On variability of the weedy characteristics in a model grass, Brachypodium distachyon.

R. Kosina, B. Kłyk, and M. Florek.

At present, Brachypodium distachyon is known as an invasive weed in various world regions, e.g., South Africa, California, and Australia. Weedy plants exhibit special traits permitting their quick reproduction and dispersal. According to the description of weediness made by Ammann et al. (2000) and Basu et al. (2004), one can ascribe to the B. distachyon plant the following characteristics of weediness:

- germination in various habitats (rocky and sandy, podsol, and fertile),
- quick growth of seedling (several days),
- longevity of seed (we noted a good germination after 28 years of seed storage at room temperatures),
- a short period between vegetative and sexual phases (2–4 weeks, varying for different types (Fig. 1A)),
- self-compatibility with possibility of chasmogamy (for some accessions we documented chasmogamy),
- adaptation to unspecialized pollinators (wind for chasmogamy),
- a large amount of seed produced (~40 seeds from one raceme spike, depending on the number of spikelets/spike), and
- the possibility of short and long dispersal (heavy diaspores and zoochory).

Some weed characteristics are not exhibited by B. distachyon. The plants do not produce seeds continuously. In the Kosina collection of 25 accessions, various forms are cultivated (Fig. 1). Some lines express a rigid spikelet rachilla (Fig. 1D), which is typical for cultivated forms, others have hairy spikelets adapted for zoochory. This mostly autogamic species creates its own population, which are composed of various pure lines. Many autogamic grasses, such as Anisantha sterilis (Green et al. 2001) or Avena barbata (Florek 2008) have similar microevolutionary potential. For such a breeding status, the selection can be very effective. Examples of various weedy characteristics of B. distachyon are provided (Fig. 1).

References.
On wheat and Brachypodium distachyon caryopsis.

R. Kosina and P. Tomaszewska.

A wheat grain has some specific properties that can be used as a discriminatory tool in cereal taxonomy and in grain processing. The caryopsis is covered by several cell layers of the pericarp and is thicker in threshable wheats and thinner in unthreshable ones. The nucellar epidermis is highly reduced and joined with outer testa, which is sometimes distinctly suberinized. The aleurone is most often composed of single layer of proteinaceous cells (Fig. 2A), however, rarely, one can note different development of this tissue in the form of two or more layers of such cells (Fig. 2B). Starchy endosperm is composed of starch granules, the large, A type, and small, B type (Fig. 2C). Filling of endosperm cavity by starch can be considered as a spectacular developmental phenomenon (Fig. 2D); it is rare and was observed in T. turgidum subsp. durum and polonicum or T. aestivum (Kosina 1979). The creation of the cavity is dependent on relations between development of outer parts of caryopsis and rate of starch synthesis. Transfer tissues (vascular bundle, pigment strand, nucellar projection), no doubt, play an important role here.

Brachypodium distachyon is considered as a relative to Triticeae cereals, and its grain presents the same general pattern of development. However, the nucellar epidermis is extremely thick in this species when compared to other species of Brachypodium (Fig. 3A, yellow arrow). This thick layer is digested during seed germination. Within the system of transfer tissues, the vascular bundle is almost invisible and no cavity is created above the aleurone layer in a shallow crease (Fig. 3A, white arrow). The endosperm tissue is poorly filled with starch. Starchy cells also have thick walls decomposed along with starch during the seedling growth (Fig. 3B). All starch granules are of single type. The aleurone exemplified in Fig. 3B is formed by one layer of cells, but quite often this tissue is composed of 2–3 layers. Digestion of hemicelluloses of nucellar epidermis and starchy cell walls is slower than that of starch, and it is probably a trait characteristic for a wild grass.

Reference.
Microstructural differentiation in Brachypodium distachyon and its relatives – a case of the lemma.

