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On breeding system in wheat and Brachypodium distachyon.

R. Kosina and P. Tomaszewska.

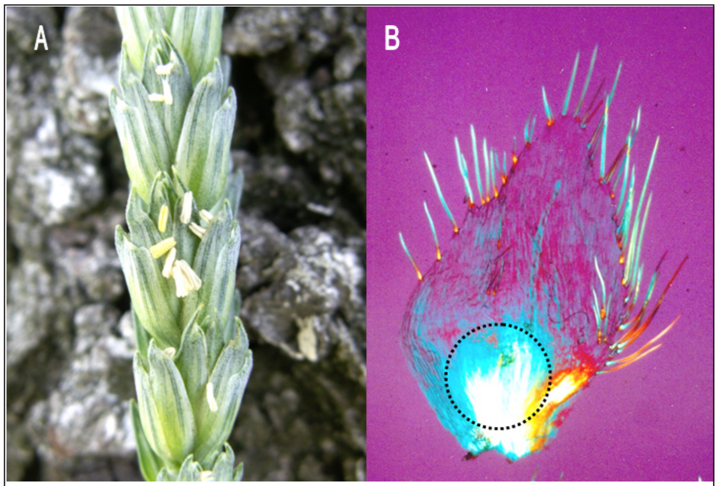

Fig. 1. Exertion of anthers in a *Triticum aestivum* cultivar (A) and a cushionless (encircled) mutation in lodicule of *T. compactum* (B).

Studies of breeding systems in many plants proved that the level of auto- or allogamy may vary in terms of time and space and can be recognized as flexible. Any conclusions on the genetic structure of cultivated or wild populations cannot be made without the knowledge regarding the plant's breeding system. Wheat is considered as an autogamic plant; however, its mating system depends on a species, ecotype and location of cultivation. Percival's data on wheat blooming in England, in the oceanic climate, showed that natural hybridization is rare, but Meister's observations from Saratov, in the continental climate, presented a large number of hybridization events between wheat and rye (Vavilov 1949/50). Wheat, being a self-pollinated plant, frequently expresses chasmogamy during early morning up to noon hours (Fig. 1A), which opens the possibility of geitonogamic mating, or close outcrossing as it was evidenced by Waines and Hedge (2003). Low or very high temperatures and rains restrict chasmogamy and, thus, closed flowers are cleistogamic. Cleistogamy can be also determined by a structural mutation, e.g., of lodicules (Fig. 1B; Kosina 2010). Keydel (1972, 1973) proved great differences in the level of chasmogamy and production of pollen grains for many cultivars of winter wheat cultivated in Bavaria, Germany.

Brachypodium distachyon, like wheat, is an autogamic plant and its chasmogamy is noted sporadically (Vogel et al. 2009; Jaroszewicz et al. 2012). Our last observations for many early blooming ecotypes of *B. distachyon* showed that chasmogamy is common in this species (Fig. 2A and B). The blooming occurred at temperatures ca 20–30°C, under windy and partially sunny weather, between 10:00 AM and 3:00 PM. The range of number of pollen grains/anther is 271–582. In *B. pinnatum*, a fully allogamic species, the range is between 5,000 and 7,640 grains. Thus, an input of pollen grains in the gene flow and heterozygosity of populations is 13 and many times larger in *B. pinnatum* than in *B. distachyon*.

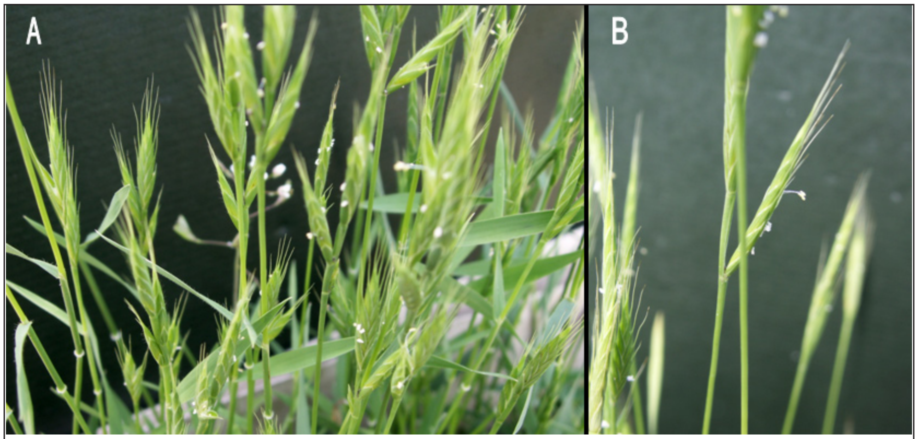

Fig. 2. Chasmogamy in an early ecotype of *B. distachyon* (A) and anther exertion in the species (B).

Therefore, chasmogamy in *B. distachyon* is common in suitable weather conditions, which occur frequently in the Mediterranean region, its area of origin. Such breeding behavior can be realized in the form of geitonogamy or allogamy. These data change our understanding on the genetic nature of *B. distachyon* populations.

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Regressional variation of wheat lodicules.

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

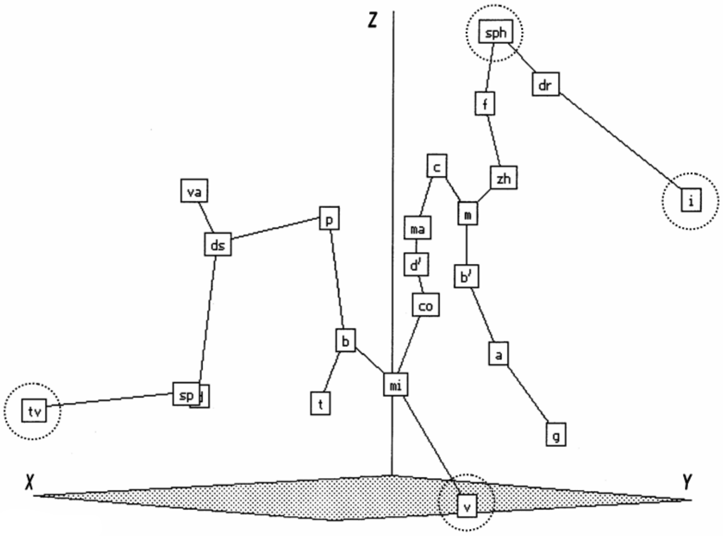

Fig. 3. A minimum spanning tree of wheat species. Operational taxonomic units (OTUs) in a nonmetric multidimensional scaling ordination space. OTUs are described by regressional parameters related to traits of lodicule (diploids, *Triticum monococcum subsp. aegilopoides* (b, b') and subsp. *monococcum* (m); tetraploids, *T. timopheevii* subsp. *armeniacum* (a), subsp. *timopheevii* (t), and subsp. *militinae* (mi), *T. turdigum* subsp. *dicoccoides* (ds), subsp. *dicoccum* (d, d'), subsp. *durum* (dr), subsp. *carthlicum* (c), subsp. *georgicum* (g), and subsp. *ispahanicum* (i); and hexaploids *T. aestivum* subsp. *aestivum* (v), subsp. *macha* (ma), subsp. *compactum* (co), subsp. *sphaerococcum* (sph), subsp. *spelta* (sp), subsp. *vavilovii* (va), subsp. *petropavlovskyi* (p), and subsp. *zhukovskyi* (zh); and octoploids *T. fungicidum* (f) and *T. timonovum* (tv)).

