



Biochemical and Physiological Response of Egyptian Wheat Genotypes to Drought Stress



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Abstract

Drought one of the imperative abiotic stresses which limits plant growth and causes numerous biochemical physiological and morphological changes in Wheat. In this study, ten Egyptian spring wheat genotypes used were obtained in cooperation with the Egyptian Gene Bank at the Agricultural Research Center. The greenhouse growth model was applied to test the morphological and physiological traits of the ten wheat genotypes in a Randomized Complete Block Design (RCBD) with three replications. The biochemical and physiological properties, total phenols, peroxidase enzyme, proline, total protein concentration, chlorophyll concentration and nutritional elements: (Ca^{+2} , Mg^{+2} , k^+ , Na^+), and total sugars were studied. The morphological traits were the plant height, flag leaf area, dry weight, fresh weight, thousand grain weight and grain yield. Results showed highly significant differences among genotypes for the measured traits under normal and drought stress. Increase in total phenols, free proline, total protein and K^+ uptake under drought. The results were classified the ten wheat genotypes in three groups, tolerant, moderate, and sensitive to drought stress. Each genotype has a feature of drought tolerance by containing certain characteristics. Genotypes L7 and L10 has genetic makeup that has more than one trait that indicates that it is drought to tolerant, while L4, L6 and L9 were moderate tolerant to the stress. The L1 and L3 were the most sensitive genotypes to drought stress. The correlation between the biochemical and physiological traits were studied and the results show significant positive correlation between Na^+ with total protein and Ca^{+2} under drought stress. However, there are high and significant correlations between total phenols with free proline under control and drought conditions.

Key words: wheat (*Triticum aestivum* L), proline, drought, chlorophyll, total phenols, protein

1. Introduction

Since the demand for food especially wheat is in fixed increase the agriculture around the world will face multiple changes year by year [1]. Wheat (*Triticum* spp.) belongs to family *Poaceae*, and it is the first important and strategic cereal crop for most of the world's population. Wheat is a magnificent health structure food and the best source of proteins, minerals, vitamins like B-group vitamins, starch, and dietary fiber [2, 3]. By 2025 there is an estimated demand increase by 60% for wheat although the

production decreased by 29% due to climate change forced ecological stresses [4]. Furthermore, of the wheat areas of spread cultivated in semiarid and arid regions. The adaptation of wheat to wide range of climatic changes is the key factor in the promising wheat outcome [5]. Wheat is the second produced cereal grain behind maize, and the worldwide exchange of wheat is larger than all other crops collective. In 2020, the whole worldwide production of wheat was 760 million tons where China India and Russia are the three main discrete wheat producers in

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the world, describing for about 41% of the global total wheat production [6]. Response of plants be contingent on developmental phase, plant period, stress cruelty and plant genotype [7]. Many biotic and abiotic stresses affect the growth and development of plants, abiotic stress such as drought, salinity, cold, heat, flooding) and biotic stress caused by fungi, bacteria, viruses, insects, etc.) can be prominent. [8]. Wheat yield in many parts of the world is reduced by biotic stress but also by abiotic stress. To handle with the water deficiency, crops tolerance might be developed across numerous approaches containing genetic engineering, vegetation cover, plant breeding, more crop lands or farm programming. although, most of these solutions are time overwhelming or cost exhaustive and may even magnify environmental problems and the climate change further [9]. Biochemical, morphological, and physiological status of plants can be affected by drought [10, 11]. Drought stress can be stated as the lack of sufficient moisture essential for a plant to cultivate normally and complete its life cycle. The absence of suitable moisture leading to drought stress is a communal incidence in rain nourished regions. [12] The root system is the primary plant organ to intellect a restriction of water resource and a relationship has been recognized between drought resistance in wheat and improved root system [13]. Leaf water impending decreased by drought in wheat because of the solute's accumulation, nevertheless variation of genotypic may survive in response to water prospective under well-watered as well as drought disorders [14]. The plant physiologically change at what time plants are exposed to drought stress to tolerate this stress, physiologically, to recognize the capability of plants to make imperative changes, drought needs a background- contingent view that assuage the influence of drought stress [15]. Drought stress may be occurred at several growth stages consequently genotypes may be verified for their drought tolerance at distinctive growth stages as many genotypes may tolerate drought at germination or seedling stage and be appropriate to drought at the flowering stage or *vice versa* [16]. Dissimilarity in photosynthetic pigment considerate the serious response of plant to drought. The key indicator to control the magnitude of photosynthesis in stress situation is distinction in contents. The drought declines the photosynthetic rate of cereals as known [17]. The Stomata closure, the activity reduction photosynthesis, the assembly of metabolites, the interchange in the integrity of cell wall and the expansion of oxidative stress, are toxic physiological responses can lead to plant death [18]. Although a lot of researchers have implemented study on drought resistance apparatus in numerous

