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Breeding and quality of soft-textured durum wheat

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Abstract. Puroindolines A (PIN-A) and B (PIN-B) encoded by genes *Pina-D1* and *Pinb-D1* on chromosome 5DS are the principal determinant factors of kernel hardness in common wheat, and exert a strong impact on several quality traits such as yield and granularity of flour, starch damage and water absorption, rheological and baking properties. Effects of grain texture on pastamaking and breadmaking quality were evaluated in soft-textured durum wheat lines (SDLs) as compared with their hard durum sister lines (HDLs). SDLs accumulated puroindolines on their starch granules and showed SKCS values significantly lower than those of their hard-textured counterparts lacking Pin-A and Pin-B. The average flour extraction rate of SDL was about 23% higher than that of HDL. Increasing kernel softness significantly affected rheological parameters, whereas spaghetti cooking quality was unaffected by kernel hardness. Loaf volume exhibited a 10% increase associated with kernel softening. In order to reduce plant height, soft durum lines with the lowest SKCS indexes were further crossed with durum wheat cv. Simeto and 17 F_6 progeny lines were evaluated in terms of stability for their short height, soft texture and gluten quality. Modulation of kernel texture in durum wheat was obtained as well by transgenic approach by inserting vromindoline genes from oats. Finally soft textured durum wheat were used in crosses with *Triticum aestivum* with the aim to obtain extra-soft common wheats that may supply breeders with a broader range of kernel texture.

Keywords. Kernel texture – Durum wheat breeding – Pasta-making quality – Bread-making quality.

Amélioration et qualité du blé dur à texture tendre

Résumé. *Les puroindolines A (PIN-A) et B (PIN-B) codées par les gènes Pina-D1 et Pinb-D1 sur le chromosome 5DS sont les principaux facteurs déterminants de la dureté du grain chez le blé commun, et exercent un fort impact sur plusieurs caractères de qualité tels que le rendement et la granularité de la farine, la dégradation de l'amidon et l'absorption d'eau, les propriétés rhéologiques et de cuisson. Les effets de la texture du grain sur la qualité de la production de pâtes et de la panification ont été évalués dans les lignées de blé dur Soft (SDL) par rapport à leurs lignées sœurs Hard (HDL). Les SDL ont accumulé les puroindolines sur leurs granules d'amidon et ont montré des valeurs SKCS significativement inférieures à celles de leurs homologues Hard sans Pin-A et Pin-B. Le taux d'extraction moyen de farine des SDL était d'environ 23% plus élevé que celui des HDL. L'augmentation de la tendreté du grain a affecté de façon significative les paramètres rhéologiques, alors que la qualité de cuisson des spaghettis n'a pas été affectée par la dureté du grain. Le volume du pain a présenté une augmentation de 10% associée au ramollissement de la graine. Afin de réduire la hauteur des plantes, les lignées de blé dur tendre avec les plus faibles indices de SKCS ont ensuite été croisées avec des blés durs cv. Simeto et 17 lignées de descendance F6 ont été évaluées en termes de stabilité pour leur faible hauteur, la tendreté et la qualité du gluten. La modulation de la texture du grain de blé dur a ainsi été obtenue par une approche transgénique, en insérant des gènes de la vromindoline de l'avoine. Enfin, le blé dur à grain tendre a été utilisé dans des croisements avec Triticum aestivum dans le but d'obtenir des blés communs extra-tendres qui peuvent fournir aux sélectionneurs une gamme plus large de texture des grains.*

Mots-clés. *Texture des grains – Amélioration du blé dur – Qualité de la production des pâtes – Qualité de la panification.*

I – Introduction

Kernel hardness is a main determinant of end product quality because of its strong effects on milling conditions, granularity of flour and starch granule integrity. In particular, common wheat

