SELECTED ITEMS OF WHEAT VARIATION – FROM PALAEOBOTANY TO MOLECULAR BIOLOGY

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(Received: August 18, 1998. Accepted: May 23, 1999)

ABSTRACT

The usefulness of data on ecotypes of wheat as well as of information about distribution of genes of hybrid necrosis for an interpretation of some questionable detections of fossil materials is emphasized. Variability of contemporary wheats is illustrated by means of morphology of lodicules, anatomical structure of caryopsis, morphology of embryo and features of epidermis of inflorescence bracts. These structures exhibit often a trend dependent on ploidy level. Discrimination of similar grains of fossil *Triticum compactum* and *T. sphaerococcum* is possible when traits of embryo are used. Wheat genomes are changed by numerous translocations and are spatially separated. This status may be detected by means of in situ hybridization of the genomic DNA. With such a spatial arrangement of the genomes the dominance of a caryopsis trait complex in hybrids between tetraploid wheats may be correlated. It may also create a part of new variation in wheat.

KEY WORDS: wheat, variation, ecotypes, micromorphology, in situ hybridization, genomes.

INTRODUCTION

The genus *Triticum* is a polyploid, much differentiated complex, and this pattern is enriched by the influence of man. Archaeobotanists have at their disposal only scarce remains of this rich source, such as spike rachis, bracts of head and grains, most often charred. There is a possibility of mistakes, while identifying such a material. Detailed studies made by Vavilov (1949/50) on old local wheat cultivars in different regions of the world, as well as Genkel's (1969) research on morphophysiological wheat types provide additional information useful for the archaeobotanist. The present review gives some new information on microstructural wheat variation displayed by endosperm structure, morphology of embryo, epidermis of flower bracts. Such data should permit a better identification of fossil material and its deeper interpretation due to knowledge of interrelation between, e.g. weather during vegetation period and the endosperm structure. For wheat students processes leading to genome differentiation are very important. They are responsible for the evolution of this plant and still play such a role. The studies made by Flavell (1985, 1989) are substantial for considerations about genome changes in wheat. Hybridization in situ of the genomic DNA or of selected nucleotide sequences, especially those of ancient origin, is very useful to detect such a reorganization. A study of relationship between the spatial distribution of the genomes in nucleus (Bennett 1984) and gene expression may clarify the course of differentiation, e.g. cultivars within a given species.

PALAEOBOTANICAL CONTRIBUTION TO THE WHEAT SCIENCE

There are many archaeological discoveries of fossil wheats but only a few are sufficiently rich to permit some considerations related to variation of the material. Such investigations include Kislev's (1979/80) study on *parvicoccum* wheat, detailed research made by Jacomet and Schlichtherle (1984) on wheats from lake settlements in a German-Swiss borderland as well as Moffett's (1991) analysis of tetraploid wheats in England. For a researcher of wheat evolution other, more spectacular reports, are also important, e.g. Rusishvili's (1991) study on the group of wheats (*T. boeoticum*, *T. timopheevi*, *T. carthlicum*, *T. macha*, *T. sphaerococcum*) from Georgia, rarely evidenced in excavations or the more recent paper by Rego and Rodriguez (1993) about *Triticum sphaerococcum* from Portugal. Kislev (1979/80) described fossil, small-grained, compact-headed, threshable wheat from the Mediterranean region as new tetraploid species, *Triticum parvicoccum*. Kosina (1991) examining the pictures of this wheat referred this material to the contemporary *Triticum durum*. From Kislev's data it may be concluded that this wheat was especially well adapted to the Mediterranean climate (exemplified by its broad distribution). The ancient man was distinctly aware of the advantage of this wheat. Jacomet and Schlichtherle (1984) proved the cultivation of tetraploid threshable wheats in a Central-European mountainous region.

Such a territory favoured the isolation and creation of differentiated populations. The authors proved also that these populations did not become mixed. Moffett (1991), studying a

rachis of tetra- and hexaploid wheats, was able to detect a cultivation of tetraploid threshable wheat, *Triticum turgidum*, in mediaeval England. Rusishvili (1991), as well as, Rego and Rodriguez (1993) reported on wheat (*T. sphaerococcum*) rather unexpected in the given regions. The materials were detected by means of grain macromorphology only. The present author, like some other archaeobotanists, is aware of the weakness of such discriminations. They appear in scientific literature and then due to citations they act as 'perpetuum mobile'.

