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INHERITANCE OF ELECTROPHORETIC PATTERNS OF HORDEIN IN BARLEY SEEDS (*HORDEUM VULGARE L*)[1].

ALICJA KAPAŁA, HENRYK PATYNA[2]

Institute of Plant Genetics, Polish Academy of Sciences, Poznań

Summary. It was observed that high-lysine mutant 1017 has a different hordein polypeptide spectrum than the initial cv. Diva. On the basis of the genetic analysis it was found that the expression of hordein loci in mutant 1017 is under an epistatic action of the recessive gene, which inhibits the synthesis of some subunits of hordein. The specific hordein spectrum of mutant 1017 is likely to be a pleiotropic effect of the recessive gene of a high lysine content.

Mutant 1017 was obtained as a result of treating barley seeds of the cv. Diva with N-nitroso-N-methylourea (MNUA) and sodium azide. It is characterized by an increased lysine content and a decreased fertility of grain. A decrease of the mutant fertility (by about 5%) is caused by a worse filling of the endosperm portion of grain (Rybiński, Patyna 1984). The high lysine content (4.1%) is an effect of the decrease in the level of hordein and the incresae in the portion of albumins, globulins, glutelins and unsoluble nitrogen (Kapała, Patyna 1986). It was also found that a high lysine content in mutant 1017 is conditioned by a single recessive gene (Kapała, unpublished data).

Comparative electrophoretic studies displayed a characteristic pattern of hordein proteins in mutant 1017 (Kapała, unpublished data). The purpose of the present paper was to determine the inheritance mode of the specific hordein spectrum in that mutant.

MATERIAL AND METHODS

PLANT MATERIAL

The subject of the studies were F_2 segregants (500 seeds) originating from two-directional crosses of pure lines of the cv. Diva and mutant 1017. F_1 hybrids (20 seeds) and 50 seeds from each initial forms were also analysed.

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[1] Received for publication: April 1986.

[2] Drs. Present address: ul. Strzeszyńska 34, 60-479 Poznań, Poland.

ANALYTICAL TECHNIQUES

The analysed seeds were halved: part of the seed with the embryo was sown out, whereas the endosperm was used for hordein extraction.

Hordein extraction. Hordein was extracted from the seed meal by 70% ethanol (in the ratio 1 : 10). The extraction was performed during 17 hours at a room temperature. After centrifugation (5 min., 13 000 g, temperature of 4°C), the hordein extract was evaporated.

Dry-evaporated hordein was treated with 70 µl of 0.05 M tris-HCl buffer (pH 6.7), which contained sodium dodecyl sulphate (SDS 2%), urea (4 M), β-mercaptoethanol (1%) and sucrose (12.5%). Hordein with disassociating mixture were incubated for 1 hour at 50°C. A disassociated and reduced hordein extract was placed on polyacrylamide gel plates and subjected to electrophoresis.

Electrophoresis in the presence of SDS and urea. Protein electrophoresis was performed in polyacrylamide gel plates like described in the previous paper (Kapała 1981).

The separating gel contained 12.5% of acrylamide, 0.13% of bisacrylamide, 0.1% of SDS and 4M of urea in 0.125 M tris-borate buffer (pH 8.9). The stacking gel contained 3% of acrylamide, 0.08% of bisacrylamide, 0.1% of SDS and 4M of urea (pH 6.7). After 0.125 M tris-borate buffer (pH 8.9) containing 0.1% of SDS and 4M of urea was used.

The proteins were fixed and stained according to Jensen et al. (1980). The separated protein bands were fixed by a mixture containing 50% of methanol and 10% of acetic acid for 17 hours, after which the gel was rinsed in distilled water for 1 hour. The protein bands were stained in a 0.1% solution of Coomassie Brilliant Blue R-250 in a 15% trichloracetic acid for 17 hours and then decoloured in 7% acetic acid.

