Impact of Planting Dates and Cultivars on the Expression of HMW-GS, LMW-GS Genes and Gluten Quality in Wheat

Abstract

A field experiment was conducted for the seasons 2022 and 2023 in Kerbala Governorate to determine the effect of some wheat varieties and planting dates on gluten protein levels, wheat quality, and expression of genes responsible for low and high molecular weight glutenin units (LMW-GS and HMW-GS).The experiment was implemented according to a randomized complete block design (RCBD) according to the split plot arrangement, as the main plots included three planting dates (November 15, December 1, and December 15) and were symbolized by the symbols (D1, D2, and D3, respectively), while the sub-plots included four wheat varieties (Latifiya, Ibaa 99, Tamuz, and Rashid).Using qPCR at the second planting date (December 15th), we investigated gene expression and discovered that the "Ibaa 99" variety displayed the greatest levels of gene expression for both LMW-GS and HMW-GS while the "Rashid" variety displayed the lowest levels. With LMW-GS levels of 17.5 ± 1.7 mg/g and HMW-GS levels roughly 15.5 ± 1.4 mg/g, "Ibaa 99" also reported the highest gluten protein content on the same planting date. Between gene expression, protein synthesis, and gluten quality, our study revealed a substantial favorable connection (r = 0.95–0.99). Gene expression therefore raises protein synthesis and gluten quality. Because of its excellent day length and mild temperatures, second planting date—December 15th—seemed suitable for gene expression and protein synthesis. These results highlight the significance of timing planting to raise wheat yield and quality as well as of employing high-gene-expression wheat varieties such as "Ibaa 99." This paper shows how choice of planting date and type improves wheat quality and yield. Selecting high-performance varieties and matching planting times to environmental conditions will let researchers and farmers increase wheat yields and quality. These findings provide guidance for improving agricultural methods in many different settings.

Keywords: Wheat (*Triticum aestivum* L.), Gene expression, Gluten proteins (HMW-GS and LMW-GS), Planting dates, Cultivars

UNDER PEER REVIEW

Introduction

Scientifically known as *Triticum aestivum* L., wheat is a foundation of world food security as it provides all around essential nutrients and protein. Greatly influences the quality of wheat is gluten, a complex mixture of proteins influencing the viscosity and elasticity of a dough. These traits determine whether flour is suitable for bread and other baked products (Filip et al,. 2023). Glutenin subunits (Sun et al., 2024) most either low or low molecular weight (LMW-GS) or high molecular weight (HMW-GS). Interactions among these proteins dictate most of the structure and use of gluten. Important actors in wheat quality are genes coding for LMW-GS and HMW-GS. Although genes mostly determine gene expression, environmental variables and agricultural methods including the cultivar choice and planting dates also affect it (Hu et al., 2020). Researchers have found environmental elements influencing the expression of these fundamental genes include temperature, humidity, and day length—which change depending on planting dates. According to Zhao et al. (2023), the change in temperature influences the synthesis of HMW-GS, therefore influencing the quality of gluten. Moreover, different types of wheat might respond differently to their environment. This suggests that various cultivars express genes linked to gluten production quite differently. Balouchi's 2010 study revealed how direct effect of genetic variants across wheat cultivars influences the quantity and quality of gluten proteins synthesized. Especially places with notable temperature variations demonstrate this difference. Gluten quality determines the worth of wheat largely as the food industry depends mostly on its properties to produce premium products. Knowing the ideal planting dates and cultivars helps farmers boost productivity and quality, therefore benefiting the more general agricultural sector. Moreover stressed by Dai et al. (2023) is this essential need. Many studies show that environmental factors obviously alter the expression of LMW-GS and HMW-GS genes. For example, high temperatures have been shown to limit the development of HMW-GS, therefore compromising the quality of gluten. Yang et al. (2022) underlined the significance of selecting appropriate wheat varieties to get optimal gluten quality by means of a research demonstrating how different wheat varieties react to different environmental conditions.

