Theor Appl Genet (2003) 106:1139–1146
DOI 10.1007/s00122-002-1162-3

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Expression of the gamma-zein protein of maize in seeds of transgenic barley: effects on grain composition and properties

Received: 8 April 2002 / Accepted: 6 August 2002 / Published online: 28 February 2003

Abstract A cDNA clone encoding the γ-zein protein of maize was expressed in developing grain of barley using the starchy endosperm cell-specific promoter from the wheat *Glu-1D-1* (HMW subunit 1Dx5) gene. Seven transgenic lines were recovered from 226 bombarded immature embryos, of which two were sterile and four tetraploid, while five were shown to express the γ-zein protein based on western blotting. Southern blot analysis showed the presence of between about three and twelve transgene insertions. Detailed comparative studies of five null and five homozygous transformed sub-lines from transgenic line A showed that γ-zein accounted for over 4% of the total prolamin fraction, corresponding to about 1.9% of the total grain N. Comparison of the proteins present in the gel protein fraction demonstrated that the γ-zein was incorporated into polymers, as in maize. However, there was no effect on grain hardness measured using the Perten Single Kernel Characterisation System or on the vitreousness measured by visual inspection. This contrasts with the situation in maize where a clear association with vitreousness has been reported.

Keywords Barley · Transgenic plants · Grain texture · Grain vitreousness · γ-Zein

Communicated by J.W. Snape

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Introduction

Grain texture has been described as "the most important characteristic that affects the functionality of a common wheat" (Pomeranz and Williams 1990), being the primary character used to separate wheats into groups and affecting both milling and end use properties. Although texture is less important as an end use determinant in barley, there is clear evidence that soft-textured grains have better malting characteristics (Brennen et al. 1996) and that low milling energy (a measure of texture) can be used as a selection criterion for malting barleys (Allison et al. 1976).

However, grain texture in barley and wheat can be considered to have two components: hardness and vitreousness. Hardness is thought to be determined by the strength of bonding between the cell contents, principally between the starch granules and matrix proteins but probably also between the matrix proteins and cell walls. The major determinant in wheat has been mapped to a single locus (designated *Ha*) on the short arm of chromosome 1D in hexaploid bread wheat (Law et al. 1978), and the absence of this locus from tetraploid pasta wheat results in an "ultrahard" phenotype. A similar locus appears to be present on chromosome 5H of barley (Powell et al. 1992; Chalmers et al. 1993; Thomas et al. 1996; Rouvés et al. 1996). In both wheat and barley the hardness loci appear to encode tryptophan-rich proteins called puroindolines and hordoindolines, respectively (Gautier et al. 1994; Beecher et al. 2001; Darlington et al. 2001), with allelic differences in the expression and sequences of the former being proposed to account for variation in hardness within bread wheat (Giroux and Morris 1998; Lillemo and Morris 2000). However, no such correlations have been reported for hordoindolines and grain texture in barley (Beecher et al. 2001; Darlington et al. 2001).

Whereas hard and soft grains appear similar to the eye, differences in vitreousness are clearly observed as steely (vitreous) or mealy (floury) grain, or regions within grain. Furthermore, vitreousness does not appear to be strongly

genetically determined in wheat and barley but can occur in most if not all genotypes, particularly when grown at high temperature and/or with high nitrogen availability. The molecular basis for vitreousness is not known, but vitreous grain contains higher levels of protein which is more densely packed, the absence of air spaces being proposed to account for the appearance. However, neither vitreous nor floury grain appear to be enriched in specific protein components.

Although it is doubtful whether a similar type of hardness to that present in wheat and barley occurs in maize and sorghum, grain of these species do contain vitreous and floury regions, whose amount and distribution are determined by genetic factors but may also be influenced by environment (Watson 1987). Thus, the five general classes of maize (flint corn, popcorn, flour corn, dent corn and sweet corn) all differ in the extent and distribution of vitreous (also called corneous or horny) endosperm.

There is also compelling evidence that vitreousness in maize and sorghum is associated with high levels of a specific group of prolamin storage proteins (the γ-zeins and γ-kafirins, respectively) that are enriched in vitreous regions of endosperms (Chandrashekhar and Mazhar 1999). The amount of γ-zeins is also increased by two- to threefold in modified lines of *opaque-2* maize in which the endosperm texture is converted from floury to vitreous (Wallace et al. 1990). The mechanism of this effect is not known but could relate to the specific location of γ-zein proteins in the outer part of the protein bodies deposited in the starchy endosperm cells (Lending and Larkins 1989; Wallace et al. 1990; Geetha et al. 1991; Lopes and Larkins 1991).

