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## ITEMS FROM POLAND

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## Instability of some endosperm traits in Triticum/Aegilops amphiploids.

## R. Kosina and D. Zając.

We studied endosperm instability in the following amphiploids: T. turgidum subsp. dicoccum/Ae tauschii, T. turdidum subps. carthlicum/Ae. tauschii, T. turgidum subsp. turgidum/Ae. tauschii, T. timopheevii subsp. timopheevii/Ae. longissima, and T. timopheevii subsp. timopheevii/Ae . umbellulata. All hybrids were obtained from the Plant Germ-plasm Institute in Kyoto, Japan. Anatomy and cytology of this material were observed under light microscopes (polarized Amplival Carl Zeiss Jena and epifluorescence Olympus BX60) and documented with the use of Fuji 400 negatives.

The normal development of endosperm in grasses ends with the creation of interior starch tissue, the subaleurone, high-protein layer, and the outer-most aleurone. Normally, the aleurone is composed of one-cell layer with a special protein encapsulated in the aleurone grains. The aleurone layer can disappear or multiply under some genetic or environmental stimuli. An interesting example of multiplication of the aleurone layer in the vicinity of the caryopsis crease is presented in Fig. 1A (p. 199) for the T. timopheevii subsp. timopheevii/Ae . umbellulata amphiploid. A multicelled layer is created as a result of several periclinal divisions. A two-celled aleurone is showed on the left (red arrow). This type of cell penetrates deeper into the starch tissue on the right (a green arrow). In these two adjacent, anticlinal rows of cells, the expression of aleurone phenotype differs distinctly. A starch phenotype can penetrate outside the starch tissue and appears, often in the form of a large, undivided anticlinally, cell between smaller aleurone cells (Fig. 1B for $T$. turdigum subsp. carthlicum/Ae. tauschii, p. 199). This tissue also is visible in the cross-section of caryopsis (Fig. 1C in T. timopheevii subsp. timopheevii/Ae. umbellulata, p. 199) where more cells of the starch phenotype develop. The most surprising development is in T. timopheevii subsp. turdigum/Ae.tauschii (Fig. 1D, p. 199), where a very long starch cell, isolated by a hemicellulosic wall expressing blue autofluorescence. grows for a long time and finally is located between
 V O L. 56. the aleurone cells. This kind of development suggests that the status of a cell wall and a lack of the last periclinal cytokinesis can be factors in determining expression of the cell phenotype and that the expression of the starch phenotype is earlier developmentally than that of the aleurone grains.

Another special feature of the aleurone layer is the development of cells with various phenotypes. These phenotypes are represented by light (a few small globoids) or dark (many small globoids) protein masses. In addition, this phenotype also could be represented by large dark granules (globoids) in aleurone grains. We observed sister associations of light and dark cells; evidence of somatic crossing-over occurring during last anticlinal divisions in aleurone layer (Fig. 2A (T. timopheevii subsp. timopheevii/Ae. umbellulata) and Fig. 2B (T. turdigum subsp. carthlicum/Ae. tauschii)). The same phenomenon is related to a pair of cells with large or small globoids (Fig. 2B). The mutant cell having large globoids can be multiplied to create a large spot (Fig. 2C (T. timopheevii subsp. timopheevii/Ae. longissima)).

These examples were observed in the form of somatic mosaicism created by mitotic crossing-over.

## Morphometry of lodicules on the diploid level in the Triticeae tribe.

## R. Kosina.

A high correlation between mating system and size of lodicule was observed for a large set of Iranian grasses (Kosina 2005). In the tribe Triticeae, at least three components of the lodicule morphology are known: dimensions, hairiness, and shape (Kosina 2006).


Fig. 1. Deviations in the development of endosperm tissue (crosssections of caryopsis). A and C: T. timopheevii subsp. timopheevii/ Ae . umbellulata, A-two series of aleurone cells filled with light protein or dark starch created by periclinal divisions along violet lines and C-two starch cells (green arrows) expressed within the aleurone layer; B-T. turgidum subsp. carthlicum/Ae. tauschii, a large cell of the starch phenotype (outlined green) within a proteinaceous aleurone layer; D: T. turgidum subsp. turgidum/Ae. tauschii, a large cell of the starch phenotype increased due to intrusive growth and penetrating the aleurone layer.


