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Developing a feed barley with more balanced aminoacid composition

M. Lange*, E. Vincze*, H. Wieser, P.B. Holm***
*Danish Institute of Agricultural Sciences, Department of Genetics and Biotechnology
Research Centre Flakkebjerg, Forsoegsvej 1, DK-4200 Slagelse, Denmark
Tel.: + 45 8999 3797, Fax: + 45 8999 3501, e-mail: mette.lange@agrsci.dk
**German Research Centre for Food Chemistry, Lichtenbergstraße 4, D-85748 Garching, Germany

SUMMARY – In this study we report the selective suppression of the synthesis of C-hordeins in barley by antisense technology. We found a reduction in the total hordein content, especially in the C-hordeins, and increases in the glutelin fraction. Amino acid analyses revealed reductions in the contents of proline, glutamic acid/glutamine and phenylalanine while the levels of all other amino acids were increased. We conclude that antisense-mediated suppression of C-hordein synthesis may be a promising approach for improving the nutritional value of barley as a feed crop while at the same time reducing the environmental nitrogen load.

Introduction

Grains of cereals such as maize, barley and wheat possess a suboptimal amino acid composition when being used for feeding livestock. This imbalance results from the abundance of prolamin storage proteins – in barley termed hordeins –that typically constitute 50% of the total grain protein. These proteins are characterized by an abundance of glutamine and proline residues with low contents of essential amino acids, especially lysine, threonine and methionine. Over the last five decades great effort was devoted to improving the amino acid composition of cereals. In barley, large scale mutagenesis programs have resulted in the identification of a range of mainly recessive mutants that confer a high lysine phenotype (Munck, 1992). However, the high lysine trait is invariably associated with pleiotropic effects that affect yield, quality and agronomic performance. The unbalanced amino acid composition has in a number of species been addressed by gene technology.

However, although the level of essential amino acids may be increased for example, by manipulation of amino acid biosynthetic pathways or the introduction of genes encoding lysine and methionine rich storage proteins, the changes are often associated with side effects that negatively affect seed quality (see review: Ferreira *et al*., 2005, Galili *et al*., 2005). In the present study we have taken a different approach. Barley hordeins consist primarily of sulphur-rich B-hordeins and sulphur-poor C-hordeins (Miflin *et al*., 1983; Shewry *et al*., 1986). As the C-hordeins in particular are deficient in essential amino acids and have high contents of proline and glutamine we have explored the possibility of improving the amino acid profile by selectively suppressing the synthesis of C-hordeins.

Results and discussion

T-DNA integration and expression

Barley immature embryos were transformed with *Agrobacterium* strain AGLO harbouring the pWBVec82b vector that contains the hygromycin resistance gene (Mathews *et al*., 2001). In this construct we inserted a gene cassette in its own T-DNA borders containing an antisense fragment of the C-hordein gene between the ubiquitin promoter and the nos terminator.

The expression of the antisense construct was verified by RT-PCR and the T-DNA integration was analysed by Southern blots. Here we report on the detailed analysis of a line that contains five integration sites of the T-DNA.

Changes in storage protein composition and amino acid profile

The effects of the antisense construct on the storage protein composition in the mature grain as well as on the relative proportions of the four different hordein fractions were evaluated by SDS-PAGE and by reversed-phase (RP) high-performance liquid chromatography (HPLC). RP-HPLC has been found to give high-resolution separations of cereal seed proteins, the resolution often being better than that obtained by other chromatographic or electrophoretic methods (Wieser *et al*., 1998). RP-HPLC resolves proteins primarily on the basis of differences in surface hydrophobicity, complementing other techniques that separate proteins on the basis of size or charge. The storage proteins were isolated from the flour. The relative amounts and proportions of albumins/globulins (AL), prolamins (PR) and glutelins (GL) were determined by integration of absorbance areas at 210 nm which have been shown to be highly correlated with the amounts of proteins (Wieser *et al*. 1998). HPLC of unreduced prolamins (PR) was used to determine the total amounts of prolamins.

As shown by the intensity of the hordein polypeptide bands in Fig. 1 it appeared that the transgenic line had a reduced C-hordein content compared to the parental variety.

