Evaluation of protein fractions as biochemical markers for identification of spelt wheat cultivars (*Triticum spelta* L.)

V. Dvořáček[1], V. Čurn[2]

[1]Research Institute of Crop Production, Prague-Ruzyně, Czech Republic
[2]Faculty of Agronomy, University of South Bohemia in České Budějovice, Czech Republic

ABSTRACT

Four protein fractions: 1 – albumins and globulins, 2 – gliadins, 3 – glutenins (extracted in NaOH), 4 – glutenins (extracted in SDS) separated by SDS-PAGE were used as biochemical markers for evaluation of polymorphism level in three spelt wheat cultivars – Hercule, Altgold and Rouquin, three new–breeders' spelt lines – H92.27, H92.28 and M92.20 (originated from hybridisation between spelt and common wheat) and reference common wheat cultivar Brea. Electrophoretic phenotypes and zymograms were evaluated by means of digital image analysis and Nei and Li coefficient of similarity was used to evaluate the relation of analysed genotypes. Entire evaluation of all four-marker systems showed differences between common wheat cultivar Brea and spelt cultivars and spelt breeders' lines. Also significant differences between old spelt cultivars (Hercule, Altgold and Rouquin) and new spelt breeders' lines were found. The reality of the mutual passing of protein fractions (gliadins and glutenins), based on Osborne extraction was confirmed. In this sense it is necessary to see both fractions as dynamic overlapping structures.

Keywords: spelt wheat; biochemical markers; seed proteins; electrophoresis; protein fractions

In the middle of nineties in Czech Republic, the ecological agriculture began to assert in the greater scale, in comparison with the previous period. Within that the other non-traditional crops began to be cultivated including spelt wheat (*Triticum spelta* L.). From 1995 to 2000 many various Swiss and German cultivars of spelt wheat were evaluated with reference to yield and quality (Moudrý 1999, Moudrý and Dvořáček 1999, Dvořáček et al. 2002). High morphological similarity of some cultivars did not guarantee the correct determination. Because of that, it was tested to achieve more exact identification with biochemical and molecular markers (Harsch et al. 1997, Šašek and Černý 1997). The high similarity, especially in old German cultivars, was also confirmed with these determination methods (Radić-Miehle et al. 1997). The seed storage proteins (gliadins and glutenins) were the most used marker system for wheat and spelt identification, albumins and globulins were used only marginally. In our previous study, the esterase isozymes were only important for the spelt cultivars identification (Dvořáček et al. 2001b). The seed storage proteins are associated with agriculturally significant traits and they are used in a legal protection of cultivars (Černý and Šašek 1996, Harsch et al. 1997, Knoblochová and Gálová 2000).

In consequence of the easy crossability between spelt and common wheat and their similar character of gliadins and glutenins pattern it is presumed an analogous localisation of genes encoding these proteins in the spelt genome (Harsch et al. 1997, Radić-Miehle et al. 1998). Canadian authors found the basic differentiating regions of spelt gliadins, in which the heaviest group of omega gliadins (100–113 kDa) and higher number of alpha gliadin bands are absent in comparison with common wheat (Abdel-Aal et al. 1996). German authors constructed ideal spelt gliadin pattern, where the heaviest group of omega gliadins was also absent, in comparison with common wheat, and additionally on the basis of A-PAGE analyses they described a new D-zone of bands, located between omega and gamma gliadins. That D-zone was not found in common wheat (Harsch et al. 1997). Radić-Miehle et al. (1998) compared the electrophoretic pattern of glutenins between spelt and wheat. This pattern was characterised with the molecular weight from 30 to 113 kDa. Common wheat showed six typical bands (44, 45, 57, 100, 106 and 112 kDa) and spelt was characterised with typical bands with molecular weight 48, 50, 60, 62, 66, 85, 92, 105, 110 and 113 kDa. Also Knoblochová and Gálová (2000) studied a wide set of 33 spelt cultivars and found the polymorphic protein pattern in six cultivars (H92.20, H92.27, KR489-11-15, Lueg, LW12, Schwabenkorn).