Fig. 2. A surface-view of mosaic aleurone layers. A: T. timopheevii subps. timopheevii/Ae . umbellulata, three sister cells filled by dark (left) and light (right) protein; B: T. turgidum subsp. carthlicum/Ae. tauschii, groups of cells of sister origin (green outlined) expressing different nature of protein; C: T. timopheevii subps. timopheevii/Ae. longissima, a clone of aleurone cells with large dark globoids.

Thirty-two diploid species of the tribe Triticeae were described by means of lodicule structure. Five characters, presenting dimensions, shape, and hairiness were used to arrange accessions within an ordination space by means of Kruskal's Nonmetric Multidimensional Scaling (nmMDS). Accessions of the following species were cultivated in the field: Ae. speltoides (Asp1, Asp2), Ae. bicornis (Ab), Ae. comosa (Aco1, Aco2), Ae. caudata (Ac), Ae. searsii (As), Ae. sharonensis (Ash), Ae. tauschii (Asq), Ae. umbellulata (Aum), Ae. uniaristata (Aun), Ag. pectiniforme (Ap1, Ap2), Am. muticum (Am1, Am2), Critesion bogdanii $(\mathrm{Cb})$, C. californicum $(\mathrm{Cc})$, C. chilense $(\mathrm{Cch})$, C. marinum $(\mathrm{Cm})$, C. stenostachys $(\mathrm{Cs})$, C. violaceum $(\mathrm{Cv})$, Dasypyrum villosum (Dv), Hordelymus europaeus (He), Hordeum spontaneum (Hs1, Hs2), H. vulgare (Hv), Heteranthelium piliferum (Het), Pseudoroegneria libanotica (Pl), Ps. spicata (Ps), Psathyrostachys juncea (Pj1, Pj2), S. afganicum (Sa), S. digoricum (Sd), S. silvestre (Ss), S. vavilovii (Sv), Thinopyrum junceum (Thj), Taeniatherum crinitum (Tae), T. monococcum subsp. aegilopoides (Tb), and T. urartu (Tu). The accessions were obtained from the collections of the Vavilov Institute in St. Petersburg (Russian Federation), IPK Gatersleben (Germany), and the USDA (USA).

A synthetic picture of the nmMDS arrangement of forms is presented in Fig. 3. Along the two ordinant axes ( x and y ) Cm , Aco 1, Dv, and Hs1 are located as extremes. For the z axis (Fig. 3) $\mathrm{Cm}(\mathrm{min})$ and $\mathrm{Hs} 1(\max )$ are the extremes. Within the ordinant space, several lodicule groups representing the main patterns of the reproduction system can be observed:

1. broad, long, and very hairy lodicules in $\mathrm{Hs}, \mathrm{Sa}, \mathrm{Sd}, \mathrm{Ss}$, and $D v$; taxa probably having a highly allogamic system of breeding,
2. bare, narrow, and short lodicules with one lobe in Cm ; cleistogamy is very probable, and
3. other intermediate forms of organs developed within a range; facultative autogamy-facultative allogamy.

In the same species, distances in the ordination space between two accessions (for example, $\mathrm{Hs} 1-\mathrm{Hs} 2, \mathrm{Aco1}-\mathrm{Aco} 2$, and $\mathrm{Pj} 1-\mathrm{Pj} 2$ ) are of the similar rank like those interspecific. These results show that grasses have a flexible mating system permitting very successful intra- and interspecific microevolution.

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## Microstructure of endosperm in some intergeneric amphiploids and their parental species of the Triticeae tribe.

R. Kosina and P. Tomaszewska.

This study was made on microscopic slides of aleurone layer (surface view). Caryopsis cross sections of the following species and hybrids were obtained: Leymus racemosus, $2 \mathrm{n}=28$, L. karataviensis, $2 \mathrm{n}=28$, L. arenarius, $2 \mathrm{n}=56 ; L$. multicaulis, 2n =28; Elymus canadensis, 2n =28; E. yezoënsis, 2n $=28$; Pseudoroegnaria libanotica, 2n $=14$; Critesion bogdanii, $2 \mathrm{n}=14$ and their amphiploids 'Ps. libanotica/E. yezoënsis', $2 \mathrm{n}=42$; 'L. multicaulis/L. karataviensis', $2 \mathrm{n}=$ 56; 'E. canadensis/Ps. libanotica', $2 \mathrm{n}=42$; 'L.arenarius/L. racemosus', $2 \mathrm{n}=84$; and ' $E$. canadensis/C. bogdanii', $2 \mathrm{n}=$ 42. The material was kindly provided by the late Dr. Douglas Dewey from the Utah State University.

