

Genome-wide association study reveals novel loci modulating pre-harvest sprouting and grain color in red-grained winter wheat *T. aestivum* L.

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Motivation and Aim: The association between pre-harvest sprouting (PHS) and grain color has long been recognized. Red-grained wheats generally are more PHS resistant compared to white-grained wheat. Nevertheless, red-grained wheat vary greatly in PHS resistance [1, 2]. The search for genes controlling PHS in a color-independent manner is of special interest. For instance, developing PHS-resistant cultivars in white-grained wheats is advantageous due to their lighter flour color and higher flour yield [3]. Research based on analysis of population with identical color allows to identify the novel gene loci involved in PHS control [4, 5]. In present work, we conducted a genome-wide association study on a panel of red-grained wheat varieties, aimed at uncovering genes that modulate PHS resistance and red color components of seed coat using digital image processing.

Methods and Algorithms: Statistical analysis of pre-harvest sprouting and grain color traits (brightness, lightness, redness, yellowness and blueness), genome-wide association analysis testing MLM, FarmCPU and BLINK models, gene prioritization using gene expression data from expVIP, functional annotation using InterPro and DAVID resources, and the literature.

Results: We used a panel of red-grained winter wheat accessions exhibiting variations from high to low in resistance to PHS as determined by the analysis of germination index at the late milk/hard dough (GI_milk) and mature grain stages (GI_mat) and the falling number at mature grain stage (FN). The heritability of all PHS-related traits was estimated above 50 %. The variability of grain color characteristics was detected by a digital phenotyping, their heritability varied from low (below 20 %) up to 50 %. The correlation analysis showed a weak, predominantly statistically insignificant relationships between grain color and PHS traits.

As a result of genome-wide association analysis we identified 12 markers associated with PHS traits at $p < 10^{-4}$ on chromosomes 1A, 1B, 1D, 3A, 4A, 5B, 6B, and 7D. Particularly, SNPs on chromosome 1B and chromosome 7D each of them explained a up to 25.3 % of the phenotypic variance. Eight loci, containing the detected markers, were considered novel. We observed 26 markers associated with seed color traits at $p < 10^{-4}$. They were located on the chromosomes 1A, 1B, 1D, 2A, 2B, 3B, 5A, 6A and 7D. Markers on chromosome 1D explained up to 50 % of brightness, redness and blueness. Some SNPs were associated with multiple traits for color characteristics.

Interestingly, loci associated with color traits did not overlap with loci associated with PHS-related traits.

As a result of gene prioritization, we detected 12 candidate genes for PHS-related traits. The most promising gene was *TraesCS6B02G147900*, encoding a protein involved in aleurone layer morphogenesis. Out of 14 genes prioritized for the grain color two genes, *TraesCS1D03G0758600* and *TraesCS7B03G1296800*, were considered as top candidates. The former is located on chromosome 1D and encodes a flavonol-synthase which synthesizes flavonols in the flavonoid biosynthesis pathway. The second gene is located on chromosome 7B and encodes a phytoene synthase which participates in one of the initial steps in carotenoid biosynthesis.

Conclusion: In our study of a population of red-grained common wheat varieties, we found that variation in coloration among these varieties does not correlate with resistance to PHS. Thus, when analyzing populations of wheat with red grain, we exclude grain color as a factor from our analysis. This exclusion allowed us to detect novel loci in the genome associated with PHS and grain color and suggest significant SNPs that could potentially enhance wheat varieties during the breeding.

Through the analysis of expression data and gene annotation within the revealed loci, we identified candidate genes conferring PHS resistance, in addition to the known genes. Our analysis suggests that the structure and composition of the aleurone layer may play a role in this process. Furthermore, we propose that variation in grain color characteristics in red-grained wheat, such as brightness, redness and blueness, is regulated by the balance between the synthesis of uncolored flavonols and colored anthocyanins, regulated by the activity of flavonol synthase. Another speculated mechanism underlying grain color is the carotenoid synthesis and accumulation pathway controlled by phytoene synthase.

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