Responses of Yield and Protein Composition of Wheat to Climate Change

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Abstract: Global wheat demand is expected to continue to increase due to the projected increase in the World’s population but regrettably, wheat yield is expected to decrease due to the progressively changing climate. Although the effects of temperature, soil moisture and nutrient absorption on the yield of wheat have been studied extensively to address the threats posed by climate change on food security, the combined effects of these factors have been studied to a lesser extent. This study thus aims to investigate the interactive effects of different regimes of fertilizer and soil moisture on the yield and amino acid composition of wheat. Twelve treatments under different regimens of soil moisture and fertilizer, replicated ten times in a randomized block design were considered in the greenhouse and in the field. The study reveals that variation in each factor had a significant effect on wheat but soil moisture was the principal factor controlling yield and protein accumulation. Application of organic fertilizer to wheat increased amino acid accumulation when the average temperature was at 18 °C, with minimum temperature (Tmin) and maximum temperature (Tmax) of −6 °C and 42 °C respectively. However, application of inorganic fertilizer to wheat enhanced amino acid accumulation when the average daily temperature was at 8 °C, with Tmin and Tmax of −10 °C and 26 °C respectively. Our results also show that a decrease in soil moisture from 100% to 30% in the greenhouse improved the quantity of amino acid in the grain by 26.4% and 56.8% for organic and inorganic treatments respectively. Also, grain amino acid concentration increased by 16.6% and 4.76% when soil moisture dropped from 100% to 30% for the organic and inorganic treatments in the field respectively.

Keywords: wheat; nutrient; soil moisture content; temperature; yield; climate change

1. Introduction

Wheat (Triticum spp) is among the first domesticated food crops in the world and it is known to be an important source of protein. According to [1], global wheat consumption is projected to increase by 13% between 2017 and 2027. Consumption in Sub Saharan Africa is also increasing rapidly and faster than for any other major cereal and it is expected to increase at an even faster rate from 770,000 MT to 1.28 million MT per year between 2020 and 2030 [2]. Regrettably, the progressively changing climate has become a major threat to wheat production and food security [3,4] particularly in sub-Saharan Africa which has been hit the most by climate change, but also at the global level [5]. Thus, wheat yields can be expected to decline markedly [6,7] without the development of suitable management practice for wheat.

Water unavailability, poor crop nutrition and the use of varieties with low yield potential are other factors that threaten wheat production [8,9]. Many empirical studies have confirmed that for
a particular level of fertility, a reduced amount of soil moisture is associated with an increase in nitrogen content of the plant tissue and a decrease in potassium content [10–13]. Temperature is another factor that plays an important role in wheat production. High temperature would require adequate soil moisture content due to high rate of evapotranspiration. At a very low temperature, the plant root is cooled and the nutrient uptake capacity of the plant is reduced compared to warmer roots. Referring to [14], the time of high temperature during grain-filling is very important, and has a specific effect on grain quality through the accumulation of protein in the wheat grain.

However, the collective effect of irrigation along with nutrition and variety can play a vital role in increasing wheat productivity [15]. The combined use of nitrogen, phosphorus and potassium (NPK) fertilizers can play an important role in increasing yield and quality of wheat. Even though there have been numerous studies on the influence of temperature, fertilizer and soil moisture on growth and yield of wheat, we are not aware of any studies that attempt to combine different regimens of all three factors in one experiment. Reference [16] investigated the effects of increased fertilizer application to wheat under high temperature without any explanation on how different levels of soil moisture could affect yield. References [17–19] also focused on the combined effects of soil moisture content and fertilizer on wheat without recognizing the additional role of temperature on yield.

Moreover, most studies comparing the response of wheat to the combined effects of water and moisture do not analyze different regimes of all two factors in a single experiment [20,21]. Consequently, the objective of this work is to investigate the relative importance of wheat performance under different regimes of fertilizer and soil moisture in the greenhouse and field, in order to understand the interactive effects of these factors on the yield and amino acid content of wheat.

