypes were studied. The multivariate evaluation of accessions is presented (Fig. 21). The accessions of *B. distachyon* are differentiated in separate clusters on the dendrogram. The perennial species also create separate clusters (Fig. 21). The biennial forms of *B. distachyon* (IRQ) are close to perennial species *B. pinnatum* and *B. phoenicoides*. The values of average taxonomic distance presented in the dendrogram prove that interpopulational (interaccessional) variation in *B. distachyon* is greater than that in the interspecific variation. In *B. distachyon*, this variation concerns highly homozygous units, whereas in the genus, it is related to differences between highly heterozygous species. In *Brachypodium* caryopses, germination can proceed in two ways: (1) root and coleoptile growth almost simultaneous and a short dormancy in annuals and (2) root growth stopped and only coleoptile grows under lemma and highly dormant in perennials. Thus, the allocation of digested stored starch and proteins during germination is different in both groups of species. Our latest observations show that in the facultatively biennial form IRQ, selecting plants expressing short dormancy is possible. Such a trait can be quickly fixed in the population due to autogamic mating in the species. Other traits of the species also can be changed quickly under selection (Bakker et al. 2009). However, because all the accessions of *B. distachyon* in the our collection express chasmogamic flowering, some level of heterozygosity can be present in the species populations (Kosina and Tomaszewska 2012).

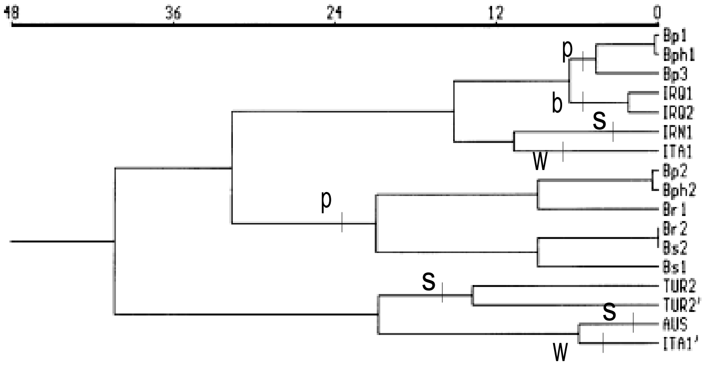

Fig. 21. Dendrogram (average taxonomic distance, UPGMA) of perennial species of the genus *Brachypodium* and accessions of *B. distachyon* described by several germination characteristics. Different accessions are marked by numbers or an apostrophe. Bp = *B. pinnatum*, Bph = *B. phoenicoides*, Br = *B. rupestre*, Bs = *B. sylvaticum*; IRQ, IRN, ITA, TUR, and AUS are accessions of *B. distachyon*; s, w, b, p are spring, winter, facultatively biennial, and perennial forms, respectively.

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A role of caryopsis tissues in germination of Brachypodium.

R. Kosina, P. Tomaszewska, and K. Kamińska.

The nucellus of the caryopsis of both annual and perennial *Brachypodium* species develops a thick epidermis. This tissue is well preserved in ripe caryopses. In perennials, the nucellus is a little thinner than in annuals (Kłyk 2005). Perennials contain more starch and aleurone protein in proportion to hemicelluloses in the nucellar epidermis. In *B. distachyon*, this proportion favors hemicelluloses. The tangential walls of epidermal cells are distinctly layered and, when observed in a polarizing microscope, are less anisotropic than pure cellulosic walls (Kamińska 2013). During germination, the nucellar epidermis is digested and the hemicelluloses are used by the developing seedling (Kosina and Kamińska 2013). The role of the aleurone layer in germination is well known. This tissue not only releases enzymes that digest starch and proteins in the endosperm but also hemicelluloses in the nucellar epidermis and, in addition, cell walls and the proteinaceous protoplast. The cell walls are isotropic in the aleurone layer, a set of aleurone grains decreases and, finally, a protoplast condenses. Starch is heavily digested before these changes. At that point, the nucellar epidermis seems to be intact (Fig. 22A). This epidermis first is digested enzymatically from the side adjacent to the aleurone layer (Fig. 22B). In the next step, the internal tangential walls disappear and adjacent aleurone cells are preserved (Fig. 22C). The nucellar epidermis becomes isotropic and the aleurone protoplasts only are remnants. The disappearance of the nucellar epidermis appears as a continuous process (Fig. 23A, B, and C) and in the lateral parts of the cross-section of caryopsis, the epidermis is most resistant to digestion. How much different is germination in annuals versus perennials in its dependence on caryopsis tissue resources? This is a question that needs further study.

