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BIOCHEMICAL AND MOLECULAR GENETICS IN BREEDING FOR QUALITY IN BARLEY

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ABSTRACT

To this day plant breeding is most successfully carried out emperically by crossing promising lines and by selecting high yielding plants with improved quality. Biochemical genetics provides increasing possibilities to determine the metabolic pathways leading to storage proteins, enzymes, flavonoids and fatty acids, compounds which give favourable or unfavourable quality characteristics to the desired plant product. Mutations blocking the pathway are of special significance in evaluating the effect of a substance on a quality parameter. Molecular genetics permits the isolation of individual genes and their multiplication in bacteria and yeast. The structure as well as expression of genes in single copy and complex loci can thereby be explored. The obtained information provides the basis for the breeding strategies to be applied.

In spite of knowledge about the biochemical pathway and the structure of genes, the selection of appropriate rare genotypes is often economically unfeasible. Genetic transformation and the application of fluorescing monoclonal antibodies in a non-destructive way are methods of overcoming these restrictions. The problems and their solutions are exemplified by breeding proanthocyanidin-free malting barley and breeding for improved nutritional quality of feeding barley.

KEYWORDS

Storage proteins, flavanoids, proanthocyanidin, monoclonal antibodies, mutation, nutritional qualities, genetic transformation.

INTRODUCTION

The potential genetic information of the haploid barley genome comprises 5 x 10^9 base pairs of DNA. If we assume that an average gene containes 10^3 nucleotides, the chromosomes of the barley plant would contain a few million genes. Each gene can occur in several or even many different allelic forms. We understand then that the number of gene combinations, which are obtainable with the existing and newly arising genotypes, is virtually unlimited; in fact it is so large that only a small portion of the total possible numbers of combinations has been realised since the existence of the crop barley. This is the reason why selection after crossing of existing lines or newly selected mutants will continue to produce improved varieties. It also demonstrates that finding rare genotypes with improved yield, disease resistance, better climatic adaptation, and a better quality of the desired plant product is like looking for a needle in a haystack.

Our knowledge of biochemical and molecular genetics has improved steadily over the last hundred years and has given us increased opportunities to apply this knowledge to plant breeding in a rational manner. The cloning of genes has vastly extended our possibilities for exploring the biochemical pathways of the plant and to identify targets for improvement. Transformation of plant cells with useful or improved genes and their regeneration into whole plants has increased the chance of tailoring plants with the desired characteristics. My ideas on the possibilities of gene technology in plant breeding have been set out in two previous articles (von Wettstein, 1983 and 1984). I would like to reiterate that genetic engineering is a technique which has been added to our arsenal of existing breeding techniques and is complementing but not replacing the conventional breeding procedures. It is frequently said that these techniques will speed up plant breeding; there is no evidence for this. Field testing of new genotypes remains the most time consuming part of plant breeding and such complex characteristics as yield or photosynthetic efficiency are unlikely to be bred using gene technology techniques in the near future.

PROANTHOCYANIDIN-FREE BARLEY FOR BREWING

Proanthocyanidins of barley grains consist of dimers and trimers of (+)− catechin and (+) gallocatechin units (Fig. 1, 2). These flavanoids, also called anthocyanogens because they can be cleaved by acid into red anthocyanidins and colourless catechin molecules, stay in the kernels during malting. During mashing these compounds dissolve in the wort and are retained during the following processes of primary and secondary fermentation of the wort. In the finished beer, proanthocyanidins precipitate proteins and thereby give rise to undesirable non-biological haze. To

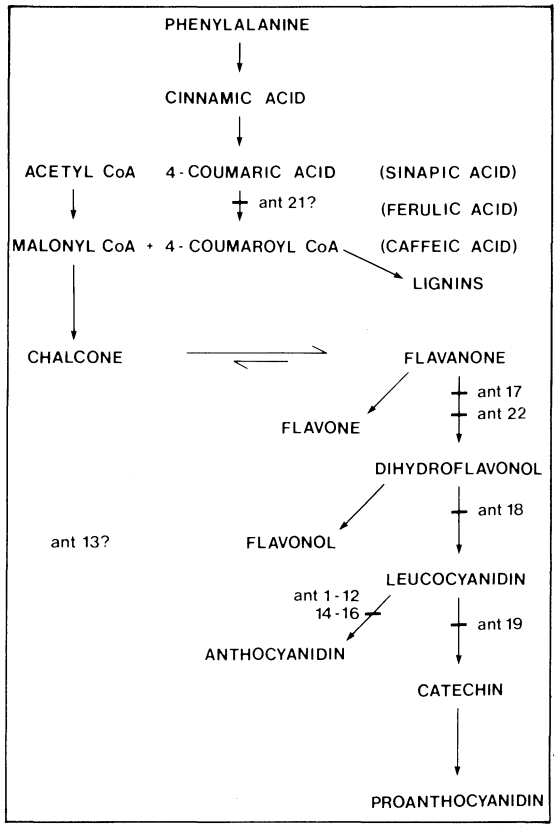

Figure 1. Biosynthetic pathway of proanthocyanidins, anthocyanidins and related compounds. The site of action for different *ant* genes in barley is indicated.

avoid this haze formation, several technologies are applied which aim at removing the proanthocyanidins (e.g. by filtering through polyvinylpolypyrrolidone-containing sheets) or degrading the proteins with proteolytic enzymes or precipitating both compounds.

While these proanthocyanidins are prominent constituents in grains of barley and millet, they are absent from kernels of maize or wheat, thus illustrating that they are not required for the survival of a crop plant. Figures 1 and 2 reveal that the red anthocyanin pigments formed by a different branch of the flavonoid pathway, the branch point being located at the 2,3-trans-3,4-cis-leucocyanidin intermediate (Kristiansen, 1984).

