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HORDEIN POLYPEPTIDE PATTERN IN RELATION TO MALTING QUALITY AND THE VARIETAL IDENTIFICATION OF MALTED BARLEY GRAIN

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A procedure is described for the extraction of hordein fractions from single seeds of barley and determination of their polypeptide composition by SDS-PAGE. Varietal differences in the hordein polypeptide pattern occur, and on the basis of this character the 28 varieties which are most widely grown in the U.K. can be divided into 11 groups. Whereas several of these groups contain varieties with similar malting quality, other groups contain both good and poor quality varieties. Examination of pilot scale malts and germinating seeds showed little change in the hordein polypeptide pattern during malting or the early stages of germination. Thus the procedure is suitable for use in the identification of malted grain as well as dry seed.

Key words: *varietal identification, hordeins, electrophoretic methods, malting quality, malts, germination.*

Introduction

Barley varieties differ considerably in malting quality. Several factors appear to be responsible including differences in the ability to produce malt enzymes[1,8] and in endosperm structure and texture.[23,11-13] More recently Wainwright and co-workers[5,23,24] have proposed that malting quality is also related to the storage protein (hordein) fraction. They analysed fractions from 16 varieties and showed that the varieties which were of higher malting quality also had relatively less of certain protein bands. They also reported differences in the hordein fractions from steely and mealy grain of Proctor, and that considerable changes occurred in the hordein patterns of several varieties during a range of malting procedures.

We have recently examined the hordein polypeptide patterns of over 200 barley cultivars bred and grown in Western Europe, and have developed a procedure for the varietal identification of single seeds on the basis of this character.[19,22] In the present paper we use the same procedure to compare hordein fractions from dry seed with those from pilot scale malts and germinating seed. In the varieties studied the polypeptide patterns remained relatively unchanged during malting, allowing the varieties to be identified.

Consideration of the hordein patterns of 28 widely grown varieties in relation to the malting scores published by the National Institute of Agricultural Botany (NIAB) shows that whereas some groups of varieties with similar hordein patterns are also similar in malting quality, other groups contain both good and poor quality varieties.

Results and Discussion

Varietal Identification of Single Seeds.—Hordein is separated by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) into two groups of polypeptides termed '*B*' and '*C*'.[9,10,18] These groups are controlled by two separate but linked loci on chromosome 5[6,7,16,20] and occur in a range of patterns in different cultivars.[14,19,22] The fraction also contains a small amount of low mol. wt. polypeptides (termed '*A*' hordein). These, however, exhibit a constant pattern in all varieties[22] and are not now generally considered to be storage polypeptides.[10]

Barley varieties can be divided into groups on the basis of the different '*B*' and '*C*' hordein patterns and combinations of these. We have previously classified the patterns which are most widespread in currently-grown European varieties using numbers for the '*B*' patterns and letters for the '*C*'.[10,22] The same classification system and exact notation for each pattern is retained in the present paper.

Twenty-eight barley varieties were selected for inclusion in the present paper on the basis of the 1977/78 seed cereal sales.

When hordein fractions from these varieties were analysed a total of 8 different '*B*' and 7 different '*C*' hordein patterns were found. One variety, Ark Royal, consisted of two types of seed with different hordein patterns. We have previously shown the presence of different seed types, or biotypes, in a number of varieties and have shown that this variation exists in seed and plants checked for morphological uniformity at NIAB.[22] On the basis of the hordein pattern the varieties can be divided into 11 groups containing between 1 and 6 varieties (Table 1). SDS-PAGE separations of fractions from one variety in each group are shown in Figure 1. There is no correspondence between these groups and those based on grain morphology.[22]
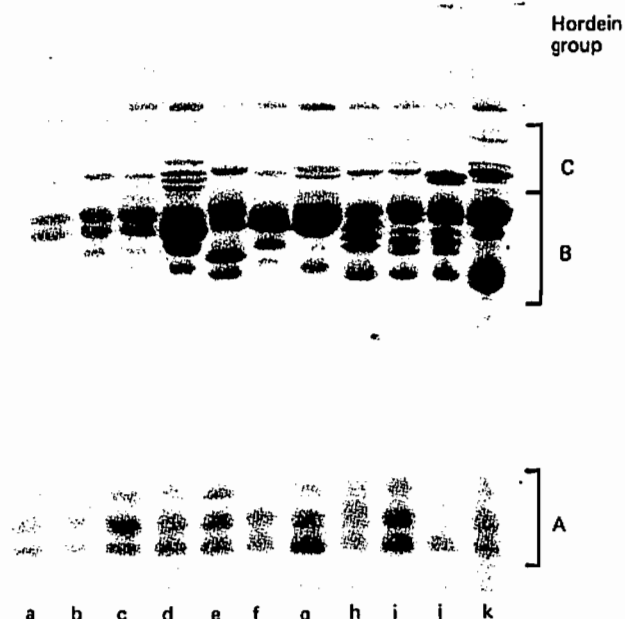

