The molecular basis for grain texture in wheat

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Abstract

Grain hardness is a major determinant of the milling and processing quality of wheat. Current theories suggest that hardness is determined by the degree of adhesion between various components of the starchy endosperm cells of the mature wheat grain, notably between starch granules and matrix (gluten) proteins but also between proteins and cell walls. Furthermore, it has been proposed that one group of proteins, called puroindolines (PINs), play a specific role by acting as “non-stick” proteins on the starch granule surface, resulting in grain softness.

In hexaploid wheat, hardness is largely controlled by the *Ha* locus on chromosome 5D. Durum wheat is null for the *Ha* locus and is therefore ultra hard. To study the role of PINs, durum and bread wheat lines have been genetically transformed with three constructs. The first two contain the *uidA* gene (encoding β-glucuronidase) driven by the *pin-a* and *pin-b* promoters, respectively; the third contains a bread wheat gene encoding PIN-b incorporating a *C*-terminal epitope tag that allows detection using a commercially-available monoclonal antibody. Near-isogenic lines of wheat differing in grain texture have also been transformed with the third construct. Microscopy will be used to study the synthesis, trafficking and deposition of the tagged PIN-b protein in the developing and mature grain of the transgenic lines using the epitope tag as a basis for immunogold labelling. Electrophoresis, western blot analysis and GUS staining have also been used to compare the patterns of accumulation of PINs in the developing grain of the transgenic and control wheats.

Media summary

Transgenic durum and bread wheats have been transformed with the gene encoding puroindoline-b resulting in increased grain softness.

Key Words

Transgenic wheat, pasta wheat, friabilin, SKCS, particle bombardment

Introduction

Of the major crop plants, hexaploid bread wheat (*Triticum aestivum* L., genome composition AABBDD) and tetraploid pasta wheat (*T. durum* Desf., genome composition AABB) have the unique ability to be used in a wide variety of foodstuffs. Hardness affects both the milling and the processing quality of wheat; the harder the wheat, the greater the damage to the starch during milling and the larger the milled particle size. To the end-user, hardness indicates the suitability of a particular flour for use in foodstuffs. Hard hexaploid wheat varieties are used for bread whereas soft wheat varieties are used for biscuits, cakes and pastries. The ultra-hard durum wheat is used for pasta. Flours milled from hard wheat have a higher baking absorption, giving higher quality and increased profit. This difference is assumed to result from increased starch damage during the milling of hard wheats, implying that the starch granules are more tightly bound to the protein matrix.

The concept that hardness is controlled by one major gene was developed by Symes (1965) from his work on Falcon and Heron crosses, these being hard and soft wheat cultivars, respectively. He also noted that minor genes were responsible for variation in hardness within classes of hard and soft wheat cultivars. Mattern *et al.* (1973) and Law *et al.* (1978) used chromosome substitution lines to determine the location of the major locus controlling hardness on the short arm of chromosome 5D. This is now known

as the *Ha* (*Hardness*) locus. Tetraploid pasta wheat lacks the D genome, including chromosome 5D, and is therefore ultra-hard.

Greenwell and Schofield (1986) extracted proteins associated with the surfaces of starch granules from hard and soft wheat cultivars using sodium dodecyl sulphate (SDS). The protein fractions were separated using SDS-PAGE revealing an intense 15 kDa band which was abundant in soft wheat, but only weakly present in hard wheats, and absent from ultra-hard durum wheat. They named this protein friabilin. Rahman *et al.* (1994) later demonstrated that friabilin was not a single 15 kDa protein, but a complex of three major components: puroindoline-a (PIN-a), puroindoline-b (PIN-b) and grain softness protein (GSP-1), all of which share a degree of sequence identity. Jolly *et al.* (1993) and Bloch *et al.* (2001) reported that the amounts of the friabilin complex do not necessarily differ between hard and soft cultivars. The precise biological function of PINs is still unknown, and is the subject of continued research, but it has been shown that, with the exception of the tryptophan-rich region, PINs have strong sequence identity to lipid transfer proteins, including similar patterns of disulphide bonds (Le Bihan *et al*., 1996; Douliez *et al.*, 2000).

Dubriel *et al.* (1998) carried out immunolocalisation studies using monoclonal antibodies raised against PIN-a and PIN-b which showed that PIN-a is mainly located in the starchy endosperm, whereas PIN-b is located in the aleurone layer, and possibly also in the starchy endosperm. PIN-a was found at the protein matrix-starch granule interface in the endosperm, whereas PIN-b was located in small inclusion bodies in the aleurone layer. The role of PIN-b in the aleurone layer is unclear and this location does not explain why PIN-b is associated with water-washed starch.