Although Osborne protein fractionation is known and has been often used for almost one century, many studies proclaim, that bounders among protein fractions are

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not sharp and method is influenced by temperature, time of extraction and intensity of shaking (Byers et al. 1983). It was found that HMW glutenin zones are passing to the pattern of gliadins and also mutual overlapping between LMW glutenins and electrophoretic pattern of alfa, beta and gamma gliadins in conditions of the SDS-PAGE (Abdel-Aal et al. 1996). Mosse and Baudet (1963) reported that about 50% of the flour protein could be extracted with water at 22°C. Whence it follows, that a considerable part of next protein fractions (globulins, gliadins and glutenin) were included in this albumin fraction. Changes in protein pattern can be influenced by external factors e.g. cultivation of spelt cultivars in conditions of low and high sulphur level in the soil or using of reducing detergents e.g. dithiotreitol as extraction buffer (Kim and Bushuk 1995).

The aim of our study was evaluation of polymorphism of three protein fractions (1 – albumins and globulins, 2 – gliadins, 3 – glutenins) and their suitability for the identification of spelt wheat cultivars.

MATERIAL AND METHODS

In this study six cultivars of spelt wheat were analysed: one traditional Swiss cultivar Altgold, two Belgian cultivars Hercule and Rouquin whose origin relates to Altgold cultivar and three Swiss new spelt hybrids from the breeding station Triemenhof-Girenbad H92.28 (spontaneous hybrid between *T. aestivum* – cv. Nyon and non identified spelt cultivar), H92.27 (spontaneous hybrid between *T. aestivum* – cv. Avalon and non identified spelt cultivar) and M92.20 (new breeding between *T. aestivum* Avalon/Altgold). The Czech cultivar of common winter wheat – Brea was analysed as the reference cultivar. Fifteen individual grains were tested of each cultivar. Individual protein fractions were extracted by the standard Osborne method from 1907 (Králová et al. 1991). Dual slab gel electrophoretic system Hoefer SE 600 was used for analyses. Protein fractions were lyophilised and extracted by the method according to Dvořáček et al. (2001b, c).

Standard ISTA SDS-PAGE was done according to Wrigley (1992). Data analysis: electrophoretic phenotypes were digitalised and evaluated using specialised software Bio-Profil 1D++ (Vilber Lourmart). Similarity matrixes were calculated using Nei and Li coefficient of genetic similarity and dendrograms were calculated using UPGA method and 95% confidence Dvořáček et al. (2001a).

RESULTS AND DISCUSSION

Pattern of proteins dissolved in Na-phosphate buffer (albumins and globulins)

Albumins and globulins were characterized by rich protein pattern – the number of bands varied from 24 (H92.28) to 32 (Hercule and M92.20) and they were defined by molecular weight 106–2 kDa (Figure 1). The protein pattern of albumins and globulins was divided into two relatively wide areas 66–23 kDa and 16–2 kDa. The electrophoretic pattern of tested cultivars showed high similarity. The differentiating areas were located to the three zones: the 1st with molecular weight 92–87 kDa, the 2nd with molecular weight 32–22 kDa, the 3rd with molecular weight 19–14 kDa.

