

UNIVERSITY OF WROCLAW

Plant Speciation Group, Institute of Experimental Biology, Przybyszewskiego 63-77, 51-148 Wrocław, Poland.

Developmental variability of the aleurone layer in some members of the tribe Triticeae.

R. Kosina, P. Tomaszewska, and D. Zając.

An important role of noncellulosic polysaccharide aleurone cell walls in grain imbibition was evidenced in *Avena fatua* (Raju and Walther 1988). Polysaccharides are abundant in these walls, and they can hold a lot of water. Imbibition in the caryopsis also can be facilitated by the thick endosperm cell walls adjacent to an aleurone layer in *Brachypodium distachyon*. (Kosina and Jaroszewicz 2007). In wheat, endosperm cell walls are mainly composed of arabinoxylans and β -glucans (Philippe et al. 2006). Arabinoxylans dominate (70%) in the wall, whereas β -glucans amount to 20% (Shewry et al. 2012). Among cereals (wheat, rye, barley, and oat), differences have been detected in the amount of both groups of polysaccharides in cell walls of the aleurone layer and those in subaleurone endosperm (Dornez et al. 2011). Arabinoxylans are stored more in cell walls in wheat, but barley and oat are rich in β -glucans (Hands and Drea 2012).

Aleurone autofluorescence of starchy endosperm cell walls was examined under an epifluorescence microscope Olympus BX60 for some species of the tribe Triticeae and in cross sections of amphiploid caryopses. Seeds of 'wheat/goatgrass' amphiploids were obtained from the Kyoto Germ-plasm Institute, and the Triticeae species were from Dr D. Dewey, Utah State University. Seeds were sown and maintained for many years by R. Kosina in his living grass collection.

In the caryopsis of a *Triticum timopheevii* subsp. *timopheevii*/*Aegilops longissima* amphiploid, a multilayered aleurone has developed locally (Fig. 1A). External and internal tangential aleurone walls show a blue fluorescence, evidence of no chemical difference between them. No change in fluorescence color or intensity is detected across the wall. The local development of groups of aleurone cells, similar to callus, confirms the mosaic pattern of the aleurone layer (endosperm) arrangement (Kosina 2007). The same walls were observed in the unilayered aleurone in *Leymus racemosus* (Fig. 1B). However, here the polysaccharides are synthesized in a great amount and the tangential walls are distinctly thicker. Mosaics of the aleurone layer can be expressed in the form of a group of very narrow cells, sometimes disappearing almost completely (see Fig. 1C in a *T. turgidum* subsp. *dicoccoides*/*Ae. tauschii*' amphiploid. In the callus-like aleurone layer, the development of polyploid cells also is observed (Fig. 1D for a lateral part of the caryopsis of *L. racemosus*). In these examples, wall fluorescence is uniform.

Sometimes, the mosaics of aleurone/endosperm tissue are composed of proteinaceous and starchy cells (Kosina 2007; Kosina and Tomaszewska 2010; Kosina and Zając 2010). Such a development is more often noted in plants of hybrid origin and less so in pure species. In *Elymus canadensis* (Fig. 2A, p. 47), a single starch cell is formed between the light aleurone cells. The wall of this cell shows the same pattern of fluorescence as is seen in a proteinaceous aleurone. The phenotype of the walls is the same, but the phenotype of the stored assimilates, protein *versus* starch, is completely different. The second example presents a different phenomenon (Fig. 2B, p. 47); the very thin walls of two starchy cells penetrating the aleurone layer do not express any fluorescence.

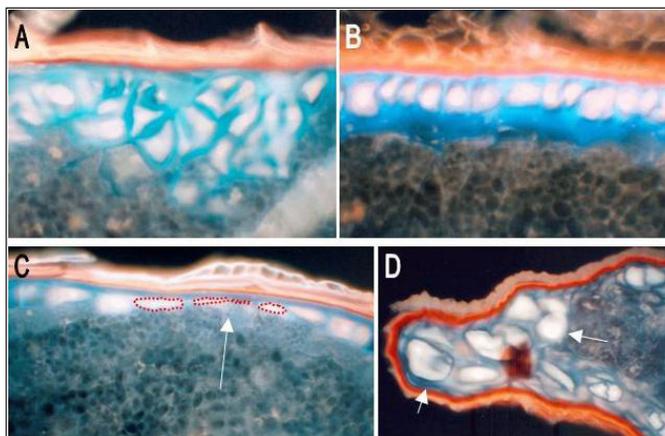


Fig. 1. Developmental events in the aleurone layer. A – amplification of aleurone cells with thick hemicellulosic walls, as in an irregular callus, in a *Triticum timopheevii*/*Aegilops longissima* amphiploid; B – unilayered aleurone cells with thick tangential walls in *Leymus racemosus*; C – disappearance of the aleurone layer into small narrow cells (arrow) in an *T. turgidum* subsp. *dicoccum*/*Ae. tauschii* amphiploid; and D – polyploidization of aleurone cells in the lateral parts of the caryopsis in *L. racemosus* (arrows). A blue fluorescence of cell walls was documented on cross sections of ripe caryopses.

Two main conclusions can be drawn:

1. The aleurone layer can disappear locally and look like a lighter dot of starchy cells. Such a development differs from the 'autonomous development' of cells with stored starch grains between aleurone cells.
2. The two main components of the starchy cell located within an aleurone layer, a cell wall and a protoplast with stored starch grains, are controlled by various genetic backgrounds.

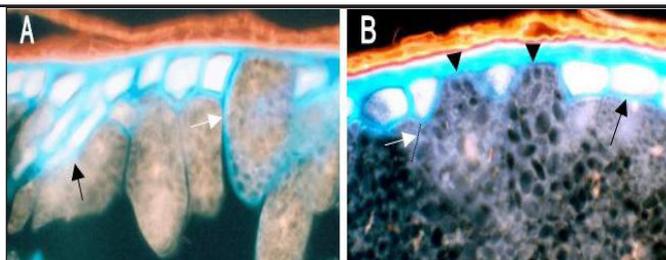


Fig. 2. Starchy phenotypes of aleurone cells expressing various cell wall phenotypes. A – a cell with an aleurone cell wall phenotype in *Elymus canadensis* (white arrow); B – two cells with starchy endosperm cell wall phenotypes (black arrowheads). The black arrow(s) shows the blue phenotype of an aleurone cell wall and the white arrow the thin, dark cell wall of a starch endosperm phenotype in a '*Triticum timopheevii* subsp. *timopheevii*/*Aegilops umbellulata*' amphiploid. Caryopsis cross-section, autofluorescence.

References.

- Dornez E, Holopainen U, Cuyvers S, Poutanen K, Delcour JA, Courtin CM, and Nordlund E. 2011. Study of grain cell wall structures by microscopic analysis with four different staining techniques. *J Cereal Sci* 54:363-373.
- Hands P and Drea S. 2012. A comparative view of grain development in *Brachypodium distachyon*. *J Cereal Sci* 56:2-8.
- Kosina R. 2007. Some topics on the grass mosaics. In: Biological issues in grasses (Frey L, Ed) W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków. Pp. 159-167.
- Kosina R and Jaroszewicz A. 2007. Mikrostrukturalne determinanty kiełkowania ziarniaków *Brachypodium distachyon* i *B. sylvaticum* (Poaceae). *Fragmenta Floristica et Geobotanica Polonica*, Suppl. 9:117-125 (In Polish).
- Kosina R and Tomaszewska P. 2010. Microstructure of endosperm in some intergeneric amphiploids and their parental species of the Triticeae tribe. *Ann Wheat Newslet* 56:200-201.
- Kosina R and Zajac D. 2010. Instability of some endosperm traits in *Triticum* x *Aegilops* amphiploids. *Ann Wheat Newslet* 56:198-199.
- Philippe S, Saulnier L and Guillon F. 2006. Arabinoxylan and (1→3),(1→4)-β-glucan deposition in cell walls during wheat endosperm development. *Planta* 224:449-461.
- Raju MVS and Walther A. 1988. Heterogeneity and behaviour of aleurone cells in the caryopsis of wild oats (*Avena fatua*). *Flora* 180:417-427.
- Shewry PR, Mitchell RAC, Tosi P, Wan Y, Underwood C, Lovegrove A, Freeman J, Toole GA, Mills ENC, and Ward JL. 2012. An integrated study of grain development of wheat (cv. Hereward). *J Cereal Sci* 56:21-30.

Polymorphism of starch granules in the endosperm of some species and amphiploids of the Triticeae L. tribe.

R. Kosina, P. Tomaszewska, and D. Zajac.

Kosina (2009) attributed variations in the microstructure of grass caryopses to the clonal development of endosperm tissue. He described the nature of the tissue in a free nuclear stage as a complex body composed of subsyncytial units, i.e., groups of nuclei of different origin. As a result, for instance in *Avena strigosa*, adjacent cells of the endosperm synthesized starch granules of different sizes. However, starch synthesis can differ in two adjacent regions of a single cellular space, the central part versus external, and cells expressing such a nature created a single clone, e.g., in *A. brevis*. The morphology and size of starch granules also depends on the distance between tissues conducting assimilates, i.e., a vascular bundle and a transfer nucellar projection and pigment strand and a region of endosperm where the starch is stored. Smaller starch granules are synthesized in parts of a caryopsis more distant from the conducting tissues, as proved in *Bromus secalinus*. The amylopectin-poor starch was detected by a Lugol's reaction or imaging in a polarized light. The amylopectin-poor mutations of single starch granules were found in a '*T. turgidum* subsp. *dicoccum*/*Ae. tauschii*' amphiploid and in *B. secalinus*. An amylopectin/amylose-poor mutant with tiny starch granules was detected in *B. racemosus*.