It can be assumed that the ancient man chose for cultivation the wheats best adapted to local climate and soils. Mistakes were single accidents of short duration. As a consequence of this conscious choice, there was a transfer of wheat ecotypic variation from ancient times up to old local cultivars, almost contemporary for us. A study of variation of such cultivars may be very useful for archaeobotany. In this respect, much was contributed by Vavilov, Flaksberger, Palmova, Jakubziner and other Russian students. Vavilov (1949/50) stressed a role of the following factors: temperature relation, day length and moisture for creation of wheat ecotypes. Wheats differ sharply in rhythm of spring development. In *Triticum vulgare* (*T. aestivum* – common wheat) five ecotypes were distinguished: **hygrophytes** with squareheaded morphology (cf. Flaksberger's proles *hygrophilum*), **hygromesophytes** closely related to the first type, **mesophytes** typical for Central Europe countries, **mesoxerophytes** well adapted to the rye soils and **xerophytes** adapted to dry climate (cf. Flaksberger's proles *hungaricum*). The last type includes the 'Banatka' wheat of *T. vulgare*, but also durums, spelts, emmers, and einkorns. E.F. Palmova (according to Vavilov 1949/50) distinguished four ecotypes of *T. vulgare*: **adapted to moist climate** (vigorous development, leafiness, high tillering capacity, large heads, large and mealy grains, high productivity, low requirement for warmth but high for day length and for air and soil moisture); **for steppe vegetation** (xerophytic traits, thin straw, average tillering, few leaves – 'Banatka' is a typical representative of this group); **from desert and semi-desert** areas with or without irrigation (low growth, stiff straw, coarse leathery leaves with waxy layer, coarse heads and awns, non-shattering grain, difficult milling, frequent brittleness, early maturing, heat resistance); **from moist high mountainous region** (leafiness, high water requirement). Vavilov (1949/50) distinguished in *Triticum durum* essentially two large groups of Abyssinian and Mediterranean origin.

The first group shows a great polymorphism in head and grain features. The second group has large heads and grains, long awns, thick and tall straw, smooth leaves. In this group Vavilov separated a Syrian-Palestine wheat with compact heads (ca. four spikelets per 1 cm). Vavilov, examining the ecotypes of *T. dicoccum*, *T. compactum*, *T. spelta* and other wheats, concluded that "the future systematist must be basically a physiologist". Such a tendency is visible in Genkel's (1969) work. His ten morphophysiological types of *T. vulgare* relate to a wide range of climates of the former Soviet Union. There are wheats having varied vegetation periods, e.g. from 70–85 days to 120–130 days, different height and length of head, various weight of 1000 grains, e.g. from 14–18 g to 38–45 g. Such types evolved under different spectra of temperature, insolation, and rainfall during all the vegetation period, however, the first stage of development was especially sensitive. Weather conditions influence e.g. chemical content of grain during plant growth. Prolongation of this period at lower temperature decreases protein synthesis in the endosperm. Therefore, the wheat ecotypes of the North-West Rus-

sia have a lower content of protein than the South-East ecotypes (Knjaginichev 1951). Differences related to the protein amount are correlated with the endosperm structure. To some extent, we have, independently of the wheat species, vitreous or mealy grain. Such a structure of endosperm is legible in charred, fossil caryopses (Kosina 1994). Considerations about ecotypes of a given region may give additional information, just on the endosperm structure – on the other hand, in fossil material this structure may reflect weather changes in a given year of cultivation.

Remnants of a head (rachis, glumes, lemmas, paleas) are sometimes studied in fossil material. Mac Key (1966), considering morphology of the head in different wheats, distinguished the following major genes: *Q* (a squarehead factor), *C* (factor of compactness), *s* (sphaerococcoid head) and *P* (polonicoid glumes). The *Q* gene is recognized to be typical for the *vulgare* wheat. Muramatsu (1986) proved, however, the presence of this super gene in *T. polonicum* (var. *vestitum*), *T. dicoccum* (var. *liguliforme*) and *T. durum*. Its presence not always induces round glumes. In many cultivars of *T. vulgare* (Ethiopia, Middle East, Nepal, Japan) keels persist down to the base of glumes. Then, the discrimination of *T. vulgare* and *T. spelta* may be difficult. Additionally, the presence of a *Tg* gene (tenacious glumes) on chromosome 2D may change the expression of *Q* (Harlan 1981; Kislev 1984). Activity of the *C* and *s* genes at tetraploid level is also possible. Bekele (1984) found, in a large spectrum of Ethiopian wheats, *Triticum turgidum* with compactoid, *dicoccum* and *sphaerococcum* genes. Stankov and Tsikov (1974) pointed to the presence of the *C* gene in a set of the AABB genomes. Genetic experiments with common wheat, cv. "Canthatch" (Kerber 1964) show also the presence of the *C* gene in such a set. The above data prove that the head morphology (loose or compact head), glume morphology (presence of keels) and the shape of grain may be insufficient criteria for distinguishing between tetra- and hexaploid wheats or within each of the series. With regard to grain shape the polymorphism of caryopses of wild and weedy forms (Mac Key 1989a; Kosina 1995a) complicates additionally the discrimination of wheats. The idea of transfer of the ancient variability into local varieties expressed in the studies of hybrid necrosis served Zeven (1980) to follow the scattering of *T. vulgare* from the Neolithic. It is important that *Ne*-alleles are neutral and displacement of man with his crops was very limited. Zeven's data are of exceptional value for the interpretation of fossil materials. For instance *Triticum sphaerococcum* is an *Ne1^w*-carrier. The distribution area of wheats with this gene is a narrow belt from Greece to Portugal. Our opinion about endemism of *T. sphaerococcum* may change, if we take into consideration the last finding of this wheat in Portugal (Rego and Rodriguez 1993). Probably numerous findings recognized by Kislev (1979/80) as *T. parvicoccum* wheat might actually be *Triticum sphaerococcum*.