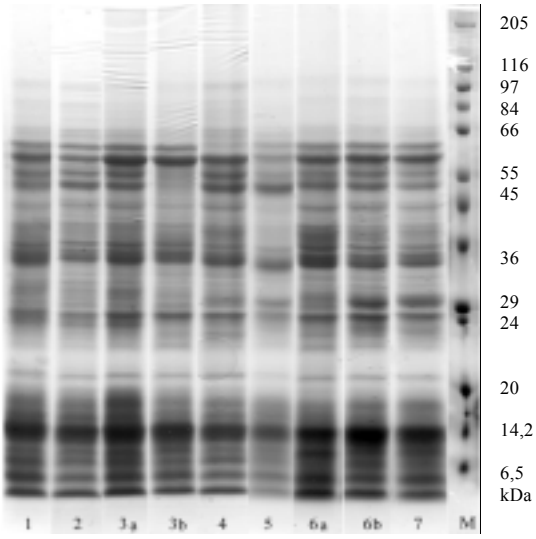

Figure 1. Albumins and globulins, SDS-PAGE

T. spelta: Altgold (1), Hercule (2), Rouquin (3), H92.27 (4), H92.28 (5), M92.20 (6), *T. aestivum*: Brea (7)

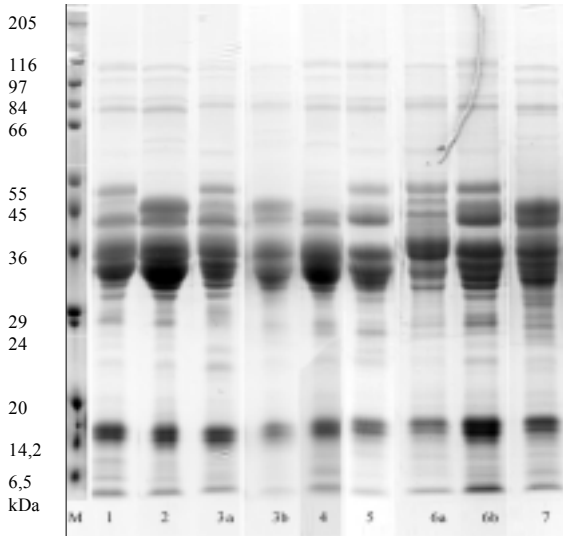

Figure 2. Gliadins, SDS-PAGE

T. spelta: Altgold (1), Hercule (2), Rouquin (3), H92.27 (4), H92.28 (5), M92.20 (6), *T. aestivum*: Brea (7)

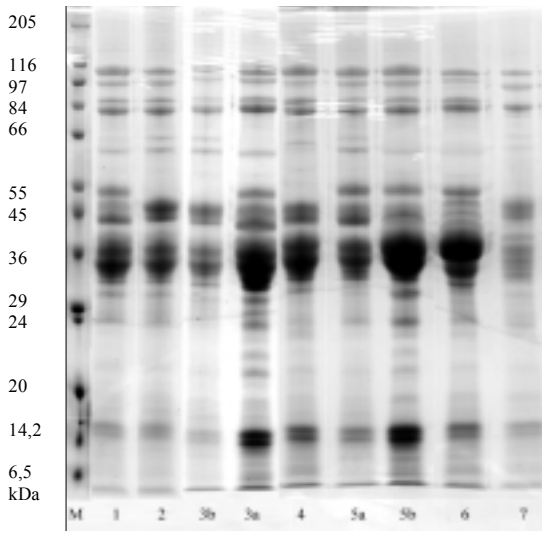

Figure 3. Glutenins (NaOH), SDS-PAGE

T. spelta: Altgold (1), Hercule (2), Rouquin (3), H92.27 (4), H92.28 (5), M92.20 (6), *T. aestivum*: Brea (7)

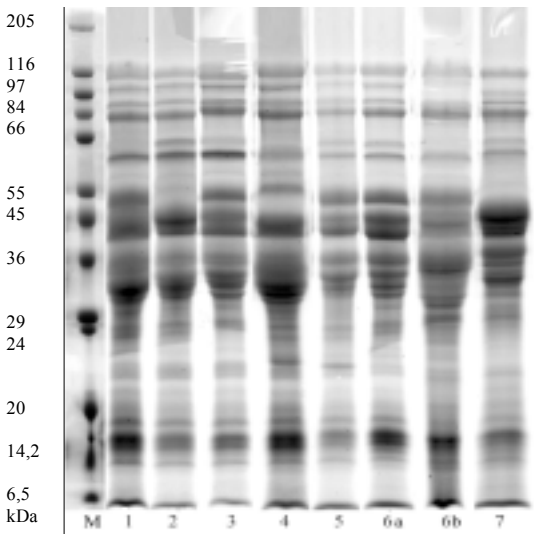

Figure 4. Glutenins (SDS), SDS-PAGE

T. spelta: Altgold (1), Hercule (2), Rouquin (3), H92.27 (4), H92.28 (5), M92.20 (6), *T. aestivum*: Brea (7)

The similarity matrix and the resulting dendrogram (Figure 5) characterised similarity coefficients and mutual position of the tested cultivars. Although the results showed high similarity, the tested cultivars were firmly identified. Rouquin and the breeders' line M92.20 were characterised by two different protein patterns that were marked with letters (a) and (b). The finding predicates higher level of the intra-varieties polymorphism in these cultivars.

The highest mutual similarity was found between cultivars Hercule and Rouquin(a) – 0.98 and also cultivars M92.20(b) and common wheat Brea showed the high mutual similarity – 0.97. On the other hand, the lowest value of the similarity coefficient was found between cultivars Brea and H92.28 – 0.80.

Pattern of proteins dissolved in 25% 2-chlorethanol (gliadins)

Gliadin pattern showed analogous number of bands ranging from 22 Rouquin(b) to 31 bands from Brea and M92.20(a) and also analogous range of a protein pattern characterised with molecular weight from 115 to 3 kDa (Figure 2), but with considerably higher polymorphism among tested cultivars. Total protein pattern was possible to separate in three zones based on their