HUESCA VII
13 y 14 de mayo de 2021 • VIRTUAL
CONGRESO NACIONAL
SOCIEDAD ESPAÑOLA DE ENFERMEDAD CELÍACA

Contenido de prolamina y peso del grano de líneas de trigo RNAi con bajo contenido en gluten bajo diferentes condiciones de temperatura y nitrógeno

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Rendimiento del grano
Calidad del grano

Llenado del grano

Temperatura (Estrés térmico; ET)
Fertilización de N

Fase de latencia

Período efectivo de llenado del grano

Fase de deshidratación

Proteína del grano — Almidón

Prolaminas 80 % PS — Proteínas no gluten (NGPs) 20 % PS

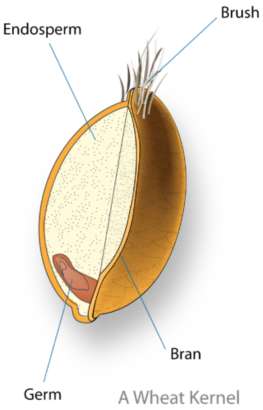
Gliadinas — **Gluteninas** — Albuminas — Globulinas

ω α γ — **HMW LMW**

Enfermedad celíaca (EC) ← **Proteínas del Gluten** → **Propiedades de la pan**

Tecnología RNAi

Líneas RNAi con bajo contenido en gluten



Objetivo:

¿Cómo se comportan las líneas RNAi bajo dos de los factores ambientales más importantes?

Efectos en el peso del grano y la composición de proteína

Líneas RNAi con bajo contenido en gluten

BW208 (WT)

D793 **D770** x **Gazul (WT)**

J631 **M959**

Materiales y Métodos

Material vegetal
Experimento de cámara
Tratamientos

Determinación de:
Peso del grano
Proteína total
Cuantificación de prolamina por RP-HPLC
NGPs

Procesamiento de datos

2 disponibilidades de N: 9 mg (+ 21 mg) N
2 tratamientos de temperatura: 25/18, 40/18 °C

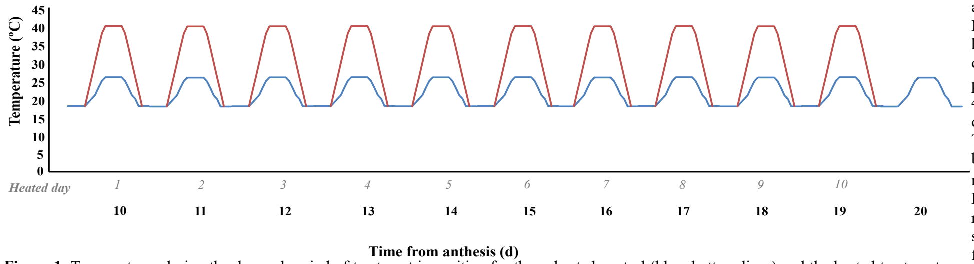


Figure 1. Temperatures during the day and period of treatment imposition for the unheated control (blue, bottom lines) and the heated treatments (red, upper lines). Temperatures were determined hourly in control and heated experimental chambers for all wheat lines.

