Response of diverse wheat genotypes to moringa leaf extract concentrations and nitrogen levels on agronomic performance and nitrogen use efficiency under arid conditions

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Abstract

Sustainable agricultural practices are crucial for enhancing crop production in arid regions. Improving growth performance and nitrogen use efficiency of wheat genotypes contributes to yield stability in challenging environments. This study investigated the effects of foliar application of moringa leaf extract (MLE) at different concentrations (0%, 3%, and 6%) and varied N fertilizer levels (142, 190, and 238 kg N/ha) on growth, yield, and NUE of three diverse wheat cultivars (Sids 14, Sakha 95, and Giza 171) under field conditions in an arid environment over two growing seasons. The experimental site soil was clay and the climate was dry with low precipitation. The results indicated that increasing MLE concentration and N level significantly improved all studied growth, yield, and quality traits. In particular, the application of MLE in concentration 6% resulted in the highest grain yield (6871 and 6888 kg/ha) and straw yield (9368 and 9310 kg/ha), representing significant increases over the untreated control in both seasons. For quality, 6% MLE improved grain N content (up to 15.09 mg/g), straw N content (up to 7.67 mg/g), and grain N uptake (up to 104.66 kg/ha), with NUE peaking at 28.53 kg/kg. High N application (238 kg N/ha) also produced a significant yield increment by increasing grain yield compared to the lowest N level (142 kg N/ha). Cultivars Sids 14 and Giza 171 surpassed Sakha 95 in most yield and N accumulation parameters. Principal component and hierarchical cluster analyses confirmed that combinations of 6% MLE and 238 kg N/ha, especially with Sids 14 and Giza 171, maximized yield and NUE traits. Certain cultivars such Sids 14 and Giza 171 are more appropriate for these practices under arid clay soil conditions. Therefore, foliar application of 6% MLE combined with 238 kg N/ha with appropriate cultivars could significantly enhance wheat yield, quality, and NUE for improving wheat productivity in arid environments.

Keywords: Genotypic variations, foliar biostimulants, nitrogen use efficiency, plant nutrition, sustainable wheat production, crop management, plant growth regulators, principal component analysis, hierarchical clustering heatmap

Introduction

Wheat (*Triticum aestivum* L.) is a vital cereal crop in global food security, supplying daily caloric intake worldwide (Mottaleb et al., 2023). In addition to energy, wheat is a significant source of essential proteins, minerals, vitamins, dietary fiber, and micronutrients such as iron and zinc, along with numerous bioactive compounds that contribute to human health (Gupta et al., 2024). This nutritional importance is particularly pronounced in populations reliant on wheat as a primary food source (Drewnowski et al., 2025). Most of wheat production is directly consumed by humans with the remainder used for livestock feed and various industrial applications (Hao and Zhou, 2025). Wheat adaptability to diverse environmental and climatic conditions reinforces its widespread cultivation across multiple regions, especially in developing countries (Yanagi, 2024). Currently, global wheat cultivation covers approximately 220.4 million hectares, producing nearly 799 million tonnes annually (FAOSTAT, 2025). However, to meet the rising food demands expected by population growth, global wheat production requires a considerable increase (Mottaleb et al., 2023). In Egypt, bread wheat is a strategically critical crop, cultivated on about 1.35 million hectares and producing around 9.7 million tonnes (FAOSTAT, 2025). Despite this substantial production, domestic output falls short of the country consumption needs, emphasizing the urgent need for productivity enhancement (Abdalla et al., 2022).

Sustainable wheat production faces increasing challenges from soil nutrient depletion caused by continuous cropping without adequate replenishment (Shah and Wu, 2019). Mineral fertilizers are indispensable in restoring soil fertility, sustaining crop growth, and maximizing yield potential (Goud et al., 2022). Among these nutrients, N plays a pivotal role in wheat development, contributing to the expansion of photosynthetically active canopy and the synthesis of grain proteins (Noor et al., 2023). Extensive research has established N fertilization as key agronomic practice that directly influences both yield quantity and quality in wheat (Zörb et al., 2018). Nonetheless, excessive N application may lead to environmental degradation (Sabina et al., 2025). Therefore, improving NUE, the capacity of crops to utilize applied N effectively, is essential for sustainable agricultural production (Govindasamy et al., 2023).

Recently, sustainable and eco-friendly agronomic inputs, such as plant biostimulants, have been used to complement traditional fertilizers (Han et al., 2024). Moringa leaf extract (MLE) is considered a promising biostimulant, rich in natural growth regulators including phenolic compounds, ascorbates, and essential minerals such as N, P, K, Ca, and

Mg (Arif et al., 2023). MLE also contains plant hormones such as gibberellins, auxins, abscisic acid, salicylic acid, and jasmonic acid, which collectively stimulate plant growth and development (Brockman et al., 2020). Foliar application of MLE, especially at critical growth stages such as tillering and booting, has been demonstrated to enhance crop productivity (Khan et al., 2020; Mashamaite et al., 2022). Several studies report that MLE not only promotes growth but also improves NUE, contributing to better nutrient assimilation (Brockman and Brennan, 2017; Mashamaite et al., 2022; Irshad et al., 2025; Irshad et al., 2025). Moreover, MLE is cost-effective, environmentally friendly, and compatible with sustainable crop management practices, making it an attractive tool for increasing wheat productivity under resource-limited conditions (Irshad et al., 2025).

The release of numerous high-yielding wheat cultivars necessitates the evaluation of their responses to N fertilization and biostimulants like MLE (Melash et al., 2023). These cultivars exhibit differential NUE and yield performance across N supply levels indicating the need for cultivar-specific management strategies to maximize productivity (Lu et al., 2015; Ayadi et al., 2022). Accordingly, this study aimed to investigate the effects of MLE at different concentrations and N fertilizer levels on growth, yield, yield components and NUE of three diverse wheat cultivars and evaluate the interactive effects of combined MLE and N fertilization on wheat agronomic performance under arid conditions.

Materials and Methods

Experimental site

Two field experiments were conducted at the Farm of Faculty of Agriculture, Zagazig University, located in Ghazala, Sharqia, Egypt (30°33′35″ N, 31°34′25″ E), during two consecutive winter growing seasons (2019–2020 and 2020–2021). The experimental site was characterized by clay-textured soil with consistent physical and chemical properties across the 0–90 cm profile (Table 1). Soil analysis revealed that the soil contained 57.59% clay, 23.70% silt, and 18.71% sand, resulting in a clay classification. The soil exhibited a slightly alkaline pH and moderate electrical conductivity. The local climate during the wheat growing seasons was characterized by dry winters with average maximum temperatures ranging from 16.96°C to 30.33°C in the first season and 21.58°C to 30.90°C in the second season (Table S1). Minimum temperatures fluctuated between 12.84°C and 19.07°C in the first season, and 13.75°C to 18.41°C in the second season. Relative humidity was generally moderate to high (61.99%–78.71%), while total monthly precipitation was low, with monthly values varying from 0.01 mm to 3.05 mm in the first season and 0.08 mm to 2.72 mm in the second. Such conditions reflect the semi-arid environment typical of the region and indicate the need for efficient water and nutrient management in wheat production.