Molecular weights of hordein subunits were determined using the following standard proteins of the "Serva" firm: myoglobin, m. wt. ca 17 200; chymotrypsinogen A, m. wt. ca 25 700; pepsin, m. wt. ca 34 500; albumin of hen's egg, m. wt. ca 43 000; glutamine dehydrogenase, m. wt. ca 53 000; bovine serum albumin, m. wt. ca 68 000.

RESULTS

Results of the analysis of single seeds of pure lines of the cv. Diva and mutant 1017 showed homogeneity of the analysed barley forms with regard to their hordein polypeptide spectra.

The electrophoretic hordein patterns have two groups of protein bands designated hordein-1 and hordein-2. The approximate molecular weights of the subfraction designated as hordein-1 are 50 000 - 67 000 and those of the subfraction designated as hordein-2 are 26 000 - 40 000.

The hordein polypeptide spectrum of mutant 1017, as compared with that of the cv. Diva, shows marked differences in the both subfractions — hordein-1 and hordein-2 (Fig. 1).

The subfraction hordein-1 in mutant 1017 was not found to have two subunits with the approximate molecular weight 54 000 and 67 000; the portion of these subunits in hordein of the initial cultivar is significant.

When comparing the spectra of the subfraction hordein-2 of the cv. Diva and mutant 1017, differences of the qualitative and quantitative type were observed. The mutant was not found to have subunits with the molecular weight ca 30 000 and 32 000, whereas the presence of other subunits with the molecular weight ca 31 000 and 36 000 was revealed. It should, however, be noticed that the portion of these subunits (m. wt. ca 31 000 and 36 000) in hordein of the mutant is insignificant. It was also found that a subunit with the molecular weight ca 26 000 shows a higher portion in hordein of the mutant than in hordein of the initial cultivar.

Table 1. F_2 segregation of electrophoretic hordein patterns in the endosperm of barley seeds (*H. vulgare*)

Cross	Analysed seed number	Electrophoretic hordein patterns	
		Diva	Mutant 1017
Diva × Mutant 1017	250	192	58
Mutant 1017 × Diva	250	186	64
Totally	500	378	122
Expected for ratio 3 : 1		375	125
	$\chi^2 = 0.096$ $(0.75 < P < 0.90)$		

Single seeds representing F_1 hybrids of the cv. Diva and mutant 1017 showed an electrophoretic pattern of hordein of the cv. Diva independently on the direction of crossing. The F_2 generation was found to have segregation of electrophoretic hordein patterns of the cv. Diva and mutant 1017 in the ratio 3 : 1 (Fig. 2 and Table 1).

DISCUSSION

Hordein is a storage protein of barley seeds and constitutes up to 50% of crude protein (Shewry et al. 1978a). In hordein we distinguish two main polypeptide fractions designated hordein-1 and hordein-2 (Doll, Brown 1979). They differ by amino acid composition and other chemical properties (Ivanov et al. 1968, Køie et al. 1976, Kapała 1983). As shown using electrophoresis the polypeptide composition of hordein-1 and hordein-2 is controlled by codominant alleles of two respective loci, *Hor* 1 and *Hor* 2, localized in the short arm of chromosome 5 (Favret 1971, Oram et al. 1975, Netsvetaev 1978, Shewry et al. 1978b, Sozinov et al. 1978a, Doll, Brown 1979, Jensen et al. 1980, Shewry et al. 1980).

It is known that mutations changing amino acid composition of barley seeds are accompanied by changes in hordein (Ingwersen et al. 1973, Ingwersen

1975, Brandth 1976, Køie et al. 1976, Doll 1977, Shewry et al. 1977, Shewry et al. 1978a, Køie, Doll 1979, Miflin, Shewry 1979, Kreis, Doll 1980, Balasaraswathi et al. 1984, Kreis et al. 1984).

