Development of durum wheat (*Triticum turgidum* ssp *durum*) lines with soft kernel texture by chromosome engineering

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ABSTRACT

Puroindolines A (Pin-A) and B (Pin-B) are the principal determinant factors of endosperm texture in common wheat and, as a consequence, a main determinant in end product quality. They are encoded by the *Pina-D1* and *Pinb-D1* loci on chromosome 5DS and, therefore, are absent in durum wheat (*Triticum turgidum* ssp. *durum*). In order to introduce puroindolines A and B into durum wheat through allosyndetic recombination, the 5D(5B) substitution line of durum wheat cv. Langdon was crossed with durum wheat line Cappelli M lacking the *Ph1* locus on chromosome 5BL. Amongst the resulting progeny, 11 F_6 recombinant inbred lines (RILs) with soft kernels were crossed as the male parent with durum wheat cv. Colosseo, and 81 F_5 progeny from this cross were analyzed for the presence and expression of puroindoline genes. Moreover, 16 F_5 lines containing Pin-A and Pin-B were compared for their kernel texture and storage protein composition. Finally, ten F_6 RILs were analysed for their chromosome structure using microsatellite sequences located on homoeologous group 5 chromosomes. Soft grain texture (SKCS value = 34.9 ± 11.2), vitreous kernels, high protein content (15.7 ± 2.15%) and good gluten quality as determined by the SDS sedimentation volume (58.4 ± 9.1 mL) were found in 16 F_6 RILs possessing a small terminal fragment of chromosome 5DS carrying *Pina-D1* and *Pinb-D1*.

INTRODUCTION

Puroindolines A (Pin-A) and B (Pin-B) are the principal determinant factor of endosperm texture in common wheat (Greenwell and Schofield 1989; Morris et al. 1994). Pin-A and Pin-B are basic polypeptides, 13 kDa in size, with a characteristic tryptophan-rich domain, which makes them unique amongst plant proteins (Blochet *et al*., 1993; Gautier *et al*., 1994). Variation in endosperm texture is probably due to affinity of the tryptophan-rich domain of puroindolines to the polar lipids of the amyloplast membranes. Because of their lipid-binding properties, puroindolines are claimed to influence loaf volume and crumb structure of bread, and protect beer against foam destabilisation (Blochet *et al*., 1993; Dubreil *et al*., 1998). Puroindolines are encoded by the *Pina-D1* and *Pinb-D1* genes closely linked to each other at the *Ha* locus in the distal part of the short arm of chromosome 5D and, therefore, are absent in durum wheat (*Triticum turgidum* ssp. *durum*) (Giroux and Morris 1997). In order to introduce the *Pina-D1* and *Pinb-D1* loci into durum wheat through allosyndetic recombination, the 5D(5B) substitution line of durum wheat cv. Langdon was crossed with durum wheat Cappelli M, a deletion line lacking the *Ph1* locus (allele *ph1c*), which prevents pairing of homoeologous chromosomes at meiosis (Giorgi, 1978). In the resulting progeny, the absence of the *Ph1* locus was found to promote pairing and allosyndetic recombination between homoeologous chromosomes 5B and 5D. Eleven F6 recombinant inbred lines (RILs) with soft kernels obtained from the cross mentioned above were crossed as the male parent with durum wheat cv. Colosseo. Here, 81 F_5 progeny from this latter cross are screened for the presence and expression of puroindoline genes, and several F_6 recombinant lines are compared for their (i) chromosome structure using microsatellite (SSR) and (ii) kernel texture, storage protein composition and technological properties.

MATERIALS AND METHODS

Plant material

The 5D(5B) substitution line of cv. Langdon was crossed with durum wheat line Cappelli M lacking the *Ph1* locus (allele *ph1c*) , and the F_1 progeny was selfed for five generations. Amongst the resulting F_6 progeny, 11 recombinant inbred lines (RILs) with soft kernels, as measured by the SKCS method, were crossed as the male parent with durum wheat cv. Colosseo. The 81 F_5 progeny from this latter cross were used for biochemical and technological analyses.

DNA extraction and PCR amplification

The presence of the *Pina-D1* and *Pinb-D1* loci was determined in DNAs extracted from leaves by the CTAB method. Puroindoline genes were amplified by PCR as described by Gautier *et al.*, (1994). SSR (Simple Sequence Repeats) sequences on 5A, 5B and 5D chromosomes (Song *et al.,* 2005) were used for microsatellite marker characterization.

Protein analyses

Puroindoline fractionation by A-PAGE (Acid-PAGE) and western blotting were performed as described previously (Gazza *et al*., 2006). SDS-PAGE and A-PAGE fractionations of storage proteins were carried out according to Pogna *et al.* (1990).

Kernel hardness and biochemical analyses

Kernel hardness was measured by the Perten Single Kernel Characterization System (SKCS 4100) following the manufacturer's operating procedure. Protein content of the F_5 progeny was determined by semi-automatic micro-Kjeldhal (N x 5.7). Gluten content and gluten index were determined according to UNI 10689 and UNI 10690, respectively. SDS sedimentation volume

was measured according to AACC Method 56-70, using a 3% SDS solution.

