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Brief report

Genetic instability of a barley shrunken mutant

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A putative transposable genetic instability in barley (*Hordeum vulgare* L.) has produced a shrunken mutation showing xenia, i.e., observable segregation in spikes of the heterozygotes. The enzymology of this mutant in a Bomi-like genetic background has previously been studied (SCHULMAN and AHOKAS 1990). Extracts of its immature shrunken endosperms show 14 % of the activity of soluble starch synthase of a normal Bomi cultivar, which may mean that the gene for soluble starch synthase has been mutated (SCHULMAN and AHOKAS 1990). Six shrunken endosperm, xenia or *sex* genes have been listed for barley in the review by SØGAARD and VON WETTSTEIN-KNOWLES (1987). Typical *sex/sex* plants are viable. A special class of endospermal mutants called *dex* causes defective endosperms, xenia, and lethality (RAMAGE and CRANDALL 1981). The present shrunken mutant displays reduced viability, and the suppressor mutant described below may fall into the *dex* category.

In barley, WISE and ELLINGBOE (1985) understood the occurrence of the resistance gene recombination between *Mla6* and *Mla13* to be caused by a transposition. SIMONS and SOMERVILLE (1988 and erratum 1990), using the same barley accessions as WISE and ELLINGBOE (1985), obtained results which differed, however. While transposable elements are rare or undocumented for barley (ROHDE et al. 1987; MOORE et al. 1989; FUERSTENBERG and JOHNS 1990), in maize, a cross-pollinating species, they are dynamic factors creating new genetic material in populations where variation is chosen (GRIEL et al. 1989).

The present mutation and different mutants, which are in effect similarly shrunken, have arisen independently in the barley stocks carrying the putative genetic instability, the shrunken mutant being one of the most common mutant types along with a mutant called light-green. Though precise assessments of the frequencies of these instabilities are wanted, it is obvious that the genetic background affects the instability frequencies in the present case. At their most frequent, they fall in the range of 1/500 to 1/1000 gametes, the occurrences probably being meiotic in origin.

The present shrunken mutant shows some instability. It can evidently mutate to a thinner shrunken form. Further, the shrunken stocks have produced a stripe-shoot mutant, and a mutant with embryos leading to inviability at germination (results not discussed here). On the other hand, plants segregating nearly plump, shrunken and sterile florets in their spikes have independently appeared and been observed three times in shrunken stocks (Fig. 1). When grown, the nearly plump grains give rise to plants segregating the two types of grains and sterility as above. The shrunken grains give rise to consistently shrunken plants. Because the segregating spikes carried mainly shrunken grains, some nearly plump grains, and rather less sterile florets, the hypothesis that the typically shrunken grains represent two mutations whose heterozygotes show partial complementation resulting in the nearly plump grains was tested, but this explanation was wrong. A suppressor gene or allele making the heterozygous grains nearly plump but causing zygotic lethality when homozygous might explain the nearly plump phenotype better.

Material and methods

Segregation data were recorded from crosses of homozygous shrunken mutant with cv. Adorra. The barley line used below has an isogeny of about 75 % with cv. Bomi, being F_6 or later generation from the cross, formula unknown/2*/Bomi. Plants were grown in the greenhouse either on Vapo B2 peat from winter to spring (environment 1) or on Kekkilä pot-filling loam from autumn to winter (environment 2). In both environments, additional fertilizers

