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Genetic variation of hordein polypeptides in grains of mutants of hulless spring barley (*Hordeum vulgare* L.) breeding line

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Abstract. Hordein polypeptides patterns of 146 mutants in M_7 from a hulless spring barley breeding line were examined. The mutants were obtained after treatment of grains of Polish hulless spring barley breeding line, 1N/86, with the chemomutagens N-nitroso-N-methylurea and sodium azide. The mutants were distinguished from the parental line with regard to morphological and yield structure traits. SDS-PAGE of the main hordein fractions, B and C, showed that the electrophoretic patterns of the majority of the analysed mutants were similar to the hordein polypeptide spectrum of the parental line. However, specific hordein patterns were obtained from six of the mutants. In the previous study, the same mutants showed a significantly better feeding value as compared to hulled cultivars and the rest of the analysed mutants.

Key words: hordein, *Hordeum vulgare*, hulless mutants, polypeptide spectrum.

Introduction

The hull constitutes 10-13% of the dry weight of barley grain and its removal reduces the crude fiber content. Thus, the level of digestibile energy (DE) of hulled barley is lower because the high content of crude fiber has a negative influence on DE for feeding purpose of livestock. In addition, in contrast to hulled barley, hulless grain has a higher protein content and a similar or better composition of amino acids (BHATTY et al. 1975, BHATTY, ROSSNAGEL 1981).

Hordeins, the major storage proteins of barley are alcohol-soluble proteins (prolamins) which are characteristic of cereal grain. They constitute a complex

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mixture of polypeptides and are classified into four groups, called B, C, D and gamma hordeins, which differ in molecular mass, isoelectric point and amino acid composition (KØIE et al. 1976, SHEWRY et al. 1978a, DOLL, BROWN 1979, SHEWRY et al. 1980a, b). There is also a considerable genotypic variation in the polypeptides present in each group (SHEWRY et al. 1978b, 1979, KAPAŁA 1981, 1983, KAPAŁA, PATYNA 1987). While D and gamma hordeins are only minor compounds, B hordeins account for 70-80% and C hordein for 10-20% of the total hordein under conditions of normal nitrogen nutrition (SHEWRY et al. 1978c). Variation in the relative amounts of B and C hordeins with time of development or nutrition suggests that, at least under some conditions, the two groups are under separate regulatory control (SHEWRY, MIFLIN 1982, SHEWRY et al. 1978b, 1979, 1983, KAPAŁA, PATYNA 1993).

Hordein production is controlled by multigene families. In cultivated barley all the hordein genes are located on chromosome 5, where they are organized in complex loci (JENSEN 1981, HEIDECER, MESSING 1986, PELGER et al. 1993). The B hordeins are encoded by a major locus, *Hor2*, and a minor locus, *Hor4* (NETSVETAEV, SOZINOV 1984, SHEWRY et al. 1988). The "C" hordeins and the B hordein – related gamma hordeins – are encoded by the *Hor1* and the *Hor5* locus, respectively (NETSVETAEV, SOZINOV 1982, SHEWRY, PARMAR 1987). These four loci are situated on the short arm of the chromosome, while the *Hor3* locus, encoding the D hordeins, is located on the long arm (BLAKE et al. 1982).

In the present study, hulless barley mutants were analysed for their electrophoretic hordein patterns to establish the range of genetic variability induced by chemical mutagenesis.

Material and methods

Plant material

146 mutants (M_7) of a hulless spring barley breeding line were obtained after treating kernels of the 2-rowed hulless Polish line 1N/86 with N-nitroso-N-methylurea and sodium azide (KAPAŁA, RYBIŃSKI 1995). Selection of the mutants was made in M_2 and the main criterion for selection was diversity of morphological and yield structure traits in comparison with the parental line.