If the pathway is blocked by a mutation before the branch point, plants result which lack the red anthocyanin pigmentation on the stem, auricles, lemma, and awns as well as the colourless proanthocyanidins in the kernels. Accordingly anthocyanin-free mutants were tested for the presence or absence of proanthocyanidins in their kernels. Among the first 42 anthocyanin-less mutants tested one, *ant 13-13*, was found to be also blocked in the biosynthesis of proanthocyanidins.

After propagation of the mutant, pilot and full scale brewing tests were performed with malt prepared from this mutant (von Wettstein *et al.*, 1977, 1980, 1981, 1985; von Wettstein, 1979; Ahrenst-Larsen and Erdal, 1979). Excellent haze stability was achieved in the beers. For the first time it was possible to test barley, malt and beer with an intrinsic absence of these polyphenols. Earlier hypotheses which regarded proanthocyanidins an important part of beer flavour and taste could now be tested scientifically.

It was discovered that beers produced with tannin-free hop extract and proanthocyanidin-free malt were indistinguishable from control beers in taste panel evaluations (Erdal *et al.*, 1980, 1983; Delcour *et al.*, 1984a). In addition, the flavour stability of proanthocyanidin-free beer over a six-month period was as good as that of the reference beers.

The use of gene mutations to eliminate individual classes of compounds in the barley grain thus provides a superior tool for evaluating the contribution of these compounds to the flavour profile and stability of beer. The classical adsorption techniques apparently also removed compounds of importance for flavour and flavour stability and thus a critical assessment of the role of proanthocyanidins was not possible.

To evaluate the haze-forming potential of individual proanthocyanidins and their possible effect on beer flavour, dimeric prodelphidin B-3, the procyanidins B-2, B-3, B-4, and B-6 as well as trimeric proanthocyanidins, were added individually or in groups to proanthocyanidin-free wort or beer (Erdal *et al.*, 1983; Outtrup and Erdal, 1983; Delcour *et al.*, 1984b). In general, the amount of haze formed by these additions was proportional to the amount of proanthocyanidins added. Procyanidin B-6 and the trimeric molecules were more haze active than the other dimeric molecules.

In terms of agronomic performance, mutant *ant 13-13* yielded about 25% less than its mother variety and was highly susceptible to infection by powdery mildew. During the past years, an extensive programme of mutation and recombination breeding has been carried out to obtain high yielding proanthocyanidin-free malting barley varieties adapted to various climates (cf. von Wettstein, 1985). As of October 1985, 476 proanthocyanidin-free mutants have been isolated after mutagenesis in 90 different spring and winter barley varieties comprising cultivars from Europe, North-America, and Japan as well as one each from New Zealand and Australia. By diallelic crosses, 274 of the recessive mutants have been localised to six gene loci (Jende-Strid, 1984 and unpub.). Evidence for the existence of three additional genes controlling the biosynthesis of flavonoids has been obtained. The gene loci are designated *ant 13*, *ant 17*, *ant 18*, *ant 19*, *ant 21*, *ant 22*. (Genes *ant 1* to *12*, and *14* to *16* control the branch leading to anthocyanins.)

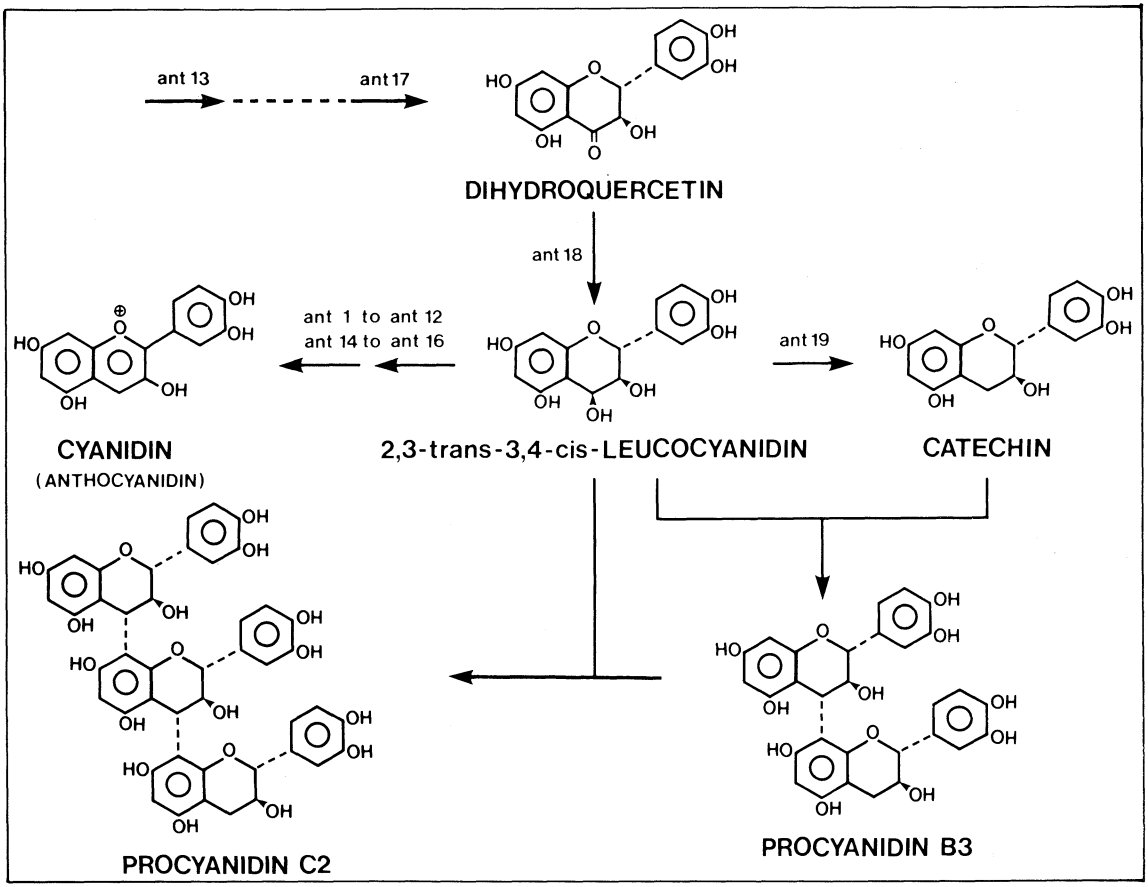

Figure 2. Pathway from dihydroquercetin to catechin and procyanidins as elucidated by studies of wild type and *ant* mutants in barley.