RESULTS AND DISCUSSION

Production of durum wheat Recombinant Inbred Lines (RILs) with soft texture

Amongst the F_2 progeny of the cross Cappelli M x Langdon 5D(5B) substitution line, 42 plants devoid of the Iodine Binding Factor (IBF) encoded by the *Ibf-D1* locus on chromosome 5DL were found to be homozygous for IBF encoded by the *Ibf-B1* locus on chromosome 5BL inherited from line Cappelli M (Gazza *et al.*, 2002). Upon PCR amplification with puroindoline-specific primers, 127 F_3 progeny from those plants were shown to contain *Pina-D1* and *Pinb-D1*, suggesting that allosyndetic recombination occurred between chromosomes 5B and 5D. Single kernel hardness readings obtained by the SKCS method revealed that the selfed progeny of 116 out of 127 F_3 plants was heterogeneous, their SKCS values ranging from 20 to 90. However, the progeny of 11 F_3 plants turned out to be homogeneous for soft grain texture, their average SKCS value being as low as 33. Those 11 plants were assumed to be homozygous at the *Pina-D1* and *Pinb-D1* loci and crossed as the male parent with durum wheat cv. Colosseo in order to reintroduce the *Ph1* locus into the resulting progeny and improve their technological (gluten quality) and morphological (reduced plant height) traits (Gazza *et al.,* 2002). The 81 F_5 Recombinant Inbred Lines (RILs) obtained form this latter cross were compared for their chromosome structure, and biochemical and technological properties.

Presence and expression of puroindoline genes

Upon PCR amplification with primers specific for *Pina-D1* and *Pinb-D1*, DNAs extracted from 49 out of 81 F_5 RILs were positive for both loci (Fig.1). Direct sequencing of the amplicons demonstrated the presence of wild-type alleles *Pina-D1a* and *Pinb-D1a*.

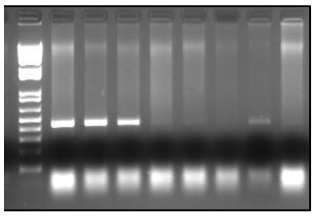

Figure 1. PCR amplicons obtained with *Pinb-D1* specific primers in eight F_5 RILs of durum wheat. The first lane on the left shows DNA size markers.

Nevertheless, when starch-bound proteins extracted from those 49 RILs were fractionated by A-PAGE, only 19 lines revealed a couple of bands that co-migrate with puroindolines in soft-textured common wheat cv. Lontra (Fig.2). The polyclonal antiserum developed against a 16-mer sequence in the C-terminal region of mature Pin-A (Gazza *et al.*, 2006) reacted strongly with protein marked with A in Figure 2, confirming that this protein is encoded by allele *Pina-D1a*. On the other hand, gene silencing in 30 RILs could be accounted for by a positional effect at the site of insertion of puroindoline genes on chromosome 5B.

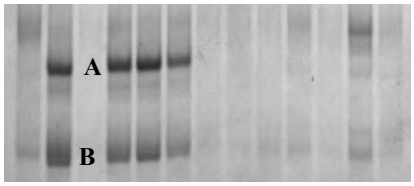

Figure 2. Fractionation by A-PAGE of starch-bound proteins from (from left to right) durum wheat cv. Colosseo, common wheat cv. Lontra and 10 F_5 RILs of durum wheat. (A) Pin-A; (B) Pin-B.

Kernel texture, storage protein composition and technological properties of durum wheat RILs

A-PAGE fractionation of gliadin from 16 F_5 RILs showed the presence of both γ-gliadin 45 and ω-gliadin 35 associated with good gluten quality (Pogna *et al.*, 1990). Upon SDS-PAGE fractionation of HMW glutenin subunits (Fig.3), these 16 lines turned out to be homozygous for either subunit pair "6+8" inherited from Langdon 5D(5B) substitution line or subunit pair "13+16" inherited from cv. Colosseo.

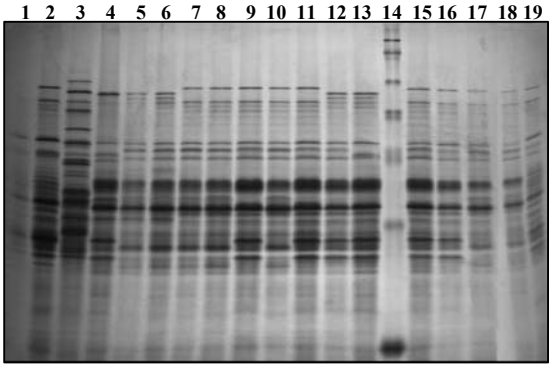

Figure 3. Fractionation by SDS-PAGE of total endosperm proteins from (1) cv. Langdon, (2) 5D (5B) substitution line, (3) common wheat cv. Chinese Spring, (4) Cappelli M, (5) cv. Colosseo and (6-13, 15-19) F_5 RILs of durum wheat. Lane 14 shows molecular weight markers.

