FUNCTIONAL MARKERS FOR GLUTAMINE SYNTHETASE AND CORRELATION WITH GRAIN PROTEIN CONTENT IN DURUM WHEAT

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Durum wheat (Triticum turgidum L. var. durum) is one of the most important cereal crops grown world-wide and provides most of the proteins in human diet, especially in the less developed countries. Seed storage proteins are directly related to the nutritional and technological value of the derived products. Several studies have attested the key-role of the glutamine synthetase enzyme in plant nitrogen metabolism. Glutamine synthetase gene encodes for an enzyme responsible of the first step of ammonium assimilation and transformation into glutamine and glutamate, essential compounds in amino acid-biosynthetic pathway. High protein content is a very important quantitative trait controlled by several genes located on wheat chromosomes. Glutamine synthetase genes are located on the homeologous chromosomes 2A, 2B, and 2D where several authors reported major QTL for protein content. The goal of the present study was to assess the linkage between GS gene and the QTL for protein content. For this purpose, the nucleotide sequence of glutamine synthetase gene acc. DQ124214 was aligned to all the wheat ESTs available in public data bases by means of BLAST tool (http://www.wheat.pw.usda.gov/GG2/blast.shtml.). The bioinformatic analysis allowed to find 40 sequences with a similarity > 94% to the GS2 gene, of which three covered the whole gene sequence (DQ124213, DQ124212 and CJ705909). For each of these sequences we designed two or three primer pairs identifying a total of 7 functional markers that were screened among the parents of three segregant populations. Mapping analysis performed by Join Map software allowed to localize the amplified polymorphic fragments and to identify 4 loci: Gs-A2, Gs-B2, Gs-A4, Gs-B4, respectively mapped on chromosome 2A, 2B, 4A and 4B. The QTL analysis for protein content was carried out in a RIL population obtained from the crossing the two durum wheat cultivars Ciccio and Svevo. Two major QTLs were identified through Composite Interval Mapping (CIM) performed by the Q-Gene software: one QTL was identified by the functional marker Gs-B2 located on chromosome 2B, and the other one was identified by the functional marker Gs-A4 located on chromosome 4A. These data were confirmed by a linkage disequilibrium analysis carried on a collection of 75 different wheat genotypes.

The present study represents the first step for the identification and sequencing of GS2 gene, which could be employed in breeding programs aimed to increase grain protein content commercial cultivars. Moreover, Gs-B2 and Gs-A4 represents functional markers that could be also efficiently used in marker assisted selection (MAS) programs and map-based cloning.