The size of starch granules is species-specific in the genus *Avena* (Kosina 2007). The same situation was discovered in the genus *Hordeum* (Baum and Bailey 1987). A bimodal size for starch granules was noted for barley, rye, and wheat, but is unimodal in millet, rice, and triticale (Tester et al. 2004).

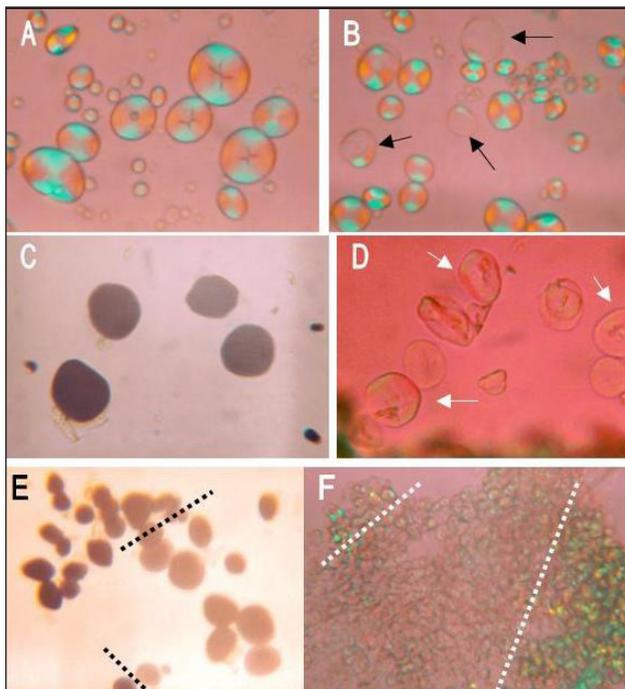


Fig. 3. Variation in starch granule reaction in polarized light and Lugol's solution. A – bimodally sized, amylopectin-rich starch granules in *Triticum timopheevii* subsp. *timopheevii* presenting a distinct 'polarizing reaction'; B – the same as in A for *Leymus racemosus*, but three granules present a very poor polarizing reaction (arrows); C – a strong Lugol's reaction for amylopectin-rich starch in *Aegilops umbellulata*; D – a negative Lugol's reaction for amylopectin-poor granules in an *Ae. umbellulata* starch mutant; E – two types of Lugol's reaction for an amylopectin-rich (dark) pool of granules and an amylopectin-poor pool (lighter, separated by dotted lines) in *L. racemosus*; and F – a pool of amylopectin-poor granules with a weak polarizing reaction (between the dotted white lines) and a second with a contrasting reaction (outside the dotted lines) in a '*L. multicaulis/L. karataviensis*' amphiploid.

large granules express a low amylopectin content seen in the form of poor coloring of outer parts of the granule. We also detected an amylopectin-free type without polarizing colors in *Ae. umbellulata* (Fig. 3D), whereas the normal type has darkly stained granules with Lugol's solution. The amylopectin-free trait in goatgrass is plant specific. A similar pool of amylopectin-free granules is found in an '*L. multicaulis/L. karataviensis*' (Fig. 3F) amphiploid, whereas an amylose-low pool is noted in *L. racemosus* (Fig. 3E).

Other examples of starch granule polymorphism are provided (Fig. 4). In a '*Triticum timopheevii* subsp. *timopheevii/Aegilops longissima*' amphiploid, the colors of polarization (amylopectin amount) are distinct and uniform (Fig. 4A); however, starch granules sometimes express stronger colors (Fig. 4B, more amylopectin). A different

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, *Risø17* and *Notch-2*, were studied by Burton et al. (2002). These mutants synthesize phytyglycogen and several starch granules develop in their plastids. In ripe caryopses, starch granules are composite. In addition, Patron et al. (2002) described some cultivars in *waxy* barley with low expressed levels of amylose in the starch granules or with starch free of amylose. The low-amylose cultivars have more amylose in the granules of the outer cells of the endosperm than in the central part of this tissue. The level of amylose depends on the activity of granule-bound starch synthase I (*GBSSI*). The low- or free-amylose types probably are of Chinese origin. Satoh et al. (2003) induced six low-amylose mutants in japonica rice using *N*-methyl-*N*-nitrosourea. All mutants were of the *waxy* type. The lowered synthesis of amylose did not change the development of normal chains of amylopectin. *Waxy* mutants expressing lower *GBSSI* activity also were obtained in *A. strigosa* (Verhoeven et al. 2004). In potato, starch granules with low *GBSSI* activity are not stained by Lugol's iodine (Edwards et al. 2002).

We stained starch granules with Lugol's solution. Nonstained starch granules also were observed under a polarizing Amplival microscope. Granules with high amounts of amylopectin stained with Lugol's iodine were dark brown, and had distinct red and blue color in a polarizing microscope. Such colors are noted for '*T. timopheevii* subsp. *timopheevii*' (Fig. 3A) and *L. racemosus* (Fig. 3B). Their granule pools are bimodal. In *L. racemosus*, several

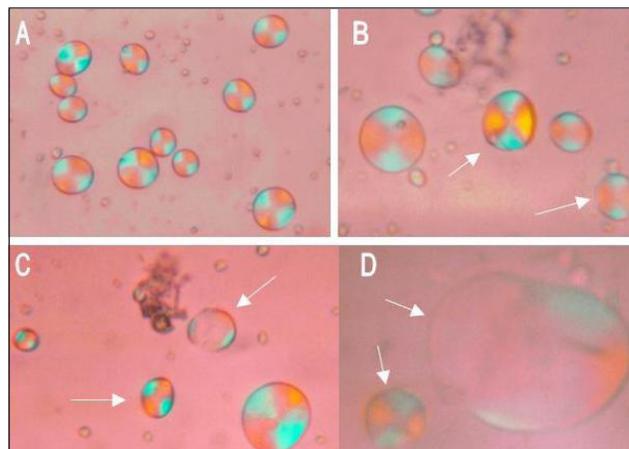


Fig. 4. The 'polarizing reaction' of starch. A – a positive reaction for amylopectin-rich granules and B – a more variable reaction, strong versus weak, in a '*Triticum timopheevii* subsp. *timopheevii/Aegilops longissima*' amphiploid; C – the same as in B for a '*T. turgidum* subsp. *carthlicum/Ae. tauschii*' amphiploid; and D – normal and giant granules presenting very poor reaction in an '*Elymus canadensis/Pseudoroegneria libanotica*' amphiploid.

level of amylopectin synthesis is detected among starch granules in a '*T. turgidum* subsp. *carthlicum*/*Aegilops tauschii* amphiploid (Fig. 4C, p. 48). A giant starch granule in an '*Elymus canadensis*/*Pseudoroegneria libanotica*' amphiploid expresses only weak a polarization reaction in outer parts (Fig. 4D, p. 48).

In conclusion, amylopectin- or amylose-low amounts in Triticeae are cell-, plant- or species-specific. Detecting natural starch mutations among accessions in the Triticeae collection is possible.

References.

- Baum BR and Bailey LG. 1987. A survey of endosperm starch granules in the genus *Hordeum*: a study using image analytic and numerical taxonomic techniques. *Can J Bot* 65:1563-1569.
- Burton RA, Jenner H, Carrangis L, Fahy B, Fincher GB, Hylton C, Laurie DA, Parker M, Waite D, van Wegen S, Verhoeven T, and Denyer K. 2002. Starch granule initiation and growth are altered in barley mutants that lack isoamylase activity. *Plant J* 31:97-112.
- Edwards A, Vincken JP, Suurs LCJM, Visser RGF, Zeeman S, Smith A, and Martin C. 2002. Discrete forms of amylose are synthesized by isoforms of GBSSI in pea. *Plant Cell* 14:1767-1785.
- Klemdal SS, Kvaale A, and Olsen OA. 1986. Effects of the barley mutants Risø 1508 and 527 high lysine genes on the cellular development of the endosperm. *Physiol Plantarum* 67:453-459.
- Kosina R. 2009. On polymorphism of starch grains in the grass endosperm. *In: Grass Research* (Frey L, Ed). W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków. Pp. 109-118.
- Patron NJ, Smith AM, Fahy BF, Hylton CM, Naldrett MJ, Rossnagel BG, and Denyer K. 2002. The altered pattern of amylose accumulation in the endosperm of low-amylose barley cultivars is attributable to a single mutant allele of granule-bound starch synthase I with a deletion in the 5'-non-coding region. *Plant Physiol* 130:190-198.
- Satoh H, Nishi A, Fujita N, Kubo A, Nakamura Y, Kawasaki T, and Okita TW. 2003. Isolation and characterization of starch mutants in rice. *J Appl Glycosci* 50:225-230.
- Tester RF, Karkalas J, and Qi X. 2004. Starch—composition, fine structure and architecture. *J Cereal Sci* 39:151-165.
- Verhoeven T, Fahy B, Leggett M, Moates G, and Denyer K. 2004. Isolation and characterisation of novel starch mutants of oats. *J Cereal Sci* 40:69-79.

Changes in the pericarp in Triticum sinskajae and T. timopheevii subsp. *timopheevii* under environmental stress.

R. Kosina.

The developing caryopsis of wheat is covered by a thick, parenchymatous pericarp. Many starch grains are in the cells of the outer layers (Simmonds and O'Brien 1981; Kosina 2014). The inner epidermis and hypodermis of the pericarp change into tube cells and cross cells, respectively (Morrison 1976). The fleshy pericarp is green and actively assimilates CO₂. Xiong et al. (2013) proved that the development and physiological role of different parts of the common wheat pericarp is cultivar-specific. The wheat pericarp is changed by apoptosis, which starts near the brush on the fourth day after fertilization (Young and Gallie 1999). At this time, the endosperm is enlarging. In unthreshable wheats, the parenchymatous pericarp is reduced in a ripe caryopsis to the outer epidermis and inner cross and tube cells (Kosina 1991, 1995). Similar changes are noted for an unthreshable oat, *Avena fatua* (Morrison and Dushnicky 1982).