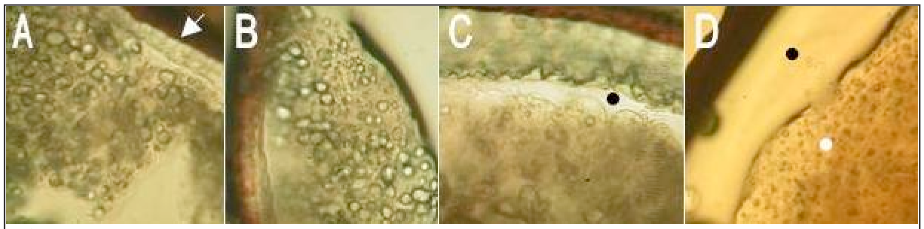

Fig. 22. Enzymatic digestion of caryopsis tissues in perennials of the genus *Brachypodium*, a dorsal part with digested starch and an intact nucellar epidermis (arrow) in *B. pinnatum* (A), digested starch in *B. pinnatum* (B), a digested nucellar epidermis and thin-walled aleurone layer with a free area (black dot) between both tissues in *B. pinnatum* (C), and highly digested starch (white dot) and an amorphous nucellar epidermis (black dot) in *B. rupestre* (D).

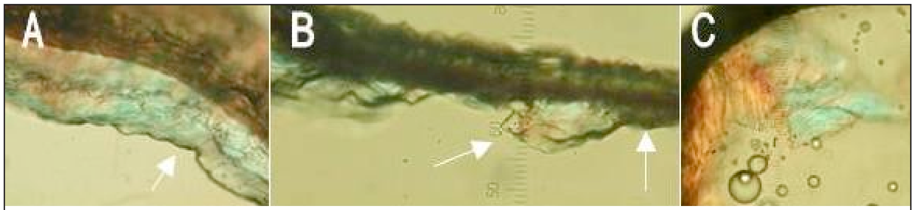

Fig. 23. Enzymatic digestion of nucellar epidermis (arrows) in perennials of the genus *Brachypodium*, *B. phoenicoides* (A), *B. phoenicoides* (B), and *B. rupestre* (C).

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Variation of winter hardiness in Brachypodium distachyon.

R. Kosina and P. Tomaszewska.

Manzaneda et al. (2012) present interesting data for *Brachypodium distachyon* about relationships between the level of polyploidy and its geographical distribution in more or less arid area in Spain. The authors concluded that polyploid cytotypes are distributed in the arid southern parts of the country. Is summer water shortage similar or comparable to that seen in the winter? What is the relationship between cold acclimation and winter water shortage? Many other questions can be posed to consider winter hardiness in *B. distachyon*. Colton-Gagnon et al. (2014) studied cold acclimation for seven, diploid accessions of *B. distachyon*. In light of Manzaneda et al. (2012) results, analyzing the winterhardiness of the species for a set of accessions of various ploidy seems to be more appropriate. Our collection is more appropriate for such a purpose, because the biotypes of *B. distachyon* represent various ploidy levels and cytogenetic stability (Jaroszewicz et al. 2012). In 2013, several accessions, from Afghanistan, Bulgaria, Italy, Iraq, and Turkey, had a prolonged vegetative period into the winter of 2013–14 (see Fig. 24). Some accessions did not bloom and some produced only few flowering shoots. The winter of 2013–14 was very mild in Wroclaw, Poland, where the collection is maintained. The lowest temperatures were recorded at the end of January, lasting nine days. Temperatures from –10°C to –14°C lasted only three days. Among the overwintering accessions, only one accession from Iraq was finally preserved. This accession is composed of two morphs, smooth and hairy glumellae. The hairy morph overwintered successfully (Fig. 24B). During the current hot and dry spring, this accession flowered and developed diaspores. In a future study, this form will be treated as a facultatively biennial biotype. Plants of the other accessions died.