Although soft texture is preferred for malting barley, hard-textured endosperms could have advantages for feed quality, restricting starch digestion in the rumen of cattle fed whole grain but increasing starch digestibility due to a higher level of damage during milling in monogastric livestock fed milled grain. We have, therefore, expressed a γ-zein protein of maize in the developing grains of barley to determine its deposition pattern and impact on grain properties.

Materials and methods

Plant materials

Plants of barley cv. Golden Promise were grown in a controlled environment growth room under a 16/8-h (day/night) photoperiod with light supplied by 400-W sodium lamps at an irradiance of approximately 300 μmol PAR. The air temperature was adjusted to 18/15 °C (day/night) with a relative humidity of 50–70%.

Production of transgenic plants

Two separate plasmids were used: pAHC25, which contained the marker genes *bar* (phosphinothricin resistance gene) and *uidA* (B-glucuronidase gene) under the control of the maize ubiquitin gene (*Ubi-1*) promoter and terminated by the nopaline synthase gene (*nos*) terminator, for selection and screening of transgenic plants (Christensen and Quail 1996), pHMWzein, which contained a γ-zein cDNA driven by the wheat high-molecular-weight (HMW) glutenin subunit *Glu-1D-1* gene promoter (Lamacchia et al. 2001) and terminated by its own 3' UTR (Fig. 1).

Plasmid pHMWzein was co-delivered with plasmid pAHC25 into immature scutellar tissue of barley using a biolistic gene delivery system (PDS 1000/He, BioRad, Hercules, Calif.), and putative transformants were selected based on their resistance to the selection agent Bialaphos.

Molecular characterization of transgenic plants

Genomic DNA was isolated from leaf tissues of primary transformants and their progenies using a modified CTAB method based on the protocol of Stacey and Isaac (1994). The presence of transgenes was determined by polymerase chain reaction (PCR) amplification of a DNA fragment using oligonucleotide primers for the marker genes *bar* and *uidA* (Cannell et al. 1999). The PCR primer set γz2 (5'-CATGCCACTACCCTACTCAA-3') and γz6 (5'-GGATG-GACTGGAGGACCAAG-3') was designed to amplify specifically a 294-bp DNA fragment from the 3' region of the γ-zein transgene. The PCR parameters were as follows: one cycle of 94 °C for 5 min, 55 °C for 1 min and 72 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min plus 3 s, with a final extension at 72 °C for 10 min.

Southern blotting was performed according to the DIG system user's guide for filter hybridization (Boehringer Mannheim, Germany). Genomic DNA (10 μg) was either digested with *Sph*I to cut once within the plasmid pHMWzein sequence, or with *Sph*I and *Xba*I to excise the γ-zein gene expression cassette. The DNA was then size-separated by electrophoresis through a 0.8% (w/v) agarose gel and blotted by capillarity onto a positively charged nylon membrane (Boehringer Mannheim). Prehybridization was carried out at 52 °C for 3 h in hybridization solution (DIG Easy Hyb, Boehringer Mannheim) and hybridization was overnight under the same conditions with the inclusion of 10 ng/ml of digoxigenin-labelled probe prepared from the coding region of the γ-zein cDNA using the PCR DIG probe synthesis kit (Boehringer Mannheim). The membrane was washed twice at room temperature

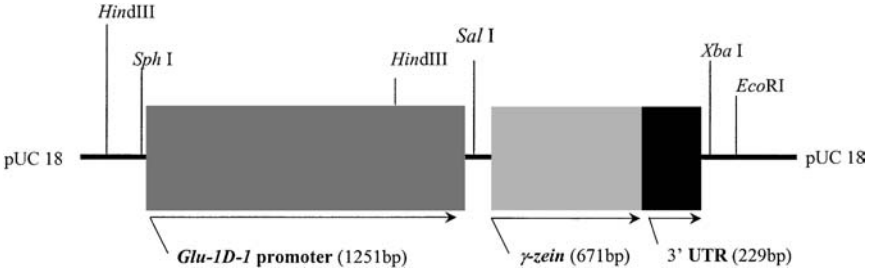

Fig. 1 Diagram of the transformation vector pHMWzein

in 2 × SSC (0.3 *M* NaCl, 0.03 *M* sodium citrate) containing 0.1% (w/v) sodium dodecyl sulphate (SDS) and twice at 68 °C in 0.1 × SSC containing 0.1% (w/v) SDS. The chemiluminescent signal was detected using the DIG luminescent detection kit for nucleic acids (Boehringer Mannheim) and recorded on X-ray film (Amersham, UK).

