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The Role of Foliar Nutrition in Tolerance of Wheat (*Triticum aestivum* L.) Varieties under Drought Stress

Azhar Taher Sleibi[1*] and Asaad Kadhim Abdullah[2]
[1,2]Department of Biology, College of Education for Pure Sciences (Ibn-Al-Haitham), University of Baghdad, Baghdad, Iraq.

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Abstract

Drought stress resulting from the depletion of available water in the soil leads to disturbances in the plant's metabolic processes, reducing productivity due to its decreased ability to absorb water and essential nutrients. This study aimed to evaluate the interactive effects of three bread wheat varieties (Adna 99, Bohooth 22, and Wafia), three drought stress levels (25% control, 50%, and 75% depletion of available water), and foliar application of micronutrients(control) iron (Fe), zinc (Zn), copper (Cu), and manganese (mn) at 100 mg L^{-1}) and no spraying on agro-biochemical characteristics. The experiment was conducted using a randomized complete block design (RCBD) with three replications. The results showed that as drought conditions got worse, from 25% to 75%, the antioxidant activity increased a lot, with superoxide dismutase (SOD) going up by 138.44%, catalase (CAT) by 81.40%, α-tocopherol by 9.88%, and glycine betaine by 11.55%. On the other hand, important factors for crop yield went down during severe drought, with the number of spikes dropping by 23%, grain yield by 20.77%, weight of a thousand grains by 9.25%, biological yield by 11.53%, and harvest index by 16.33%. Among the genotypes tested, Adna 99 performed the best under severe drought when foliar micronutrients were applied, followed by Bohooth 22, while Wafia was very sensitive to both drought and lack of micronutrients. These results suggest that using foliar micronutrients along with drought-resistant genotypes could be an effective way to improve wheat production when water is scarce. Future research should focus on the molecular validation of antioxidant-related gene expression involved in drought tolerance mechanisms.

Keywords: Drought stress, Wheat varieties, Micronutrient foliar application, Antioxidants (enzymatic, non-enzymatic)

1.Introduction

Drought is a major challenge in plant growth, significantly affecting the uptake and transport of essential nutrients such as iron, copper, magnesium, and zinc and leading to disruptions in physiological processes such as metabolism and reduced productivity. (1) Wheat (*Triticum aestivum* L.) is one of the most important cereal crops globally, cultivated on large areas and ranking first worldwide. Bread wheat is a staple food for millions, including the Iraqi population, and holds strategic importance in achieving food security (2). Drought water stress threatens wheat production, requiring strategies to enhance drought tolerance. Micronutrients (Fe, Zn, Cu, and Mn) play crucial roles in metabolism and stress tolerance resistance by physiological and molecular mechanisms regulation (3). TThey improve seed germination, water use efficiency, membrane stability, stomatal regulation, and photosynthesis; they interact with hormones; they enhance stress protein expression; and they activate antioxidant enzymes (4, 5). Studies also indicate that applying iron and zinc in nanoparticle form, combined with bacterial and amino fertilizers, can further enhance drought tolerance in crops like wheat (6, 7). Drought stress increases reactive oxygen species (ROS) production, causing oxidative damage to lipids, proteins, and DNA. Antioxidants like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione neutralize ROS, preserving cellular functions. Water stress also triggers physiological and morphological changes (8), stimulating antioxidant enzyme activity in wheat (*Triticum* spp.) to enhance stress resistance. Drought stress acts as a primary physiological constraint that impairs water and nutrient uptake, disrupts stomatal regulation, and alters photosynthetic efficiency, ultimately leading to oxidative stress. These disruptions trigger significant changes in biochemical markers, such as antioxidant enzyme activities and osmolyte accumulation, and negatively impact agronomic traits, including grain yield, spike number, and biomass accumulation (9, 10). Moreover, studies have highlighted the roles of non-enzymatic antioxidants such as proline and α-Tocopherol in enhancing plant stress tolerance. These compounds maintain cellular water balance and protect membranes from free radical damage. Foliar spraying with α-Tocopherol has been shown to improve antioxidant activity and boost drought tolerance (11). Genetic variation also influences wheat varieties' ability to withstand water stress, guiding the selection of stress-resilient varieties with effective antioxidant traits (12).Despite extensive research on the physiological and biochemical effects of drought stress in wheat, there remains a lack of integrated studies that simultaneously evaluate genotype-dependent responses and the role of foliar micronutrient application in enhancing antioxidant defense mechanisms. This study addresses this gap by investigating the interactive effects of drought severity, genotype variation, and micronutrient supplementation on antioxidant activity and yield traits in wheat under controlled conditions. It is hypothesized that foliar application of Fe, Zn, Cu, and Mn will improve the biochemical and agronomic performance of drought-tolerant genotypes, particularly under severe water deficit. This study stands out because it looks at two stress factors at the same time, drought and lack of micronutrients, allowing for a clear understanding of how different wheat types respond when both stresses are present. It aims to analyze the interactive effects of these factors on yield-related traits and antioxidant activity (enzymatic and non-enzymatic) to identify the most tolerant genotypes and distinguish them from the sensitive ones.