We focused on several aspects of caryopsis development and structure; the duration of the cell cycle, clonal mosaics, expression of starch phenotype in the aleurone layer, and frequency of mitotic crossing-over. Cells in aleurone layer can be highly polyploidized, especially those of globular shape, and are very large (Fig. 4A and B). Sometimes they express a special type of intrusive growth, such as fibers, penetrating deeply into the starch endosperm. Such a growth can sometimes divide the endosperm into areas with different starch synthesis. Many patterns of


Fig. 4. Anomalous development of the aleurone layer viewed in the cross sections of caryopsis. A and B 'Pseudoroegneria libanotical Elymus yezoënsis' amphiploid, A, a highly polyploidized aleurone cell (green arrow) and B, a group of aleurone cells growing intrusively into the starchy endosperm; C 'Leymus arenarius/L. racemosus', a clone of a small aleurone cell (along the green line) surrounded by cells of a normal cell cycle.

endosperm development composed of clones or cell mosaics are initiated by subsyncytial nature of this tissue (Kosina 1996). An example of a short cell cycle in the aleurone layer is presented in Fig. 4C (p. 200). A spot of small cells developing after anticlinal and periclinal divisions surrounded by larger ones is visible.

A mosaic pattern of development can be observed in various tissues of caryopsis. For example, large dark and light spots of aleurone cells were noted in the 'E. canadensis/Ps. libanotica' amphiploid (Fig. 5A). These cells differ in the nature of the globoids in the aleurone grains and such mutations have been documented in barley (Ockenden et al. 2004). Another kind of mosaic was the expression of a starch phenotype in the aleurone layer (Fig. 5B and C). We documented numerous cases of mitotic crossingover, which caused 'double-spots' of sister origin. Exchanges in chromosomes created pairs of cells with different phenotypes, such as starch vs. protein, starch amylopectin rich vs. starch


Fig. 5. Mosaicism of the aleurone layer; surface views in an 'Elymus canadensis/Pseudoroegneria libanotica' amphiploid. A, two large spots of dark and light aleurone layer (blue arrows); B, cells of starch phenotype (light) scattered among proteinaceous aleurone cells; and C, a clone of aleurone cells (outlined in black) with phenotypes having large (ls) and small (ss) starch granules or light (lp) and dark (dp) aleurone grains. amylopectin poor, large starch grains vs. small starch grains, and light protein (not numerous small globoids) vs. dark protein (many large globoids). Numerous cross-overs within one cell clone and high instability of the tissue can be seen in Fig 5C.

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## Morphology of embryo in 32 species of the Triticeae tribe.

## R. Kosina.

Thirty-two species of various ploidy levels of all taxa from the Triticeae tribewere evaluated for five morphological traits of the embryo describing the epiblast, coleorhizal papilla, and relative dimensions of coleoptile. These five characters were used to arrange OTUs within an ordinate space by means of Kruskal's Nonmetric Multidimensional Scaling (nmMDS). Accessions of the following species were cultivated: Ae. cylindrica (Aec), Ae. triuncialis (Aet), Ag. cristatum subsp. cristatum (Acc), Ag. cristatum subsp. desertorum (Acd), Critesion californicum (Cc), C. chilense (Cch), C. hystrix (Ch), Elymus breviaristatus subsp. scabrifolius (Ebs), E. caninus (Ec), E. dahuricus (Ed), E. gmelinii (Eg), E. hystrix (Eh), E. mutabilis (Em), E. nutans (En), E. trachycaulus (Etr), E. tsukushiensis (Et), Elytrigia intermedia subsp. graeca (Elig), Eremopyrum bonaepartis (Erb), Hordelymus europaeus (Hee), Hordeum vulgare subsp. sponta-