One of the main determinants of the quality and volume of the harvest is definitely the dates of wheat planting. A balance between the plant's demands for ideal environmental conditions including temperature, humidity and illumination, and the several physiological phases of wheat development depends on the timing of planting. Timing wheat planting guarantees the exploitation of the ideal climatic conditions during the growth season, thereby improving the plant's capacity to photosynthesize and attain the maximum performance in the process of nutrient absorption (Abbas et al., 2019). Therefore, choosing the suitable dates for planting wheat depends on accurate knowledge of the needs of the cultivated variety, the dominant climatic conditions in the region, and the type of soil, which helps to achieve a balance between quantity and quality to obtain a profitable and sustainable economic crop (Alamgeer et al., 2022).

This work aims to improve understanding of how the choice of cultivar and planting times affects the expression of HMW-GS and LMW-GS genes in wheat, therefore affecting gluten quality. The outcomes of this effort will provide farmers with practical knowledge on how to maximize planting times and cultivar choice, thereby boosting the quality, nutritional value, and financial worth of their wheat crops. Moreover, given the complex interaction of environmental and genetic components, we want to contribute to the development of more ecologically friendly agricultural practices.

This study thus aims to investigate the effect of different planting dates on the expression of LMW-GS and HMW-GS genes in wheat, together with the choice of different cultivars. We want to pinpoint the ideal cultivars and planting seasons to improve gluten quality. Over several planting seasons, we will do field experiments involving several wheat types. Using PCR and qPCR molecular biology approaches, we will track environmental elements and quantify gene expression. Moreover, we will explore how variations in gene expression affect gluten quality by means of protein analysis—including gluten—using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and Methods

We carried out a field experiment on one Kerbala Governorate farm during the 2022–2023 growing season. The main actors in gluten synthesis in wheat (*Triticum aestivum* L.), hence we aimed to investigate how different planting dates and different wheat types impact the expression of HMW-GS and LMW-GS genes. We planned the experiment utilizing a randomized complete block design (RCBD) with a split-plot layout and three replicas in order to guarantee consistent findings.

UNDER PEER REVIEW

Wheat varieties served as the sub-plot element while planting dates dominated our experiment's design. Three distinct planting dates were specifically D1 (the first date, November 15th), D2 (the second day, December 1st), and D3 (the third date, December 15th). Regarding the kinds of wheat, we selected four different cultivars: Latifiya, a modern cultivar with good gluten quality; Ibaa 99, a modern cultivar recognized for its high yield; Tamuz, a local variety lauded for its high yield; and Rashid, a cultivar noted for resistance to numerous fungal diseases.

Field preparation was accomplished with conventional agricultural techniques. We split the ground into different experimental plots after first plowing and leveling it. Every one of our three planting dates was allocated to a main plot, which was subsequently split into four sub-plots to enable the random distribution of our four various wheat varieties. We three times repeated every therapy to guarantee the accuracy of our findings.

Using verified seeds of the wheat varieties we had selected, we hand-rolled every seed. With 20 cm between rows and 5 cm between individual plants, we sowed the seeds at a pace of 120 kg per acre hoping to provide the greatest growth circumstances.

Controlling the Experiment: We closely controlled the trial using all advised farming methods. This comprised consistent irrigation, nitrogen and phosphate fertilizers used, and suitable pesticide management of pests and diseases. We also closely tracked environmental factors including temperature and humidity across the whole growth period.

Gathering leaf and spike samples from every treatment at various growth phases—especially during blooming and maturity—was very challenging. We promptly stored these samples in liquid nitrogen to guarantee their integrity and then shipped them to the lab for gene expression analysis. Standard extraction methods let us extract RNA from the samples in the lab. We then reverse transcriptase an RNA into complementary DNA (cDNA). Using specifically primers tailored to target the HMW-GS and LMW-GS genes, we measured their expression levels using quantitative polymerase chain reaction (qPCR). At last, we applied data analysis tool to compare the gene expression levels among the several treatments.

Strengthening gluten proteins: Using a specific buffer, we separated the mature wheat grains' gluten proteins. We next ran LMW-GS and HMW-GS proteins under sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine their relative concentrations. This allows us to compare the protein content among the numerous cultivars and planting seasons (Ma et al., 2019).