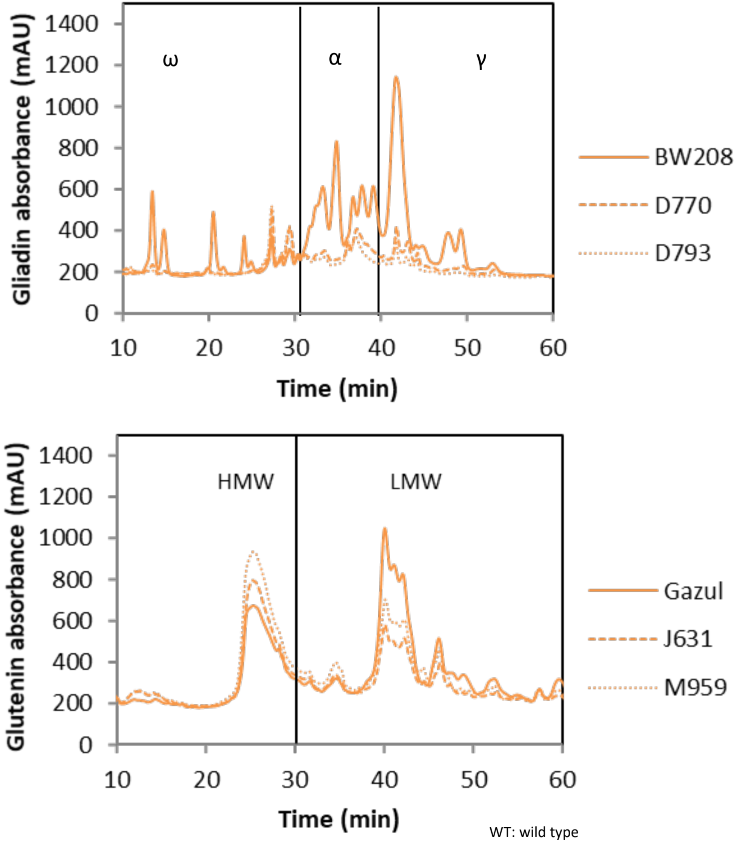


Figure 2. RP-HPLC chromatograms of gliadin and glutenin fractions of all genotypes and tratments conditions. N_0: no N application after heading, N_1, N application after heading; control: 25/18 ºC during whole grain filling period, Heat stress HS: 40/18 ºC for ten days during grain filling period. The signals were obtained by RP-HPLC software and represented with Microsoft Excel. The intervals of retention time used for the separation of prolamin fractions peaks are indicated according to Wieser et al. (1998)

WT: wild type
RP-HPLC: Reverse Phase-High Performance Liquid Chromatography

ET y tratamiento de N en el peso del grano y el contenido de proteína total

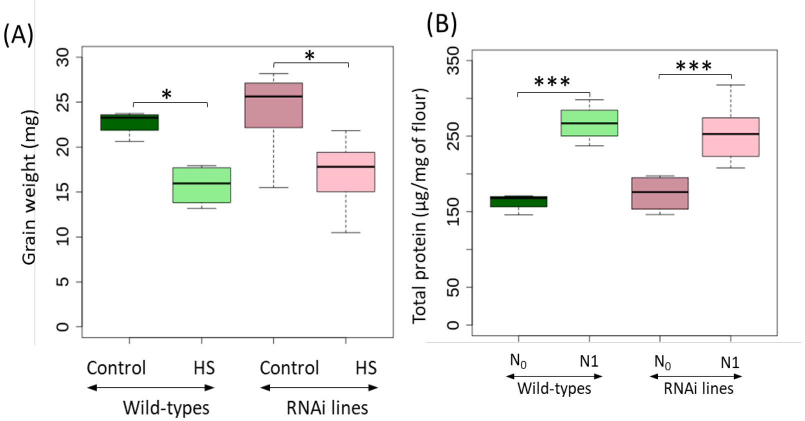


Figure 3. Grain weight and total protein content for wild-types and RNAi lines under control and heat stress temperature treatments **(A)** and nitrogen availability **(B)**. N_0: no N application after heading, N_1: N application after heading; control: 25/18°C during whole grain filling period, Heat stress (HS): 40/18°C for 10 days during grain filling period. The black line represents the median value. * above the bars indicates significant difference (*$P \leq 0.05$; ***$P \leq 0.001$) between treatments according to the variance analysis.