molecular weight. The 1st zone was characterised by molecular weight ranging from 115 to 80 kDa and it was created with four thin bands with specific changes in molecular weight among cultivars. The 2nd zone showed the highest polymorphism and it was characterised by molecular weight from 53 to 24 kDa. The 3th zone was uniform with range 16–3 kDa.

The resulting dendrogram (Figure 6) showed, in comparison with albumin and globulin pattern, the wider range of similarity coefficient (0.98 –0.62) among tested cultivars. It follows much better suitability of gliadins for identification of tested cultivars. Analogous to the previous marker system it was found two variants of gliadin pattern in cultivar Rouquin and the breeders' line M92.20.

Pursuant to gliadins, the traditional Swiss and Belgium cultivars were classified together except Rouquin(b). These groups differed significantly from all the breeders' lines and from the cultivar Brea. The lowest value of the similarity coefficient was found, analogous to pattern of albumins and globulins, between the breeders' line H92.28 and the cultivar Brea – 0.62.

Pattern of proteins dissolved in 0.02M NaOH (glutenins)

Glutenin pattern was almost uniform with above-mentioned pattern of gliadins and only minimal qualitative differences were found in area of molecular weight 36–20 kDa (Figures 3 and 10). Some differences could be originated with different bands intensity of both patterns. The similarity coefficient showed, analogous to gliadin pattern, high value of polymorphism among cultivars (0.96–0.64). Some polymorphism differences were found within tested cultivars. In accord with the gliadin pattern two heterogeneous bands were found within pattern of cultivar Rouquin and newly in the breeders line H92.28. On the contrary, the cultivar M92.20 appeared in pattern of glutenins as uniform. These changes of polymorphism within cultivars evoked differences in the resulting den-