2. Materials and Methods

2.1. Study Site

The field experiment was conducted in the Biology Department, College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad, during the 2024 season.

2.2. Experiment factors

Three wheat varieties, Adna 99, Bohooth 22, and Wafia, were planted using seeds from the Agricultural Research Department (Abu Ghraib), the National Wheat Development Program in Iraq, and the Seed Inspection Department. A split-split plot design within RCBD was used with three replicates. Factors included variety of wheat, water stress (25%, 50%, and 75% soil water depletion), and micronutrient spraying (Fe, Zn, Cu, Mn at 100 mg/L^{-1} or no spraying). Water stress (D1, D2, D3) was applied at 30 days based on field capacity (13), and at 45 days, plants were sprayed with $ZnSO_4.7H_2O$, $MnSO4.H_2O$, EDTAFeNa, and $CuSO_4.5H2O$.

2.3. Studied characteristics

2.3.1. Yield characteristics and components

The average length of 10 main spikes per experimental unit (m²) was measured, The average grain number of five spikes, one from each line of the experimental unit, was manually counted. Based on the total spike count per m², 100 grains were randomly selected from each unit's yield and converted to 1000-grain weight (14), the number of spikelets (spikelet·spike^{-1}) from the average number of spikes for five spikes per experimental unit. Grain yield was calculated per m² and converted to ton·ha^{-1}. The harvested plants per unit were dried and converted to biological yield (ton·ha^{-1}). The harvest index was estimated as (grain yield/biological yield) × 100 (15).

2.3.2. Enzymatic antioxidants

2.3.2.1. SOD enzyme (Absorption Unit·ml^{-1})

Efficacy was estimated by Beyer and Fridowich (16).

2.3.2.2. POD enzyme (Absorption Unit·ml^{-1})

The estimate was made using the method described by Müftügil (17).

2.3.2.3. CAT enzyme (Absorption Unit·ml^{-1})

It was estimated according to the method of Bergmeyer (18). Each enzyme was measured using three biological replicates per treatment.

2.3.3. Non-enzymatic antioxidants

2.3.3.1. Proline acid concentration (µg·g^{-1})

The concentration of proline acid was estimated by the method of Bates *et al.* (19).

2.3.3.2. α-Tocopherol concentration (µg·g^{-1})

The estimate was made using the method described by Rosenberg (20).

2.3.3.3. Glycine Betaine Concentration (µg·g^{-1})

Estimated by the method described by Grieve and Grattan (21). Each compound was measured from three biological replicates per treatment.

2.4. Statistical analysis and experimental design.

Data were subjected to two-way and three-way ANOVA using the SAS (Statistical Analysis System) program to analyze data and examine the effects of drought stress, Micronutrients spray, and their interaction on the studied characteristics of selected varieties (22) Significant differences between means were tested using the Least Significant Difference (LSD) test at a 0.05 probability level. Additionally, RStudio version 4.3.2 (2024) was employed for Principal Component Analysis (PCA) to identify variables with the greatest impact on overall variance. The software also streamlined statistical processes, revealed patterns and key relationships among variables, and supported precise conclusions (23).

3. Results

3.1. Yield characteristics and components

Table 1 results showed significant differences in spike length due to water stress, with 25% stress producing the longest spikes (17.81 cm) and 75% stress the shortest (15.16 cm), a 17.48% decrease. Spraying micronutrients increased spike length (16.92 cm) compared to non-sprayed plants (16.23 cm), a 4.25% rise. Adna 99 had the longest spike (19.01 cm), surpassing Wafia (12.47 cm) by 52.45%. The highest spike length (19.95 cm) was recorded in sprayed Bohooth 22 under 25% stress, while the shortest (9.40 cm) was in non-sprayed Wafia under 75% stress.

Table 1. Effect of drought, foliar application, varieties and interaction on yield characteristics and components of wheat

Treatments	Parameters							
	Spike length (cm)	**Number of grains (grain. spike-1)**	**Weight of 1000 grain (g)**	**Number of spikes (spike. m^{-2})**	**Number of spikelets (spikelet. spike^{-1})**	**Grain yield (tons. ha^{-1})**	**Biological yield (ton. ha^{-1})**	**Harvest Guide (%)**
Variety								
Adna 99	19.01	49.72	43.86	398.58	23.54	5.91	11.70	49.65
Bohooth 22	18.24	44.78	41.87	293.69	19.82	4.94	10.74	46.11
Wafia	12.47	40.48	38.46	225.77	14.71	3.51	9.22	37.98
L. S. D	0.99	1.88	3.23	18.09	2.57		0.91	1.02

Drought	Spike length (cm)	Number of grains (grain. spike^{-1})	Weight of 1000 grain (g)	Number of spikes (spike. m^{-2})	Number of spikelets (spikelet. spike^{-1})	Grain yield (tons. ha^{-1})	Biological yield (ton. ha^{-1})	Harvest Guide (%)
D1	17.81	49.16	43.52	354.49	20.81	5.20	11.12	48.92
D2	16.76	44.35	41.13	298.27	19.15	4.23	10.56	43.94
D3	15.16	41.48	39.54	265.28	18.11	4.12	9.97	40.93
L. S. D	0.99	1.88		3.23	18.09	2.57	0.91	1.02

