ITEMS FROM POLAND

UNIVERSITY OF WROCLAW
Plant Speciation Group, Institute of Experimental Biology, Kanonia 6/8, 50-328 Wroclaw, Poland.

Genomes of wheat and other grasses during the cell cycle and apoptosis.

R. Kosina and P. Tomaszewska.

In nature, some plants of hybrid origin cannot easily be distinguished from their known or supposed parental species, mother or father, in terms of their gross morphology. This means that both parental genomes act unequally, being either expressed or suppressed. In the literature, this is known as uniparental dominance. This phenomenon has been well exemplified for sets of microstructural characters in the hybrid progeny of wheat tetraploids and within the tribe Triticeae for amphiploids (Kosina 1995, 1996). For instance, a set of caryopsis traits of *T. turgidum* subsp. *polonicum* dominates over similar ones in *T. turgidum* subsp. *carthlicum*. Another important process that defines genome relationships in plant hybrids is apoptosis (Tomaszewska and Kosina 2013). Apoptosis is a programmed phenomenon eliminating bad or useless nuclei, cells, or tissues. A question arises: Is the apoptotic role of parental genomes equal or unequal in a hybrid body? Many examples of apoptosis can be observed on the embryonic path of a plant. In antipodals, in differentiating xylem vessels, or in an aging root cap, nuclei are condensed (Fig. 1) and next a chromatin and DNA are cleaved by nucleases.

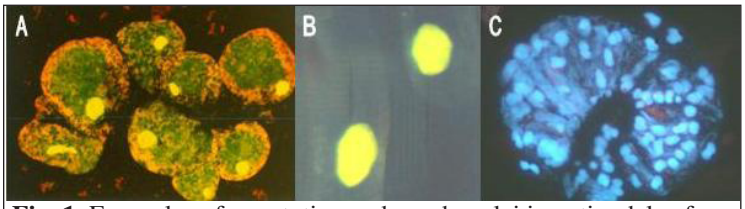

Fig. 1. Examples of apoptotic condensed nuclei in antipodals of *Leymus glaucus* (A), in young xylem vessels of an amphiploid *T. turgidum* subsp. *carthlicum* / *Ae. tauschii* (B), and in a root cap of *Brachypodium distachyon* (C).

Two main arrangements of parental genomes are recognized in plant hybrids: a sectorial one (Fig. 2A) or side-by-side, where both types of genomes can be expressed – hybrids are, most often, intermediate to parents, a concentric one (Fig. 2B), where the genome of one parent is enclosed by that of the second parent, and the outer genome is expressed – hybrids present uniparental dominance. Surprisingly, a change in the genome arrangement is noted in nuclei entering apoptosis (Fig. 2B).

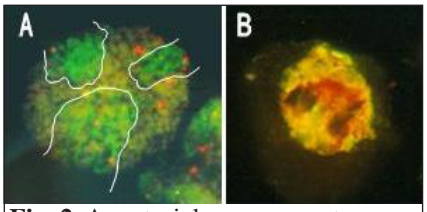

Fig. 2. A sectorial arrangement of parental genomes (green and brown) in a root prophase nucleus of an amphiploid *T. turgidum* subsp. *durum* / *Thinopyrum distichum* / *Lophopyrum elongatum* and a concentric one (yellow and red) in an apoptotic nucleus of the same amphiploid.

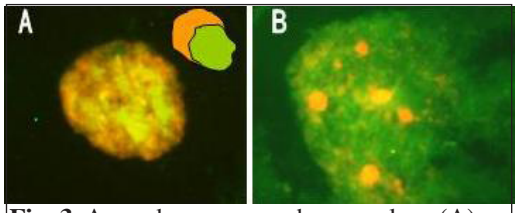

Fig. 3. An endosperm prophase nucleus (A) in an amphiploid *Avena barbata* (AABB) / *A. nuda* (AA) with probed A genomes (green) and B genomes (red) – genomes are arranged side-by-side, and an early apoptotic endosperm nucleus (B) of the same amphiploid – the B genome of *A. barbata* is highly condensed and situated within a green sphere of A genomes of both parental species.

Two parental genomes in an *Avena* amphiploid were identified by GISH and they are arranged side-by-side in a living endosperm nucleus (Fig. 3A). When the nucleus approaches apoptosis, one selected genome, here B, is condensed and not expressed within green A genomes belonging to both parents (Fig. 3B).

These results prove that the roles of parental genomes in a hybrid plant are different during a normal cell cycle and during apoptosis. In apoptosis, the choice of a functional genome is not species, but genomically, specific.

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Kosina R. 1996. Parental dominance in some Triticeae amphiploids. *In:* Abstracts of the Vth International Congress of Systematics and Evolutionary Biology, Budapest, p.201.
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Cytogenetic events in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid with native and demethylated genomes.

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

In the above amphiploid, the expected number of chromosomes is 42. The number observed most often is 41, in both, native and demethylated. Telocentrics also were noted for both types of genomes (Fig. 4A). Divided telocentrics were noted as laggards during telophase and, as a consequence, two micronuclei were eliminated. The most important phenomenon in the amphiploid with native genomes was the appearance of fully decondensed, anaphase chromosomes. In an amphiploid with demethylated genomes, we observed both elongated metaphases in the form of 'multi-Rabl' (Fig. 4B) and increased export of RNA from nucleoli into cytoplasm. Cytomixis is common here (Fig. 4C), and this is not for the native state. Cytomixis results in an unequal distribution of chromatin (genes) in sister nuclei and asymmetric condensation of chromatin is linked with this. Telocentrics also were observed in other amphiploids of the Triticeae, and this resulted in a higher variation of the chromosome number (Kosina and Heslop-Harrison 1996). Enhanced RNA export is correlated with the synthesis of abundant RNA after demethyhlation of genomes, as previously reported (Kosina and Markowska 2010). The frequency of the most common number of nucleoli in the main and lateral roots also is changed after demethylation (Kosina and Markowska 2011).

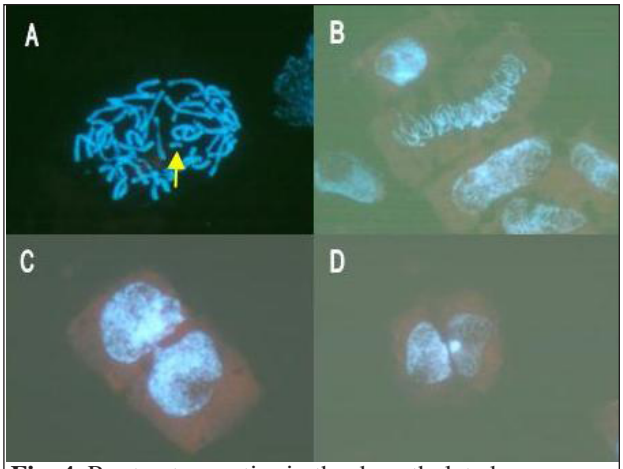

Fig. 4. Root cytogenetics in the demethylated amphiploid. A – metaphase with telocentric chromosome (arrow), B – an elongated Rabl configuration in prophase–metaphase, C – cytomictic nuclei, and D – asymmetric chromatin condensation in cytomictic nuclei. All pictures in DAPI fluorescence.