Statistical Analysis

Data were analyzed using the SAS statistical software (version 9.4). Analysis of variance (ANOVA) was applied to evaluate the effects of planting dates and cultivars on gene expression and gluten quality. The least significant difference (LSD) test was used to compare means at a significance level of 0.05 (Al-Rawi and Khalafallah, 2000).

Results and Discussion

We found some really notable variations in the relative gene expression of LMW-GS and HMW-GS genes across several wheat cultivars, during several planting seasons. Clearly, gene expression levels were much influenced by the kind of wheat as well as the planting timing. Over all planting seasons, the

UNDER PEER REVIEW

cultivar "Ibaa 99" routinely had the greatest gene expression for both LMW-GS and HMW-GS. We assessed LMW-GS expression at 2.10 ± 0.15 and HMW-GS at 2.00 ± 0.18 at the D2 planting date, for example. By comparison, the cultivar "Rashid" constantly showed the lowest gene expression levels during all the planting times. Its HMW-GS expression at the D3 planting date was 1.20 ± 0.09 whereas LMW-GS expression was 1.30 ± 0.08.

When we investigated how timing of planting affected gene expression, we discovered that, among D1 and D3, the D2 planting date (December 1st) regularly produced the greatest HMW-GS and LMW-GS gene expression. Consider the "Latifiya" variety: HMW-GS expression was 1.90 ± 0.16 at D2, LMW-GS expression was 2.00 ± 0.14. These values dropped, nevertheless, at D3 to 1.60 ± 0.12 and 1.70 ± 0.11 respectively (Table 1).

Genetic diversity of their HMW-GS and LMW-GS genes is probably responsible for the difference in gene expression among many cultivars. Given that "Ibaa 99" displayed such high gene expression levels relative to other cultivars, it appears that it could have stronger variants of these genes. Conversely, "Rashid" could be more sensitive to its surroundings or might simply lack some of the advantageous genetic features encouraging increased gene activation. Furthermore, the D2 planting date—December 1st—seem to be the ideal for gene expression. Long days and moderate temperatures at this period most probably produced perfect conditions for beginning gene expression. The D3 planting date most obviously lowered the activity of the enzymes required for gene transcription, therefore affecting the expression of genes, given the cooler temperatures and shorter days on December 15th. Still more fascinating were the relationships between the cultivars and planting seasons. This showed how differently depending on when they were grown specific cultivars react to environmental variables. For example, "Latifiya" appears to thrive under the ideal conditions in D2 as its gene expression was substantially higher than in D1 and D3. Day length, temperature, and humidity all influence the synthesis of HMW-GS and LMW-GS genes, as other investigations have indicated. The D2 planting date's mild temperatures most likely allow the enzymes involved in gene transcription to operate more effectively, hence raising gene expression. Reduced enzyme activity brought on by lower temperatures on the D3 planting date adversely affecting gene expression (Li et al., 2020) These results stress the need of combining suitable planting dates with high genetic potential types to increase the expression of HMW-GS and LMW-GS genes, therefore improving the gluten quality of wheat. The "Ibaa 99" variety seems to offer the best balance of robust gene expression and good gluten quality at the D2 planting date. Using these results to maximize farming techniques and choose suitable cultivars will enable researchers and farmers to raise wheat yield and quality under many different environmental conditions.

Table 1: Relative Gene Expression of HMW-GS and LMW-GS Genes Under the Influence of Planting Dates and Different Cultivars

Planting Date	Cultivar	HMW-GS Expression (Relative Units)	LMW-GS Expression (Relative Units)
D1	Tamuz	0.12 ± 1.45	0.10 ± 1.60
D1	Ibaa 99	0.15 ± 1.80	0.12 ± 1.75
D1	Rashid	0.10 ± 1.30	0.11 ± 1.50
D1	Latifiya	0.14 ± 1.70	0.13 ± 1.85
D2	Tamuz	0.13 ± 1.60	0.12 ± 1.70
D2	Ibaa 99	0.18 ± 2.00	0.15 ± 2.10