Micronutrients	Spike length (cm)	Number of grains (grain. spike-1)	Weight of 1000 grain (g)	Number of spikes (spike. m^{-2})	Number of spikelets (spikelet. spike^{-1})	Grain yield (tons. ha^{-1})	Biological yield (ton. ha^{-1})	Harvest Guide (%)
-M	16.23	43.75	40.39	291.43	18.53	4.58	10.40	43.35
+M	16.92	46.25	42.40	320.59	20.19	4.99	10.70	45.81
L. S. D	0.43	2.34	1.78	17.54	3.09		1.44	0.94
				0.34				

Vari*Drought*Micro	Spike length (cm)	Number of grains (grain. spike-1)	Weight of 1000 grain (g)	Number of spikes (spike. m^{-2})	Number of spikelets (spikelet. spike^{-1})	Grain yield (tons. ha^{-1})	Biological yield (ton. ha^{-1})	Harvest Guide (%)
Adna 99*D1*-M	19.43	51.43	44.44	436.12	23.30	6.17	12.04	51.24
Adna 99*D2*-M	18.87	47.22	42.62	382.78	23.00	5.47	11.62	47.07
Adna 99*D3*-M	18.20	45.55	40.25	324.75	21.55	4.61	11.00	41.90
Adna 99*D1*+M	19.90	52.80	45.83	448.44	25.44	6.72	12.24	54.90
Adna 99*D2*+M	19.22	51.24	45.31	410.40	25.00	6.49	12.18	53.28
Adna 99*D3*+M	18.44	50.12	44.74	389.02	23.00	5.52	11.14	49.55
Bohooth*D1*-M	18.69	48.30	43.20	322.29	20.00	5.89	11.02	53.44
Bohooth*D2*-M	17.75	43.20	40.40	287.86	18.22	4.66	10.63	43.83
Bohooth*D3*-M	17.00	39.40	40.00	227.40	18.00	4.14	10.13	40.86
Bohooth*D1*+M	19.95	48.31	44.73	321.03	23.22	6.14	11.67	52.61
Bohooth*D2*+M	18.37	46.30	42.77	306.33	20.00	4.85	10.89	44.53
Bohooth*D3*+M	17.15	43.22	40.15	297.26	19.52	4.25	10.14	41.91
Wafia*D1*-M	13.90	45.86	40.50	295.42	15.00	3.95	9.78	40.38
Wafia*D2*-M	12.85	37.67	37.13	177.15	15.33	3.23	8.96	36.04
Wafia*D3*-M	9.40	35.12	35.02	169.15	12.39	3.00	8.46	35.46
Wafia*D1*+M	15.00	48.26	42.45	303.65	17.95	4.10	10.00	41.00
Wafia*D2*+M	13.50	40.50	38.56	225.12	13.40	3.55	9.12	38.92
Wafia*D3*+M	10.22	35.52	37.12	184.15	14.22	3.25	9.00	36.11
L. S. D	2.43	4.62	7.93	44.33	6.31	2.09	2.23	3.49

Table 1 results showed significant differences in grains per spike, decreasing by 18.52% from 49.16 to 41.48 (grain. Spike^{-1}) as water stress increased from 25% to 75%. Spraying led to a 5.41% increase, reaching 46.25 (grain. Spike^{-1}) compared to 43.75 (grain. Spike^{-1}) without spraying. Adna 99 had the highest count at 49.72 (grain. Spike^{-1}), exceeding Wafia at 40.48 (grain. Spike^{-1}) by 22.82%. The highest value of 52.80 (grain. Spike^{-1}) was recorded in sprayed Adna 99 under 25% stress, while the lowest, 35.12 (grain. Spike^{-1}), was in non-sprayed Wafia under 75% stress.

The results in **Table 1** showed a significant effect of water stress on the weight of 1000 grains, as increasing stress to 75% reduced the weight to 39.54 g compared to 43.52 g at 25% stress, a decrease of 9.25%. Micronutrient treatments also had a significant impact, with spraying yielding a higher weight of 42.40 g compared to 40.39 g without spraying, an increase of 4.97%. Among varieties, Adna 99 significantly outperformed, recording the highest weight of 43.86 g, while Wafia had the lowest 38.46 g, an increase of 10.24%. The triple interaction showed no significant differences, but the highest weight of 45.83 g was recorded for sprayed Adna 99 at 25% stress, while the lowest weight of 35.02 g was observed for non-sprayed Wafia at 75% stress. The results of **Table 1** indicated a significant effect of available water depletion levels on the number of spikes (spike. m^{-2}). Exposure to 75% stress resulted in the lowest average of 265.28 (spike. m^{-2}), while 25% stress recorded the highest average of 345.49 (spike. m^{-2}), a decrease of 23%. Spraying micronutrients significantly increased the number of spikes to 320.59 (spike.m^{-2}) compared to 291.43 (spike. m^{-2}) without spraying, an increase of 10%. Varietal differences were also significant, with Adna 99 achieving the highest value of 398.58 (spike. m^{-2}), while Wafia had the lowest at 225.77 (spike. m^{-2}), an increase of 77%. The triple interaction showed significant differences, as spraying Adna 99 with micronutrients under 25% stress resulted in the highest number of 448.44 (spike. m^{-2}), while not spraying Wafia under 75% stress led to the lowest value of 169.15 (spike. m^{-2}).

