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Brief report

High-lysine gene segregation distorted in the barley cross Risø 1508 × Crypt CI 1090: Patterns of endosperm proteins by an electrophoretic method

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The barley cross between the Indian line Crypt (CI 1090) and 1508 (Risø high-lysine mutant no. 1508 in cv. Bomi) shows, in F_1 spikes, an aberrant segregation of about 1:63 of shrunken to plump grains instead of the expected ratio of 1:3 (AHOKAS 1978, 1979a). A different kind of distortion was observed in the cross of cv. Glacier and 1508 (AHOKAS 1979b). The Glacier suppression, which seems to be promising to breeding, will be described later. Progress in breeding of 1508 grain plumpness and yield has been reported by BANG OLSEN et al. (1986). The present article deals with the case of Crypt.

Below, the crosses are marked according to PURDY et al. (1968), and the generation of seed, according to AHOKAS (1976). When an F_1 spike of Crypt/1508 or the reciprocal cross is pollinated with 1508, the BC-$F_{(1)}$ grains segregate shrunken and plump in a ratio of 1:1 with experimental results 58:50 for the cross Crypt/2*1508 ($P>0.30$) and 35:36 for 1508/Crypt//1508 ($P\sim1$). Thus the mutant gene for shrunken phenotype segregates normally in this F_1, though its phenotypic effect is masked in selfed F_1 spikes, leading to 1:63 ratio in the $F_{(2)}$ grains. Additionally, when the frequency of the mutant gene (or the proportion of 1508 genome) reaches 75 % in the zygotes on a Crypt/1508 heterozygous plant, the distortion is no longer apparent. It was of interest to know then, whether the genotypically homozygous shrunken grains with a plump phenotype on F_1 spikes have a mutant-like protein pattern in endosperms. This question will be answered below.

Sample preparation for SDS-PAGE from single grains

All removable husks were pealed off with finger nails from the grain. The embryo was cut away with a scalpel (its total removal may be checked under stereomicroscope). The grain was fitted into a transparent plastic pipette-tip (of range 50 to 200 µl) to serve as a grip while drilling. Excess plastic was cut away. The endosperm was ground to a powder by drilling with an F1 cutting edge attached to a miniature drill (Mini Plus or equivalent). Meal samples of 15 to 25 mg were weighed in a 1.5 ml Eppendorf tube and suspended in a modified LAEMMLI (1970) sample buffer containing 65.8 mM Tris-HCl (pH 7.0), 2.1 % SDS, 9.2 % glycerol and 0.001 % bromophenol blue. The amount of sample buffer (µl) used to the suspension was 14.25 times the milligrams of the meal. The meal was suspended in the evening with a micropipette-tip and by vortexing, and allowed to stand for less than half-hour. The tubes were then spun for 5 seconds in a microcentrifuge (12500 rpm) to avoid extensive swelling during the subsequent heating for 2 min in boiling water. The caps were pierced before boiling. The boiled tubes were left over-night at 18–22°C. Any interruption in boiling may lead to anomalies in the protein pattern following electrophoresis with some high-molecular weight proteins totally missing. The result is similar, if boiling is omitted. On the following morning, 2-mercaptoethanol was added (µl) to 0.75 times the weight of the meal in milligrams, and the pellet was broken and partially resuspended as above. Before loading, the samples were clarified by spinning in microcentrifuge at 12000 to 14000 rpm for 10 min at room temperature. The dissolving process can also be done during a couple of hours before beginning the gel run without any great effect on the banding pattern. The gels used here were essentially according to LAEMMLI (1970) with 9.5 % acrylamide in the separation gel. After electrophoresis, the gels were stained with Brilliant blue R (Sigma) following a modification of FAIRBANKS et al. (1971). The gels were agitated in solution 1 for 5 h, solution 2 for 10 h, and in several changes of solution 4 to clarify the background. In my hands, the method above reveals more protein bands than the rapid one described by SMITH and PAYNE (1984) and