2. Materials and Methods

2.1. Experimental Site

The study was conducted at the experimental site of the College of Agriculture and Life Sciences, Kyungpook National University in South Korea, to evaluate the effects of fertilizer and soil moisture on the growth of “Baegjoong” wheat cultivar in the greenhouse and field conditions.

2.2. Treatments Application and Planting

Plants were subjected to different regimes of fertilizer and soil moisture in the greenhouse covered with polyethylene film, and in the field: (1) Fertilizer: organic and inorganic fertilizers; (2) Water: 30%, 60% and 100% moisture levels. The details of the treatment application are presented in Table 1.

Table 1. Treatments application.

| Treatment | Growth Condition | Fertilizer Type | Soil Moisture Content (%) |
|-----------|-----------------|----------------|--------------------------|
|           | Greenhouse      | Field          | Organic | Inorganic | 30 | 60 | 100 |
| T1        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T2        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T3        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T4        | ✓               | ✓              | ✓       | ✓         | ✓  | ✓  |     |
| T5        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T6        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T7        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T8        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T9        | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T10       | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T11       | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
| T12       | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
|           | ✓               | ✓              | ✓       | ✓         | ✓  |    |     |
The greenhouse treatment was performed under a controlled temperature regime (Figure 1), while the field treatment was performed under ordinary daily temperature (Figure 2). Tmin (minimum temperature) and Tmax (maximum temperature) from sowing to the start of heading were at –6 °C and 26 °C respectively. From heading to physiological maturity, Tmin and Tmax in the greenhouse were at –6 °C and 42 °C respectively. Tmax at 42 °C was maintained throughout the heading, anthesis, and physiological maturity stages. Tmin and Tmax before the heading stage in the field were at –10 °C and 15 °C respectively. Tmin increased to –3 °C, 8 °C and 13 °C throughout the heading, anthesis and maturity stages, respectively. Tmax also increased to 17 °C, 19 °C and 26 °C during the heading, anthesis and maturity stages, respectively.

Figure 1. Minimum and maximum temperature in the greenhouse with respect to day of measurement. GH: Greenhouse.

Figure 2. Minimum and maximum temperature in the field with respect to day of measurement.
A range of nitrogen (1.52 g), phosphorus (3.53 g) and potassium (0.75 g) fertilizer was applied per pot. Full doses of phosphorus, potassium and half dose of nitrogen (0.76 g) were applied per pot before sowing for the inorganic treatment, and the remaining half dose of N (0.76 g) was then top dressed at heading stage. Three hundred and fifty g of compost organic fertilizer (1.31% N, 1.94% P and 1.99% K) containing 70% wood, 25% plant and 5% fecal matter was also applied per pot once before planting for the organic treatment.

Twelve treatments replicated ten times were considered in this experiment (Table 1). Four wheat seeds were sown (15 cm apart × 2 cm deep) per pot (30 cm diameter × 20 cm deep) with two seeds per hole. Pots spacing within the rows was at 30 cm to allow for stress-free movement during water treatment and easy collection of data. After planting, all pots were treated with 100% soil moisture content but not waterlogged until the day of three leaflets. Treating all pots at 100% moisture was to ensure proper germination of the seed as this stage is one of the most important life-stages of a plant. Plants were then watered twice daily at 6:00 am and 6:00 pm according to our treatments until the research was terminated. Soil moisture levels were measured each time before irrigation using a soil moisture sensor (WET Sensor Kit WET-2-K1) to determine how much water is required by each pot.

2.3. Experimental Design

The pots were arranged in a randomized complete block design (RCBD) with 10 single-treatment replications.