Table 1 showed significant differences in spikelet number (spikelet. Spike^{-1}) under different water stress levels. Increasing stress to 75% reduced the number to 18.11, compared to 20.81 at 25% stress— a 12.97% decrease. Micronutrient addition had no significant effect, with an average of 20.19 compared to 18.53 without addition— an 8.96% increase. Adna 99 recorded the highest average (23.54), while Wafia had the lowest (14.71), a 59.42% difference. The triple interaction was significant, with Adna 99, addition, and 25% stress yielding the highest value (25.44), while Wafia, no addition, and 75% stress recorded the lowest (12.39).

The results of **Table 1** showed a significant effect of water stress on grain yield, as increasing stress to 75% reduced yield to 4.12 (tons.ha^{-1}) compared to 5.20 (tons.ha^{-1}) at 25% stress, a decrease of 20.77%. Spraying micronutrients increased yield to 4.99 (tons.ha^{-1}) compared to 4.58 (tons. Ha^{-1}) without spraying, an increase of 8.95%. The variety Adna 99 recorded the highest yield at 5.91 (tons.ha^{-1}), while Wafia had the lowest at 3.51 (tons.ha^{-1}), an increase of 68.37%. The triple interaction showed significant differences, with the combination of spraying, Adna 99, and 25% stress achieving the highest yield of 6.72 (tons.ha^{-1}), while the combination of no spraying, Wafia, and 75% stress recorded the lowest at 3.00 (tons.ha^{-1}). The results of **Table 1** showed a significant effect of water stress on the biological yield of wheat, with yield decreasing from 11.12 (tons.ha^{-1}) at 25% stress to 9.97 (tons.ha^{-1}) at 75% stress, a reduction of 10.34%. Spraying micronutrients increased yield to 10.70 (tons.ha^{-1}) compared to 10.40 (tons.ha^{-1}) without spraying, an increase of 2.8%. The variety Adna 99 recorded the highest yield at 11.70 (tons. Ha^{-1}), while Wafia had the lowest at 9.22 (tons.ha^{-1}), an increase of 26.90%. The triple interaction showed variations in biological yield, with the combination of spraying, Adna 99, and 25% stress achieving the highest yield of 12.24 (tons.ha^{-1}), while the combination of no spraying, Wafia, and 75% stress recorded the lowest at 8.46 (tons. ha^{-1}).

The results of **Table 1** indicated significant differences in the harvest index due to varying water stress levels. Increasing water stress from 25% to 75% reduced the harvest index from 48.92% to 40.93%, a decrease of 16%. Micronutrient treatments show significant differences, but the addition treatment recorded a slightly higher average of 45.81% compared to 43.35% in the non-addition treatment, an increase of 5.68%. The variety Adna 99 significantly outperformed Wafia, with a harvest index of 49.65% compared to 37.98%, an increase of 30.73%. The triple interaction revealed significant differences, with Adna 99 under 25% water stress and micronutrient addition achieving the highest value of 54.90%, while Wafia under 75% stress without micronutrient addition recorded the lowest value of 35.46%.

3.2. Determination of enzymatic and non-enzymatic antioxidants

Table 2 shows a significant increase in SOD enzyme activity under drought stress, reaching 76.04 (absorption unit.ml-^{1}) at 75% stress, compared to 31.89 at 25% stress, with a 138.44% increase. Enzyme activity was higher under non-addition of micronutrients (56.32 absorption unit.ml^{-1}) than with addition (44.27 absorption unit.ml^{-1}), increasing by 27.21%. Among varieties, Adna 99 showed the highest SOD activity (78.95 absorption unit.ml^{-1}), significantly outperforming Wafia (18.76 absorption unit.ml^{-1}) by 320.84%. Under 75% stress without micronutrients, Adna 99 recorded the highest activity (161.50 absorption unit.ml^{-1}), while Wafia showed the lowest (15.00 absorption unit.ml^{-1}).

The results in **Table 2** show a significant increase in POD under 75% stress (26.20 absorption unit.ml^{-1}) compared to 25% stress (25.79 absorption unit.ml^{-1}), with a 1.59% rise. Micronutrient application reduced enzyme activity (25.00 absorption unit.ml^{-1}) compared to without-spraying (27.00 absorption unit.ml^{-1}), showing an 8% increase. Adna 99 recorded the highest activity (30.33 absorption unit.ml^{-1}), surpassing Wafia (18.64 absorption unit.ml^{-1}) by 62.71%. Under 75% stress without spraying, Adna 99 showed the highest activity (35.30), while Wafia had the lowest (11.83) under the same stress with spraying.