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On interrelations between a placental xylem and a nucellar projection in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid

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

A placental vascular bundle, composed of xylem and phloem, develops on the abaxial side of the caryopsis. The phloem is most often divided into two separate strands and is hardly seen in a ripe caryopsis. Xylem vessels (proto and meta) are

recognized clearly by means of a polarizing or fluorescence microscope. Cellulosic vessels are either optically active in polarizing light or express light blue auto-fluorescence. In *T. timopheevii* subsp. *timopheevii*, 7 to 20 (minimum–maximum) vessels were identified, and they were close to the pigment strand. Smaller caryopses of *Ae. umbellulata* have narrower bundles with 5–9 (minimum–maximum) vessels. In the amphiploid with native genomes, we noted 4–14 (minimum–maximum) vessels, and with demethylated genomes the range was almost the same, i.e., 4–11 (minimum–maximum). The amphiploid xylem is an intermediate between its parents (Fig. 5A,B). Among wheat tetraploids, *T. timopheevii* subsp. *timopheevii* has the richest placental bundle (Kosina 1988). Wang et al. (2008) provided data showing that xylem and phloem apoptosis in such a bundle is different. In the last stages of caryopsis development, both elements of the vascular bundle are dead.

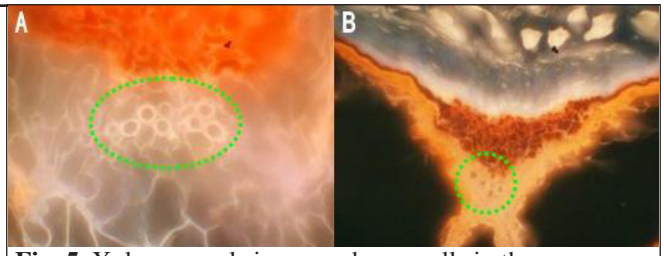

Fig. 5. Xylem vessels in parenchyma cells in the demethylated amphiploid (A), and vessels (outlined) in an acellular, obliterated tissue below a brown pigment strand in the native amphiploid (B). A and B – different magnifications.

Chalazal tissues, pigment strand and nucellar projection, are paths for assimilates, which flow into endosperm. The pigment strand, even when it is suberized, has active plasmodesmata and is open to assimilates (Oparka and Gates 1982). The gross morphology of nucellar projection in both parental species is different, and this kind of variability is illustrated (Fig. 6A and B) as two extreme variants occurring in the amphiploid. In the cross-section, the nucellar projection can be either narrow and high (Fig. 6A) or broad and low (Fig. 6B). Most often, a high nucellar projection is formed by elongated cells of the chalazal region. High correlations between the size of caryopsis and the number of xylem vessels were found; large caryopses developing in lower parts of the spikelets have richer vascular bundles. Another important correlation ($r = 0.77$; significant at $\alpha = 0.001$) exists between the number of xylem vessels and the height of nucellar projection. This means that an abundant placental vasculature positively affects both the growth of nucellar projection as transfer tissue and the caryopsis as a whole. As a reverse example, we noted a nucellar projection in *Ae. umbellulata* with a degraded cellular structure, which strongly influenced the development of caryopsis.

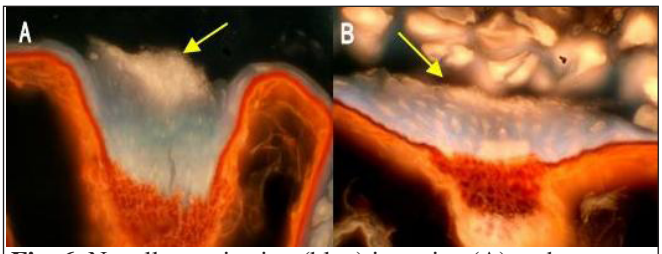

Fig. 6. Nucellar projection (blue) in native (A) and demethylated (B) types of amphiploid. Light apoptotic remnants of nucellar tissue shown by arrows

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Variability of pigment strand in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid and related species.

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

The pigment strand is a caryopsis structure which develops between the placental vascular bundle and nucellar projection, i.e., a changed area of chalaza. The pigment strand is connected with a pigment layer surrounding the caryopsis and is a component of the testa. Koźlik (2013) offered the first description of the pigment strand structure in a *Triticum*/*Aegilops* amphidiploid with native and demethylated genomes. Kosina (1995) proved that the gross morphology of the

pigment strand is genotypically significantly differentiated in wheat tetraploids. Also, its shape greatly varies in wheats and goat grasses. In grasses, cell walls and protoplasts of the pigment strand are often changed by suberin, phytomelans, carotenoids and phenols, which determine, among other factors, seed dormancy. Despite the suberinization of cell walls, the pigment strand has long active plasmodesmata and its role as transductory tissue continues (Oparka and Gates 1982). In the *Triticum*/*Aegilops* amphiploid, cell walls of the strand show light fluorescence of cellulose and a brown, suberized layer inside. This is recognized as an initial stage of suberinization, without synthesis of the subsequent layers of cellulose and suberin. Kosina and Tomaszewska (2011) discovered that, in interspecific *Avena* amphiploids, a transfer complex (pigment strand + nucellar projection) is highly diversified. The variability of the pigment strand in the studied *Triticum*/*Aegilops* amphiploid is presented (Fig. 7). The pigment strand can either be very narrow and composed of a few cells (Fig. 7A) or can be broad and multi-celled (Fig. 7B). Its cells are like puzzles (Fig. 7C), and such a structure distinctly increases the plasmolemma surface, the number of plasmodesmata and the transductory potential of this tissue. On the other hand, its cells can be round and their conductive role is then weaker (Fig. 7D). Suberinization of the strand occurs unequally within this structure and a kind of mosaic with lighter and darker parts is documented (Fig. 7E,F). Koźlik (2013) found that only the width of the pigment strand is positively correlated with the size of caryopsis and this proves its role as a conductive gate for developing endosperm.

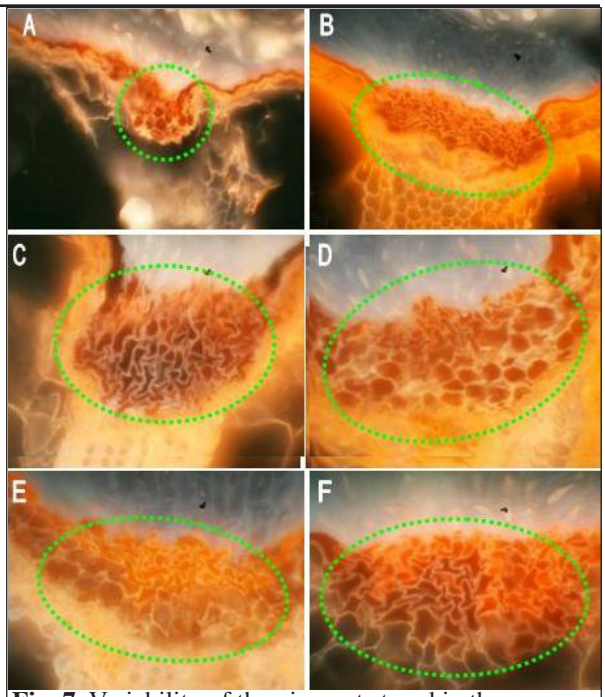

Fig. 7. Variability of the pigment strand in the amphiploid with native (A-E) and demethylated (F) genomes. The strand is outlined.