Kosina (2004) proved that description of plant populations, individuals or other types of OTUs (Operational Taxonomic Units) by means of not only positional statistics (averages) but also by covariation parameters (correlation, regression) reveals new information on variability of studied objects. Such an approach was applied for wheat lodicule morphology, shapes of lodicule body and its main lobe (Kosina 2011a). The studied wheat species were as follows: diploids, *Triticum monococcum subsp. aegilopoides* (b, b') and subsp. *monococcum* (m); tetraploids, *T. timopheevii* subsp. *armeniacum* (a), subsp. *timopheevii* (t), and subsp. *militinae* (mi), *T. turdigum* subsp. *dicoccoides* (ds), subsp. *dicoccum* (d, d'), subsp. *durum* (dr), subsp. *carthlicum* (c), subsp. *georgicum* (g), and subsp. *ispahanicum* (i); and hexaploids *T. aestivum* subsp. *aestivum* (v), subsp. *macha* (ma), subsp. *compactum* (co), subsp. *sphaerococcum* (sph), subsp. *spelta* (sp), subsp. *vavilovii* (va), subsp. *petropavlovskyi* (p), and subsp. *zhukovskyi* (zh); and octoploids *T. fungicidum* (f) and *T. timonovum* (tv) (symbols in brackets in Fig. 3). *Triticum fungicidum*, *T. monococcum subsp. aegilopoides*, and *T. turgdum* subsp.*carthlicum* were extreme OTUs when described by regressional parameters related to the lodicules shape. The same three species also were the extremes for 'shape of the main lobe', however, they were positioned in different points of the ordination space. A diagram shows positions of the same 23 wheats described by regression parameters of a relation between lengths of two lobes of the lodicule (Fig. 3). In fact, the ratio of both lengths presents differences in cell shapes of both lobes. Kosina (2011b) evidenced that these cells are different, depending on the number of cytokineses along main axis of the lodicule, and their longitudinal growth. *Triticum timonovum* (tv), *T. aestivum* subsps. *aestivum* (v) and *sphaerococcum* (sph), and *T. ispahanicum* (i) are extremes in the ordination space for the longitudinal development of the lodicule lobes. The visibility of structures is our common perception, but invisible covariation characteristics are no less important in plant biology considerations.

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On caryopsis crease and endosperm cavity in wheat and Brachypodium distachyon.

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

For researchers studying internal structure of wheat caryopsis, it is obvious that shape of a crease is variable and dependent on the development of the main mass of starchy endosperm. The shapes of the crease and adjacent endospermal cavity (ec) dominate in exemplary wheats (Fig. 4). The crease is a complex structure composed of an outer pericarp, a pigment strand with a vascular bundle, a nucellar projection being a modified nucellus in the chalaza region, an endosperm cavity filled by substances derived from apoptosis of nucellus, and a modified aleurone layer adhering to endosperm cavity. In various wheats, this region can be organized as a symmetric or asymmetric structure, flat or penetrating deeply into the starchy endosperm, such as in *T. trugidum* subsp. *durum*. The endosperm cavity can have limited volume or can be large and filled by starch granules as in the cultivar Purdue. Hands and Drea (2011) expressed an opinion that the endosperm cavity is not developed in caryopsis of *Brachypodium distachyon*. Our observations of more than 20 accessions of this grass showed that in some ecotypes, the endosperm cavity is present (Fig. 5A and 5B). The crease is visible on the cross-sections of caryopsis as a symmetric structure (Fig. 5A) with two, separate, small cavities (see arrows) or with one cavity developed between nucellar projection and modified aleurone layer (Fig. 5B). The aleurone layer penetrates far between starchy cells, and its cell multiplication takes place in this area. In conclusion, the caryopsis creases of wheat and *B. distachyon* are qualitatively similar but quantitatively different.

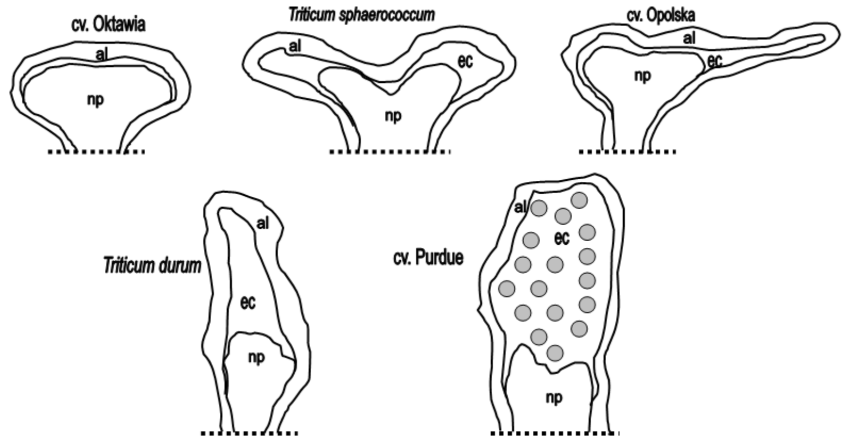

Fig. 4. Most common types of endosperm cavity (ec) in common wheat cultivars and in *T. aestivum* subsp. *sphaerococcum* and *T. turgidum* subsp. *durum* (np = nucellar projection, al = aleurone layer). In the endosperm cavity is filled by starch grains in the cultivar Purdue.

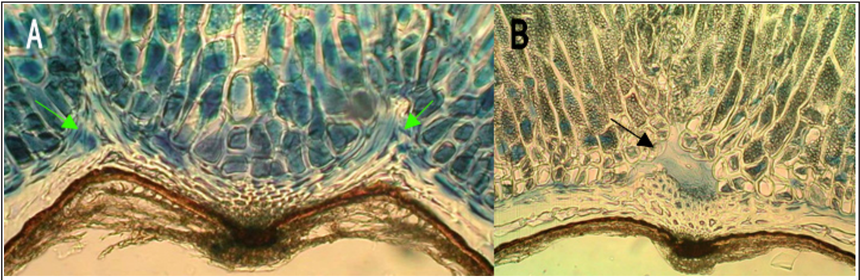

Fig. 5. Endosperm cavities in *B. distachyon*: A – two symmetrical cavities on the sides of the crease and B – one cavity penetrating the starchy endosperm.

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On caryopsis developmental events in wheat and Brachypodium distachyon.

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

Development of wheat caryopsis varies between threshable and unthreshable forms. A 'floret cavity' formed by the lemma and palea determines the organization of caryopsis tissues. The development of ovary walls are especially restricted by sclerified glumellae. Such an interaction between inflorescence leaves and the young fruit results in the depression of the pericarp in unthreshable wheats. The pericarp in common or durum wheats is thicker and composed of more cell layers. The nucellar epidermis and integuments are restricted to thin, sometimes suberinized, layers and in the area of chalaza, they form pigment strand and nucellar projection. An endosperm is composed of unilayered aleurone and cylindrical (Fig. 6A) and isodiametric (Fig. 6B) starchy cells. The cell walls in the starchy endosperm are thin.