(*Triticum aestivum* L.) cultivars can be divided into three endosperm-texture classes based on their average SKCS (Single Kernel Characterization System) values, i.e. soft, medium hard and hard. On the contrary, all durum wheat (*T. turgidum* ssp *durum*) cultivars are characterized by an extra-hard kernel texture with SKCS index >90. This extremely hard texture is mainly due to the absence of PIN-A and PIN-B, two basic, tryptophan- and cysteine-rich polypeptides encoded by two closely linked genes named Pina-D1 and Pinb-D1, located in the distal part of the short arm of chromosome 5D (Mattern *et al*., 1973; Gautier *et al*., 1994) and consequently absent in AB-genome durum wheat. Extra-hard durum wheat grain is mainly ground to make semolina for the production of pasta and cous-cous, and in Mediterranean countries it is also used for breads of all types (Quaglia, 1988; Palumbo *et al*., 2000). Breeding programs have focused on selecting durum wheat genotypes with superior pastamaking quality because of its primary commercial importance, and selection for baking quality has been applied to a minor extent (Boggini and Pogna, 1989; Peña *et al*., 1994; Boggini *et al*., 1995; Liu *et al*., 1996; Palumbo *et al.*, 2000). To make a durum bread, semolina is reground to reduce its particle size and provide sufficient starch damage to assure appropriate gassing power during the fermentation process (Quaglia, 1988). Because of the extreme hardness of durum wheat grain, semolina regrinding can result in excessive starch damage, which alters alveogram and farinogram shapes, and exerts detrimental effects on baking performance (Dexter *et al*., 1994).

In order to insert puroindoline genes into durum wheat, Gazza *et al*. (2003) used durum wheat line "Cappelli M" lacking the Ph1 locus (Giorgi, 1978) as the female parent in a cross with the 5D(5B) substitution line of durum wheat cv. Langdon carrying wild-type alleles Pina-D1a and Pinb-D1a. The resulting soft-textured plants devoid of chromosome 5D were used as the male parent in crosses with commercial durum wheat cv. Colosseo (Gazza *et al*., 2008) and three F6 plants emizygous at the Pina-D1/Pinb-D1 locus from these crosses were self-pollinated for three generations to develop six F9 lines, i.e. three Soft Durum Lines (SDL) homozygous for wild-type alleles Pina-D1a and Pinb-D1a, and three Hard Durum Lines (HDL), lacking the Pina-D1 and Pinb-D1 genes.

Here, soft-textured and hard-textured durum wheat lines are compared for their milling properties, rheological characteristics, pastamaking and breadmaking quality. In addition, in order to reduce plant height a selected SDL line was crossed with durum wheat cv. Simeto and 17 F6 progeny lines were evaluated in terms of stability for their short height, soft texture and gluten quality. Modulation of kernel texture was also obtained in transgenic durum wheat cv. Svevo containing vromindolines, two puroindoline-like proteins bound to starch granules, and likely responsible of the extra-soft texture of oat kernels. Production of extra-soft common wheat lines deriving from a cross between SDLs and common wheat is discussed as well.

II – Material and methods

DNA was extracted from leaves by the CTAB method. Puroindoline genes were amplified by PCR as described by Gautier *et al*. (1994). SSR (Simple Sequence Repeat) sequences on chromosome 5D were used for microsatellite marker characterization (Somers *et al*., 2004; Song *et al*., 2005).

Starch-bound proteins were extracted with 50mM NaCl and 50% (v/v) propan-2-ol from 50 mg of air-dried starch granules as described previously (Corona *et al*., 2001). A-PAGE at pH 3.1 of starch-bound proteins was carried out as described by Corona *et al*. (2001). Reduced endosperm proteins were fractionated by SDS-PAGE as described previously (Pogna *et al*., 1990).

Kernel hardness was performed on 300 kernels-sample by the Perten SKCS 4100 (Springfield, IL, USA) following the manufacturer's operating procedure. The instrument was set in a range of hardness values between -40 and +120. Samples (3Kg) from soft-textured and hard-textured lines were milled with (i) the MCK Buhler experimental mill for durum wheat, (ii) the MLU 202 Buhler experimental mill for common wheat or (iii) the Bona 4RB (Bona, Italy) experimental mill for common wheat.