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Structural characteristics of grass hybrid endosperm development.

R. Kosina, M.K. Bureś, M. Florek, A. Grabińska, P. Kawa, B. Kłyk, Ł. Kochmański, A. Koźlik, A. Kurek, J. Skowrońska, P. Tomaszewska, and D. Zając.

The idea on hybrid endosperm development presented below is drawn from many studies of the grass genera, *Triticum*, *Avena*, *Bromus*, *Lolium*, and *Brachypodium* and intergeneric hybrids of the tribe Triticeae. Ivanovskaja (1983) presented data on the differentiation of isolated nuclei and cells in a young hybrid grass embryo sac or nuclear endosperm. A cytogenetic approach identified these nuclei and cells as subsyncytial units (Kosina 1996). Kosina (1992, 2012) proved that grass endosperm is composed of developmentally distinct units, i.e., cell clones. One of the most intriguing development issues is the relationship between the development of nucellar chalaza and endosperm. The apoptotically changed chalaza is preserved in the form of a long and narrow border running along the axis of the caryopsis and reaching the dorsal aleurone layer (Fig. 8A, p. 117). Potentially, it forms two large endosperm domains which cover the two halves of the caryopsis. These two halves can develop independently and can differ structurally. However, their persistence over time can have its beginning in the nuclear endosperm and the present status can be a result of earlier nuclear differentiation, including subsequent intrusive growth of chalazal tissue. We have observed the same growth behavior, as in chalaza, in the nucellar epidermis or in the aleurone layer (Fig. 8B, p. 117). Cells growing intrusively always separate endospermal

areas, i.e. domains. The subsyncytial nature of nuclear endosperm and its later domainal development are two basic processes creating a mosaic endosperm. It is especially frequent in grass hybrids (Kosina 2007, Koźlik 2013). Very large domains (Fig. 10A) or smaller cell clones (Fig. 11A) can be distinguished in both endospermal tissues, aleurone and starchy. Each characteristic of endosperm relating to metabolism, structure or function can be mutationally changed; for instance: synthesis of cell wall components (Fig. 9B), expression of starch phenotype in the aleurone layer (Fig. 9A,10B), polyploidization (Fig. 11A), synchronized cell cycle (Fig. 11B), and protein, starch or globoids synthesis. This extremely variable nature of endosperm and its simple tissue composition, aleurone plus starchy cells, creates the best research model subject in the area of applied plant biology.

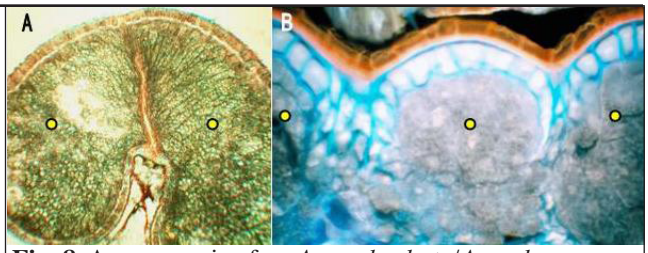

Fig. 8. A - caryopsis of an *Avena barbata*/*A. nuda* amphiploid with a light line of chalazal remnants penetrating deeply into the starchy endosperm; two possible domains are marked by yellow dots. B - caryopsis of an *Elymus canadensis*/*Pseudoroegneria libanotica* amphiploid with an aleurone layer (blue) growing deeply into starchy endosperm; three possible domains are marked by yellow dots.

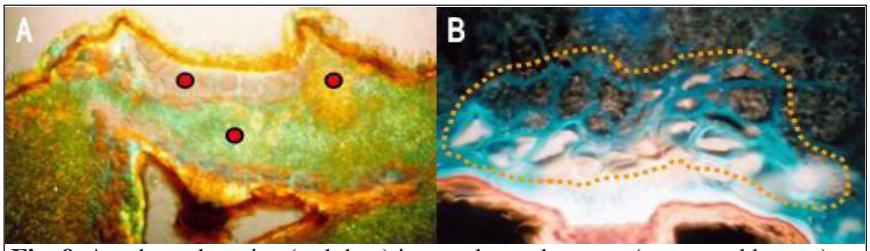

Fig. 9. A – three domains (red dots) in starchy endosperm (green and brown) and in an aleurone layer (pink) in a *T. turgidum* subsp. *dicoccum*/*Ae. tauschii* amphiploid. B – a thick-walled domain (outlined) of the callus-like aleurone layer in a *T. timopheevii* subsp. *timopheevii*/*Ae. umbellulata* amphiploid.

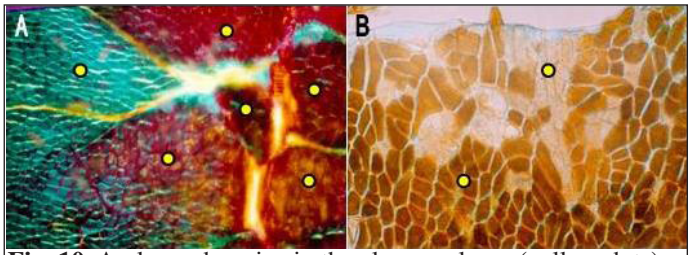

Fig. 10. A - large domains in the aleurone layer (yellow dots) with distinct developmentally disturbed borders between them in a *T. turgidum* subsp. *orientale*/*Ae. tauschi*i amphiploid- B – large starch domains (lighter) in the aleurone layer (brown) in an *Elymus canadensis*/*Pseudoroegneria libanotica* amphiploid.

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Endospermal domains in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

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

The following information is part of an MSc thesis written by Koźlik (2013).

The clonal substructure of grass endosperm has been documented already (Kosina 1992, 2012), and can be realized in different ways, e.g., by changes of metabolism or growth. In the above amphiploid, an intrusive growth of aleurone cells into starchy endosperm divides

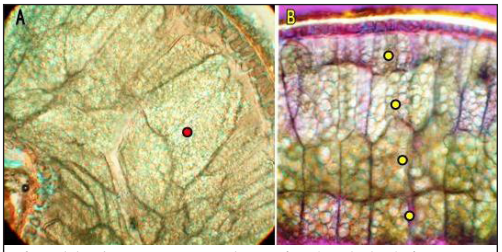

Fig. 11. A – a mega-domain (red dot) in starchy endosperm composed of highly polyploidized cells in an *Avena barbata*/*A. nuda* amphiploid. B – horizontal starchy domains created by simultaneous periclinal cytokineses in *Lolium temulentum* (yellow dots).

this tissue into several domains (Fig. 12A). These domains are composed either of aleurone and starchy cells, or, within the aleurone layer, only a starchy phenotype is expressed (Fig. 12B). In intrusively growing aleurone cells, all the assimilates support synthesis of the components of cell walls, cellulose and hemicelluloses, and cells do not store any protein (Fig. 12B). Such aleurone cells also can be treated as a domain presenting changed metabolism and growth. The domainal bordering role of aleurone cells also is exemplified (Fig. 12C), where a group of starchy cells is separated by aleurone cells surrounding them. Two special domains have been recognized in *Ae. umbellulata*, a paternal species of the amphiploid (Fig. 12D). The first change concerns the expression of a starchy phenotype instead of the aleurone one. The second change relates to starch metabolism; there are two starch grain phenotypes in adjacent cells, large and small. If we accept a sister origin for both types of cells, then we can conclude that this change is caused by somatic (mitotic) crossing-over. These two types of cells can also be of different origin, they will then be recognized as two various clones. The process of somatic crossing-over has often been documented in grasses (Kosina 2007).