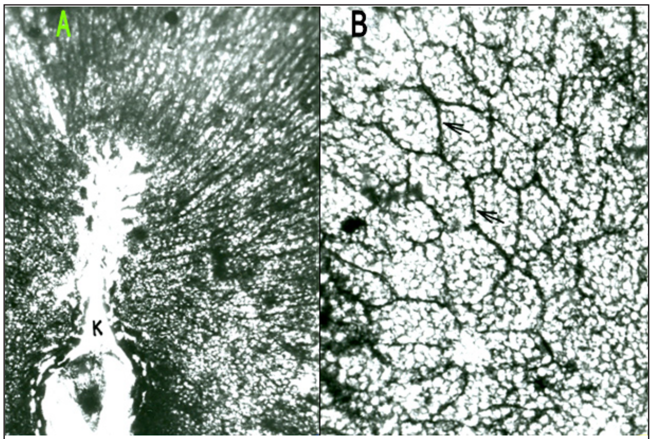

Fig. 6. Starchy endosperms in wheats: A – in *T. turgidum* subsp. *durum* with a dominance of cylindrical cells in the area of endosperm cavity, and B – in *T. aestivum* subsp. *aestivum* with isodiametric cells in a lateral part of caryopsis.

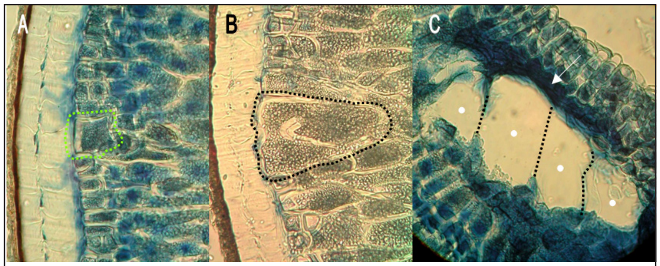

Fig. 8. Developmental events in a *Brachypodium distachyon* caryopsis: A – a large (polyploid ?) aleurone cell on the dorsal side, B – a large (polyploid ?) cell with starch granules on the dorsal side in the aleurone layer, and C – a clone of huge polyploid cells in the starchy endosperm, pressing the high-protein subaleurone layer (dark blue).

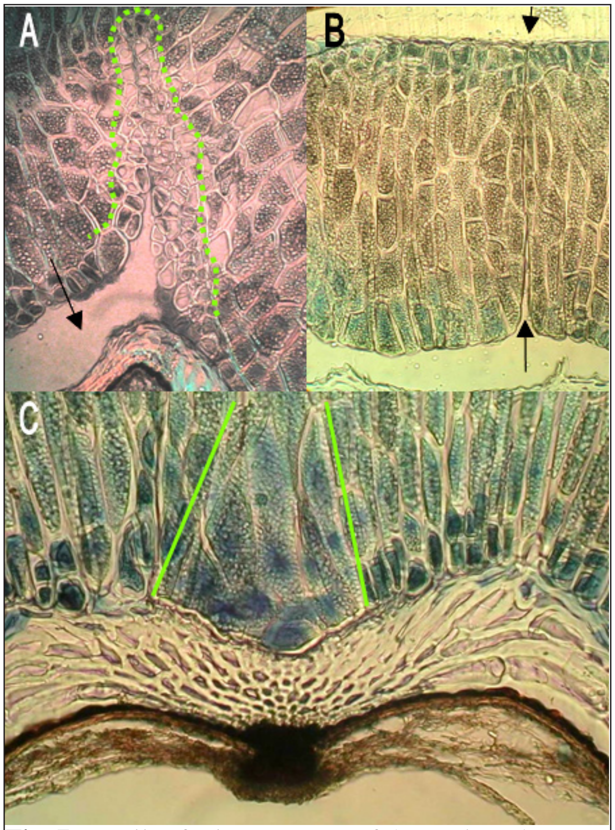

Fig. 7. Details of microstructure of the *B. distachyon* caryopsis: A – an endosperm cavity deeply penetrating starchy endosperm by multiplied aleurone cells, B – two large domains of starchy endosperm, and C – a large starchy cell segment adjacent to nucellar projection and a lack of aleurone layer in this area.

The development of the caryopsis in *Brachypodium distachyon* is a little different. The nucellar epidermis and starchy cell walls are very thick. Several developmental phenomena evidence the peculiarity of the caryopsis structure, which are that the aleurone layer in the crease area can deeply penetrate starchy endosperm and aleurone cells are multiplied there by means of frequent cytokineses (Fig. 7A). The starchy endosperm can be developmentally divided into macro-domains, which are of the same structure (or different) (Fig. 7B). This phenomenon is very rare. An island of large starchy cells is often formed instead of aleurone layer in the crease area (Fig. 7C, p. 197). At the dorsal side, large cells are formed, probably polyploidized, being of aleurone or starchy nature (Fig. 8A and 8B, p. 197). In the starchy endosperm, a set of huge cells were observed (Fig. 8C, p. 197), which by pushing the adjacent proteinaceous subaleurone layers, ceased their development. It evidences an independent development of some parts of *B. distachyon* caryopsis. We suggest that such a fruit can be a good model for study of mosaicism.

Embryo relationships among wheat hexaploids.

R. Kosina.

The embryo of wheat hexaploids was described by 11 traits (scutellum, embryo axis, epiblast, coleoptile, and radicle). OTUs (wheats) were set into an ordination space (Fig. 9) using values of the first three principal components of each OTU. Cultivars of common wheat (labels with v plus large letters) are located in the center of the diagram together with items of *T. aestivum* subsps. *compactum* (co), one form of *petropavlovsky*i (p1), *vavilovii* (va), and an original form of *spelta* having a tough rachis (see an arrow). Variability of *T. aestivum* cultivars is quite large. *T. zhukovskyi* (zh), *T. aestivum* subsps. *sphaerococcum* (sph), *macha* (ma), and another accession of *T. petropavlovskyi* (p2) are situated outside the center. The spelta form with fragile spike rachis (sp2) is close to unthreshable wheats. *T. zhukovskyi* with genomes AAGGGG has a narrowest epiblast among hexaploids, while this organ is shortest in *T. sphaerococcum* (Kosina 1999). The latter has also the shortest radicle. The epiblast of *T. zhukovskyi* is similar to that in AAGG wheat tetraploids (Kosina 1995).

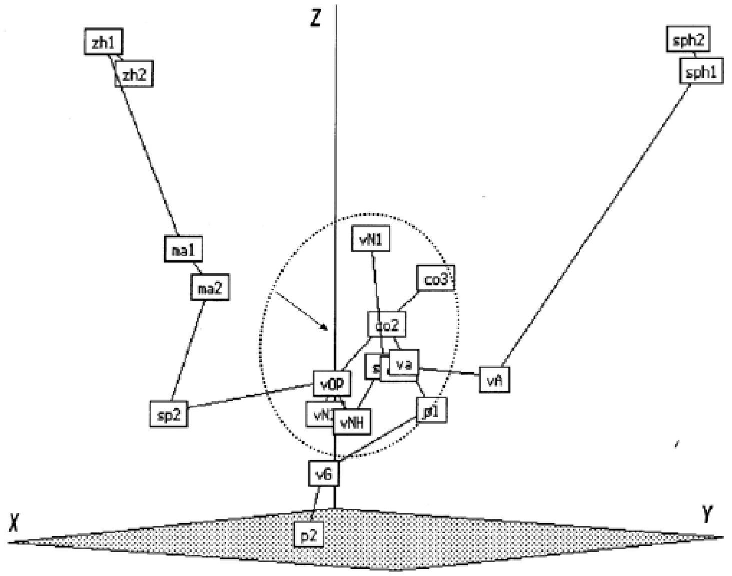

Fig. 9. A minimum spanning tree of wheat haxaploids. OTUs in a nonmetric multidimensional scaling ordination space. OTUs are described by arithmetic averages of embryo traits (*T. aestivum* subsps. *compactum* (co), *macha* (ma), *petropavlovsky*i (p1 and p2), a form of *spelta* having a tough rachis (see an arrow), *sphaerococcum* (sph), *vavilovii* (va), and *T. zhukovskyi* (zh).

