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## UNRAVELING THE GENETIC MECHANISM OF PURPLE GRAIN PIGMENTATION IN DURUM WHEAT

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Pigmented cereals have gained considerable attention in recent years, especially for the development of products with functional and health-promoting properties. Among the pigmented varieties, purple wheat grains are of particular interest for the presence of anthocyanins, a class of natural pigments widely recognized for their antioxidant and anti-inflammatory properties. From the genetic point of view, three main loci have been identified for purple pericarp (Ppgenes) in wheat. The *Pp-A3* has been mapped to chromosome 2A both in bread (*Triticum aestivum* L.) and durum wheat (*T. durum* Desf.), whereas *Pp1* has been positioned on chromosomes 7B ( *Pp-B1*) in durum and on 7D (*Pp-D1*) in bread wheat. Unfortunately, although these loci were mapped in durum wheat through molecular markers, the causative genes and functional and not functional alleles are still unknown. To uncover this gap, a recombinant inbred line (RIL) population, derived from two contrasting durum wheat genotypes (purple vs yellow), was genotyped using a 25K SNP array and phenotyped for two growing seasons (2019-2020 and 2021-2021) for color indices (L*, a*, and b*). Additionally, high-density genotyping with a 400K SNP array was performed on two contrasting bulks to better define regions of interest. Three major QTL

regions on chromosomes 2A, 3A, and 7B were identified. The QTLs explained the highest phenotypic variation (more than 50%) and the regions were further confirmed using bulked segregant analysis (BSA). Leveraging the Svevo (v1.0) reference genome and the latest insights into the functional role of *Pp-A3* and *Pp-B1*, we successfully annotated a *basic helix-loop-helix* (*bHLH*) gene on chromosome 2A and a *MYB* on the short arm of chromosome 7B as candidates for *Pp-A3* and *Pp-B1* in durum wheat. Notably, we identified a non-functional allele of *Pp-B1* characterized by a significant insertion (~1.6 kb) in the first exon in yellow genotypes, while the coding sequence of the functional allele in purple durum wheat was elucidated for the first time. Additionally, we found six 261-bp tandem repeats in the promoter region of *Pp-A3* in purple varieties, compared to only one repeat unit in non-purple ones, thus allowing us to design valuable functional markers to be employed in breeding programs. Furthermore, our investigation extended to the identification of structural genes involved in the anthocyanin pathway, including *Dihydroflavonol-4-reductase* (*DFR*) and *Cinnamoyl-CoA reductase* (*CCR*), which were found under the QTLs of interest. Our findings offer new molecular markers to accelerate breeding programs aimed at developing purple wheat genotypes. Furthermore, the molecular characterization of promoter regions will shed new light on the genetic makeup of purple wheat, enabling an improved understanding of its underlying regulatory mechanisms.