The pathway is depicted in Figure 1. The flavan skeleton is synthesised from coumaroyl CoA and malonyl CoA. The immediate precursor of anthocyanidins and proanthocyanidins is a leucocyanidin which is derived from a dihydroflavonol. This latter part of the pathway is drawn in Figure 2.

Two approaches have been used to locate the steps in the synthesis of proanthocyanidins which are blocked in the mutants (Kristiansen, 1984). (1) After establishing the time course for catechin and procyanidin synthesis in developing barley grains, *ant* mutants were analysed for an accumulation of precursors. (2) Radioactive dihydroquercetin was prepared, fed to barley wild type and mutant testa-pericarp tissue at a time optimal for proanthocyanidin synthesis, and the radioactive products were analysed.

- Mutant *ant 18-102* accumulated significant amounts of dihydroquercetin, while no such accumulation was observed in wild type grains or mutants in the *ant 13*, *ant 17*, and *ant 19* genes. This indicates that mutations in this gene block the pathway at the step converting dihydroquercetin into leucocyanidin (Fig. 2). Taste panels could not distinguish the flavour in pilot beer brewed with *ant 18-102* malt from that of Nordal malt. Thus, the significant but small accumulation of dihydroquercetin in *ant 18* mutants does not affect beer flavour.
- Radioactive dihydroquercetin was produced biologically in the flower buds of the white Petunia hybrida mutant W78 by incubating buds in radioactive acetate. The purified radioactive dihydroquercetin was fed to testa-pericarp tissue of developing barley grains. The wild type barley metabolised the radioactive dihydroquercetin into catechin, procyanidin B-3, and procyanidin C-2 and the intermediate 2,3-trans-3,4-cis-leucocyanidin (cf. Fig. 2). Mutant *ant 17-139* metabolised dihydroquercetin into catechin and procyanidin B-3, a behaviour expected from mutants

blocked in the synthesis of dihydroquercetin but not in the subsequent steps. Mutants in the *ant-13* gene failed to yield radioactive products and are considered to have an early block in flavonoid synthesis. Mutant *ant 19-109* forms anthocyanins in many tissues of the plant and is therefore considered to have a block after the branch point to anthocyanidins.

The amount of phenolic acids and other side products in the flavonoid pathway were analysed by Jende-Strid (1985). Mutants in the gene *ant 21* contained significantly more caffeic acid than the wild type indicating an early block in the pathway, while mutants in the gene *ant 17* and *and 22* accumulated naringenin-related compounds, and *ant 13* is specifically deficient in flavones. Key enzymes of the pathway such as dihydroquercetin reductase, 3,4-cis-diol (leucocyanidin) reductase, and procyanidin synthase are now isolated from barley grains. We wish to identify the structural genes for these enzymes and see if the genes mutated in the proanthocyanidin-free mutants encode such enzymes.

The agronomic performance of some of the raw mutants and of various recombinant lines has been recently very encouraging (von Wettstein *et al.*, 1985). Yields of 7 to 8 t/ha and resistance to diseases have been attained which are in line with those of modern varieties. The mutant line *ant 17-148* isolated in the excellent malting barley variety Triumph is on the Danish National list of approved varieties under the name Galant and is marketed in Denmark.

Brewing tests have shown that beer produced with proanthocyanidin-free barley has a better haze stability and shelf-life than that achieved with present technology (Ahrenst-Larsen and Erdal, 1979). This has two important consequences. If the brewer used proanthocyanidin-free hops extract, thus avoiding the addition of proanthocyanidins from hops, he can blend proanthocyanidin-free malt with malt containing proanthocyanidins in quantities up to 50% and still achieve a shelf life of the beer corresponding to present day standards. If he wishes to use hop pellets or hop cones this can be done with varieties which have a low proanthocyanidin to α-acid ratio, preferably one below 0.4 (Erdal *et al.*, 1985; von Wettstein *et al.*, 1985).

The malster and the brewer who has bought proanthocyanidin-free barley would like to sell off as feed the screenings comprising thin grain below 2.5 mm width or barley that happens not to meet his malting or brewing specifications. Chick performance trials have therefore been carried out on diets composed of proanthocyanidin-free barley.

It was found that broiler chickens fed on proanthocyanidin-free barleys gained equivalent or better weight than when fed on check varieties containing these polyphenols. The feeding quality of barley is not influenced negatively by the absence of proanthocyanidins. Therefore this property can in the future be a standard characteristic of all barley varieties.

Until then the farmer, the trade and the malsters would like to be able to determine in a short time the purity of a lorry with proanthocyanidin-free grain or malt. The finding that the proanthocyanidins are located in the seed coat (testa) of the mature grain (Aastrup *et al.*, 1984) has permitted the development of a procedure, which can determine the frequency of proanthocyanidin-containing grains in 15 minutes (Aastrup, 1985). One hundred grains are fixed in a block of thermoplastic clay, half the kernel is sanded off and the sanded grains are stained with a 1% vanillin-6M-HCl solution. The testa of proanthocyanidin-containing grains stains red and can be recognised by eye. Recently this procedure has been refined to allow the staining of testa tissue without damaging the embryo. Large scale non-destructive screenings for proanthocyanidin-free mutants in M_3 populations or recombinants in F_3 generations have thereby become feasible.