VARIATION PATTERNS IN WHEAT MICROMORPHOLOGY

Morphology of lodicules

Wheats were here described with use of five traits, best informing about the lodicule morphology as a whole. The following traits were selected by means of multiple regression analysis: length of frontal lobe, length of hairs of back lobe, hairiness of dorsal lobe, width ratio of frontal and back lobe, shape of back lobe. Wheats were ordinated along the first two

Fig. 1. Scattering of taxa of the genus *Triticum* along the first two principal components with application of five traits (see text) best describing the morphology of lodicules. For Figs. 1-4: axes are described by loading values $\times 10$; within each diagram the level of taxa variation displayed by three successive components (PCI, PCII, PCIII) is presented.

principal components which include 61% of information (PC I and PC II) about variation of taxa described by given traits (Fig. 1). Tetra- and hexaploid wheats form a vast complex in which *Triticum sphaerococcum* and *T. ispahanicum* are extremes, especially when values of the second principal component are taken into consideration. The largest differentiation of lodicules within this complex is visible in *Triticum dicoccoides*. The positive values of the second principal component describe threshable wheats while the negative ones refer to the unthreshable ones. Such a distinction may be correlated with the type of floral cavity. Millet (1988) found a correlation between this cavity and grain weight. The lodicules are an essential element of the cavity. *Triticum petropavlovskiyi* and wheat diploids are extremes along the first principal component axis. The large variation of *T. boeoticum* is distinct and it is the same for its other traits (Kosina 1995a). Ranges of variation of *T. boeoticum* and *T. monococcum* overlap partly, which is consistent with Mac Key's (1981a) taxonomic treatment. A large distance of *Triticum urartu* from the mentioned diploids is well correlated with the study of Johnson and Dhaliwal (1976) on reproductive isolation between *T. boeoticum* and *T. urartu*, as well as, with the data of Gill and Waines (1978) on the development of grain in hybrids of these wheats. Summarizing the values of two components we may conclude about some trend between morphology of lodicule and ploidy level, such as: diploids – tetra- and hexaploids – octoploids. An indirect status of *Triticum polonicum* between *T. dicoccum* and threshable wheats is confirmed by the head morphology expressed in quantitative traits (Kosina

1981), as well as, caryopsis traits (Kosina 1995a). In the genus *Triticum* as a whole, *T. boeoticum*, *T. urartu*, *T. ispahanicum*, *T. fungicidum*, *T. georgicum*, *T. petropavlovskiyi* and *T. sphaerococcum* have extremely different lodicules. The central position in the diagram is occupied by *Triticum macha*. The separateness of *T. ispahanicum* in relation to *T. carthlicum* finds also reflection in their caryopsis structure (Kosina 1995a). Endemic wheats having narrow variation, such as *T. georgicum*, *T. urartu*, *T. sphaerococcum* and *T. ispahanicum*, as well as young hybrid forms like *T. zhukovskiyi* and *T. fungicidum* or wheats supposed also to be macromutants like *T. ispahanicum*, *T. petropavlovskiyi* and *T. sphaerococcum* seem to be the extremes. Head mutations including change of floral cavity may distinctly alter the morphology of lodicule (Kosina 1998). The lodicules as separate organs mutate also, e.g. lodicule without dorsal lobe found in some cultivar of *Triticum compactum*. Such a change may increase the level of self-pollination (flowers are only a little open or closed like in cleistogamy).