UNDER PEER REVIEW

D2	Rashid	0.11 ± 1.50	0.12 ± 1.65
D2	Latifiya	0.16 ± 1.90	0.14 ± 2.00
D3	Tamuz	0.10 ± 1.30	0.09 ± 1.40
D3	Ibaa 99	0.14 ± 1.70	0.13 ± 1.80
D3	Rashid	0.09 ± 1.20	0.08 ± 1.30
D3	Latifiya	1.60 ± 0.12	1.70 ± 0.11

Our study of protein levels found some striking variations in the quantity of gluten proteins (HMW-GS and LMW-GS) across many wheat cultivars and planting dates. Independent of planting date, it was abundantly evident that the "Ibaa 99" cultivar had the greatest gluten protein content. For instance, the second planting date saw LMW-GS reach 17.5 ± 1.7 mg/g and HMW-GS reach 15.5 ± 1.4 mg/g, therefore producing a total gluten concentration of 33.0 ± 2.3 mg/g. By contrast, the "Rashid" variety often displayed the lowest gluten protein levels. Its HMW-GS on the D3 planting date was 10.5 ± 1.0 mg/g and LMW-GS was 13.5 ± 1.3 mg/g, so combining just 24.0 ± 1.7 mg/g.

Fascinatingly, compared to the other two planting dates (D1 and D3), the second, December 1st, planting date regularly produced the greatest gluten protein levels. On the "Latifiya" cultivar, at the D2 planting date the LMW-GS concentration was 16.5 ± 1.6 mg/g and the HMW-GS concentration was 14.5 ± 1.5 mg/g. By the D3 planting date, these values had dropped accordingly to 13.0 ± 1.2 mg/g and 15.5 ± 1.5 mg/g. This highly implies that, on the D2 planting date, circumstances were ideal for the synthesis of gluten proteins (Table 2).

Variations in the genes coding for LMW-GS and HMW-GS help to mostly explain the variable levels of gluten proteins seen in various cultivars. The "Ibaa 99" cultivar seems to have more efficient forms of these genes, which would help to explain why its protein levels were far greater than those of other types. Conversely, the "Rashid" cultivar exhibited the lowest gluten protein levels, maybe because it is more susceptible to environmental changes or because fewer efficient gene versions are involved. Furthermore, the second planting date, December 1st, had the maximum gluten protein accumulation most likely due to the perfect environment for protein synthesis: namely, mild temperatures and longer days. As seen in past experiments, the lower temperatures and less hours of sunshine at the D3 planting date (December 15) seem to have adversely damaged gluten quality and reduced protein accumulation (Qiu et al., 2022).

Furthermore, indicating that they react differently to the same environmental conditions is a statistically significant interaction between planting dates and cultivars. For instance, the "Latifiya" variety flourished in the circumstances supplied by D2 as it displayed somewhat greater amounts of gluten proteins at D2 compared to D1 and D3. Strong scientific data point to environmental elements like temperature, humidity, and day duration as major determinants of gluten protein synthesis. Moderate temperatures most likely encouraged best activity for the enzymes in charge of protein synthesis on the D2 planting date, therefore generating higher protein accumulation. On the D3 planting date, the lower temperatures most likely decreased enzyme activity, which would have detrimental effects on protein accumulation (Naeem et al., 2012).
Finally, our results show that wheat quality may be improved by means of optimal planting date paired with cultivars with great genetic efficiency, hence boosting gluten protein buildup. Especially the D2 planting date and the "Ibaa 99" cultivar grabbed out as the ideal combo for achieving the best gluten

UNDER PEER REVIEW

protein concentrations and outstanding quality generally. This data offers perceptive study of how farmers and academics could enhance agricultural techniques and select the finest cultivars to increase wheat yields and quality under many scenarios.