Fig. 1. SDS-PAGE of PE hordein in fractions from single seeds of 11 barley varieties with different polypeptide patterns.
a, Golden Promise (1A); b, Wing (1B); c, Astrix (1G); d, Igri (2C); e, Tern (3B); f, Hoppel (4E); g, Midas (5F); h, Mosane (6G); i, Hassan (7A); j, Maris Mink (7H); k, Julia (15A).

Fourteen of the 16 varieties analysed by Baxter and Wainwright[5] are included in Table 1, the exceptions being Keg (pattern 3B) and Mala Abed (pattern 1A). However, comparison of our results with theirs shows considerable differences. For example, Baxter and Wainwright report that Hassan, Maris Mink, Athos and Porthos differ in both '*B*' and '*C*' patterns. We have analysed hordein fractions from these varieties by SDS-PAGE, isoelectric focusing (IEF) and a two-dimensional (2-D) system combining both procedures[14] and shown that Maris Mink, Athos and Porthos have almost

TABLE 1. 28 widely-grown barley varieties divided into groups on the basis of hordein polypeptide pattern.

1A	Armelle (5), Golden Promise (6), *Maris Otter* (7), *Proctor* (6), Tyra (1), Varunda (1)
1B	Ark Royal type 1 (8), Mazurka (2), Wing (7)
1G	*Astrix* (1)
2C	*Igri* (1), *Malta* (3-4), *Sonja* (1)
3B	Ark Royal type 2 (8), Tern (5)
4E	*Hoppel* (1)
5F	Midas (3)
6G	Mosane (7)
7A	Hassan (6)
7H	Aramir (4), Athos (4), Maris Mink (5), *Maris Trojan* (5), Porthos (6)
15A	Abacus (1), Georgie (1), Julia (2), Lofa Abed (2), Sundance (4)

The varieties included accounted for 1% or more of the total seed sales of spring or winter varieties in 1977/78. NIAB malting scores are given in brackets. Winter varieties are in italics.

identical patterns while Hassan differs in the pattern C only. Analysis of the parentage of these varieties suggests that '*B*' pattern 7 and '*C*' hordein pattern H were introduced from the exotic variety Arabische, which was included in breeding programmes as a source of mildew resistance.[19] Athos and Porthos are, in fact, sister lines from the cross line 207 × Emir.[19] Baxter and Wainwright[5] also reported differences in the '*B*' patterns of Wing and Mazurka, which we have again shown to be identical by 2-D analysis.[19]

We ascribe these differences to the procedures used. Whereas Baxter and Wainwright[5] separated unalkylated protein using an acid gel system which separates primarily on charge with a secondary separation on molecular size and shape due to sieving through the gel matrix, we used reduced and pyridylethylated fractions and an SDS-PAGE system which separates on molecular weight. Although we have shown that an acid gel system different to that used by Baxter and Wainwright[5] may show minor differences between the '*B*' hordein patterns of some varieties which appear identical by SDS-PAGE, we also found that it gave poor resolution of the 'B' hordein, especially in the absence of alkylation.[14,17] Consequently we have used SDS-PAGE as the primary system of dividing varieties into electrophoresis groups.