Two wheats, *T. sinskajae* (AA), a diploid, free-threshing mutant from *T. monococcum* subsp. *monococcum*, and *T. timopheevii* subsp. *timopheevii* (AAGG), an unthreshable tetraploid species, were used in a starvation experiment. Plants of both wheats were cultivated in a greenhouse in pots filled with sand and on small plots in the field. In the greenhouse, plants were watered poorly. During the development of caryopsis, temperatures reached more than 60°C in the insulated room. In the field, plants were sown in soil of moderate fertility and were regularly watered. The development of the outer part of the caryopsis pericarp and inner layer of cross cells was examined under a microscope on a cross section of the middle part of the caryopsis.

In more favorable field conditions (Figs. 5A and 6A), the outer epidermis of the pericarp is thicker, with better preserved cell lumina in free-threshing *T. sinskajae* than it is in unthreshable *T. timopheevii* subsp. *timopheevii*. The same difference has been noted between *T. timopheevii* subsp. *timopheevii* and its threshable mutant, *T. militinae* Zhuk. & Migush. (Kosina 1995). The inner cross cells are well preserved in both wheats, but in *T. timopheevii* subsp. *timopheevii* they are thicker. One should remember that here species of different ploidy levels, $2n$ versus $4n$, are compared. In starva-

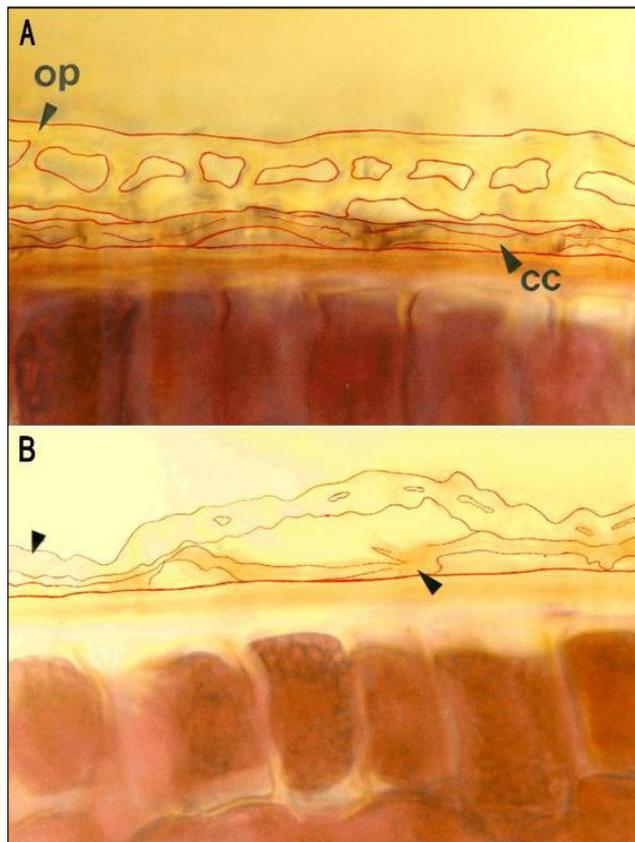


Fig. 5. The covering layers of a *Triticum sinskajae* caryopsis. A – field cultivation and B – stress cultivation in a greenhouse (op = outer pericarp and cc = cross cells; layers are indicated by arrowheads).

- Kosina R. 1995. Tetraploids of the genus *Triticum* in the light of caryopsis structure. Acta Universitatis Wratislaviensis 1785, Prace Botaniczne 66. Wydawnictwo Uniwersytetu Wrocławskiego, Wrocław, pp. 146.
- Kosina R. 2014. On caryopsis xylem and its interactions. In: Advances in Medicine and Biology 75 (Berhardt LV, Ed), Nova Science Publishers, New York. Pp. 43-63.
- Morrison IN. 1976. The structure of the chlorophyll-containing cross cells and tube cells of the inner pericarp of wheat during grain development. Bot Gaz 137:85-93.
- Morrison IN and Dushnicky L. 1982. Structure of the covering layers of the wild oat (*Avena fatua*) caryopsis. Weed Sci 30:352-359.
- Simmonds DH and O'Brien TP. 1981. Morphological and biochemical development of the wheat endosperm. Adv Cereal Sci Tech 4:5-70.
- Xiong F, Yu XR, Zhou L, Wang F., and Xiong AS. 2013. Structural and physiological characterization during wheat pericarp development. Plant Cell Rep 32:1309-1320.
- Young TE and Gallie DR. 1999. Analysis of programmed cell death in wheat endosperm reveals differences in endosperm development between cereals. Plant Mol Biol 39:915-926.

tion conditions, high temperatures, poor sandy soil and deficiency of water (Fig. 5B and 6B), pericarp tissues in *T. sinskajae* are thin and cell lumina disappear. A very strange change is seen in pericarp of *T. timopheevii* subsp. *timopheevii*. This unthreshable wheat has a thick outer pericarp epidermis with well preserved cell lumina, and the epidermis is not collapsed. The cross cells are collapsed. This difference between both wheats in terms of their reaction to stress conditions seems difficult to explain. In Fig. 6B, the pericarp and nucellar tissue cover a dark layer of aleurone with cells not fully filled by protein bodies. Such a picture suggests poor endosperm development. Between both wheats, the possibility of ovary development within 'a floral cavity' is limited by the level of sclerification of glumellae, lemma and palea, and their pressure against ovary tissues. Under starvation conditions, endosperm develops badly and leaves free space for pericarp. Surprisingly, pericarp epidermis is less stressed in *T. timopheevii* subsp. *timopheevii*.

References.

- Kosina R. 1991. Embryo structure of some grasses – its application to fossil materials. Acta Interdisciplinaria Archaeologica 7:177-185.

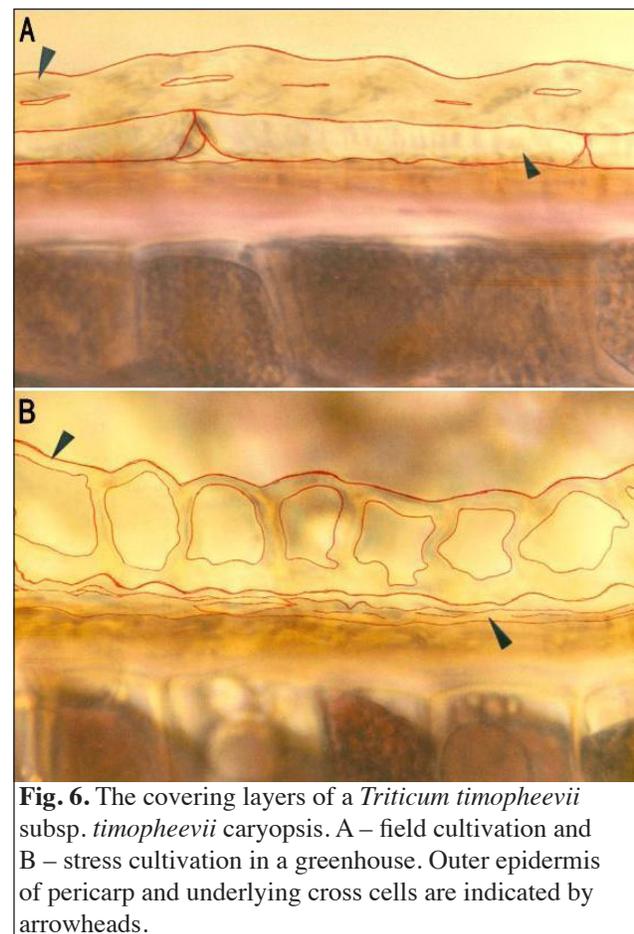


Fig. 6. The covering layers of a *Triticum timopheevii* subsp. *timopheevii* caryopsis. A – field cultivation and B – stress cultivation in a greenhouse. Outer epidermis of pericarp and underlying cross cells are indicated by arrowheads.

On the glumeless form of Agropyron pectiniforme Roem. et Schult.

R. Kosina.

The inheritance of many traits is complex, however, morphological variations in plants are often under the control of only a few genes (Gottlieb 1984). Expression of these genes does not induce abnormal development, but their activity is shown in pleiotropic effects. The study of morphological deviation in the development of the grass inflorescence has mainly been on artificial mutants. Different mutagens have been applied. Cummings et al. (1978) used ethyl methanesulfonate (EMS) to induce morphological mutations in oats. They obtained mutations related to inflorescence bracts, inter alia, a *semiglumeless* form. A *palealess* mutant was described in rice (Luo et al. 2005). The palea was considered a homologous leaf to sepals in eudicot plants, but the lemma is recognized as a different organ. Expression of the *Os-MADS22* gene in transgenic *Oryza sativa* results in the anomalous development of the palea and glume (Sentoku et al. 2005). Any change in the number of mutated organs is called a meristic mutation, which was described by Bossinger et al. (1992) within a barley spike composed of phytomers. The meristic addition of spikelets in *Brachypodium distachyon* is under the control of the *MORE SPIKELETS1* gene (Derbyshire and Byrne 2013). An increase in the number of spikelets also was noted in mutants of *Lolium multiflorum* (Jauhar 1993). The change of flowers into spikelets was discovered by Ceynowa-Giełdon (1997) in *Festuca polesica*. She interpreted new spikelet bracts as lemmas, however, a microscopic examination proved their nature as glumes (Kosina 1998). This natural mutation shows a complex nature, homeotic and meristic. New glumes are homologous to lemmas. The mutation in *F. polesica* increases the potential for assimilation by adding new green organs in the inflorescence. The fertility of the plant is not reduced, therefore, this form can be active in the speciation process (Kosina 1999).

