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Improvement of technological and nutritional quality in durum wheat: achievements and perspectives

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Abstract. Durum wheat represents one of the main food source for numerous countries of the Mediterranean area. It is mainly used for pasta production, but also in an array of other regional foods such as flat bread, cous cous, burghoul, etc. Breeding activities for quality in durum wheat have been mainly targeted towards the production of high-yielding cultivars with superior pasta-making characteristics. The role played by the gluten proteins in influencing processing characteristics of semolina, and in particular by the low-molecular weight glutenin subunits, has been elucidated. This has resulted in the development of breeding strategies for modifying protein composition in a predictable way and releasing durum wheat cultivars capable of satisfying processors and consumers requirements. More recently, the strong evidences between diet and health are leading to focus breeding activities on nutritional aspects and enhancement of wheat kernel components of health value. Among these, starch composition represents an important target due its role in influencing both technological and nutritional aspects of wheat end-products. In particular, high amylose starch can play an important role on human health preventing the onset of important diseases. In this paper, the manipulation of proteins and starch with the final goal to tailor novel durum wheat cultivars with improved technological and nutritional characteristics will be presented.

Keywords. Quality – Glutenin subunits – Starch – RNA interference – TILLING.

Amélioration de la qualité technologique et nutritionnelle du blé dur : réalisations et perspectives

Résumé. Le blé dur représente l'une des principales sources de nourriture pour de nombreux pays de la région méditerranéenne. Il est essentiellement utilisé pour la production de pâtes, mais aussi pour l'élaboration de plusieurs autres produits régionaux tels que le pain plat, le couscous, le burghoul, etc. Les activités de sélection pour la qualité du blé dur ont été principalement orientées vers la production de cultivars à haut rendement avec des caractéristiques supérieures pour la fabrication des pâtes alimentaires. L'influence des protéines de gluten sur les caractéristiques de traitement de la semoule a été élucidée, en examinant en particulier le rôle joué par les sous-unités gluténines de faible poids moléculaire. Des stratégies d'amélioration ont donc été élaborées pour modifier la composition des protéines d'une manière prévisible et obtenir des cultivars de blé dur capables de satisfaire les exigences des transformateurs et des consommateurs. Plus récemment, les indications évidentes du rapport étroit entre alimentation et santé conduisent à concentrer les activités de sélection sur les aspects nutritionnels et l'amélioration des composants du grain de blé bénéfiques pour la santé. Parmi ceux-ci, la composition de l'amidon représente une cible importante dans la mesure où elle intervient dans les propriétés technologiques et nutritionnelles des produits finis du blé. En particulier, la haute teneur en amylose de l'amidon peut jouer un rôle important dans la santé humaine pour prévenir l'apparition de maladies redoutables. Dans cet article, nous allons présenter la manipulation des protéines et de l'amidon pour la production de nouveaux cultivars de blé dur avec des caractéristiques technologiques et nutritionnelles améliorées.

Mots-clés. Qualité – Sous-unités gluténines – Amidon – Interférence ARN – TILLING.

Amylose/amylopectin ratio (1:3 in normal wheats) is one of the most important parameters that affect the chemical-physical properties of the starch; modulating the activity of key enzymes involved in starch biosynthesis, both low and high amylose starch have been produced in durum wheat (Lafiandra *et al.*, 2010; Sestili *et al.*, 2010a,b; Hogg *et al.*, 2013; Bovina *et al.*, 2013; Botticella *et al.*, unpublished).

Recent studies have demonstrated the existence of positive correlation between amylose content in flour and resistant starch (RS) in food products. RS has been shown to escape digestion to glucose in the stomach and plays a prebiotic role in the large bowel. In fact its fermentation produces small molecules, known as short chain fatty acids (SCFAs), representing important metabolites capable to promote optimal function of the viscera (Topping and Clifton, 2001). RS has a role similar to dietary fibre, protecting against diet-related diseases as colon cancer, type II diabetes, obesity and osteoporosis (Nugent, 2005). In addition, semolina containing high amylose amount improves the quality of pasta, increasing the firmness and reducing the stickiness and water absorption during the cooking (Soh *et al.*, 2006).

Different strategies have been applied to obtain high amylose in durum and bread wheat: silencing of genes involved in amylopectin biosynthesis (Starch synthase IIa or Starch granule protein 1 and Starch branching enzymes IIa), through use of natural mutant or TILLING (natural or induced mutants) and biotechnology tools (RNA interference silencing).

1. Natural mutants: Creation of a Starch granule protein 1 (Sgp-1) null collection in durum wheat

Single mutations for *Sgp-A1* (Chousen 30) and *-B1* (Kanto 79) genes, previously identified by Yamamori *et al.* (2000), were introgressed in the durum wheat cv Svevo with the aim to produce a complete Sgp-1 null line. An extensive SDS-PAGE electrophoresis of starch granule proteins was used to select the progeny of interest. Backcrosses between the parental cultivar and Sgp-1 null genotype (Chousen 30/Svevo/Svevo)/(Kanto 79/Svevo/Svevo) produced 144 Sgp-1 sister lines, that were characterized either for qualitative or quantitative traits. A set of fourteen sister lines, showing good or poor agronomic traits, was chosen and grown in two different years. These lines highlighted an increase in amylose content (AC) ranging between 36-45 %, but this result was also associated to a drastic loss in grain yield and starch content. Similar, but not identical effects have been previously reported in bread wheat and barley (Yamamori *et al.*, 2000 and 2006; Morell *et al.*, 2003). In barley, the lesion of the *Sgp-1* gene produced a strong increase in AC, (~70% of total starch), while in bread wheat the increase was more modest (~36%).

