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FUNCTIONAL MARKERS FOR GRAIN POLYPHENOL OXIDASE ACTIVITY IN A WHEAT COLLECTION

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Polyphenol oxidase (PPO) activity in wheat grains causes undesiderable darkening of various end-products. In the last years, the knowledge of genetic control of PPO activity has substantially accelerated development of wheat cultivars with low PPO activity to reduce flour darkening in wheat. One of the most powerful tools to evaluate and validate the association between PPO activity and the flour darkening is the identification of PPO allelic variations and the development of functional markers. In comparison to genomic markers, candidate genes are more powerful because they are directly involved with known biological function or they regulate the developmental processes of the investigated traits, which could be confirmed by evaluating the effects of the allelic variants in association analysis.

The aims of the present study were 1) to analyze the genetic diversity for PPO activity, 2) to develop new genome-specific functional markers for PPO genes and 3) to validate molecular markers for PPO activity in a tetraploid wheat collection.

A collection of 231 tetraploid wheat accessions (*Triticum turgidum* L.), including 128 old and modern cultivars of durum wheat (*T. turgidum* L. var. *durum*) and 103 wild and domesticated tetraploid wheats, was evaluated for PPO activity in kernels. The analysis of variance for PPO activity showed high significant differences ($P \leq 0.001$), with an average of 0.63 (measured at A_{405} nm) ranging between 0.15 and 1.88, and high heritability of the trait ($h^2 > 80\%$).

To obtain B genome-specific sequences, several primers pairs were designated based on the sequence of PPO gene (GenBank Accession Number, GQ303713.1). Two primer pairs, physically mapped on chromosome 2B, amplified a single product of 500-bp and 900-bp, respectively, in the cultivar Chinese Spring.

Some of the reported molecular markers for PPO activity have been analyzed in the tetraploid wheat collection previously described. Interestingly, *PPO18* marker, mapped on chromosome 2A by Sun *et al*., 2005, showed a polymorphic electrophoretic pattern: it amplified a 730 bp fragment in the cultivars with low PPO activity, a 850 bp fragment in the cultivars with high PPO activity, and null allele in several accessions. Association analysis validated that the 730 bp fragments was associated with higher PPO activity than the 850 bp fragments and null allele.