R. Kosina and B. Kłyk.

Arithmetic average is the most common statistical method used to describe any unit in agronomy or biology. However, in fact other measures are of the same rank from the scientific point of view, such as variation of a given character and covariation of characters and its statistics coefficient of correlation and regression parameters. For the same set of Brachypodium OTUs (B. distachyon, B. sylvaticum, B. retusum, B. pinnatum, and B. phoenicoides) described by traits of abaxial epidermis of the lemma, the following statistics were calculated: arithmetic average, median, min-max values, coefficient of variation, coefficients of correlation, parameters of equation of regression (linear or curvilinear), and parameters of regression variation. Arrangements of the OTUs within an ordination space (nonmetric multidimensional scaling) were different for each group of parameters. The main conclusion is that each group of statistics is of equal value and independent for OTU characterization. For instance, arithmetic averages discriminated a B. sylvaticum group from other taxa quite well (Fig. 4). When using coefficients of correlation to characterize the OTUs, we obtained completely different arrangement of species (Fig. 5). Two accessions of B. distachyon are especially remarkable in this respect. They occupy two extreme points in the ordination space. Such a sharp intraspecific difference in traits interrelations is probably caused by genetic variation created among pure lines of this autogamic species.

Reproduction in Brachypodium distachyon and related species.

R. Kosina and B. Kłyk.

Differentiation of the mating system within a range of auto- and allogamy exists among species of the genus Brachypodium. Our study was made for accessions of the species B. pinnatum, B. phoenicoides, B. sylvaticum, B. retusum, and the model grass, B. distachyon. Morphometric analysis of the male structures proved that in Brachypodium two patterns of anther development were observed:

- long anthers with a large amount of small pollen grains in allogamic units and
- short anthers with a small amount of large pollen grains in autogamic units.

The OTUs of these two breeding patterns are well discriminated within an ordination space (Fig. 6, p. 251). Brachypodium pinnatum appeared as the most allogamic species. Others, such as B. phoenicoides and B. sylvaticum, are in the intermediate position between allo- and autogamy. Accessions of the model grass, B. distachyon, are in a distinct position in the diagram, which is described by smallest values of ordination axes. Distances between these accessions...
are small; however, some chasmogamy, typical for allogamy, was also observed.

Nucleolar variation in Brachypodium distachyon.

R. Kosina and B. Kłyk.

An Ag-NOR technique plus DAPI staining were used for the identification of active rDNA loci. The research was conducted for root mitoses of several accessions of \textit{B. distachyon}. Two unequal nucleoli are the most common arrangement and this suggests that two pairs of SAT chromosomes produce nucleolar proteins. Neighboring nuclei have the same nucleolar pattern, which proves the clonal nature of root nuclei. Nucleoli are mirrored in sister nuclei. Terminal Ag-NOR signals are typical for one pair of chromosomes and interstitial signals were observed in two other pairs. An important input into Ag-NOR variability is caused by association of NORs with telophase bridges. Active rDNA loci can be symmetrically located on a bridge (Fig. 7A) or a picture (Fig. 7B) can show distinct asymmetry, and Ag-NOR proteins can be stretched along a bridge. Finally, the Ag-NOR signal is included into one telophase nucleus and a sister nucleus is lacking it (Fig. 7C). The presented data prove an extreme cytogenetic potential existing in the species.

On the cytogenetic variability in Brachypodium distachyon.

R. Kosina and B. Kłyk.

For several accessions of \textit{B. distachyon}, DAPI karyotypes were studied. Some of them were diploid (Fig. 8A), and others were tetraploid. Many intermediate karyotypes, between 20 and 30 chromosomes, were documented. All the studied karyotypes were bimodal, composed of long, 3–4 pairs of chromosomes and short ones (Fig. 8A, white arrows). The long chromosomes appeared to be allocyclic (Fig. 8A, green arrows) with one decondensed arm. Small chromosomes have mostly strong DAPI fluorescence of an AT-rich heterochromatin. The genome of \textit{B. distachyon} exhibited many disturbances (Fig. 8B, C, and D). There were noted laggards (Fig. 8B) and multiple bridges (Fig. 8C), sometimes numerous, associated...
with rDNA and ring chromosomes (Fig. 8D, p. 252). The above-mentioned behavior creates unequal sister nuclei and their dysfunction finalized by apoptosis. This cytogenetic instability should be considered along with developmental anomaly of the grass caryopsis, which is visible in the form of mosaic of two endosperm phenotypes, starchy and aleurone cells. Both, cytogenetic and developmental anomalies suggest the activity of transposons in *B. distachyon*.