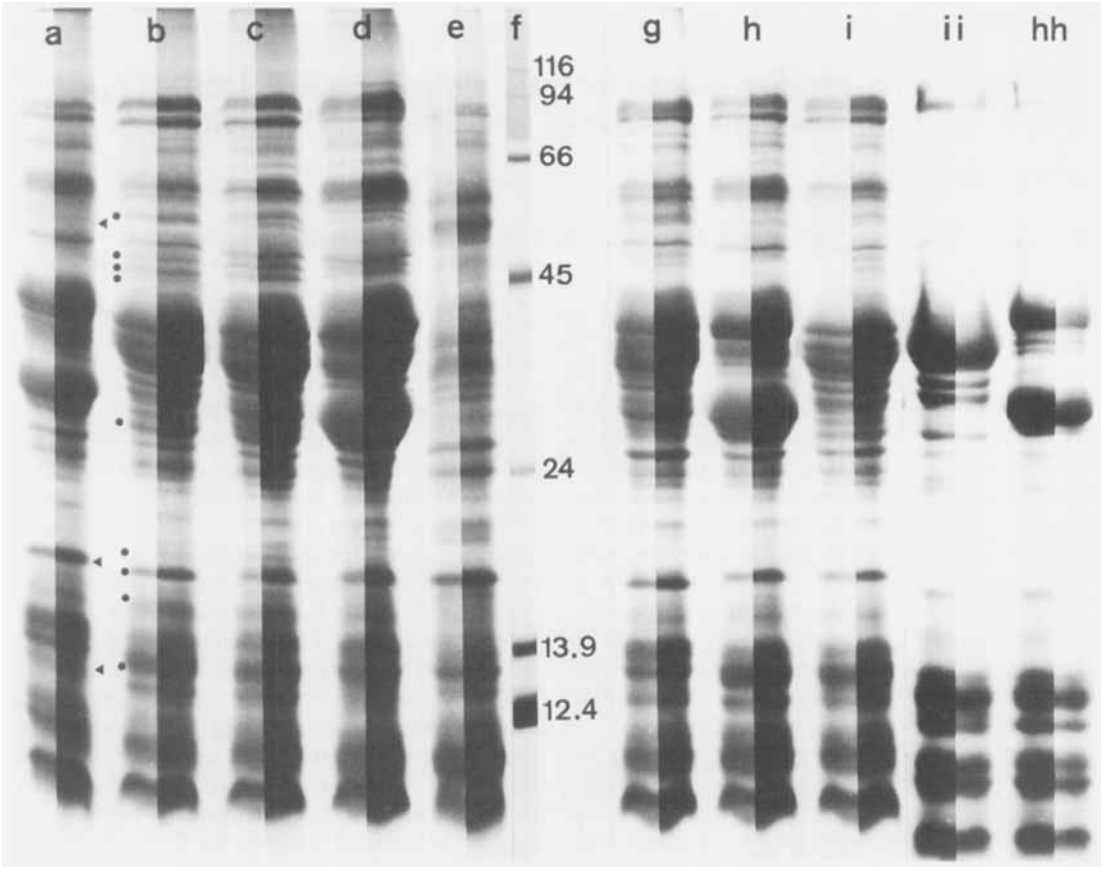

Fig. 1. SDS-PAGE distribution of endosperm proteins from meals with the present method (*a–e, g–i*) or with the method of SMITH and PAYNE (1984) on lanes *ii* and *hh*. Solution volumes used for extraction were the same on a meal-weight basis. 60 µl was loaded per lane. Lane halves were printed with two different exposure times. Lane *a* = Bomi, *b* = Crypt, *c* = Crypt/1508 $F_{(1)}$, *d* = 1508/Crypt $F_{(1)}$, *e* = 1508, *f* = molecular weight markers in kilodaltons (kd), *g-h-i* = $F_{(2)}$ endosperms from Crypt/1508 cross with major proteins between 24–45 kd of $F_{(1)}$-, Bomi- and Crypt-like patterns, respectively. Lanes *ii* and *hh* = split samples of meals of *i* and *h*, correspondingly, with less bands visible and with different mobilities than with the present method. Angles and dots indicate unique bands or mobility differences between Bomi and Crypt. Some of the mobility differences are expressed codominantly in $F_{(1)}$'s of Crypt and 1508.