cereals, the wheat enhancement for the drought tolerance is inadequate for several limitations. The objectives of the current study are to consider the probable variations in the physiological and morphological traits of wheat due to the drought stress and plant tolerance apparatus to the stress.

2. Materials and methods:

In wheat growing season, 125 accession of Egyptian wheat germplasm taken from the National Gene Bank of Egypt, Agriculture Research Center, were evaluated in separate experiments in greenhouse. Ten genotypes were chosen according to the results obtained from previous testing. In the 2019-2020 growing season, the ten selected wheat genotypes were planted in early November till harvesting in pot experiments in a Randomized Complete Block Design (RCBD) in greenhouse at National Gene Bank of Egypt, Agriculture Research Center. with three replicates, three pots per replicate and two plants per pot. After 70 days the plant height and flag leaf area were measured, and plants sample taken for the physiological and biochemical tests. Wheat genotypes were subjected to two water treatments, normal (control) and drought stress conditions. Water holding capacity of soil was determined, and pots were watered three times per week to 25% (stressed) and 100% (well-watered) of 100% of soil water holding capacity. The range of temperature was 20-22°C during day and 17-19°C during night and the light was adjusted for 16 hours daily. The soil was prepared by adding 50% sand and 50% peatmoss. The code number, name, and origin of the studied wheat genotypes were recorded in Table (1).

Table1

The Accession number, name, Origin/collection location of the ten wheat genotypes

landrace	Acc. no.	Origin	Collection location
L1	8	Giza	International genebanks (USA)
L2	36	Giza	International genebanks (USA)
L3	191	Sohag	National genebank
L4	47	Sharqia	International genebanks (USA)
L5	55	Giza	International genebanks (USA)
L6	30	Giza	National genebank
L7	148	Manufi a	National genebank
L8	111	Giza	National genebank
L9	172	Qena	National genebank
L10	223	Sohag	International genebanks (USA)

2. 1.Morphological traits

Flag leaf area (cm²), plant height (cm), dry weight of whole plant (g), fresh weight of whole plant (g), 1000 grain weight (g) and grain yield (g/plant) were

measured, and data recorded as a morphological trait for the ten Egyptian wheat genotypes.

2.2. Physiological and biochemical response

The study of physiological response of shoot and root of the ten wheat Landrace genotypes to drought after 70 days measured by chemical analysis including chlorophyll concentration, free proline, total sugars, total protein, mineral elements (Potassium- Calcium- Magnesium - Sodium) and antioxidant properties (peroxidase and total phenols).