Experimental design and treatments

A split-split plot design with three replications was implemented in both seasons. The main plots were assigned to exogenously applied MLE at different concentrations, with subplots assigned to wheat cultivars, and sub-subplots allocated to N fertilizer rates. The MLE was prepared by soaking 40 g of fresh young Moringa leaves in 1350 ml of 80% ethanol with continuous stirring using a homogenizer following the method of Makkar and Becker (1996). The crude extract was filtered through Whatman No. 2 filter paper, then divided and diluted to the desired concentrations immediately before each application. Chemical composition of the extract is presented in Table 2. Wheat plants were foliar sprayed three times with two concentrations of MLE (3% and 6%), alongside untreated control treatment sprayed with distilled water. The applications were performed at growth stages GS22 (tillering), GS32 (stem elongation), and GS60 (anthesis) using a hand-held compressed air sprayer (Zadoks et al., 1974). The volume of spraying solution applied per plot was approximately 5 liters for the first two applications and 6 liters for the final application. The N fertilizer treatments comprised three rates: 142, 190, and 238 kg N/ha, applied in the form of ammonium nitrate. Nitrogen was split into three doses: 15% at sowing, 50% at GS22, and 35% at GS35 for each fertilizer level. Three bread wheat cultivars (*Triticum aestivum* L.); Sids 14, Sakha 95, and Giza 171, were evaluated. Wheat seeds were sown on November 20th of each season in 5-m rows with 15 cm apart at seeding rate of 350 seeds/m². Experimental plots were 25 m² (5 × 5 m), separated by 1-m borders. The preceding crop for both seasons was maize. Surface irrigation was performed according to the standard practice in the studied region, with four irrigation events applied each season. Harvesting was during the last week of April in both seasons.

Measurements

At heading (GS59), flag leaf area was measured on ten randomly selected flag leaves per plot using the formula: length × maximum width × 0.80 according to Voldeng and Simpson (1967). At harvest, ten random tillers per plot were collected to record plant height (cm), spike length (cm), grain number per spike, and 1000-grain weight (g). All plants within one-square-meter area of each plot were counted to determine spike number per m². Three-square-meter area in each plot was harvested to determine grain yield and straw yield and then converted to kg/ha.

At maturity, ten plants were randomly sampled per plot and separated into grain and straw components, oven-dried at 70°C until constant weight. Dry samples underwent wet digestion with perchloric and sulfuric acids ($HClO_4 + H_2SO_4$). Nitrogen content (mg/g) of grain and straw was determined using the micro-Kjeldahl method. The following N-related parameters were calculated: Grain N uptake (kg/ha) = Grain yield × Grain N content. Nitrogen use efficiency (kg/kg) = Grain yield / (Soil available N + Applied N fertilizer)

Statistical analysis

Data from each season were analyzed separately using analysis of variance (ANOVA) for split-split plot design with R programming software. Treatment means were compared using the least significant difference (LSD) test at the 5% probability level ($p \leq 0.05$). Principal component analysis was carried out employing packages of ggplot2, factoextra, FactoMineR and ggrepel, while the hierarchical clustering used gplots.

Results

Growth traits

The effects of MLE concentrations and N fertilizer levels on flag leaf area and plant height of wheat cultivars during two seasons are presented in Table 3. The analysis of variance displayed significant effects of MLE concentrations, N levels, and wheat cultivars on flag leaf area and plant height in both seasons. Increasing the concentration of MLE from 0% (untreated control) to 6% significantly enhanced flag leaf area and plant height in both seasons. The highest flag leaf area and plant height were recorded under 6% extract. Flag leaf area increased by 28.99% and 24.97%, plant height increased by 10.41 % and 16.47% respectively, in both seasons. Increasing N levels exhibited significant positive effect on studied growth traits. The highest values were recorded at 238 kg N/ha, with flag leaf area reaching 37.93 cm², and plant height 105.09 cm. Likewise, in the second season, the highest values were recorded at 238 kg N/ha, with flag leaf area 40.22 cm², plant height 109.25 cm. Nevertheless, low N level exhibited reduced growth and yield components. The high N level 238 kg N/ha increased flag leaf area by 17.24% and 19.48% and plant height 12.87% and 18.10% in the first and second seasons in the same order. The evaluated cultivars displayed significant variations in growth traits. Sids 14 and Giza 171 exhibited the highest flag leaf area and taller plants. Most two and three-way interactions among MLE concentrations, N levels, and cultivars were not significant for flag leaf area and plant height. The three-way interaction between MLE, N fertilizer and wheat cultivars for flag leaf area and plant height is presented in Figure 1. Increases in flag leaf area and plant height across treatments were attributed to the effects of higher MLE concentration, increased N level, and cultivar differences. The highest flag leaf area and plant height were observed with 6% MLE and 238 kg N/ha, particularly in Giza 171.

Yield attributes

The analysis of variance revealed that MLE concentration, wheat cultivar, and N level significantly influenced all measured wheat yield components across both seasons (Table 4). Application of MLE at 6% produced the highest spike length, grain number per spike, 1000-grain weight, and spike number per m², significantly surpassing both the untreated control and 3% treatments. Spike length showed the greatest increment at high extract level by 16.75 % and 16.19% compared to the untreated control in both seasons, respectively. Similarly, grain number per spike increased by 8.95% and 14.17%, 1000-grain weight by 6.1% and 9.1%, and spike number per m² increased by 9.9% and 8.4% in both seasons, respectively. The evaluated cultivars displayed significant variations in yield components. Giza 171 and Sids 14 exhibited the highest spike length and grain number per spike. Moreover, Sids 14 produced the heaviest 1000-grain weights in both seasons, followed by Giza 171 and Sakha 95. Besides, Sakha 95 had the highest number of spikes per area, followed by Sids 14 and Giza 171. Increasing N levels significantly improved spike length, grain number per spike, 1000-grain weight, and spike number per m². The highest values were obtained at 238 kg N/ha while the lowest was at 142 kg N/ha. At 238 kg N/ha, spike length increased by 33.07% and 36.49%, grain number per spike 13.30% and 8.95%, 1000-grain weight by 5.53% and 9.14%, and spike number per m² by 12.62% and 15.84% compared to 142 kg N/ha in the first and second seasons, respectively. Most two and three-way interactions among MLE concentrations, N levels, and cultivars for yield components were mostly non-significant. The three-way interaction for spike length, number of grains per spike, 1000-grain weight, and number of spikes per m² is presented in Figure 2. Increases in yield components across treatments were attributed to the effects of higher MLE concentration, increased N application, and cultivar differences. For all traits, higher values were obtained with 6% MLE and 238 kg N/ha, especially in high-performing cultivars like Sids 14 and Giza 171. Spike length, grains per spike, 1000-grain weight, and spike number all improved significantly with these higher input levels.

Grain and straw yields

The results in Table 5 indicate that MLE concentration, wheat cultivar, and N fertilizer level significantly affect grain and straw yields of wheat across two winter seasons. Higher MLE concentration (6%) produced the highest grain and straw yields in both seasons compared to the untreated control and 3% concentration. The untreated control showed the lowest yields for both grain and straw in all seasons. Grain yield increased by 23.53% and 31.97%, and straw yield increased by 8.25% and 6.95% respectively, in the first and second seasons. Among cultivars, Sids 14 had the highest grain yield in both seasons, followed by Giza 171 and Sakha 95. For straw yield, Sakha 95 produced the highest, while Giza 171 produced the lowest. Increasing N from 142 kg N/ha to 238 kg N/ha led to the highest values for both grain and straw yields in all seasons. The lowest N level gave the minimum grain and straw yield. The high N level 238 kg N/ha, increased grain yield by 52.74% and 55.11% and straw yield by 11.82% and 16.88% in the first and second seasons in the same order. Most interactions among MLE concentration, wheat cultivar, and N fertilizer level were not significant. Figure 3 presents the three-way interaction among the three studied factors. Increases in both grain and straw yields were observed across treatments due to the effects of higher MLE concentrations, increased N levels, and cultivar differences. The highest grain and straw yields were achieved with 6% MLE and 238 kg N/ha, particularly in Sids 14 and Giza 171.

