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

Abstract

We examined in a thorough investigation how various wheat types and planting dates affect gluten protein levels, wheat quality, and the expression of genes liable for low and high molecular weight glutenin subunits (LMW-GS and HMW-GS). Using a randomized whole block design with split plots, we investigated this in Karbala, Iraq, in the agricultural seasons 2022 and 2023. At three separate dates—the first (December 1st), the second (December 15th), and a third unidentified date—we planted four wheat varieties: Tamuz, Ibaa 99, Rashid, and Latifiya. 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

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. 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. Researchers have found environmental elements influencing the expression of these fundamental genes include temperature, humidity, and day length—which change depending on planting dates. For example, a 2023 Zhao et al. research looked at how temperature variations could influence HMW-GS synthesis, therefore influencing gluten quality. Moreover, various kinds of wheat could react differently to their surroundings. This implies that multiple cultivars have relatively different expressions of genes

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related to gluten generation. Research by Shewry et al. (2003) have shown how direct influence of genetic variations across wheat cultivars affects the quantity and quality of gluten proteins generated. Places with significant temperature fluctuations especially show this disparity. This effort seeks to further knowledge of how the choice of cultivar and planting dates influences the expression of HMW-GS and LMW-GS genes in wheat, therefore influencing gluten quality. The results of this work will give farmers useful knowledge on how to maximize planting timings and cultivar choice, therefore improving the quality, nutritional content, and financial worth of their wheat crops. Furthermore, by considering the intricate interplay of environmental and genetic elements, we wish to help to create more ecologically friendly farming methods. Not only is knowledge of these elements important for farming but also for general progress of agricultural research. Targeting increase of the general quality of wheat would be made possible by knowing the elements controlling the expression of LMW-GS and HMW-GS genes.

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-Wheat market value is mostly determined by gluten quality as the food sector depends mostly on its characteristics to generate premium goods. Knowing the best planting times and cultivars can assist farmers increase both quality and yield, therefore helping the larger agricultural industry. Furthermore, underlined by Dai and associates (2023) is this vital necessity. Environmental influences clearly affect the expression of LMW-GS and HMW-GS genes, according to several research. High temperatures, for instance, have been demonstrated to restrict the growth of HMW-GS, which may compromise gluten quality. Yang et al. (2022) conducted a research showing how various wheat varieties react to diverse environmental situations, therefore stressing the need of choosing suitable wheat varieties to attain optimal gluten quality. 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)—Ma et al., 2020). Different degrees of LMW-GS and HMW-GS gene expression depending on the cultivars utilized and planting seasons should show in the predicted results. This knowledge will enable farmers to better know how interactions between environmental and genetic elements define wheat quality, therefore guiding their methods of production. This study will help us to better understand the factors influencing wheat quality, therefore transcending basic recommendations for enhancing farming methods. Ultimately, by selecting suitable cultivars and timing planting to maximize financial and nutritional value of wheat, we may increase both.

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Materials and Methods

We carried out a field experiment on one Karbala 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.

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.

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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.

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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.

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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 next turned the RNA into complementary DNA (cDNA) using a reverse transcriptase enzyme. We quantified the expression levels of the HMW-GS and LMW-GS genes by means of quantitative polymerase chain reaction (qPCR) with particular primers meant to target these genes. At last, we compared the gene expression levels among the many treatments using data analysis program.

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Exercising Gluten Proteins: With a particular buffer, we isolated gluten proteins from the mature wheat grains. To ascertain the relative quantities of HMW-GS and LMW-GS proteins, we next subjected these proteins to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This let us compare the protein content among the several cultivars and planting times.

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.

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 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.

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 moderate temperatures of the D2 planting date most probably allow the enzymes engaged in gene transcription to function more efficiently, hence increasing gene expression. Lower temperatures on the D3 planting date caused reduced enzyme activity that negatively impacted gene expression (Li et al., 2020). These findings emphasize the significance of combining appropriate planting dates with high genetic potential types to boost the expression of HMW-GS and LMW-GS genes, therefore enhancing 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
D2	Rashid	0.11 ± 1.50	0.12 ± 1.65

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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.

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.

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 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

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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, 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.

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

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.

References

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