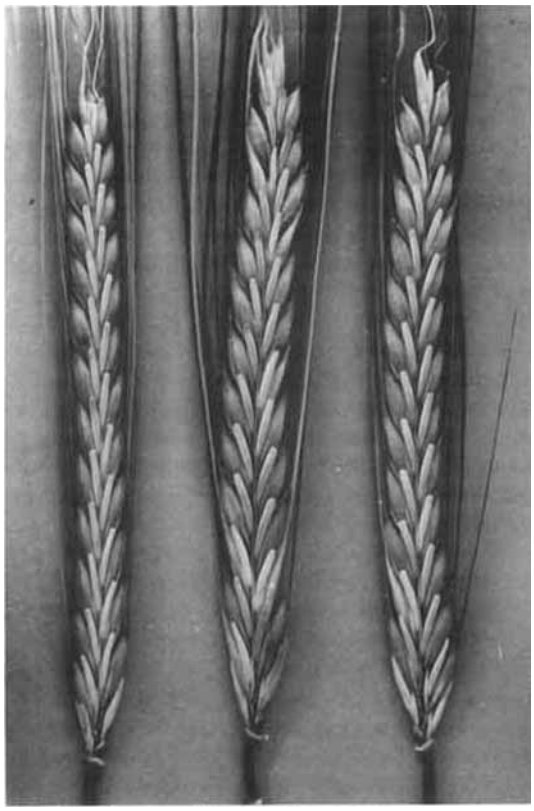

Fig. 1. Spikes of barley at late dough stage. — Left: a spike with all grains shrunken, caused by the typical shrunken mutant. — Middle and right: spikes segregating shrunken and nearly plump grains and sterile florets as a result of carrying the suppressor gene.

were supplied to avoid nutritional stresses, and supplementary light was applied with sodium vapour lamps. Emasculations and pollinations were made by the author, who has almost 30 years of experience of crossing barley. For SDS-PAGE (sodium dodecyl sulphate, polyacrylamide gel electrophoresis), endosperms of individual de-embryonated, mature endosperms were sampled from segregating spikes and ground as described elsewhere (AHOKAS 1988). De-embryonated endosperms at the incipient yellow-maturity stage were homogenized in 1.5-ml Eppendorf tubes with Eppendorf micropestles in LAEMMLI'S (1970) sample buffer without 2-mercaptoethanol. To determine the necessary buffer volume in microlitres, the fresh weight in milligrams was multiplied by 7.4. The samples were heated at 92°C for 2 min, after which 2-mercaptoethanol was added to make 5 % of the total volume. The previously reported SDS-PAGE procedure (AHOKAS 1988) was followed in other respects.

Results and discussion

Heterozygotes as well as F_1 plants display a 1:3 ratio of segregation of shrunken to normal grains in their spikes. The F_1 spikes of the shrunken mutant × cv. Adorra displayed 769 shrunken grains and 2371 plump grains; $\chi^2 = 0.435$, $P>0.50$. When these plump grains were planted, there was a segregation in the F_2 of all-plump to shrunken-and-plump grained plants in the ratio of 71 to 137 which fits with 1:2, $\chi^2 = 0.060$, $P>0.70$. The shrunken grains resulted in F_2 plants with slow growth, low fertility and stunted appearance. These homozygous shrunken plants make for better growth under greenhouse conditions than in the field. The Bomi-like genetic background is relatively fruitful in the greenhouse environment. The lowest grain in a spike may rarely show a plump phenocopy. The following shrunken mutants exhibiting xenia, Risø 13 in Bomi, Risø 56 in Carlsberg II, Risø 1508 in Bomi, and *amo1* in High-Amylose Glacier were not found to be allelic with the present mutant as judged from the F_1 spike segregation. Further, the F_1 plants grown in the field did not display stunted growth.

The nearly plump grains of plants segregating shrunken, nearly plump and sterile (Fig. 1) were grown in the greenhouse in two environments. Environment 1 resulted in 820 shrunken, 674 nearly plump grains, and 230 sterile florets. Environment 2 resulted in 828 shrunken, 555 nearly plump grains, and 227 sterile florets, the respective totals being 1648, 1229, and 457. Assuming that the sterile florets contain a proportion of sterility induced by the lowered adaptation of the genotype, and that the gametic transmission of the gene making heterozygotes nearly plump is lowered, the seed population may follow Hardy's and Weinberg's equation, $p^2:2pq:q^2 = 1648: 1229: [457 - (\text{number of sterile florets due to other reasons})]$. The predicted value for q^2 would be 229, and the amount of sterility due to other reasons would be 6.8 %. The ratio 1648:1229:229 deviates significantly from 1:2:1 in a way that the gametic transmission of the suppressor factor leading to nearly plump grains and sterile florets must be reduced.