Table 2 shows a significant increase in CAT enzyme activity with higher stress levels, reaching (21.06 absorption unit.ml^{-1}) at 75% stress compared to (11.61 absorption unit.ml^{-1}) at 25% (81.40% rise). Enzyme activity was higher under non-spraying (16.51 absorption unit.ml-^{1}) than spraying (15.88 absorption unit.ml^{-1}). Adna 99 recorded the highest activity (21.63 absorption unit.ml^{-1}), while Wafia had the lowest (11.17 absorption unit.ml^{-1}), with a 93.64% increase. The triple interaction showed Adna 99 under 75% stress without micronutrient addition had the highest value (33.24 absorption unit.ml^{-1}), whereas Wafia under the same stress with addition had the lowest (10.11 absorption unit.ml^{-1}).

The result of **Table 2** shows a significant increase in proline from 25% to 75% led to a significant increase in the average concentration of proline from 91.64 to 95.82 (µg.g^{-1}) respectively, with an increase of 5%. The averages of micronutrients showed significant differences in proline concentration, increasing from 93.10 (µg.g^{-1}) with spraying to 93.90 (µg.g^{-1}) without spraying, a rise of 0.85%. The variety Adna 99 had the highest proline content (100.49 µg.g^{-1}), while Wafia recorded the lowest 87 (µg.g^{-1}), with a 15.51% increase. The triple interaction showed significant differences, as Adna 99 under 75% stress without spraying had the highest proline activity ,107.39 (µg.g^{-1}), whereas Wafia under the same stress with spraying had the lowest, 85.01(µg. g^{-1}).

Table 2. Effect of drought, foliar application, varieties and interaction on antioxidants concentration of wheat.

Treatments	Parameters					
	SOD unit. ml^{-1}	**POD unit. ml^{-1}**	**CAT unit. ml^{-1}**	**Proline µg. g^{-1}**	**α-Tocopherol µg. g^{-1}**	**Glycine Betaine µg. g^{-1}**
Variety						
Adna 99	78.95	30.33	21.63	100.49	27.14	65.90
Bohooth 22	53.16	29.01	15.79	93.01	22.56	61.06
Wafia	18.76	18.64	11.17	87.00	12.27	45.21
L. S. D	2.17	0.26	0.82	1.29	1.88	6.34
Drought	SOD unit. ml^{-1}	POD unit. ml^{-1}	CAT unit. ml^{-1}	Proline µg. g^{-1}	α-Tocopherol µg. g^{-1}	Glycine Betaine µg. g^{-1}
D1	31.89	25.79	11.61	91.70	19.88	55.12
D2	42.95	26	15.92	93.04	20.24	55.56
D3	76.04	26.20	21.06	95.73	21.84	61.49
L. S. D	0.76	0.66	0.61	1.09	0.84	2.15
Micronutrients	SOD unit. ml^{-1}	POD unit. ml^{-1}	CAT unit. ml^{-1}	Proline µg. g^{-1}	α-Tocopherol µg. g^{-1}	Glycine Betaine µg. g^{-1}
-M(with spry)	56.32	27.00	16.51	93.90	21.91	56.72
+M(without spry)	44.27	25.00	15.88	93.10	19.40	58.05
L. S. D	0.62	0.54	0.50	0.49	0.69	1.75
Vari*Drought*Micro	SOD unit. ml^{-1}	POD unit. ml^{-1}	CAT unit. ml^{-1}	Proline µg. g^{-1}	α-Tocopherol µg. g^{-1}	Glycine Betaine µg. g^{-1}
Adna 99*D1*-M	23.50	26.66	11.22	97.19	28.57	59.08
Adna 99*D2*-M	89.50	32.43	21.32	100.33	28.65	62.80
Adna 99*D3*-M	161.50	35.30	33.24	107.39	30.98	77.11
Adna 99*D1*+M	54.40	27.83	10.68	96.94	19.34	50.13
Adna 99*D2*+M	38.00	28.16	20.20	99.35	25.55	59.19
Adna 99*D3*+M	106.75	31.65	33.13	102.09	29.77	87.10
Bohooth*D1*-M	31.50	25.83	12.38	90.62	23.15	58.80
Bohooth*D2*-M	64.25	32.86	16.00	91.31	20.16	63.15
Bohooth*D3*-M	80.25	32.43	20.12	96.90	30.45	69.47
Bohooth*D1*+M	34.50	26.50	11.46	90.23	20.67	57.71
Bohooth*D2*+M	31.25	25.61	15.44	93.39	22.73	56.92
Bohooth*D3*+M	77.25	30.86	19.35	95.66	18.12	60.32
Wafia*D1*-M	25.15	24.63	12.34	87.58	13.72	49.29
Wafia*D2*-M	16.25	17.75	11.56	85.90	11.23	38.40
Wafia*D3*-M	15.00	15.15	10.42	87.88	10.30	32.46
Wafia*D1*+M	22.19	23.33	11.60	87.67	13.86	55.73
Wafia*D2*+M	18.50	19.16	11.03	87.98	13.12	52.91
Wafia*D3*+M	15.50	11.83	10.11	85.01	11.42	42.52
L. S. D	31.87	4.63	1.50	2.67	5.07	8.26

The results shown in **Table 2** show a significant increase in α-tocopherol when the level of water stress increased from 25% to 75%, if the concentration of α-tocopherol increased from 19.88 to 21.84 (µg.g^{-1}) with an increase of 9.86%. Statistically significant differences were observed in α-tocopherol concentration due to micronutrient treatments, where spraying reduced its average from 21.91 (µg.g^{-1}) to 19.40 (µg.g^{-1}), a decrease of 12.9%. The variety Adna 99 recorded the highest concentration 27.14 (µg. g^{-1}), while Wafia had the lowest at 12.27 (µg.g^{-1}), with an increase of 121.18%. In the triple interaction, Adna 99 without spraying under 75% stress gave the highest value 30.98 (µg.g^{-1}), while Wafia under the same conditions recorded the lowest of 10.30 (µg.g^{-1}).