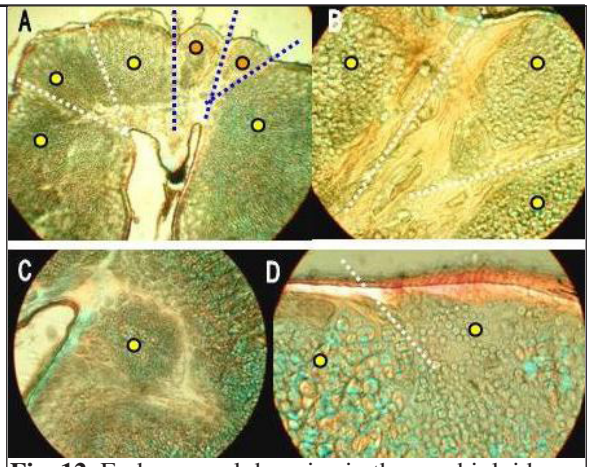

Fig. 12. Endospermal domains in the amphiploid (A,B,C) and its paternal species, *Ae. umbellulata* (D). Dotted lines and dots separate and mark the domains. A – two domains (brown dots) presenting the expression of a starch phenotype in the aleurone layer.

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Variability in the endosperm cavity in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

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

Kosina (1984) distingushed two main types of endospermal cavity in fossil and contemporary wheats, similar to the letters, T or I. Most often, the cavities are of small volume, but sometimes a large body filled by nucellar apoptotic remnants is formed. Deformed and collapsed cells are often identified just above the nucellar projection. An excess of starch grain is located in the cavity, and it is varietal specific (Kosina et al. 2012). The cavity develops just above the nucellar projection as a symmetric or asymmetric structure, and penetrates the starchy endosperm. In *T. timopheevii* subsp. *timopheevii*, the cavity is narrow and long as the letter I (Fig. 13A); in *Ae. umbellulata*, it is like the letter T or letter I (Fig. 13B). For the amphiploid with native or demethylated genomes, different cavities were documented, and they developed as large symmetric or asymmetric letters T (Fig. 14, p. 119). The asymmetry was sometimes coupled with developmental abnormalities of the nucellar epidermis (Fig. 14C, p. 119) or aleurone and starchy endosperm (Fig. 14D, p. 119). Nucellar epidermis anomalies appeared as its polyploidization and the location of assimilates in its very thick walls (hemicelluloses). The morphology of cavities is uniform in parental species and its variation is greatly increased in the amphiploid.

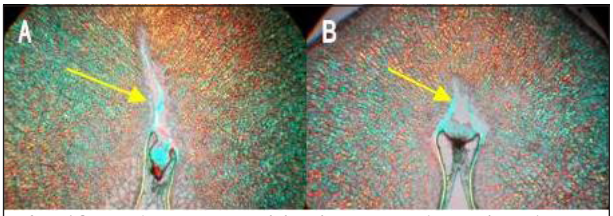

Fig. 13. Endosperm cavities in parental species: A – *T. timopheevii* subsp. *timopheevii*; B – *Ae. umbellulata*.

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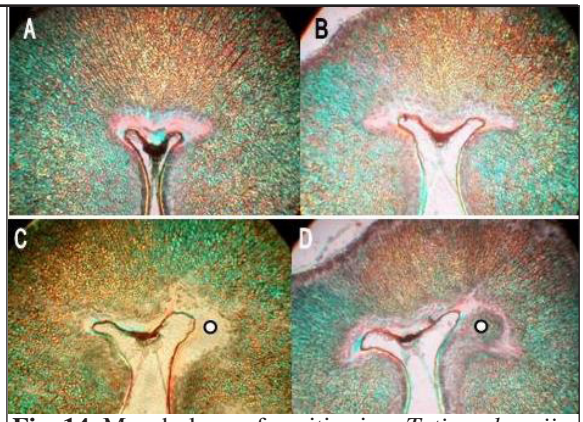

Fig. 14. Morphology of cavities in a *T. timopheevii* subsp. *timopheevii*/*Ae. umbellulata*. amphiploid. In C and D, white dots mark asymmetric cavities filled by nucellar remnants and by a starch-aleurone body, respectively.

Asymmetry of aleurone layer development in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

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

The final periclinal cell divisions in the outer layer of young starchy endosperm provide a new aleurone cell phenotype. In wheat and rye, this layer is one-celled, but in barley and, e.g. in *Thinpyrum distichum*, it is 2-4-celled (Kosina 2012a) and in *Brachypodium distachyon* it is 1-4-celled. Around nearly all the caryopsis the aleurone layer is uniform, but in the area of the crease, the cells have a different morphology. Aleurone cells covering an embryo are of a different structure and function. In the amphiploid, the aleurone layer is structurally more variable than in parental species. The layer in the dorsal part of the caryopsis has cells of different size and shape, sometimes intrusively elongated into starchy endosperm. Periclinal divisions locally increase the number of cells within the layer. Callus-like assemblages of aleurone cells are created by multi-directional cytokineses. In the amphiploid, we documented rare developmental events in the layer. In two halves of the same caryopsis, the following developmental patterns of aleurone cells occurred on both sides of the crease:

- cells grow tangentially, without expected cytokineses and do not grow radially; when looking at the surface of the layer, we can see a set of large cells (Fig. 15A).
- cells frequently divide anticlinally and their tangential growth is not observed. A group of small cells is visible when looking at the surface (Fig. 15B).
- development of the aleurone layer is similar to that in Fig. 15A (Fig. 15C)
- development of the aleurone layer is similar to that in Fig. 15B, but with radial growth of aleurone cells towards the starchy endosperm (Fig. 15D).

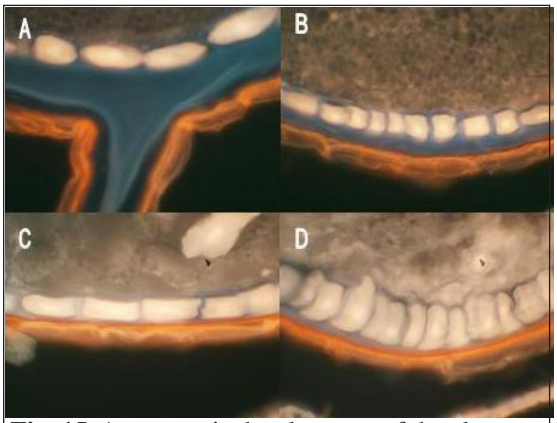

Fig. 15. Asymmetric development of the aleurone layer in two (A, B and C, D) caryopses.