Fig. 6. An MST diagram in a nonmetric multidimensional scaling ordination space presenting a scattering of Triticeae species characterized by means of embryo morphology.
neum (Hvs), Heteranthelium piliferum (Htp), Leymus paboanus (Lp), L. racemosus (Lr), Lophopyrum nodosum (Lon), Pseudoroegneria strigosa subsp. jacutorum (Prsj), Psathyrostachys juncea (Pj), S. silvestre (Ss), Th. bessarabicum (Thb), Taeniatherum caput-medusae (Tacm), triticale (Tle), T. monococcum subsp. monococcum (Tb), and T. fungicidum (Tf). The accessions were obtained from the collections of the Vavilov Institute in St. Petersburg (Russian Federation), IPK Gatersleben (Germany), and the USDA (USA).

In the MST diagram (Fig. 6, p. 201), some characteristic groups of species can be distinguished. In a large group described by high z axis values and low x and y values are species having embryos without an epiblast (Hee, Ebs, Lp, Eg, Prsj, Elig, Tacm, Eh, and En). Distant from this group are the species of Triticum and Hordeum with large epiblasts and distinct coleorhizal papilla. Other species, such as Tle, Ss, Tb, Tf, Etr, and Hee, are characterized by delayed longitudinal growth of coleoptile and are scattered between both of the above-mentioned groups. In annual and perennial species of Brachypodium (Kosina and Jaroszewicz 2007), the coleorhizal papilla play an important role during seed germination. Kosina (1995) described a short coleoptile as typical for AAGG wheats, whereas in AABB wheats, the growth of the coleoptile is more dynamic. A clear difference in embryo morphogenesis between annual or biennial (Triticum, Aegilops, and Hordeum) and perennial grasses can be seen (Fig. 6, p. 201), with the perennial grasses are separated on the left. Such a discrimination supports data on germination differences in the Brachypodium genus (Kosina and Jaroszewicz 2007).

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## DNA RAPD profiles in Brachypodium distachyon, a model grass related to the Triticeae tribe.

## R. Kosina and A. Jaroszewicz.

RAPD markers are commonly used to determine varietal or accession variation in gene banks. The level of variation expressed in electrophoretic DNA bands is different and depends on the PCR primer used in amplification. We estimated RAPD variation in a collection of 21 accessions of $B$. distachyon of various origin. This species, at present, is studied in detail, because its biology is well related to biology of members of the Triticeae tribe, including a broad spectrum of the genus Triticum. Twenty 10 -nucleotide primers were used for PCR amplification. Some were poor (B-3), but others were rich and polymorphic (B20). Determining the level of variation was done by two approaches; counting only strong bands or including all bands, even the very weak, for comparison.

Most of the bands were 500-1,000 bp. Two examples of primer amplification show that the pattern of DNA bands are accessionspecific (Fig. 7). However, some similarity in banding patterns also was observed, e.g., B-3 for Pakistan-Morocco and Slovakia-Iran. No adequate accession was found for the item from Denmark Botanic Garden. Such an analysis can be helpful to identify material of unknown origin collected in the botanical gardens. Primer B-20 gave
more accession-specific bands. Poor patterns were identified for accessions from Spain and Pakistan. Rich banding patterns were found for accessions from Slovakia, Australia, Iran, and Turkey. The data are being evaluated numerically at present.

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

R. Kosina and K. Markowska.

Random amplified polymorphic DNA (RAPD) patterns are used not only to determine inter- and intra-population variation but also to study relationships between parental species and their hybrid progeny. We noted RAPD variation patterns for two parental species, T. timopheevii subsp. timopheevii (Tt) and Ae. umbellulata $(\mathrm{Au})$ from IPK Gatersleben, Germany, and two amphiploid two accessions from the Plant Germ-plasm Institute, Kyoto, Japan. We used 40 10-nucleotide primers in the PCR amplification. Strong and very weak bands can be used to determine the level of variation.