2.2.1. Chlorophyll concentration

According to Lichtenthaler's method [19] Chlorophyll a, b and total chlorophylls taken from fresh leaves of each replicate; leaves tissue was ground and supernatant solution is transferred into 25 ml volumetric flask and made up to 25 ml using 80% acetone. Color intensity of the green pigment is read at 645nm and 663 nm for chlorophyll a and chlorophyll b using Lichtenthaler's (1987) equations by spectrophotometry and expressed as mg/g fresh weight FW.

$$\text{Chl a} = 12.7 A_{663} - 2.69 A_{645}$$

$$\text{Chl b} = 22.9 A_{645} - 4.69 A_{663}$$

$$\text{Total Chl (a+b)} = 20.2 A_{645} + 8.02 A_{663}$$

2.2.2. Free proline

Free proline concentration was extracted from 200 mg of dry shoot and root samples according to the method of [20]. With 10 ml of 3% Sulfosalicylic acid, for 30 min at 70°C. An aliquot of 1 ml of the extract was mixed with 1 ml of acid ninhydrin and of 1 ml glacial acetic acid. The mixture was heated at 90°C for 1 h in water bath. The organic toluene phase containing the chromophore was separated and absorbance of red color developed was read at 520 nm. by using calibration curve the proline concentration was determined as $\mu\text{mol proline g}^{-1}$ dry weight (DW).

2.2.3. Mineral elements

According to [21] method Potassium Calcium, and Sodium samples from the dry shoots and roots was digested by 0.1 M HCl solution and determined using the flame photometer apparatus (CORNING M 410, Halstead, UK) and expressed as mg/g dry weight (DW). Magnesium in shoot and root samples was digested and determined using atomic absorption spectrophotometer with fuel and air-acetylene (PyeUnicam, model SP-1900, Cambridge, UK) and expressed as mg/g dry weight DW.

2.2.4. Total Sugars

Total sugars were determined using [22] method. Extraction of 100 mg of dry shoot and root samples using 80% ethyl alcohol, then 1ml of extract was taken and added 1ml of 5% phenol and immediately followed by the addition of 5 ml of concentrated sulfuric acid rapidly then the mixture was shaken gently and left to cool. OD of greenish brown color

developed was taken at 490 nm in spectrophotometer. The quantity of sugars was calculated against the standard curve prepared by using pure glucose (10-100 $\mu\text{g/ml}$) and expressed as mg g^{-1} dry weight (DW).

2.2.5. Total protein

According to Kjeldahl method [23] the dried and homogenized 0.5 g of the shoot and root samples is digested with sulfuric acid in a suitable Kjeldahl tube titanium dioxide/copper sulfate is used as a catalyst and potassium sulfate is added to rise the temperature. After adding sodium hydroxide to the digestion solution the produced ammonium from all nitrogen species is evaporated by distillation with ammonia. This is condensed in a conical flask with boric acid solution. with sulfuric acid the amount is titrated against indicator then multiply with factor 6.25 and expressed as mg/g dry weight (DW).

2.2.6 Antioxidant properties

2.2.6.1. Total Phenols

The total phenols of the extract were measured by the Folin-Ciocalteu method [24]. 1ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added to 200 μl of extracts. Finally, the absorption of all samples (after storage for 1.5h at 30°C and darkness) was determined by spectrophotometer at 750nm. The concentration of phenols in the shoots and roots sample was calculated using a standard curve and expressed as milligrams of gallic acid equivalents $\mu\text{g/mg}$ fresh weight (FW).

2.2.6.2. Peroxidase (POD)

According to [25] method peroxidase was determined. Shoot and root samples (500 mg fresh weight) were homogenized with a prechilled mortar and pestle with 2 ml of ice-cold trichloroacetic acid TCA (0.1%, w/v) and centrifuged at 15,000 \times g for fifteen min at 4°C. Assay mixture containing 2 ml aliquot of supernatant and 2 ml of (0.67%, w/v) thiobarbituric acid (TBA), was heated at 95°C for 20 min and then cooled rapidly in ice bath. The samples were centrifuged (10,000 \times g for ten min at 4°C) and the supernatant absorbance was measured at 532 and 600 nm and expressed as $\mu\text{mol.g}^{-1}$ fresh weight (FW).