It will be interesting to obtain larger numbers of productive mutants which form anthocyanins in the vegetative parts of the plant but lack the proanthocyanidins in the testa. Perhaps this will facilitate the replacement of our present cultivars with proanthocyanidin-free ones.

MOLECULAR GENETICS AND NUTRITIONAL QUALITY OF BARLEY

Since the beginning of this century it has been known that barley and maize are nutritionally poor food for animals and humans. In both cereals the essential amino acid lysine is wanting. Therefore proteins from soybeans or other sources of lysine have to be added if pigs are to be fed with barley. An ancient Ethopian variety Hiproly and certain mutants such as Riso 1508 (*lys 3a*) show dramatic elevations of lysine content. In the latter mutant the increase amounts to 45% and in large scale trials it was shown that pigs fed with the mutant were ready for slaughtering after 100 days while the animals fed with present day barley attained the proper size only after 200 days. Indeed the growth rate of pigs on *lys 3a* diet without lysine addition approaches the growth rate of pigs on full feed diet. It has been difficult by breeding efforts to overcome the yield depression caused by low starch contents in the high lysine mutants. Recently some significant yield gains and improved starch content have been obtained by recombination breeding of *lys 3a* with Triumph and Nery (Munck *et al.*, 1985).

For a comprehensive review on the molecular analysis of storage protein synthesis in barley and corn, the reader is referred to an earlier publication (von Wettstein, 1983). Here I will list the major points of interest to the plant breeder and focus on some discoveries, which provide new ideas for the breeder.

Figure 3 is a diagram of the developing endosperm cell during grain filling. Starch is synthesised in the amyloplasts. The major storage proteins, the lysine deficient hordein polypeptides C, B, and D are encoded in three complex loci Hor-1, Hor-2, and Hor-3 on Chromosome 5. Only in endosperm nuclei is messenger RNA transcribed from these loci. The mRNA is translated on the ribosomes of the endoplasmic reticulum into larger

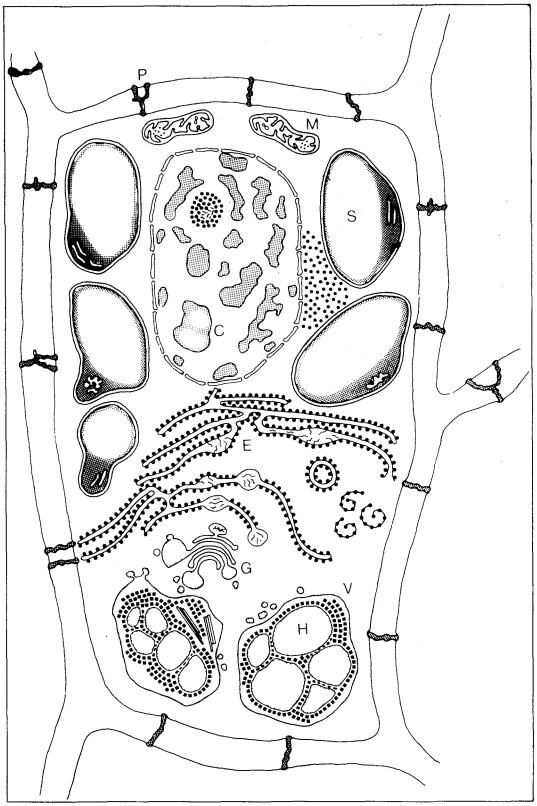

Figure 3. Developing endosperm cell of barley grain. C = chromosomes in nucleus; E = endoplasmic reticulum with attached ribosomes; G = Golgi apparatus; H = hordein; M = mitochondrion; P = plasmodesmata traversing cell wall; S = starch in plastid; V = vacuole.

sized precursor polypeptides which are cotranslationally transferred into the lumen of this membrane system. There the amino-terminal signal peptide is cleaved off and thereafter the hordein polypeptides are directed into the vacuoles of the cell, where they precipitate out with other polypeptides as protein bodies.

The alcohol-soluble hordein polypeptides are separable by electrophoresis into three groups of polypeptides. The largest, D, has an apparent molecular weight around 80 000, the C hordein polypeptides range in molecular weight from 48 000 to 67 000 and the B hordein polpeptides range from 27 000 to 38 000. Amino acid analyses, peptide mapping and sequencing together with characterisation of cDNA clones encoding B and C hordein polypeptides (Rasmussen *et al.*, 1983; Forde *et al.*, 1985a) reveal significant amino acid homologies but also distinct differences apart from size. Thus the B hordein polypeptides have a 3% content of cysteine while the C hordein polypeptides have a low amount of this amino acid. In C hordein polypeptides, the glutamine and proline content can reach 70% as compared to about 50% overall in the B1 hordein.

The complete structure for two Hor-2 genes is now known, i.e. two genes encoding two hordein polypeptides with the lowest apparent molecular weights (Brandt *et al.*, 1985; Forde *et al.*, 1985b). The primary structure of the gene designated λhor 2-4 and its deduced amino acid sequence is presented in Figure 4.

The gene encodes a 271 amino acid long polypeptide. The first 19 amino-terminal residues (marked off by a verticle arrow above the sequence) comprise the putative signal sequence cleaved away by the signal peptidase after transfer of the newly synthesised polypeptide into the lumen of the endoplasmic reticulum.

Characteristic features of signal peptides are a charged lysine residue next to the initiator methionine and a core of hydrophobic amino acids. The B1 polypeptide of 252 amino acids (MW 28 786) can be divided into three domains. In the 53 residue amino-terminal region there are nine blocks of four to six prolines and glutamines (Fig. 4, italicised) with a preferred core sequence -pro-gln-gln-pro- separated by one or two other amino acids. This gives a glutamine-proline content of 78%.