Detection of transgene expression

Expression of the γ-zein in transgenic seeds was demonstrated by SDS - polyacrylamide gel electrophoresis (PAGE) (Shewry et al. 1995) and by protein dot and western blotting (Fido et al. 1995), using anti-γ-zein rabbit polyclonal antiserum. The primary γ-zein antibody was used at a dilution of 1:6250, and the secondary anti-rabbit alkaline phosphatase conjugate (Sigma, St. Louis, Mo., A9919) at 1:6250.

Grain composition

The total nitrogen (N) contents were determined using the Dumas method on a combustion analyser (LECO, FP/NCS-2000) for flour and by Kjeldahl analysis for protein fractions.

The total contents of sulphur (S) were determined according to Zhao et al. (1994) using inductively coupled plasma atomic emission spectrometry (ICP-AES) with prior HNO_3–$HClO_4$ digestion.

The composition of alcohol-soluble storage proteins was determined by SDS-PAGE (Shewry et al. 1995) and the pattern quantified by volume analysis using a BioRad Gel Doc 1000 gel documentation system.

Protein polymers were determined as gel protein as described by Smith and Lister (1983) and by size-exclusion high-performance liquid chromatography (SE-HPLC). For SE-HPLC analysis, barley flour was mixed with 50 m*M* sodium phosphate/0.5% (w/v) SDS buffer (pH 6.9) at a final concentration of 10 mg/ml. The flour sample was then thoroughly wetted by vortex mixing for 30 s, and total protein was extracted by sonication for 30 s. The suspension was then centrifuged at 20,000 rpm for 5 min. A 50-μl aliquot of the supernatant was injected into a Phenomenex BIOSEP-SEC-S4000 column (Phenomenex, Torrance, Calif.) in a Beckman Gold Noveau SE-HPLC system consisting of a model 507e autosampler, a model 126 solvent module and a model 166 UV detector. Flow rate was 0.5 ml/min for a total run time of 30 min. Samples were eluted with 50% acetonitrile in water (v/v) containing 0.05% trifluoroacetic acid (TFA) (v/v). Detection was at 214 nm.

Grain hardness was determined on 300 single grains of each line using the Perten single kernel characterization system (SKCS 4100, Perten Instruments, Reno, Nev.). Vitreousness was determined by visual inspection of intact and cut grains, according to ICC-Standard No. 129.

Results and discussion

Production and characterization of transgenic plants

Identification of lines expressing the γ-zein transgene

Bombardment of 226 immature embryos of barley cv. Golden Promise led to the regeneration of seven lines which were PCR-positive for both the marker (*bar* and *uidA*) and γ-zein genes. Two of these (F and G) were sterile, while four (B, D, E and G) were identified as tetraploid on the basis of morphological characteristics. [Compared with normal diploid plants, tetraploid plants have broader and thicker leaves which attract more insects, thicker and generally shorter culms, thicker roots, delayed growth rate, partial fertility with spikes partially emerged from the leaf sheath and longer and thinner seeds (Choi et al. 2000)]. Thus, only lines A and C appeared to be normal, fertile diploid plants. The overall transformation efficiency was 3.1% and the recovery of fertile diploid transformants 0.9%.

T_1 seed from the five fertile lines (A–E) were screened for γ-zein expression by dot blot analysis followed by SDS-PAGE and western blotting (Fig. 2). This showed the highest levels of expression in lines A and B with a lower level in line E and only traces in lines C and D. Segregation was also observed between single seeds of lines A, C and D. Lines A and B were derived from the same bombarded embryo, and Southern blotting (see below) showed identical patterns of transgene integration. It was therefore concluded that they were diploid and tetraploid derivatives, respectively, from the same transformation event. The γ-zein encoded by the transgene co-migrated with endogenous barley proteins in the total protein extracts shown in Fig. 2 but can be seen as increased band intensity in lines A and B (see arrow in Fig. 2). Subsequent studies with alcohol-soluble protein fractions (see below) allowed the identity of the band in line A to be confirmed by microsequencing; the sequence obtained (Thr.His.Thr.Ser.Gly.Gly.Cys.Gly.Gln) being identical to that of the mature protein encoded by the γ-zein sequence (Reina et al. 1990). The separations in Fig. 2 also showed that the expression of γ-zein in lines A, B and E was accompanied by less intensely stained bands corresponding to D hordein, the barley homologue of the wheat HMW glutenin subunits. This is quantified for line A below.