Table 2 results show a significant increase with increasing drought (25% to 75%) raised glycine betaine concentration from 55.12 to 61.49 (µg. g^{-1}), an 11.55% increase. Micronutrient application had a significant effect, as sprayed plants exhibited a higher glycine betaine concentration (58.05 μg·g^{-1}) compared to non-sprayed plants (56.72 μg·g^{-1}). Adna 99 recorded the highest concentration (65.90 μg. g^{-1}), while Wafia had the lowest (45.21 μg.g^{-1}). The triple interaction's highest concentration (87.10 μg.g^{-1}) was recorded in the triple interaction of the sprayed Adna 99 variety under 75% water, while non-sprayed Wafia under the same stress had the lowest (32.46 μg.g^{-1}).

3.3. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) reduces variables by summarizing data into key components, identifying major variance factors, simplifying analysis, and revealing patterns. **Table 3** results show that PC1 strongly reflects growth and yield traits, with high positive loads on spikelet count (0.987), spike count (0.926), biological yield (0.969), and grain yield (0.941), making it a key yield indicator. PC2 captures stress response variability, linked to antioxidant enzymes CAT (0.789) and SOD (0.746). PC3, PC4, and PC5 contribute minimally, representing secondary traits.

Table 3. Results of PCA for Five Principal Components of Study Traits under the Main Effects of Experimental Factors.

Trait	PC1	PC2	PC3	PC4	PC5
CAT	**0.582**	**0.789**	**0.173**	**0.052**	**-0.079**
POD	0.913	0.255	-0.309	-0.075	0.002
SOD	0.657	0.746	0.051	-0.001	0.090
Proline	0.855	0.481	0.183	-0.063	-0.027
α-Tocopherol	0.921	0.355	-0.112	-0.112	0.024
Glycine betaine	0.877	0.438	-0.094	0.167	0.039
spike L.	0.949	-0.188	-0.242	0.016	-0.068
Grain N.	0.857	-0.489	0.152	-0.021	0.048
spike N.	0.926	-0.290	0.231	-0.070	0.011
spikelet N.	0.987	-0.129	0.073	0.043	-0.039
1000 grain	0.886	-0.450	0.041	0.103	0.000
Grain.Y	0.941	-0.338	-0.003	-0.005	0.005
B.Y	0.969	-0.241	0.003	-0.043	-0.043
H. index	0.916	-0.394	-0.051	0.032	0.044
Eigenvalues	10.870	2.699	0.328	0.074	0.030
Variance %	77.643	19.277	2.340	0.528	0.212
Cumulative %	77.643	96.920	99.260	99.788	100.000

Figure 1, which illustrates the five principal components derived from the analysis of the studied traits, indicates that the distribution of the wheat varieties varied across the principal components. The variety Adna *99* was represented in both PC1 and PC2, reflecting superior performance in both productivity and physiological traits under stress conditions, thereby confirming its high tolerance to drought and micronutrient deficiency. In contrast, the variety Bohooth *22* was positioned near the center of the plot, close to the intersection of the two axes, which may be interpreted as a balanced response in terms of yield performance and oxidative stress. The variety Wafia, on the other hand, was located in the quadrant associated with PC2, closer to traits linked with oxidative stress tolerance and farther from productivity-related traits.

Regarding the levels of soil-available water depletion, the 75% treatment—representing the most severe water stress—was associated with physiological traits along PC2, indicating a clear activation of antioxidant responses. The 50% depletion level occupied an intermediate position, while the 25% treatment was located closer to yield-related traits on PC1, reflecting relatively optimal growing conditions. As for the effect of micronutrient application, the spraying treatment aligned with enhanced productivity traits (PC1), highlighting the role of micronutrients in promoting growth and yield. In contrast, the non-sprayed treatment was positioned toward the physiological defense traits (PC2), indicating the activation of antioxidant pathways in the absence of nutrient supplementation.

