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Poster Abstract – B.06

ISOLATION AND CHARACTERIZATION OF TWO GENES CODING STARCH BRANCHING ENZYME IIA IN WHEAT

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Manipulation of cereal starch is receiving an increasing attention due to the demonstration of its important role in influencing quality characteristics of end products. In particular, altering the amylose/amylopectin ratio of starch results in the possibility to obtain products with novel technological and nutritional characteristics. In particular, high amylose starch -HAM- is believed to have beneficial effects on human health, lowering the risk of serious diseases, due to the increase of "resistant starch".

Researcher's efforts to produce cereal starches with an increased amylose content are focusing on the manipulation of genes involved in the biosynthetic pathway. Synthesis of the two starch polymers starts from a common substrate, the ADP-glucose, following different pathways governed by several enzymes classes. The complex structure of amylopectin results from the cooperation of at least three enzymes classes: the starch synthases (SSII), starch branching enzymes (SBE) and debranching enzymes (DBE). The attempts of researchers to obtain increased level of amylose by the suppression of enzymes involved in amylopectin production have been successfully. Particularly, the lack of the three SSII isoforms in natural or induced bread wheat mutants, has been shown to increase amylose content from 25 % to 35 %, whereas the silencing of the starch branching enzyme SBEIIa, through RNAi, in bread wheat line, has resulted in an increase of amylose content to 70 % of total starch.

Chemical and physical mutagenesis represent a powerful tool to generate novel genetic variation which has been widely used in crop improvement. In particular, EMS treatment of seeds induces point mutations which can result in gene silencing. Recently, mutagenesis coupled with PCR detection of specific mutation, has resulted in a novel approach termed TILLING (Targeted Induced Local Lesions in Genomes). The PCR conditions to allow homoeologous allele-specific amplification for a polyploid species such as wheat is based on knowledge of DNA sequences of target genes. Hexaploid wheat is characterized by the presence of three homoeoalleles coding SBEIIa enzyme, one for each genomes, localized on the long arm of chromosomes 2. Rahman *et a.l* (2001) have characterized the structure of the gene coding SBEIIa from *Aegilops tauschii*, the donor of the D genome to wheat -*wSBEII-DA1*. The gene consists of 22 exons, which vary in size from 43 to 384 bp and cover a region of 10.5 kb. The size of the introns vary from 83 bp to 1019 bp. The genes associate to the A and B genomes are not available yet, limiting the possibility to screen mutagenised wheat populations for the presence of knockout genes for SBEIIa protein

In the present work we have realized the isolation and sequencing of the two homoeoalleles coding the enzymes SBEIIa from *Triticum aestivum* cv Chinese Spring . The two genes *wSBEII-AA1* and *wSBEII-AB1,* are located respectively on the A and B genome. The exon-intron structure of these genes result the same as *wSBEII-DA1*gene; the three genes show a level of sequence

similarity of 91% to one another. The nucleotide sequences of the two genes *wSBEII-AA1* and *wSBEII-AB1* and that one of *wSBEII-DA1* from *Ae. tauschii* have been aligned by ClustalW to determine regions proper to design genome specific oligonucleotides. The identified primers will be used in the screening of a EMS treated *Triticum aestivum* population for the selection of wheat lines characterized by the presence of single nucleotide polymorphisms (SNPs) leading to the alteration of the activity of SBEIIa isoenzymes.