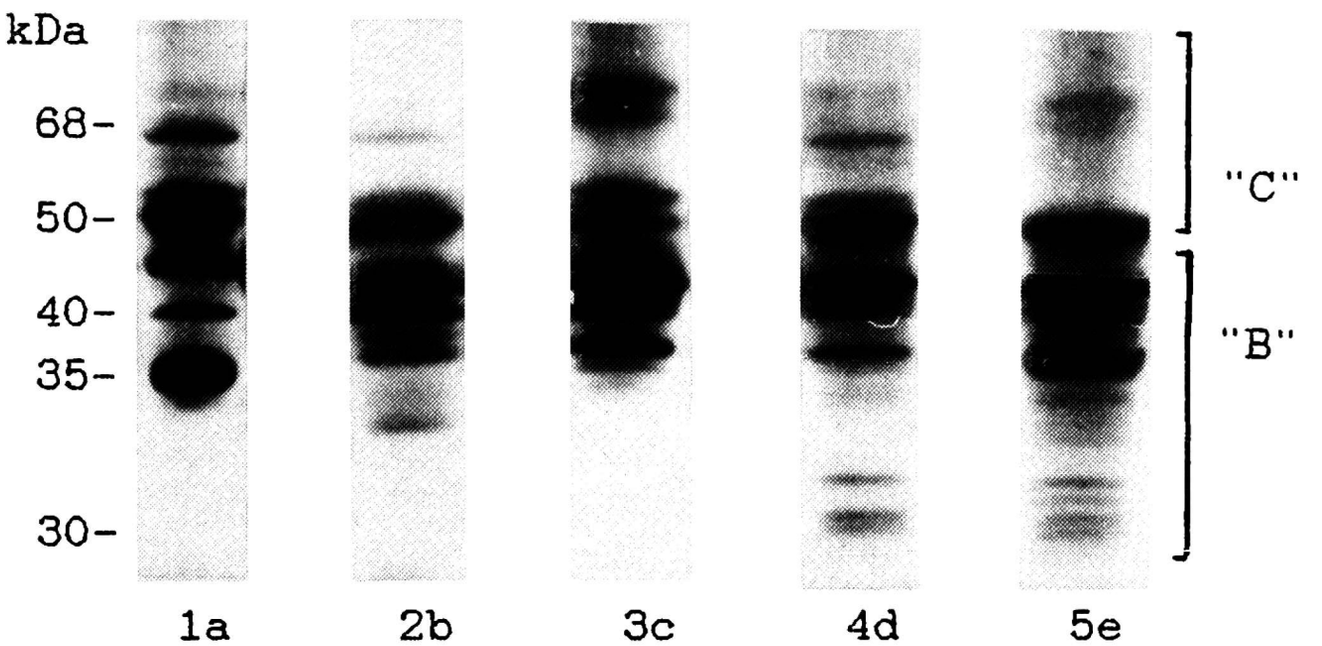

Fig. 1. Electrophoretic patterns of hordein proteins found in mutants of a hulless spring barley breeding line (*Hordeum vulgare*)

Patterns of the following lines are presented: 1a – parental line 1N/86, 2b – mutant 9, 3c – mutant 31 and 36, 4d – mutant 12, 5e – mutant 41 and 52

Analytical technique

Hordein extraction

Hordein was extracted from the seed meal with 70% ethanol (in the ratio 1:10) for 17 hours at a room temperature. After centrifugation, the hordein extract was evaporated.

Dry-evaporated hordein was treated with 0.05 M Tris-HCl buffer (pH 6.7), containing 2% sodium dodecyl sulphate (SDS), 4 M urea, 2% 2-mercaptanol and 12.5 M sucrose for 1 hour at 50°C. A dissociated and reduced hordein extract was subjected to polyacrylamide gel electrophoresis (PAGE).

SDS-PAGE in the presence of urea

Protein electrophoresis was performed as described in the previous paper (KAPAŁA 1981).

The separating gel contained 12.5% acrylamide, 0.13% bis-acrylamide, 0.1% SDS and 4M urea in 0.125 M Tris-borate buffer (pH 8.9). The stacking gel contained 3% acrylamide, 0.08% bis-acrylamide, 0.1% SDS and 4M urea (pH 6.7). The separation was performed using 0.125 M Tris-borate buffer (pH 8.9) containing 0.1% SDS with the initial current of 10 mA/ gel slab, and then, upon penetration of samples into the separating gel – with 15 mA/slab. During the whole separation time (5-6 h) the apparatus was cooled with cold air. To control protein migration during electrophoresis, an aqueous solution of amido black 1% (w/v) was used.