These examples provide evidence that, within the aleurone layer, selected groups of cells develop independently, having specific cell cycles and types of growth. They appear in the form of a mosaic. The larger the segment of cells, the older it is and vice versa, and this depends on the clonal behaviour of endosperm (Kosina 2012a). Such phenomena prove the domainal structure of this tissue, especially in hybrid forms (Kosina 2007, Kosina 2012b, Koźlik 2013).

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A high protein subaleurone layer in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

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

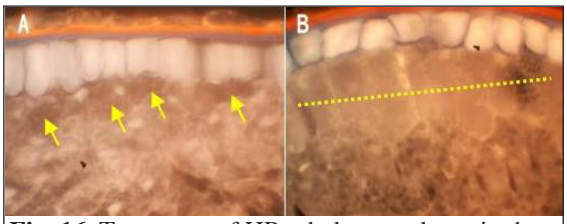

Fig. 16. Two types of HP subaleurone layer in the amphiploid with native genomes.

Development of a high-protein (HP) subaleurone layer has been documented in many grasses, including cereals; the variation in wheats was studied by Kosina (1988). Kosina and Tomaszewska (2012) reported on the variability of the layer in some 'wheat/*Thinopyrum distichum*' amphiploids. Koźlik (2013) documented such a layer in a '*Triticum*/*Aegilops*' amphiploid. In *T. timopheevii* subsp. *timopheevii*, the HP layer is poorly developed, but in *Ae. umbellulata*, it is created by 1-2 rows of cells with a large amount of protein and with few starch grains. Despite the highly diploidized nature of the amphiploid and the expected small variation, its HP layer is very variable. The layer can be poor, expressed in the form of scattered small cells (Fig. 16A) or rich and uniform with a large amount of protein (Fig. 16B). The layer is often composed of several rows of cells or isolated callus-like groups of cells. The HP layer is especially thick in the lateral sides of caryopsis, adjacent to the crease. In the study, we also noted a mega domain of the HP layer covering half of the caryopsis. The development of the subaleurone layer may be further complicated by the creation of highly polyploidized cell clones assisted by apoptosis of adjacent starch tissue, such as can be noted in *Brachypodium distachyon* (Kosina et al. 2012).

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Numerical taxonomy of a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid and its parents.

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

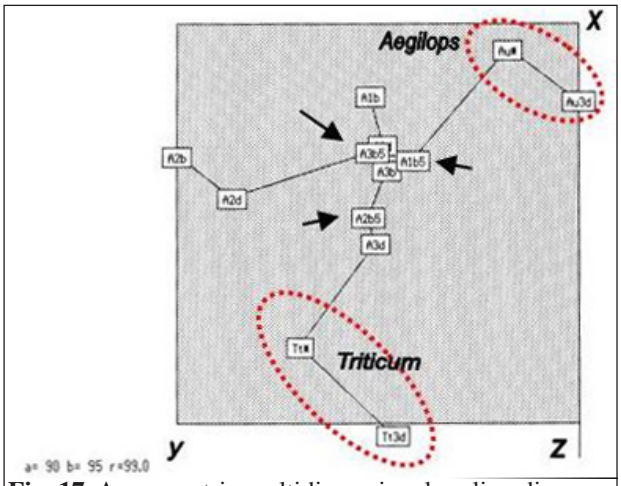

Fig. 17. A non-metric multidimensional scaling diagram with *Triticum*, *Aegilops*, and an amphiploid OTUs described by twelve characteristics of caryopsis.

Plants of the amphiploid and its parents were cultivated in two environments, marked b and d in the diagram (Fig. 17). The amphiploid was studied in two forms, with native and demethylated genomes (Koźlik 2013). Caryopses of the amphiploid and its parental species were evaluated by twelve characteristics. The mean taxonomic distance was calculated between each pair of OTUs (operational taxonomic units). The matrix of taxonomic distances was used in a non-metric multidimensional scaling to put OTUs within an ordination space (axes x, y, and z). The group of amphiploid forms is strictly intermediate between parents and their distance in this minimum spanning tree is 0.93. The intraspecific

variation of two *Aegilops* accessions is larger (a distance of 1.36) than that of two accessions of *Triticum*, which have a distance of 0.87. All the amphiploid forms are characterized by a range of distances from 0.07 to 0.91. The demethylated forms are situated in the centre of the diagram (arrows). No significant distance is noted for both types of amphiploid. A study of character covariation proved that demethylation shows not significant positive interaction with the development of starch cells instead of the protein phenotype in the aleurone layer, and with the thickness of the aleurone layer in the dorsal part of caryopsis. This study adds new data on the interparental variation of hybrid progeny and its expression in changing genomes (Kosina 1996, Tomaszewska and Kosina 2013).

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Notes on Brachypodium distachyon cytogenetics.

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

The cytogenetic status of *B. distachyo*n is not simple. A unique variation exists in the species regarding the number of chromosomes in the intra- and interpopulational space (Jaroszewicz et al. 2012). This variation is caused by multiple cytogenetic anomalies in the form of bridges, rings and laggards (Kosina and Kłyk 2011a). Bridge behavior determines increased nucleolar variability (Kosina and Kłyk 2011b).

Metaphases (Fig. 18A, B, and C) show that the chromosome numbers in the species are from 2n = 10 to 2n = 60, from diploid (2x) to dodecaploid (12x). Also, some aneuploid numbers are common. In the literature, some basic numbers are reported: 10, 20 and 30. If we look at the chasmogamic breeding system found in all studied accessions (Kosina and Tomaszewska 2012), the species is open to hybridization, and a new cytogenetic variation can be created. Dodecaploids (Fig. 18B) are formed after an endomitotic process. Bimodality related to chromosome length is confirmed here (Fig. 18A). The side-by-side separation of short and long chromosomes, frequently observed in metaphases (Fig. 18A), proves that both chromosomal sets can be of different species origin, and their expression can be possible without suppression of any loci.

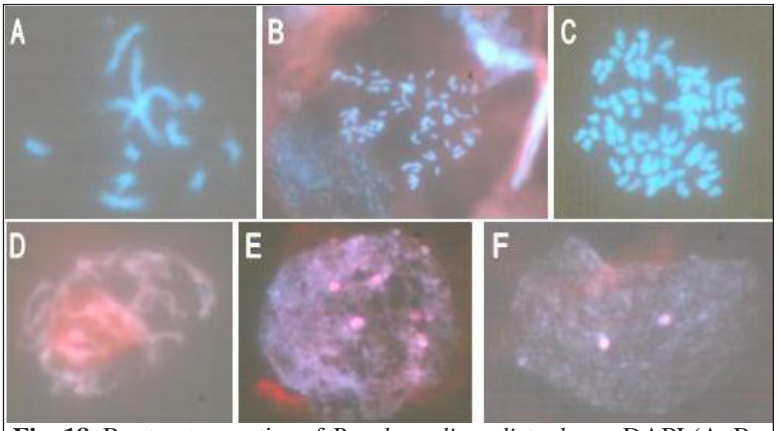

Fig. 18. Root cytogenetics of *Brachypodium distachyon*. DAPI (A, B, and C) and DAPI-PI (D, E, and F) staining. A, 2n = 12; B, 2n = ~60; and C, 2n = 53. D–F, red staining of RNA.