A meristic *glumeless* mutant of *Agropyron pectiniforme* (Fig. 7A) was found on the edge of a cultivated field in the Sudety Mountains, in southwest Poland. The plant was gathered before blooming. The flowers have three, well developed, long anthers, approximately 6 mm. Such long anthers are typical for an allogamous breeding system. The lack of glumes in all spikelets is a meristic defect of the spike. About 10 spikelets in the lower part of spike are underdeveloped. Additional flowers developed in the upper part of spike on the overgrown rachillae of spikelets. In *Triticum jakubzineri* (*T. turgidum*), four glumes develop at the base of each spikelet (Dorofeev 1987). This wheat also expresses a meristic mutation, but it is a reverse mutation compared to that in *A. pectiniforme* (Kosina 1998). The examined plant of *A. pectiniforme* has a smaller surface of assimilation and lower pollen grain production because of underdeveloped lower spikelets and a lack of glumes. The selective advantage of the population also would be lower (Kosina 2015).

References.

- Bossinger G, Lundqvist U, Rohde W, and Salamini F. 1992. Genetics of plant development in barley. *Barley Genet* 6:989-1022.
- Ceynowa-Giełdon M. 1997. Interesting lemmas in *Festuca polesica* fo. *anomala* (Poaceae). *Fragm Flor et Geobot* 42:233-237.
- Cummings DP, Stuthman DD, and Green CE. 1978. Morphological mutations induced with ethyl methanesulfonate in oats. *J Hered* 69:3-7.
- Derbyshire P and Byrne ME. 2013. *MORE SPIKELETS1* is required for spikelet fate in the inflorescence of *Brachypodium*. *Plant Physiol* 161:1291-1302.
- Dorofeev VF. 1987. *Pshenicy mira*. VO Agropromizdat, Leningrad (In Polish).
- Gottlieb LD. 1984. Genetics and morphological evolution in plants. *Am Nat* 123:681-709.
- Jauhar PP. 1993. Cytogenetics of the *Festuca-Lolium* complex. Relevance to breeding. Springer-Verlag, Berlin.

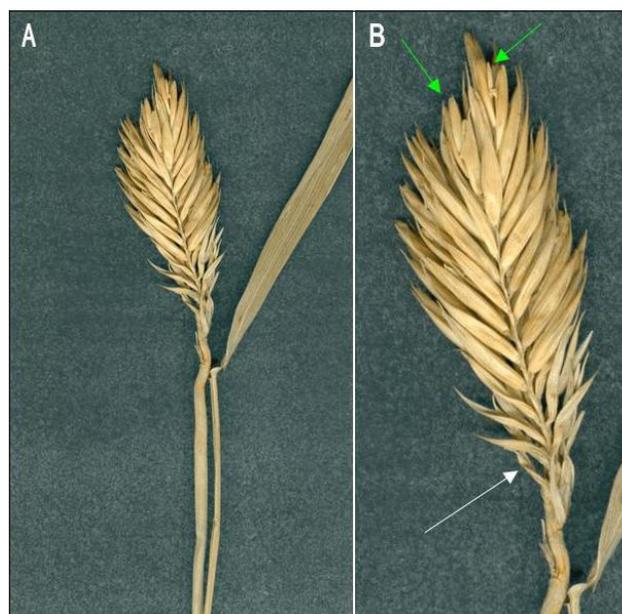


Fig. 7. Morphology of a glumeless spike in *Agropyron pectiniforme*. A – a general view and B – details of underdeveloped spikelets in the lower part of the spike (white arrow) and overgrown spikelets in the upper part (green arrows).

- Kosina R. 1998. O zmienności anomalnej traw. *In*: III Ogólnopolskie Spotkanie „Taksonomia, kariologia i rozmieszczenie traw w Polsce”, Kraków. P. 8 (In Polish).
- Kosina R. 1999. Ranga taksonomiczna morfologicznych aberracji traw. *In*: Materiały Ogólnopolskiej Konferencji „Genetyka a taksonomia roślin”, Poznań. Pp.18-19 (In Polish).
- Kosina R. 2015. Grass inflorescence mutations and their role in speciation. *Mod Phytomorph* 8:7-9.
- Li A, Zhang Y, Wu X, Tang W, Wu R, Dai Z, Liu G, Zhang H, Wu C, Chen G, and Pan X. 2008. DH1, a LOB domain-like protein required for glume formation in rice. *Plant Mol Biol* 66:491-502.
- Luo Q, Zhou K, Zhao X, Zeng Q, Xia H, Zhai W, Xu J, Wu X, Yang H, and Zhu L. 2005. Identification and fine mapping of a mutant gene for palealess spikelet in rice. *Planta* 221:222-230.
- Sentoku N, Kato H, Kitano H and, Imai R. 2005. *OsMADS22*, an *STMADS11*-like MADS-box gene of rice, is expressed in non-vegetative tissues and its ectopic expression induces spikelet meristem indeterminacy. *Mol Genet Genomics* 273:1-9.

On the spirodistichous form of *Lophopyrum elongatum* (Host) Á. Löve.

R. Kosina.

At least one nullisomic 3D plant with a spike expressing a compact arrangement of spikelets in the upper part and a few loose spikelets in the lower part can be recognized within the Sears' nullisomic series of Chinese Spring wheat (Schulz-Schaeffer 1980). Similar types, called 'squarehead', were exemplified by Mac Key (1966) in a hybrid progeny of '*Triticum aestivum* subsp. *aestivum*/*T. aestivum* subsp. *macha*' and '*T. aestivum* subsp. *aestivum*/*T. aestivum* subsp. *vavilovii*'. The *Q* factor (gene) is responsible for a free-threshing feature and square spikes (Mac Key 1964; Simons et al. 2006). This genetic control is closely related to other simple morphological inheritance patterns, which were exemplified by Gottlieb (1984). The *Q* gene exhibits dosage effects on spike compactness, but modifiers regulate its pleiotropic influence on the development of square spikes. In transgenic wheats, a high expression of the *Q* gene, due to its dosage, was detected (Faris et al. 2005). The gene was localized on chromosome 5A in *T. aestivum* (Jantasuriyarat et al. 2004). The *QQ* genotype was detected in *T. turgidum* subsps. *carthlicum*, *polonicum*, *dicoccum*, and *durum*. In these subspecies, dense compact spikes were described (Faris et al. 2005). Species with compact spikes also are known in diploid wheat diploids (*T. sinskajae*), in AAGG tetraploids (*T. militinae*), and in hexaploids (*T. aestivum*, subsps. *compactum* and *sphaerococcum*) (Dorofeev 1987; Goncharov 2002). Wheats with compact spikes, such as *T. vulgare antiquorum*, also were identified in fossil materials (Schiemann 1932).

A spontaneous, spirodistichous mutant of *Lophopyrum elongatum* found in the grass collection of R. Kosina shows a gross spike morphology similar to that mentioned above. The lower part of its spike is distichous (Fig. 8A). In some spikes, three spikelets are developed at each rachis segment, above the distichous part. In the short upper part, a spiral phyllotaxis is expressed (Fig. 8B). Flowers of *L. elongatum* have well-developed anthers, ~4–5 mm long, including in the spiral part of the spike. Spikelets are very densely packed in this section, and the morphology of glumes and glumellae are changed (Kosina 1998). Pollen grains often are reduced and no seeds are formed in the upper part (Kosina 1999, 2015). Lodicules are anomalous and flowers behave as cleistogamic (Kosina et al. 2012). *Triticum aestivum* subsp. *compactum* with square heads and *QQ* gene formulae also can lose the possibility of flower chasmogamy due to lodiculae mutation (Kosina and Tomaszewska 2012). In the hybrid progeny '*Triticum*/*L. elongatum*', no compact heads or similar parts were described by Tsitsin (Tsitsin 1978).



Fig. 8. Morphology of a spirodistichous spike in *Lophopyrum elongatum*. A – a general view (arrows indicate a distichous part of a spike and an upper part with a spiral phyllotaxis) and B – details of the spiral arrangement of spikelets.

References.

- Dorofeev VF (1987) Pshenicy mira. VO Agropromizdat, Leningrad (In Russian).
- Faris JD, Simons KJ, Zhang Z, and Gill BS. 2005. The wheat super domestication gene *Q*. *Wheat Inf Serv* 100:129-148.
- Goncharov NP. 2002. Comparative genetics of wheats and their related species. Siberian University Press, Novosibirsk (In Russian).
- Gottlieb LD. 1984. Genetics and morphological evolution in plants. *Am Nat* 123:681-709.
- Jantasuriyarat C, Vales MI, Watson CJW and Riera-Lizarazu O. 2004. Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). *Theor Appl Genet* 108:261-273.
- Kosina R. 1998. O zmienności anomalnej traw. *In: III Ogólnopolskie Spotkanie 'Taksonomia, kariologia i rozmieszczenie traw w Polsce'*, Kraków. P. 8 (In Polish).
- Kosina R. 1999. Ranga taksonomiczna morfologicznych aberracji traw. *In: Materiały Ogólnopolskiej Konferencji 'Genetyka a taksonomia roślin'*, Poznań. Pp. 18-19 (In Polish).
- Kosina R. 2015. Grass inflorescence mutations and their role in speciation. *Mod Phytomorph* 8:7-9.
- Kosina R, Florek M, and Tomaszewska P. 2012. Lodicule variability in a spiro-distichous spike of *Lophopyrum elongatum*. *Ann Wheat Newslett* 58:201.
- Kosina R and Tomaszewska P. 2012. On breeding system in wheat and *Brachypodium distachyon*. *Ann Wheat Newslett* 58:194-195
- Schiemann E. 1932. Entstehung der Kulturpflanzen. Handbuch der Vererbungswissenschaft Band III. Verlag von Gebrüder Borntraeger, Berlin (In German).
- Schulz-Schaeffer J. 1980. Cytogenetics. Plants, animals, humans. Springer-Verlag, New York. 446 pp.
- Simons KJ, Fellers JP, Trick HN, Zhang Z, Tai YS, Gill BS, and Faris JD. 2006. Molecular characterization of the major wheat domestication gene *Q*. *Genetics* 172:547-555.
- Tsitsin NV (1978) Mnogoletnyaya pshenitsa. Nauka, Moskva (In Russian).