Anatomy of caryopsis and embryo morphology in di- and tetraploid wheats

The following characters: width of scutellum, width of epiblast, length of coleoptile, thickness of external part of pericarp, shape of scutellum, shape of embryo axis and shape of nucellar projection were chosen with use of the Wilk's lambda criterion. They best distinguish diploid and tetraploid species of *Triticum* and *Aegilops* (Fig. 2). An extensive range is noted for *T. boeoticum*, *T. dicoccoides*, *T. dicoccum*, *T. tur-*

Fig. 2. Ordination of wheat diploids and tetraploids as well as two goatgrass species along the first two principal components with use of seven traits of grain anatomy and embryo morphology (see text) best distinguishing the taxa.

gidum, *T. polonicum* (cf. lodicule variation in Fig. 1). Variation of *T. ispahanicum* overlaps with that of *T. dicoccoides* like in the case of lodicules. *T. ispahanicum* and *T. carthlicum* as well as *T. boeoticum* and *T. urartu* are very different taxa. Cultivated *T. monococcum* is close to *T. dicoccoides*. A trend from wild *Ae. speltoides* through wild 2x and 4x wheats up to cultivated 4x wheats is distinct. *Ae. squarrosa* (DD genomes) is well distinguished from the wheats and from other goatgrass.

Embryo morphology of wheat hexaploids

By means of multiple regression the following traits: width of coleorhizal papilla, length of embryo axis, width ratio of base and upper part of epiblast, shape of coleorhizal papilla, shape of epiblast, length ratio of radicle and coleoptile, and distance of coleoptile top to internal scale of scutellum were selected as describing best the embryo. *Triticum zhukovskiy*, *T. sphaerococcum* as well as a large complex of *compactum-vulgare* including *T. vavilovii*, *T. macha*, *T. petropavlovskiy*

TABLE 1. Means of embryo traits for some contemporary species of the genus *Triticum*.

Traits of embryo	<i>T. urartu</i>	<i>T. monococcum</i>	<i>T. timopheevi</i>	<i>T. dicoccoides</i>	<i>T. dicoccum</i>	<i>T. turgidum</i> s.s.	<i>T. durum</i>	<i>T. ispahanicum</i>	<i>T. carthlicum</i>	<i>T. zhukovskiy</i>	<i>T. vulgare</i>	<i>T. compactum</i>	<i>T. sphaerococcum</i>	<i>T. spelta</i>
Width of scutellum	1.27	1.84	2.14	2.10	2.06	2.30	2.20	2.22	2.13	2.07	2.51	2.17	1.98	2.56
Width of embryo axis	0.86	1.16	1.15	1.32	1.29	1.36	1.29	1.49	1.31	1.27	1.35	1.29	0.98	1.44
Width of epiblast	0.49	0.60	0.22	0.74	0.76	0.78	0.75	0.83	0.85	0.40	0.69	0.76	0.47	0.65
Length of scutellum	2.30	2.60	3.00	2.69	3.20	2.60	2.81	3.48	2.52	2.86	2.94	2.67	2.02	3.20
Length of embryo axis	1.89	2.20	2.72	2.53	2.46	2.29	2.45	3.14	2.24	2.38	2.33	2.16	1.55	2.73
Height of epiblast	0.35	0.37	0.21	0.40	<u>0.37</u>	0.42	0.38	0.51	0.43	0.29	0.42	0.41	<u>0.25</u>	<u>0.36</u>
Length of coleoptile	1.55	1.65	2.17	1.58	<u>1.98</u>	1.38	1.43	2.20	1.36	1.71	1.56	1.41	<u>0.97</u>	<u>1.91</u>
Length of radicle	0.69	0.78	0.90	<u>1.09</u>	1.02	0.98	1.05	1.11	0.93	0.98	0.89	<u>0.86</u>	<u>0.66</u>	0.98
Shape of epiblast	1.43	1.64	1.09	<u>1.88</u>	2.03	1.87	2.00	<u>1.65</u>	<u>2.01</u>	1.38	1.64	1.88	<u>1.88</u>	<u>1.79</u>
Shape of scutellum	0.55	0.71	0.72	0.78	0.64	0.89	0.78	<u>0.64</u>	<u>0.85</u>	0.73	0.85	0.82	<u>0.98</u>	<u>0.80</u>
Shape of embryo axis	0.46	0.53	0.43	0.53	<u>0.53</u>	0.59	0.53	<u>0.47</u>	0.58	0.54	<u>0.58</u>	0.60	<u>0.63</u>	0.53

Dimensions are given in mm. Extreme accessions among AABB tetraploids and among AABBDD hexaploids are underlined.