It is possible that the suppression of D hordein expression could have resulted from the use of the wheat HMW subunit promoter, which is related in sequence to the D hordein promoter [the HMW subunit and D hordein promoters show more than 90% sequence identity over the upstream regions that have so far been sequenced, up to –372 for D hordein (Shewry et al. 1999)]. To explore this possibility, we also carried out SDS-PAGE analyses of eight lines in which the HMW subunit gene promoter was used to drive two different gene coding regions: six lines expressing a protein kinase gene (SnRK1) (Zhang et al. 2001) and two lines expressing the *uidA* reporter gene which enclodes β-glucuronidase (GUS) (authors' unpublished results). Analysis of five individual T_1 and T_2 seeds of these lines showed no evidence of D hordein suppression (results not shown), indicating that the use of the HMW subunit promoter was not responsible for the suppression of D hordein in the γ-zein lines.

Molecular analyses of transgenic events and inheritance

Genomic DNA isolated from five transgenic lines, already shown by PCR to contain the γ-zein transgene, was analysed by Southern blotting. DNA from lines A–D

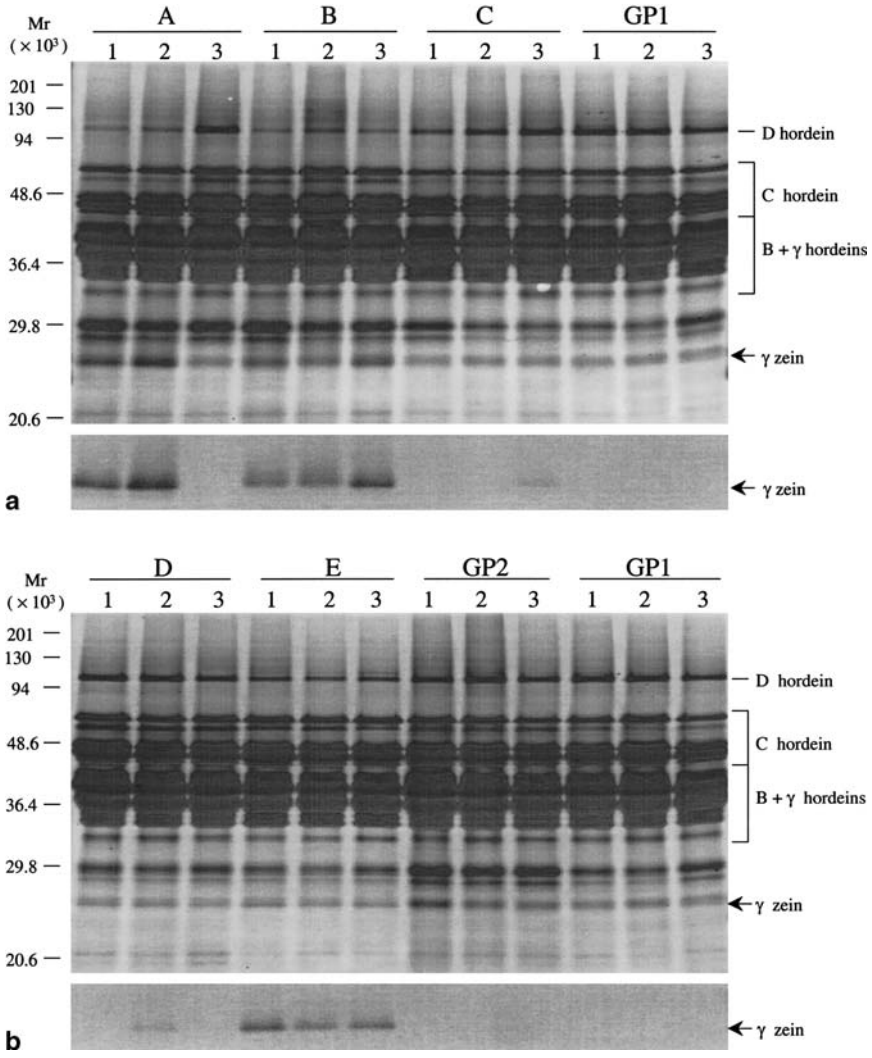

Fig. 2a, b SDS-PAGE (*upper panels*) and immunoblotting (*lower panels*) analyses of storage proteins from individual T_1 seeds of transgenic lines *A–E*. *GP1* and *GP2* are controls from donor and tissue culture-derived plants, respectively

and F was digested with either *Sph*I and *Xba*I, known to excise a 2.15-kb fragment of the γ-zein transgene, or *Sph*I, known to cut once within the sequence. Plasmid DNA (pHMWzein) and genomic DNA from a non-transformed plant were included as controls (Fig. 3).