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Caryopsis microstructure in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

R. Kosina, K. Markowska, and A. Koźlik.

Observations were made on semi-permanent microscopic slides of caryopsis cross-sections mounted in glycerine. Polarized (Amplival) and epifluorescence (Olympus) microscopes were used. Seeds of amphiploid parents were obtained from the IPK Gatersleben collection (Germany) and an amphiploid from Plant Germ-plasm Institute collection in Kyoto (Japan). In grains of demethylated amphiploid progeny, some changes in the pigment strands (suberinization) also have been recognized previously (Kosina and Markowska 2010). Differences between the parents (*Triticum* and *Aegilops*) and the amphiploid are noted for other fruit structures (Fig. 10, p. 199). The epidermis of the amphiploid pericarp is of an intermediate form (white arrows) between epidermis observed in either parent; a little thicker than that in *T. timopheevii*, but cellular walls and lumina are more similar to that of wheat. Aleurone cells are larger than those in *Ae. umbellulata*, but not so high as in *T. timopheevii* subsp. *timopheevii*. Expression of the aleurone layer traits in the amphiploid is intermediate between both parents. The amphiploid expresses some paternal traits in the starchy endosperm structure. In the subaleurone layer, more protein is accumulated, as in *Ae. umbellulata* (see green arrows in Fig. 10). Both parents synthesize two types of starch granules, large and small, commonly identified in the literature as A and B, but variation in the diameter of the starch granules should be described quantitatively. In *Ae. umbelluata*, starch granules of the type A are significantly larger than those in *T. timopheevii*. Very small starch granules are synthesized additionally in *T. timopheevii*. The amphiploid synthesizes large granules, such as those in *Aegilops*, some of B type and, in addition, very small ones, such as those in *Triticum*. The starchy endosperms of the amphiploid and *Aegilops* look darker in epifluorescence because of the lack of fluorescence of abundant large starch granules (compare pictures in Fig. 10A with 10B and 10C). Cross-sections also show the accumulation of endospermal protein (a rose fluorescence) between starch granules in *T. timopheevii* and in the subaleurone layer in amphiploid and *Ae. umbellulata*. Demethylation did not change significantly the caryopsis structure in the amphiploid, with the exception of pigment strand.

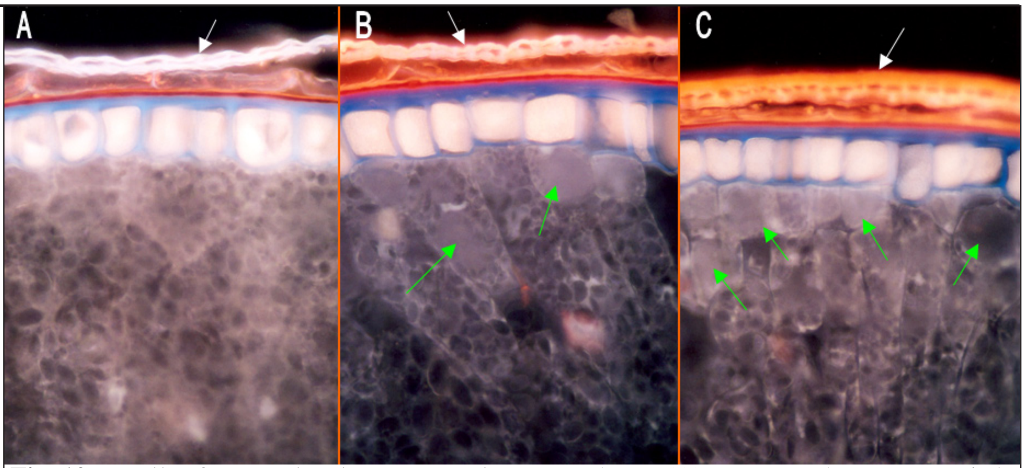

Fig. 10. Details of caryopsis microstructure in *T. timopheevii* (A), a *T. timopheevii*/*Ae. umbellulata* amphiploid (B), and *Ae. umbellulata* (C). White arrows show an outer layer of pericarp, green arrows for the high-protein subaleurone layer.

Reference.

Kosina R and Markowska K. 2010. Patterns of variation in *Triticum timopheevii* x *Aegilops umbellulata* amphiploid after demethylation of genomes. Ann Wheat Newslet 56:207-208.

RAPD relationships in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

R. Kosina and K. Markowska.

We studied patterns of RAPD variation in two species, *T. timopheevii* subsp. *timopheevii* and *Ae. umbellulata*. Both species were obtained from the IPK collection in Gatersleben, Germany. Two accessions of amphiploid progeny *T. timopheevii* / *Ae. umbellulata* (A1 and A2) were kindly given by the Plant Germplasm Institute in Kyoto, Japan, and also investigated. Forty, 10-nucleotide primers were used to amplify DNA in an MJ Research thermocycler. Expressed bands were of 750–500 bp.

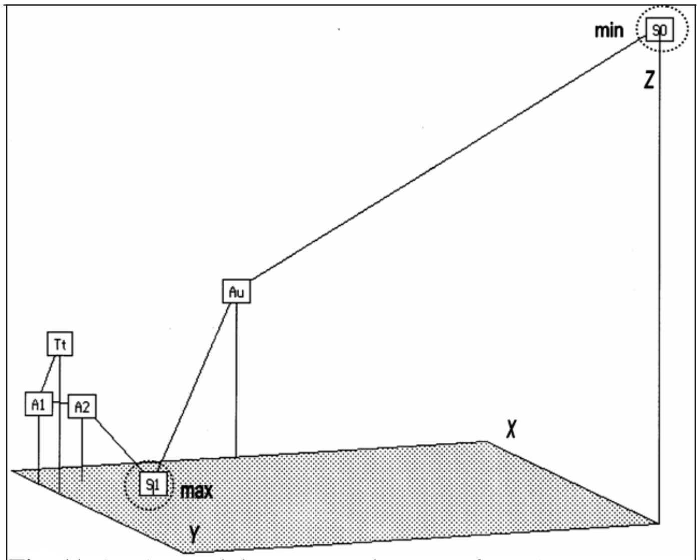

Fig. 11. A RAPD minimum spanning tree of two '*T. timopheevii* (Tt) / *Ae. umbellulata* (Au)' amphiploids (A1 and A2) and parents. The Operational Taxonomic Units (OTUs) are in a nonmetric multidimensional scaling ordination space. OTUs are described by Jaccard's coefficients calculated from 0, 1 RAPD data.

We found found species-specific, RAPD bands for both parental species (Kosina and Markowska 2010). Data from the 40 primers were elaborated numerically by nonmetric multidimensional scaling. The similarity of the Operational

Taxonomic Units (OTUs) was calculated in the form of Jaccard's coefficients. For comparison, new OTUs, S0 with zero bands and S1 with maximal number of bands, were introduced into an ordination space (Fig. 11, p. 199). Both the A1 and A2 amphiploids are located close to *T. timopheevii,* the maternal parent. *Aegilops umbellulata* is closer to the OTU S0, which means that this species is less variable, with regard to RAPD markers. *Triticum timopheevii* and both forms of the amphiploid are more variable. These results are evidence of matroclinal dominance of RAPD patterns in the amphiploid.

Reference.

Kosina R and Markowska K. 2010. DNA RAPD variation in a *Triticum timopheevii* / *Aegilops umbellulata* amphiploid. Ann Wheat Newslet 56:203.