SMITH et al. (1986). Also multiple bands are revealed where the latter displays a single diffuse band (Fig. 1). Ground meal of barley can also be used, if whole-grain proteins are to be studied. The method described here carried out on individual grains is a good test for varietal purity of seeds of small grain cereals.

Results and discussion

With SDS-PAGE, Bomi shows two major protein bands between 24 and 45 kd (Fig. 1 *a*), which are also spparent when alkylated B hordein preparations of Bomi are electrophoresed (DOLL and ANDERSEN 1981; ULLRICH et al. 1986). Crypt has a composite of three major bands at the B hordein position (Fig. 1 *b*). $F_{(1)}$ endosperms between 1508 and Crypt are intermediary between Bomi and Crypt at their 24–45 kd bands, where 1508 is devoid of voluminous bands (Fig. 1 *e*). The $F_{(1)}$ endosperms, being triploid, show some dosage effect in the reciprocal crosses (Fig. 1 *c* and *d*). The $F_{(1)}$ pattern with 1508 as seed parent (Fig. 1 *d*) distinctly displays, also at the B hordein position, the presence of most Bomi bands not appearing in 1508 (Fig. 1 *e*). This is in accordance with the lack of deletions in the B hor-

dein locus of 1508 (FORDE in KREIS et al. 1983) and with the appearance of B hordein mRNA in 1508 (THOMPSON and BARTELS 1983) as well as with some immunoreactive end products of B hordeins in 1508 (ULLRICH et al. 1986).

When $F_{(2)}$ grains of the cross Crypt/1508 segregating about 1:63 shrunken to plump were studied on gels, no 1508-like banding pattern was observed among the 100 plump endosperms studied. These grains are expected to segregate 1:3, or probably more exactly $(\frac{16-1}{64}):(\frac{3\times16}{64})$ or 1:3.2 of mutant to non-mutant phenotypes ($P<0.001$). Instead, the three patterns of the major proteins (Fig. 1, *g, h, i*) were found. All the rare shrunken $F_{(2)}$ endosperms studied had the 1508-like pattern. Thus, the phenotypically plump, but genotypically shrunken endosperms are not ghost-grains devoid of proper storage proteins.

The 1508 allelic Risø mutants no. 18 and 19 (JENSEN 1979) were also distorted in the $F_{(2)}$ grain segregation in crosses with Crypt. Some other old Indian barley lines, CI 1085, CI 1086, CI 1087, CI 1088, CI 1089, CI 1091, CI 2229, all from Cawnpore as is Crypt, and CI 3405 from Quetta (presently in Pakistan) failed to show distortion with 1508.

The shrunken grains of BC-F_1 spikes (see introduction) were the source for a search for the putative homozygous suppressors. Supposing recombination to occur between shrunken gene and putative complementary suppressor genes (AHOKAS 1978), the shrunken BC-$F_{(1)}$ seeds should give rise to plants segregating shrunken and plump BC-$F_{(2)}$ grains. Such plants showing about 1:15 segregation of shrunken to plump were detected. Their descendents were selected for stable suppressors during several seasons. Such derivatives from 1508/Crypt//1508 cross are still environmentally somewhat sensitive, small grained, six-rowed, with pedicellate laterals (Fig. 2). Their endosperm protein patterns on SDS gels are mostly similar to those of 1508, with three differences concerning the major proteins (results not described in this paper). Presently, some backcross lines based on the suppressive genotype of cv. Glacier are under study. These Glacier/1508 derivatives have better grain and plant characteristics than the Crypt derivatives, and are expected to be useful as high-lysine feeding barley.

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Fig. 2. Grain sample of a high-lysine derivative from the cross 1508/Crypt//1508. Natural size.

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