We have shown that the hordein polypeptide pattern is constant at the later stages of seed development[21] and is unaffected by fungicidal seed dressings.[22] The only recorded effect of growth conditions is an increase in the relative amount of '*C*' hordein with increasing seed N.[9] However we find that this effect is relatively small, an increase from 1·26% to 2·03% N resulting in an increase in '*C*' hordein from 13·0% to 18·5% of the total hordein fraction of Julia.[17] Although we have not specifically studied grains differing in texture (mealy and steely or vitreous), we have found little variability between single seeds in the ratio of *B*:*C* hordein. It should be noted, however, that whereas Baxter and Wainwright[5] extracted hordein with hot 70% ethanol without a reducing agent, we used 50% propan-1-ol + 2% 2-mercaptoethanol, which we have found to give more complete extraction of '*B*' hordein.[15] We therefore conclude that any variation in the ratio of *B*:*C* hordein is not sufficiently great to be apparent using our extraction and separation procedures.

Changes in Hordein Pattern during Malting and Germination.—We have examined malted grain of a number of varieties. Seed of Maris Otter, Ark Royal, Wing, Porthos and Hassan were examined during the first 4 days of germination during a pilot scale malting process. Fractions from these seed are compared with those from dry unmalted grain in Fig. 2. In most cases malting was accompanied by an increase in low mol. wt. polypeptides. However in all cases the patterns were similar to those of the dry seed, allowing the varieties to be correctly assigned to the electrophoresis groups in Table 1. Pilot scale 4 day malts of Igri, Golden Promise, Sonja and
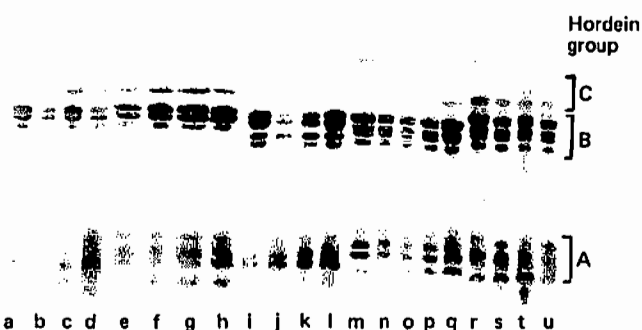

Fig. 2. SDS-PAGE of PE Hordein from dry seed (0 day) and 1, 2, 3 and 4 day malted grain
a-d, 0, 1, 2 and 4 day Maris Otter; e-h, 0, 2, 3 and 4 day Wing; i-l, 0, 2, 3 and 4 day Ark Royal Type 2; m-q, 0, 1, 2, 3 and 4 day Hassan; r-u 0, 1, 3 and 4 day Porthos.

Maris Trojan also gave similar patterns to those of the unmalted seed. In all cases the reproducibility between seeds was good, as shown by the separation of 10 separate seeds of Igri in Fig. 3.

We also analysed experimental micromalts of Porthos and Sundance. Although the seed was grown on a range of sites near the Plant Breeding Institute, Cambridge, and differed considerably in respect of 19 different malting characteristics (Gothard pers. comm.), the patterns of all the samples within each variety were identical and similar to those of the dry seed.