In the amino acid sequence deduced from the following domain of 492 nucleotides, the frequency of these two amino acids drops to 41% but they still tend to be organised in blocks separated by longer peptides containing other residues. In this domain are found seven of the eight cysteine residues encoded by the gene. The 35 carboxy-terminal amino acids encoded by the gene lack glutamine residues and contain only three proline residues.

If we compare this primary structure of the coding region in the λhor 2-4 gene with that of the other B1 hordein gene (HvBH3.4) sequenced (Forde *et al.*, 1985b) it is found to be 23 amino acids longer having additional proline-glutamine blocks in the amino-terminal domain. This may indicate that insertions and deletions of such blocks in this domain are the major source for size polymorphisms within the B hordein polypeptide family. Fifty-four additional nucleotide changes in the remaining coding region result in 24 amino acid substitutions, seven of these change a neutral amino acid to a charged residue. Thus, point mutations in the second domain with the scattered glutamine-proline blocks may be the major reason for the charge heterogeneity observed among B hordein polypeptides. This is supported by the analysis of the primary structure of three partial cDNA clones (Brandt *et al.*, 1985) which differ from the λhor 2-4 gene by deletion of one triplet and by 21, 13, and 14 amino acid substitutions. They represent three additional genes of the Hor-2 locus.

The clustering of glutamine-proline blocks in the amino-terminal region is not a feature of the maize storage protein, zein (Hu *et al.*, 1982; Pedersen *et al.*, 1982) or the high molecular weight glutenin of wheat (Forde *et al.*,

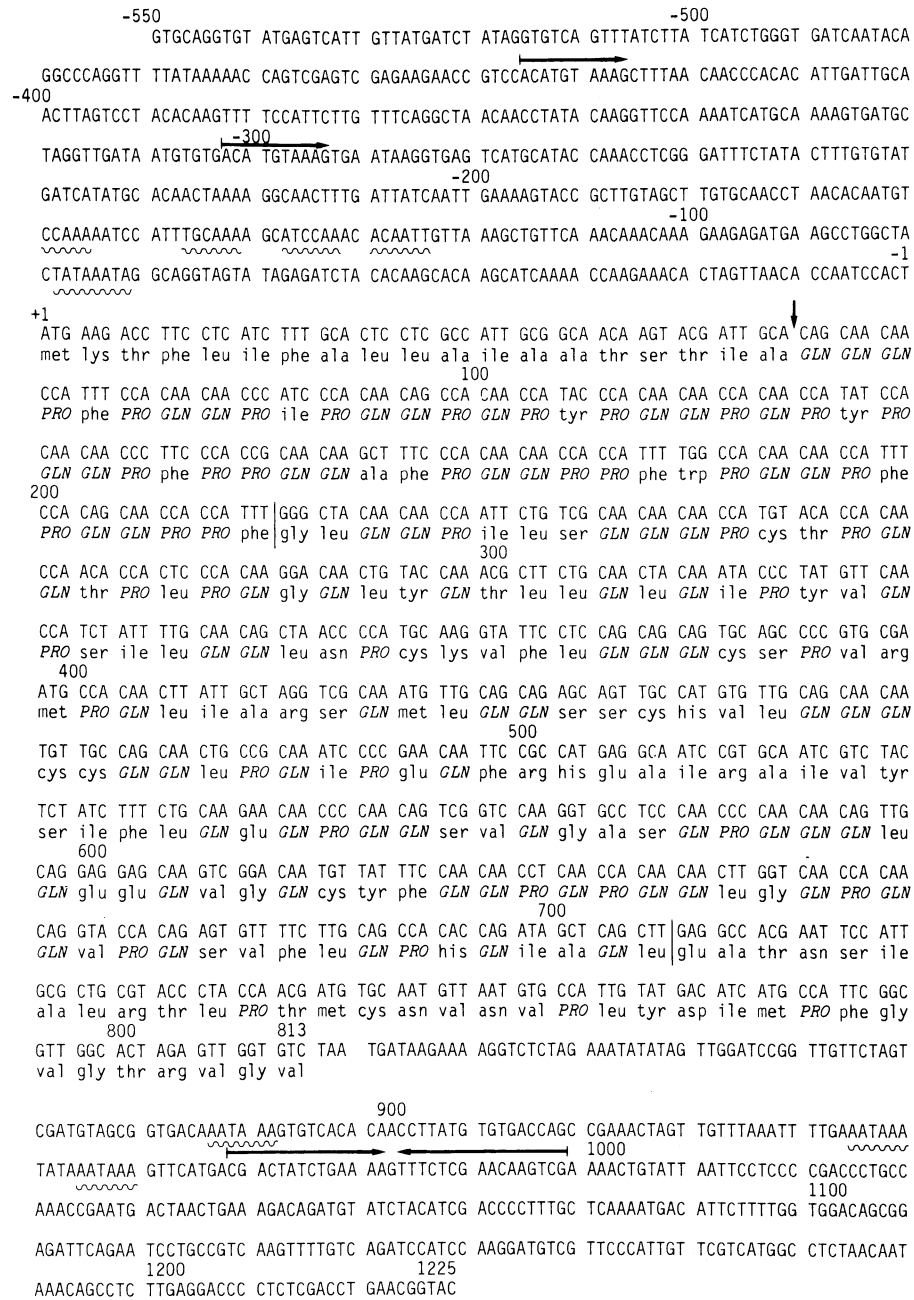

Figure 4. The primary structure of the λhor 2-4 encoding a B1 hordein polypeptide and its deduced amino acid sequence.

1985c; Thompson *et al.*, 1985). On the other hand, the α/β gliadin storage proteins of wheat have different variations of glutamine-proline blocks in the amino-terminal polypeptide part (Anderson *et al.*, 1984; Rafalski *et al.*, 1984; Summer-Smith *et al.*, 1985). The coding region of the cereal storage proteins exhibits extensive polymorphisms and we begin to see that incorporation of lysine in certain domains instead of existing residues should be feasible.