2. Transgenic approach: RNA interference silencing of Starch Branching enzyme IIa (SBEIIa)

Regina *et al.* (2006) used the RNAi technology to knock out the starch branching enzyme genes (*SBEIIa*) and increase the amylose content in bread wheat. Suppression of the activity of SBEIIa enzymes resulted in lines with amylose content above 80%. The same approach has been used by Sestili *et al.* (2010) in durum wheat, using two different cultivars (Svevo and Ofanto). Although two different methods were used for the genetic transformation, biolistic for cv Svevo and *Agrobacterium* for cv Ofanto, similar effects were observed on amylose content, granule morphology and starch composition in RNAi seeds. Amylose content was significantly increased in all the transgenic RNAi lines, but it varied between 31 and 75%. This result was probably due to the efficiency of gene suppression depending on transgene copy number and its position on genomic DNA. The value of resistant starch was also strongly increased in transgenic starch and resulted notably higher than in durum wheat cultivar Svevo (≈12% of total starch in transgenic line MJ16-112; 0.4% in Svevo). Regarding to starch granule phenotype, it was markedly affected in SBEIIa-silenced lines compared to the reference cultivars. In particular type-A granules were

smaller and deflated, whereas type-B granules lost their normal spherical shape and became more extended, similarly to that observed by Regina *et al.* (2006) in bread wheat.

With the aim to investigate the effects of biolistic and *Agrobacterium*-mediated transformation methods, a comparative proteomic approach has been undertaken to compare the proteome (starch granule and metabolic proteins) of mature and immature kernels of untransformed and transgenic durum wheat (Sestili *et al.* 2013). This study highlighted subtle differences, most of them considered as “predictable unintended effects” due to the silencing of *SBEIIa* genes. In conclusion, the comparison of the proteome of the transgenic lines obtained by two different transformation methods has shown only some small differences, that might depend on the different varietal responses.

3 Mutagenesis: a TILLING approach to suppress *SBEIIa* gene activity

T Mutagenesis represents a very effective strategy to generate novel genetic variation and its widespread use has resulted in the release of over 3000 crop cultivars with improved quality characteristics. Combination of the power of mutagenesis and a high throughput screening, based on PCR, to identify induced mutations in a target gene has resulted in the development of a powerful non transgenic technology, termed TILLING (Targeting Induced Local Lesions IN Genomes). Recently the TILLING strategy has also been applied in durum wheat and used to modify starch composition (Slade *et al.*, 2005, 2012; Hazard *et al.*, 2012; Bovina *et al.*, 2014).

In particular, Bovina *et al.* (2014) have developed a mutagenized population by treating seeds of the durum wheat cultivar Svevo with Ethyl-Methan-Sulfonate (EMS). The M_1 generation was advanced by Single Seed Descent (SSD) to obtain M_3 seeds, whereas genomic DNA was extracted from each of the M_2 individual lines obtained. The entire M_3 population, consisting of 2601 families, was field-sown for both seed multiplication and phenotypical evaluation. Field phenotypic screening showed a high frequency of morpho-physiological alterations (ca. 22%). After harvesting, the M_4 seeds were also characterized for quality characteristics as yellow pigment, protein content and Sodium-dodecyl-sulfate (SDS) sedimentation test. Alterations of seed morphology, as kernel size/shape or colour were also identified.

In order to develop high-amylose durum wheat genotypes starting from knock-out mutants for the *SBEIIa* homeologous genes (*Sbella-A* chr 2AS; *Sbella-B*, chr. 2BS), the genomic DNA, isolated from the M_2 lines of the mutagenized population of Svevo, was screened by a TILLING approach. High Resolution Melting was applied to identify functional SNPs in the two homoeologous genes encoding *SBEIIa-A* and *-B* enzymes, using the strategy and primer pairs described by Botticella *et al.* (2011).

TILLING analysis permitted to identify 45 novel allelic variants: 39 for the gene *SBEIIa-A* and 6 for *SBEIIa-B*. Sequencing analysis confirmed that the mutations were G to A or C to T transitions as expected from alkylation by EMS. Note of worth a non-sense mutation for each homoeoalleles was identified. These single *null* mutants for *SBEIIa-A* and *-B* were crossed to obtain a complete *null* genotype. The screening of F_2 plants is in progress.

III – Conclusions

Technological and nutritional improvement of wheat is more feasible thanks to the possibility to integrate classical and novel approaches in both research and breeding. Increasing components present in the wheat kernel capable to exert beneficial effects on the onset of chronic diseases will open the possibility to develop novel end products with important added value and social benefits.

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