N accumulation parameters

MLE concentration, wheat cultivar, and N fertilizer level exhibited significant effects on grain N content, straw N content, grain N uptake, and NUE (Table 6). Increasing MLE concentration from 0% (untreated control) to 6% significantly improved all studied N-related traits in both seasons. The highest values for grain N content, straw N content, grain N uptake, and NUE were consistently recorded with 6% MLE application, compared to the untreated control. The use of 6% MLE compared to the untreated control caused increases in grain N content by 7.37 and 8.02%, straw N content by 7.12% and 3.82%, grain N uptake by 30.06% and 41.68%, and NUE by 25.36% and 28.24% in the first and second seasons, respectively. Among wheat cultivars, Giza 171 and Sids 14 exhibited the highest nitrogen content and uptake values, while Sakha 95 showed lower values. Increasing nitrogen fertilizer level from 142 to 238 kg N/ha resulted in significant increases in grain and straw nitrogen content, uptake, and NUE in both seasons, with the 238 kg N/ha level achieving the highest means for all traits. Applying the highest nitrogen level (238 kg N/ha) instead of the lowest (142 kg N/ha) resulted in increased grain nitrogen content by 10.82% and 11.86%, straw nitrogen content by 2.19% and 4.23%, grain nitrogen uptake by 68.78% and 72.74%, while NUE decreased by 14.77% and 11.63% in the first and second seasons, respectively. The three-way interaction between MLE, nitrogen fertilizer, and wheat cultivars for grain N content, straw N content, grain N uptake, and NUE was statistically non-significant (Figure 4). Improvement in these nitrogen-related traits resulted from the effects of increasing MLE concentration, higher nitrogen application levels, and cultivar differences. The highest values for grain and straw N content, N uptake, and use efficiency were recorded with 6% MLE and 238 kg N/ha, particularly in cultivars Sids 14 and Giza 171.

Principal component and heatmap analyses

The principal component analysis biplot in Figure 5 presents the relationships among treatments and measured agronomic and nitrogen-related traits across different MLE concentrations, N fertilizer levels, and wheat cultivars. The first principal component (PC1) explained 77.26% of the total variance, while the second principal component (PC2) accounted for 13.7%. Most measured traits, including grain yield (GrainY), straw yield (StrawY), plant height (PH), flag leaf area (FLA), spike length (SL), number of grains per spike (GNS), 1000-grain weight (TGW), number of spikes per m² (SNM), grain N content (GrainN), straw N content (StrawN), grain N uptake (GNUp), and N use efficiency (NUE), are positively aligned with PC1. The PCA separates treatments according to MLE concentration and N level, with the highest input combination (6% MLE and 238 kg N/ha) producing the highest performance in yield, growth, and N traits. Treatments with 6% MLE (6-) and high nitrogen (238), particularly in Sids14 and Giza171 cultivars, are located furthest in the positive direction along PC1, closely associated with increased values for all agronomic and N accumulation and efficiency traits. Conversely, treatments with no MLE (Ct-) and low N (142), mainly in Giza171 and Sids14, are located on the negative side of PC1, indicating lower performance in measured traits. Treatments with intermediate MLE levels (3-) and moderate N levels (190) show an intermediate distribution, confirming their moderate trait values.

The heatmap with hierarchical clustering presents the patterns of major agronomic and nitrogen-related traits across treatments differing in MLE concentration, nitrogen fertilizer level, and wheat cultivar. Each cell represents the value of specific trait for each treatment, with blue tones indicating higher values and red tones indicating lower values. Treatments with 6% MLE and 238 kg N/ha (6-S14-238 and 6-G171-238) consistently cluster together at the top, showing the deepest blue colors across mostly all traits, indicating maximized values for grain yield (GrainY), straw yield (StrawY), number of spikes per m² (SNM), grain N uptake (GNUp), plant height (PH), flag leaf area (FLA), and N use efficiency (NUE). Untreated control plots with low nitrogen (Ct-S95-142, Ct-G171-142, Ct-S14-142) cluster at the bottom, characterized by the strongest red across traits, signifying the lowest performance for these variables. Intermediate treatments (*e.g.*, 3-S14-238, 6-S14-190, 3-G171-190) display moderate blue coloration, reflecting a gradual increase in trait values with increasing MLE concentration and N level. The clustering of traits reveals strong associations among yield, spike number, N uptake, and vegetative traits, as these group closely together and show similar response

Discussion

The application of biostimulant foliar sprays and optimized N fertilization is crucial for wheat production in arid and semi-arid environments. In these regions, enhancing crop nutrient use efficiency translates to improved yield stability and sustainability. The present study investigated the effects of foliar application of MLE at different concentrations (0%, 3%, and 6%) and varied N fertilizer levels (142, 190, and 238 kg N/ha) on yield, yield attributes and N efficiency of diverse wheat cultivars (Sids 14, Sakha 95, and Giza 171) under field conditions in an arid environment over two growing seasons. The results demonstrated that MLE concentration, N fertilizer level and wheat cultivar play significant roles in enhancing agronomic performance, yield, and NUE in wheat under field conditions. The application of MLE, particularly at a concentration of 6%, significantly improved growth traits, yield components, and quality parameters in wheat across two growing seasons. The observed increments in flag leaf area and plant height with increasing MLE concentrations indicate that moringa-derived bioactive compounds could enhance leaf expansion and promote cell division as elucidated by Rehman et al. (2017) and (Irshad et al., 2025). MLE contain natural growth regulators such as cytokinins, auxins, vitamins, and minerals, which enhance cell division, expansion, and overall plant vigor (Arif et al., 2023). In this context, Brockman and Brennan (2017) and Khan et al. (2020) reported that MLE foliar spray significantly increased wheat plant height and leaf area leading to improved photosynthetic capacity and stimulating vegetative growth. Moreover, application of 6% MLE also elevated yield attributes including spike length, grain number per spike, 1000-grain weight, and spike density, confirming the role of moringa as a growth enhancer. The growth-promoting substances in moringa likely stimulate the grain filling stage, positively influencing yield components (Tahir et al., 2022). In this context, Jhilik et al. (2018), Brockman et al. (2020) and Merwad (2020) studied the comparable effectiveness of MLE and found improvements in wheat yield components using MLE foliar application, attributing enhancements to increased nutrient uptake and hormonal stimulation. The application of 6% MLE significantly enhanced both grain and straw yields of wheat across the two growing seasons, as evidenced by the highest recorded values compared to the untreated control and 3% MLE treatments. These improvements can be attributed to the bioactive compounds present in MLE, such as growth hormones, vitamins, and antioxidants, which promote enhanced photosynthetic activity, nutrient uptake, and overall plant growth (Mashamaite et al., 2022). The findings align with previous studies of Brockman and Brennan (2017), Khan et al. (2020) and Khan et al. (2021), who reported that foliar application of MLE improved biomass production and grain yield in cereal crops by improving physiological processes. Increased grain N content, uptake, and NUE with MLE application indicate improved N assimilation and metabolic efficiency likely due to bioactive compounds enhancing nutrient transport and enzymatic activities (Rashid et al., 2021). MLE has also been reported to boost antioxidant enzyme activities and improve photosynthesis rates which can indirectly support N metabolism (Hafeez et al., 2022).

Nitrogen remains the most critical nutrient limiting wheat production, and the improvements obtained by increasing N levels to 238 kg N/ha are consistent with extensive agronomic research (Zhang et al., 2015). The enhanced flag leaf area and plant height with higher N supply reflect N key role in vegetative growth and canopy development (Ju et al., 2022). Elevated spike length, grain number, and grain weight with increased N reflect its pivotal importance during reproductive growth stages for protein synthesis and grain filling (Yu et al., 2018). The grain and straw yield increments at the highest N level compared to the lowest confirm the critical threshold nutrient requirements of wheat for optimal productivity (Wang et al., 2017). However, N efficiency parameters, including NUE, showed some decreases at the highest N levels, which is a common phenomenon due to luxury consumption and increased losses (Govindasamy et al., 2023). The decline in NUE at higher N levels despite increased uptake confirms the commonly observed trade-off between yield potential and resource efficiency at high fertilization (Ciampitti and Lemaire, 2022). The optimal balance observed with combined 6% MLE and 238 kg N/ha application demonstrates a beneficial strategy for raising yields while maintaining relatively high N efficiency. Hence, synergistic approaches that enhance N uptake and utilization, such as combined use of bio-stimulants like moringa, can be valuable for sustainable intensification.