Lines C, D and F had relatively simple and unique banding patterns, indicating they were derived from independent transgenic events, with approximately three to five transgene copies in each. Line C showed three distinct bands after digestion with *Sph*I, indicating a minimum of three transgene copies. However, two of the three bands released by *Sph*I were smaller than the 4.8 kb expected from a complete pHMWzein plasmid, and neither of the two bands released after double digestion was of the expected size (2.15 kb) for the expression cassette. This indicated that some of the γ-zein transgene insertions had undergone deletions or rearrangements resulting in the loss of either *Sph*I and/or the *Xba*I restriction sites. In line D, four bands were released after *Sph*I digestion of genomic DNA, indicating at least four transgene copies. Double digestion with *Sph*I and *Xba*I released three distinct bands of the expected size or larger, confirming that line D contained at least one intact transgene expression cassette. Line F showed four distinct bands after digestion with *Sph*I, indicating at least four transgene copies. An intense band of the expected size was released after double digestion together with a second band that was smaller than the expected size and probably derived from transgene rearrangement and/or modification. Lines A and B had identical but complicated banding patterns, indicating that they were derived from the same transgenic event with multiple DNA insertions. Digestion with *Sph*I released a total of 12 identifiable bands, the most intense of which was the same size as the linearized plasmid, indicating the presence at least 12 transgene copies, with some concatenation. Nine bands were released after double digestion, with a band of the expected size being the most intense. The presence of other bands larger than the 2.15-kb excision product, suggested that some rearranged and/or modified transgenes were also present. Comparison with the density of the plasmid control bands, calculated to contain the genomic equivalent of 20 transgene copies, indicated the copy numbers of lines A and B to be between 20–30.

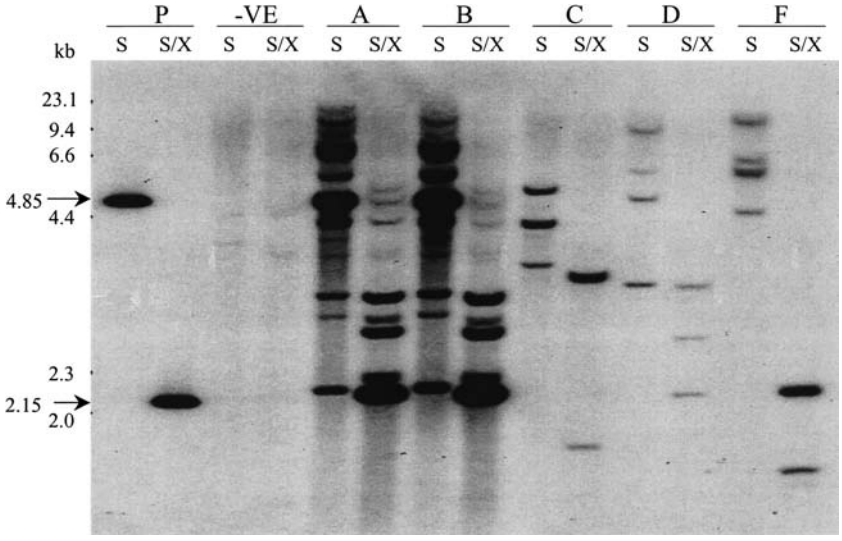

Fig. 3 Southern blot analysis of primary transgenic lines. Genomic DNA (10 μg) of five transgenic lines (*A*, *B*, *C*, *D*, and *F*) was either singly restricted by *Sph*I (*S*) or double restricted by *Sph*I and *Xba*I (*S/X*). *P* Plasmid control, *-VE* donor plant control

Table 1 Comparison of grain characteristics and alcohol-soluble (prolamin) protein contents of transgenic and control lines of cv. Golden Promise