Shrunken seeds from the original mutant plant segregating shrunken, nearly plump and sterile florets were raised for two successive generations as lines, proving to be stable and yielding only shrunken grains. Crosses were done to test whether these lines would actually be carriers of two kinds of shrunken mutants with different mutation sites or representing different states of a transposable element (SCHWARZ-SOMMER et al. 1985), resulting

in a nearly plump grain when heterozygous. The seven shrunken lines were crossed in 18 of the 21 possible different pairs. Had the two mutants occurred in a 1:1 ratio among the seven shrunken lines, either of the mutants should appear at least in one of the lines at $P = 0.99$. Five F_1 plants of each cross were grown and proved to be all-shrunken, the lines thus carrying the typical shrunken gene. This was also evidenced by crossing two of the lines with the typical shrunken carrier line. Two of the lines were crossed as a pollen parent with plants segregating shrunken, nearly plump and sterile florets. Because emasculated without cutting the husks, the $F_{(1)}$ hybrid grains were well-formed and permitted the following classification: twenty shrunken, eight nearly plump and five sterile. The segregation was also confirmed by the segregation of the corresponding F_1 generation spikes. The hypothetical aa:Aa numbers of 20:8 fit well with the ratio of the extracted values of 1648:(1229/2) from the selfing data above ($\chi^2 = 0.002$, $P > 0.95$).

There are several quantitative differences between nearly plump and shrunken endospermal protein bands revealed by SDS-PAGE both in immature and mature grains (Fig. 2). The SDS-PAGE also shows two qualitative differences. The blue band of 66.4 kg mol^{-1} is present in shrunken and absent from nearly plump endosperms. Nearly plump grains have a purple staining band of 61.3 kg mol^{-1}, which zone is represented by a blue band in shrunken endospermal proteins. The staining procedure used permits visual distinction of the two shades of colour on gels. The accumulation of the major hordeins ranging from 33 to 46 kg mol^{-1} is greater in nearly plump grains than in shrunken grains (Fig. 2).

The present data indicate that the suppressor gene causing the nearly plump phenotype has a lethal effect when homozygous, and has a decreased transmission through gametes. Two gametophytic factors causing aberrant segregation ratios have been described in barley (Tabata 1961; Konishi and Matsuura 1988; Konishi and Abe 1990). The effect of the suppressor gene in a non-shrunken genetic background is not known. It cannot be concluded yet, whether the suppressor gene is allelic with the typical shrunken allele, or whether it occupies another locus. This can probably be resolved when the corresponding genes have been cloned.

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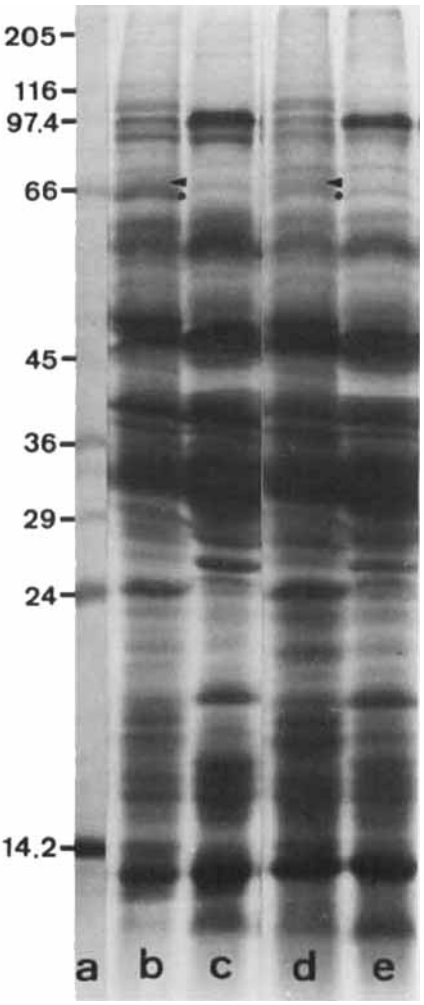

Fig. 2. Shrunken (**b and d**) and nearly plump (**c and e**) endospermal proteins fractionated with SDS-PAGE. Track **a** shows the molecular weight standards in kg mol^{-1}. The arrow head indicates the 66.4 kg mol^{-1} band, and the dot, the 61.3 kg mol^{-1} band, which stains blue in shrunken and purple in plump samples.

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