and *T. spelta* with tough rachis (*spelta tr*) are extremes in the first two principal components plane. *T. spelta* having a brittle rachis (*spelta br*) is in the central position of the diagram (Fig. 3). The head and embryo morphology in both spel-tas may vary according to the expression of their *Q* and *Tg* genes. Differentiation of *Triticum vulgare* cultivars is pronounced. Each of these cultivars is of a very different origin ('Ostka Popularna' – Poland, 'Gigas' – Israel, 'Nap Hal' – Himalayas, 'Atlas 66', 'Nebraska 11029' – USA). The 2x, 4x and 6x wheats were described with embryo characters having a significant genetic variation (Kosina 1995a). Short comparative data are included in Table 1. Among the AABB tetraploids *Triticum ispahanicum* and *T. carthlicum* are best differentiated, but *T. dicoccum* also has extreme values of some traits. There is, however, a similarity between Emmer and *T. durum*. Among the 6x wheats *T. sphaerococcum* and *T. spelta* are most contrasted. Differences between embryos of *T. sphaerococcum* and *T. compactum* are very distinct when the linear characters: width of embryo axis, width of epiblast, length of scutellum, length of embryo axis, height of epiblast, length of coleoptile and length of radicle are used for comparison. The ratios describing embryo shapes are not useful for this purpose. An embryo undergoes small alterations during charring of fossil grain (dimensions), however, some deformation is visible (coleoptile is curved towards pericarp). The usefulness of embryo morphology for discrimination of fossil wheats has been pointed out by Kosina 1991. Morphology of embryo distinguishes well e.g. species of *Bromus* from different sections (Kosina 1996). The discrimination between *T. sphaerococcum* and *T. compactum* by this way could be useful to check the materials described by Rego and

Rodriguez (1993), because a general morphology of grain fails when more cultivars of both wheats are compared. Discrimination of wild and cultivated diploids and the AAGG wheats is rather simple.

Morphology of glume, lemma and palea epidermis

Observations were made in the middle intercostal region of each bract. The abaxial epidermis contains long cells with sinusoid walls frequently pitted; short cells: well visible cork cells and silica cells with thinner wall, low round papillae with numerous pits and short or longer hairs. The variability of shapes and types of cells depending on their location within a bract is indisputable. Upwards of the organ the papillae are often changed into hairs. Sideways the walls are thinner and the frequency of short cells is altered. Change of dimensions of some epidermal cells is correlated with ploidy level. Such a trend is especially clear for epidermis of lemma and palea (Table 2). This tendency may be caused by different doses of the *Q* gene altering size of cell and chemical composition of the wall (Mac Key 1989b). Some wheats may differ qualitatively from others, e.g. papillae were not observed in glume epidermis of *T. spelta* and *T. dicoccum*, while in *T. zhukovskyi* cork cells are absent in lemma epidermis. *T. zhukovskyi* (AAAAGG genomes) differs clearly from the AABB and AABBDD wheats (Fig. 6). Differences in epidermis structure are rarely of qualitative nature. They are most often quantitative (see Table 2). Cells of glume epidermis (Fig. 5) have a different wall thickness (thin in *T. dicoccum*), various folding of walls perpendicular to long cells (cf. *T. monococcum* and *T. dicoccum*), different shape of cork and silica cells (see *T. durum* and *T. sphaerococcum*). The cork cells in *T. monococ-*

Fig. 3. Ordination of wheat hexaploids along the first two principal components with application of eight traits (see text) best describing the morphology of embryo.

Fig. 4. Scattering of reciprocal hybrids of F₁ generation and their parents, *Triticum carthlicum*, *T. durum*, *T. polonicum* and *T. dicoccum*, along the first two principal components with use of six traits of embryo morphology and caryopsis anatomy best discriminating parents and offspring. The traits: length of coleoptile, width of pigment strand, thickness of external part of pericarp, length ratio of coleoptile and radicle, shape of scutellum, and shape of embryo axis display significant genetic variation.

cum are distinctly smaller than in other wheats and this is also true of the silica cells, not visible in Fig. 5. In the epidermis of lemma (Fig. 6) the shapes of short cells and of perpendicular walls are very differentiated among wheats. Tubb et al. (1993) indicated that papillae and their pits are useful for discrimination of fossil cereals. It is corroborated by Fig. 7, where the difference between diameters of papilla pits is very clear in relation to *T. compactum* and *T. sphaerococcum*. In the latter they are almost twice larger. This may be an additional tool for discrimination of sphaerococcoid wheats from Portugal (Rego and Rodriguez 1993).

On the other hand, Consaul and Aiken (1993) concluded about the small utility of palea characteristics for the taxonomic interpretation of *Festuca* species. In wheats these traits may be used for the identification of species, but one should know their variability within a given organ, between organs, and between cultivars of the given species. For instance, the hairiness of inflorescence changes the structure of epidermis. Sometimes variation of some characters may overlap, being less useful for the researcher. Between wheats and other cereals, as well as, wild grasses there are distinct qualitative dif-

TABLE 2. Dimensions of characters in inflorescence bract epidermis given for some contemporary species of the genus *Triticum*.

