Multiomic approaches identified a novel region for grain protein content in European winter wheat populations

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ABSTRACT: Wheat stands as a fundamental crop for billions across Europe, South Asia, and Africa, contributing up to 20% of global caloric intake and reaching as high as 50% in specific regions. Beyond meeting daily carbohydrate requirements, wheat offers a significant supply of protein, vitamins, dietary fibers, and phytochemicals crucial for health. The quality traits of wheat grains play a pivotal role in determining both their end-use and monetary value. The protein content in wheat grains, known as grain protein content (GPC), influences the gliadin to glutenin ratio, ultimately shaping the viscoelastic properties and bread-making quality of dough. High protein levels in wheat not only satisfy nutritional needs but also maximize returns for farmers. Given its impact on both grain yield (GY) and GPC, improving the latter is a primary goal in virtually every wheat breeding program. In our research, a genome-wide association study (GWAS) was conducted using a panel of elite lines from the Scandinavian region (STRAW), revealing a novel locus on chromosome 2 of the B sub-genome. This finding was validated independently through GWAS using a panel of European elite lines (GABI-WHEAT). Furthermore, the identified region was linked to an introgression from *T. timopheevii*, underscoring the significance of introgression breeding in enhancing quality traits in wheat. Ongoing transcriptomic analysis aims to explore the expression patterns of candidate genes associated with this region, providing valuable insights for potential interventions to enhance GPC in wheat.

	Genotyping	and Phenotyping	
wo Panels:	a	b	
STRAW: 255	0.04 —		

PC2 (4.2

• GABI-WHEAT (Gogna et al., 2022): 325

Array Name	#SNPs	#Samples	Panel
7K	6,731	41	STRAW
15K	13,006	25	STRAW
20K	17,267	23	STRAW
25K	24,145	20	STRAW
25K	24,145	146	STRAW
Total	11,219	255	STRAW
35K-90K	118,439	9325	GABI-WHEAT

Phenotyping for GPC and GY:

STRAW panel:

- GPC and GY data collected from 37 different locations and for years 2000 to 2023 were downloaded from SortInfo (<u>https://sortinfo.dk</u>).
- BLUPs: Pheno ~ (1|genotype) + (1|location) + (1|year) + (1|sample: location).
- Grain Protein Deviation (GPD): deviation from GY ~ GPC line.
- GABI-WHEAT panel:
 - BLUEs for GPC and GY datasets downloaded from public repository (Gogna et al., 2022) and GPD values were calculated.



PC1 (6.9 %)

Figure 1: **a)** Distribution of elite lines from STRAW and GABI-WHEAT panel on the diversity space of 9K winter wheat accessions described in Schulthess, et al., 2022.**b)** Location-wise and **c)** Year-wise distribution of GPC in selected accessions.

D

Negative effect of *T. timopheevii* introgression on GPC ²⁸

cluster01



e

RAW Panel



Figure 2: **a)** Manhattan plots showing GWAS results for GPD based on STRAW and GABI-WHEAT panel. **b)** clustering of accessions based on the significant SNP identified on chr2B in STRAW panel. **c)** GPC and GPD distribution among the clusters. **d)** Clustering of GABI-WHEAT accessions based on T. *timopheevi* introgression on chr2B and **e)** GPC and GY distribution in two major clusters identified in GABI-WHEAT panel.





Figure 3: a) Principal component analysis (PCA) of normalized expression levels of all transcripts. b) Differentially expressed genes (DEGs) identified between same stages from accessions with positive and negative GPD values. c) Clustering of log2FC values of DEGs identified from comparison of G10 stage. d) Clustering of DEGs identified from G10 and G20 stages in from accessions with positive and negative GPD values.

Conclusions:

- SWAS identified a SNP at position 680,573,507 on chr2B associated with GPC and GPD in STRAW panel.
- The region was validated independently using accessions from GABI-WHEAT panel.
- The region coincides with an introgression from T. *timopheevi* (675-725 Mb) on long arm of chr2B.
- Downstream analysis showed negative effect of T. timopheevi introgression on GPC and GPD in the winter wheat population.
- Transcriptomic analysis identified clusters of DEGs with consistently high and low expressions in accessions with positive and negative GPD values.
 References:
- 1. Gogna, Abhishek, et al. "Gabi wheat a panel of European elite lines as central stock for wheat genetic research." Scientific Data 9.1 (2022): 538.
- 2. Schulthess, Albert W., et al. "Genomics-informed prebreeding unlocks the diversity in genebanks for wheat improvement." Nature Genetics 54.10 (2022): 1544-1552,