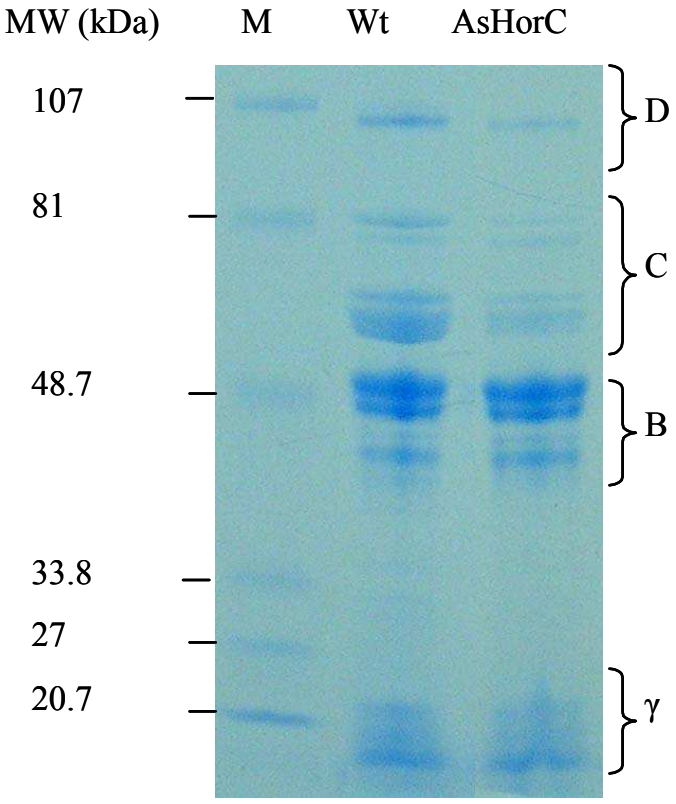

Fig. 1. SDS-page of hordein fractions extracted from wild type (Wt) and the antisense (AsHorC) lines of barley. The molecular weights are given in kDa. The positions of the D-, C-, B- and γ-hordeins are indicated. Molecular marker (M).

The HPLC results showed an overall reduction in the prolamin content. In the parental cultivar, prolamins constituted 52.3% of the total storage proteins, while in the transgenic line the prolamin content was reduced to 41.3%. In contrast there was an increase in the glutelin fraction from 38% in the parental line to 50.1% in the transgenic line. Likewise, there were minor changes in the proportion of the albumin/globulin fraction in the transgenic line compared to the parental line. The albumins decreased from 9.2% to 8.6% and the globulin fraction increased from 38.5% to 50.1%. In the transgenic line the C-hordeins accounted for 31% of the total prolamins compared to 35.4% in the

parental line. The relative amount of B-hordeins was was increased to 60% in the transgenic line compared to 58.5% in the parental line. Smaller relative increases in the proportions of D- and γ-hordein also occurred, from 4.9% and 1.2%, respectively, in the parental line to 7.0% and 1.4% in the transgenic line.

Amino acid analysis

The seed weight, protein content and content of the individual amino acids in the transgenic line and the parental line were measured. The mean seed weight of the transgenic line was 37.6 mg compared to 35.2 mg for the control, based on samples of 20 seeds. Apart from this weight difference the seeds appeared very similar in their external morphology. The protein content of the transgenic line was 15.7% while the parental line had a protein content of 17.1%. We attribute these differences and the high protein content to the fact that the plants were grown in a greenhouse where, in our experience, it is difficult to obtain a low and consistent grain protein content.

The amino acid profile (not including tryptophan) was determined following hydrolysis of total proteins. Glutamine and asparagine are accordingly included in the glutamate and aspartate fractions. The profile revealed a number of differences between the transgenic line and the parental variety. In the transgenic line glutamine/glutamate, proline and phenylalanine were less abundant than in the parental line. The percentage differences were 5.9% for glutamine/glutamate, 12.1% for proline and 6.3% for phenylalanine. The reductions in the contents of the three amino acids are in good agreement with the general reduction in the prolamin contents of the transgenic lines as well as the selective suppression of C-hordeins that contain high proportions of phenylalanine. All other amino acids were more abundant per unit protein in the transgenic lines than in the parental line. The contents of the sulphur-containing amino acids cysteine and methionine were increased by up to 18.2% and 11.8% while there were also increases in other essential amino acids including lysine, threonine, isoleucine, leucine and valine by 14.7%, 8.8%, 5%, 8.1% and 11.1%.

Conclusion

A conventional antisense approach was used to generate transgenic lines expressing an antisense C-hordein construct. While prolamin formation was suppressed and the amounts of albumins decreased, the amounts of glutelins/globulins were increased. Within the prolamin fraction there was a relative reduction in the content of C-hordeins and relative increases in B-, D- and γ-hordeins resulting in reduced contents of proline, glutamine/glutamate and phenylalanine and increases in the proportions of all other amino acids. In future studies we will evaluate the agronomic performance and nutritional quality of the antisense lines grown in field trials and assess potential pleitropic effects on the transcriptome and proteome imposed by the antisense suppression.

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