Wheats	Characters of bract epidermis											
	External width of long cell			External diameter of papilla			Internal width of cork cell			Amplitude of wall sinusoid		
	Glume	Lemma (x)	Palea (x)	Glume (x)	Lemma (x)	Palea (x)	Glume	Lemma	Palea (x)	Glume	Lemma (x)	Palea
<i>T. monococcum</i>	38	23	26	22	22	24	8	10	16	17	10	16
<i>T. dicoccum</i>	38	30	36	no	34	38	22	22	26	10	13	13
<i>T. durum</i>	50	32	32	48	34	44	26	20	28	21	17	18
<i>T. spelta</i>	34	44	48	no	44	50	26	26	30	13	17	18
<i>T. compactum</i>	44	46	50	42	44	48	18	26	32	20	18	18
<i>T. sphaerococcum</i>	38	38	40	40	38	46	24	20	30	18	14	16
<i>T. zhukovskyi</i>	48	30	28	32	30	34	18	no	20	21	14	13

Dimensions are given in micrometers. x – a charakter displaying the trend with ploidy level

Fig. 5. Abaxial epidermis of glumes of: A – *Triticum monococcum*, B – *T. dicoccum*, C – *T. durum*, D – *T. sphaerococcum*. For Figs 5-7: CC – cork cell, SC – silica cell, P – papilla; bar 40 micrometers.

Fig. 6. Abaxial epidermis of lemmas of: A – *T. dicoccum*, B – *T. spelta*, C – *T. compactum*, D – *T. zhukovskyi*.

Fig. 7. Abaxial epidermis of paleas of: A – *T. dicoccum*, B – *T. durum*, C – *T. compactum*, D – *T. sphaerococcum*. In C and D pits in papillae are outlined.

ferences (Kosina 1995b). Such differences were also evidenced by Parry and Smithson (1966).

MOLECULAR APPROACH TO WHEAT VARIATION

Molecular analyses of ctDNA may show donors of cytoplasms of allopolyploid wheat. Ogihara and Tsunewaki (1983) studying restriction fragment patterns of ctDNA distinguished three types of chloroplast genomes in wheat: 1. *monococcum-urartu*, 2. *timopheevi-zhukovskyi*, 3. *diccoides-dicoccum-aestivum-selta*. The authors stated the identity of cytoplasm from *T. timopheevi* and *Aegilops longissima*. The latter, closely related to *Ae. speltoides*, has cytoplasm of identical pattern of restriction fragments as Emmer and common wheat. Dvorak and Zhang (1990) analysed the variation in repeated nucleotide sequences of the genomic DNA and proved that *Ae. speltoides* was the closest relative of the G genome of *T. timopheevi* and B genome of *T. turgidum*. They suggest also that the *T. turgidum* lineage should be more ancient than the *T. timopheevi* lineage. Additionally Daud and Gustafson (1996) applied the *Ae. speltoides*-specific DNA sequence, pSp89.XI, and showed by the dot blot technique and Southern hybridization the presence of this sequence in genomes of *T. turgidum* and *T. aestivum*. Application of the RFLP method (restriction fragments length polymorphism of DNA) provided evidence that the A genome of both tetraploid series, AABB and AAGG, is of *T. urartu* origin (Dvorak and Zhang 1991). Jiang and Gill (1994a) came to a similar conclusion examining dislocations of rRNA gene loci in genomes of polyploid wheats.

Analyses of wheat genomes complete the data on the amount of repeated DNA in plant genomes which is in excess of 75% of the total DNA (Flavell 1985). Less than 1% of the total DNA is in the transcribed genes (Flavell 1989). The linear organization of related sequences varies between species. The variants of sequences in related species are amplified. The products of amplification, deletion, turnover and transposition events produce major chromosomal structural differences between diverging species (Flavell 1985). The differentiation of translocations is another characteristic feature of wheat genomes. Kawahara (1984), using methods of classical cytogenetics, found differences in type and amount of translocations between wild tetraploids of both series, AABB and AAGG. Rayburn and Gill (1988) analysed in both groups distribution patterns of a highly repeated 120 bp DNA sequence on chromosomes by in situ hybridization. This sequence is recognized as ancient. Distinct differences in hybridization patterns were established between the B and G genomes. The authors concluded that the divergence of the tetraploid wheats is caused by changes in location, but not in amount of the repeated sequence. Jiang and Gill (1994b), by means of a sequential N-banding of chromosomes and genomic in situ hybridization, found species-specific translocations in *T. timopheevi* and *T. turgidum*. In *T. turgidum* they relate to 4A, 5A and 7B chromosomes. The authors concluded then about diphyletic origin of both wheats. Chromosome translocations are also genome-specific. Kawahara et al. (1996) discovered in G genome of the highly polymorphic wild *Triticum araraticum* several times more chromosome breakpoints than in A genome. The G one is distinctly more heterochromatic. An important supplement into our knowledge on evolution of wheat genomes was given by Liu et al. (1998). They found that wheat allopolyploid formation is connected with rapid and nonrandom elimination of specific low-copy, noncoding

DNA sequences (0.206-2.8 kb). These sequences are present in all diploid progenitors. The authors proved also that such changes of DNA are not related with DNA methylation process. This new way of the genome change may create the diploidization system in polyploid wheats.

