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IDENTIFICATION OF CANDIDATE GENES FOR TRAITS OF RELEVANT BREEDING VALUE TRANSFERRED FROM A WILD RELATIVE TO WHEAT

GENNARO A., CEOLONI C.

Department of Agrobiology and Agrochemistry, University of Tuscia, Via S. Camillo de Lellis, s.n.c., 01100 Viterbo (Italy)

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Two valuable genes for durum wheat breeding, i.e. *Lr19* (leaf-rust resistance) and *Yp* (yellow endosperm pigmentation), closely linked on the 7AgL chromosome arm of the perennial wheatgrass species *Thinopyrum ponticum,* have been transferred to the 7AL durum wheat arm by chromosome engineering. Several 7AL-7AgL recombinant lines were obtained which incorporated alien segments of different size and gene content, and from them near-isogenic recombinant lines (NIRL) were produced and used in differential analyses. No previous knowledge was available on the structural and functional characteristics of *Lr19* and *Yp.* To identify candidate genes for such relevant traits, different approaches were followed.

For the *Lr19* gene, given its characteristic hypersensitive response to the pathogen, disease resistance (R) genes encoding proteins with nucleotide-binding site (NBS)-leucine-rich repeat (LRR) domains, were considered likely candidates. Structural conservation within the NBS domain was used in a 'NBS profiling' assay, a motif-directed PCR-based approach, to identify specific DNA fragments tightly linked to *Lr19*. The NBS profiling of two NIRLs, one carrying and the other lacking *Lr19,* were compared and a polymorphic fragment, located within the 1% 7AgL chromatin differentiating the two lines and containing *Lr19,* was isolated. Such polymorphic band was cloned and sequenced, and new primers were designed which allowed development of a codominant marker for *Lr19,* useful for its marker-assisted selection in breeding programs. The initial 212bp 7AgL polymorphic band was extended by rapid amplification of cDNA ends (RACE). A full-length sequence of 4121bp, containing a coding region of 3774bp, was obtained. BLASTX showed very high similarity with other Triticeae R genes. The deduced amino acid sequence revealed that the *Lr19* candidate gene product corresponds to a protein with CC-NBS-LRR characteristic domains for monocot R genes. Expression of the isolated sequence was investigated by quantitative PCR on mRNA extracted from leaf-rust infected and uninfected *Lr19+* wheat seedlings. The results confirmed for the *Lr19* candidate gene a constitutive type of expression, usually observed for R genes. The isolated full-length sequence is currently being employed in a stable transformation assay to verify its actual correspondence with *Lr19.*

To identify a candidate gene for *Yp,* determining a considerable increase in semolina carotenoid pigment content, a comparative genomics approach has been followed. On the basis of a partial nucleotide sequence for the phytoene synthase (*Psy*) wheat genes located on group 7 chromosomes, specific primers were developed which enabled identification of the 7AgL *Psy* orthologue. Given the observed association between the 7A/7Ag *Psy* polymorphism and the low

(7A) vs. high (7Ag) carotenoid pigment content of the different durum wheat-*Th. ponticum* recombinant lines, such key enzyme in carotenoid biosynthesis appears as a strong candidate for the yellow pigmentation determined by this wild wheat relative, as it seems to be the case for several grass species.