2.3. Statistical Analysis

The analysis of variance was done according to [26] if overall F-test was significant, SD at P level of 0.05 and 0.01% were used to mean comparison test. The statistical analysis of coefficient of variance (C.V%), reduction percentage (R%) and Pearson correlation coefficients was performed using 'GraphPad- prism' software (version 9.0, www.graphpad.com).

3. Results and discussion

The ten genotypes performance affected by drought stress, which becomes more evident when data analysed the morphological traits and physiological response. The imperilled crops to the drought stress illustrate altered behaviour, various crops are

resistance to drought whereas others are susceptible [27]. Significant difference was found between the 10 wheat genotypes presented in Table (2).

3.1. The Morphological traits

Results of the morphological traits revealed that distinctive drought intensities had altered effects on dry and fresh matter assembly. In Figure (1), the landrace genotypes L1 and L2 showed decrease in the dry weight and fresh weight than normal conditions as sensitive genotypes to drought stress conditions, while L4, L8 and L9 are tolerant genotypes by low decrease in the dry weight and fresh weight obtained under drought stress. The moderate tolerance landrace genotypes are L3, L5, L6 and L7. Among ten wheat genotypes studied under drought conditions grain yield ranged from 13.45 to 22.32 (g) / plant, The mean grain yield was 17.46 (g / plant), with genotypes L7, L10 and L8 as the highest yielding genotypes, and L1 and L3 as the lowest yielding genotypes. The results of the morphological and yield traits studied showed that three genotypes L4, L6 and L10 had high 1000 grain weight under normal conditions, while four genotypes L4, L7, L8 and L10

were desirable had high mean performance under drought conditions. The last four bread wheat genotypes L4, L7, L8, and L10 are considered drought-tolerant genotypes. Numerous studies signified that the rising in water stress could reduce the plant dry weights [28, 29, 30, 31, 32]. The present results disagree with Colom et al., [33] who revealed that dry weight of the plants were negatively linked to drought stress in plants. The results also showed that flag leaf area and plant height are in similar trend with dry weight and that's contract with Bather et al., [34] who indicated that greater soil water stress decreased plant height and total fresh and dry weights of crops. The thousand grain weight decrease may be due to efficiency of nutrient uptake distribution and translocation of photosynthesis within the wrinkled grains produced due to rushed maturity. This is possible because the deficiency of moisture which force plant to complete its grain creation in comparatively lesser interval [35].

Table 2

Mean performance, LSD and F-test for the morphological traits: plant height, flag leaf area, fresh weight and dry weight at 70 days from sowing, while 1000 grain weight and grain yield at harvesting time for the 10 wheat genotypes studied under control and drought stress conditions

Genotype	Plant height (cm)		Flag leaf area (cm ²)		Dry weight (g)		Fresh weight (g)		1000 grain weight (g)		Grain yield (g/ plant)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	drought
L1	55.40	28.40	7.80	4.96	5.24	2.18	26.20	10.90	30.82	26.56	17.55	7.28
L2	48.40	34.80	14.24	6.20	7.33	3.82	36.65	19.10	37.23	31.21	19.36	7.68
L3	54.60	29.50	9.88	9.35	5.63	6.02	28.15	30.10	33.82	28.33	13.45	7.15
L4	45.20	20.50	7.50	4.10	6.15	5.97	30.75	29.85	34.78	32.12	16.32	10.68
L5	42.40	22.20	8.50	7.63	7.75	5.91	38.75	29.55	31.28	28.59	14.61	8.29
L6	57.40	31.60	15.90	5.75	7.88	5.50	39.40	27.50	35.39	31.81	14.83	9.08
L7	52.40	48.20	9.44	4.80	7.33	6.42	36.65	32.10	38.46	32.37	22.32	14.67
L8	44.20	40.00	10.16	7.20	7.98	7.46	39.90	37.30	39.10	32.31	19.38	11.61
L9	45.40	35.60	9.76	9.78	8.23	8.56	41.15	42.80	39.17	31.68	18.97	9.54
L10	61.40	49.80	11.30	5.80	7.46	5.62	37.30	28.10	36.11	32.95	17.84	14.05
Mean	50.68	34.06	10.45	6.56	7.10	5.75	35.49	28.73	35.62	30.79	17.46	10.00
F-test	**	**	**	**	**	**	**	**	**	**	**	**
LSD 0.05	0.67	0.58	0.80	0.68	0.54	0.64	0.57	0.68	0.77	0.91	0.67	0.84