The proteins were fixed as previously described with a mixture containing 50% methanol and 10% acetic acid for 17 hours (KAPAŁA, PATYNA 1987). After rinsing in distilled water for 1 hour, the gels were stained in a 0.1% solution of Coomassie Brilliant Blue R-250 in 15% trichloroacetic acid for 17 hours and then destained in 7% acetic acid.

Molecular masses of hordein subunits were determined using the following standard proteins (Serva) myoglobin, 17200 Da; chymotrysinogen A, 25700 Da; pepsin, 34500 Da; OV albumin 43000 Da; glutamine dehydrogenase, 53000 Da; bovine serum albumin, 68000 Da.

Results

An analysis of single seeds from the breeding line 1N/86 and from 146 mutants showed homogeneity of the studied barley lines with regard to their hordein polypeptide patterns. The protein patterns of individuals representing a given barley line were almost indistinguishable.

Electrophoretic hordeins patterns had two major groups of protein bands designated hordeins B and C. The relative molecular masses in the subfraction B were 30-46 kDa and those in the subfraction C were 48-68 kDa.

The hordein patterns obtained from a broad majority of the analysed mutants were similar to that of the parental line 1N/86 (Fig. 1, Fig. 2-1a). However,

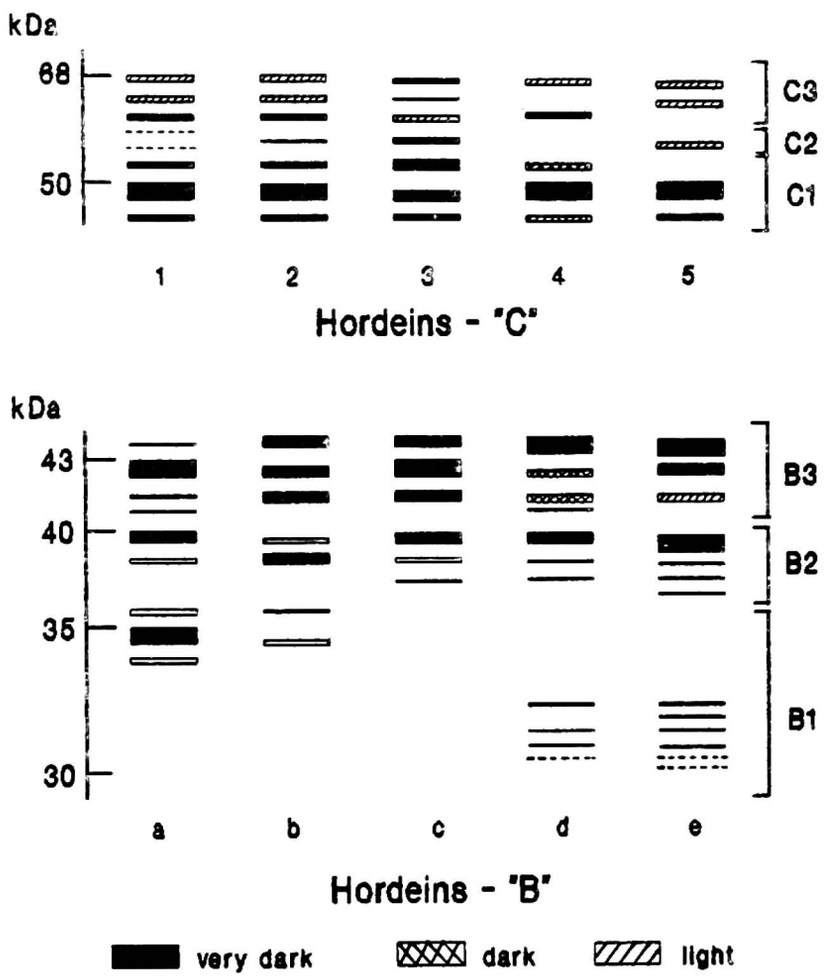

Fig. 2. Diagram of electrophoretic subunit patterns of the hordein subfractions B and C from mutants of a hulless spring barley breeding line (*Hordeum vulgare*)
Patterns of the following lines are presented: 1a – parental line 1N/86, 2b – mutant 9, 3c – mutant 31 and 36, 4d – mutant 12, 5e – mutant 41 and 52