Fig. 24. Variability of winterhardiness in some accessions of *Brachypodium distachyon*, a selection from accession TUR2 (Turkey, A) and an overwintered part (arrow) of an IRQ tuft (Iraq, B). A matchbox is included for comparison.

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Variability in germination in 'Triticum turgidum subsp. diccocum / Aegilops tauschii' amphiploids.

R. Kosina and M. Bureś.

Seeds of two amphiploids, K222 and K221-13, from a '*T. turgidum* subsp. *dicoccum* / *Ae. tauschii*' hybrid, were obtained from the Plant Germ-Plasm Institute in Kyoto, Japan. K222 appeared to be a highly preharvest sprouting biotype; K221-13 did not express this unfavorable trait. Some germination characteristics, such as thegrowth of main and lateral roots and the development of coleorhizal hairs, were studied for both amphiploids (Bureś 2008). K221-13 had polymorphism in both spike and grain color (dark and light). The coleorhizal hairs seem well developed in germinating dark

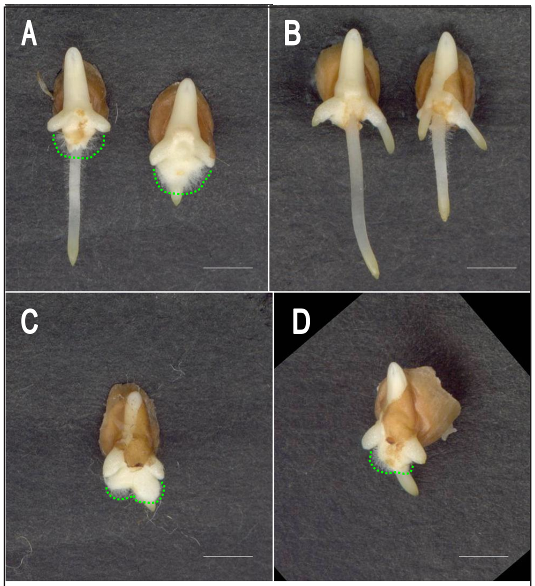

Fig. 25. Germination characteristics in the '*Triticum turgidum* subsp. *dicoccum* / *Aegilops tauschii*) hybrid K221-13, dark grains from a mother plant with a dark phenotype (A), light grains from a mother plant with a dark phenotype (B), dark grains from a mother plant with a light phenotype (C), and light grains from a mother plant with a light phenotype (D).

grains (Fig. 25A, p. 115) but not in light grains (Fig. 25B). Both types of seed developed on plants with dark spikes. The maternal influence on germination is shown (Figs. 25Cand 25D). The dark and light seeds gathered from plants with light spikes developed coleorhizal hairs, but germination in both types was slow. K222, a non preharvest sprouting form, exhibits dynamic growth of the main and lateral roots but does not develop coleorhizal hairs (Fig. 26).

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Fig. 26. Germination in the preharvest sprouting '*Triticum turgidum* subsp. *dicoccum* / *Aegilops tauschii*' hybrid K222.

Is the microstructure of palea in Brachypodium distachyon a good taxonomic tool ?

R. Kosina and P. Tomaszewska.

Microstructure of the grass palea is scarcely used in taxonomy. Ammann (1981) applied characteristics of palea in taxonomy of *Bromus hordeaceus* and *B. racemosus*. He considered both the shape and hairiness of this organ. Skowrońska (2005) performed a more detailed study regarding the variability of a tip of palea in a distinct group of *Bromus* species, *i.e.*, *B. secalinus*, *B. commutatus*, and *B. racemosus*. Many differences were observed in the morphogenesis of the upper part of palea in *B. secalinus* as well as in the *B. commutatus-racemosus* species complex. In the genus *Brachypodium*, variability of palea morphology was presented by Kłyk (2005), who discovered an original morphogenesis of hair and cilia bases.