Inflorescence epidermal microstructure in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

R. Kosina and K. Markowska.

Variation of epidermal traits in the grass inflorescence is species specific and can be used for the taxonomic determination of contemporary and fossil materials (Kosina 1999a, b). The abaxial epidermis of the lower glume and lemma were analyzed in a '*T. timopheevii* subsp. *timopheevii* / *Ae. umbellulata*' amphiploid and its parental species. In the amphiploid, the epidermal traits of both parents are expressed. The following cells are typical in a lower glume epidermis: *T. timopheevii,* stomata, papillae, and duplexes composed of cork cells and micropapilla; *Ae. umbellulata*, stomata, macropapillae, hooks, duplexes composed of cork cells and large silica cells, and duplexes of macropapilla and cork cells; the amphiploid, stomata, macropapillae, and duplexes composed of cork cells and micropapilla (Fig. 12). Two types of cells, macropapillae, and duplexes with micropapillae were expressed in the amphiploid due to maternal and paternal inheritance.

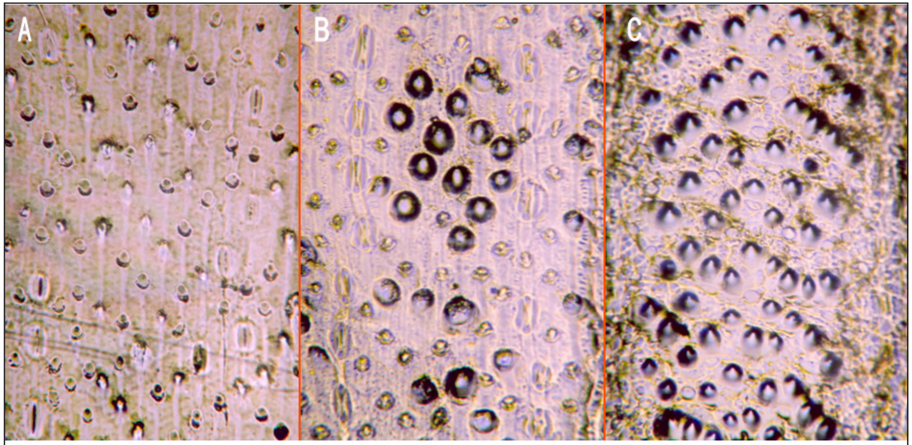

Fig. 12. Varnish replicas of the abaxial epidermis of the lower glume in *T. timopheevii* subsp. *timopheevii* (A), a *T. timopheevii*/*Ae. umbellulata* amphiploid (B), and *Ae. umbellulata* (C). Quantitative and qualitative differences between the replicas are seen.

The structural organization of the lemma epidermis seems to be simpler; *T. timopheevii,* papillae and small hooks; *Ae umbellulata*, macropapillae and single cells; and in the amphiploid, macropapillae and duplexes with micropapillae. Macropapillae are smaller than those in *Aegilops* and duplexes are rarely scattered when compared with those in the glume of *Triticum*. Such a cellular pattern of the lemma of amphiploid is intermediate between both parents, involving patterns of both organs, glume and lemma. Demethylation of amphiploid genomes increases the frequency of duplexes with micropapillae in lemma, however, this phenomenon should be presented quantitatively.

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Kosina R. 1999b. Selected items of wheat variation – from palaeobotany to molecular biology. Acta Societatis Botanicorum Poloniae 68:129-141.

Lodicule variability in a spiro-distichous spike of Lophopyrum elongatum.

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

We noted a specimen of Lophopyrum elongatum with altered morphogenesis of spikes in a collection of Triticeae. In the lower part of the rachis, two spikelets instead of one developed. In the upper part of the inflorescence, densely packed spikelets were arranged spirally. *Lophopyrum elongatum* is an allogamic species with very distinct chasmogamy and the exertion of anthers on long filaments. There are no doubts that the opening of the grass flower is well correlated with the structure and metabolic nature of lodicules (Kosina 2005). In normal *Lophopyrum* plants, lodicules are very active and flowers open wide. Further progeny of the changed plant continued this type of morphogenesis, proving the genetic nature of this morphology.

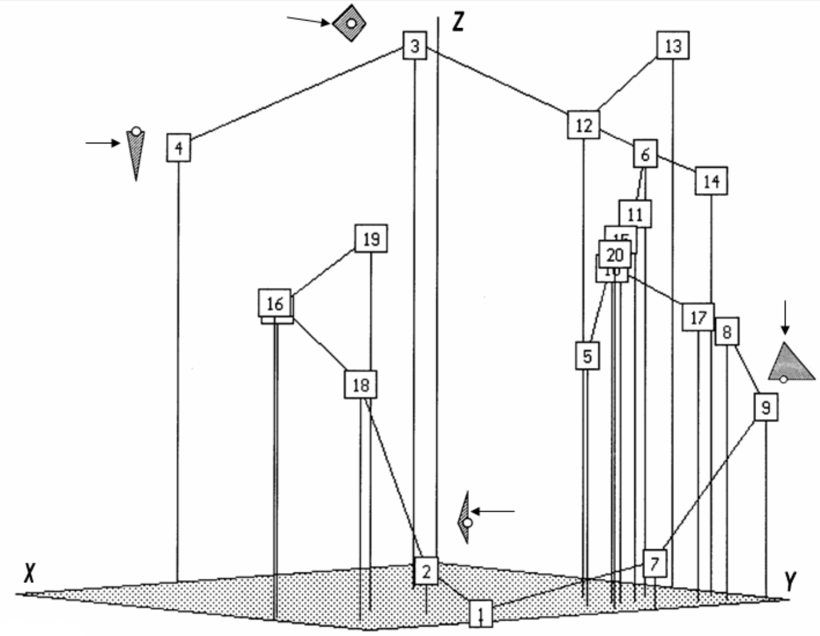

Fig. 13. A minimum spanning tree of *Lophopyrum elongatum* lodicule (numbers) Operational Taxonomic Units (OTUs) in a nonmetric, multidimensional scaling ordination space. OTUs are described by arithmetic averages of the lodicule traits. Polygonal graphs are shown for four OTUs.

The morphology of lodicules isolated from the changed spike was the purpose of this study. Lodicules were evaluated by four characters related to their dimension, shape, and hairiness. Morphotypes of lodicules are illustrated in the form of polygonal graphs (shown for extreme specimens in Fig. 13 by arrows). Lodicules (Operational Taxonomic Units (OTUs) were clustered into a dendrogram using Ward's method. The maximum linkage distance (Euclidean distance) was 170. Lodicules were scattered widely in the ordination space, created by the use of nonmetric, multidimensional scaling (see numbers in Fig. 13). Such a picture proves that the lodicules developed in the spirodistichous spike are very variable. They often present dysfunction because of a distinctly changed morphology. Spirally packed flowers remained closed like cleistogamic flowers. Such a changed structure decreases the number of pollen grains and ultimately the reproductive output of seeds. Such a type of inflorescence mutation can be a very good object for the study of morphology and physiology of the lodicule as well as its behavior during chasmogamy.

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Lodicule variability in Elymus repens.