The SKCS values of these RILs were typical of a population segregating for kernel texture, showing a bimodal distribution of the single kernel hardness readings. However, amongst the F_6 progeny of 36 single plants belonging to two F_5 RILs (L482 and L504), 16 lines showed a mean SKCS value of 34.9 ± 11.2, which is typical of soft kernels homozygous for the puroindoline genes. Moreover, 10 F_6 lines produced extra-hard kernels (mean SKCS value = 89.6 ± 11.8), whereas the remaining 10 lines showed a bimodal distribution with a mean SKCS value of 58.5 ± 24.1. All the 16 F_6 RILs with soft kernels exhibited very high protein content (15.7 ± 2.1%) and a mean SDS sedimentation volume as high as 58.4 ± 9.1 mL. Furthermore, they showed vitreous kernels, confirming that kernel texture and vitreousness are independent traits.

SSR analysis

Molecular marker analysis was carried out on 10 F_6 progeny of lines L482 and L504. PCR amplification with primers specific for microsatellite *Xbarc130* localized at 0 cM on the short arm of chromosome 5D produced amplicons in eight soft-textured progeny, all of them possessing the *Pina-D1* and *Pinb-D1* loci (Table 1). The remaining two F_6 progeny (L482-5 and L504-5) with hard kernels gave no amplification product for both *Xbarc130* and puroindoline genes. Microsatellites *Xbarc205* (18.4 cM), *Xbarc322* (126.0 cM), *Xbarc110* (140.4 cM) and *Xgmw169* (169.0 cM) were absent in all the progeny analysed except line L482-1. This latter soft line revealed the presence of *xbarc110* and *Xgmw169* localized at the distal end of the long arm of chromosome 5D, suggesting that this genotype inherited two intergenomic exchanges between chromosomes 5B and 5D. Seven soft lines were found to contain *Xgmw190* located at 8.6 cM on the short arm of chromosome 5D (Table 1). On the contrary, soft line L504-1 gave no amplification product with primers specific for *Xgmw190,* suggesting that a very short fragment of chromosome 5DS occurs in this genotype.

Table 1. PCR amplification of the selfed progeny of lines L482 and L 504 with primers specific for six SSRs on chromosome 5D.

SSR	L482					L504				
	1	2	3	4	5	1	2	3	4	5
Chromosome 5DS										
Xbarc130 (0 cM)	+	+	+	+	-	+	+	+	+	-
Xgmw190 (8.6 cM)	+	+	+	+	-	-	+	+	+	-
Xbarc205 (18.4 cM)	-	-	-	-	-	-	-	-	-	-
Chromosome 5DL										
Xbarc322 (126.0 cM)	-	-	-	-	-	-	-	-	-	-
Xbarc110 (140.4 cM)	+	-	-	-	-	-	-	-	-	-
Xgmw169 (169.0 cM)	+	-	-	-	-	-	-	-	-	-

+ present; - absent

Several SSR sequences located on chromosomes 5A and 5B were polymorphous, giving amplicons of different sizes in the parental genotypes Cappelli M, Langdon 5B(5D) substitution line and Colosseo (Fig. 4). PCR amplifications with primers specific for polymorphous SSR sequences revealed that the F_6 progeny of RILs L482 and L504 inherited chromosome arms 5AL and 5BL from cv. Colosseo (data not shown).

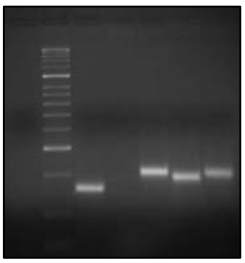

Figure 4. PCR amplification with primers specific for polymorphous microsatellite *xbarc4* on chromosome 5B. Lanes show (from left to right) DNA size markers, common wheat cv. Chinese Spring, Langdon 5D (5B) substitution line, F_6 line 482-1, Cappelli M and durum wheat cv. Colosseo.

Previous findings have shown that the presence of wild-type alleles *Pina-D1a* and *Pinb-D1a* is the principal determinant factor of soft kernel texture in common wheat (Greenwell and Schofield 1989; Morris *et al*. 1994; Corona *et al*. 2001). Puroindolines modulate grain texture and, as a consequence, they exert a strong indirect influence on several technological parameters such as flour yield, starch damage, water absorption, farinograph peak time and stability, alveograph P, L and W parameters. Moreover, they influence directly loaf volume and crumb structure of bread. Protein content and kernel weight were found to explain a large proportion of the variation in kernel hardness due to environment and genotype x environment interaction (Gazza *et al*., 2008). Here, evidence has been provided that introgression of wild-type Pin-A and Pin-B through a non-transgenic approach resulted in durum wheat genotypes with soft kernel texture without any negative effect on grain vitrousness. These genotypes are currently being analysed for their pastamaking and breadmaking properties.

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