The tested wheat cultivars (Sids 14, Giza 171, Sakha 95) exhibited significant variation in response to both MLE and N fertilization. Sids 14 and Giza 171 regularly outperformed Sakha 95 regarding yield and N assimilation traits, reflecting significant genetic differences in resource use efficiency, source-sink strength, and stress adaptability. The superior performance of Sids 14 in particular for both yield and grain N content suggests its greater responsiveness to combined biostimulant and N management. Such genotype-dependent responses reveal necessity of agronomic recommendations and the potential for targeted cultivar selection to maximize the benefits of innovative input strategies. Similar results were reported by Liu et al. (2019), Congreves et al. (2024) and Wang et al. (2025) who found cultivar-specific responses to nitrogen fertilizer levels in wheat, with clear differences in yield and nitrogen use efficiency among varieties. The results indicated that mostly non-significant interactions among MLE concentrations, N levels, and wheat cultivars, except for few traits. The relatively minor interaction effects among these factors for most parameters suggest that MLE benefits are broadly applicable but can be maximized with suitable cultivar selection and agronomic context (Irshad et al., 2025). However, the optimum combination of 6% MLE, 238 kg N/ha, and high-performing cultivars like Sids 14 could be applied for maximum productivity.

The integration of results from PCA and heatmap clustering analyses provides complete view of treatment effects. The positive association of higher MLE concentration and N rates most measured traits, especially grain yield, straw yield, spike number per m², and NUE reinforces the benefits of combining bioactive foliar sprays with optimal fertilization. Treatments combining 6% MLE and 238 kg N/ha, particularly in Sids 14 and Giza 171, clustered together as the highest performing groups across agronomic and N-related traits. This additive effect indicates the essential relationship between plant growth regulators in moringa and basic mineral nutrition. Moreover, the heatmap analysis revealed strong trait associations, indicating that improvements in wheat productivity are highly associated with enhancements in vegetative and N uptake characteristics. This clustering indicates that the boosting source capacity (flag leaf area, plant height), reproductive potential (spike length, grains per spike), and nutrient assimilation reinforce higher wheat productivity. The visualization approach using PCA and heatmap clustering analyses aligns with previous studies recommending multivariate analyses to capture complex trait interactions in crop nutrition (Abrar et al., 2024; Meena et al., 2025).

Conclusion

The results of this study demonstrate that the combined application of MLE and optimized N fertilizer rates has a considerable positive effect on wheat growth, yield, and NUE, particularly under arid field conditions. Increasing MLE concentration up to 6% and raising N inputs to 238 kg N/ha significantly improved all agronomic and yield traits across two seasons, with cultivars Sids 14 and Giza 171 exhibiting the highest responses. These enhancements increase wheat productivity, encompassing greater grain and straw N content, N uptake, and improved resource use efficiency. The benefits of MLE and N were validated by principal component and cluster analyses. Therefore, integrating biostimulant foliar applications such as MLE with appropriate N fertilization, presents sustainable and eco-friendly effective strategy to maximize wheat productivity and nutrient utilization under arid environments.

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Table 1. Physical and Chemical properties of the experimental field soil.

Depth (cm)	**Particle distribution**			**Textural class**	**Bulk density ($g\ cm^{-1}$)**		
	Sand (%)	Silt (%)	Clay (%)				
0–30	18.81	23.44	57.75	Clay	1.49		
30–60	18.75	23.60	57.65	Clay	1.51		
60–90	18.56	24.06	57.38	Clay	1.53		
Depth (cm)	**Organic matter (%)**	**pH**	**EC ($dS\ m^{-1}$)**	**Available nutrient ($mg\ kg^{-1}$ Soil)**			
				Nitrogen	Phosphorus		Potassium
0–30	2.55	7.98	1.60	29.85	12.90		182.50
30–60	2.37	7.91	1.57	27.20	11.25		168.00
60–90	2.22	7.88	1.54	25.30	10.05		153.38
Depth (cm)	**Soluble cations and anions in the soil paste extract (mmolc L^{-1})**						
	Calcium	Magnesium	Sodium	Potassium	Bicarbonate	Chloride	Sulphate
0–30	5.67	4.52	3.16	2.64	6.47	4.36	5.16
30–60	5.66	4.44	3.21	2.46	6.09	4.69	4.99
60–90	5.54	4.24	3.26	2.39	5.96	4.68	4.79

Table 2. Chemical composition of *Moringa oleifera* leaves per dry weight (dw).

Component	**Value**
Protein	273 g/kg dw
Phosphorus (p)	3. 90 g/kg dw
Potassium (K)	21.70 g/kg dw
Calcium (Ca)	24.0 g/kg dw
Magnesium (Mg)	4.5 g/kg dw
iron (Fe)	0.582 g/kg dw
Vitamin A (β-carotene)	163 mg/kg
Vitamin B_1 (thiamine)	26 mg/kg
Vitamin B_2 (riboflavin)	210 mg/kg
Vitamin B_3 (nicotinic acid)	800 mg/kg
Vitamin C (ascorbic acid)	1700 mg/kg
Vitamin E (tocopherol acetate)	1130 mg/kg

Table 3. Effects of moringa leaf extract at different concentrations, wheat cultivars, and N fertilizer levels on flag leaf area and plant height across two growing seasons.

Studied factor		**Flag leaf area (cm^2)**				**Plant height (cm)**			
		First season		Second season		First season		Second season	
Moringa leaves extract (M)									
Untreated Control		31.07	c	33.19	c	94.63	b	93.84	c
3%		34.94	b	36.11	b	101.2	a	103.3	b
6%		40.08	a	41.48	a	104.5	a	109.3	a
Cultivars (C)									
Sids 14		36.22	a	37.81	a	98.84	b	99.80	b
Sakha 95		33.61	b	35.36	b	100.4	a	102.9	a
Giza 171		36.26	a	37.61	a	101.1	a	103.7	a
Nitrogen fertilizer levels (N)									
142 kg N / ha		32.35	c	33.66	c	93.10	c	92.50	c
190 kg N / ha		35.80	b	36.90	b	102.2	b	104.7	b
238 kg N / ha		37.93	a	40.22	a	105.1	a	109.3	a
ANOVA	**df**	**Mean squares and significance level**							
M	2	551.3	**	476.8	**	681.4	**	1639	**
C	2	62.18	**	49.82	**	37.05	**	115.1	**
M×C	4	15.17	**	8.500	**	2.530	ns	10.91	ns

N	2	214.5	**	290.4	**	1056	**	2025	**
M×N	4	0.270	ns	3.690	ns	0.190	ns	1.180	ns
C×N	4	0.770	ns	2.320	ns	0.560	ns	2.790	ns
M×C×N	8	0.130	ns	2.030	ns	0.140	ns	0.310	ns

Means followed by different letters within the same factor are significantly different according to LSD ($p < 0.05$).
ns: Not significant; *: $P < 0.05$; **: $P < 0.01$

Table 4. Effects of moringa leaf extract at different concentrations, wheat cultivars, and N fertilizer levels on yield components across two growing seasons