2.4. Measurement of Temperature and Yield Components

Air temperature in the greenhouse and field conditions was recorded at two days intervals using a thermometer. Max, min and daily average temperatures (Figures 1–3) were determined for each day of measurement using the Six’s Maximum and Minimum thermometer and a data logger. Plant height was measured from the base of the pot with a line gauge and tiller number per hole was also counted. We counted the tiller number per hole because we could not differentiate the stems of the two seeds that were sown in each hole. Records on plant height and tiller number were taken after every ten days from the day of seedling until growth stopped. At physiological maturity, plants were then off-rooted and weighed using an electronic scale (All-in-1 Series, ACCUTECK). Three panicles per pot with a total of thirty panicles per treatment were randomly chosen for the measurement of panicle length. The average length was then calculated to have the panicle length for each treatment. Panicles were then rumpled gently, and records on the number of grains panicle$^{-1}$ and grain weight panicle$^{-1}$ for each treatment were collected. The yield was then calculated by measuring the grain weight plant$^{-1}$ for each treatment.
2.5. Amino Acid Analysis

The concentration of free amino acids was determined by an amino acid analyzer (L8900, Hitachi, Tokyo, Japan). The following steps were involved: 1 milliliter of 6N HCl was poured into 0.05 g of sample in the glass vial. The sample was then charged with nitrogen gas to remove air and then tightly capped. The sample was hydrolyzed at 110 °C for 24 h. After hydrolysis, the sample was allowed to cool for 30 min. The cap was then opened and the sample dried at 80 °C for 24 h. After cooling for 30 min, 1 milliliter of 0.02N HCl was added to the sample in the vial and then extracted by sonication-vortex method. The ample was filtered by 0.45 µm cellulose acetate membrane filter and then diluted with 0.02N HCl after filtration. The separated amino acids were reacted with ninhydrin acids and detected at 570 nm except for proline, which was detected at 440 nm.

2.6. Analysis of Variance (ANOVA)

The collected data was analyzed statistically by the analysis of variance technique using SPSS version 22. T-test for equality of means was used for data with two factors. For experiments that require the evaluation of all possible pairs treatment means, the Duncan Multiple Range Test (DMRT) test is recommended [22]. DMRT test was applied for data with more than two factors at 5% probability level, to test the significance of treatments means. $P < 0.05$ was taken to indicate statistical significance.

3. Results and Discussion

3.1. Organic and Inorganic Fertilizers

In Table 2, all the data from greenhouse and field were pooled. The result showed that fertilizer type had no significant impact on plant height, tiller number hole$^{-1}$, plant weight, panicle length and the number of grains panicle$^{-1}$ but a significant impact on grain weight panicle$^{-1}$, grain yield plant$^{-1}$ and harvest index at 5% probability level.

Inorganic fertilizer produced a higher plant height (73.60 cm), tiller number hole$^{-1}$ (11), plant weight (17.30 g), panicle length (7.22 cm), number of grains panicle$^{-1}$(29.3), grain weight panicle$^{-1}$ (1.1 g), grain yield plant$^{-1}$ (6.29 g) and harvest index (37%) than the organic fertilizer (73.55 cm, 10, 16.55 g, 7.16 cm, 25.8, 1.0, 4.81 g and 30%, respectively). This is because inorganic fertilizers are known to have the uniqueness of fast release of their nutrient contents. The remaining half dose of nitrogen (0.76 g) that was top-dressed at the heading stage significantly produced a longer
panicle length for the inorganic treatment. The longer panicle length in turn increased the number of grains panicle$^{-1}$ and grain weight panicle$^{-1}$. According to [23], the release of nutrients from organic fertilizer is slow compared to inorganic fertilizer. Consequently, nutrients were not readily available throughout the growing period for organic treatment. Also, leaching possibly might have occurred since organic fertilizer was applied only one time before planting. Although the yield plant$^{-1}$ for the inorganic treatment was 13.3% better than that for the organic treatment, the continuous use of these fertilizers can however cause a decrease in crop yield over time. Excessive use of chemical fertilizer can cause serious soil degradation through nitrogen leaching and reduction in soil organic matter, and consequently decrease in crop yield over time [24]. Therefore, the combined use of chemical fertilizer and organic matter is recommended to enhance soil fertility and increase the yield of crops [25].