The activity of rDNA loci can be measured by identification of associated proteins using the Ag-NOR method (Kosina and Kłyk 2011b) or by localization of RNA in NORs or outside these loci, e.g. 5S rDNA, in the DAPI – propidium iodide (PI) technique. The latter method involves DAPI staining first and then PI (Fig. 18D, E, and F). The red RNA is discriminated as one large body associated with a nucleolus in a late prophase (Fig. 18D) and such a picture dominates in the studied material – one large nucleolus is common in the DAPI stained chromatin. In earlier prophases two 'RNA loci' on one pair of SAT-chromosomes (Fig. 18F) are identified. The RNA loci are also seen on several pairs of chromosomes (Fig. 18E). The DAPI-PI method also showed the location of RNA loci on bridges.

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Intraspecific variation of caryopsis microstructure in Brachypodium distachyon.

R. Kosina and K. Kamińska.

In *Brachypodium distachyon*, a large variation of gross morphology has been documented (Jaroszewicz et al. 2012). Many of its traits can be considered as weedy (Kosina et al. 2011). The caryopsis structure is related to that in wheat (Kosina and Tomaszewska 2011, Kosina et al. 2012a,b).

Caryopses of the species (22 accessions) were evaluated by 15 microstructural characters. Plants were cultivated for three years (2004, 2006, and 2009), and OTUs in the diagram are marked with one, two, or three dots, respectively. The accessions are species units from a broad geographical range. The analyzed characters were as follows: width and height of caryopsis on cross-section, cell number between ventral and dorsal parts of caryopsis, number of single periclinal divisions in a 10-celled aleurone dorsal segment, number of double periclinal divisions in a 10-celled aleurone dorsal segment, a high protein subaleurone layer (+, –), grain filling by starch cells (+, –), thickness of nucellar epidermis, width and height of pigment strand, height of nucellar projection, thickness of dorsal aleurone layer, thickness of anticlinal cell walls in starchy endosperm, starch cells adjacent to nucellar projection (+, –), amd cylindrical cells in the middle part of caryopsis (+, –).

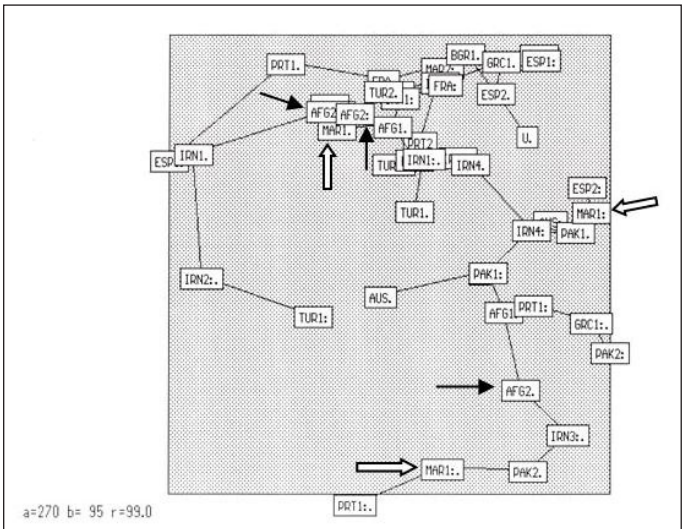

Fig. 19. An nmMDS and MST (non-metric multidimensional scaling and minimum spanning tree) diagram for different accessions of *Brachypodium distachyon* from a geographical range between Portugal (PRT) and Afghanistan (AFG).

Numerical taxonomy of multivariate units (OTUs) proved that intraspecific relationships between them vary in different years. We noted only a partial geographical relationship for some accessions. The individual accessions show different seasonal variation. Two examples, MAR1 from Morocco and AFG2 from Afghanistan, are provided (Fig. 19). Three Moroccan units are more distant from each other than is the case in Afghanistan. These data prove that an ample variation exists in the species and interactions within it are worthy of further research.

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Nucellar projection types in Brachypodium distachyon.

R. Kosina and K. Kamińska.

In a crease of wheat caryopsis, we recognized various tissues, such as parenchyma of the pericarp, phloem-xylem bundles, zig-zag puzzle cells of the pigment strand, transfer cells of the nucellar projection, an apoptotic body of the endosperm cavity, and specific cells of the aleurone layer. Association and interaction of these cells and tissues are based on the broad variation of microstructures (Kosina 1984, 1995). These crease structures determine the nutritional value of the caryopsis and the starch/protein ratio in it (Kosina 1988). The crease structure is highly variable in *Avena* amphiploids (Kosina and Tomaszewska 2011a). In the set of tissues, the nucellar projection plays a very important role. Koźlik (2013) discovered in a '*Triticum*/*Aegilops*' amphiploid that this projection has an intermediate morphology to parents, and is even transgressive to paternal species. In *B. distachyon*, some data related to microstructure of the crease region have been presented by Kosina and Tomaszewska (2011b) and Kosina et al. (2012a,b).

Analyses of the nucellar projection were made under epifluorescence and polarizing microscopes. A blue cellulose autofluorescence was stronger in thick, transfer walls in cells adjacent to the pigment strand. A part of the nucellar projection close to starch endosperm presents patterns of correlated development of the projection and starch tissue, open like a fan or collapsed. Our structural data prove that the size and shape of the nucellar projection depend on nucellar tissue preserved between the chalaza and enlarging embryo sac. In *B. distachyon*, we determined three basic types of nucellar projections (Fig. 20)

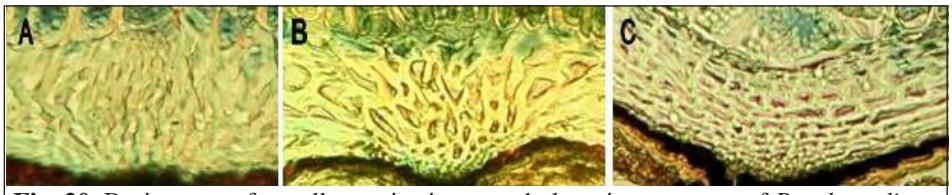

Fig. 20. Basic types of nucellar projection morphology in caryopses of *Brachypodium distachyon*.

- a high projection, elongated between the pigment strand and starchy endosperm (This structure is composed of elongated cells. Such a structure promotes the transport of assimilates through relatively few cell walls and plasma membranes (Fig. 20A)),
- an intermediate structure with shorter and multi-directionally oriented cells (Fig. 20B), and
- a low projection formed by many small cells, such as bricks in the wall (Fig. 20C, Here, the assimilates must flow through more cell walls and plasma membranes).

The two extreme types of nucellar projection differ in the number of cell divisions and cell growth. Anticlinal cytokineses and elongated growth dominate in the first type, and periclinal divisions and tangential growth dominate in the third type.

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Koźlik A. 2013. Zmienność mikrostrukturalna owocu amfiploida *Triticum timopheevi*i x *Aegilops umbellulata*. MSc Thesis, Institute of Experimental Biology, University of Wroclaw, Wroclaw (In Pollish).

The role of nucellar epidermis during the germination of Brachypodium distachyon.