The milled samples were analyzed with the Chopin Alveograph (Chopin, Villeneuve La Garenne, France) according to the manufacturer's instructions as modified by D'Egidio *et al*. (1990). In addition, the flour samples obtained with the MLU 202 Buhler experimental mill were analyzed with the Brabender (South Hackensack, NJ) farinograph. Flour and semolina obtained from each soft-textured or hard-textured line with the MCK Buhler experimental mill for durum wheat were combined and mixed with tap water to reach a dough water content of 24.5% (for SDLs) or 30% (for HDLs). The dough was processed into spaghetti (1.7 mm in diameter) using a laboratory press. After drying at 50°C for 20 h, spaghetti (100 g) were cooked and evaluated for firmness, stickiness and bulkiness by a trained panel of three experts as described by D'Egidio *et al.* (1990).

Bread was baked according to the AACC Method 10-10B with minor modifications (Cattaneo and Borghi, 1979), using flour samples obtained with the milling for common wheat. Loaf volume was determined by rapeseed displacement.

All data are the means of at least duplicate determinations. Data were statistically evaluated by Student's *t* test or analysis of variance.

III – Results and Discussion

PCR amplifications of genomic DNA with primer pairs specific for seven microsatellites located on 5D chromosome suggested that SDLs contain only a small 5DS fragment, inferior to 14.4 cM in size, likely translocated to homoeologous chromosome 5BS. Upon A-PAGE fractionation, soft textured durum wheat lines were found to accumulate PIN-A and PIN-B on the surface of their starch granules in amounts comparable to those observed in soft-textured common wheat cultivars. Accumulation of puroindolines reduced SDLs mean SKCS indexes to 19.9 - 23.6, which are typical of soft-textured common wheat cultivars whereas hard-textured durum wheat lines HDL were similar to durum wheat varieties in lacking both puroindolines. According to SDS-PAGE fractionation, all durum wheat lines produced in the present study exhibited LMW-2 glutenin subunits, which are associated with superior gluten strength (Pogna *et al*., 1990), and inherited HMW glutenin subunit pair 6+8 from Langdon 5D(5B) substitution line.

SDLs revealed that grain hardness has a strong influence on several quality-related traits at the tetraploid level as well. In particular, the average flour extraction rate of SDLs was approximately 24% higher than that of HDLs, and even greater (about 60%) after milling with the MCK Buhler mill for durum wheat. Grain softness strongly decreased farinograph water absorption and consequently resulted in inferior dough tenacity (P), strength (W) and P/L ratio of SDLs with respect to HDLs. Moreover, the lower starch damage accounts for the higher farinograph dough stability and mixing tolerance of SDL milling products, which likely derives from their lower water absorption. It is noteworthy that the substantial variation in water absorption and rheological properties associated with the contrasting kernel textures of the durum wheat lines did not significantly affect firmness, stickiness and bulkiness of spaghetti. In addition, HDLs and SDLs did not differ significantly for their pasta-making quality as determined by the global quality score, and were comparable with high-quality durum wheat cultivars grown in Italy. On the other hand, soft-textured lines showed a small, but significant, increase of the bread loaf volume (approximately 10%) compared with their hard-textured counterparts. These results suggest that modulation of kernel hardness in durum wheat does not impair its pasta-making potential, and may improve its baking performance.

As compared with HDLs, the soft texture of SDLs resulted in significant lower yellow and brown indexes in both flour and semolina fractions obtained with the MCK Buhler mill. This suggest that color was strongly related to the particle size of the milling fractions, yellowness b* and brownness (100 - L*) being consistently and significantly lower in the finer flour and semolina of SDLs.

proteins sharing a tryptophan-rich domain of four tryptophan residues. A total of 24 T_1 plants and 50 T_2 plants expressing both *Vin-2* and *Vin-3* genes were grown in growth chambers, and characterized for their genetic structure and kernel texture.