3.2. Fertilizer and Soil Moisture

It is prominent that adequate water in the soil allows efficient uptake of nutrients by the plant. According to [26], yield and yield components of plants in drying soil are reduced even in tolerant genotypes and soil moisture affects the growth of tillers, while grain yield depends on number of tillers, spike length, seed per ear and grain size. In Table 3, all the data from greenhouse and field were pooled. It was noted that tiller number amongst all yield components did not have a significant difference under different regimes of fertilizer and soil moisture at 5% probability level.

The highest plant height (79.0 g), tiller number hole$^{-1}$ (12), plant weight (21.49 g), panicle length (7.60 cm), number of grains panicle$^{-1}$ (38), grain weight panicle$^{-1}$(1.35 g), grain yield plant$^{-1}$ (8.07 g) and harvest index (38%) were observed in the treatment with inorganic fertilizer and 100% moisture content. Reference [27] also noticed that, the application of irrigation increased grain and straw yield, number of tiller and thousand kernel weights. Treatment with organic fertilizer and 30% moisture content had the lowest plant height (68.05 cm), tiller number hole$^{-1}$ (9), plant weight (11.98 g), number of grains panicle$^{-1}$ (2), grain weight panicle$^{-1}$(0.87 g), grain yield plant$^{-1}$ (3.09 g) and harvest index (28%). The lowest panicle length (6.78 cm) was observed in the treatment with inorganic fertilizer and 30% soil moisture. The poorest yield and yield components were noticed in the treatment with 30% soil moisture because plants experienced drought stress. Reference [28] observed this type of performance in their work.

Moreover, [29] pointed out that N fertilization reduces grain yield in unirrigated plots by decreasing the number of heads, apparently by increasing the moisture stress of the plant. Reference [30] also witnessed moisture stress and reduced dry matter production for plants receiving more N fertilizer under dryland conditions by anthesis. Consequently, the reduced yield and plant weight seen in the treatment with 30% soil moisture and inorganic N fertilization during the heading stage confirms previous studies. However, the yield plant$^{-1}$ for wheat with 30% soil moisture and inorganic fertilizer was better than wheat treated with 30% soil moisture and organic fertilizer. Grain yield plant$^{-1}$ for wheat treated under 100% soil moisture was 15.1% and 26.7% better than the yield for wheat treated under 60% and 30% moisture treatments, respectively.

3.3. Growth Condition, Fertilizer Type and Soil Moisture Content

This research revealed that the interactive effects of fertilizer and soil moisture were significant on yield and all yield components of wheat in the greenhouse and field at 5% probability level (Table 4). T6 produced the largest tiller number hole$^{-1}$ (15), plant height (82.00 cm), plant weight (26.46 g), grain weight panicle$^{-1}$(1.4 g) and grain yield plant$^{-1}$ (9.56 g) because during the germination, tillering and stem elongation stages, the average temperature in the greenhouse was at 10 °C, whereas the average temperature in the field was at 2.7 °C. Warming in the greenhouse made wheat to attain physiological maturity 15 days earlier than wheat in the field. Agreeing with [31], wheat grew faster under warming temperature during re-greening, and this advantage was maintained till maturity. T12 also produced the best panicle length (8.41 cm), number of grains panicle$^{-1}$ (41.5) and harvest index (40%) because at the time of panicle growth and grain filling stage, the temperature in the field had risen
to 19 °C, whereas the greenhouse had a temperature of 42 °C. According to [32], high temperatures during grain filling may reduce the grain growth period by shortening the duration of photosynthetic tissue. Since high temperatures shortened the duration of photosynthesis in our research, the amount of carbohydrate available for panicle growth was also reduced. This result tie with that of [14,33] also noted that the maximum temperature during the grain-filling stage for wheat lies between 33.4 and 37.4 °C. The results observed in T6 and T12 was also because the inorganic source of NPK fertilizer was readily released. Additionally, the readily available fertilizer was easily absorbed by plants treated with 100% soil moisture. The uptake of nutrients by plants in T6 enhanced growth of stems, tillering, grain weight and consequently, best yield plant$^{-1}$. T12 had the highest number of grains panicle$^{-1}$ because $T_{\text{max}}$ of 19 °C in the field was favorable for grain filling. This result ties with that of [33], who revealed that the optimal and maximum temperature required for grain filling should be 20 °C and 35 °C respectively. Reference [34] also noted in their study that, increase in grain number per ear is the result of fertilized spikes which is determined by moisture levels that influence nutrient uptake. Our result in T6 (largest plant height and tiller number hole$^{-1}$) is in line with that of [35].