R. Kosina and K. Kamińska.

In cereal grains, the nucellar epidermis is preserved as a very thin, light layer. It is rather acellular, cell lumina can be rarely seen. In wheat octoploids, remnants of this epidermis create a thicker layer (Kosina and Bureś 2011). In wild grasses, the nucellar epidermis is thick, but simultaneously their starchy endosperm is poorer than in cereals. One can recognize two developmental paths and two directions of assimilate storage by

starchy and aleurone endosperm develops via metabolism of assimilates and nucellar apoptotic products and

assimilates and nucellar apoptotic products, the latter only in part, excluding the nucellar epidermis, are used for endosperm and nucellar epidermis development and differentiation.

The second path was recognized in *B. distachyon*. In this species, the nucellar epidermis is very thick (Kosina and Tomaszewska 2011), but also interaccessional variation is noted (Kosina and Jaroszewicz 2007; Jaroszewicz et al. 2012). The same interaccessional (interpopulational) variation is exhibited by a perennial species, *B. sylvaticum*. In *B. distachyon*, there is a balance between storage of assimilates in endosperm as starch and protein, and in nucellar tissue as hemicelluloses. The hemicelluloses are mainly stored in tangential walls of epidermis cells (Fig. 21A).

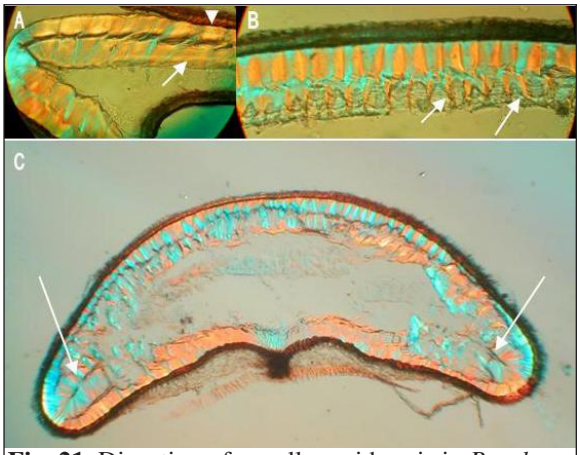

Fig. 21. Digestion of nucellar epidermis in *Brachypodium distachyon* under germination (polarized microscope). A – arrows show thick tangential, hemicellulosic walls in epidermis cells, B – arrows show anticlinal, not digested more cellulosic walls, C – arrows show undigested closed up lateral parts, when starchy endosperm is not fully developed.

Germination of unripe and ripe caryopses of *B. distachyon* was studied over three periods, after 3, 7, and 14 days. Starchy endosperm and aleurone protein are digested first; however, the integrity of the aleurone cells is long maintained. The nucellar epidermis is digested first in the dorsal part (Fig. 21B,C) and this relates to internal tangential walls. Anticlinal walls have more cellulose and they are more persistent (Fig. 21B). In the lateral parts of caryopsis, which can be closed up (see Fig. 21B), when there is some scarcity of starchy endosperm, hemicelluloses are digested with more difficulty. Probably, due to the lower activity of the aleurone layer in the crease area, the nucellar epidermis there is slowly digested. The data show that, in grasses, there are two strategies for the storage of assimilates, germination of diaspores, and seedling growth.

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Jaroszewicz A, Kosina R, and Stankiewicz P. 2012. RAPD, karyology and selected morphological variation in a model grass, *Brachypodium distachyon* (L.) Beauv. Weed Res 52:204-216.

Structural types of endosperm in Brachypodium distachyon.

R. Kosina and K. Kamińska.

The endosperm starts to develop from nuclei scattered inside an embryo sac, contiguous to its wall. Alveolar units are pushed to the center by successive periclinal cytokineses. Finally, these units fill the endospermal space in the form of clonal pyramids. This has been well exemplified for *Thinopyrum distichum* (Kosina 2012). In *B. distachyon*, regular columns of starch cells develop between dorsal and ventral parts of caryopsis (Kosina and Tomaszewska 2011). Such a structure is determined by cell divisions and their growth. Here, one can recognize three types of cytokineses: periclinal,

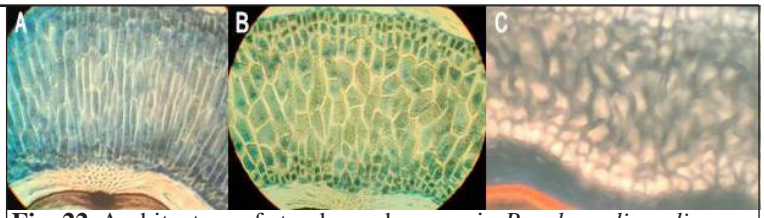

Fig. 22. Architecture of starchy endosperm in *Brachypodium distachyon*. A – cylindrical endosperm, B – isodiametric endosperm, C – poorly developed endosperm with a changed ratio between starch and protein levels.

anticlinal and diagonal, and three types of cell growth: tangential, radial and intrusive. These divisions and growth create two main patterns of endosperm in cereals: cylindrical and isodiametric. The first type is characteristic for the central part of caryopsis, the second is in lateral parts. However, we found an intraspecific variability of endosperm architecture in B. distachyon. The cylindrical or isodiametric cell arrangement was observed in the whole volume of endosperm. In the cylindrical endosperm (Fig. 22A), a few periclinal cytokineses and radial cell growth dominate. Sometimes, an intrusive growth formed very long and narrow starch cells. In the isodiametric endosperm (Fig. 22B), multiple periclinal and diagonal cytokineses are associated with poor or a weak radial cell growth. Isodiametric endosperm is also formed in plants with poor assimilation, and then starch synthesis in endosperm is suppressed. These two processes, assimilation and starch synthesis, can be independent. Such anomalous endosperm is composed of irregular cells with a small amount of starch and increased protein levels (see the pink fluorescence of protein) (Fig. 22C). The data show that an unknown, new type of endosperm variability is detected in *B. distachyon*.

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Variability of caryopsis micromorphology in Brachypodium distachyon.

R. Kosina and K. Kamińska.

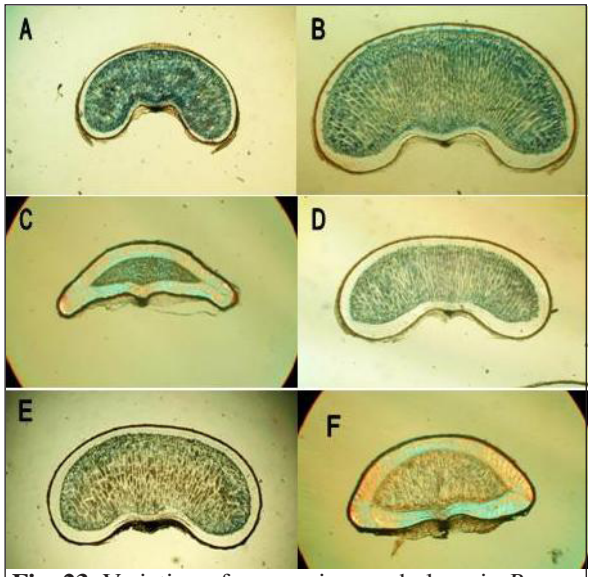

Fig. 23. Variation of caryopsis morphology in *B. distachyon*. A and B – small vs large, C and D – unfilled vs. filled by starch, E and F – variation of a starch/hemicelluloses ratio.