The genomic in situ hybridization was applied in the present paper for detection of translocations between A and B genomes in an amphiploid composed of *T. turanicum* and *Ae. squarrosa* (see Fig. 8A). The identification of such a translocation is possible by use of a probe of the genomic DNA from diploid wheat (*T. monococcum*). Application of an rDNA probe makes also possible localization of the NOR sequences. Two NORs are in terminal position on *Ae. squarrosa* chromosomes. The A-B translocations and other cytogenetic anomalies are related in this amphiploid to morphological instability – somatic mutations of spike and spikelet colour (Kosina 1994). In trigeneric amphiploid including genomes of *T. durum*, *Lophopyrum elongatum* and *Thinopyrum distichum* the centromeric fissions occur, giving numerous telocentrics and then translocations of the whole arms of chromosomes (Kosina and Heslop-Harrison 1996). Besides telocentrics of *T. durum* origin there are also green ones of *Th. distichum* origin (see Fig. 8B). The above indicates a distinct gross reorganization of wheat genomes which may lead i.a. to saltatory morphological and cytogenetic differentiation, e.g. *T. timopheevi*-like mutant derived from *T. diccoides* (Kushnir and Halloran 1983a, b).

A spatial organization of genomes in nucleus and different expression of their genes are other fascinating phenomena which may create new variation in wheat plant. In such a case the genes of the outer genome have a tendency to dominate in a plant phenotype. It was proved that the genomes of allopolyploid plants are separated in interphase nucleus and during karyokinesis (Leitch et al. 1991; Bennett and Bennett 1992; Kosina and Heslop-Harrison 1996). Heslop-Harrison and Bennett (1984) relate a chromosome order to such developmental processes as root/shoot differentiation or juvenile/mature stages of development. The genomic DNA in situ hybridization in trigeneric hybrid (Fig. 8C) exhibits not only the separation of the *Th. distichum* genome from wheat genomes, but also the same between A and B genomes. The green A genome is separated from the brown B genome by the red *Th. distichum* genome. The different expression of genes depending on the genome location in nucleus leads often to dominance of a trait complex of one parent (parental dominance or genome imprinting) in progeny (Heslop-Harrison 1990). Kosina (1997) found such relationships in some intergeneric amphiploids of the Triticeae. It is also clear in Fig. 4 where hybrids of the F₁ generation between four tetraploid wheats are located close to *T. dicoccum*. They are described by several caryopsis traits displaying significant genetic variation (Kosina 1995a). Then, a *Triticum dicoccum* group, like a natural hybrid swarm, is created. The above shows dominance of a trait set from *T. dicoccum* as well as a recessive status of *T. carthlicum*, *T. durum* and *T. polonicum*. It may relate to spatial organization of A and B genomes and their different expression. It may also indicate that genomes of the same kind are not equivalent with each other (compare Liu et al. 1998) in different species or even varieties of wheat. Genomes of *T. dicoccum* are older than those of the other three tetraploids. It was evidenced by chlorophyll mutations in wheats of different geographic origin (Mac Key 1981b) as well as by the frequency of duplicate non-allelic interactions (Kosina 1995a). Different age of the genomes in various wheats influences differentiation of the DNA sequences to the different

Fig. 8. Data on the genomic DNA and rDNA sequence in situ hybridization.

Fig. 8A. A root metaphase of the *Triticum turanicum* and *Aegilops squarrosa* amphiploid. The *Ae. squarrosa* genomic DNA probe (anti-biotin-streptavidin Cy3) marks orange chromosomes of goatgrass (dotted on diagram). The *Triticum monococcum* genomic DNA probe (FITC anti-digoxigenin) marks chromosomes of the A genome of wheat by light green. Chromosomes of the B genome of wheat are dark green. The A-B translocation is arrowheaded. The rDNA sequence, pTA71, marks sites of NOR activity by a vivid green colour.