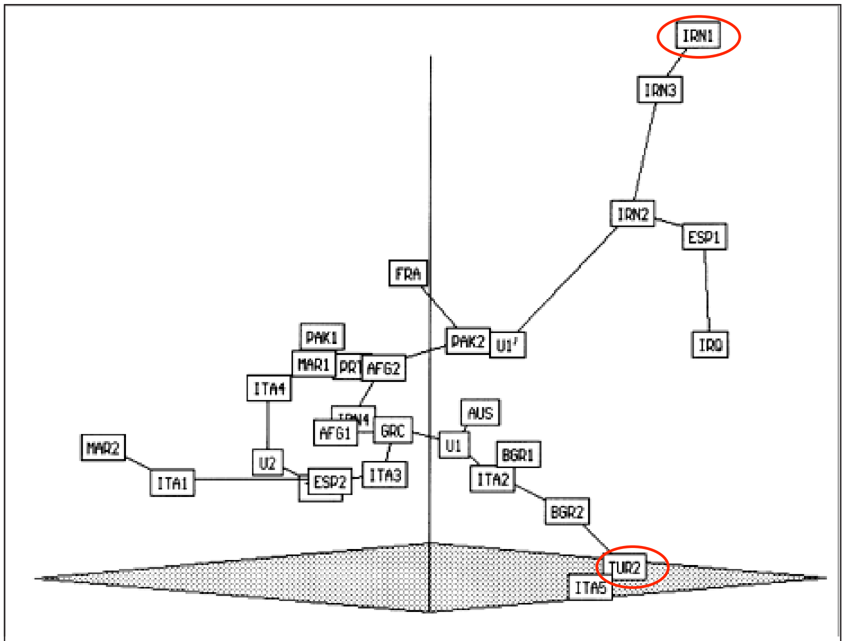

Fig. 27. Minimum spanning tree of accessions of *Brachypodium distachyon* described by traits of the palea tips. The tree was constructed after calculation of the average taxonomic distances and the use of nonmetric multidimensional scaling. Two examples of extreme accessions are circled in red and depicted in Fig. 28.

We studied variability of the tip of palea in *B. distachyon*, a model system for grasses. Twenty-nine accessions of the species were cultivated under the same soil-climatic conditions. Random samples of paleas were analyzed for seven characteristics, width and shape of tip, length of lateral cilia, distance between cilia, presence of microcilia, and the presence of hooks in the central, upper, and lower parts of the palea. The following accessions (OTU, operational taxonomic unit) were detected as extremes: IRN1 (Iran), TUR2 (Turkey), MAR2 (Morocco), ITA1 (Italy), FRA (France), and AUS (Australia). Accessions IRN1 and TUR2 are marked in a minimum spanning tree (Fig. 27) and pictured (Fig. 28). For separate traits, additional OTUs appeared as extremes: U1 (unknown origin), IRQ (Iraq), BGR2 (Bulgaria), and PAK1 (Pakistan).

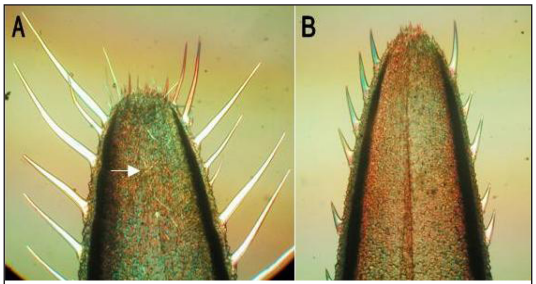

Fig. 28. Microstructure of palea tips in two extreme accessions of *Brachypodium distachyon*, IRN1 from Iran (an arrow shows short hairs on the surface of the palea) (A) and TUR2 from Turkey (B).