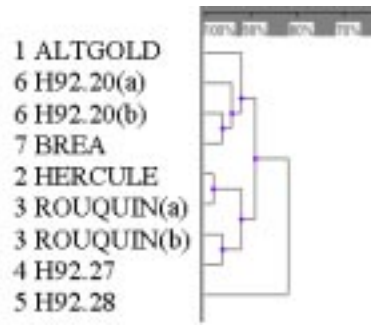

Figure 5. Dendrogram – albumins and globulins, Nei and Li coefficient

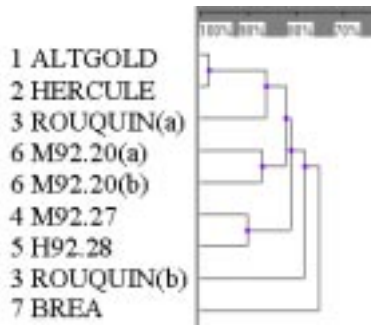

Figure 6. Dendrogram – gliadins, Nei and Li coefficient

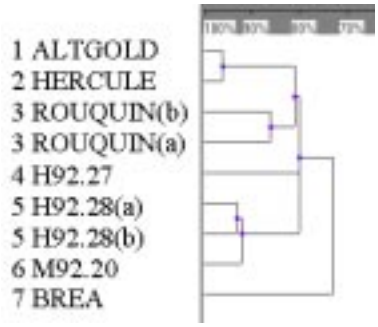

Figure 7. Dendrogram – glutenins (NaOH), Nei and Li coefficient

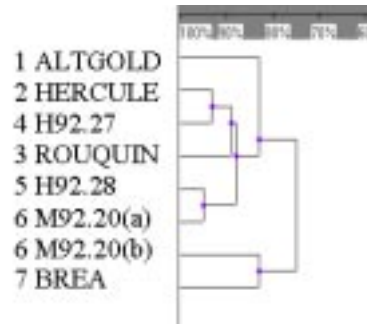

Figure 8. Dendrogram – glutenins (SDS), Nei and Li coefficient

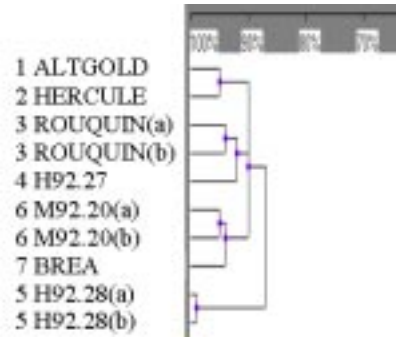

Figure 9. Dendrogram – entire evaluation of all four marker systems, Nei and Li coefficient

drogram in comparison with the result of gliadins, nevertheless the main principle of clusters construction was the same (Figure 7). The three cultivars Altgold, Rouquin (both variants) and Hercule were fused again together and they differed significantly from all breeders' lines and the cultivar Brea.

Pattern of proteins dissolved in SDS-extraction buffer (glutenins)

This remaining protein pattern, that was obtained after a separation of albumins, globulins and gliadins, had a analogous character of the pattern as previous gliadin and glutenin pattern with the range of the molecular weigh 115–4 kDa (Figure 4) and with number of bands ranging from 24 (H92.28) to 30 (H92.27). Only the breeders' line M92.20 was characterised with pair of polymorphic patterns. The lowest similarity coefficients were obtained between cultivars Brea vs. Altgold (0.69) and Brea vs. M92.20(a) (0.73).

Entire evaluation

The four marker systems (inclusive cultivars with non-uniform protein pattern) were analysed for a complex similarity evaluation of tested cultivars. The resulting dendrogram (Figure 9) confirms a close relationship of both Belgian cultivars Rouquin and Hercule and also their reference to Swiss cultivar Altgold. From the comparison of both spontaneous hybrids H92.27 and H92.28 it is possible to presume with a high probability, that genetic origin of both cultivars is different. The breeders' line H92.27 was inserted to the clusters with traditional spelt cultivars, while the breeders' lines H92.28(a + b) created isolated cluster different from other cultivars. The breeders' line M92.20 is also interesting. It did not show uniform pattern and in comparison with both breeders' lines (H92.28 and H92.27) was in a close relationship with the cultivar Brea. Non-uniform protein pattern was found in the cultivar Rouquin and in the

breeders' lines H92.28 and M92.20. These findings agreed (in case of the breeders' line M92.20) with Slovak authors (Knoblochová and Gálová 2000), the cultivar Rouquin was totally homogenous.

The seed proteins are very suitable and useful genetic markers, they are not influenced by external conditions and so they enable to identify tested plant genotypes (Černý and Šašek 1996). These proteins are divided to three groups: the 1st – storage proteins – gliadins and glutenins, the 2nd – proteins with a high metabolic activity – albumins and globulins, the 3th protein groups are structural proteins, very difficult to dissolve and fixed in a cell structure (Králová et al. 1991).