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

Spikes of *Elymus repens* were collected along two kilometers of field roads in the vicinity of Wroclaw, Poland. The material was gathered from several populations. Lodicules separated from flowers were investigated under a polarized microscope and described with four characteristics, dimensions of the organ, shape, and hairiness. Flowers of *E. repens* open by lodicules, show chasmogamy, and produce highly allogamic progeny. Lodicules (Operational Taxonomic Units (OTUs) were clustered into a dendrogram using Ward's method. The maximum linkage distance (Eulidean distance) was 255; 1.5 times greater than that of *Lophopyrum elongatum*. OTUs (plant number) were scattered in an ordination space after application of nonmetric, multidimensional scaling (Fig. 14, p. 202). An arrangement of OTUs is very regular and directional, from minimum values in the x and z axes and maximum values in the y axis to maximum values in the y and z axes and minimum values in the x axis. This original arrangement can be a good characteristic of a 'lodicule pattern' in *E. repens* populations. Three polygonal graphs illustrating lodicule morphology in extremes and in an intermediate specimen (Fig. 14). Considering the importance of lodicule traits for breeding systems, we conclude that OTUs located in the upper part of the diagram are examples of obligatory allogamy and, vice versa, those in the lower part, such as 28, 24, 20, and 4, express facultative allogamy or even lack of chasmogamy.

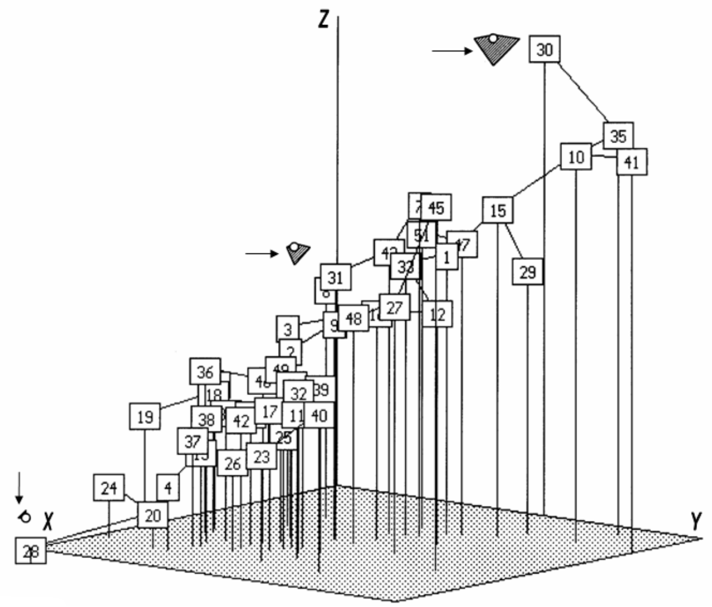

Fig. 14. A minimum spanning tree of *Elymus repens* lodicules (numbers). Operational Taxonomic Units (OTUs) are in a nonmetric, multidimensional scaling ordination space. OTUs are described by arithmetic averages of the lodicule traits. Polygonal graphs are shown for three OTUs.

Lodicule variability in Dasypyrum villosum.

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

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Lodicules of *D. villosum* were separated from spikes of one plant. The studied material is a small random sample. *Dasypyrum villosum*, like *Lophopyrum elongatum* and *Elymus repens*, is an allogamic species presenting widely opened flowers with anthers on long filaments. An investigation of its lodicules was made to compare a range of variability of three objects; *L. elongatum* (one spike), *E. repens* (several populations), and *D. villosum* (one plant). Lodicules isolated from spikes were observed in an Amplival polarized microscope and evaluated with four characteristics, length and width of lodicule, shape, and length of hairs developed on the top. Lodicules (Operational Taxonomic Units (OTUs)) were clustered in a dendrogram using Ward's method. Maximal linkage distance (Euclidean distance) was 56; 4.5 times smaller than that in *E. repens* and 3.0 times smaller than that for *L. elongatum*. OTUs (lodicule number) were scattered in an ordination space after application of the nonmetric, multidimensional scaling (Fig. 15). Polygonal graphs (see arrows in Fig. 15) describe the large allogamic (No. 3) and small lodicule (No. 1). An arrangement of lodicules in an ordination space is not irregular as in *L. elongatum* (see above) but is directional and can be described by a significant regression.

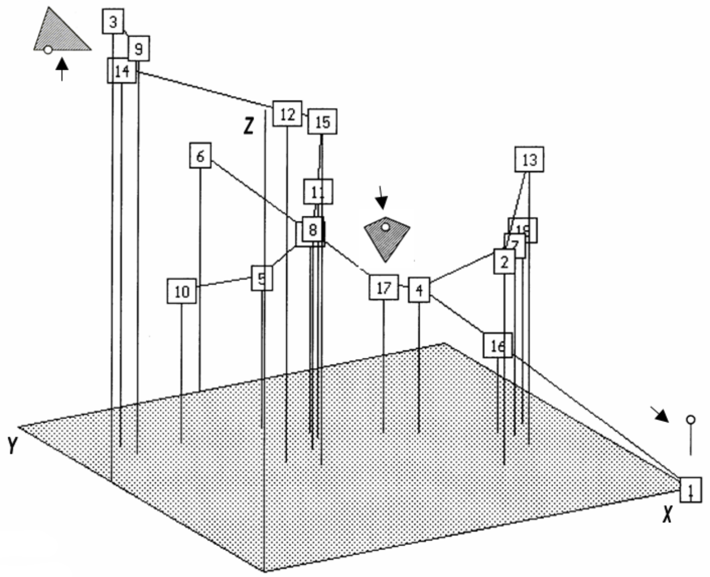

Fig. 15. A minimum spanning tree of *Dasypyrum villosum* lodicules (numbers). Operational Taxonomic Units (OTUs) are in a nonmetric, multidimensional scaling ordination space. OTUs are described by arithmetic averages of the lodicule traits. Polygonal graphs are shown for three OTUs.

On caryopsis development in Thinopyrum distichum versus wheat.

R. Kosina.

Several structures, formed by different tissues, can be identified in a wheat caryopsis, the pericarp, thicker and better preserved in threshable forms and thinner in unthreshable wheats; nucellar remnants, seen as a thin layer, commonly without cell lumina in embryo sac; and a unilayered aleurone in the embryo sac, cylindrical and isodiametric starch cells.

The aleurone layer is differentiated into three forms, one encompassing starchy cells, a second modified in the crease, and a third covering the embryo. Starchy cells develop due to periclinal cytokineses that produce new cells from the ventral and dorsal parts of the caryopsis. As a rule, isodiametric starch cells are formed in lateral parts of caryopsis. For a given species, variety, or cultivar, the volume ratio between cylindrical and isodiametric cells is characteristic. Caryopses of *Th. distichum* were kindly provided by R. de V. Piennar, the University of Stellenbosch, Republic of South Africa. Details of the structure and development were observed on microscopic slides in cross-sections of the caryopsis. Development of a multilayered aleurone is typical for this grass (Fig. 16A and 16B); however, this trait was not fully expressed in all the sections. Drawings present cellular clones in which sequential cytokineses form smallest (youngest) aleurone cells outside. Both clones, dorsal (A) and ventral (B), are of the same size, but the number of cytokineses differs, 17 versus 10, respectively.

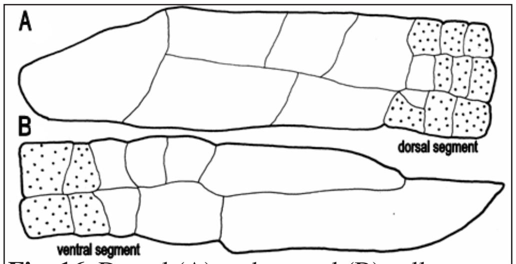

Fig. 16. Dorsal (A) and ventral (B) cell segments of endosperm in caryopsis of *Thinopyrum distichum*. The aleurone layer is dotted.