The 5' non-coding regions of the two hordein B1 genes are very similar, differing in less than 10% of the nucleotides. At position -79 is found a typical TATA box, TATAATA, which may function as an RNA polymerase binding site. Variants of the CAAT-motives for transcription are found at -130 to -160 (Fig. 4). Marked in Figure 4 is the repeated sequence ACATGTAAG which is homologous to the core sequence of the SV 40 enhancer present not only in front or the hordein genes but also in the wheat α/β gliadin genes (Rafalski *et al.*, 1984; Summer-Smith *et al.*, 1985), in the maize zein genes (Hu *et al.*, 1981; Pedersen *et al.*, 1982) and as a variant, TCTAAG, in a wheat high molecular weight glutenin gene (Forde *et al.*, 1985c; Thompsen *et al.*, 1985). Part of this sequence has been first identified by Lycett *et al.* (1985) in three legumine encoding genes of pea. In the 3' flanking region the gene contains three putative polyadenylation signals, AATAAA, (Fig. 4) and an almost perfect inverted repeat, which by virtue of a stable secondary structure may function in the termination of transcription.

The gene copy number of the Hor-2 locus derived from Southern blot analysis of barley DNA gives estimates of more than 10 members (Kreis *et al.*, 1983; Brandt *et al.*, 1985). Crossing over analysis has not permitted the separation of these genes. On the other hand, no genomic clones containing two adjacent Hor-2 genes have been found so far. Most likely the members of the gene family are several kilobases of DNA apart from each other. For the breeder, the most important task is to analyse the structure of such complex loci. The extensive sequence conservation of the flanking regions of the two hordein genes may reflect the importance of these sequences for the coordinated expression of the members of the gene families. In this regard it is of interest that probing of the expression or hordein B and C polypeptides with appropriate cDNA clones as well as the expression of protein Z and β-amylase revealed that mRNA levels for these proteins can be enhanced by the supply of ammonium nitrate to cut developing spikes (Giese and Hopp, 1984). While expression of C hordein and protein Z increased linearly even at high doses of nitrogen supply, that of the B hordein polypeptides reached saturation at intermediate levels of nitrogen supply.

It will be a challenging task to identify the regulatory sequences responsible for controlling gene expression with respect to tissue specificity, temporary specificity, and nutritional regulation.

Three of the high lysine mutants have been analysed as to molecular characteristics. Mutant Risø 56 (*hor 2ca*) fails to produce mRNA for B hordein polypeptides (Hopp *et al.*, 1983) and appears to be the result of a large deletion including the entire Hor-2 locus (Kreis *et al.*, 1983). The absence of B hordein polypeptides prevents the proper packaging of protein in the vacuole. Mutant Risø 1508 (*lys 3a*) in a gene on Chromosome 7 produces diminished levels of hordein mRNA.

Functional reconstitution of rough microsomes (endoplasmic reticulum) from wild type barley polysomes and smooth microsomal membranes permits the cotranslational transport of newly synthesised hordein precursors and signal peptide cleavage. If wild type polysomes are reconstituted with mutant *lys 3a* membranes vectorial discharge and signal peptide cleavage takes place only to a very limited extent indicating that this mutant produces a defective membrane transport component (Weber and Brandt, 1985). In experiments testing functional reconstitution of polysomes from barley with membranes from *Phaseolus vulgaris* or *Vicia faba*, transport and processing of hordein polypeptides was limited. Likewise vicilin precursor polypeptides — a storage protein from *Vicia faba* cotyledons — were not processed by barley or bean membranes.

The differences in amino acid sequence of the signal peptides of hordein and vicilin are apparently recognised by the species or tissue specific membrane components involved in the processing of the precursors. Knowledge of the function of signal sequences and their targets is important if one wishes to express and deposit gene products in species or tissues where they do not normally occur.

Transgenic expression has been achieved with the gene encoding the small subunit of ribulose bisphosphate carboxylase from pea in Petunia (Broglie *et al.*, 1984) and with the phaseolin gene from *Phaseolus vulgaris* in tobacco cotyledons (Sengupta-Gopalan *et al.*, 1985). In both cases the foreign genes are expressed in the correct tissue under the developmental control of the 5' flanking region. On the other hand only transcripts were detected, when a zein gene was introduced into sunflower tissue (Matzke *et al.*, 1984). Possibly the sunflower endoplasmic reticulum is unable to process the precursor for the zein polypeptide, whereby the transport pathway of the zein to the vacuolar protein body is obstructed and translation suppressed. The lack of transformation systems and plant regeneration from transformed protoplasts or cells in barley prevents as yet the use of storage protein genes which are redesigned by site-directed mutagenesis.

There is an alternative way of breeding for improved nutritional quality. The increased lysine content of Hiproly (*lys 1*) is primarily due to an elevated production of lysine rich proteins such as chymotrypsin inhibitor CI-2 (MW 9000, 10% lysine), protein Z and α-amylase (Hejgaard and Boisen, 1980; Svendsen *et al.*, 1980; Hejgaard *et al.*, 1985). Rasmussen (1985) has shown that monospecific antibodies directed against chymotrypsin inhibitor 2 can be conjugated with flourescein isothiocyanate and reacted with the paraformaldehyde fixed surface of abraded grains in which

the endosperm has been exposed. The emitted flourescence is proportional to the amount of chymotrypsin inhibitor present and Hiproly kernels with a high content can be picked out by eye from wild type kernels because of their higher flourescence. The embedding of 100 seeds at a time in a block of thermoplastic clay in such a manner that the embryo is protected, allows the screening out of a single kernel among large populations of kernels and its germination thereafter. This procedure is applicable to any protein for which antibodies are available. Mutants or rare recombinants elevating the content of a desired protein or lowering the amount of an undesired protein can be screened out in such a way.