Where *, ** significant at 0.05 and 0.01 levels of probability, respectively

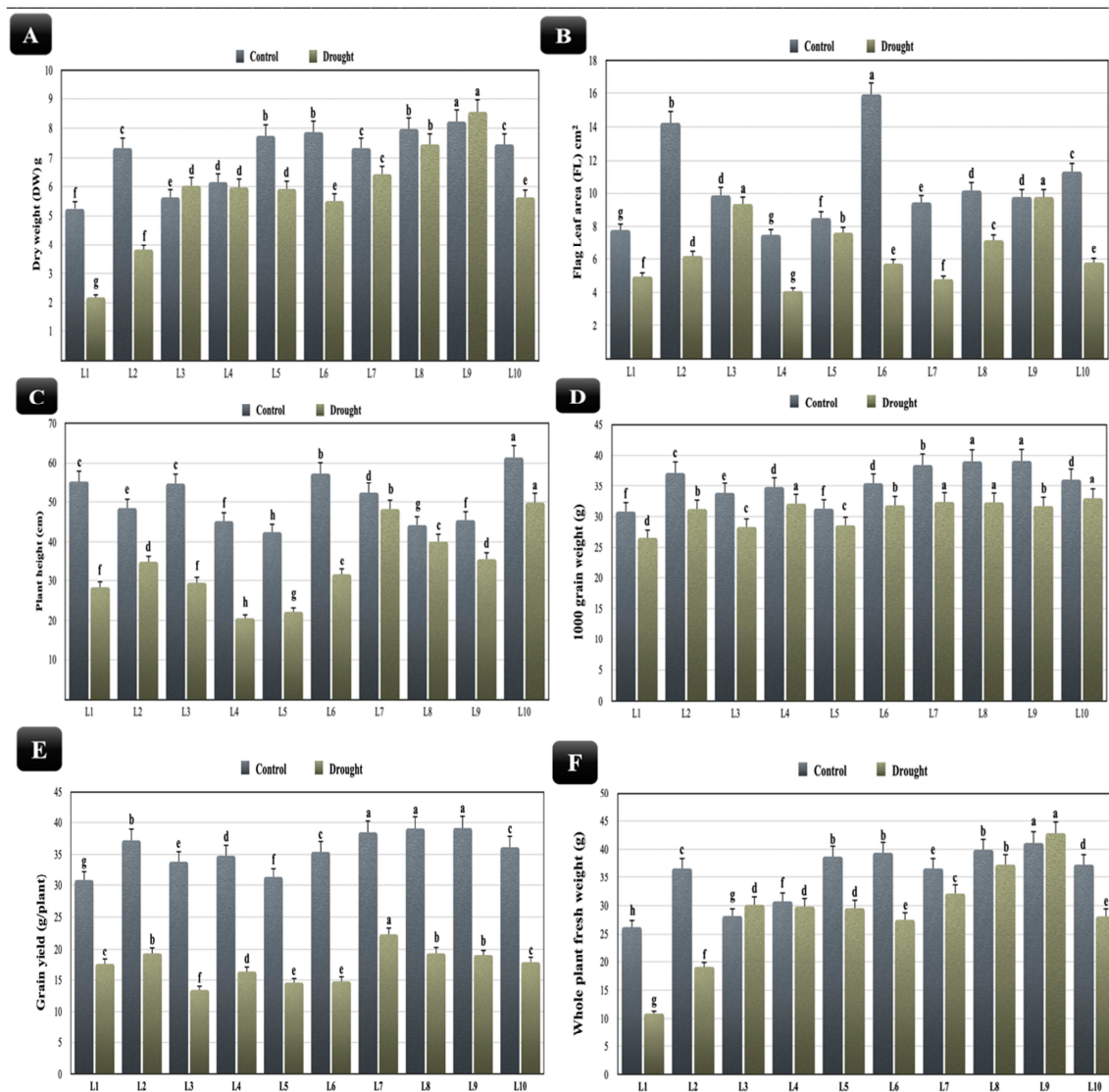


Fig. 1. Morphological traits variations in the ten landrace genotypes under drought stress conditions. (A) dry weight of whole plant (B) flag leaf area (C) plant height (D) 1000 grain weight (E) grain yield (F) fresh weight of the whole plant of the ten landraces genotypes under control and drought conditions. The different letters mean significant differences among treatments and genotypes range, $p < 0.05$

3.2. Physiological and biochemical response

3.2.1 Chlorophyll concentration

The results revealed the alterations in photosynthetic chlorophyll concentration in the leaves of the ten wheat genotypes reacting to drought treatment. Commonly in Table (3) significant reduction in chlorophyll concentration was found in all genotypes. In Figure (2) the results of chlorophyll (a) at drought stress conditions compared to the normal conditions shows light decrease for L3, L6 and L9 as tolerant genotypes for the stress, while L7, L8 and L10 show moderate adaptation to the stress, and highly decrease in chlorophyll a concentration in L1, L2 and L4

genotypes as sensitive one to the drought. Ten wheat genotypes were divided into three categories based on the chlorophyll (b) concentration in leaves i.e. sensitive L4, L7 and L8 its contains high values, moderate L5 and L10 slight increase, tolerant L3 and L9 the lowest value as a compared by control. The results of total chlorophyll concentration for L3, L6 and L8 genotypes slightly decrease in drought conditions than normal conditions as indicator for highly tolerance genotypes to drought stress, while moderate decrease for L1, L5 and L10 genotypes and for L2 and L4 are very sensitive for drought with high decrease in total chlorophylls. The results obtained here agree with Larkunthod et al., [11] who stated that strict drought stress constrains the photosynthesis of plants by alterations in chlorophylls concentration, chlorophyll components