Character	Transgenic segregants[a]	Null segregants[a]	GP1[b]	GP2[b]
Grain weight (mg)	43.43±0.87	41.11±1.93	39.01	42.19
Grain diameter (mm)	2.62±0.04	2.54±0.04	2.54	2.56
Grain N				
mg/g DW	22.81±0.75	21.56±0.82	22.97	20.09
mg/grain	0.876±0.034	0.781±0.035	0.792	0.753
Grain S				
mg/g DW	1.616±0.070	1.549±0.064	1.695	1.464
Alcohol-soluble N				
% total N	42.9±1.0	38.7±0.8	42.16	39.18
mg/g DW	9.76±0.30	8.36±0.39	9.72	7.87
mg/grain	0.376±0.018	0.303±0.019	0.335	0.295
Grain hardness	61.83±2.40	63.85±2.58	63.38	65.02

[a] Values for transgenic and null segregants are based on analysis of five sub-lines each of line A and are ± the standard error
[b] GP1 and GP2 are the donor cultivar and a tissue culture-derived line, respectively

T_1 progeny of line A were analysed by PCR for transgene inheritance. The genes for *bar*, *uidA*, and γ-zein were inherited together and present in 14 out of 20 T_1 plants analysed. This is consistent with a 3:1 Mendelian segregation for one genetic locus ($\chi^2 = 0.025 < \chi^2_{0.05} = 3.84$).

Effects of γ-zein on grain composition and properties

Line A was selected for detailed analysis based on its "normal" (i.e. diploid) phenotype and high expression of γ-zein. Five homozygous T_2 sub-lines (A4, A5, A9, A11 and A17) and five null segregant T_2 sub-lines (A8, A13, A16, A18 and A19) were initially selected based on SDS-PAGE of grain proteins, with the presence or absence of the γ-zein transgene subsequently being confirmed by PCR analysis. A randomised block experiment was then set up in a containment glasshouse, with 12 pots of each sub-line (each containing a single plant) and 12 additional pots each of the donor cultivar (GP1) and a tissue culture-derived line (GP2) as negative controls. Seeds from the 12 plants of each sub-line were bulked for analysis and the results from the sub-lines combined for statistical analysis (Table 1).

The transgenic grain gave significantly higher values for seed weight ($P<0.05$) and grain diameter ($P<0.01$). Similarly, the N content of the transgenic sub-lines was significantly increased when expressed as milligrams per gram dry weight ($P<0.05$) or per grain ($P<0.005$). The sulphur contents of the transgenic sub-lines were also higher than those of the null sub-lines but the differences were not statistically significant.

The increased N contents of the transgenic sub-lines were associated with statistically significant increases in the amount of N soluble in 50% (v/v) propan-*l*-ol + 2% (v/v) 2-mercaptoethanol, expressed on the basis of milligram per gram DW ($P<0.001$), milligram per seed ($P<0.001$) or percentage total grain N ($P<0.001$). This fraction comprises prolamin storage proteins: the endogenous hordeins of barley and the γ-zein transgene product. Reduction of disulphide bonds in this fraction and

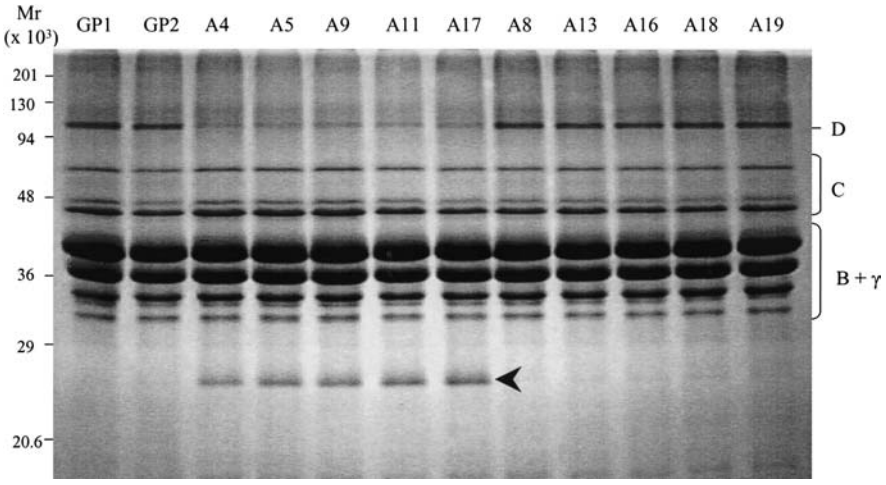

Fig. 4 SDS-PAGE of alcohol-soluble proteins extracted from T_3 seeds of five homozygous transgenic sub-lines (*A4*, *A5*, *A9*, *A11*, *A17*) and five null sub-lines (*A8*, *A13*, *A18*, *A19*) of cv. Golden Promise. *GP1* and *GP2* are control seeds from a donor plant and a plant derived from tissue culture, respectively. *Arrowhead* γ-Zein band, *B+γ*, *C* and *D* hordein groups