**Nucleolar variability in a Triticum timopheevii subsp. timopheevii / Aegilops umbellulata amphiploid.**

R. Kosina and K. Markowska.

For a ‘*T. timopheevii* subsp. *timopheevii* / *Aegilops umbellulata*’ amphiploid and its parents, an Ag-NOR analysis in mitoses of main and lateral roots was performed. In addition, the status of Ag-nucleoli in interphase nuclei was studied in three forms of the amphiploid: control, roots from demethylated caryopses (5-azaC-I), and roots from caryopses harvested from mother plants, which were demethylated in the stadium of germinated caryopses and subsequently cultivated on plots (5-azaC-II). Polymorphism of amphiploid caryopses, light versus dark, is inherited from its paternal species. Sixty types of nucleoli arrangement were noted in the amphiploid (Fig. 9). The largest number of types is expressed in main roots from dark caryopses for the 5-azaC-II treatment. The NOR-activity of two unequal pairs of SAT-chromosomes is most probable in all the studied forms. Nucleoli of these two pairs can join independently giving two bodies of unequal size or nucleoli of two different chromosomes can be merged and two equal bodies are visible. These two types of nucleoli arrangement are most common in all studied plants. The number of nucleoli is within a range of 1–7, which means that in the amphiploid, some parental Ag-NOR loci are suppressed. Demethylation changes frequency of the most common type of nucleoli arrangement between the main and lateral roots when compared to the control and it is similar to the status noted for parental species.

**Lodicule micromorphology in an autogamic grass, Brachypodium distachyon.**

R. Kosina and K. Pietrzak.

Grass lodicules play a decisive role in chasmogamic activity of these plants. Their structure and function is very original and complex (Kosina 2005, 2006). *Brachypodium distachyon* is, as a rule, recognized as an autogamic unit (Kłyk 2005); however, some chasmogamy was also noted for it (Kosina, unpublished
The description of *Brachypodium* lodicules was made by Kłyk (2005) and Pietrzak (2007). The most interesting properties of the *B. distachyon* lodicule are presented in Fig. 10 (p. 253). The lodicule is differentiated structurally and functionally along its own axis. At the base is a distinguished layer of cells with thick cellulosic walls (Fig. 10A, arrow, p. 253); however, the other parts of this organ are also differentiated developmentally, going toward the apex. In the lower part of the lodicule, a cushion, many thin phloem veins are developed (Fig. 10B, p. 253). The main metabolite in the upper part of lodicule, a lobe, are composite starch granules, which are observed before anthesis (Fig. 10C, p. 253). In addition, a lot of calcium oxalate crystals are noted around the border area cushion-lobe. After the anthesis, cells of the lobe contain many lipid globules (Fig. 10D, p. 253). In lodicules one can show some characteristics typical for leaves, such as short epidermal cells and stomata (Kosina 2010). In *B. distachyon*, short epidermal cells, such as papillae, were found (Fig. 10E, p. 253). Development of stomata in lodicules in *B. distachyon* (Fig. 10F, p. 253) is very rare. In a collection of more than 20 accessions of the species, only two expressed this trait, probably of mutational origin.

