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Genetic modification of starch composition in wheat

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SUMMARY – Starch, accounts for 65-75% of wheat grain dry weight and it is composed of two different glucan chains, amylose and amylopectin. Enzymes involved in starch synthesis and, particularly, the role of five isoforms of starch synthases (SS) have been identified. Four of these are involved in amylopectin synthesis, along with branching and debranching enzymes; whereas the granule bound starch synthases (GBSSI or waxy proteins) are responsible for amylose synthesis in storage tissues. Electrophoretic analyses of common and durum wheat have led to the identification of partial *waxy* mutant lines, characterised by the lack of one or two waxy proteins. Crossing of these materials has permitted the combination of the different *null* alleles detected to produce the entire set of partial and complete waxy lines. Similarly, a different set of mutant lines (*SSIIa nulls*) has been used to produce durum and bread wheat varying in number of SGP-1 proteins.

Introduction

Starch is the predominant carbohydrate reserve of wheat grain and accounts for 65-75% of wheat grain dry weight in the wheat endosperm. Starch is composed of two different glucan chains, amylose and amylopectin. The relative amounts of amylose and amylopectin, which in normal wheat starch vary from 1:3 to 1:5, are responsible for its unique physical and chemical properties with strong influences on functional properties of flour or semolina and on its specific uses in the food and manufacturing industries (Zeng *et al*., 1997; Yoo and Jane, 2002). Enzymes involved in starch synthesis and, particularly, the role of five isoforms of starch synthases (SS) have been identified (Rahman *et al.*, 2005). Four of these are involved in amylopectin synthesis, along with branching and debranching enzymes; whereas the granule bound starch synthases (GBSSI or waxy proteins) are the starch synthases responsible for amylose synthesis in storage tissues.

By altering level of key enzymes involved in the regulation of starch synthesis, it is possible to generate novel starches with new unique properties. In this work we have focalised the attention on GBSSI and SGP-1 (SSII) proteins. In bread wheat (AABBDD) three different waxy protein isoforms are present which are encoded by three genes designated as *Wx-A1*, *Wx-B1* and *Wx-D1* located on chromosome arms 7AS, 4AL and 7DS (Chao *et al*., 1989; Miura *et al*., 1994; Yamamori *et al*., 1994); similarly, three homoeologous SSII genes, located on chromosome arm 7AS, 7BS and 7DS, control the synthesis of the SGP-1 proteins (SGP-A1, SGP-B1 and SGP-D1) (Yamamori and Endo, 1996).

Production and characterisation of low-amylose wheat lines

SDS-PAGE analyses of granule-bound proteins present in bread and durum wheat have led to the identification of partial *waxy* mutant lines, characterised by the lack of one or two waxy proteins (Yamamori *et al*., 1992; Urbano *et al*., 2002). Crossing of these materials has permitted the combination of different *null* alleles in a durum wheat cultivar (Svevo) and a bread wheat line (N11) along with the production of the entire set of partial an total waxy lines (Fig. 1).

Comparison of amylose content of partial and complete *waxy* wheats we have produced with their normal genotype show this effect. In fact, there is a difference of 1-7% amylose content between wild-type and partial *waxy* mutants, whereas the total *waxy* genotype presents a drastic reduction of amylose content (0-1% of total starch). Semiquantitative RT-PCR experiments (data not shown) on durum wheat kernels collected at different development stages show that the functional *waxy* gene compensated for the lack of other isoforms in partial *waxy* mutant lines by producing more transcript

and consequently more enzyme, but it was not possible to discriminate between *Wx-A1* and *Wx-B1* allele.

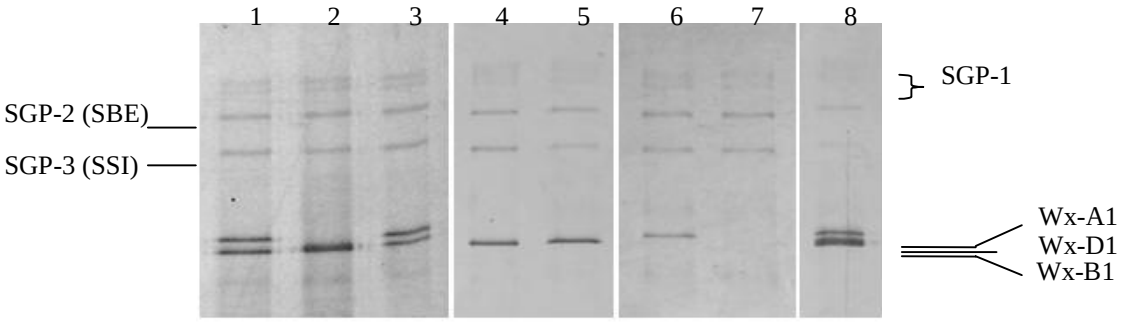

Fig. 1. SDS-PAGE of granule-bound starch proteins of partial and complete bread waxy wheat: 1) Genotype *Wx-D1*$^-$, 2) *Wx-A1*$^-$, 3) *Wx-B1*$^-$, 4) *Wx-A1*$^-$*/D1*$^-$, 5) *Wx-A1*$^-$*/B1*$^-$, 6) *Wx-D1*$^-$*/B1*$^-$, 7) *Wx-A1*$^-$*/B1*$^-$*/D1*$^-$, 8) *Wt.*

Table1. Amylose content of total and partial waxy lines of bread(1) and durum(2) wheat

Genotypes	*Wt*	*Wx-A1*$^-$	*Wx-B1*$^-$	*Wx-D1*$^-$	*Wx-A1-/B1*$^-$	*Wx-A1-/D1*$^-$	*Wx-B1*$^-$*/D1*$^-$	*Wx*
(1)Amylose content (%)	25.6	23.2	22.9	24.2	22.7	21.2	20.2	1.2
(2)Amylose content (%)	29	26.4	22.2	------	------	------	-----	1.5