When grown under chamber conditions, the 34 T_1 progeny of two transgenic T_0 individuals with expression of both *Vin-2 and Vin-3* were indistinguishable from control cv. Svevo for their morpho-physiological traits and fertility. Transgenic plants produced T_2 kernels significantly softer (mean SKCS = 37.1±12.5) than their sister plants without vromindoline transgenes (mean SKCS = 82.2±12.9, Table 2). *As expected, heterozygous T_1 plants* generated seeds with contrasting hardness characteristics, with SKCS values ranging from 0.4 to 115.7. These results suggest that vromindolines VIN-2 and VIN-3 are able to modulate grain texture in durum wheat as well.

Soft-textured F_6 lines of durum wheat containing PIN-A and PIN-B were crossed with common wheat cvs Eridano and Bilancia. The hard-textured cv. Eridano contains null allele *Pina-D1b* and wild-type allele *Pinb-D1a*, whereas the soft-textured cv. Bilancia has wild type alleles *Pina-D1a/ Pinb-D1a*. Amongst the F_2 progeny of cv. Eridano, individuals with three doses of puroindolines, *i.e*. one of PIN-A and two of PIN-B, were selected by PCR amplification with primers specific to *Pina-D1b* or *Pina-D1a* (Fig.2A), and found to have a mean SKCS value of 45 (Fig.2B). Moreover, the F_2 progeny of cv. Bilancia showed a mean SKCS values of 22, with extra-soft individuals exhibiting an SKCS index as low as -3.

Table 2. SKCS values of kernels produced by 34 T_1 plants from two T_0 plants of durum wheat cv. Svevo expressing *Vin-2* and *Vin-3.*

Genotype of T_1 plants	No. of T_1 plants	No. of T_2 kernels	Mean SKCS value ± SD	SKCS Range Min	Max
Homozygous	9	210	37.1 ± 12.5**	-11.3	72.2
Heterozygous	20	666	58.3 ± 23.4	0.4	115.7
Null	5	152	82.2 ± 12.9	49.3	117.6
Control cv. Svevo	5	300	88.8 ± 15.2 ns	41.1	110.2

** *t-value significant at P < 0.01 with respect to the null T_2 progeny; ns, t-value not significant with respect to the null T_2 progeny.*

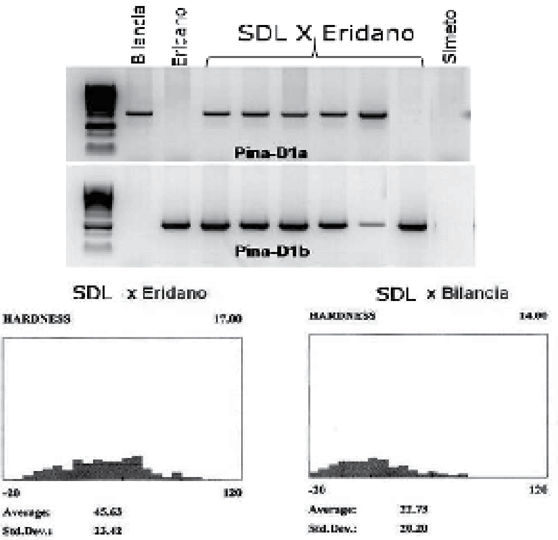

Figure 2. Results of PCR amplification of F2 SDL lines using allele PinaD1a or Pina D1b specific primer.

IV – Conclusions

Durum wheat lines homozygous for a <14.4 cM terminal fragment of chromosome 5D containing the Pina-D1a/Pinb-D1a alleles showed SKCS values typical of soft-textured kernels. Softening effect resulted in a about 24% higher flour extraction rates compared with hard-textured lines. Spaghetti cooking quality was unaffected by kernel hardness, whereas loaf volume exhibited a 10% increase associate with kernel softening. Availability of soft-textured durum wheat genotypes may have important practical and useful implications for breeding multiple-purpose durum wheat (pasta, bread, biscuits and other oven products), and for technological operations of industrial interest.

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