Variability of germination in Brachypodium distachyon and B. sylvaticum evaluated with a variance-covariance matrix.

R. Kosina.

Various coefficients of similarity or dissimilarity are useful to describe numerical relationships among plant operational taxonomic units (OTUs) (Sneath and Sokal 1973). The coefficients are measures of taxonomic distances. Correlation coefficients are some examples. Most often, arithmetic means of the characters are used to calculate the difference between any pair of OTUs. An average taxonomic distance (ATD) based on character data is applied in biology (Rohlf 1994). The ATD is a generalized Euclidean distance (Sneath and Sokal 1973). The ATD was used to set OTUs belonging to two species of the genus *Brachypodium*, an annual autogamic *B. distachyon* (Bd) and perennial allogamic *B. sylvaticum* (Bs), in an ordination space by means of non-metric multidimensional scaling (Kosina and Jaroszewicz 2007). OTUs were described by germination data and arranged in two- or three-dimensional diagrams. Accessions of *B. distachyon* are scattered widely, whereas those of *B. sylvaticum* create a compact cluster. In the *x* and *y* ordinants, the points of OTUs are highly correlated. One conclusion is such that interaccessional variation in the autogamic species is larger than that between allogamic populations.

Sneath and Sokal (1973) pointed out that the weighting of data is important during the clustering process, because clusters are either less or more distinct and can be of different shape. Rohlf (1970) proposed some weighting by means of a generalized distance function, where the variance-covariance matrix (var-cov) of *n* variables is used. Rohlf's measure takes into account size and shape trends existing in each cluster. These trends change when adding successive OTUs into the cluster. A difference between clusters can be easily illustrated by a difference between, e.g., hyperellipsoid and hyperspheroid. Within two such geometric clouds of OTUs, correlations and covariances between OTUs will be different. The var-cov method was applied to germination data according to Rohlf's approach (Rohlf 1994). The eigenvalues and eigenvectors are calculated from the var-cov matrix (Rohlf 1970). Nonmetric multidimensional scaling was used to arrange OTUs into an ordination space (Figs. 9 and 10, p. 54). They are visually different from the results obtained with the application of ATD (Kosina and Jaroszewicz 2007). The OTUs belonging to *B. distachyon* create a compact cloud in the center, three Bd accessions are more distant from the center. Accessions of *B. sylvaticum* are located outwards (Fig. 9, p. 54). The distribution of OTUs on the *x*-*y* axes do not have any significant correlation (Fig. 10, p. 54). In fact, the relationships between Bd and Bs accessions are similar to the situation pertaining when ATD was applied. The main difference is shown by the reverse compactedness of Bs versus Bd OTUs. Looking at the variance-covariance

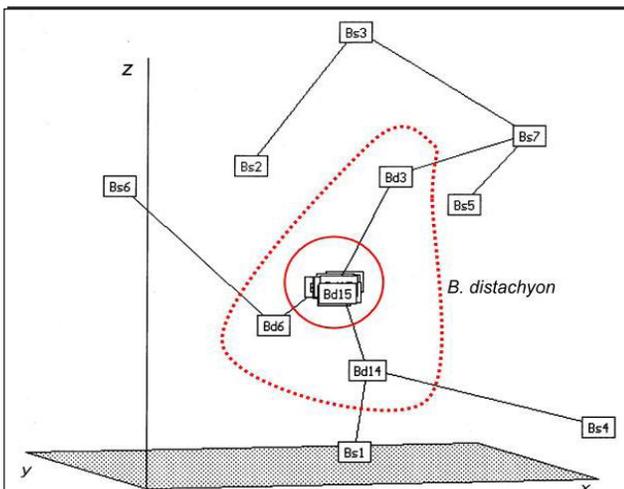


Fig. 9. Minimum spanning tree of accessions (operational taxonomic units (OTUs)) of *Brachypodium distachyon* (Bd, in the center of diagram) and *B. sylvaticum* (Bs) located around it. The tree was constructed after calculation of the variance-covariance matrix and the use of non-metric multidimensional scaling. The OTUs are scattered along three ordination axes (x, y, z).

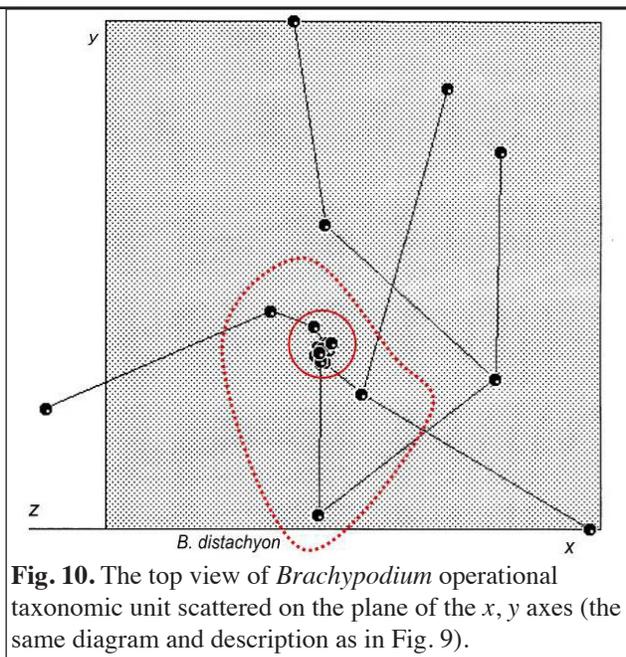


Fig. 10. The top view of *Brachypodium* operational taxonomic unit scattered on the plane of the x, y axes (the same diagram and description as in Fig. 9).

matrix, it is remarkable that Bd OTUs plotted in the center of diagrams are characterized by greater variances and covariances of the examined germination traits and in outer Bs accessions these values are smaller. The above statement means that each examined caryopsis of *B. distachyon* is different from another. A normal occurrence when the progeny of an autogamic species is a set of many homozygous lines. Both kinds of results not only are visually different but biologically, they are consistent with each other.

References.

- Kosina R and Jaroszewicz A. 2007. Mikrostrukturalne determinanty kiełkowania ziarniaków *Brachypodium distachyon* i *B. sylvaticum* (Poaceae). *Fragmenta Floristica et Geobotanica Polonica*. Suppl. 9:117-125 (In Polish).
 Rohlf FJ. 1970. Adaptive hierarchical clustering schemes. *Syst Zool* 18:58-82.
 Rohlf FJ. 1994. NYSYS-pc v. 1.80. Applied Biostatistics Inc, New York.
 Sneath PHA and Sokal RR. 1973. Numerical taxonomy. Freeman, San Francisco.

Annuality-perenniality and dormancy in the genus Brachypodium.

R. Kosina and P. Tomaszewska.

We know a lot about the germination of grass diaspores. Within the grass family, one can distinguish two extreme phenotypes related to breeding system, autogamic versus allogamic, with many intermediate types. Related to life habit, annual versus perennial, with some intermediates. Cereals from the Triticeae tribe are mostly annuals, but their mating system can be different, for instance, in autogamic wheat and allogamic rye. Wild grasses seem to be more differentiated. We will consider the relationships within a wild genus *Brachypodium*, which comprises annuals and perennials, auto- and allogamic and self-compatible and self-incompatible types. Dormancy, a life history component, is not well elucidated in the grass life habit.

In *Brachypodium*, the annual *B. distachyon* essentially expresses autogamy, however, in suitable weather conditions, can be chasmogamic and capable of cross-pollination (Kosina and Kłyk 2011; Kosina and Tomaszewska 2012; Kosina and Tomaszewska 2014b). Other species are perennials and self-incompatible, but *B. sylvaticum* is self-compatible facultatively (Khan and Stace 1999; Kłyk 2005). Differences in the germination potential between cereals and annual-perennial species of *Brachypodium* are created by the level of dormancy, caryopsis microstructure, and assimilate storage pattern. In general, annuals are nondormant or less dormant versus dormant perennials. However, examples of the annual *Avena fatua* with fewer or more dormant diaspores and wild goatgrasses and diploid wheats expressing dormancy

polymorphism in diaspores, contradict the above general assumption. Annual and perennial species of *Brachypodium* store assimilates in the form of endosperm starch and protein and, in addition, a large amount in the form of cell wall hemicellulosic compounds (Jaroszewicz et al. 2012; Kosina and Jaroszewicz 2007; Kosina and Kamińska 2013a and 2013b). In cereals, starch and protein are the main storage substances. Germination is more facilitated in *Brachypodium* due to the capillary potential of its coleorhizal hairs, than is the case in cereals (Kosina and Tomaszewska 2014a). Dormancy-germination behavior exhibits a large variation in *Brachypodium* (Kosina and Tomaszewska 2014c).