An absence of PIN-a or mutations in the sequence of PIN-b have been correlated with hard endosperm texture (Giroux and Morris, 1997 & 1998). Giroux and Morris (1997) reported a single nucleotide change in the *pin-b* gene that may change the secondary or tertiary structure of PIN-b preventing the binding to starch granules, resulting in a hard grain texture. These results, together with those of Giroux and Morris (1998) showed that of 13 hard wheat cultivars analysed, nine had the PIN-b mutation and the remaining four cultivars had a *pin-a* 'null' mutation resulting in absence of PIN-a. However, such mutations are not universally present in hard wheat varieties and, although a correlation has been reported between grain texture and PINs, the mechanism by which this is effected has not been established.

The aim of this work is to determine the basis for differences in grain texture between hard and soft wheats using a transgenic approach and the use of near-isogenic lines of hexaploid wheat.

1. Durum wheat and bread wheats (including near-isogenic hard and soft bread wheats) have been transformed with a gene encoding PIN-b incorporating a *C*-terminal epitope tag. These plants will be used to study the synthesis, trafficking and deposition of the tagged PIN-b protein in the grain using the epitope tag as a basis for immunogold labelling.

2. Durum wheat and bread wheat have been transformed with the gene encoding β-glucuronidase under the control of the *pin-a* and *pin-b* promoters. This will allow GUS assays to be carried out on developing grain to determine the patterns of *pin* gene expression.

Methods

Transformation

Scutella isolated from early-milk stage grains of *Triticum aestivum* L. (cvs. Cadenza and Avalon x Hobbit lines [DWA lines 5.1, 9.2, 11.9 and 36.3]) and *T. durum* Desf. (cvs. Ofanto and Venusia) were pre-cultured on induction media (details on request) prior to bombardment. These scutella were bombarded with two plasmids in an equimolar ratio precipitated onto submicron gold particles. One plasmid contained a marker gene (*bar*); the other contained the gene of interest (see Table 1 for details of the plasmids and genes used). Plantlets were regenerated on selective media containing PPT. Putative transgenic plants that survived selection were analysed for the presence of transgenes by PCR.

Table 1: Marker gene plasmid and gene-of-interest plasmids used for bombardment

Plasmid	Promotor::Gene	Function	Size (kb)
pAHC20	*ubi1::bar*	Selectable marker gene - PPT resistance	5.52
ppaxs23	*pina::uidA*	Gene of interest - β-glucoronidase gene under the control of the PIN-a promoter	7.11
pPBI-1	*pinb::uidA*	Gene of interest - β-glucoronidase gene under the control of the PIN-b promoter	6.98
pinb-cmyc	*pinb::pinb-cmyc*	Gene of interest – PIN-b promoter driving *pin-b* gene incorporating a *C*-terminal epitope tag	5.74

Seed texture measurement

The seed texture of transgenic lines transformed with the pinb-cmyc plasmid and control lines was determined using the Single Kernel Characterisation System, SKCS 4100 (Perten Instruments, Springfield, IL, USA). SKCS analyses were performed on approx. 100 T_1 seeds per line (Cadenza and DWA lines only).

Histochemical GUS analysis

Expression of the *uidA* reporter gene was determined in seeds transformed with the ppaxs23 or pPBI-1 plasmids by incubating half-seeds in X-Gluc buffer. Blue staining was visually assessed after incubation overnight at 37 ?C.

Preliminary Results

Transformation

Table 2 summarises the 60 transgenic plants that have been generated and confirmed as PCR-positive for the gene-of-interest and marker gene.

Table 2: Background/gene combinations of transgenic plants.

Gene of interest	Cadenza	Durum		DWA lines[1]			
		Ofanto	Venusia	5.1	9.2	11.9	36.3
pinb::pinb-cmyc	9	3	2	2	1	2	0
pina::uidA	7	16	0	N/A			

pinb::uidA	9	9	0	N/A

[1]These are Avalon x Hobbit crosses recombinant for the *Ha* locus. 5.1 and 11.9 are hard; 9.2 and 36.3 are soft.

SKCS analysis

Samples of approx. 100 seeds from T_0 plants of Cadenza and DWA lines transformed with *pinb-cmyc* were analysed using SKCS and compared with seed from non-transformed, glasshouse-grown plants. Insufficient T_0 seed of the transgenic durum wheat lines were available to analyse using this method. A number of the transgenic lines exhibited altered phenotypes compared with the controls used and demonstrated segregating populations of hard and soft kernels (Figure 1 shows 3:1 phenotypic segregation of a transgenic line, confirmed by PCR). T_1 seed from these lines were sown and analyses will be carried out on T_2 seed from homozygous and null lines.