Table 2 Comparison of the amounts and proportions of hordeins and γ-zein in transgenic and control lines of cv. Golden Promise

Alcohol-soluble protein	Transgenic segregants[a]	Null segregants[a]	GP1[b]	GP2[b]
γ-zein				
% alcohol-soluble protein	4.34±1.04	0.31±0.06	0.36	0.30
% total N	1.86±0.44	0.12±0.03	0.15	0.12
μg/g DW	423.86±104.99	26.23±6.06	34.98	23.62
D hordein				
% alcohol-soluble protein	1.79±0.23	5.18±0.17	5.41	5.29
% total N	0.77±0.11	2.01±0.09	2.29	2.06
μg/g DW	174.51±25.08	433.10±24.85	525.66	416.44
C hordein				
% alcohol-soluble protein	14.46±1.58	14.06±1.03	15.77	12.58
% total N	6.21±0.71	5.44±0.45	6.67	4.93
μg/g DW	1,409.95±154.11	1,176.68±123.03	1,532.29	990.34
B hordein				
% alcohol-soluble protein	79.43±0.52	80.55±0.89	78.45	81.76
% total N	34.08±0.79	31.18±0.55	33.16	32.03
μg/g DW	7,748.70±255.27	6,729.68±269.43	7,622.59	6,436.39

[a] Values for transgenic and null segregants are based on analyses of five sub-lines each of line A and are ± the standard error
[b] GP1 and GP2 are the donor cultivar and a tissue culture-derived line, respectively

alkylation of the free sulphydryl groups gave sharper banding patterns on SDS-PAGE (Fig. 4) that facilitated quantification by gel scanning (Table 2). This showed that γ-zein accounted for about 4% of the total prolamins in the transgenic sub-lines but that the proportion of D hordein was significantly reduced from about 5% to less than 2% total prolamin N ($P<0.001$). The small amounts of γ-zein determined by gel scanning of the null sub-lines (0.31% total N) resulted from the presence of a minor endogenous barley protein of similar mobility. There were no significant differences between the amounts of C hordeins in the transgenic and null segregant sub-lines when expressed as percentage total prolamins or percentage total grain N. However, when expressed as micrograms per gram grain DW, the amounts of C hordein in the transgenic sub-lines were significantly higher than that in the null sub-lines ($P=0.029$). The amounts of B hordein were significantly lower in the transgenic sub-lines (79.43%) than in the null sub-lines (80.55%) when expressed as percentage total prolamins ($P=0.04$) but significantly higher in the transgenic sub-lines than in the null sub-lines when expressed as percentage total grain N or micrograms per gram grain DW ($P<0.001$). This is due to the larger amounts of total prolamins present in the transgenic sub-lines than in the null sub-lines (Table 1).

γ-Zein is incorporated into protein polymers

γ-Zein is not extracted from maize grain in the absence of a reducing agent due to its presence in insoluble protein polymers stabilized by inter-chain disulphide bonds (Coleman and Larkins 1999). Two different approaches were therefore used to determine whether γ-zein was incorporated into polymers in the transgenic barley line.

Gel protein is a polymeric fraction which can be prepared by stirring milled grain with 1.5% (w/v) SDS followed by centrifugation. The gelatinous upper layer