To investigate the influence of the single *waxy* alleles and their different combination on starch pasting properties, RVA (Rapid Visco Analyzer) analysis has been performed on the set of *null* lines for waxy proteins (Fig. 2). Clear differences between normal and *waxy* genotypes are evident, while partial *waxy* genotypes have intermediate characteristics. In particular, from Fig. 2 it appears that *waxy* mutants present higher breakdown, lower setback and lower final viscosity. This results suggest that total *waxy* genotypes have granules softer and more fragile than wild type after swelling, and collapse drastically producing a large drop in the starch viscosity. Moreover, starch properties, as defined by the parameters previously reported, are more influenced by *Wx-B1* allele from comparison of lines null for this particular allele and remaining lines. Therefore, the amylose content appears to be the most important factor in controlling the starch properties.

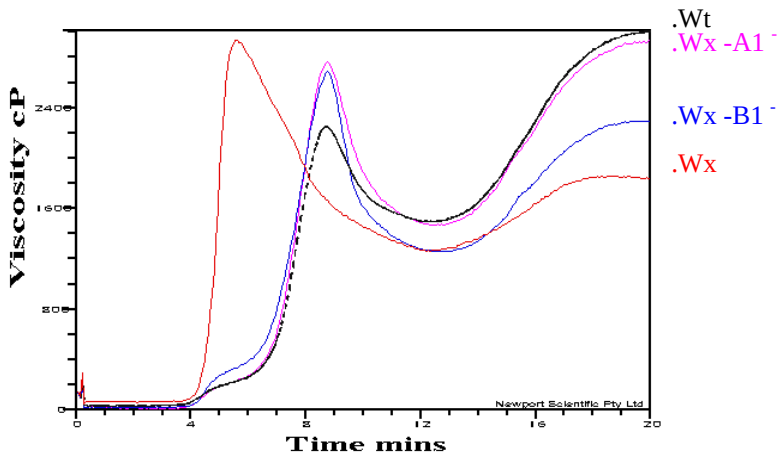

Fig. 2. RVA analysis on partial and total waxy durum wheat.

Production and characterisation of high-amylose wheat lines

Similarly to waxy proteins, mutant lines lacking one of the three possible SGP-1 proteins (Starch synthase II) have been identified (Yamamori *et al.*, 2000). Bread lines lacking all the three SGP-1 proteins have being produced (Yamamori *et al.*, 2000). In these lines the A-type starch granules are deformed, the structure of amylopectin results to be altered (the short chains are increased) and the apparent amylose content is significantly higher than that of normal starches (Yamamori *et al*., 2006). High amylose wheat flours show lower swelling and lower peak viscosity than waxy and normal wheat flours. Products made from SGP-1-deficient wheats with amylose contents of 35-40% contain slightly increased levels of resistant starch. The resistant starch has a role similar to dietary fiber; it can be used as substrate by the intestinal microflora during the fermentation. One of the principal products of this process is the butyrate that may also play a role in promoting lowering of the risk of colorectal cancer (Brouns *et al.,* 2002). Moreover, high-amylose starch appears to impart a slight improvement in cooked pasta firmness and is low in energy and in total carbohydrate content.

The same mutants are being used to produce new *null* lines in the bread lines N11 and durum cultivar Svevo. SDS-PAGE experiments reveal that the levels of two starch granule proteins, SGP-2 (starch branching enzyme) and SGP-3 (starch synthase I enzyme), decrease considerably in the SGP-1 *null* wheat, whereas the waxy proteins remain unaltered (Fig. 3). This result suggests that SGP-1 proteins are important for the binding of other SGPs on the starch granules.

Further analyses on amylose content and starch properties will be performed.

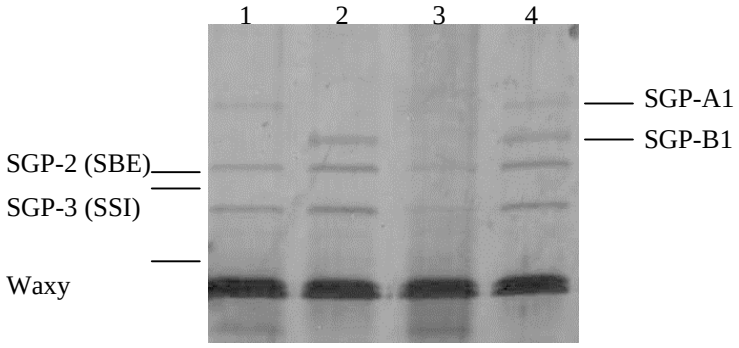

Fig. 3. SDS-PAGE of granule-bound starch proteins of durum wheat 1) genotype *Sgp-B1*$^-$, 2) *Sgp-A1*$^-$, 3) *Sgp-A1*$^-$*/B1*$^-$, 4) *Wt.*

Conclusions

The identification of mechanisms that control starch biosynthetic pathway provides the possibility of manipulating starch composition by altering the expression of genes encoding enzymes that are involved in starch synthesis. This is possible selecting for appropriate mutants available. A second approach to obtain novel starches takes advantage of modern biotechnology. For example, Regina *et al.* (2006) have produced a transgenic wheat with high amylose (>70%) using RNA interference technology. The technological properties of novel starches, so far realised, have revealed many possible uses for wheat breeders and food processors; manipulation of additional genes involved in starch biosynthesis will lead to the possibility to obtain a wide array of starches with different composition and functionalities broadening its use in food and non food applications.

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