Differences in the morphogenesis of hair and cilia can play some role in the possibility of capturing of self and/or foreign pollen grains. When a flower is closed, the upper parts of both glumellae, haired or ciliated lemma, and the palea, stop foreign pollen grains and they fall on the stigma. If the flower expresses chasmogamy, both organs create an additional screen for pollen. We suppose that the more hairy and ciliated organs decrease the level of cross-pollination. A high level of interaccessional variation of palea microstructure can be suitable for taxonomy and to study intraspecific evolution.

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Morphometry of lodicules in Brachypodium distachyon and its application for taxonomy.

R. Kosina and P. Tomaszewska.

For a broad spectrum of Iranian grasses, Kosina (2005) discovered a trait syndrome of the stamens and lodicules that was different for auto- and allogamy. The activity of lodicules is based on metabolism of starch, calcium oxalate, and callose. In the next step of a lodicule study (Kosina 2006), a morphogenetic separateness of lodicule cushion and lodicule lobe was detected. Stomatous biotypes were found in *B. distachyon*. Kłyk (2005) described different morphs of a lodicule in the genus *Brachypodium*, and stated that *B. rupestre* and *B. sylvaticum* have them most different. Pietrzak (2007) presented a role of callose and various tissues at the base of lodicule during *B. distachyon* flowering. Some characteristics of the lodicule show that this organ is a reduced leaf. Kosina (2010) presented new traits for lodicule leafyness. Traits of the main lobe of the lodicule appeared to be the best discriminants of wheat species. *Triticum urartu* appeared to be an extreme in ordination space (Kosina 2011a). Finally, for taxonomic ordination of wheats described by lodicule characteristics, some parameters of correlation and regression were used beyond the arithmetic means (Kosina 2011b).

The lodicules of 29 accessions (OTUs, operational taxonomic units) of *B. distachyon* were described with eight characteristics. OTUs were set in an ordination space using a nonmetric multidimensional scaling. The following accessions were distinguished as extremes: GRC (Greece), ITA (Italy), ESP (Spain), IRN (Iran), and TUR (Turkey); two accessions, ITA5 and IRN4, are covered with the label IRN1 and are marked in red (Fig. 29). The early flowering, spring types are situated in the lower left part of Fig. 29 and the semiwinter, winter, or biennial types in the upper right. Such a surprising result indicates a difference between summer chasmogamic flowers versus autumn cleistogamic ones. In the first, chasmogamic flowers, large lodicules are developed (Fig. 30B, p. 118), and in the second ones the lodicules are smaller (Fig. 30A). However, the lodicule of ITA5 also has a distinct cushion, an

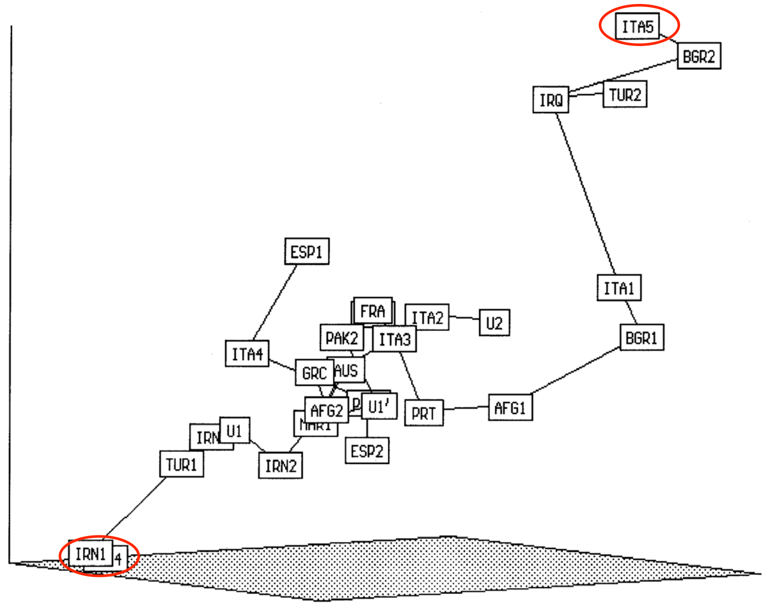

Fig. 29. Minimum spanning tree of accessions of *Brachypodium distachyon* described by traits of the lodiculae. The tree was constructed after calculation of average taxonomic distances and the use of a nonmetric multidimensional scaling method. Two examples of extreme accessions are outlined in red and depicted in Fig. 30.

organ that opens the flowers. In that case the above-mentioned conclusion can be ambiguous.