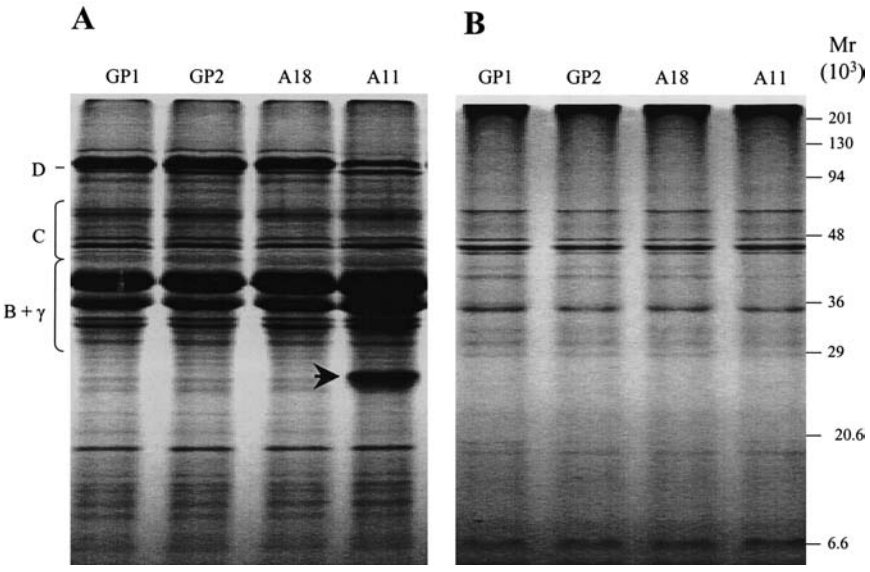

Fig. 5 SDS-PAGE under reducing conditions (**A**) and without reduction (**B**) of gel proteins extracted from seeds of control lines (*GP1*, *GP2*), a null sub-line (*A18*) and a transgenic sub-line (*A11*). *Arrowhead* γ-Zein band, *B+γ*, *C* and *D* hordein groups

can then be removed with a spatula for SDS-PAGE analysis. Furthermore, the proportion of the total grain N present in the gel protein is inversely correlated with malting quality (Smith and Simpson 1983). The amount of gel protein recovered from 1 g of milled grain of the transgenic sub-line A11 was 186.7 ± 28.6 mg, which did not differ significantly from that recovered from the null sub-line A18 (189.3 ± 51.2 mg) and from GP1 (199.2 ± 46.0 mg). Comparison of the gel protein fractions by SDS-PAGE under reducing (Fig. 5A) and non-reducing (Fig. 5B) conditions showed that they comprised mainly polymeric B hordein, D hordein and, in line A11 only, γ-zein, with the unreduced samples showing only trace amounts of monomeric C hordeins that were probably entrapped in the gel protein matrix.

γ-Zein has no effect on grain texture in barley

The initial aim of expressing γ-zein in barley was to manipulate the texture of the grain. The hardness of the transgenic and null sub-lines was, therefore, determined using a Perten Single Kernel Characterisation System. This showed that the mean hardness of the transgenic sub-lines was slightly lower than that of the null sub-lines (giving a value in arbitrary units of 61.83 ± 2.40 compared with 63.85 ± 2.58), although this difference was not statistically significant (Table 1). Similarly, no differences were observed in the degree of vitreousness of the grains, as determined by visual inspection of transversely cut grain.

General discussion

Expression of γ-zein in seeds of barley using a wheat HMW subunit promoter resulted in a high level of accumulation, up to almost 2% of the total grain nitrogen in the best line. Furthermore, the γ-zein protein was incorporated into protein polymers, as in maize. However, the transgene had no effect on the texture or vitreousness of the mature grain, unlike the situation in maize where γ-zein is associated with vitreousness (Chandrashakhar and Mazhar 1999) and is increased in amount when soft *opaque-2* lines are converted to normal (i.e. hard, translucent) texture through the activity of genetic modifiers (Wallace et al. 1990; Geetha et al. 1991; Lopes and Larkins 1991). This difference could result from quantitative effects. γ-Zein accounted for about 4% of the total alcohol-soluble (hordein) proteins in our transgenic barley line. In contrast, Dombrink-Kurtzman and Bietz (1993) separated zeins from hard and soft parts of kernels of eight maize lines by reverse phase-high performance liquid chromatography, demonstrating that the areas of peaks corresponding to γ-zeins (M_r 16,000 and M_r 27,000 forms) together accounted for between about 14% and 21% of the total absorption at 210 nm in the hard parts and from about 15% to 54% in the soft. Much higher levels of transgene expression would therefore be required to provide definitive results on the potential for γ-zein to affect endosperm texture in barley and other small grain cereals.

Acknowledgements Long Ashton Research Station and Rothamsted Research receive grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. We are grateful to Dr. D. Ludevid (Consejo Superior de Investigacions Cientificas, Barcelona, Spain) for provision of the γ-zein cDNA and antibody. Finally, we would like to thank the Lawes Agricultural Trust for financial assistance to Y. Zhang.

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