A question arises: What are the relationships between the dormancy pattern and life habit in the genus *Brachypodium*? Germination tests were performed for annual, spring, and winter accessions of *B. distachyon* of different geographic origin (Australia, Italy, Iran, Iraq, and Turkey) and perennials *B. pinnatum*, *B. phoenicoides*, *B. rupestre*, and *B. sylvaticum*. Accessions, evaluated by several germination traits and numerical methods (Rohlf 1994), were set into an ordination space using a non-metric, multidimensional scaling. An interpretation of a two-dimensional (x and y axes of the ordination space) diagram (Fig. 11) was conducted, and regression parameters were calculated for a set of operational taxonomic units (OTUs) (Fig. 12). The arrangement of OTUs along the x and y axes is not random and seems to be highly correlated (Fig. 11). The variation in regression is highly significant, and the regression line has a slope close to zero (Fig. 12). The correlation coefficient is highly significant ($r = 0.89$). The results prove that dormancy is highly correlated with perennial habit, whereas nondormant types mostly are annual.

References.

- Jaroszewicz AM, Kosina R, and Stankiewicz PR. 2012. RAPD, karyology and selected morphological variation in a model grass, *Brachypodium distachyon*. *Weed Res* 52:204-216.
- Khan MA and Stace CA. 1999. Breeding relationships in the genus *Brachypodium* (Poaceae: Pooideae). *Nord J Bot* 19:257-269.
- Kłyk B. 2005. Zmienność mikrostrukturalna niektórych gatunków rodzaju *Brachypodium* P. Beauv. PhD thesis, University of Wrocław, Wrocław (In Polish).
- Kosina R and Jaroszewicz A. 2007. Mikrostrukturalne determinanty kiełkowania ziarniaków *Brachypodium distachyon* i *B. sylvaticum* (Poaceae). *Fragmenta Floristica et Geobotanica Polonica*, Suppl. 9:117-125 (In Polish).
- Kosina R and Kłyk B. 2011. Reproduction in *Brachypodium distachyon* and related species. *Ann Wheat Newslet* 57:251-252.
- Kosina R and Kamińska K. 2013a. Variability of caryopsis micromorphology in *Brachypodium distachyon*. *Ann Wheat Newslet* 59:125-126.
- Kosina R and Kamińska K. 2013b. The role of nucellar epidermis during the germination of *Brachypodium distachyon*. *Ann Wheat Newslet* 59:124.
- Kosina R and Kłyk B. 2011. Reproduction in *Brachypodium distachyon* and related species. *Ann Wheat Newslet* 57:251-252.

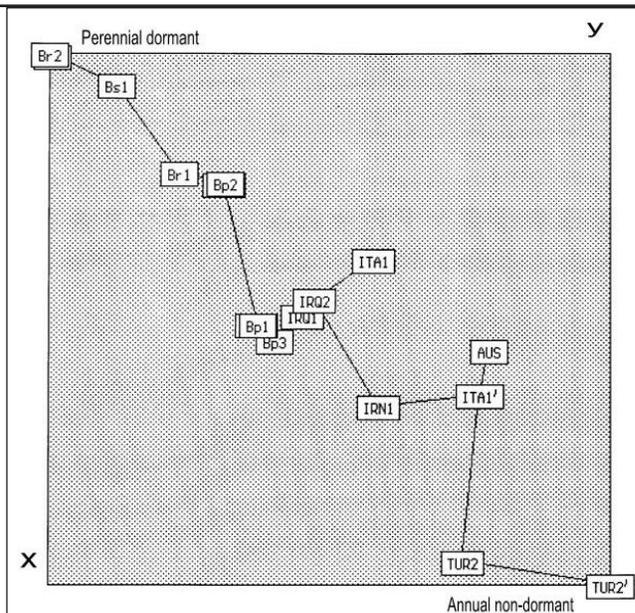


Fig. 11. The top view of the minimum spanning tree of annual and non-dormant accessions (operational taxonomic units, OTUs) of *Brachypodium distachyon* and perennial dormant species. The tree was constructed after calculating the matrix of average taxonomic distances and the use of non-metric multidimensional scaling. The OTUs are scattered along two ordination axes (x , y) (AUS = Australia, ITA = Italy, IRN = Iran, IRQ = Iraq, and TUR = Turkey).

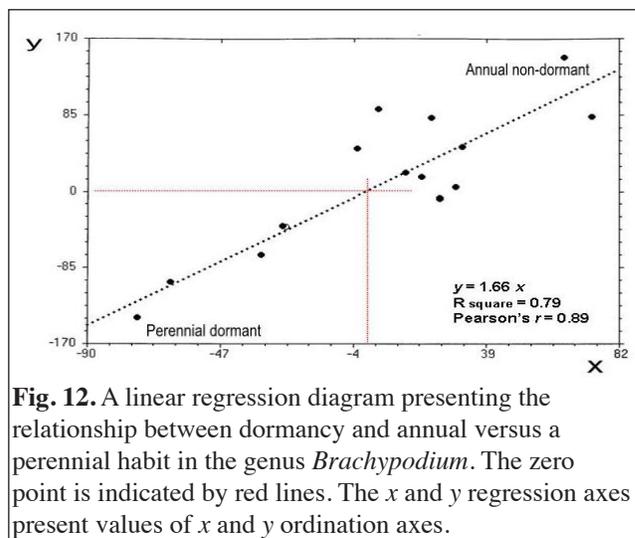


Fig. 12. A linear regression diagram presenting the relationship between dormancy and annual versus a perennial habit in the genus *Brachypodium*. The zero point is indicated by red lines. The x and y regression axes present values of x and y ordination axes.

- Kosina R and Tomaszewska P. 2012. On breeding system in wheat and *Brachypodium distachyon*. Ann Wheat Newsletter 58:194-195.
- Kosina R and Tomaszewska P. 2014a. Variability of coleorhizal hairs in Triticeae and *Brachypodium*. Ann Wheat Newsletter 60:110-112.
- Kosina R and Tomaszewska P. 2014b. Questions about the nature of an anomalous plant in *Brachypodium distachyon*. Ann Wheat Newsletter 60:109-110.
- Kosina R and Tomaszewska P. 2014c. Interpopulational and interspecific variation of germination in *Brachypodium*. Ann Wheat Newsletter 60:113-114.

Mosaics in the aleurone layer in some members of Triticeae L.

R. Kosina.

Plant development through cell lineages is commonly accepted. The lineages are well recognized in roots or a leaf epidermis (Lyndon 1990). Some kinds of cell lineages can be observed in the grass endosperm. The endosperm is a very variable tissue. Multipolar anaphases and telophases are its cytogenetic phenomenon. Such a cytogenetic behavior also was noted in root mitoses, for instance, in *Bromus secalinus* (Skowrońska 2005), but is especially frequent in the triticale (\times *Triticosecale*) endosperm (Kaltsikes et al. 1975). Multiple telophases or nuclei connected by chromatin threads are present in triticale. In the *Avena* amphiploids, such cytogenetics gives nuclei of various ploidy levels. Surprisingly, numerous nuclei are diploid, not triploid (Tomaszewska and Kosina 2015). Programmed cell death is another phenomenon that occurs during endosperm development (Young and Gallie 2000), however, this can relate not only to starchy cells but also to many defective nuclei at the free-nucleolar stage (Kosina and Tomaszewska 2013). This elimination of many nuclei results in isolation of the remaining, which differ from each other. In the later stages of endosperm development, large units (endospermal domains) of isolated cells are distinguished by thick cell wall ingrowths. Ivanovskaja (1983) presented such isolated groups of nuclei for '*Triticum aestivum* subsp. *aestivum*/*Leymus arenarius* F₁ hybrids. Subsycytial nuclear units of antipodal or a central embryo sac origin (Kosina 1992, 1996) are created by the same endospermal events. The clonal architecture of the grass endosperm was described by Kosina (1992, 2012) and, due to many earlier cytogenetic disorders, the clones can be different and observed in the form of mosaics (Kosina 2007; Kosina et al. 2013a and 2013b). Some clones can be highly polyploid (Kosina et al. 2013b).

The following examples show various mosaics/domains in the aleurone layer in some species and amphiploids of the Triticeae tribe. The aleurone