The evolutionary younger proteins (gliadins and glutenins), or some isozyme systems are suitable for the genetic identification, because they are specific for the determination of subspecies (Brdička 1981, Nielsen 1985, Černý and Šašek 1996). This experience is confirmed with the comparison of polymorphism among water soluble protein (albumins and globulins) and seed storage proteins (gliadins and glutenins) or with our previous study (Dvořáček et al. 2001b) characterising also some complexes of isozymes.

Patterns of albumins and globulins among cultivars were very similar and their character corresponded with the detection by American authors, who used water dissolved proteins for identification of two common wheats in non-reduction conditions (Kim and Bushuk 1995).

The protein groups of gliadins and glutenins (with similarity coefficient 0.6) are possible to consider as dominant determinant markers. The sum of homologous pattern is, in an entire analysis of several protein patterns, higher than in comparison with a partial analyse, and that is why resulting mutual similarity coefficient has the higher value. On the contrary, this result has for geneticists the more predicative value, because it includes the higher ratio of the tested genome (Kovach 1995).

The high similarity of gliadins and glutenins (NaOH) was already mentioned – Figures 2, 3 and 10. The reality of the mutual LMW-glutenins and gliadins overlapping or the ability of HMW-glutenin fraction passes to the gliadin fraction in conditions of SDS-PAGE was published very often (Pomeranz et al. 1988, Abdel-Aal et al. 1996, Ovesná et al. 2001) and from this viewpoint it is also possible to register a historical development. Miflin et al. (1983) recommended that all wheat endosperm proteins should be called gliadins, while Field et al. (1982) named the two major storage protein groups aggregative gliadins (glutenins) and non-aggregative gliadins. The other studies (based on combination of HPLC and SDS-PAGE methods, or two dimensional electrophoresis, genetic studies with monosomic lines and studies of amino-acids sequences in separated fractions) allowed to divide protein fragments and located them in a genome. Therefore, it happens, the declaratory reinterpretation of Osborne conception of the protein fractionations is based on exactly defined bounders of the protein solubility in the corresponding solvent. It is possible to suppose that protein patterns in the Figures 2, 3 and 10 are created with overlapping mixture of gliadins and glutenins. Analogous to Abdel-Aal et al. (1996) we identified four thin bands with the high molecular weigh in the range 115–80 kDa. This component is considered generally as HMW-fraction of glutenins. The other marked zones 2 (54 –24 kDa) and 3 (16–3kDa) will be probably compounded with fraction of gliadins and LMW-glutenins.

The SDS extracted glutenin fraction (SDS-glutenin) after previous pre-extraction of albumins, globulins and gliadins contents the other protein fractions, insoluble