Most of the bands were $500-750 \mathrm{bp}$. Some general observations were seen in DNA profiles (Fig. 8). If bands of both parents are present in the amphiploid, they often are of intermediate strength (Fig. 8, red arrow). For three profiles, some variation between the two amphiploid accessions were observed including the weak bands (Fig. 8, white arrow). Several primers had amphiploid-specific bands. The T. timopheevii subsp. timopheevii genome bands in the amphiploid are larger; DNA profiles for Ae. umbellulata are poor. We found species-specific bands for both parents. A RAPD analysis also will be made for the amphiploid after demethylation of the genomes. The data currently are being numerically evaluated.

## Parental dominance of lemma and palea epidermal microstructure in some amphiploids of Triticeae.



Fig. 8. Electrophoretic patterns of RAPD markers for four selected primers (OPB2, OPB5, OPB13, and OPB20). Lanes are 1, DNA ladder; 2, Triticum timopheevii subsp. timopheevii; 3, amphiploid 1; 4, amphiploid 2; and 5, Aegilops umbellulata.

## R. Kosina.

Uniparental complex dominance is well known in hybrid plants (Grant 1981; Heslop-Harrison 1990). For Triticeae hybrids, such data were presented by Kosina (1996). We studied inflorescence bract morphology described by nine characters of the highly differentiated abaxial epidermis. The set of parental species and their amphidiploids were Leymus racemosus (Lr), L. karataviensis (Lk), L. arenarius (La), L. multicaulis (Lm), Elymus canadensis (Ec), E. yezoënsis (Ey), Pseudoroegnaria libanotica (Pl), Critesion bogdanii (Cb), 'Ps. libanoticalE. yezoënsis' (PlxEy), 'L. multicaulis/L. karataviensis' (LmxLk), 'E. canadensis/Ps. libanotica' (EcxPl), 'L. arenarius/L. racemosus' (LaxLr), and 'E. canadensis/C. bogdanii' (EcxCb). Research material was provided by the late Dr Douglas Dewey from the Utah State University, chairman of the past International Triticeae Cooperative.

The investigated taxa (operational taxonomic units, OTUs) are scattered within a space created by means of Kruskal's Nonmetric Multidimensional Scaling (Fig. 9, p. 204). The genus Leymus is characterized by larger z axis values; other species have lower values, thus distinguishing both groups of species. A distinct, complex, maternal dominance is recognized only for two amphiploids, LaxLr and EcxCb. Parents of the latter are connected immediately with their hybrid. Weaker paternal dominance is noted for amphiploid EcxPl and weak expression of the maternal phenotype is noted for the PlxEy hybrid. OTUs are scattered along a positive regression line between the $x$ and $y$ axes. The OTUs form an ellipsoid sphere that rises from low to high values of the $z$ axis. The shape of this sphere and its behavior is an
 additional characteristic of the OTUs studied. The original variation patterns enabled us to extend the research of these forms and the results are presented here by Kosina and Tomaszewska.

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## Expression of parental variation in caryopsis structure of some amphiploids in the Triticeae tribe



Fig. 9. An MST diagram in a nonmetric multidimensional scaling ordination space presenting a uniparental dominance ( $\mathrm{m}=$ maternal and $p=$ paternal) of glumellae epidermal morphology in Triticeae amphiploids.

## R. Kosina.

We analyzed a set of operational taxonomic units (OTUs) comprising parental species and some interspecific and intergeneric hybrids progeny, Leymus racemosus (Lr), L. karataviensis (Lk), L. arenarius (La), L. multicaulis (Lm), Elymus canadensis (Ec), E. yezoënsis (Ey), Pseudoroegnaria libanotica (Pl), Critesion bogdanii (Cb), 'Ps. libanotica/E. yezoënsis' (PlxEy), ‘L. multicaulis/L. karataviensis’ (LmxLk), 'E. canadensis/Ps. libanotica' (EcxPl), 'L. arenarius/L. racemosus' (LaxLr), and ' $E$. canadensis/C. bogdanii' (EcxCb).

