Article

## Effects of Nitrogen Application in the Wheat Booting Stage on Glutenin Polymerization and Structural–Thermal Properties of Gluten with Variations in HMW-GS at the *Glu-D1* Locus

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## Supplementary data



**Figure S1**. Separation and identification of gliadins and glutenins. **A**, Gliadins from the two NILs separated by A-PAGE: lane 1, *Glu-1Dd*; lane 2, *Glu-1Da*. **B**, Glutenins separated by SDS-PAGE from the two near-isogenic lines (NILs)and five wheat varieties as controls: lane 1, *Glu-1Dd*; lane 2, *Glu-1Da*; lane 3, Pompei (null, Bx6+By8, Dx2+Dy12); lane 4, Jin47 (null, Bx7+By9, Dx2+Dy12); lane 5, Guadalupe (null, Bx13+By19, Dx5+Dy10); lane 6, Lankao Teaizao (Ax1, Bx7+By8, Dx2+Dy12). **C**, Separation of the glutenins from the two NILs differs at *Glu-D1* locus by RP-HPLC.



**Figure S2**. Plant nitrogen (N) uptake, recovery efficiency, nitrogen use efficiency (NUE) –total protein yield, NUE–GMP yield, grain yield, and NUE–grain yield of the wheat lines with Glu-1Dd and Glu-1Da under different N management strategies. Same letter in the different columns for each material indicate no significant difference (p > 0.05). Data are present as mean of two years.

	GMP	β-sheets	Intermole cular β-sheets	α-helices	α-helix/β- sheet ratio	Denaturat ion peak temperatu re	Enthalpy of thermal transition	Weight loss	Degrada tion tempera ture	Disulfide bonds	Dough develop ment time	Dough stabilit y time	Protein weakeni ng	Lacuna rity	Branch rate
β-sheets	0.998**	1													
Intermolecular $\beta$ -sheets	0.960**	0.966**	1												
α-helices	-0.966**	-0.970**	-0.963**	1											
$\alpha$ -helix/ $\beta$ -sheet ratio	-0.991**	-0.992**	-0.965**	0.989**	1										
Denaturation peak	0.926**	0.912**	0.891**	-0.868**	-0.901**	1									
temperature						1									
Enthalpy of thermal	0.905**	0.881*	0.802*	-0.875**	-0.903**	0.891**	1								
transition							1								
Weight loss	-0.937**	-0.953**	-0.960**	0.739	0.950**	-0.782*	-0.740	1							
Degradation temperature	0.893**	0.899**	0.884*	-0.888**	-0.882**	0.765	0.749	-0.853**	1						
Disulfide bonds	0.893**	0.897**	0.915*	-0.884**	-0.911**	0.870**	0.808*	-0.896**	0.680	1					
Dough development time	0.967**	0.964**	0.904**	-0.962**	-0.971**	0.853**	0.920*	-0.891**	0.927**	0.814*	1				
Dough stability time	0.721*	0.727*	0.828*	-0.781*	-0.759*	0.809*	0.639	-0.718*	0.543	0.878**	0.625	1			
Protein weakening	0.883**	0.874**	0.874**	-0.869**	-0.899**	0.803*	0.877**	-0.863**	0.720*	0.930**	0.857**	0.725*	1		
Lacunarity	0.972*	0.962*	0.882	-0.894	-0.932	0.933	0.974*	-0.793	0.904	0.757	0.962*	0.611	0.947*	1	
Branch rate	0.963*	0.966*	0.984*	-0.985*	-0.983*	0.913	0.866	-0.921*	0.769	0.970*	0.957**	0.878*	0.919**	0.892	1
End-point rate	-0.955*	-0.948*	-0.925	0.933	0.949	-0.983*	-0.950*	0.816	-0.745	-0.909	0.850	0.848*	0804	-0.935	-0.972

Table S1 Pearson correlation coefficients between GMP, different secondary structures, thermal stability, disulfide bond concentrations, dough mixing properties, and the parameters of protein network analysis.

\* And \*\* indicate significant correlations at p < 0.05 and p < 0.01, respectively.