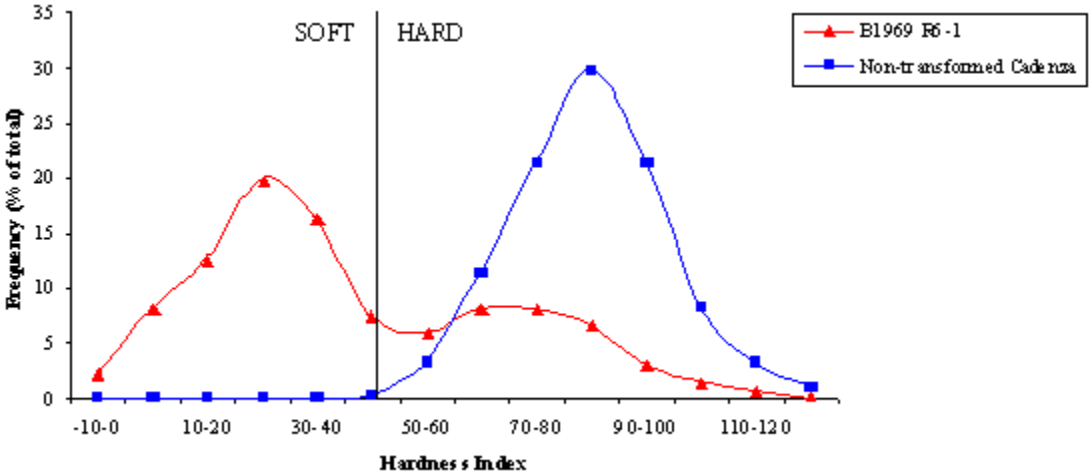

Figure 1: Histogram showing distribution of kernel hardness within a transgenic T1 and a control population (Cadenza transformed with pinb-cmyc [B1969 R6-1] and non-transformed Cadneza respectively).

Histochemical GUS staining

Half seeds from 15 mature T_0 plants transformed with ppaxs23 (8 Ofanto and 7 Cadenza) and 13 T_0 plants (5 Ofanto and 8 Cadenza) transformed with pPBI-1 were histochemically assayed for GUS activity together with a number of negative controls (seed from plants that had survived selection but were PCR-negative for *uidA*). Of these, 26 transgenic lines exhibited GUS activity (Figure 2). T_2 seeds from GUS-positive plants will be used to determine expression during grain development.

Conclusions and Future Work

Preliminary data have shown that transformation with a construct containing the *pin-b* gene under the control of its own promoter resulted in decreased grain hardness in T_1 seed from primary transgenic lines. Detailed analyses of T_2 seed using immunolabelling, microscopy and western blotting will be carried out providing an insight into the synthesis, trafficking and deposition of PIN-b throughout seed development.

T_2 seed from wheat transformed with the *uidA* gene under the control of the *pin-a* and *pin-b* promoters will be used to investigate the activity of these promoters using histochemical GUS analysis at various developmental stages.

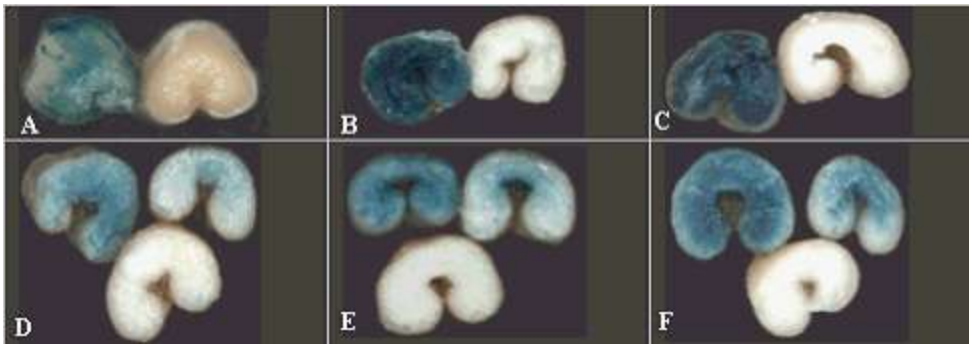

Figure 2: Histochemical GUS expression in T1 transgenic seed. A – C are *pina::uidA* lines and each shows one positive (left) and one negative (right) seed from the same parent [A: B1855 R5P1; B: B1937 R9-2; C: B1970 R5-2A]. D – F are *pinb::uidA* lines and each shows one strong expresser (top left), one weak expresser (top right) and one negative (bottom) from the same parent [D: B1971 R2; E: B1998 R3-1; F: B1998 R3-2].

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