Monoclonal antibodies have been produced against chymotrypsin inhibitor 2 and shown to be useful in the same way as the rabbit-elicited antibodies. Monoclonal antibodies have two advantages for the plant breeder: they can be produced in unlimited amounts over and over again once an appropriate hybridoma cell line has been isolated; they recognise single epitopes on a protein and can therefore be used to distinguish closely homologous polypeptides produced by individual members of multigene families, in casu different B hordein polypeptides. Screening with monoclonal antibodies in this non-destructive way opens the way to breeding for increases of individual high lysine proteins and compensating decreases of individual hordein polypeptides.

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REFERENCES

Aastrup, S. 1985. A test for presence or absence of proanthocyanidins in barley and malt. *Carlsberg Research Communications 50:* 37-42.

Aastrup, S., Outtrup, H., Erdal, K. 1984. Location of the proanthocyanidins in the barley grain. *Carlsberg Research Communications 49:* 105-109.

Ahrenst-Larsen, B., Erdal, K. 1979. Anthocyanogen-free barley — A key to natural prevention of beer haze. *Proceedings European Brewery Convention Congress,* Berlin (West), 1979, pp. 631-644.

Anderson, O.D., Litts, J.C., Gautier, M.F., Greene, F.C. 1984. Nucleic acid sequence and chromosome assignment of a wheat storage protein gene. *Nucleic Acids Research 12:* 8129-8143.

Brandt, A., Montembault, A., Cameron-Mills, V., Rasmussen, S.K. 1985. Primary structure of a B1 hordein gene from barley. *Carlsberg Research Communications 50:* 333-345.

Broglie, R., Coruzzi, G., Fraley, R.T., Rogers, S.G., Horsch, R.B., Niedermayer, J.G., Fink, C.L., Flick, J.S., Chua, N.-H. 1984. Light-regulated expression of a pea ribulose-1, 5-bisphosphate carboxylase, small subunit gene in transformed plant cells. *Science 224:* 838-843.

Delcour, J.A., Vandenberghe, M.M., Dondeyne, P., Schrevens, E.R., Wijnhoven, J., Moerman, E. 1984a. Flavour and haze stability differences in unhopped and hopped all-malt pilsner beers brewed with proanthosyanidin-free and with regular malt. *Journal Institute Brewing 90:* 67-72.

Delcour, J.A., Schoeters, M.M., Meysman, E.C., Dondeyne, P., Moerman, E. 1984b. The intrinsic influence of catechins and procyanidins on beer haze formation. *Journal Institute Brewing 90:* 381-384.

Erdal, K., Ahrenst-Larsen, B., Jende-Strid, B. 1980. Use of proanthocyanidin-free barley in beer brewing. *International Conference on Cereals for Food and Beverages: Recent Progress in Cereal Chemistry and Technology.* Copenhagen, pp. 365-379.

Erdal, K., Outtrup, H., Ahrenst-Larsen, B. 1983. The role of proanthocyanidins in beer flavour and flavour stability. *Proceedings European Brewery Convention Congress,* London, 1983, pp. 557-564.

Erdal, K., Outtrup, H., Ahrenst,-Larsen, B. 1985. Barley and hop proanthocyanidins and the colloidal stability of beer. *Proceedings European Brewery Convention Congress, Helsinki,* 1985, pp. 459-466.

Forde, B.G., Kreis, M., Williamson, M.S., Fry, R.P., Pywell, I., Shewry, P.R., Bruce, N., Miflin, B.I. 1985a. Short tandem repeats shared by B- and C-hordein cDNAs suggest a common evolutionary origin for two groups of cereal storage protein genes. *The EMBO Journal 4:* 9-14.

Forde, B.G., Heyworth, A., Pywell, I., Kreis, M. 1985b. Nucleotide sequence of a B1 hordein gene and the identification of possible upstream regulatory elements in endosperm storage protein genes from barley, wheat and maize. *Nucleic Acids Research 13:* 7327-7339.

Forde, I., Malpica, I.-M., Halford, N.G., Shewry, P.R., Anderson, O., Greene, F.C., Miflin, B.I. 1985c. The nucleotide sequence of a HMW glutenin subunit gene located on chromosome 1A of wheat (Triticum aestivum L.). *Nucleic Acids Research 13:* 6817-6832.

Giese, H., Hopp, E. 1984. Influence of nitrogen nutrition on the amount of hordein, protein Z and β-amylase messenger RNA in developing endosperms of barley. *Carlsberg Research Communications 49:* 365-383.

Hejgaard, J., Boisen, S. 1980. High-lysine proteins in Hiproly barley breeding: Identification, nutritional significance and new screening methods. *Hereditas 93:* 311-320.

Hejgaard, J., Rasmussen, S.K., Brandt, A., Svendsen, I. 1985. Sequence homology between barley endosperm protein Z and protease inhibitors of the α_1-antitrypsin family. *FEBS Letters 180:* 89-94.

Hopp, H.E., Rasmussen, S.K., Brandt, A. 1983. Organization and transcription of B1 hordein genes in high lysine mutants of barley. *Carlsberg Research Communications 48:* 201-216.

Hu, N.-T., Peifer, M.A., Heidecker, G., Messing, I., Rubenstein, I. 1982. Primary structure of a genomic zein sequence of maize. *The EMBO Journal 1:* 1337-1342.

Jende-Strid, B. 1984. Co-ordinator's Report: Anthocyanin genes. *Barley Genetics Newsletter 14:* 76-79.

Jende-Strid, B. 1985. Phenolic acids in grains of wild-type barley and proanthocyanidin-free mutants. *Carlsberg Research Communications 50:* 1-14.

Kreis, M., Shewry, P.R., Forde, B.G., Rahman, S., Miflin, B.J. 1983. Molecular analysis of a mutation confering the high-lysine phenotype on the grain of barley (*Hordeum vulgare*). *Cell 34:* 161-167.

Kristiansen, K.N. 1984. Biosynthesis of proanthocyanidins in barley: Genetic control of the conversion of dihydroquercetin to catechin and procyanidins. *Carlsberg Research Communications 49:* 503-524.