T7 had the least tiller number hole$^{-1}$ (5), plant weight (8.02 g) and grain yield plant$^{-1}$ (2.48 g), whereas, T1 had the least grain weight panicle$^{-1}$ (0.85 g), number of grains panicle$^{-1}$ (17.2) and harvest index (23%). T4 and T10 also had the least panicle length (6.62 cm) and plant height (66.80 cm), respectively. Reference [36] also found the corresponding decrease in the number of tillers with dryland. The least plant weight (T7) and plant height (T10) for plant treated with 30% moisture content in the field were observed because the soil moisture content (30%) and $T_{\text{min}}$ of −10 °C at the early growth stage were not suitable for stem elongation. Referring to [37] temperature is a key factor that affects plant development and preceding studies [38] confirmed that, irrespective of how favorable light and moisture content may be, plant growth stops when the temperature drops below a certain minimum value. Further, wheat phenology and fertility of pollen grains during the reproductive phase are also affected by low temperature leading to the reduction in wheat yield [39]. The average field temperature at 2.7 °C was also low for the stem elongation and tillering stages. This consequently led to a reduced number of panicles plant$^{-1}$ and consequently a decrease in yield plant$^{-1}$. This confirms the work of [40] who stated that $T_{\text{min}}$ of 4 °C is required from sowing to emergence in wheat. T4 had the worst panicle length, while T1 had the least number of grains panicle$^{-1}$ and weight of grain panicle$^{-1}$. This is because both treatments had the lowermost soil moisture content (30%) and combined with rising temperature (42 °C), the efficiency of nutrient uptake was reduced at the heading stage. Although T12 produced the best panicle length, number of grains panicle$^{-1}$ and harvest index, the overall best yield plant$^{-1}$ was witnessed in T6. This is because $T_{\text{min}}$ (−4 °C to 6 °C) and $T_{\text{max}}$ (16 °C to 26 °C) during the early vegetative stage in T6 was suitable for tillering. This in turn produced the highest tiller number plant$^{-1}$, grain number panicle$^{-1}$, grain weight panicle$^{-1}$ and consequently the best yield plant$^{-1}$. 
### Table 2. Mean comparison of growth and yield components of wheat under different types of fertilizers.

| Treatment | Plant Height (cm) | Tiller Number Hole−1 | Plant Weight (g) | Panicle Length (cm) | Grain Number Panicle−1 | Grain Weight Panicle−1 (g) | Grain Yield Plant−1 (g) | Harvest Index (%) |
|-----------|-------------------|----------------------|------------------|--------------------|------------------------|----------------------------|-----------------------|------------------|
| Organic   | 73.55 a           | 10.00 a              | 16.55 a          | 7.16 a             | 25.80 a                | 1.00 b                     | 4.81 b                | 30.00 b          |
| Inorganic | 73.60 a           | 11.00 a              | 17.30 a          | 7.22 a             | 29.30 a                | 1.10 a                     | 6.29 a                | 36.00 a          |

Means followed by the same letter in a column are not significantly different at the 5% level based on T-test for equality of means.