Studied factor		**Spike length (cm)**				**Number of grains/spike**			
		First season		Second season		First season		Second season	
Moringa leaves extract (M)									
Untreated Control		11.10	c	10.93	b	37.65	c	36.32	c
3%		11.92	b	11.74	b	39.60	b	38.17	b
6%		12.96	a	12.70	a	41.02	a	41.47	a
Cultivars (C)									
Sids 14		13.59	a	13.07	a	41.37	a	40.41	a
Sakha 95		10.74	c	10.34	c	37.71	c	37.13	c
Giza 171		11.65	b	11.96	b	39.20	b	38.42	a
Nitrogen fertilizer levels (N)									
142 kg N / ha		10.16	c	9.81	c	37.12	c	36.84	c
190 kg N / ha		12.30	b	12.17	b	39.10	b	38.98	b
238 kg N / ha		13.52	a	13.39	a	42.06	a	40.14	a
ANOVA	**df**	**Mean squares and significance level**							
M	2	23.58	**	21.22	**	77.20	**	183.5	**
C	2	57.32	**	51.12	**	91.43	**	73.81	**
M×C	4	0.050	ns	0.090	ns	6.140	ns	13.10	**
N	2	78.34	**	89.08	**	166.7	**	75.72	**
M×N	4	0.030	ns	1.600	**	1.390	ns	0.440	ns
C×N	4	0.210	ns	3.060	**	2.730	ns	0.760	ns
M×C×N	8	0.330	ns	1.640	ns	0.720	ns	2.900	ns
Studied factor		**1000-grain weight (g)**				**Number of spikes per m^2**			
		First season		Second season		First season		Second season	
Moringa leaves extract (M)									
Untreated Control		39.33	b	37.13	c	380.4	c	387.3	c
3%		41.19	a	39.02	b	402.1	b	407.0	b
6%		41.74	a	40.50	a	417.9	a	419.8	a
Cultivars (C)									
Sids 14		42.96	a	41.45	a	382.9	c	388.6	c
Sakha 95		38.76	c	37.04	c	418.1	a	421.4	a
Giza 171		40.53	b	38.15	b	399.4	b	404.1	b
Nitrogen fertilizer levels (N)									
142 kg N / ha		39.60	c	36.89	c	374.8	c	373.3	c
190 kg N / ha		40.86	b	39.50	b	403.4	b	408.5	b
238 kg N / ha		41.79	a	40.26	a	422.2	a	432.4	a
ANOVA	**df**	**Mean squares and significance level**							
M	2	42.85	**	76.96	**	9563	**	7225	**
C	2	120.2	**	142.2	**	8365	**	7308	**
M×C	4	0.370	ns	6.310	**	230.1	**	185.6	**
N	2	32.84	**	84.27	**	15322	**	23874	**
M×N	4	0.610	ns	1.450	ns	444.9	**	111.9	**
C×N	4	1.070	ns	4.370	**	158.1	ns	283.3	**
M×C×N	8	1.500	ns	1.380	ns	0.770	ns	1.320	ns

Means followed by different letters within the same factor are significantly different according to LSD ($p < 0.05$).
ns: Not significant; *: $P < 0.05$; **: $P < 0.01$

Table 5. Effects of moringa leaf extract at different concentrations, wheat cultivars, and N fertilizer levels on grain and straw yields across two growing seasons.

Studied factor		Grain yield (kg /ha)				Straw yield (kg /ha)			
		First season		Second season		First season		Second season	
Moringa leaves extract (M)									
Untreated Control		5563	c	5219	c	8654	c	8705	c
3%		6356	b	6018	b	9013	b	9054	b
6%		6871	a	6888	a	9368	a	9310	a
Cultivars (C)									
Sids 14		6468	a	6486	a	8715	c	8835	b
Sakha 95		6081	b	5713	c	9434	a	9309	a
Giza 171		6241	b	5928	b	8886	b	8926	b
Nitrogen fertilizer levels (N)									
142 kg N / ha		4873	c	4636	c	8414	c	8175	c
190 kg N / ha		6474	b	6300	b	9213	b	9339	b
238 kg N / ha		7443	a	7190	a	9408	a	9555	a
ANOVA	**df**	**Mean squares and significance level**							
M	2	11734914	**	18803986	**	3437877	**	2493468	**
C	2	1017350	**	4296482	**	3808589	**	1707539	**
M×C	4	194561	ns	101584	**	17585	ns	26216	ns
N	2	45475654	**	45403815	**	7499975	**	14882536	**
M×N	4	392942	**	369956	**	49000	ns	2654	ns
C×N	4	85604	ns	111144	**	18090	ns	70132	ns
M×C×N	8	1.740	ns	7.250	**	0.330	ns	0.17	ns

Means followed by different letters within the same factor are significantly different according to LSD ($p < 0.05$).
ns: Not significant; * $P < 0.05$; ** $P < 0.0$

Table 6. Effects of moringa leaf extract at different concentrations, wheat cultivars, and N fertilizer levels on grain N content, straw N content, grain N uptake and N use efficiency across two growing seasons.

Studied factors		Grain N content (mg/g)				Straw N content (mg/g)			
		First season		Second season		First season		Second season	
Moringa leaves extract (M)									
Untreated Control		13.98	c	13.97	c	7.16	c	7.33	c
3%		14.62	b	14.64	b	7.32	b	7.41	b
6%		15.01	a	15.09	a	7.67	a	7.61	a
Cultivars (C)									
Sids 14		14.94	a	15.02	a	7.50	a	7.55	a
Sakha 95		14.04	c	14.12	c	7.22	c	7.33	c
Giza 171		14.63	b	14.56	b	7.43	b	7.47	b
Nitrogen fertilizer levels (N)									
142 kg N / ha		13.77	c	13.66	c	7.30	c	7.33	c
190 kg N / ha		14.57	b	14.75	b	7.39	b	7.39	b
238 kg N / ha		15.26	a	15.28	a	7.46	a	7.64	a
ANOVA	**df**	**Mean squares and significance level**							
M	2	7.33	**	8.57	**	1.87	**	0.56	**
C	2	5.65	**	5.50	**	0.59	**	0.35	**
M x C	4	0.20	**	0.12	**	0.01	**	0.01	**
N	2	14.89	**	18.51	**	0.19	**	0.74	**
M x N	4	0.29	ns	0.52	**	0.01	ns	0.01	**
C x N	4	0.33	**	0.48	**	0.01	ns	0.01	**
M x C x N	8	0.65	ns	2.12	ns	3.19	**	6.35	**
Studied factors		**Grain N uptake (kg /ha)**				**N use efficiency (kg /kg)**			
		First season		Second season		First season		Second season	
Moringa leaves extract (M)									
Untreated control		78.60	c	73.87	c	22.82	c	21.44	c
3%		93.57	b	88.94	b	26.16	b	24.98	b
6%		103.80	a	104.66	a	28.28	a	28.53	a

Cultivars (C)									
Sids 14		97.47	a	98.43	a	26.56	a	26.81	a
Sakha 95		86.43	c	81.84	c	25.00	b	23.64	c
Giza 171		92.07	b	87.20	b	25.70	b	24.50	b
Nitrogen fertilizer levels (N)									
142 kg N / ha		67.43	c	63.86	c	25.01	c	23.98	c
190 kg N / ha		94.73	b	93.30	b	26.66	a	26.11	a
238 kg N / ha		113.81	a	110.31	a	25.59	b	24.85	b
ANOVA	**df**	**Mean squares and significance level**							
M	2	4333.86	**	6398.98	**	204.73	**	339.46	**
C	2	823.03	**	1933.36	**	16.49	**	72.71	**
M x C	4	20.04	ns	24.73	**	3.64	ns	1.32	ns
N	2	14670.76	**	14908.89	**	18.95	**	30.84	**
M x N	4	101.51	**	59.11	**	6.71	**	9.92	**
C x N	4	28.66	ns	34.68	**	0.78	ns	0.30	ns
M x C x N	8	1.00	ns	5.75	**	1.43	ns	3.61	**

Means followed by different letters within the same factor are significantly different according to LSD ($p < 0.05$).
ns: Not significant; * $P < 0.05$; ** $P < 0.01$.