Nine caryopsis anatomy characters describing pericarp, testa, aleurone layer, pigment strand, nucellar projection, and starch grains were studied and calculated as a mean taxonomic distance between the OTUs. A matrix of taxonomic distances was recalculated by means of the non-metric multidimensional scaling to arrange OTUs into an ordination space (Fig. 10). Comparing parents and progeny showed a complex paternal dominace of caryopsis structure is characteristic for the LaxLr and EcxPl amphiploids. Maternal dominance is noted for the PlxEy amphiploid. The amphiploids are separated well from most of the parental species (a red line) and their interspecies variation is very large. However, two species, L. racemosus and $L$. arenarius, have a special position within a diagram. All OTUs are scattered along a negative regression line between the $x$ and $y$ axes, and this picture is completely


Fig. 10. An MST diagram in a nonmetric multidimensional scaling ordination space presenting a uniparental dominance ( $\mathrm{m}=$ maternal and $p=$ paternal) of caryopsis structure in Triticeae amphiploids.

A N N U A L W H $\mathcal{L} A \tau N \in W S L \in \tau \in R$
V L. 56. different when compared to that for lemma and palea epidermal traits (see contribution on p. 205). An ellipsoid sphere created by the OTUs starts from low values on the x and z axes and high values on y and rises to low values on the y axis and high values on x and z axes.

## Variation of meristemoidal activity in abaxial epidermis of glumellae in 32 species of the Triticeae tribe.

R. Kosina.

Thirty-two species of the tribe Triticeae were described by means of morphology of well-differentiated epidermis of lemma and palea. Ten characteristics that describe morphogenesis of original epidermal short cells were used to arrange accessions within an ordination space by means of Kruskal's Nonmetric Multidimensional Scaling (nmMDS). Accessions of the following species were obtained from the Vavilov Institute, St. Petersburg, Russian Federation, IPKGatersleben, Germany, and the USDA, USA, and were cultivated in the field: Ae. cylindrica (Aec), Ae. triuncialis (Aet), Ag. cristatum subsp. cristatum (Acc), Ag. cristatum subsp. desertorum (Acd), Critesion californicum (Cc), C. chilense (Cch), C. hystrix (Ch), Elymus breviaristatus subsp. scabrifolius (Ebs), E. caninus (Ec), E.dahuricus (Ed), E. gmelinii (Eg), E. hystrix (Eh), E. mutabilis (Em), E. nutans (En), E. trachycaulus (Etr), E. tsukushiensis (Et), Elytrigia intermedia subsp. graeca (Elig), Eremopyrum bonaepartis (Erb), Hordelymus europaeus (Hee), Hordeum vulgare subsp. spontaneum (Hvs), Heteranthelium piliferum (Htp), Leymus paboanus (Lp), L. racemosus (Lr), Lophopyrum nodosum (Lon), Pseudoroegneria strigosa subsp.jacutorum (Prsj), Psathyrostachys juncea (Pj), S. silvestre (Ss), Th. bessarabicum (Thb), Taeniatherum caput-medusae (Tacm), triticale (Tle), T. monococcum subsp. aegilopoides (Tb), and T. fungicidum (Tf).

In the abaxial epidermis of glumellae, lemma, and palea, the frequency of short, specialized cells such as papillae, duplexes of silica and cork cells, triplexes (duplex + papilla), hairs, and other meristematic cytokineses were observed. Species in the MST diagram (Fig. 11), elaborated by means of nonmetric multidimensional scaling, are scattered according to activity of their epidermal meristemoids. Critesion californicum (Cc) express a very high activity of meristemoids and they are distinctly higher in lemma. A reverse relationship between activity in both glumellae is noted for Ta. caput-medusae (Tacm). A second pole of an ordinant space is occupied by species characterized by low activity of epidermal meristemoids, such as Ae. cylindrica (Aec) and L. racemosus (Lr). Interspecific patterns of epidermal morphogenesis are differentiated by various proportions of specialized cells within one organ and between both lemma and palea. These differences are demonstrated not only in a quantitative but also qualitative way. In addition, they appear as a presence or absence of a given kind of epidermal cell. This study of epidermal morphogenetic patterns is of high value for the taxonomic approach in the Triticeae tribe.


Fig. 11. An MST diagram of the Triticeae species described by means of meristemoidal activity of glumellae epidermis presented in a nonmetric multidimensional scaling ordination space.