### Table 3. Mean comparison of growth and yield components of wheat under different regimes of fertilizer and soil moisture.

| Treatment | Plant Height (cm) | Tiller Number Hole−1 | Plant Weight (g) | Panicle Length (cm) | Grain Number Panicle−1 | Grain Weight Panicle−1 (g) | Grain Yield Plant−1 (g) | Harvest Index (%) |
|-----------|-------------------|----------------------|------------------|--------------------|------------------------|----------------------------|-----------------------|------------------|
| O30       | 68.05 c           | 9.00 a               | 11.98 d          | 6.91 de            | 20.40 d                | 0.87 c                     | 3.09 c                | 26.00 c          |
| O60       | 74.05 b           | 11.00 a              | 17.17 bc         | 7.11 cd            | 24.50 cd               | 0.95 c                     | 4.92 bc               | 29.00 c          |
| O100      | 78.55 a           | 12.00 a              | 20.39 ab         | 7.46 ab            | 32.70 ab               | 1.20 b                     | 6.41 ab               | 31.00 bc         |
| I30       | 67.30 c           | 10.00 a              | 13.73 cd         | 6.78 e             | 22.40 cd               | 0.95 c                     | 4.56 bc               | 33.00 b          |
| I60       | 74.50 b           | 11.00 a              | 16.67 bc         | 7.29 bc            | 27.70 bc               | 1.10 b                     | 6.23 b                | 37.00 a          |
| I100      | 79.00 a           | 12.00 a              | 21.49 a          | 7.60 a             | 37.90 a                | 1.35 a                     | 8.07 a                | 38.00 a          |

Post Hoc Tests: Duncan multiple comparison. Values followed by the same letter in a column are not significantly different (p < 0.05). O30: Organic fertilizer + 30% soil moisture content; O60: Organic fertilizer + 60% soil moisture content; O100: Organic fertilizer + 100% soil moisture content; I30: Inorganic fertilizer + 30% soil moisture content; I60: Inorganic fertilizer + 60% soil moisture content; I100: Inorganic fertilizer + 100% soil moisture content.
Table 4. Summarized effects of temperature, fertilizer and soil moisture on yield attributes of wheat.

| Treatment | Plant Height (cm) | Tiller Number Hole$^{-1}$ | Plant Weight (g) | Panicle Length (cm) | Grain Number Panicle$^{-1}$ | Grain Weight Panicle$^{-1}$ (g) | Grain Yield Plant$^{-1}$ (g) | Harvest Index |
|-----------|------------------|----------------------|-----------------|---------------------|-----------------------------|---------------------------------|-----------------------------|---------------|
| T1        | 68.80 $^c$       | 13.00 $^b$           | 15.94 $^{de}$   | 7.07 $^{def}$       | 17.20 $^f$                  | 0.85 $^d$                        | 3.72 $^{fgh}$               | 23.00 $^e$    |
| T2        | 75.20 $^b$       | 14.00 $^{ab}$        | 21.65 $^b$      | 7.034 $^{ef}$       | 21.00 $^{ef}$               | 0.96 $^{bcd}$                    | 5.65 $^{de}$                | 26.00 $^{de}$ |
| T3        | 81.30 $^a$       | 15.00 $^a$           | 25.88 $^a$      | 7.54 $^{bc}$        | 29.20 $^{cd}$               | 1.28 $^a$                        | 7.15 $^{bc}$                | 28.00 $^{cde}$|
| T4        | 67.80 $^c$       | 14.00 $^{ab}$        | 19.25 $^{bc}$   | 6.62 $^{g}$         | 17.80 $^f$                  | 0.93 $^{cd}$                     | 6.13 $^{cd}$                | 32.00 $^{bcd}$|
| T5        | 76.20 $^b$       | 14.00 $^{ab}$        | 20.87 $^b$      | 6.71 $^{fg}$        | 25.20 $^{de}$               | 1.09 $^{bc}$                     | 7.47 $^b$                   | 36.00 $^{ab}$ |
| T6        | 82.00 $^a$       | 15.00 $^a$           | 26.46 $^a$      | 6.80 $^{efg}$       | 34.20 $^{bc}$               | 1.40 $^a$                        | 9.56 $^a$                   | 36.00 $^{ab}$ |
| T7        | 67.30 $^c$       | 5.00 $^e$            | 8.02 $^e$       | 6.74 $^{fg}$        | 23.70 $^{def}$              | 0.90 $^d$                        | 2.48 $^h$                   | 31.00 $^{bde}$|
| T8        | 72.90 $^b$       | 7.00 $^{cd}$         | 12.90 $^{ef}$   | 7.18 $^{cde}$       | 28.00 $^{cd}$               | 0.94 $^{bcd}$                    | 4.20 $^{fg}$                | 33.00 $^{abc}$|
| T9        | 75.80 $^b$       | 8.90 $^c$            | 14.91 $^{def}$  | 7.38 $^{cd}$        | 36.10 $^{ab}$               | 1.11 $^b$                        | 5.67 $^{de}$                | 38.00 $^{ab}$ |
| T10       | 66.80 $^c$       | 5.90 $^{de}$         | 8.21 $^g$       | 6.94 $^{efg}$       | 27.00 $^{de}$               | 0.96 $^{bcd}$                    | 2.95 $^{gb}$                | 36.00 $^{ab}$ |
| T11       | 72.80 $^b$       | 7.00 $^{cd}$         | 12.47 $^f$      | 7.87 $^b$           | 30.20 $^{bcd}$              | 1.11 $^b$                        | 4.67 $^{ef}$                | 37.00 $^{ab}$ |
| T12       | 76.00 $^b$       | 8.00 $^c$            | 16.52 $^{cd}$   | 8.41 $^a$           | 41.50 $^a$                  | 1.30 $^a$                        | 6.58 $^{bcd}$               | 40.00 $^a$    |