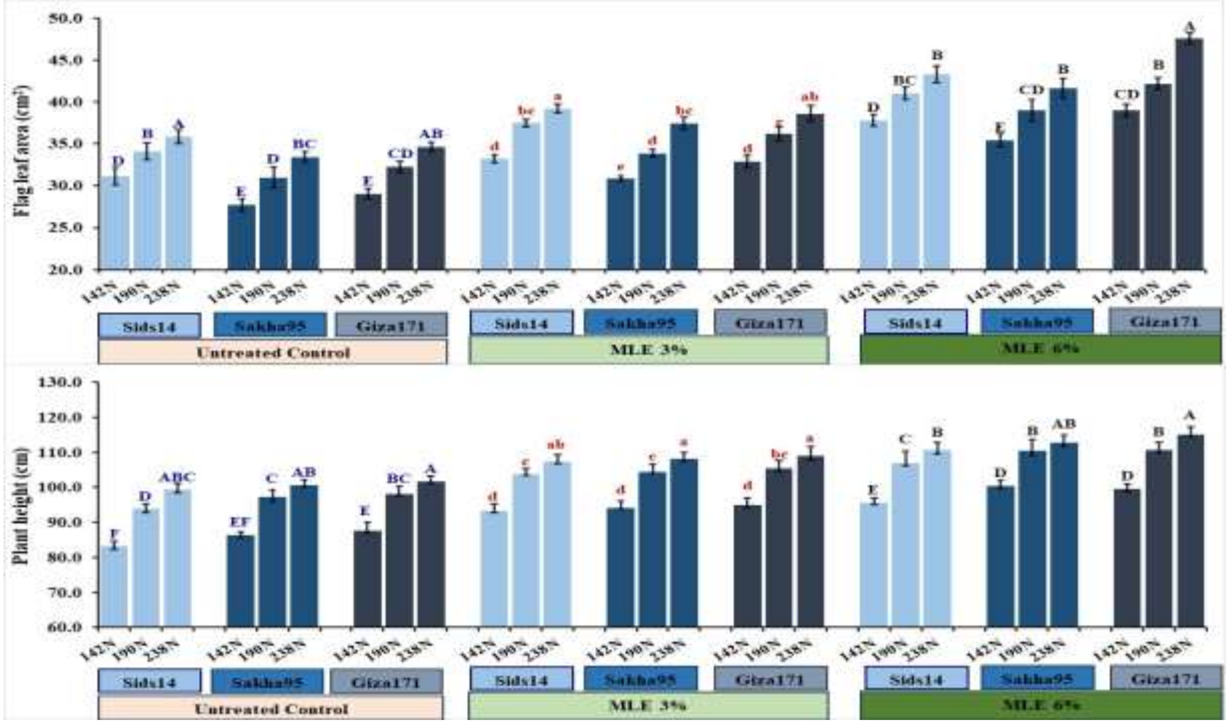

Figure 1. Effect of MLE and N fertilizer levels on flag leaf area and plant height in different wheat cultivars over two growing seasons. The bars on each column represent ± standard error. Uppercase blue letters correspond to significant differences among untreated control treatments, lowercase red letters represent significant differences among 3% MLE treatments, and uppercase red letters signify significant differences among 6% MLE treatments according to LSD at $p \leq 0.05$.

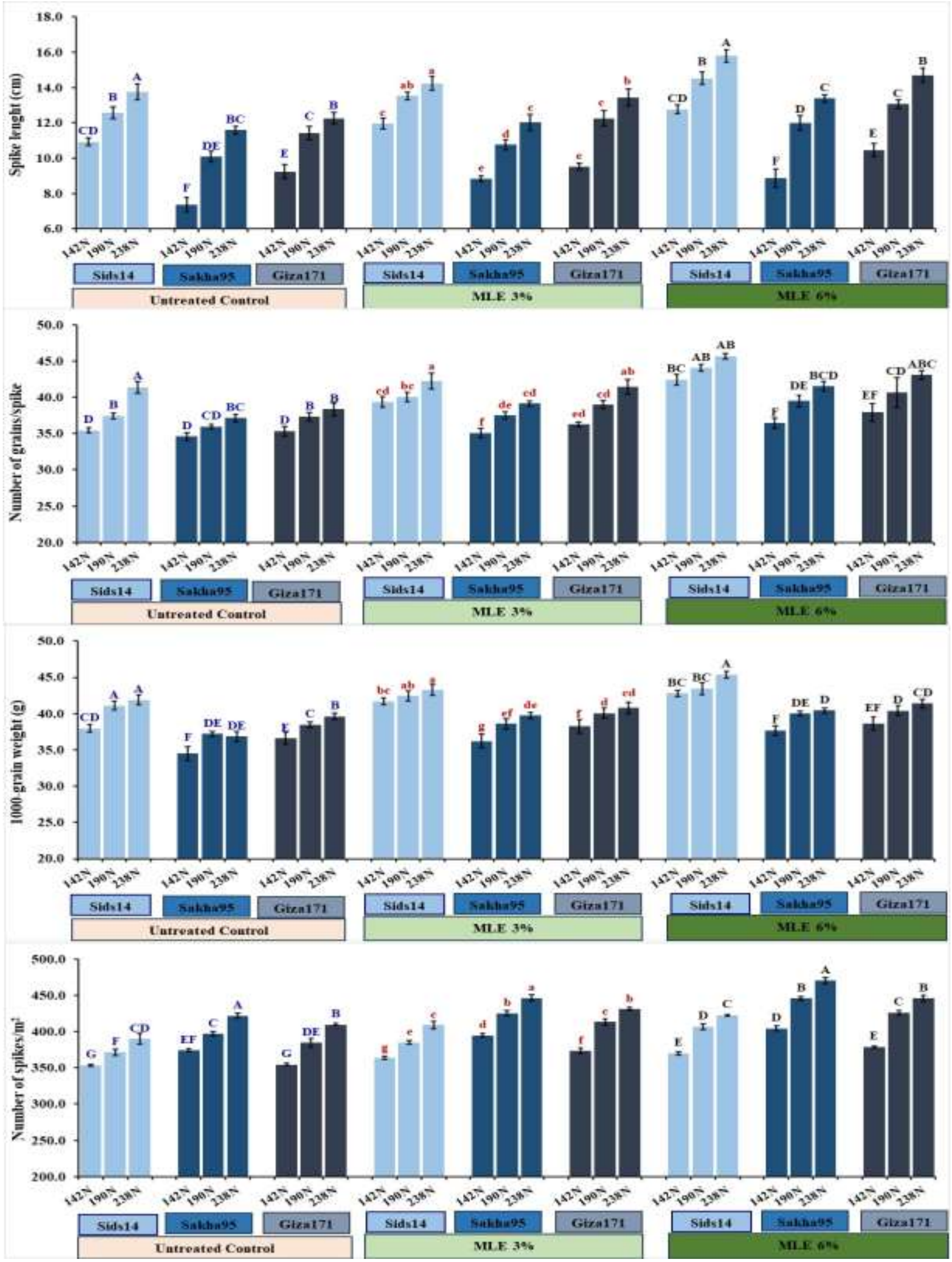

Figure 2. Interactive Effects of Moringa leaves extract, N fertilizer levels, and cultivar on wheat yield components; spike length, number of grains/spike, 1000-grain weight, and number of spikes/m² over two growing seasons. The bars on each column represent ± standard error. Uppercase blue letters correspond to significant differences among untreated control treatments, lowercase red letters represent significant differences among 3% MLE treatments, and uppercase red letters signify significant differences among 6% MLE treatments according to LSD at p ≤ 0.05.