Five varieties with different hordein patterns were selected to study the effects of germination. These were Golden Promise (pattern 1A), Keg (3B), Jupiter (5F), Maris Mink (7H) and Julia (15A). The varieties differed in their germination rate, so Julia, Jupiter, and Maris Mink were sampled at 2 and 4 days germination and Golden Promise and Keg at 3 and 5 days (Fig. 4). In the 4 and 5 day samples the plumule emergence was 50% or greater. Even so digestion of the hordein fraction had proceeded to a greater extent in some varieties than in others. However, it is clear from Fig. 4 that the hordein polypeptide pattern is virtually unaffected during the three days of germination, but becomes increasingly diffuse as germination proceeds. There is some evidence from the 4 and 5 day samples, particularly of Maris Mink and Julia, that the '*C*' polypeptides remain more distinct than the '*B*'. Baxter and Wainwright[5] also reported the '*B*' hordein was more rapidly degraded than '*C*' hordein during malting.
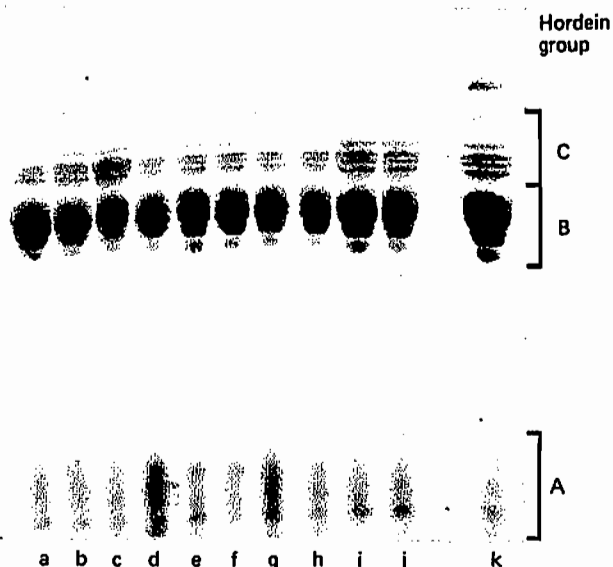

Fig. 3. SDE-PAGE of PE Hordein from single 4-day malted grain (a-j) and one single seed (k) of barley.
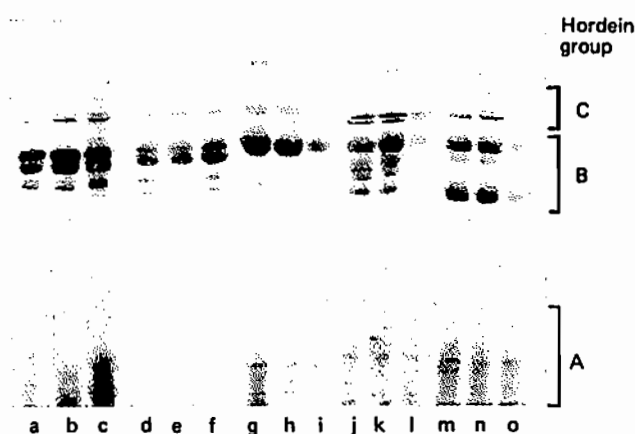
Fig. 4. SDS-PAGE of PE Hordein from single dry seed (0 day) and germinating seeds.
a-c, 0, 3 and 5 day Golden Promise; d-f, 0, 3 and 5 day Keg; g-i, 0, 2 and 4 day Jupiter; j-l, 0, 2 and 4 day Maris Mink; m-o, 0, 2 and 4 day Julia

Although we found little change in the hordein pattern during the early stages of malting and germination, Baxter and Wainwright[5] reported that the pattern was considerably altered, even 'after only one day of germination. It is possible that their acid gel system was more sensitive to small changes resulting from peptidase activity than was our SDS-PAGE system. Autran and Scriban[4] have also used a separation based on charge, starch gel electrophoresis (SGE), and shown that the hordein pattern can be used to test the purity and provide a partial identification of malted grain. However, they again used unalkylated samples which give poor resolution of ‘B’ hordein, especially on SGE.[10] Consequently their identification was based mainly on ‘C’ polypeptides which, although they are well resolved by SGE, show less variability in European varieties than ‘B’ hordein.[19,22] We have also been informed that Sowah, E. A. and Slaughter, J. C. have also found little change in band pattern over 5 days of germination (Slaughter, J. C. personal communication).

On the basis of these three studies we would suggest that SDS-PAGE of reduced and alkylated hordein is the preferred system for the varietal identification of commercial malts.