the patterns found in six mutants (mutants 9, 12, 31, 36, 41 and 52) show variability with respect to the number, molecular mass and amounts of subunits, in particular in the subfraction B (Fig. 1 and Fig. 2 – 2b, 3c, 4d, 5e).

In the B hordein region of the parental line 1N/86, the B1 polypeptide group is a triplet (34.5, 35 and 37 kDa), the B2 group is a doublet (39 and 40 kDa) and the B3 group is quadruplet (from 42 to 46 kDa). In the C region of the line 1N/86, the C1 (48, 50 and 52 kDa) and C3 groups (63, 65 and 68 kDa) are triplets, whereas the C2 group constitutes two faint bands (55 and 56 kDa).

The electrophoretic hordein pattern of mutant 9, shows differences in comparison with the line 1N/86 primarily for the subfraction B in the regions B1, B2 and B3. Changes in the region B2 and B3 concern mainly the subunit amount. In the B1 polypeptide group, the 34 kDa subunit was not observed in mutant 9. Mutant 31 and 36 show the same type of hordein pattern. The mutants differ from the line 1N/86 mainly in the B subfraction; the B1 subunits were absent in these mutants.

Mutant 12 shows pronounced differences in comparison with the parental line, especially in the B1 polypeptide region. The B1 group of this mutant was found to not contain three subunits with approximate molecular masses of 34.5 kDa, 35 kDa and 37 kDa. In contrast, the presence of four different subunits with molecular masses from 30-33 kDa was revealed.

Mutants 41 and 52 show similar patterns of hordein proteins. In comparison with the spectrum of hordein proteins from the parent line, differences concern the subfraction B and C. In the B1 region of these mutants, six faint bands became visible (30, 30.5, 31, 31.8 and 33 kDa). The hordein B2 region of these mutants, showed 4 bands, among which the 40 kDa band was the strongest. The alterations in the B3 region concern the quantities of subunits. Among the C1 hordeins from these mutants, the subunit with the molecular mass of 52 kDa was not observed.

Discussion

Hordeins are storage proteins and constitute up to 50% of total protein in barley seeds (SHEWRY et al. 1978c, DOLL, ANDERSEN 1981, KAPAŁA, PATYNA 1986, KAPAŁA, RYBIŃSKI 1995). A high proportion of hordein in barley seed protein and its amino acid composition have a bearing on the nutritional value of barley grain. Mutations changing the amino acid composition in barley seeds are accompanied by changes in hordein (INGVERSEN et al. 1973, KØIE et al. 1976, SHEWRY et al. 1978c, KREIS et al. 1984, KAPAŁA, PATYNA 1986, KAPAŁA, YANKULOV 1994). Several high-lysine genes were also found to affect the composition of the complex mixture of hordein polypeptides normally present in wild type barley endosperms (ORAM et al. 1975, KØIE et al. 1976, KAPAŁA 1981, SHEWRY et al. 1979, KREIS et al. 1984, KAPAŁA, PATYNA 1987).

A previous study of mutants of hulless spring barley showed a higher content of total protein of the same or better nutritional value as compared to hulled cultivars (KAPAŁA, RYBIŃSKI 1995). In addition, the present study shows a variation in the hordein polypeptide composition of the mutants. Six mutants proved to be particularly interesting and consequently received special atten-

tion; these mutants showed specific hordein polypeptide spectra. It should be underlined that the same mutants are characterized by better feeding values (KAPAŁA, RYBIŃSKI 1995). As a conclusion, the presented results indicate that chemical mutations besides affecting the proportion of the main protein fractions also caused changes in the hordein polypeptide composition of mutants of hulless spring barley.

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