Proceedings of the LXV SIGA Annual Congress
Piacenza, 6/9 September, 2022
ISBN: **978-88-944843-3-5**

Poster Communication Abstract – 4.10

IDENTIFYING GENETIC VARIANTS ASSOCIATED WITH VARIABILITY IN POLYPHENOL OXIDASE PROFILES IN TETRAPLOID WHEAT (TRITICUM TURGIDUM L. SSP.)

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durum wheat, PPO activity

Polyphenol oxidases (PPO) enzymes cause the loss of yellow color in semolina and pasta and determine the rate and degree of enzymatic browning. Understanding the genetic basis of PPO-related traits is crucial for durum wheat breeding. Although the biochemical reactions catalyzed by PPOs are well known, their molecular/physiological role is poorly understood. Several lines of evidence indicate that most modern varieties are characterized by a lower PPO activity than old varieties and domesticated/wild accessions. This suggests that breeding directly affected this trait. Indeed, reducing PPO activity in wheat has only become a breeding target in recent years. Therefore, the variability in PPO profiles could be probably ascribed to the selective pressure exerted on other traits of interest. In this scenario, the investigation of the genetic

variability and profiles of PPOs in tetraploid wheats could be a useful strategy to identify novel SNP markers and to understand the effects of allelic variations in PPO loci in *Triticum turgidum* L. ssp.

Our work aimed to identify haplotypes associated with different PPO profiles and discover natural allelic variants for the gene *Ppo-A1* in a large panel of tetraploid wheats phenotyped for PPO activity. A genome-wide association study (GWAS) was performed on a collection of 220 accessions, consisting of ssp. *durum*, ssp. *turanicum*, ssp. *polonicum*, ssp. *turgidum*, ssp. *carthlicum*, ssp. *dicoccun* and ssp. *dicoccoides*. Association tests based on 21,347 polymorphic SNPs returned 23 marker-trait associations (MTA), found primarily on chromosome 2A and 2B, flanking the *Ppo-A1*, *Ppo-A2* and *Ppo-B2* genes. Among the 23 MTAs, a reliable marker (WA572) was validated for marker-assisted selection, as it is able to discriminate between two haplotypes associated with high/low PPO activity. In addition, the 23 SNP markers were used to assess the genetic divergence (FST > 0.25) between the *T. turgidum* subspecies, providing important new information for understanding the domestication process of *Triticum turgidum* ssp. and in particular of ssp. *carthlicum*.

These promising results prompted the search for allelic variants within the *Ppo-A1* gene. The functional marker PPO-18, previously designed to identify polymorphic fragments of the *Ppo-A1* gene (Sun et al. 2005), was used to analyze allele profiles of the accessions collected. Three polymorphic PCR products were detected by automated capillary electrophoresis and cloned, resulting in *Ppo-A1f* (685-bp), *Ppo-A1e* (716-bp) and *Ppo-A1g* (null allele) alleles, which in turn were associated with high, moderate and low PPO activity. PCR experiments revealed that the null allele, clearly prevalent in modern varieties, includes an additional region over 3k in size amplified by PPO-18 marker. Preliminary bioinformatic analysis suggested that this large insertion could affect PPO activity. This result will be validated by collecting expression profiles in those wheat accessions carrying the *Ppo-A1g* allele and, could help understanding the origin of this insertion in modern durum wheat varieties.