**References.**


**Caryopsis microstructure in *Triticum kiharae* and *T. fungicidum*.**

R. Kosina and M.K. Bureś.

Both *T. kiharae* (*T. timopheevii* subsp. *timopheevii* / *Ae. tauschii*) and *T. fungicidum* (*T. turgidum* subsp. *carthlicum* / *T. timopheevii* subsp. *timopheevii*) wheats are artificial amphiploids with highly sclerified spikes. Development of any caryopsis is dependent on the architecture of transfer tissues (nucellar projection and pigment strand) that are the main path for assimilates supplied into the endosperm. In *T. kiharae* (Fig. 11A), these tissues have larger volume and can be a better gate for assimilates. A vascular bundle is located below the pigment strand. In a ripe caryopsis, only the xylem vessels are recognizable, while phloem is obliterated. The xylem bundles have various numbers of vessels, and in *T. kiharae* it is composed of ~15 vessels, in *T. fungicidum* ~5. As a consequence of such a differentiation of transfer tissues and vascular bundle, the development of other tissues of the caryopsis is also variable. Differences are related to the nature of suberinized testa (Fig. 11E and F, see arrows), thickness of nucellar epidermis (yellow layers) and aleurone cell shapes (stars). The ratio of starch protein is the most important parameter for wheat grain processing. This proportion is conditioned by the system of transfer tissues (Kosina 1988). Expression of a starch phenotype among aleurone cells is another
observed phenomenon, which can be caused by somatic crossing-over, which is noted in grass endosperm surprisingly often (Kosina 2007).

Reference.

Structural differentiation of embryo in the genus Brachypodium, including Brachypodium distachyon.

R. Kosina.

Measurements of scutellum, embryo axis and epiblast were used for the description of Brachypodium OTUs (Fig. 12). In an ordination space, embryos of B. distachyon are distinctly separated from other species; however, there is some overlapping of variation spheres of B. distachyon and B. sylvaticum. In the taxonomy of the genus Brachypodium, both species are recognized as extremes. There are even some proposals to discriminate B. distachyon in a separate section or a new genus Trachynia. Brachypodium distachyon is qualitatively distinguished from other species of the genus by morphology of its epiblast. In the diagram (Fig. 12), B. distachyon, B. retusum, B. pinnatum, and B. phoenicoides are at extreme points (see arrows). One can conclude that at least for some accessions, embryo characteristics well discriminate the species.

Endosperm cytogenetics in ‘Triticum / Aegilops tauschii’ amphiploids.

R. Kosina.

Free nuclear endosperm of the amphiploids ‘T. turgidum subsp. dicoccum / Ae. tauschii’, ‘T. turgidum subsp. carthlicum / Ae. tauschii’, and ‘T. turgidum subsp. turanicum / Ae. tauschii’ was studied by means of in vivo acridine orange fluorescence as well as by GISH and FISH methods (Kosina 1995). These forms were obtained from the Plant Germ-plasm Institute in Kyoto, Japan. Polyploidization of the syncytium was realized on two paths:

1. an increase in the volume of a given nucleus and amplification of rDNA signals in it and
2. an increase in the number of nuclei having the same number of rDNA loci.

Multiple bridges in anaphases are typical for these amphiploids. The cell cycle of groups of nuclei is synchronized...
is evidence of the clonal nature of syncytial endosperm. A karyokinetic spindle was disturbed and multipolar telophases were formed. The modal number of nucleoli was 4–6. In each nucleolus, one to several rDNA signals were observed (Fig. 13A, p. 255). Signals of rDNA were mirrored in telophase. However, some signals were also located on bridges or laggards and were lost giving an rDNA asymmetry in anaphase–telophase nuclei. The rDNA signals in a nucleolus decondense inside and outside of its body. Antipodal polytenic chromosomes create in the later stadia some nuclear masses with amplified rDNA loci, in amphiploids and in parental species (Fig. 13B, p. 255).

Reference.

**Nucleolar characteristics of endosperm in Triticeae.**

R. Kosina.

Embryo sacs were excised from 3–5-day-old caryopses, and subsequently free nuclei of endosperm, were mounted on slides in acridine orange. This technique was applied for the following OTUs: *Ae. juvenalis*, an amphiploid *Triticum/Thinopyrum/Lophopyrum*, an amphiploid *Triticum/Aegilops*, *Critesion jubatum*, *C. bogdanii*, *C. bulbosum*, *Dasypyrum villosum*, *Elymus caninus*, *E. yezoensis*, *E. mutabilis*, *E. hystrix*, *Elytrigia pungens*, *E. repens*, *Eremopyrum bonaepartis*, *Hordeum vulgare* subsp. vulgare, *H. vulgare* subsp. *spontaneum*, *Secale arenarius*, *Lophopyrum elongatum*, *Secale sylvestre*, *S. montanum*, triticale, *T. fungici-dum*, *T. kiharae*, and *T. aestivum*. The OTUs were described by 22 classes of distributions of nucleoli frequency. The best dendrogram was obtained for the Canberra distance and UPGMA clustering (Fig. 14). Diploids, such as species of *Secale* or *Dasypyrum*, presented short distribution and small variation of nucleoli contrary to some polyploid taxa located in lower position of the dendrograms. The lower cluster of the dendrograms is complex and it also contains some diploids like *H. vulgare*.