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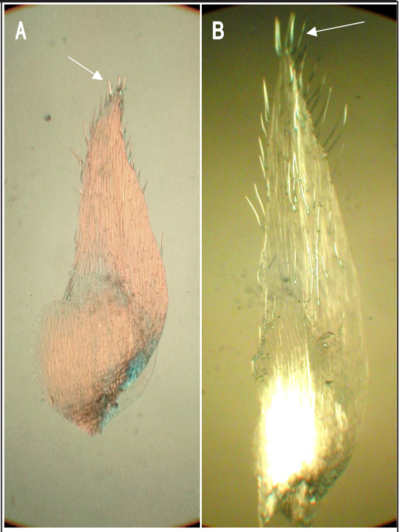

Fig. 30. Variability of lodiculae morphology in two extreme accessions of *Brachypodium distachyon*, ITA5 from Italy (A) and IRN4 from Iran (B). Arrows show short hairs on the top of the

Microstructural variability of endosperm in Triticeae versus Avena.

R. Kosina, P. Tomaszewska, and M. Florek

Kosina (2012) showed a clonal development of a starchy-aleurone endosperm in caryopses of *Thinopyrum distichum*. The last cytokineses are known to be tangential to the surface of the previous embryo sac. In the center of the caryopsis, cells are large and often polyploidized, while in the outer layers, cells are smaller and lack or are less polyploidized. Such a developmental pattern is often expressed in the form of mosaics, which have been observed in the endosperm tissue (Kosina and Tomaszewska 2010, Kosina and Zając 2010). These mosaics are related to various components of caryopsis structure. The somatic crossing-over are a process leading to a 'sister mosaic'. Developmental irregularities were noted in pure species but relatively more often in the progeny of various hybrids. In the hybrids, endosperm development is less balanced and exhibits some original properties (Kosina et al. 2013a, b). Such a hybrid endosperm has a domainant structure. Here we present some new data related to some Triticeae members and from the genus *Avena*. The aleurone cells can apparently be maintained at the basic level of polyploidy (3n). Such a level is characteristic for a young endosperm of any plant progeny (see Fig. 31A, here for *Leymus racemosus*, a pure species). The excess of assimilates can be located either in aleurone grains or in hemicellulosic