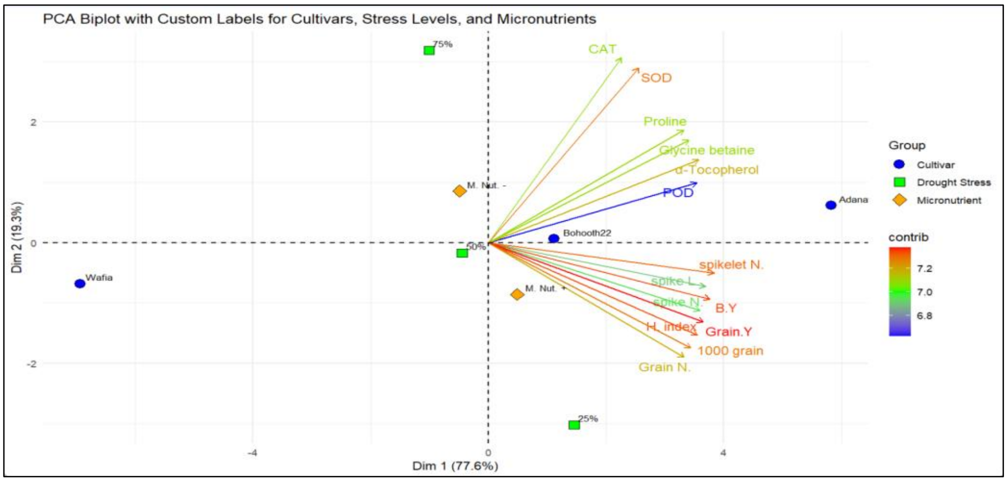

Figure 1. Two-Dimensional Plot of Principal Component Analysis (PCA) Illustrating the Relationship Between Studied Traits for Three Wheat Varieties Under + and - Micronutrient Treatments and Three Levels of Water Stress (25%, 50%, 75%)

4. Discussion

The results in **Table 1** show that Adna 99 significantly outperformed the other varieties in yield characteristics, followed by Bohooth 22. The drought tolerance of these varieties is attributed to high gene expression of *SOD* and *BADH*, which neutralize free radicals and reduce oxidative stress. Resistance to micronutrient deficiencies may be linked to increased expression of genes like ZIP7, COP1, and DMAs, which enhance the absorption of zinc, iron, and copper, and improve nutrient uptake. While the variety Wafia showed the opposite of what was mentioned, as it exhibited a significant decrease in all studied yield traits, this aligns with previous studies conducted by Zhang *et al.* (24). High water stress reduces spike length, ear and grain numbers, grain yield, and harvest index by inhibiting photosynthesis and carbon metabolism, limiting nutrient accumulation, and increasing reactive oxygen species (ROS), which damage proteins and cell membranes (25). Furthermore, water stress reduces dry matter accumulation by limiting shoot and root growth, increasing toxic ion concentrations, and causing stomatal closure, which restricts nutrient absorption and disrupts vital processes, ultimately lowering biological yield (26). The study by Hashim *et al.* (27) confirmed that wheat tolerance to water stress and yield are linked to physiological efficiency, with micronutrients like Fe, Zn, Cu, and Mn playing key roles. Their deficiency under stress reduces photosynthesis, respiration, and enzyme regulation, leading to poor growth. However, micronutrient application improves yield by enhancing physiological processes, enzyme activity, energy production, and antioxidant function (28). Studies also confirm that spraying zinc and iron on soft wheat increases spike length and grain count per spike compared to untreated plants (29). Wang *et al.* (30) found that micronutrient availability enhances plant resource efficiency, improving growth and yield even under stress. Tolerant varieties regulate gene expression to maintain high yields despite water and micronutrient deficiencies. *Adna 99* excelled across all water stress levels (25%, 50%, 75%), particularly under 75% stress without micronutrient addition, showing the highest yield traits. *Bohooth 22* followed closely, while *Wafia* performed poorly. *Adna 99*'s resilience highlights its suitability for water-limited regions. Its superiority may stem from better micronutrient availability, which supports key enzymes (Cu/Zn-SOD), ferredoxin activity, chlorophyll synthesis, and electron transport efficiency. (31). The interaction of copper and manganese enhances plant resilience to water stress by improving metabolism, energy production, and cell protection, leading to increased spike length, spikelet number, and overall yield. (32). Yield-related traits such as grain yield, biological yield, number of spikelets per spike, and number of spikes per unit area are key contributors to overall wheat productivity, as they collectively determine the plant's capacity to accumulate and allocate assimilates under both optimal and stress conditions. Adna 99's strong association with PC1, influencing yield, highlights its high productivity potential. The 1000-grain weight and harvest index also contribute to yield improvement (33). The 1000-grain weight boosts yield by increasing grain size, while the harvest index measures resource efficiency in yield production. Traits such as grain yield, biological yield, spikelet, and spike number align with PCA results, where Adana 99's strong PC1 association underscores its yield potential. Wafia's stress-response mechanisms confirm the trade-off between stress tolerance and productivity (34 -36). This supports the notion that balancing yield-improving traits with stress tolerance is crucial in breeding programs.

Antioxidant enzyme activity is positively associated with plant tolerance to abiotic stress. In the present study, the variety Wafia exhibited a marked reduction in yield-related traits (PC1), accompanied by a significant increase in antioxidant-related traits (PC2). This indicates a physiological trade-off, where the plant allocates more resources to stress defense mechanisms at the expense of productivity. While such a strategy enhances survival under adverse conditions, it compromises yield efficiency. Therefore, identifying and selecting genotypes that maintain a balance between stress tolerance and high yield potential is essential for sustainable crop improvement. Recent studies, such as those (37), highlight the significance of genetic variability and the application of Principal Component Analysis (PCA) in discriminating drought-tolerant genotypes. These findings support the integration of PCA-based selection approaches into breeding programs aimed at improving crop resilience.