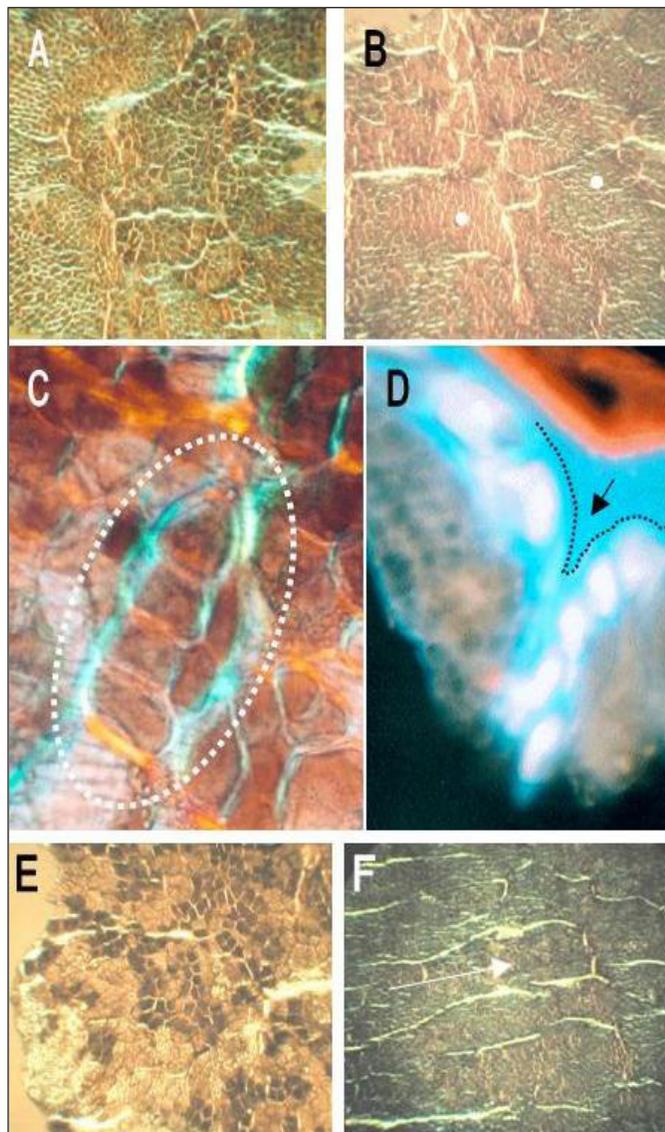


Fig. 13. Details of aleurone layer mosaics. A, large domains in the aleurone layer in *Triticum turgidum* subsp. *turanicum* bordered by thick light lines of nucellar and aleurone cell wall ingrowths; B, the same domains as in A in a '*T. turgidum* subsp. *dicoccoides*/*Aegilops tauschii*' amphiploid (two types of polarizing color domains are marked by white dots); C, a small clone-domain of aleurone cells bordered by their thick non-cellulosic walls in a '*T. turgidum* subsp. *turanicum*/*Ae. tauschii*' amphiploid; D, details of nucellar (arrow) and aleurone cell wall ingrowths in *Elymus canadensis*, the border between both types of walls is marked by a dotted line; E, partly bordered domains in a '*Triticum turgidum* subsp. *turanicum*/*Ae. tauschii*' amphiploid that are composed of dark aleurone cells and lighter starchy cells; and F, domains with directionally ordered borders in a '*T. turgidum* subsp. *dicoccoides*/*Ae. tauschii*' amphiploid.

domains are separated by thick light lines, which are cell walls seen in a polarizing microscope (Fig. 13A, p. 56). These lines are created in regions of the aleurone layer having different colors (Fig. 13B, p. 56). These colors are a polarizing effect of the cell walls, indicating that aleurone cells are arranged differently in the layer. The thick aleurone wall can separate a group of cells of common origin, a clone (Fig. 13C, p. 56). The light borders of domains are created not only by an intrusive growth into starchy endosperm of thick-walled aleurone cell (Kosina et al. 2013b), but also by ingrowths of the thick walls of the nucellar epidermis (Fig. 13D, p. 56). Light borders can limit groups of aleurone cells with protein, but a mosaic of aleurone-protein and aleurone-starch cells also can be separated (Fig. 13E, p. 56). For cell characteristics, aleurone-protein versus aleurone-starch, see a report on variability of the aleurone layer (Kosina et al.). The original development of an aleurone layer in which domain borders are directional is noted for a dorsal surface of caryopsis (Fig. 13F, p. 56). Such an arrangement of borders is related to the I- or T-type of cell division by anticlinal walls. This developmental problem was discussed by Barlow (1991).

References.

- Barlow PW. 1991. From cell walls networks to algorithms. *Protoplasma* 162:69-85.
- Ivanovskaja EV. 1983. Citoembriologicheskoe issledovanie differencirovki kletok rastenij. Izdatel'stvo Moskovskogo Universiteta, Moskva (In Russian).
- Kaltsikes PJ, Roupakias DG, and Thomas JB. 1975. Endosperm abnormalities in *Triticum-Secale* combinations. I. *x Triticosecale* and its parental species. *Can J Bot* 53:2050-2067.
- Kosina R. 1992. On endosperm structure in some grasses. *In: V Ogólnopolska Konferencja 'Mechanizmy regulacji morfogenezy roślin'*, Rogów. Pp. 139-140 (In Polish).
- Kosina R. 1996. Nucleolar variation in grass endosperm. *Acta Soc Bot Pol* 65:190.
- Kosina R. 2007. Some topics on the grass mosaics. *In: Biological issues in grasses* (Frey L, Ed). W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków. Pp. 159-167.
- Kosina R. 2012. On caryopsis development in *Thinopyrum distichum* versus wheat. *Ann Wheat Newslet* 58:203.
- Kosina R, Bureś MK, Florek M, Grabińska A, Kawa P, Kłyk B, Kochmański Ł, Koźlik A, Kurek A, Skowrońska J, Tomaszewska P, and Zajac D. 2013a. Structural characteristics of grass hybrid endosperm development. *Ann Wheat Newslet* 59:116-117.
- Kosina R, Koźlik A, and Markowska K. 2013b. Endosperm domains in a '*Triticum timopheevii* subsp. *timopheevii* / *Aegilops umbellulata*' amphiploid. *Ann Wheat Newslet* 59:117-118.
- Kosina R and Tomaszewska P. 2013. Genomes of wheat and other grasses during the cell cycle and apoptosis. *Ann Wheat Newslet* 59:113-114.
- Koźlik A. 2013. Zmienność mikrostrukturalna owocu amfiploida *Triticum timopheevii* x *Aegilops umbellulata*. MSc Thesis, Department of Cytogenetics and Plant Speciation, Institute of Experimental Biology, University of Wrocław, Wrocław (In Polish).
- Lyndon RF. 1990. Plant development. The cellular basis. Unwin Hyman, London.
- Skowrońska J. 2005. Mikrostrukturalna i cytogenetyczna analiza grupy *Bromus secalinus* – *B. commutatus* – *B. racemosus*. MSc Thesis, Plant Cytogenetics Laboratory, Institute of Plant Biology, University of Wrocław, Wrocław (In Polish).
- Tomaszewska P and Kosina R. 2015. The A, B, C and D genomes in endosperm of oat amphiploids. *Chromosome Res* 23, Suppl. 1:128.
- Young TE and Gallie DR. 2000. Programmed cell death during endosperm development. *Plant Mol Biol* 44:283-301.

External pressure on a dorsal surface of caryopsis changes the development of wheat endosperm.

R. Kosina.

The active cells inside the apical meristems of plants are isodiametric and their divisions are not regular (Lyndon 1990). The tree cambium is composed of two types of cells, parenchyma-like initials in the rays and axially elongated initials between the young differentiating elements of the xylem and phloem. The elongated initials are arranged orderly in the form of tangential strips. This order was examined by Steeves and Sussex (1989) and Lyndon (1990) and proved that pressure caused by expanding xylem and external phloem and other secondary tissues is important for an orderly cytokineses. In the cambium isolated from a tree, elongated initials start to divide like in a callus, but when external pressure is applied, cells divide tangentially to a pressed surface. The youngest endosperm tissue is located at the embryo sac wall, and the last tangential or anticlinal cytokineses occur there, giving an aleurone layer. Such divisions create regular endosperm cell clones (Kosina 1992, 2012). Thus, we justify the aleurone layer as an 'endosperm cambium'. The

cambial architecture of the aleurone layer is found in many different grasses (Mares et al. 1977; Simmonds and O'Brien 1981; Fineran et al. 1982).

Kosina (1989) reported an experiment with artificial pressure used against the endosperm cambium in several grasses. A spheroidal seed of clover was placed between a young, green pericarp and the lemma before cellularization of the endosperm. Pressure caused by the seed was maintained until maturity of the grass diaspore. Results for one of the examined grasses, *Triticum turgidum* subsp. *turanicum* are presented (Fig. 14). The distinct amplification of aleurone cells is visible beneath and around the pressure point (Fig. 14A). New tangential divisions occur in smaller larger groups of aleurone cells (Fig. 14B). Locally, single cells with thick walls can be isolated and induced into an original development; the spherical cell shape and its larger size suggests that polyploidization occurred here (Fig. 14C). Pressure also caused starch cells of endosperm not to develop, and aleurone cells from the dorsal part were connected with those from a crease region (Fig. 14D).

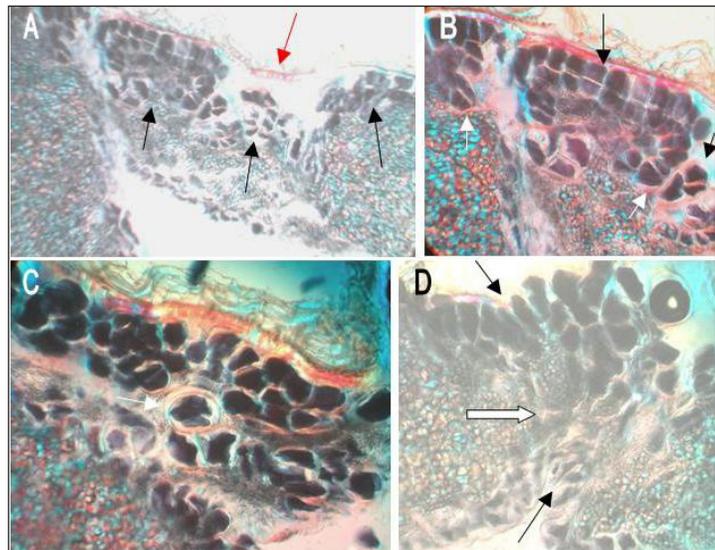


Fig. 14. Microstructure of *Triticum turgidum* subsp. *turanicum* changed by artificial pressure. A, a red arrow shows the point of the clover seed setting and black arrows indicate abundant amplification of aleurone cells; B, black and white arrows show amplification of periclinal (tangential) aleurone cell divisions; C, a white arrow indicates a polyploidized, callus-like, aleurone cell with a thick wall; and D, upper, dorsal aleurone cells and ventral ones in the bottom (black arrows) are connected (white arrow) in the area of pressure influence.

In conclusion, external pressure induced the following changes:

1. multiplication of the aleurone cells is due to increased tangential cytokineses,
2. multiplication of the aleurone cells by divisions is typical for callus, and
3. anomalous, poor development of starchy endosperm beneath the pressure point and, in consequence, connecting both aleurone layers, dorsal and ventral.