Generally known is a drastically changed hordein composition in Risö mutant 1508. Recessive gene, *lys* 3 *a*, controlling high lysine content reduces very strongly the quantity of the both hordein subfractions, hordein-1 and hordein-2 (Køie et al. 1976, Shewry et al. 1979, Kreis et al. 1984). It was also revealed that the expression of hordein loci is under epistatic action of that gene. The electrophoretic hordein spectrum of Risö mutant 1508 is a pleiotropic effect of the same recessive gene of a high lysine content (Oram et al. 1975, Sozinov et al. 1978b).

In the present studies it has been found that in mutant 1017 like in Risö mutant 1508 the expression of hordein loci is under the epistatic action of the recessive gene, which inhibits the synthesis of some hordein subunits. The characteristic electrophoretic hordein spectrum of mutant 1017 is probably a pleiotropic effect of the recessive gene of high lysine content.

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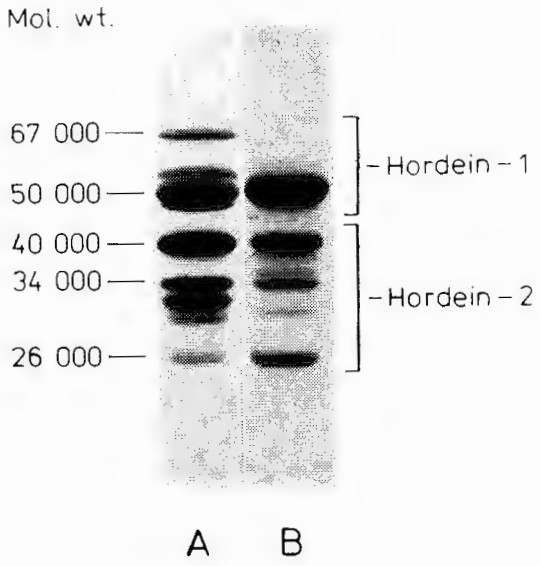

Fig. 1. Electrophoretic patterns of hordein:
A – initial cultivar Diva, B – mutant 1017