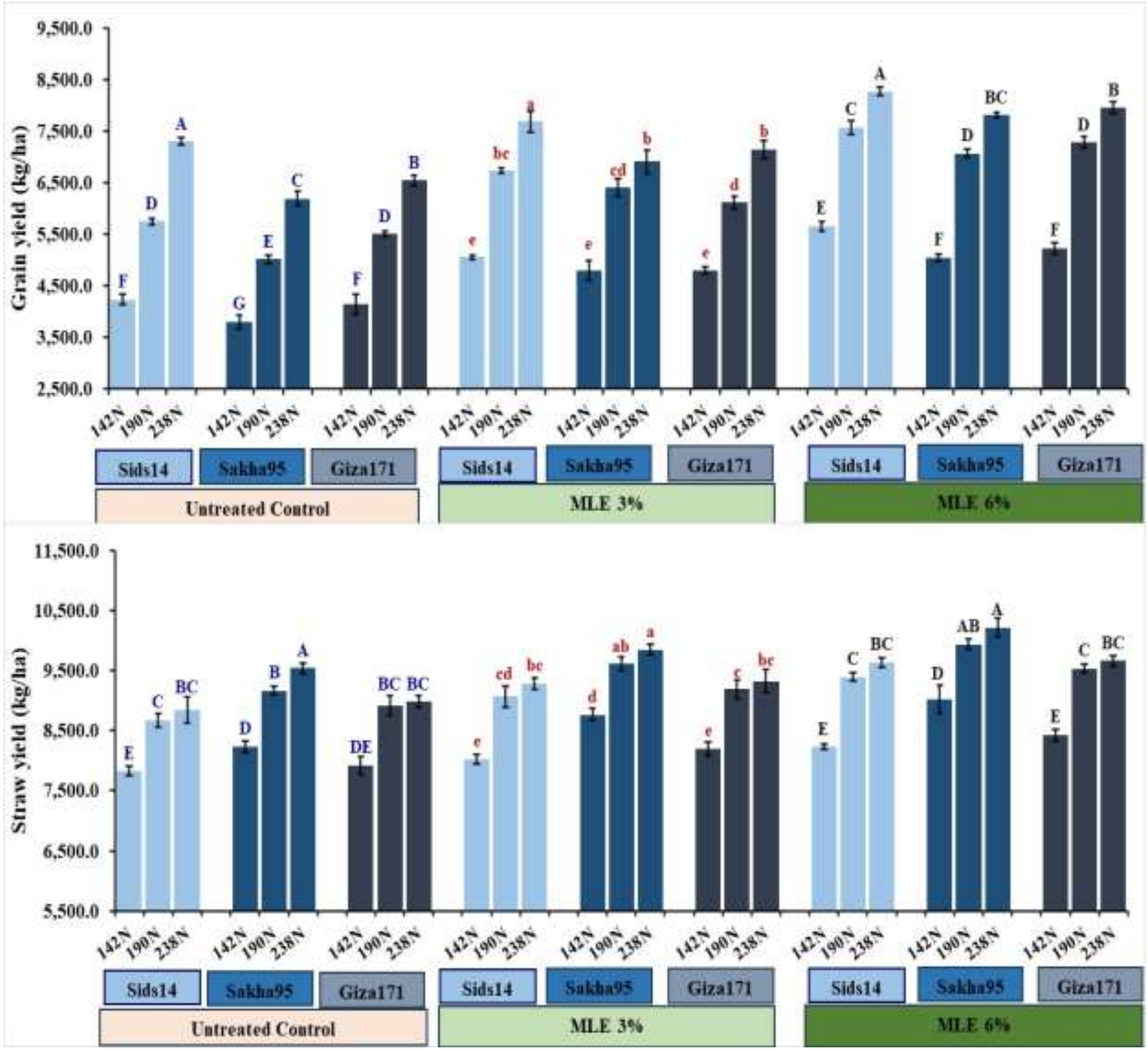

Figure 3. Grain and straw yield of wheat as affected by moringa leaf extract, N fertilizer levels, and cultivar over two growing seasons. The bars on each column represent ± standard error. Uppercase blue letters correspond to significant differences among untreated control treatments, lowercase red letters represent significant differences among 3% MLE treatments, and uppercase red letters signify significant differences among 6% MLE treatments according to LSD at $p \leq 0.05$.

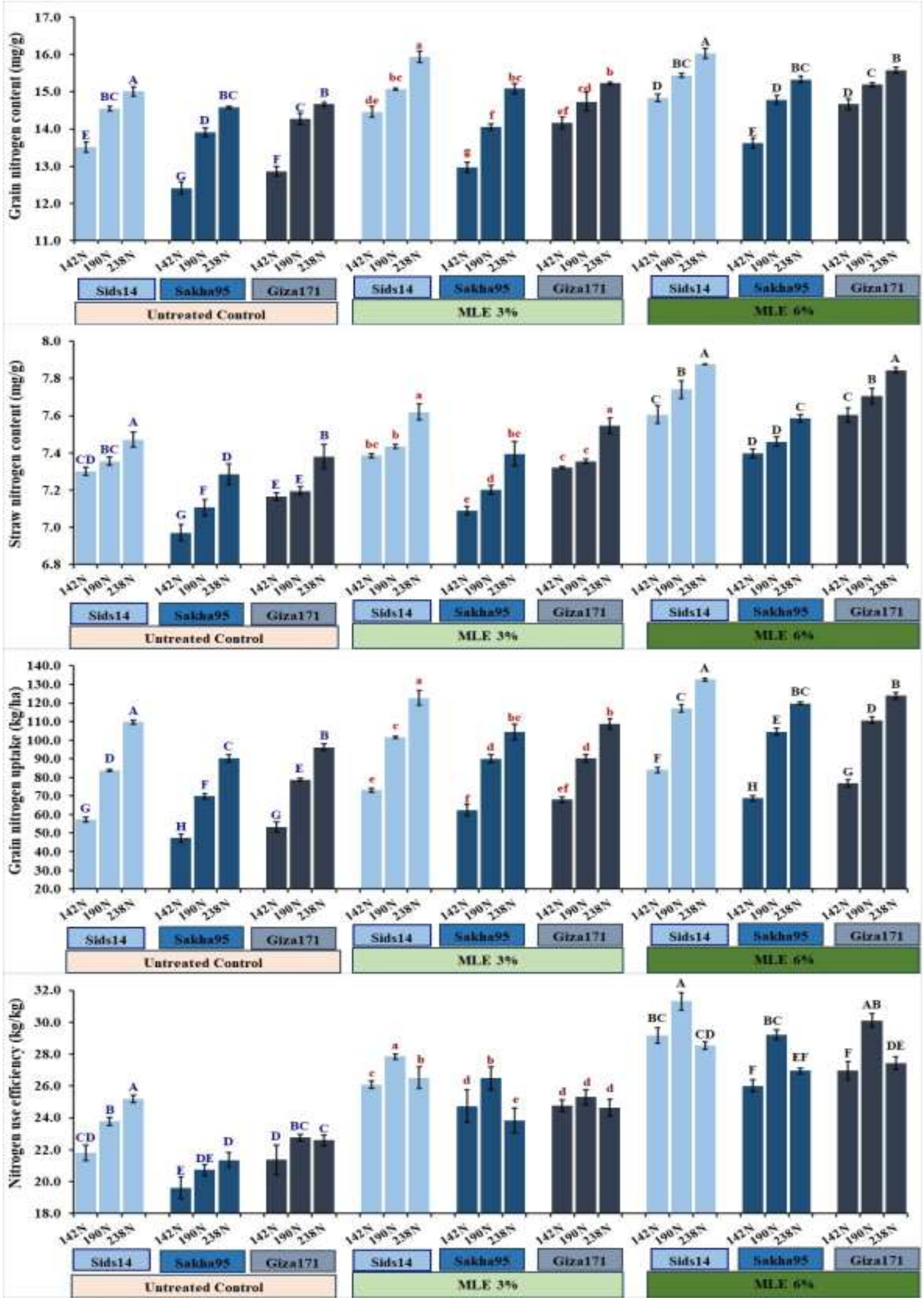

Figure 4. Impact of moringa leaf extract and nitrogen fertilizer levels on grain nitrogen content, straw nitrogen content, grain nitrogen uptake, and NUE in wheat cultivars over two growing seasons. The bars on each column represent ± standard error. Uppercase blue letters correspond to significant differences among untreated control treatments, lowercase red letters represent significant differences among 3% MLE treatments, and uppercase red letters signify significant differences among 6% MLE treatments according to LSD at $p \leq 0.05$.