Lycett, G.W., Croy, R.R.D., Shirsat, A.H., Richards, D.M. Boulter, D. 1985. The 5'-flanking regions of three pea legumin genes: comparison of the DNA sequences. *Nucleic Acids Research 13:* 6733-6743.

Matzke, M.A., Susani, M., Dims, A.N., Lewis, E.D., Rubenstein, I., Matzke, J.M. 1984. Transcription of a zein gene introduced into sunflower using a Ti plasmid vector. *The EMBO Journal 3:* 1525-1531.

Munck, L., Bang Olesen, K., Stilling, B. 1985. Genome adjustment of breeding to balance yield defects in high-lysine mutants of barley. *Proceedings International Symposium EUCARPIA,* Genetic manipulation in plant breeding, Sept. 8-13, 1985, Berlin (West) (in press).

Outtrup, H., Erdal, K. 1983. Haze forming potential of proanthocyanidins. *Proceedings European Brewery Convention Congresss London, 1983:* 307-314.

Pedersen, K., Devereux, I., Wilson, D.R., Sheldon, E., Larkins, B.A. 1982. Cloning and sequence analysis reveal structural variation among related zein genes in maize. *Cell 29:* 1015-1026.

Rafalski, J.A., Scheets, K., Metzler, M., Peterson, D.M., Hedgeoth, C., Soil, D. 1984. Developmentally regulated plant genes: The nucleotide sequence of a wheat gliadin genomic clone. *The EMBO Journal 3:* 1409-1415.

Rasmussen, S.K., Hopp, H.E., Brandt, A. 1983. Nucleotide sequences of cDNA clones for B1 hordein polypeptides. *Carlsberg Research Communications 48:* 187-199.

Rasmussen, U. 1985. Immunological screening for specific protein content in barley seeds. *Carlsberg Research Communications 50:* 83-93.

Sengupta-Gopalan, G., Reichert, N.A., Barker, R.F., Hall, T.C., Hemp, J.D. 1985. Developmentally regulated expression of the bean β-phaseolin gene in tobacco seed. *Proceedings National Academy Science USA 82:* 3320-3324.

Summer-Smith, M., Rafalski, J.A., Sugiyama, T., Stroll, M., Soll, D. 1985. Conservation and variability of wheat α/β-gliadin genes *Nucleic Acids Rexearch 13:* 3905-3916.

Svendsen, I., Martin, B., Jonassen, I. 1980. Characteristics of Hiproly barley III. Amino acid sequences of two lysine-rich proteins. *Carlsberg Research Communications 45:* 79-86.

Thompson, R.D., Bartels, D., Harberd, N.P. 1985. Nucleotide sequene of a gene from chromosome 1D of wheat encoding a HMW-glutenin subunit. *Nucleic Acids Research 19:* 6833-6846.

Weber, E., Brandt, A. 1985. Species specific signal peptide cleavage of plant storage protein precursors in the endoplasmic reticulum. *Carlsberg Research Communications 50:* 299-308.

Wettstein, D. von. 1979. Biochemical and molecular genetics in the improvement of malting barley and brewers yeast. Proceedigs 17th European *Brewery Conventional Congress*, Berlin (West), 1979, pp. 587-629.

Wettstein, D. von. 1983. Genetic engineering in the adaptation of plants to evolving human needs. *Experientia 39:* 687-713.

Wettstein, D. von. 1984. European initiatives in the improvement of agricultural plants and micro-organisms by genetic engineering. *Trends in Biotechnology 2:* 149-152.

Wettstein, D. von., Jende-Strid, B., Ahrenst-Larsen, B., Sorensen, J.A. 1977. Biochemical mutant in barley renders chemical stabilization of beer superfluous. *Carlsberg Research Communications 42:* 341-351.

Wettstein, D. von., Jende-Strid, B., Ahrenst-Larsen, B. Erdal, K. 1980. Proanthocyanidin-free barley prevents the formation of beer haze. *MBAA Technical Quarterly 17:* 16-23.

Wettstein, D. von., Larsen, J., Jende-Strid, B., Ahrenst-Larsen, B., Sorensen, J.A., Erdal, K. 1981. Fortschritte in der Zuchtung von proanthocyanidin-freien Braugersten. *Brauwelt 121:* 760-767.

Wettstein, D. von., Nilan, R.A., Ahrenst-Larsen, B., Erdal, K., Ingversen, J., Jende-Strid, B., Nyegaard Kristiansen, K., Larsen, J., Outtrup, H., Ullrich, S.E. 1985. Proanthocyanidin-free barley for brewing: Progress in breeding for high yield and research tool in polyphenol chemistry. *MBAA Technical Quarterly 22:* 41-52.

SYMPOSIUM DISCUSSION

Dr W. Bushuk, University of Manitoba

At what stage is your high lysine barley development programme? Is there a commercial hybrid or is it still under development?

von Wettstein

There is one of the recombinant lines under official testing in Denmark. The yield of this line is similar to the barley yields of 5 or 6 years ago, but today's lines are higher yielding. However, due to tremendous

surpluses in Europe, the EEC is contemplating putting stringent quality requirements on grain, and yields may be reduced — there could well be more emphasis on quality and less on quantity.

Bushuk

Will that variety be covered by any patents?

von Wettstein

No. Varieties bred in Europe are covered by royalties but there are no patents.

Mr G.D. Coles, Crop Research Division, DSIR

I understand that the chymotrypsin inhibitor which is the source of high lysine and high proline has a low molecular weight. Is there any future in considering the possibility of increasing quality by increasing the availability of free lysine in the endosperm?

von Wettstein

We do not know yet where the protein is located — whether it is in the storage protein bodies, or whether it is in the cytoplasm. I consider that the classical seed proteins, the albonens can be used to improve the composition.