## Meristemoidal activity in abaxial epidermis of glumellae in Triticum-Aegilops amphiploids and some Triticeae species.

## R. Kosina.

Species and amphiploid lines were obtained from the Kyoto Plant Germ-plasm Institute, Japan; R. de Pienaar, Republic of South Africa; Vavilov Institute, St. Petersburg, Russian Federation; IPK, Gatersleben, Germany; and the USDA, USA. Plants were cultivated in the field under uniform soil-climatic conditions. The abaxial epidermis of the lemma and palea was isolated and studied under light microscopy in Ae. caudata (Ac), Ae. uniaristata (Au), Ae. sharonensis (As), Ae. tauschii (At1, At2), Ae. umbellulata (Aum1, Aum2), Lophopyrum elongatum (Loe), S. cereale (Sc), Th. bessarabicum

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(Thb), triticale (Tle), T. monococcum subsp. aegilopoides (Tb1, Tb2), T. turgidum subsp. carthlicum (Tc), T. turgidum subps. durum (Td), T. fungicidum (Tf), T. kiharae (Tk), T. timonovum (Ttm), T. timopheevii subsp. timopheevii (Tt), T. turanicum ( Ttr ), and the amphiploids $\mathrm{Au} / \mathrm{Tb}, \mathrm{Tb} / \mathrm{Au}, \mathrm{Au} / \mathrm{At}$ (two forms), Aum/At, As/Aum, Aum/Au, Au/Aum, Ac/Aum, Tc/At, Ttr/At, and Td/Thb/Loe.

In this material, the frequency of short epidermal cells, such as papillae and duplexes, the latter composed of silica and cork cells, and the sum of all meristematic cytokineses, separately for lemma and palea, were calculated. A minimum spanning tree was obtained after using nonmetric multidimensional scaling (Fig 12). For many OTUs, morphogenesis of papillae dominates in the lemma and palea. However, S. cereale is completely different, where only duplexes in both glumellae were observed. For the species, a higher activity of epidermal meristemoids is characteristic but is low for both Aegilops species and the amphiploids. Paternal dominance of the studied traits was observed in the amphiploid Ttr/At. For the Td/Thb/Loe amphiploid, Th. bessarabicum (Thb) and T. turgidum subsp. durum ( Td ) are closer to their progeny, whereas the Lo. elangatum (Loe) parent is very distant. Distinct dominance is observed as a maternal or paternal component for many amphiploids created with Ae uniaristata.


Fig. 12. An MST diagram in a nonmetric multidimensional scaling ordination space presenting a uniparental dominance ( $\mathrm{m}=$ maternal and $\mathrm{p}=$ paternal) of meristematic activity of glumellae epidermis for selected Triticum-Aegilops amphiploids.

## Parental patterns of variation of embryo morphology in some amphiploids of the Triticeae tribe.

## R. Kosina.

A set of operational taxonomic units (OTUs) comprised of parental species and their interspecific and intergeneric hybrids were used to study variation of embryo structure included Leymus racemosus (Lr), L. karataviensis (Lk), L. arenarius (La), L. multicaulis (Lm), Elymus canadensis (Ec), E. yezoënsis (Ey), Pseudoroegnaria libanotica $(\mathrm{Pl})$, Critesion bogdanii $(\mathrm{Cb}), ~ ' P s$. libanotica/E. yezoënsis' (PlxEy), 'L. multicaulis/L. karataviensis' (LmxLk), 'E. canadensis/Ps. libanotica' (EcxPl), 'L. arenarius/L. racemosus' (LaxLr), and 'E. canadensis/C. bogdanii' (EcxCb). All lines were received from the Douglas Dewey collection maintained at the Utah State University, Logan. The embryo was described by four characteristics related to shape of the embryo, width of the scutellum, and length of the embryonic axis.

An MST diagram (minimum spanning tree, Fig. 13) presents the arrangement of OTUs within an ordination space constructed by means of nonmetric multidimensional scaling. Two extreme species, L. multicaulis and C.bogdanii, are 'epiblast' taxa. Distinct, complex parental dominance, maternal or paternal, is expressed by all amphiploids, a sign of differential expression of their genomes and, therefore, this material is very suitable for the study of nuclear architecture by means of GISH. The parental genomes very probablly occupy separate domains in the hybrid nuclei.


Fig. 13. An MST diagram in a nonmetric multidimensional scaling ordination space presenting a uniparental dominance ( $\mathrm{m}=$ maternal and $\mathrm{p}=$ paternal) of embryo morphology in Triticeae amphiploids.
 V L. 56. Expression of parental dominance in the lodiculae morphology of some Triticeae amphiploids.
R. Kosina.