Table 2 demonstrates that the variety Adna *99* significantly excelled in the effectiveness of enzymatic antioxidants, as the levels of defense enzymes increased compared to the rest of the varieties. This superiority is attributed to the high gene expression of *SOD* (superoxide dismutase), *COP* (copper transport 1), and *ZIP* (zinc/iron-regulated transporter-like protein). The *SOD* gene contributes to reducing oxidative damage and enhancing the plant's ability to withstand water stress (38). The *COP* gene promotes the production of catalase (CAT), an enzyme responsible for eliminating hydrogen peroxide, thereby mitigating its toxic effects (39). Additionally, the *ZIP* gene enhances zinc uptake, which is essential for CAT activity, helping reduce ROS accumulation and protect cells from damage caused by dehydration and micronutrient deficiency (40).

As shown in **Table 2**, high water stress (75%) increased enzymatic antioxidant activity (SOD, POD, CAT), which enhanced cellular defense and minimized oxidative damage. This activation helps neutralize ROS, thereby protecting proteins, lipids, and nucleic acids while maintaining the stability of critical physiological processes and improving drought tolerance (41). These findings are consistent with previous reports emphasizing the vital role of enzymatic antioxidants such as SOD and CAT in protecting plant cells against oxidative stress under drought conditions (42). The study conducted by Sun *et al*. (43) also confirmed that these enzymes are essential in ROS regulation and stress resilience.

Micronutrients are crucial for the functionality of antioxidant enzymes. In their absence, oxidative stress intensified, stimulating a compensatory increase in enzyme activity.

However, foliar application of micronutrients significantly reduced ROS levels and stabilized enzymatic activity (44). Under 75% water stress without micronutrient application, *Adna 99* displayed higher levels of enzymatic antioxidants than *Wafia*, attributed to more robust gene expression. In contrast, *Wafia* exhibited weaker antioxidant responses, reflecting its susceptibility to combined drought and micronutrient deficiency stress. These results are in agreement with other studies highlighting the synergistic role of micronutrients in improving enzymatic antioxidant activity and enhancing drought tolerance (45). The results of **Table 2** also showed that the variety Adna *99* significantly outperformed the other varieties in its content of non-enzymatic antioxidants, namely proline, α-tocopherol, and glycine betaine. This increase is attributed to the high gene expression of key regulatory genes responsible for the biosynthesis of these compounds, which enhance the plant's ability to adapt to environmental stress. The elevated levels of non-enzymatic antioxidants contribute to the stabilization of cellular membranes and the protection of photosynthetic systems under drought conditions (46). These findings are in line with previous studies that have confirmed the role of alpha-tocopherol in binding free radicals and protecting them from harmful interactions with phospholipids in cell membranes, enhancing the stability of these membranes, and protecting photovoltaic systems within cells from oxidative damage (47). Proline accumulation increases osmotic negativity, aiding water balance, maintaining turgor pressure, protecting proteins and membranes, and enhancing drought adaptation while counteracting ROS damage (48). The BADH gene stimulates glycine betaine production, aiding water balance, protecting proteins from oxidation, and enhancing respiration and ion transport under stress. Studies confirm that α-tocopherol, glycine betaine, and proline levels increase with stress, improving drought tolerance and plant defense. (49) Glycine betaine and proline are crucial for drought tolerance, especially with microelement deficiencies. Glycine betaine maintains water balance, stabilizes membranes, and protects against oxidative damage from iron and zinc deficiencies. Proline accumulates with zinc and copper shortages, compensating for the lack of antioxidants by stabilizing membranes and protecting proteins. (50). This is consistent with other studies that have shown that non-enzymatic antioxidant deficiencies may cause an increase in plant sensitivity to water stress due to the plant's failure to form an appropriate defense response against ROS accumulation (51). In addition, microelements contribute to the regulation of many metabolic pathways and cellular signaling that enhance a plant's ability to adapt to water stresses, protect cell membranes from oxidation, and help convert ROS into harmless compounds, improving membrane stability and integrity (52). The results show that Adna 99 increases proline, α-tocopherol, and glycine betaine concentrations under 75% water stress without micronutrient spraying, indicating a strong defense response. In contrast, Wafia showed lower levels of these compounds, reflecting its sensitivity. These findings align with studies confirming that stress-tolerant varieties elevate non-enzymatic antioxidant compound levels to counteract cadmium stress effects (53).

5. Conclusion

Availability revealed clear genotype-dependent variation. Adna 99 demonstrated the highest adaptive capacity, characterized by enhanced yield performance and antioxidant defense mechanisms. Bohooth 22 exhibited intermediate resilience, whereas Wafia showed pronounced sensitivity, suggesting a limited ability to mitigate oxidative damage under stress conditions. The application of micronutrients (Fe, Zn, Cu, and Mn) played a critical role in strengthening both enzymatic and non-enzymatic antioxidant systems, thereby contributing to improved physiological stability and yield potential under water-limited environments. These findings underscore the value of integrating foliar micronutrient strategies with tolerant genotypes to enhance wheat performance under drought. Future studies are recommended to explore gene-level regulatory mechanisms underlying antioxidant responses across diverse agro-ecological zones.

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Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this research.

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