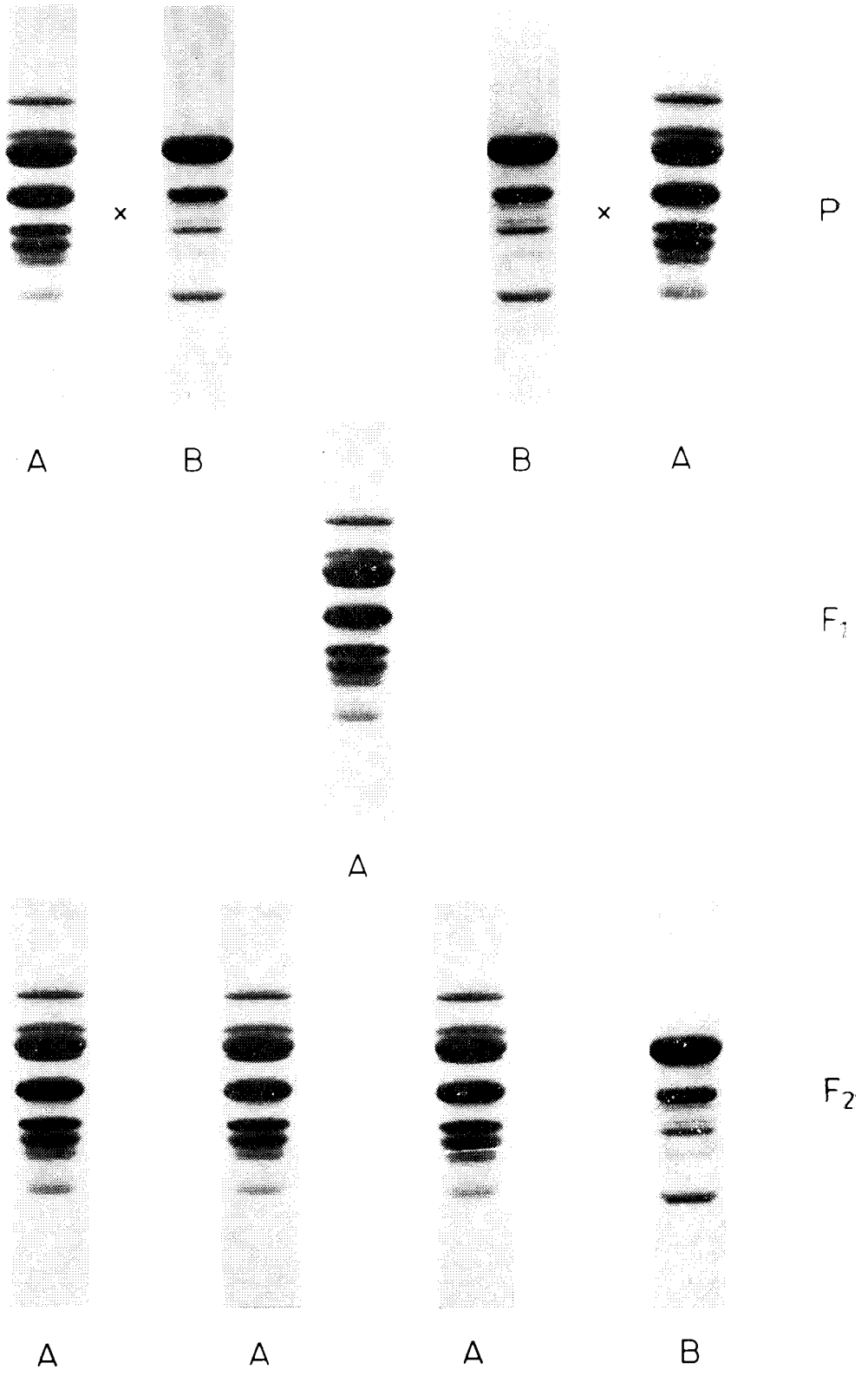

Fig. 2. The inheritance of electrophoretic hordein patterns in barley
A – initial cultivar Diva, B – mutant 1017

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DZIEDZICZENIE ELEKTROFORETYCZNYCH OBRAZÓW HORDEINY W ZIARNIAKACH JĘCZMIENIA (*HORDEUM VULGARE* L.)

Streszczenie

U wysokolizynowego mutanta 1017 obserwowano inne niż u odmiany Diva polipeptydowe spektrum hordeiny. Mieszańce F_1, pochodzące z krzyżowania mutanta 1017 z odmianą Diva, wykazują — niezależnie od kierunku krzyżowania — elektroforetyczne spektrum hordeiny odmiany

Diva. Stwierdzono, że w pokoleniu F_2 występuje segregacja w stosunku 3 : 1 elektroforetycznych obrazów hordeiny odmiany Diva i mutanta 1017. Uzyskane wyniki wskazują, że ekspresja hordeinowych loci jest pod epistatycznym oddziaływaniem recesywnego genu mutanta, który hamuje syntezę niektórych podjednostek hordeiny. Charakterystyczne spektrum hordeiny mutanta 1017 jest, prawdopodobnie, plejotropowym efektem recesywnego genu wysokiej zawartości lizyny.

НАСЛЕДОВАНИЕ ЭЛЕКТРОФОРЕТИЧЕСКИХ СПЕКТРОВ ХОРДЕИНА В ЗЕРНОВКЕ ЯЧМЕНЯ (*HORDEUM VULGARE* L.)

Резюме

Замечено, что у мутанта 1017 с высоким содержанием лизина полипептидный спектр хордеина отличается от такого спектра у исходного сорта Дива. На основании генетического анализа было установлено, что экспрессия локусов хордеина у мутанта 1017 находится под эпистатическим воздействием рецессивного гена, который тормозит синтез некоторых подединиц хордеина. Специфический спектр хордеина мутанта 1017 является по всей вероятности плейотропным эффектом рецессивного гена высокого содержания лизина.