Post Hoc Tests: Duncan multiple comparison. Values followed by the same letter in a column are not significantly different ($p < 0.05$).
3.4. Amino Acid Content of Wheat Grain

The amino acid composition of wheat was intensively affected by different regimes of fertilizer and soil moisture. Wheat treated with T10 and T6 had the best and least total amino acid content respectively (Table 5).

Table 5. Amino acid composition of wheat grain under different regimes of temperature, fertilizer type and soil moisture content.

| Amino Acids (mg/g) | T1   | T2   | T3   | T4   | T5   | T6   | T7   | T8   | T9   | T10  | T11  | T12  |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| Aspartic acid     | 4.497| 3.543| 3.027| 4.047| 3.124| 0.889| 2.048| 1.731| 1.734| 5.692| 4.678| 4.262|
| Threonine         | 2.471| 1.852| 1.529| 2.149| 1.584| 0.264| 0.770| 0.609| 0.638| 3.723| 2.229| 2.034|
| Serine            | 2.775| 2.086| 1.673| 2.469| 1.741| 0.334| 0.720| 0.599| 0.729| 3.865| 2.308| 2.092|
| Glutamic acid     | 26.983| 18.835| 13.905| 23.609| 14.458| 1.988| 6.883| 5.665| 5.665| 23.434| 23.514| 23.217|
| Glycine           | 2.319| 1.586| 1.216| 1.827| 1.214| 0.140| 0.257| 1.283| 0.262| 2.827| 1.245| 1.095|
| Alanine           | 5.301| 4.345| 3.849| 4.939| 4.075| 2.916| 5.148| 4.592| 3.638| 8.246| 6.958| 6.764|
| Cystine           | 0.832| 0.549| 0.428| 0.664| 0.434| 0.397| 0.561| 0.511| 0.480| 0.732| 0.864| 0.781|
| Valine            | 5.265| 4.088| 3.431| 4.729| 3.586| 1.971| 3.598| 3.184| 2.614| 5.997| 5.579| 5.517|
| Methionine        | 1.257| 0.928| 0.772| 1.025| 0.778| 0.188| 0.544| 0.460| 0.338| 1.001| 1.199| 1.124|
| Isoleucine        | 4.530| 3.346| 2.715| 3.998| 2.843| 1.512| 2.955| 1.965| 4.215| 4.666| 4.604|
| Leucine           | 8.457| 6.258| 5.045| 7.536| 5.294| 2.162| 4.872| 4.256| 3.226| 7.569| 8.588| 8.435|
| Tyrosine          | 2.476| 1.811| 1.398| 2.199| 1.422| 0.568| 1.502| 1.345| 0.974| 2.759| 3.278| 3.197|
| Phenylalanine     | 5.315| 3.738| 2.937| 4.808| 3.100| 1.037| 2.684| 2.335| 1.787| 4.692| 5.415| 5.296|
| Lysine            | 2.410| 1.949| 1.765| 2.102| 1.806| 0.247| 0.606| 0.502| 0.646| 1.489| 1.916| 1.760|
| Ammonia           | 4.052| 2.994| 2.357| 3.658| 2.496| 2.817| 4.025| 3.631| 2.577| 5.517| 5.429| 4.996|
| Histidine         | 2.476| 1.805| 1.425| 2.166| 1.498| 0.423| 1.000| 0.827| 0.673| 2.894| 2.487| 2.231|
| Arginine          | 4.841| 3.798| 3.276| 4.385| 3.409| 1.532| 2.988| 2.546| 2.197| 4.228| 4.956| 4.808|
| Proline           | 11.280| 8.018| 6.087| 10.240| 6.412| 4.441| 8.081| 7.323| 5.094| 13.493| 12.985| 12.666|
| Total amino acid  | 97.54| 71.53| 56.84| 86.55| 59.23| 23.83| 49.24| 42.29| 35.24| 104.37| 98.29| 94.88|