These results confirm experiments with tree cambium (Steeves and Sussex 1989; Lyndon 1990) and justify use of the term 'endosperm cambium'.

References.

- Fineran BA, Wild DJC and Ingerfeld M. 1982. Initial wall formation in the endosperm of wheat, *Triticum aestivum*: a reevaluation. *Can J Bot* 60:1776-1795.
- Kosina R. 1989. Triticeae studies III. *Ann Wheat Newslet* 35:106-107.
- Kosina R. 1992. On endosperm structure in some grasses. *In: V Ogólnopolska Konferencja „Mechanizmy regulacji morfogenezy roślin”*, Rogów. Pp. 139-140.
- Kosina R. 2012. On caryopsis development in *Thinopyrum distichum* versus wheat. *Ann Wheat Newslet* 58:203.
- Lyndon RF. 1990. *Plant development. The cellular basis*. Unwin Hyman, London.
- Mares DJ, Stone BA, Jeffery C, and Norstog K. 1977. Early stages in the development of wheat endosperm. II Ultrastructural observations on cell wall formation. *Aust J Bot* 25: 599-613.
- Steeves TA and Sussex IM. 1989. *Patterns in plant development*. Cambridge University Press, Cambridge.

Variation of winterhardiness in Brachypodium distachyon (2).

R. Kosina.

Brachypodium distachyon expresses wide variation in terms of flowering and vernalization time. Schwartz et al. (2010) mainly identified spring and winter forms within a set of diploid and polyploid accessions of various geographical origin. Polyploid accessions from Spain appear to be intermediate between spring and winter types. The vernalization period of

4°C for 3-week-old seedlings ranged from 2 to 6 weeks. In addition, Manzaneda et al. (2011) proved that, under drought stress, *B. distachyon* polyploids are more efficient than diploids in the use of water. Winter and heat survival in *Avena sativa* and *A. byzantina* varieties seem to be positively correlated (Levitt 1972). Tolerance for freezing and drought stresses are correlated, both via water stress. Supposedly, the same is true for *B. distachyon*, and the polyploid types will be more tolerant. However, Li et al. (2012) discovered that *B. distachyon* lacks genes for fructan synthesis and accumulation during cold stress. In this reaction, *B. distachyon* differs from that of other Poaceae grasses and both are components of freezing tolerance variation in the Poaceae family. Colton-Gagnon et al. (2014) presented contradictory data on fructan synthesis in *B. distachyon* treated by freezing stress. The authors also described some intermediate forms between spring and winter diploids of *B. distachyon*. The species requirements for vernalization in low temperatures showed a great variation (Woods et al. 2014).

To know precisely the winter hardiness in *B. distachyon*, new data are presented. After the summer of 2014, six accessions grew vegetatively into the winter of 2014–15; ITA3 (Italy), ITA3s (a selection from ITA3), TUR2 (Turkey), TUR2s (a selection from TUR2), BGR2 (Bulgaria), and BGR2s (a selection from BGR2). The plants were protected from severe frost by fleece, however, the winter of 2014–15 was especially mild. Minimum temperatures were a little lower than –8°C and were interrupted by periods of higher temperatures. The highest temperatures occurred on 20 December (14°C), 10 January (16°C), and 15 February (11°C) (see Fig. 15).

An increased vegetative growth and loose tuft habit were noted accession ITA3. The ITA3 plants develop prostrately rooted shoots and additional axillary tillers, such as in ITA1 (Fig. 16A and Kosina and Tomaszewska 2014a). Other accessions growing in as short, dense tufts, such as TUR2s, BGR2, and BGR2s, died during the winter (Fig. 16A, B). Only the accession TUR2 overwintered in lateral parts of the tuft (Fig. 16C). Several accessions resowed from falling diaspores. Seedlings of the IRQ accession not protected by fleece overwintered very well (see Fig. 16D). All overwintered forms headed on 15 May, but some differences were observed in the rate of this process (Fig. 17). The experiment concerning the wintering of all accessions of *B. distachyon* will be continued during winter 2015–16.

References.

Colton-Gagnon K, Ali-Benali MA, Mayer BF, Dionnel

R, Bertrand A, Do Carmo S, and Charron J-B. 2014. Comparative analysis of the cold acclimation and freezing tolerance capacities of seven diploid *Brachypodium distachyon* accessions. *Ann Bot* 113:681-693.

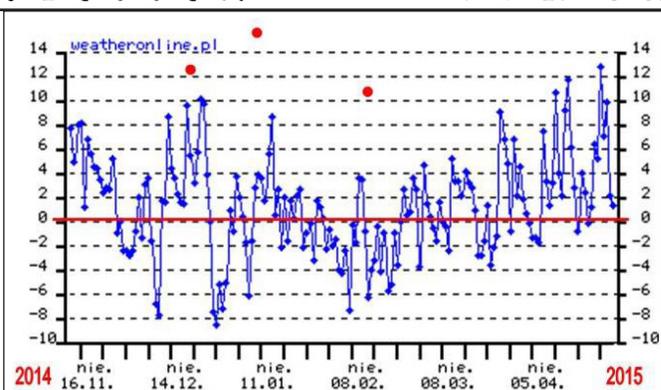


Fig. 15. Diagram of winter temperatures during 2014–15 in the area of *Brachypodium distachyon* cultivation. The temperature °C and the maximums are marked in red. According to *weatheronline.pl*.



Fig. 16. Accessions of *B. distachyon* of various geographical origin in a wintering experiment. Clumps of ITA3 (Italy, A) and BGR2 (Bulgaria, B) that died. A partly overwintered (yellow arrows) clump of TUR2 (Turkey, C). Overwintered, self-sowing seedling (yellow arrows) of the IRQ accession (Iraq, D). A blue, plastic bottle cap is included for size comparison.

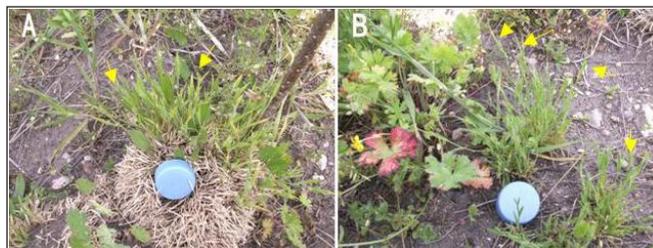


Fig. 17. Plants of *B. distachyon* at heading stage (yellow arrows) after overwintering in 2014–15. A, TUR2 (Turkey) and B, FRAs, a selection from FRA (France). A blue plastic bottle cap is included for size comparison.

Jaroszewicz AM, Kosina R, and Stankiewicz PR. 2012. RAPD, karyology and selected morphological variation in a model grass, *Brachypodium distachyon*. *Weed Res* 52:204-216.

Kosina R and Tomaszewska P. 2014a. Variability of vegetative propagation in *Brachypodium distachyon*. *Ann Wheat Newslet* 60:108-109.

Kosina R and Tomaszewska P. 2014b. Variation of winter hardiness in *Brachypodium distachyon*. *Ann Wheat Newslet* 60:115.

Levitt J. 1972. Responses of plants to environmental stresses. Academic Press, New York.

Manzaneda AJ, Rey PJ, Bastida JM, Weiss-Lehman C, Raskin E, and Mitchell-Olds T. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytol* 193:797-805.

Li C, Rudi H, Stockinger EJ, Cheng H, Cao M, Fox SE, Mockler TC, Westereng B, Fjellheim S, Rognli OA, and Sandve SR. 2012. Comparative analyses reveal potential uses of *Brachypodium distachyon* as a model for cold stress responses in temperate grasses. *BMC Plant Biol* 12:65 (doi:10.1186/1471-2229-12-65).

Schwartz CJ, Doyle MR, Manzaneda AJ, Rey PJ, Mitchell-Olds T, and Amasino RM. 2010. Natural variation of flowering time and vernalization responsiveness in *Brachypodium distachyon*. *Bioenergy Res* 3:38-46.

Woods DP, Ream TS, and Amasino RM. 2014. Memory of the vernalized state in plants including the model grass *Brachypodium distachyon*. *Front Plant Sci* 5:99 (doi: 10.3389/fpls.2014.00099).

ITEMS FROM THE RUSSIAN FEDERATION

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

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

The influence of a translocation with the combination Lr19+Lr25 on grain productivity and bread-making quality in the spring bread wheat cultivar Dobrynya.

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

At the Agricultural Research Institute for the South-East Regions (ARISER), NILs based on the Saratov-bred, spring bread wheat cultivar Dobrynya and carrying translocations with the combination *Lr19+Lr25* were produced and studied. The data from 2012–14 indicate that the interaction of these trans-

Table 1. Grain productivity and gluten values of near-isogenic lines (NILs) of the spring bread wheat cultivar Dobrynya, average for 2012–14. Gluten strength was evaluated by using the gluten deformation index.

NIL	Grain yield (kg/ha)	Gluten value	
		Content (%)	Strength
Dobrynya (<i>Lr19</i>)	3,164	38.03	71
Dobrynya (<i>Lr19+Lr25</i>)	3,323	38.87	76
LSD	NS	NS	NS

Table 2. Bread-making qualities of near-isogenic lines (NILs) and spring bread wheat cultivar Dobrynya (average for 2012–14).

NIL	Physical trait of dough (alveograph)			Bread-making quality		
	Dough extensibility (P)	P/L	Flour strength (W)	Loaf volume (cm ³)	Porosity	Crumb color
Dobrynya (<i>Lr19</i>)	142.3	2.33	368.7	847	4.9	yellow
Dobrynya (<i>Lr19+Lr25</i>)	126.3	1.83	351.0	920	4.9	yellow
LSD	NS	NS	NS	50	NS	