In caryopses showing some anomaly in development, this difference can be more pronounced (Fig. 17A and 17B) and it is due mainly to more periclinal and anticlinal cytokineses in outer parts of the caryopsis. The length of the cell cycle in wheat vs. *Thinopyrum* endosperm is not known. One can suggest that *Thinopyrum* caryopses are more active cytokinetically and express shorter cellular growth. There is a directional change of diameter of starch granules (S–S4), from the outside to the inside of the endosperm, vice versa of the protein content (Fig. 17B). Inner starch granules are older and distinctly larger, outer starchy cells form a subaleurone layer with a high protein content. In the developing grass endosperm, starch and protein sysnthesis are complementary.

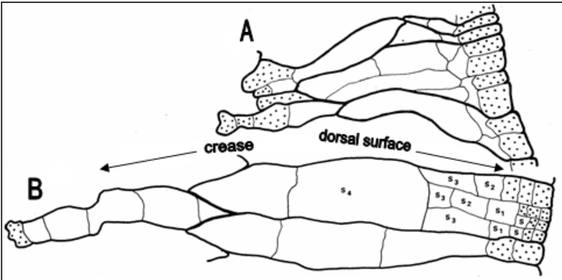

Fig. 17. 'Crease-dorsal surface' fragments of caryopsis cross-sections in *Th. distichum*. A, for some anomalous grain; B, showing a directional trend of starch synthesis (from S (small granules) to S4 (large granules)). Aleurone cells are dotted and cell segments are outlined by thick lines.

On caryopsis development in 'wheat/Thinopyrum distichum' true and partial amphiploids.

R. Kosina and P. Tomaszewska.

A study of caryopsis structure was made for amphiploids obtained from hybridization between *Triticum turgidum* subsp. *durum* (Td), *T. aestivum* subsp. *aestivum* (Ta), and *Th. distichum* (Thd) in the following associations: partial amphiploids, Td//Td/Thd, Td/Thd//Td, Td//Td/Thd/3/Agrotricum, and Ta/Thd//Ta, and true amphiploids, Td/Thd and Ta/Thd. All material was provided by R. de V. Piennar, the University of Stellebosch, Republic of South Africa.

It is well known that distant hybrids exhibit various developmental anomalies, observed also in caryopsis structure. Crosses of durum and common wheat with *Th. distichum* are interesting, because the wild grass is salt resistant. Their genomes were distinguished distinctly by GISH (Kosina and Heslop-Harrison 1995). Anomalous development of amphiploid caryopsis are known. A lack of a large part of the aleurone layer in the crease is often noted in partial amphiploids (Fig. 18A, 18B,and 18C, p. 204). The endosperm cavity deeply penetrates starch endosperm. All contact with nucellar projection lacks the aleurone (Fig. 18C, p. 204). A multilayered high protein subaleurone develops in this area. Anomalous development of the endosperm cavity (Fig. 18D, p. 204) was observed in the form of a multiplication of small, defected aleurone cells inside the cavity.

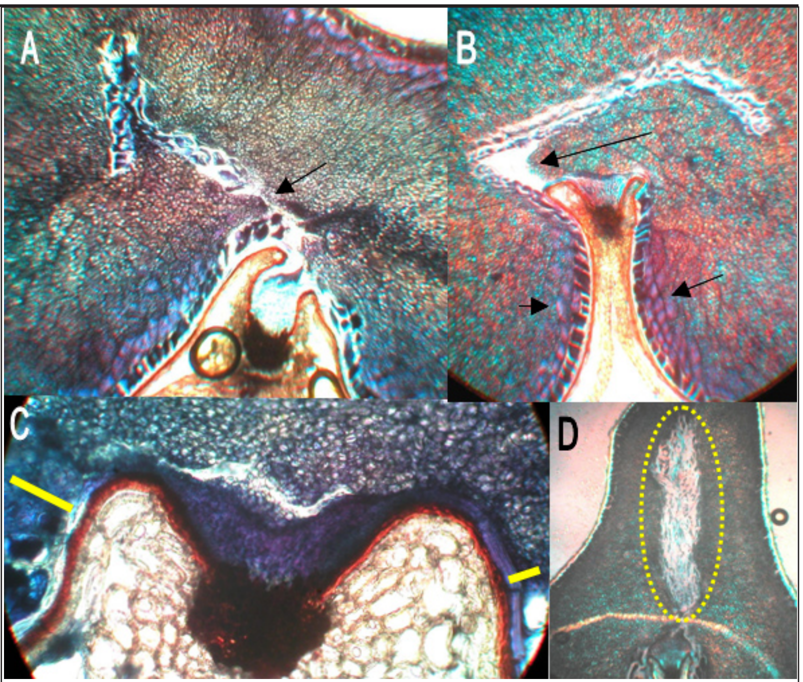

Fig. 18. An interruption of endosperm cavity (arrow) in '*Triticum turgidum* supbs. *durum* (Td)/*Thinopyrum distichum* (Thd)//Td' (A); a lack of aleurone layer in the area of endosperm cavity (a long arrow) and a multilayered, high-protein subaleurone layer adjacent to the crease (short arrows) in 'Td//Td/Thd/3/*Agrotricum'* (B); a large segment of starchy cells (see yellow lines), instead of an aleurone layer, adjacent to the nucellar projection in 'Td/Thd//Td' (C), and a large endosperm cavity (encircled), filled by anomalous aleurone cells, in a poor caryopsis of 'Td/Thd//Td (D)'.

An extremely thick, high-protein subaleurone layer develops on the dorsal side of endosperm (Fig. 19A, yellow line). Such a development is correlated with very thick, unilayered aleurone (note the green line). These aleurone cells express prolonged longitudinal growth and a lack of periclinal divisions. A multilayered aleurone, which is typical for *Th. distichum*, is partly expressed in the amphiploids. The development of the endosperm often occurs in the form of isolated clones of cells differing sharply from the adjacent tissue (Fig. 19B). Large, probably polyploid, cells of the aleurone are formed near the crease. In the endosperm, we can observe a mosaic of tissues, which is a result of its very variable development (Kosina 2007). Many defects in the development of the endosperm resulted in poor caryopses, were more often noted in partial and less in true amphiploids.

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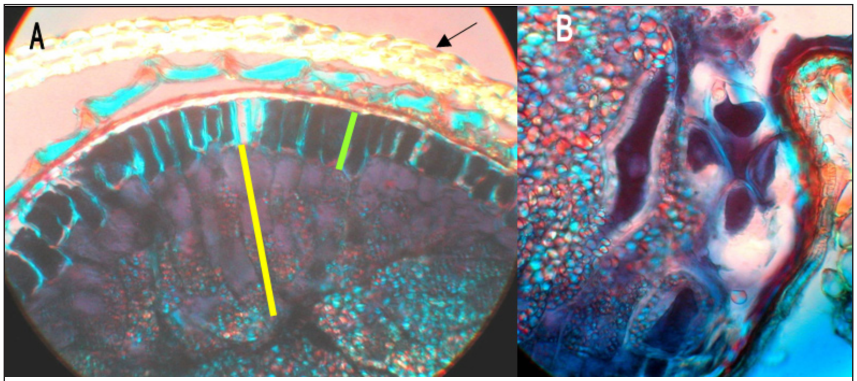

Fig. 19. A thick pericarp (light yellow layer), high aleurone cells (green line), and s thick high-protein subaleurone layer (yellow line) in a '*Triticum turgidum* subsp. *durum* (Td)/*Thinopyrum distichum* (Thd)//Td' cross (A) and huge, polyploid aleurone cells in the area of the crease in a '*T. aestivum* subsp. *aestivum* (Ta)/Thd//Ta' cross (B).