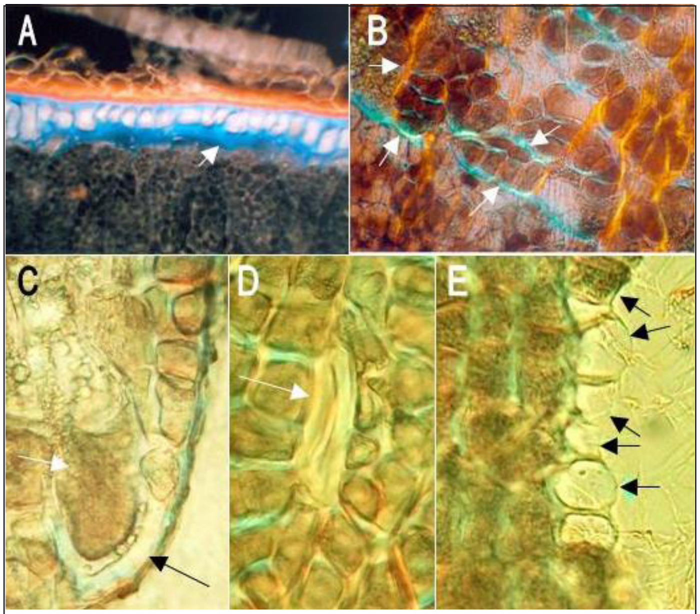

Fig. 31. Variability of endosperm microstructure. The aleurone layer in cross-section of caryopsis *Leymus racemosus*, thick hemicellulosic cell walls show blue natural fluorescence (arrow) (A) and the aleurone layer domains in a '*Triticum aestivum* subsp. *orientale* / *Aegilops tauschii*' amphiploid, domains are bordered by thick walls (arrows) seen under a polarizing microscope (B). The aleurone layer developmental events in the amphiploid '*Avena barbata* / *A. sativa* ssp. *nuda*' identified using a polarizing microscope, arrows show a polyploid cell filled by an aleurone protoplast and enclosed by thick hemicellulosic walls (C), an aleurone polyploid empty cell with thick hemicellulosic walls (D), and an aleurone layer at the edge of a domain, formed by thin-walled aleurone cells (arrows) (E).

cell walls (see blue walls of small aleurone cells in Fig. 31A, p. 118). In a '*Triticum turgidum* subsp. *turanicum* / *Aegilops tauschii*' amphiploid, in an aerial view of the aleurone layer, some distinct sectors enclosed by thick cell walls can be noted, these sectors are domains in the aleurone layer. In the *Avena* amphiploid, a highly polyploid aleurone cell (Fig. 31C, p. 118) with an abundant matrix of aleurone grains and a very thick hemicellulosic wall was detected. This case is evidence that amphiploid assimilates are located in both the protoplast and cell wall. A similar development in the form of a clone of polyploid aleurone cells was documented in wheat/*Th. distichum* amphiploids (Kosina and Tomaszewska 2012). The second case (Fig. 31D) shows that assimilates are located only in cell walls. The third case (Fig. 31E) presents unique development at the edge of the aleurone layer domain, cells have thinner walls and finally disappear. On the right of this disappearing domain in the endosperm exists a free space that can be occupied by ingrowths of starchy or aleurone tissue or by nucellar epidermis. The above-mentioned data prove that in members of different grass tribes, development of the caryopsis tissues is similar. The development of fruit is an evolutionary conservative process and, therefore, is useful in many procedures in genetics and taxonomy.

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Architecture of transfer tissues and endosperm cavity in the caryopsis crease – Triticeae and Avena cases.

R. Kosina, M. Florek, A. Grabińska, A. Koźlik, and K. Markowska.

Kosina et al. (2012) demonstrated that size and shape of nucellar projection and adjacent to it an endosperm cavity is intra- and interspecifically variable in the genus *Triticum*. The nucellar projection in *T. kiharae* and *T. fungicidum* developed as a U- or V-shaped structure, respectively (Kosina and Bureś (2011). Kosina and Tomaszewska (2012) documented in wheat/*Thinopyrum distichum* amphiploids that a very irregular narrow endosperm cavity penetrated deeply into endosperm tissue. Kosina et al. (2013a) proved in a Triticeae amphiploid that demethylation of DNA changes the shape of nucellar projection. The development of this transfer structure is correlated with a number of xylem vessels neighboring the pigment strand. The endosperm cavity in the amphiploid varies from that in the parental species. Relationships within a set of a transfer complex in caryopsis are recently presented by Kosina (2014).

The above citations reveal that the cavity and adjacent transfer complex composed of phloem and xylem bundles, pigment strands, plus a nucellar projection are difficult to present in the form of ideograms (Fig. 32). In a '*T. timpoheevii* subsp. *timopheevii* / *Aegilops umbellulata*' amphiploid, the nucellar projection and endosperm cavity complex is horizontal (Fig. 32B, blue rectangle), whereas this structure is more or less vertical in the parental species. Undoubtedly, the size of any structure depends on size of caryopsis. The shape of the pigment strand