Fig. 8B. A root metaphase of the trigeneric, *Triticum durum*, *Thinopyrum distichum* and *Lophopyrum elongatum* amphiploid. The *Th. distichum* genomic DNA probe (FITC anti-digoxigenin) paints its chromosomes in green (dotted on diagram). The *T. durum* genomic DNA probe (anti-biotin-streptavidin Cy3) marks wheat chromosomes as green-yellow. The sites of NOR activity (the pTA71 sequence probe) are red. Telocentric chromosomes of wheat are arrowheaded. C – a root prophase of the trigeneric amphiploid. Three probes were used: the genomic DNA from *Triticum monococcum* and rDNA sequence (FITC anti-digoxigenin) revealed chromosomes of the A genome and sites of NOR activity as green; the genomic DNA from *Lophopyrum elongatum* (anti-biotin-streptavidin Cy3) marks chromosomes of the wheatgrass as red. Chromosomes of the B genome remain brown. Distribution of genomes in some parts of a nucleus is arrowheaded. In the diagrams the sites of the NOR-activity are shown as black.

level. Comparing a spikelet structure in *T. urartu*, *Ae. speltoides* and *T. dicoccum* it is visible that rather the A genome of *T. urartu* is dominating in *T. dicoccum*. The same should be expected in the mentioned hybrids. The A genome is just outside in the trigeneric amphiploid (see Fig. 8C). A spatial arrangement of the A, B and D genomes in different cultivars may hide some variation and creates new its patterns.

CONCLUSIONS

1. The data on plant morphology (head, grain) related to wheat ecotypes, as well as, information on ways of species scattering, derived from a study of gene necrosis in old local cultivars, supplement considerably our considerations on variation of fossil wheats.

2. Morphology of wheat lodicules displays a trend dependent on ploidy level: 2x-4x and 6x-8x. Among 4x and 6x wheats the differentiation of morphology is related to threshability. Young polyploids, types like mutation or endemics, are recognized as extremes.
3. *Triticum dicoccoides*, *T. dicoccum*, *T. turgidum* (sensu stricto) exhibit large variation of traits related to grain anatomy and embryo morphology. *T. carthlicum*, *T. ispanicum*, *T. urartu*, *Ae. speltoides* and *Ae. squarrosa* appeared in this analysis as extremes. Both goatgrasses are distant from wheats.
4. *T. zhukovskyi*, *T. sphaerococcum* and *T. vulgare* are extremely differentiated with regard to embryo morphology. Such a differentiation is also visible between cultivars of common wheat as well as a threshable and unthreshable *T. spelta*. Embryo traits enable the distinction of fossil *T. compactum* and *T. sphaerococcum* as well as other species..
5. Epidermis of inflorescence bracts in wheat may differ qualitatively and quantitatively. Some traits of the epidermis are correlated with ploidy level.
6. Wheat genomes are changed by translocations of minor fragments or whole arms of chromosomes. These changes may be detected in detail by means of the genomic DNA in situ hybridization.
7. A and B genomes in a nucleus are spatially separated. It may induce a different expression of their genes and as a consequence there appears a parental dominance in hybrids or one diploid progenitor in the polyploid species. The differentiation of cultivars may be directed by this phenomenon.

ACKNOWLEDGEMENTS

This work has been financed by a grant (Nr 6 P204 032 05) from KBN (State Committee for Scientific Research). The kind cooperation with Prof. Dr. J.S. (Pat) Heslop-Harrison from John Innes Institute, Norwich provided data on genomic DNA in situ hybridization.

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WYBRANE ASPEKTY ZMIENNOŚCI PSZENICY – OD PALEOBOTANIKI DO BIOLOGII MOLEKULARNEJ

STRESZCZENIE

W przeglądzie wskazano na użyteczność danych na temat ekotypów pszenicy oraz rozprzestrzeniania genów nekrozy dla interpretacji materiałów kopalnych, których oznaczenie jest dyskusyjne. Przedstawiono na przykładach oryginalnych badań zmienność mikrostrukturalną (łuszczyki, anatomia ziarniaka, zarodek, epidermy liści kwiatostanów) współczesnych pszenic. Dane te również są użyteczne w studiach materiałów kopalnych. Zmienność wymienionych organów rośliny wykazuje często trend zbieżny z poziomem ploidalności. Dyskryminacja kopalnych form *Triticum compactum* i *T. sphaerococcum* jest możliwa przy wykorzystaniu cech zarodka. Zmienność pszenicy kreowana jest przez ewolucję jej genomów, które podlegają m.in. licznym translokacjom chromosomów. Genomy pszenicy są w jądrze przestrzennie rozdzielone. Ten stan wykazano metodą hybrydyzacji in situ genomowego DNA. Stwierdzono dominację jednorodzielską kompleksu cech ziarniaka w mieszańcach pszenic tetraploidalnych. Ten typ genomowego imprintingu może być zależny od przestrzennej organizacji genomów – część zmienności zostaje ukryta oraz tworzone są jej nowe wzory.

SŁOWA KLUCZOWE: pszenica, zmienność, ekotypy, mikromorfologia, hybrydyzacja in situ, genomy.