**Microstructural variation in selected cereals under environmental stress.**

R. Kosina.

*Triticum* amphiploids having genomes AAGGAA and AABDDD, and two cultivars of *Hordeum vulgare* (HV3 and HV5) and *Bromus secalinus* (BS) were cultivated in two extremely different environments: in a glasshouse with diurnal temperatures above 50°C in pots with pure sand and crucial irrigation (symbol s in Fig. 15, p. 256), and in outdoor podsol plots with diurnal temperatures of approximately 20–30°C and sufficient irrigation (symbol z in Fig. 15, p. 257).

OTUs (cereals) were described by eight characters of highly differentiated abaxial epidermis of palea and lemma as well as by four traits of lodicules. Both classes of characters (glumellae and lodicules) are well separated in den-
In the dendrogram (Fig. 15), three groups of grasses, namely the amphiploids, *H. vulgare*, and *B. secalinus*, are distinctly separated. Appropriate pairs (s vs. z) also are distinctly separated. Only the AGA amphiploid is differentiated more by the environmental stress. Development of the abaxial epidermis of palea and lemma was most often disturbed in amphiploids. Under drought conditions, high temperature, and starvation, the grass plant developed only one tiller with short spike or poor panicle. Tissues were highly sclerified. Under sufficient watering, the plants were often infested by fungi and setting of caryopses was defective. In conclusion, both environments can create a stress of various nature. Thus, the microstructure of cereals is shifted under heavy stress, but its general pattern is preserved. Then, this pattern can be a good basis for any taxonomic comparisons.

**ITEMS FROM THE RUSSIAN FEDERATION**

**AGRICULTURAL RESEARCH INSTITUTE FOR THE SOUTH-EAST REGIONS**
Department of Genetics, Laboratory of Genetics and Cytology, 7 Toulakiev St., Saratov, 410010, Russian Federation.

*The evaluation of spring bread wheat cultivars, NILs, and introgression lines in the hard, drought conditions of 2009–10.*


For the recommendation of introgression lines with identified combinations of genes for resistance to pathogens in practical breeding some prebreeding research is necessary. These research includes determining resistance to abiotic stresses and bread-making qualities. The conditions of the growing periods of 2009 and 2010 allowed estimating the set of introgression lines for drought resistance. The two-year-old data for grain productivity in NILs in the extremely hard drought conditions have shown the following results. The combination of *Lr*9+*Lr*19-translocations in the genotypes of cultivars L503, Dobrynya, and line L2032, do not depress yielding ability, but *Lr*19+*Lr*26 significantly improves grain productivity, and *Lr*19+*Lr*24 and *Lr*19+*Lr*25 significantly depresses yield ability. A neutral reaction for grain productivity in the introgression lines with substitution 6Agi (6D) is detected. The incorporation of genetic variability from *T. turgidum* subsp. *dicoccoides* and *dicoccum* to the spring bread wheat cultivars Saratovskaya 58 and Saratovskaya 55 (lines L196 and L2870) does not depress drought resistance, but incorporation of genetic variability from durum wheat (cultivars Saratovskaya zolotistaya, Lyudmila, and Saratovskaya 57) to bread wheat (lines L200/09 and L211/09), and their combination improves this trait. The NILs with combinations of translocations *Lr*9+*Lr*19, *Lr*19+*Lr*24, *Lr*19+*Lr*25, substitution 6Agi (6D), and also lines L196, L2870, L200/09, and L211/09 have good bread making quality at the level of cultivars. The NIL of L503 with combination of *Lr*19+*Lr*26 translocations was exception in which the flour strength was reduced.