Table 2: Gluten Protein Concentrations (mg/g) Under the Influence of Planting Dates and Different Cultivars

Planting Date	Cultivar	HMW-GS Concentration (mg/g)	LMW-GS Concentration (mg/g)	Total Gluten (mg/g)
D1	Tamuz	1.2 ± 12.5	1.5 ± 15.0	2.0 ± 27.5
D1	Ibaa 99	1.3 ± 14.0	1.6 ± 16.5	2.2 ± 30.5
D1	Rashid	1.0 ± 11.0	1.4 ± 14.0	1.8 ± 25.0
D1	Latifiya	1.3 ± 13.5	1.5 ± 16.0	2.1 ± 29.5
D2	Tamuz	1.2 ± 13.0	1.5 ± 15.5	2.1 ± 28.5
D2	Ibaa 99	1.4 ± 15.5	1.7 ± 17.5	2.3 ± 33.0
D2	Rashid	1.1 ± 12.0	1.4 ± 14.5	1.9 ± 26.5
D2	Latifiya	1.3 ± 14.5	1.6 ± 16.5	2.2 ± 31.0
D3	Tamuz	1.1 ± 11.5	1.4 ± 14.5	1.8 ± 26.0
D3	Ibaa 99	1.2 ± 13.5	1.5 ± 16.0	2.1 ± 29.5
D3	Rashid	1.0 ± 10.5	1.3 ± 13.5	1.7 ± 24.0
D3	Latifiya	13.0 ± 1.2	15.5 ± 1.5	28.5 ± 2.0

Using Pearson correlation coefficients, our investigation exposed some really strong and positive relationships between many elements: total gluten content, gluten protein concentrations, and LMW-GS gene expression. The concentration of HMW-GS proteins and their corresponding gene expression levels clearly and strongly correlated (r = 0.98). We also observed a pretty strong positive link between the concentration of LMW-GS proteins and their corresponding gene expression with a correlation value of r = 0.97. This data supports what Rai and Han (2023) suggested: the frequency of expression of these genes seems to directly affect the generated protein amount.

Furthermore, the expression of HMW-GS genes amply demonstrated a substantial positive correlation (r = 0.96) with the general gluten content. Additionally present are similar positive relationships between the expression of LMW-GS genes and the overall gluten content (r = 0.95). Roy et al. (2018) stress these results, which highlight even more how important the expression levels of many genes are for determining both the quality and amount of gluten in wheat. Having correlation values of 0.99 and 0.98 respectively (Table 3), we also found relatively strong positive correlations between total gluten content and the concentrations of LMW-GS and HMW-GS proteins. This makes obvious since larger total gluten levels simply follow from higher protein concentrations. These findings particularly emphasize the crucial role LMW-GS and HMW-GS proteins perform in defining overall wheat quality (Mao et al., 2013).

UNDER PEER REVIEW

Ultimately, our results support further evidence for the hypothesis that two key factors considerably influence the quality of wheat: the expression of LMW-GS and HMW-GS genes as well as the quantity of gluten proteins. Knowing these important links enables us to choose cultivars with high gene expression and begin to improve agricultural techniques, therefore improving general quality as well as wheat output.

Table 3: Pearson Correlation Coefficients Between Gene Expression of HMW-GS and LMW-GS, Gluten Protein Concentrations, and Total Gluten Content

Variable 1	Variable 2	Correlation Coefficient (r)	Interpretation
HMW-GS Gene Expression	HMW-GS Concentration	0.98	Very strong positive relationship
LMW-GS Gene Expression	LMW-GS Concentration	0.97	Very strong positive relationship
HMW-GS Gene Expression	Total Gluten Content	0.96	Strong positive relationship
LMW-GS Gene Expression	Total Gluten Content	0.95	Strong positive relationship
HMW-GS Concentration	Total Gluten Content	0.99	Very strong positive relationship
LMW-GS Concentration	Total Gluten Content	0.98	Very strong positive relationship

UNDER PEER REVIEW

Conclusions

Our findings amply show a strong positive association between gluten protein levels and the expression of LMW-GS and HMW-GS genes. This clearly implies that an elevated concentration of these crucial proteins results from enhanced gene expression directly. Most importantly, our results also show that the total gluten concentration is much influenced by the amounts of HMW-GS and LMW-GS proteins, thereby underlining their indispensable importance in deciding general wheat quality. These findings essentially offer a strong justification for choosing wheat cultivars with high gene expression for these important genes, therefore improving wheat quality, raising productivity, and improving its nutritional content.

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UNDER PEER REVIEW

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UNDER PEER REVIEW