A case of endosperm mosaic in Triticum aestivum subsp. spelta.

R. Kosina and P. Tomaszewska.

The microscopic study was made for cross-sections of caryopsis of *T. aestivum* subsp. *spelta* var. *viridarduini* (VIR 45767). The sections were stained by bromophenol blue to show localization of the total protein. An original endosperm development was discovered in one caryopsis of the small random sample of seeds. In a large sector of the caryopsis, almost one-half of the seed (Fig. 20A, p. 205), there were extensive spots within the aleurone layer. The spots lacked aleurone cells. In this area, aleurone cells spread by a tangential growth with no anticlinal cytokineses and reached large dimensions (Fig. 20B, p. 205). This development also was associated with a differentiation of the aleurone cells inside a starchy endosperm and their strong vacuolization (Fig. 20C, p. 204). Similar observations were earlier provided by Becraft and Asuncion-Crabb (2000) in the genetics of aleurone development in mutated seeds of maize. Becraft

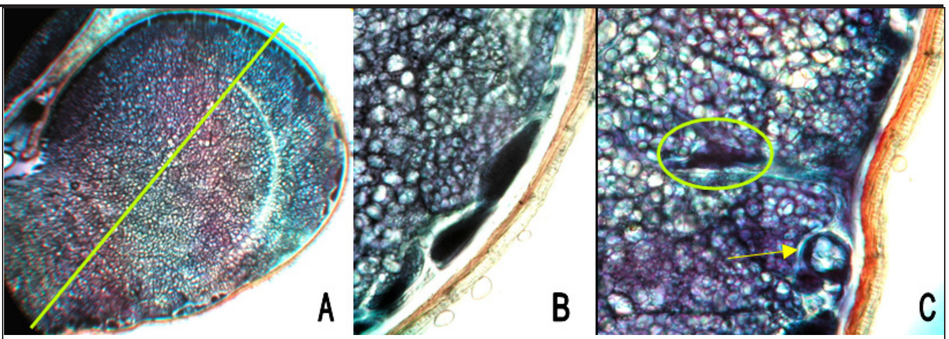

Fig. 20. A large segment of *Triticum aestivum* subsp. *spelta* caryopsis showing a patched aleurone layer (A), aleurone cells tangentially enlarged (B), an aleurone cell differentiated inside the starchy endosperm (outlined with a green line), and the aleurone cell with large vacuole (yellow arrow) (C).

and Asuncion-Crabb (2000) suggested a hierarchy of gene functions, which determine the fate of aleurone cells. Large, mutated sectors of caryopsis also were observed in *Brachypodium retusum* (Kłyk 2005), '*Bromus commutatus/racemosus* (Skowrońska 2005), and *Bromus secalinus* (Kurek 2007; Kochmański 2008). As a rule, such sectors were formed at some level of tissue isolation and growth interaction between starchy and aleurone endosperm. Mutations in a grass endosperm can be evidenced in many aspects of the tissue development and metabolism, e.g., interaction between starch-protein cell phenotypes, vacuolization of aleurone cells, length of cell cycle, cellulose vs. hemicellulose or callose metabolism in cell walls, and synthesis of globoids (Kosina 2007; Kosina and Tomaszewska 2010; Kosina and Zając 2010).

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A mosaic aleurone layer in wheat and irradiated Hordeum vulgare.

R. Kosina.

We noted different cell segments on the surface of an isolated aleurone layer in *Triticum timopheevii* subsp. *timopheevii*. These segments, which expressed phenotypes in the form of small and large cells, were easily recognized (Fig. 21). Such a phenomenon also is seen in other wheat species, and we can correlate it with increased or decreased cytokinetic activity of the layer and some level of instability, typical for endospermal tissue. Under different stress conditions, this instability can be increased (Kosina 1989).

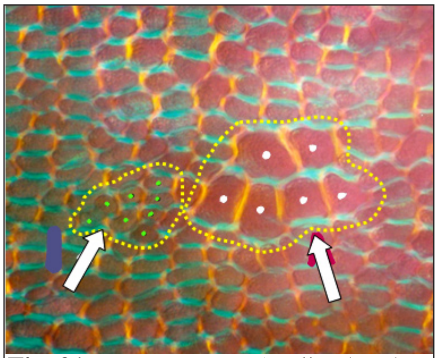

Fig. 21. Two segments (outlined and shown by arrows) of small and large aleurone cells in *Triticum timopheevii* subsp. *timopheevii*.

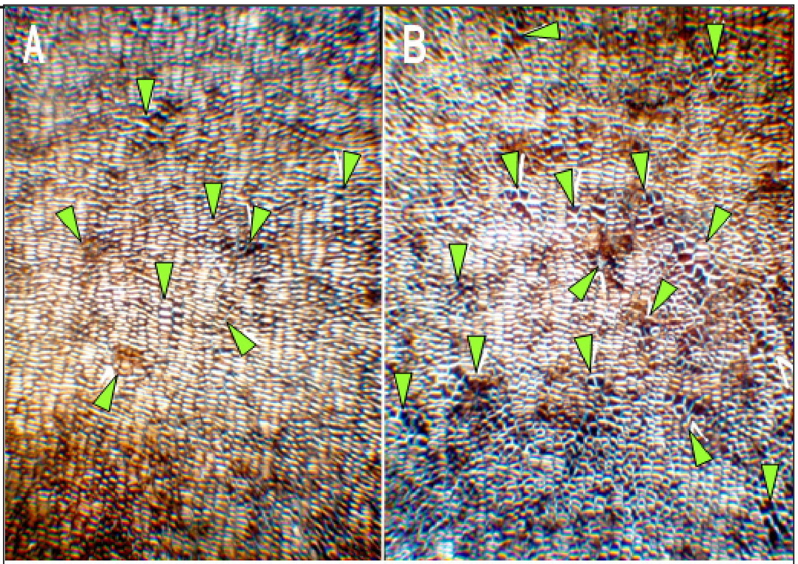

Fig. 22. Two pieces of aleurone layer isolated from *Hordeum vulgare* caryopses of normally growing (A) and irradiated (B) plants. Segments of cells with a changed cell cycle are indicated with arrowheads.

The same observations were made for plants of five old cultivars of Hordeum vulgare obtained from the IHAR collection in Radzików, Poland, which were irradiated with a dose of 0.03 Gy (X-rays). The irradiation was made at 8 DAF, at still existing cytokineses in "endosperm cambium" of main ears. Many morphological and anatomical changes were observed after irradiation. Looking at the surface of aleurone layer, several groups of smaller or distinctly larger cells were developed. The frequency of such, changed by irradiation, cell clones was significantly larger than in non-irradiated cultivars (compare A and B in Fig. 22). As it is visible in Triticum timopheevii (Fig. 21), an additional cytokinesis is typical for the segment of small aleurone cells – the length of their cell cycle is twice shorter. In the segment of large cells, the lack of two cytokineses is most often seen - the length of their cell cycle is three times longer. The visible morphological mosaic is, in fact, a metabolic mosaic of the cell cycle. Our data proved that the hybrid status of a plant or radiation environmental stress can cause the same changes related to metabolism, physiology, morphology or behaviour of any plant.

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