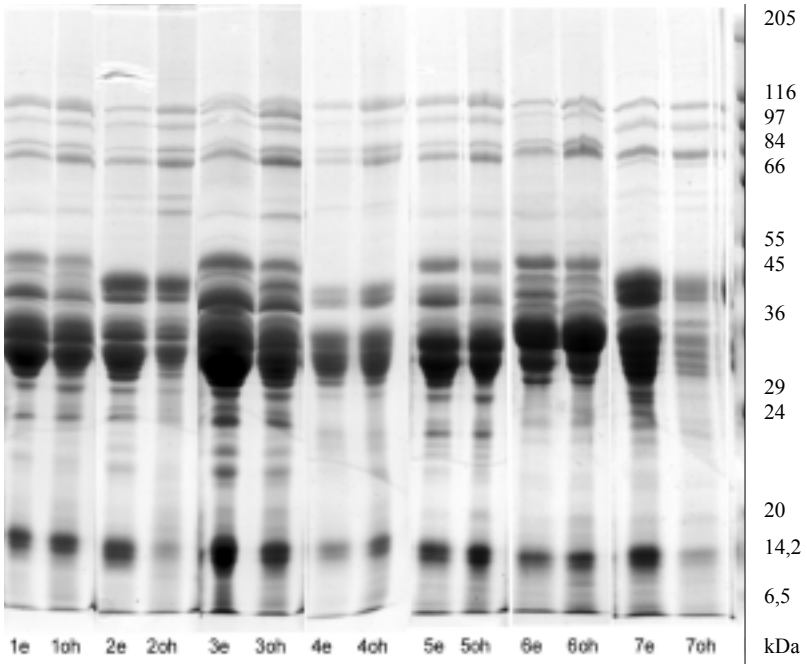

Figure 10. Comparison of gliadin and glutenin fractions (NaOH) in selected genotypes, SDS-PAGE

e – gliadins, oh – glutenins NaOH

T. spelta: Altgold (1), Hercule (2), Rouquin (3), H92.27 (4), H92.28 (5), M92.20 (6), *T. aestivum*: Brea (7)

in conditions of 0.02M NaOH (Figure 4). These protein fractions probably overlapped some differential areas, especially in breeders' lines or the cultivar that showed non-uniform band pattern in the other protein fractions.

Separation of protein fractions, based on their different solubility, and their utilisation as genetic markers seems to be very suitable for the identification of spelt cultivars. It succeeded to identify analysed cultivars and to classify traditional cultivars Altgold, Hercule and Rouquin to close clusters in according with German authors (Radić-Miehle et al. 1997). In the breeders' lines H92.27 and H92.28 we confirmed their different genetic origin. The reference cultivar of common wheat – Brea was markedly different from spelt cultivars Altgold, Hercule and Rouquin. On the other hand, the most similar cultivar to the common wheat was the breeders' line M92.20. Within three cultivars (Rouquin, H92.28 and M92.20) were found two different protein patterns predicative of their lower homogeneity. Nevertheless these groups of genotypes had no greater signification and they were situated side-by-side as the most similar clusters of the dendrogram.

We confirmed also the reality of the mutual protein fractions passing (gliadins and glutenins), based on Osborne extraction, and in this sense it is necessary to see both fractions as dynamic overlapping structures.

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ABSTRAKT

Hodnocení bílkovinných frakcí jako biochemických markerů pro identifikaci odrůd pšenice špaldy (*Triticum spelta* L.)

Čtyři bílkovinné frakce (1 – albuminy a globuliny), 2 – gliadiny, 3 – gluteniny (extrahované pomocí NaOH), 4 – gluteniny (extrahované pomocí SDS) byly děleny v podmínkách SDS na polyakrylamidovém gelu a použity jako biochemické markery pro hodnocení stupně polymorfismu u tří odrůd pšenice špaldy – Hercule, Altgold a Rouquin a tří novošlechtění – H92.27, H92.28 a M92.20 (vytvořených křížením pšenice špaldy s pšenicí setou) a referenční odrůdy pšenice seté Brea. Výsledné elektroforeogramy byly digitalizovány a pomocí Nei a Li koeficientu podobnosti byl vyhodnocen vztah mezi sledovanými genotypy. Celkové hodnocení všech čtyř markerovacích systémů vykazovalo diference mezi pšenicí setou Brea, odrůdami špaldy i hybridními liniemi. Rovněž byly zjištěny významné diference mezi původními starými odrůdami pšenice špaldy (Hercule, Altgold a Rouquin) a hybridními liniemi pšenice špaldy. V podmínkách Osbornovy extrakce byla potvrzena skutečnost vzájemného přechodu proteinových frakcí (gliadinů a gluteninů). Za těchto podmínek je nutné na obě bílkovinné frakce pohlížet jako na dynamicky prolínající se bílkovinné struktury.

Klíčová slova: pšenice špalda; biochemické markery; bílkoviny zrna; elektroforéza; bílkovinné frakce

Corresponding author:

Ing. Václav Dvořáček, Ph.D., Výzkumný ústav rostlinné výroby, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 418, fax: + 420 233 022 286, e-mail: dvoracek@vurv.cz