Many microstructural characteristics of caryopsis are linked with ploidy levels, and this has been well exemplified in wheat by Kosina (1984, 1995, 1999). Ample cytogenetic variation in *B. distachyon* (Jaroszewicz et al. 2012) is the basis for ploidal differentiation. So, one can expect a similar variability of micromorphology as in wheats. This study was conducted on caryopsis cross-sections for 22 accessions of *B. distachyon*. Small and large caryopses (Fig. 23A and B) are also differentiated by ploidy levels. The volume of endosperm also shows the difference between wild and cultivated grasses. This difference can also be discussed within the scope of the weediness of the species (Kosina et al. 2011). The difference in starch synthesis appeared for unfilled and filled caryopses (Fig. 23C and D). As an annual and wild species, *B. distachyon* locates assimilates into nucellar tissue (hemicelluloses in epidermis) and in endosperm (starch and protein). These syntheses are not simultaneous. The possible sequence of syntheses could be as follows: hemicelluloses in nucellar cell walls – starch – aleurone protein. A difference in a ratio between hemicelluloses and starch synthesis is shown (Fig. 23E and F). These 'hemicellulosic' or 'starchy' caryopses add new components to intraspecific variability. The cross-section types of caryopses show that this variation creates the possibility of the adaptation of the species to different climates and disturbed habitats, which could be effected by differentiation of the competitive ability of seedlings using caryopsis energy resources.

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Aleurone starch domains in Brachypodium distachyon.

R. Kosina and K. Kamińska.

Kosina (2007) presented a review of mosaic structures in grasses. Mosaics can be expressed in various plant organs and structures, and also in caryopsis. There are two main causes of this phenomenon: the activity of transposons and somatic crossing-over. Within the aleurone layer, two phenotypes are expressed, mainly aleurone cells in the outer layer of endosperm and, rarely, starch cells situated between the aleurone ones. Examples of such a phenomenon have been found in the Triticeae tribe (Kosina and Tomaszewska 2010, Kosina and Zając 2010), in the genus *Avena* (Kosina and Tomaszewska 2011) and in *B. distachyon* (Kosina et al. 2012). Most often, this change of phenotype occurs in plants of hybrid origin. In the 22 accessions of *B. distachyon* studied, we found such a change to be too frequent to be random. Mosaics rarely occurred in the dorsal part of caryopsis, in the form of single starch cells (Fig. 24D) and frequently in the area adjacent to the nucellar projection. Many scattered starch cells were noted, sometimes with thick walls (Fig. 24A), and often there were large starch segments composed of many cells (Fig. 24B and C), sometimes with increased levels of protein (Fig. 24C). This original development is probably dependent on several factors; spatial relationships between the chalazal part of the nucellus and the enlarging embryo sac, conductive ability of transfer tissues in the crease, and the total time available for supply of assimilates.

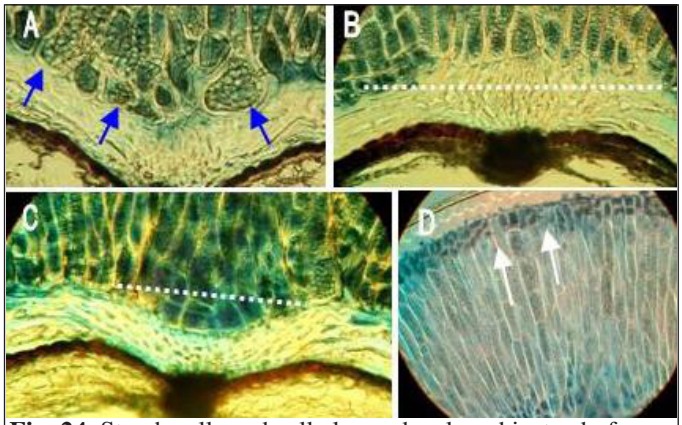

Fig. 24. Starch cells and cell clones developed instead of an aleurone layer in a crease region in *B. distachyon*. A – a group of scattered cells contiguous to the nucellar projection (blue arrows), B – a large segment of starch cells close to the nucellar projection (blue dotted line), C - a large segment of starch cells close to the nucellar projection (dotted line) with increased levels of protein, D – two starch cells in the dorsal aleurone layer (arrows).

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Expression of aleurone starch phenotypes in cereal endosperm.

R. Kosina and A. Koźlik.

Commonly, the development of endosperm is finished by periclinal cytokineses separating the cells of the aleurone layer. The layer is uniform, especially in the dorsal part of caryopsis (Kosina 1992, 2012). The layer is a one- or 2-4-celled structure. A mosaic of aleurone – starch cells has been described in the layer (Kosina 2007). Depending on tissue spatial relationships in endosperm and on its clonal nature, the aleurone cells can develop according to a callus pattern (Fig. 25A). An amphiploid Triticum timopheevii / Aegilops umbellulata will serve here to show various paths of expression of the starch phenotype within the aleurone layer (Koźlik 2013). In Fig. 25B,C,D, the disappearing aleurone cells are presented (see arrows). Such a phenomenon relates to the amphiploid with native genomes (Fig. 25B,C) as well as that with demethylated genomes (Fig. 25D). These pictures prove that the aleurone cells can be apoptotically eliminated during endosperm development – first by digestion of their aleurone protein (Fig. 25B,D) and secondly by digestion of their walls (Fig. 25C). The sites released by them are occupied by cells of starchy endosperm which are additionally developmentally enhanced by apoptotic metabolites. In the second path of starch cell occurrence in the aleurone layer, there is a change of phenotype expression after the last periclinal cytokinesis. Therefore, mistakes in the interpretation of the nature of the starch-aleurone cells are possible. The variability presented here probably exists in other grasses.

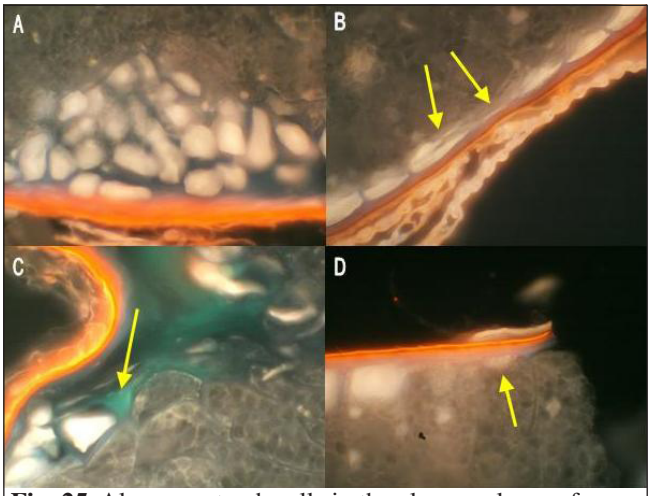

Fig. 25. Aleurone starch cells in the aleurone layer of a *Triticum timopheevii*/*Aegilops umbellulata* amphiploid. Natural fluorescence with the use of a triple-band filter under an epifluorescence microscope. A–C, the amphiploid with native genomes; D, with demethylated genomes. A, a callus-like aleurone layer; B, apoptotically changed flat aleurone cells; C, wall remnants of an aleurone cell contiguous to a starch cell, on the right; and D, remnants of an aleurone protein in the digested part of the aleurone layer.

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