Operational taxonomic units (OTUs) represented by parental species and their interspecific and intergeneric hybrids were studied for variation in lodiculae structure. The OTUs were Leymus racemosus (Lr), L. karataviensis (Lk), L. arenarius (La), L. multicaulis (Lm), Elymus canadensis (Ec), E. yezoënsis (Ey), Pseudoroegnaria libanotica (Pl), Critesion bogdanii (Cb), 'Ps. libanoticalE. yezoënsis' (PlxEy), 'L. multicaulis/L. karataviensis' (LmxLk), 'E. canadensis/ Ps. libanotica' (EcxPl), 'L. arenarius/L. racemosus' (LaxLr), and 'E.canadensis/C. bogdanii' (EcxCb). All the materials were grown under the same climate and soil. Five characters were used to describe lodicule morphology. The uniparental dominance exhibited by the amphiploids is clear (Fig. 14). This uniparental dominance is of two types, maternal or paternal. In the 'E. canadensis/Ps. libanotica' (EcxPl) and 'E. canadensis/C. bogdanii' (EcxCb) amphiploids, the maternal dominance of $E$. canadensis is well expressed in the lodiculae and embryo morphology. A separation of some parental species $(\mathrm{Lr}, \mathrm{Lm}, \mathrm{Pl}$, and Cb$)$ and hybrids also is visible in the ordination space, which could indicate a change of mating system in the amphiploids.

## Patterns of variation in a Triticum timopheevii subsp. timopheevii-Aegilops umbellulata amphiploid after demethylation of genomes.

R. Kosina and K. Markowska.

Matroclinal or patroclinal parental dominance can be observed in natural hybrids and synthetic amphiploids. Kosina (1996) has reported such a phenomenon for distant hybrids in the Triticeae tribe. Additionally, a variation pattern expressed in hybrids also is ruled by an epigenetic arrangement of parental chromosomes in nuclei of an amphiploid (Kosina and Heslop-Harrison 1996). In this study, we investigated the patterns of variation of gross morphology of spike and spikelet, caryopsis anatomy, and changes in expression of RNA in wild and demethylated genomes of a T. timopheevii subsp. timopheevii-Ae. umbellulata amphiploid. Some comparisons were made with the parental species. Two amphiploid plant progeny (Fig. 15) produced polymorphic dark and light ( d and l ) grains. The gross morphology of the spike and spikelets were arranged inside of the ordination space by means of nonmetric multidimensional scaling. This picture very clearly shows that forms having wild genomes are intermediate between demethylated extremes. Demethylation does not offer a directional change in the variation pattern but causes some increase in the existing variability.


Fig. 14. An MST diagram in a nonmetric multidimensional scaling ordination space presenting a uniparental dominance ( $\mathrm{m}=$ maternal and $\mathrm{p}=$ paternal) of lodiculae morphology in Triticeae amphiploids.


Fig. 15. A minimum spanning tree (MST) diagram representing the scattering of amphiploid progeny (A) with wild (red outline) and demethylated (red arrows) genomes described by means of gross morphology of the spike and spikelets. The progeny is located within a nonmetric multidimensional scaling ordination space. a or $b=$ progeny of two plants, $d$ or $1=$ dark or light caryopses for demethylated progeny, $\mathrm{a}=$ aza-cytidine progeny.

Additional observations were made for cross sections of the caryopsis. Demethylation changed the cytological status of the pigment strand area where strong suberization was detected (Fig. 16B, dark brown layer). This development can influence the synthesis of starch and protein in the endosperm and modify the proportion. After demethylation, the synthesis of RNA increased (compare Fig. 16C and D).

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Fig. 16. The wild status of the amphiploid genomes (A, C) and after demethylation (B, D). A, B - autofluorescence of cross-sections of caryopsis transfer tissues; C, D - DAPI and PI fluorescence of root interphase nuclei. Strong changes in the pigment strand (blue arrows) are visible, which is a part of the tissue transfer system providing assimilates to endosperm as well as of RNA (red signals) synthesis in root interphase nuclei.

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