ET y tratamiento de N en el contenido de gliadinas

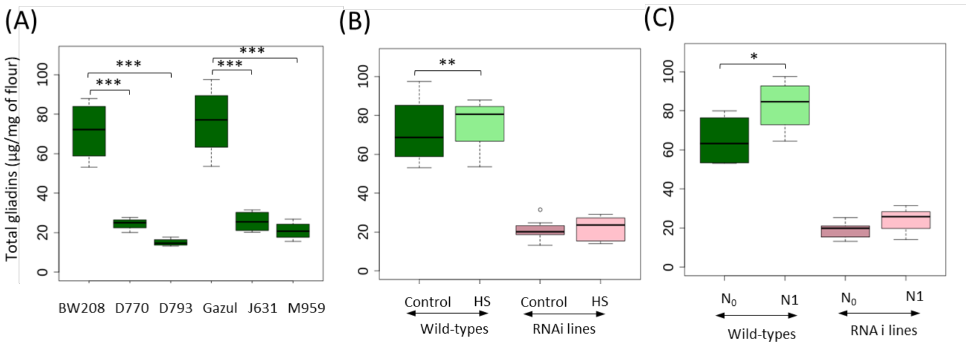


Figure 4. Content of total gliadin content for different genotypes with comparisons between RNAi lines and their wild-type by Dunnett's test **(A)**, wild-types and RNAi lines under different temperature treatments **(B)** and nitrogen availability **(C)**. N_0: no N application after heading, N_1: application after heading; control: 25/18°C during whole grain filling period, Heat stress HS: 40/18°C for 10 days during grain filling period. The black line represents the median value. * above the bars indicates significant difference (*$P \leq 0.05$; **$P \leq 0.01$) between treatments according to the variance analysis.

ET y tratamiento de N en el contenido de las fracciones de gliadinas

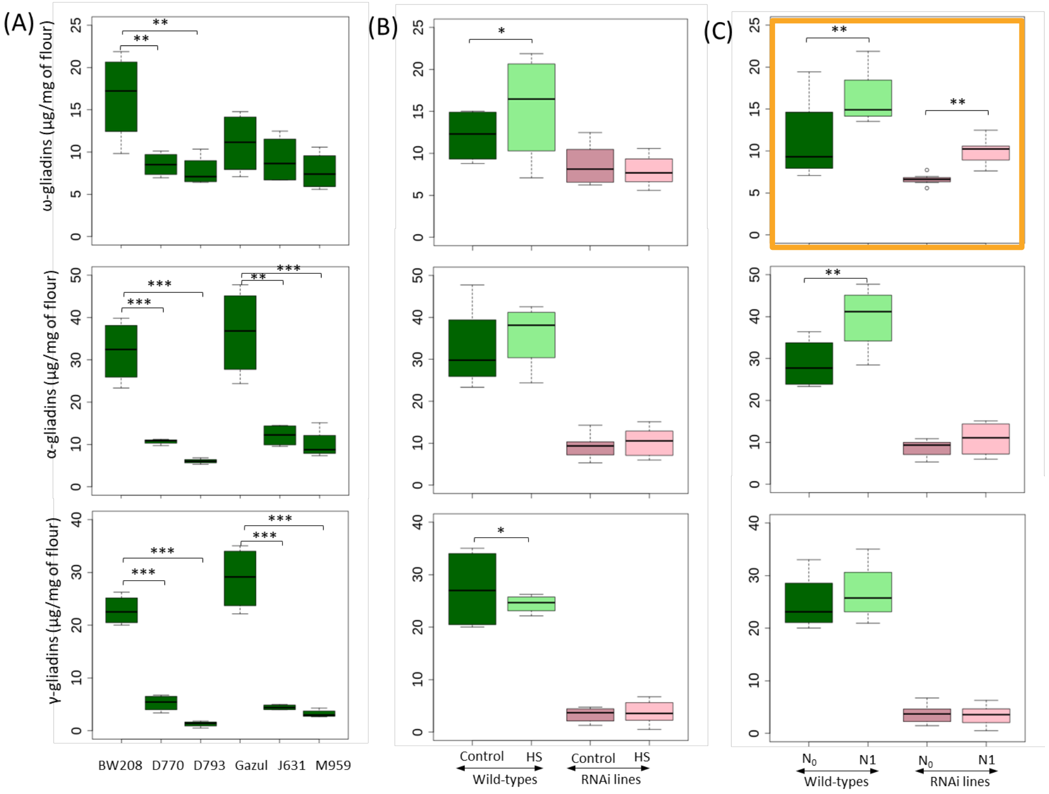


Figure 5. Content of ω-, α- and γ-gliadins under Heat stress (HS) and N availability. (A) Comparisons between RNAi lines and their wild-type by Dunnett's test, and for wild-types and RNAi lines under HS (B) and nitrogen availability (C). N_0: no N application after heading, N_1, N application after heading; control: 25/18ºC during whole grain filling period, HS: 40/18 ºC for ten days during grain filling period. The black line represents the median value. * above the bars indicates significant difference (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$) between treatments according to the variance analysis.