Hordein Pattern and Malting Quality.—Baxter and Wainwright[5] suggested on the basis of their separations of hordein from 16 varieties that good malting quality was associated with the absence of fast moving ‘B’ polypeptides. We have shown that the ‘B’ polypeptides which migrate faster in an acid-urea gel system correspond to the low mol. wt. ‘B’ polypeptides in our SDS-PAGE system.[14,16] Consideration of the larger number of varieties in Table 1, however, suggests that there is no clear cut relationship between these polypeptides and malting quality. For example, the winter barleys in group 2C (Igri, Malta, Sonja) which lack low mol. wt. ‘B’ bands are all poor malting types while group 3B in which such bands are present contains not only Ark Royal (NIAB grade 8), Tern (grade 5), Dram (grade 5) and Keg (grade 8). Low mol. wt. ‘B’ polypeptides are also present in groups 7A and 7H which contain varieties of intermediate quality, and in Group 15A which contains a mixture of poor (Abacus, Georgie, Julia) and moderate (Sundance) varieties. In contrast group 1A which lacks these bands contains not only the good quality varieties studied by Baxter and Wainwright[5] (Proctor, Golden Promise, Maris Otter, Imber, Mala Abed) but also the two very poor quality varieties Tyra and Varunda (both NIAB grade 1).

We would therefore conclude that although there is some correlation between hordein pattern and malting quality this is not universal. In many ways this is perhaps not very surprising since malting quality is such a complex property and the NIAB scores can at best be only an approximate assessment of many different attributes. However this general conclusion is not materially affected by considering a large number of varieties[19,22] or by comparison with published milling energy or sedimentation data[2,3,11,13] rather than NIAB scores. Further, any loose correlation does not, of course, prove that it is the hordein pattern itself that affects malting. Indeed this seems unlikely as we have shown that patterns 1B, 3B, 7H and 5F have been selected for linked mildew and rust resistances.[19] It is more likely that the apparent association of malting quality and hordein pattern in groups 1B, 3B, and 7H is a result of common ancestry. The converse is probably true for group 1A where the varieties differ widely in background and in malting quality. However, a direct involvement of hordein in some aspect of malting quality, for example by affecting endosperm structure, cannot be completely ruled out at present.

Irrespective of any direct link between hordein and malting quality, the polypeptide pattern can be used as a powerful aid to the varietal identification of malted grain. Where a hordein group contains varieties with similar malting quality it may often be unnecessary to completely identify a grain or malt sample. In other instances where complete identification is required it can be used together with morphological procedures. In either case we suggest that the techniques described here may be of considerable value in the regulation of trading in seed barley, malting barley and malts.

EXPERIMENTAL

A single dry or malted seed was crushed with a hammer and placed in a 1·5 ml polypropylene test tube with 200 μl of 50% (v/v) propan-1-ol + 2% (v/v) 2-mercaptoethanol and suspended in an ultrasonic bath for 30 minutes. After centrifugation the supernatant containing the extracted hordein was reduced to dryness at 40°C, dissolved in 200 μl buffer containing 8-M urea and 1% (v/v) 2-mercaptoethanol and alkylated with 4-vinylpyridine (3·0 μl). The alkylated solution was dialysed against a solution of 1% (w/v) sodium dodecyl-sulphate (SDS) and electrophoresed on 17·5% acrylamide gels containing 0·1% SDS at pH 8·9. The method has been described in detail previously.[22] All variety identification of dry seed were based on analyses involving, as a minimum, replicate runs using 10 individual seeds each time. The various malted barley analyses were done on at least duplicate samples of 5 seeds each. These were taken at 1, 2, 3 and 4 days during pilot scale malting of Maris Otter, Wing, Ark Royal, Porthos and Hassan and after 4 days for Igri, Golden Promise, Sonja and Maris Trojan. Further analyses were made of 26 different micromalts of Porthos and Sundance.

To study changes during germination 100 seeds of each variety were allowed to imbibe water for 12 h at 4°C and then placed on moistened filter paper in petri dishes and placed in the light at 20°C. Ten seeds were removed at daily intervals, frozen in liquid N_2, lyophilized and analysed. Germination was allowed to proceed until approximately 50% of the plumules had emerged (4 or 5 days depending on the variety).

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