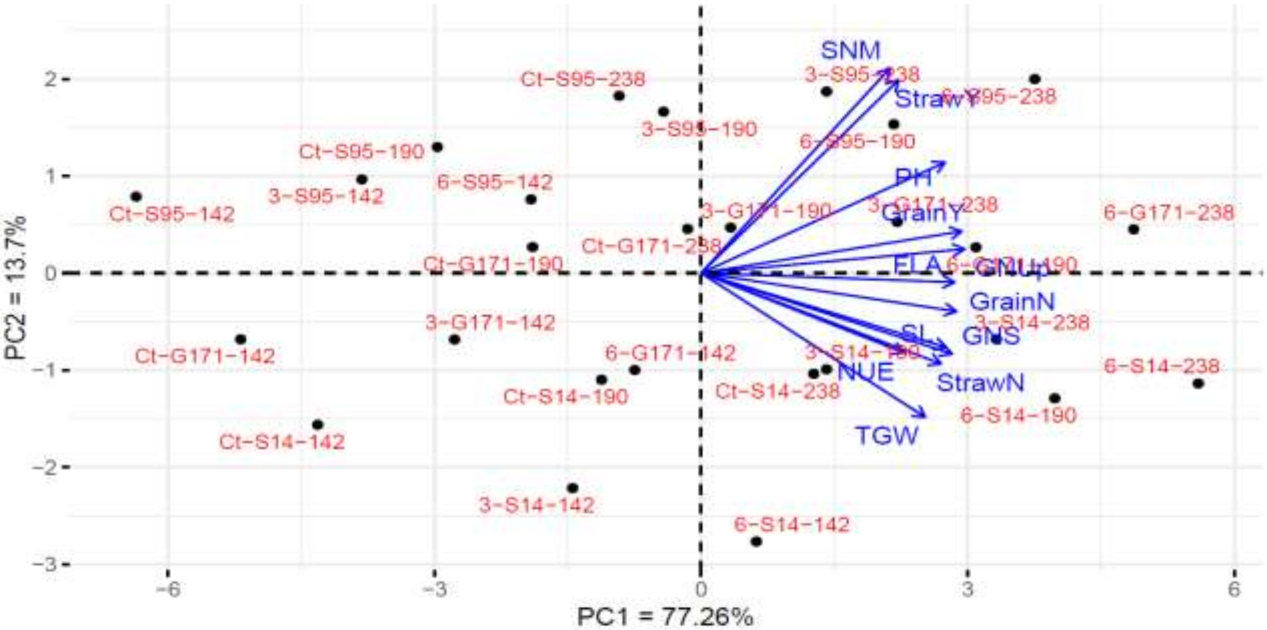

Figure 5. Principal component analysis biplot of agro-physiological and nitrogen traits under combined MLE and nitrogen fertilizer treatments in wheat over two growing seasons. The applied treatments in the PC biplot are abbreviated as follows: 6 indicates 6% MLE, 3 indicates 3% MLE, and Ct refers to the untreated control (0% MLE). S14 is Sids 14 cultivar, S95 is Sakha 95, and G171 is Giza 171. The numbers 142, 190, and 238 represent the applied nitrogen fertilizer levels of 142, 190, and 238 kg N/ha, respectively. SNM: Number of spikes per m², StrawY: Straw yield, GrainY: Grain yield, PH: Plant height, FLA: Flag leaf area, SL: Spike length, GNS: Number of grains per spike, TGW: 1000-grain weight, GrainN: Grain nitrogen content, StrawN: Straw nitrogen content, GNUp: Grain nitrogen uptake, and NUE: Nitrogen use efficiency.

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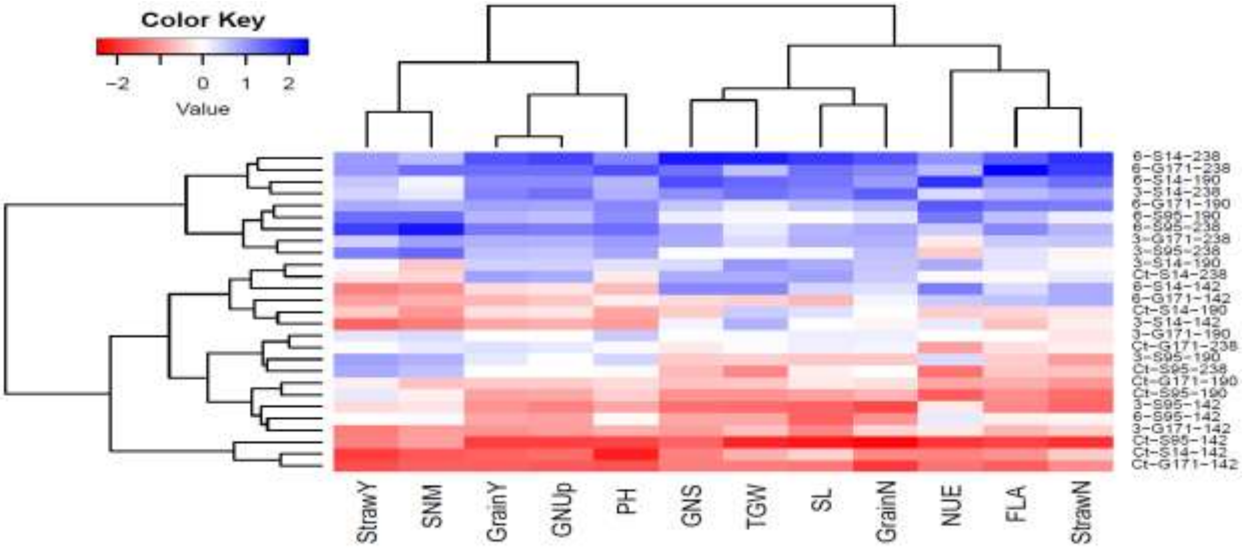

Figure 6. Hierarchical clustering heatmap of wheat agronomic and nitrogen-related traits across MLE, nitrogen, and cultivar treatments over two growing seasons. The applied treatments in the PC biplot are abbreviated as follows: 6 indicates 6% MLE, 3 indicates 3% MLE, and Ct refers to the untreated control (0% MLE). S14 is Sids 14 cultivar, S95 is Sakha 95, and G171 is Giza 171. The numbers 142, 190, and 238 represent the applied nitrogen fertilizer levels of 142, 190, and 238 kg N/ha, respectively. SNM: Number of spikes per m², StrawY: Straw yield, GrainY: Grain yield, PH: Plant height, FLA: Flag leaf area, SL: Spike length, GNS: Number of grains per spike, TGW: 1000-grain weight, GrainN: Grain nitrogen content, StrawN: Straw nitrogen content, GNUp: Grain nitrogen uptake, and NUE: Nitrogen use efficiency.

Table S1. Weather data for the experimental site during the wheat growing seasons of 2019-2020 and 2020-2021.

Month	**Maximum temperature (°C)**	**Iinimum emperature (°C)**	**elative umidity (%)**	**otal recipitation (mm)**
	irst season (2019-2020)			
November	7.10	9.07	1.99	.01
December	0.45	5.89	8.02	.45
January	6.96	3.19	0.88	.05
February	9.55	2.84	3.05	.10
March	3.32	3.46	2.82	.18
April	0.33	5.40	0.76	.01
	econd season (2020-2021)			
November	3.56	8.41	7.47	.01
December	1.74	6.19	7.09	.12
January	0.58	4.50	1.10	.81
February	2.07	4.99	8.71	.37
March	3.03	3.75	9.03	.72
April	0.90	5.05	6.93	.08