The amino acid content in wheat grain increased with decreasing soil moisture at the detriment of grain yield [41–43]. Similarly, [44] reported increased protein content of wheat with high moisture stress and nitrogen fertilization. Reference [45] also noticed in their work that soil moisture more strongly influenced the nitrogen content of wheat grain than available nitrogen in the soil. The low protein content in wheat grains as a result of high moisture content (T6) was caused by yield dilution effects on grain protein [46]. Also, low protein content of high yield lines (T10) was caused by either a limited amount of protein deposited in a large number of kernels, or it was a result of a limited amount of proteins diluted by a larger mass of carbohydrates [47]. In this research, a decrease in soil moisture from 100% to 30% in the greenhouse enhanced the quantity of amino acid in the grain by 26.4% and 56.8% for organic and inorganic treatments respectively. Also, a decrease in soil moisture from 100% to 30% in the field enhanced the quantity of amino acid in the grain by 16.6% and 4.76% for the organic and inorganic treatments respectively.

Compared to inorganic fertilizer, applying organic fertilizer to wheat in the greenhouse increased amino acid content by 5.96%, 9.41% and 40.9% for the 30%, 60% and 100% soil moisture treatments respectively. Whereas, compared to the organic treatment, applying inorganic fertilizer to wheat in the field increased amino acid content by 35.9%, 39.8% and 45.8%, for the 30%, 60% and 100% moisture treatments respectively.

4. Concluding Remarks

The study revealed that elevated levels of soil moisture produced a significant increase in grain yield but in contrast, a decrease in grain amino acid content. Low temperatures at the early vegetative stage marred stem elongation and tillering, while high temperatures at the heading stage hindered panicle growth and grain filling in wheat. The main results of the current work are as follows:
Wheat treated with inorganic fertilizer and 100% soil moisture in the greenhouse produced the best yield. Grain amino acid content was dependent on the uptake of mineral nutrient and nutrients uptake was meaningfully related to the amount of soil moisture.

Amino acid accumulation was best for wheat treated with inorganic fertilizer in the field, with decreasing soil moisture than wheat treated with organic and inorganic fertilizers in the greenhouse, and wheat treated with organic fertilizer in the field.

Wheat treated with organic fertilizer and 60% soil moisture in the greenhouse and wheat treated with inorganic fertilizer and 60% soil moisture in the field had a balance in yield and protein content.

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