Análisis PCA

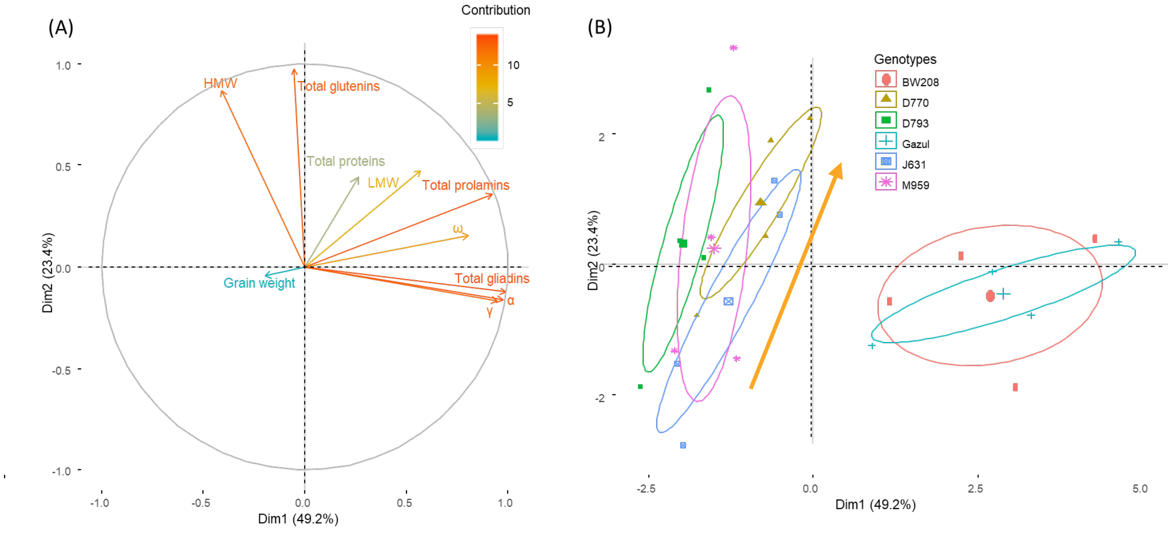


Figure 6. Principal Components Analysis (PCA). Effect of genotypes, temperature and N availability treatments on the variation of the protein fractions and grain weight **(A)**. The high values in the color scale indicates a high contribution to the PCA. The direction and the size of the vectors indicate the relationship between all variables and their contribution to each axis. **(B)** Individuals are represented on the PCA axes with the 95% confidence ellipses showed for each genotype. The largest point for each genotype indicates the intersection of ellipse axes.

Conclusiones

- Las líneas RNAi **responden** como sus líneas control al tratamiento de **N** para las **ω-gliadinas**.
- Las **γ-gliadinas** y **α-gliadinas**, la fracción más inmunogénica, **no responden** en las líneas RNAi ante ninguno de los tratamientos.

frontiers in Plant Science
ORIGINAL RESEARCH
published: 20 March 2020
doi: 10.3389/fpls.2020.00314

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Prolamin Content and Grain Weight in RNAi Silenced Wheat Lines Under Different Conditions of Temperature and Nitrogen Availability

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Temperature and nitrogen (N) availability are two important environmental factors that may produce important changes in grain composition during grain filling of bread wheat. In this study, four wheat lines with the down-regulation of gliadins by means of RNA interference (RNAi) have been characterized to determine the effect of thermal stress and N availability on grain weight and quality; with focus on gliadin and glutenin protein fractions. Grain weight was reduced with heat stress (HS) in all RNAi lines, whereas gliadin content was increased in the wild-types. With respect to gliadin content, RNAi lines responded to HS and N availability differently from their respective wild-types, except for ω-gliadin content, indicating a very clear stability of silencing under different environmental conditions. In a context of increased temperature and HS events, and in environments with different N availability, the RNAi lines with down-regulated gliadins seem well suited for the production of wheat grain with low gliadin content.

Keywords: gluten proteins, heat stress, transgenic lines, celiac disease, *Triticum aestivum*

Muchas gracias por su atención

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Front. Plant Sci., 20 March 2020 | https://doi.org/10.3389/fpls.2020.00314