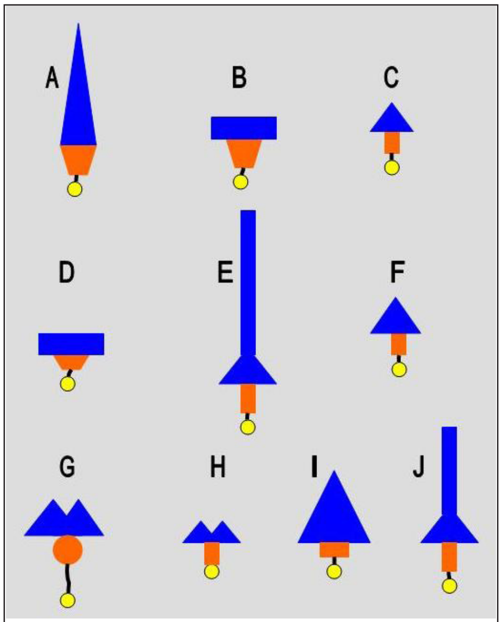

Fig. 32. Ideograms of the endosperm cavity and transfer tissues in the caryopsis of some Triticeae and *Avena* members (endosperm cavity + nucellar projection in blue, pigment strand in orange, xylem bundle in yellow). *Triticum timopheevii* subsp. *timopheevii* (A), a '*T. timopheevii* subsp. *timopheevii* / *Aegilops umbellulata* amphiploid (B), *Ae. umbellulata* (C), *A. barbata* (D), an '*A. barbata* / *A. sativa* ssp. *nuda*' amphiploid (E), *A. sativa* ssp. *nuda* (F), *A. brevis* (G), *A. hirtula* (H), and *A. strigosa* (I and J).

also is different in the depicted taxa. In the genus *Avena* (Fig. 32 D–J, p. 119), some additional shapes of the nucellar projection + endosperm cavity were detected (Fig. 32E and J). Ideograms (Fig. 32D, E, and F) prove that in an *Avena* amphiploid a change in the shape of nucellar projection + endosperm cavity structure occurs compared to the parental species. In *Avena* species, M-shaped endosperm cavities were noted (Fig. 32G and H). Xylem vessels can develop near the pigment strand (Fig. 32H) or at some distance (Fig. 32G). This difference could mostly affect the development of endosperm tissue.

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ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE FOR THE SOUTH-EAST REGIONS (ARISER)

Department of Genetics, Laboratory of Genetics and Cytology, 7 Toulaikov St., Saratov, 410010, Russian Federation.

The influence of alien genetic materials for grain productivity and bread-making qualities in NILs of spring bread wheat.

S.N. Sibikeev and A.E. Druzhin.

We have obtained sets of spring bread wheat NILs carrying alien Lr-genes at the Genetics and Cytology Laboratory at ARISER. These NILs have the following genetic material: *Lr19+Lr9*, *Lr19+Lr24*, *Lr19+Lr25*, *Lr19+Lr26*, *Lr19+Lr37*, and wheat–*Thinopyrum intermedium* substitution 6Agi (6D) and wheat–*Thinopyrum elongatum* substitution 3Age (3B). These sets of NILs have the *Lr* genes from *T. turgidum* subsps. *dicoccum* and *dicoccoides*. The vegetative period in 2013 was very wet and moderate leaf rust epidemics were observed. In these conditions, grains yield of the NILs carrying *Lr19+Lr9*, *Lr19+Lr24*, *Lr19+Lr25*, *Lr19+Lr26*, and *Lr19+Lr37* and the NILs with *Lr* genes from *T. turgidum* subsp. *dicoccum* were similar to those of other cultivars and lines, but the NILs with *Lr* genes from *T. turgidum* subsp. *dicoccoides* and the 3Age (3B) and 6Agi (6D) substitutions significantly higher at 2.89, 2.96, and 3.22 t/ha, respectively. For complex bread-making qualities, the NILs with *Lr19+Lr25* and *Lr19+Lr37* were the best. All the NILs were excellent for gluten content and strength. For flour strength (W), the minimum value was 196 (cultivar L503) and the maximum was 667 (L2032 (*Lr19+Lr24*)). As a whole, the presence of alien genetic material in the NILs has not worsened bread-making qualities and was estimated as good and excellent.