

Molecular mechanisms regulating protein and gluten accumulation in grain of spring vs winter wheat

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Motivation and aim: Common wheat (*Triticum aestivum* L.) breeding programs prioritize the development of high grain protein content (GPC) varieties, as GPC directly enhances milling efficiency and end-use quality. Additionally, the composition and quality of wheat grain storage proteins, particularly gluten, critically determine the functional properties of flour. Despite this, the molecular basis of protein and gluten accumulation in wheat grains remains insufficiently understood, especially in the context of genetic differences between spring and winter varieties. The aim of this study is to investigate the allelic variation of key genes regulating these traits, identify novel molecular markers, loci and candidate genes, associated with protein and gluten content, and perform a comparative analysis of them in spring versus winter wheat varieties.

Materials and methods: The study utilized a panel of 93 spring and 179 winter wheat varieties, representing diverse genetic backgrounds from domestic and international breeding programs. Plants were cultivated under field conditions across two contrasting environments for 2–3 growing seasons to account for genotype-by-environment interactions. Grain protein and gluten content were quantified using an infrared analyzer (OmegAnalyzer G, Bruins Instruments, Germany). SNP genotyping was conducted with the Illumina Infinium 15K/25K Wheat Array (TraitGenetics, Germany). Genome-wide association mapping was performed using the GAPIT R package (v.3.0) with multiple models, including MLM, FarmCPU and BLINK, to minimize false positives. Significant loci were annotated using the IWGSC RefSeq v.1.1 genome assembly (GrainGenes database), and candidate genes within these loci were prioritized based on tissue-specific expression profiles at key developmental stages, analyzed via the expVIP platform. For the most significant SNPs, KASP markers were designed and verified in a biparental population.

Results: Genome-wide association analysis revealed distinct chromosomal loci associated with protein and gluten accumulation in spring and winter wheat. In the spring wheat panel, most significant markers for grain protein content (GPC) were localized on chromosome 6A ($p < 0.001$), while gluten content showed associations across chromosomes 1D, 2A, 2B, 3D, 5A, 6A, 7B, and 7D. For winter wheat, 27 markers linked to GPC and 24 to gluten content ($p < 0.001$) were identified, with the strongest signals on chromosomes 4A and 2D. Two candidate genes, *TraesCS4A02G235900* (4A) and *TraesCS2D02G517200* (2D), were prioritized based on their expression profiles and functional annotations. Allelic variation at the *NAM-A1* locus significantly impacted GPC, with varieties carrying the NAM-A1d allele showing lower protein content compared to *NAM-A1c/a* carriers ($p < 0.01$), consistent across both growth types. Gluten content was strongly associated with the *Glu-A1* locus: varieties harboring the Ax0 subunit exhibited an increase in gluten levels relative to other alleles.

Conclusions: The chromosomal distribution of loci differed between spring and winter wheat, suggesting divergent genetic regulation of these traits. This comparative analysis highlights potential targets for marker-assisted selection in breeding programs focused on grain quality improvement.

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