distribution and harming the photosynthetic tool. Also, Tayeb [36] reported that total chlorophyll concentration is reduced under water stress conditions and reduced in tolerant genotypes faster than insensitive ones. In wheat with increase the drought stress, the chlorophylls cause larger decrease, as the thylakoids membrane degenerates with cell dehydration. The photosynthetic capacity determines by the amount of leaf chlorophyll [37]. The photosynthetic capacity of plants decreases under drought stress by decreasing the total chlorophyll and chlorophyll (a, b) affects thylakoid membrane integrity and chloroplast membrane permeability [38].

Nikolaeva et al., [39] indicated that, under drought stress conditions, chlorophyll synthesis stimulation was due to enzyme activation of the biosynthesis. The decrease in chlorophyllide was activated with chlorophyllase which results gathering of enzymes in activation of chlorophyll biosynthesis. Ashraf and Harris, [40] who reported that the reduction of chlorophyll b is greater than of chlorophyll a and its more sensitive under drought stress conditions, in wheat there were reported a significant decrease in chl a/b ratio in the susceptible cultivars and slight increase in tolerant cultivars under drought stress.

Table 3

Mean performance, LSD and F-test for chlorophyll concentration trait (total chlorophyll, chlorophyll a, chlorophyll b and chl a/b ratio) for the ten wheat genotypes studied under control and drought stress conditions

Genotype	Total chlorophyll (mg/g FW)		Chlorophyll a (mg/g FW)		Chlorophyll b (mg/g FW)		Chlorophyll a/b ratio	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
L1	0.300	0.175	0.050	0.075	0.250	0.100	0.200	0.429
L2	0.875	0.425	0.350	0.150	0.525	0.275	0.667	0.335
L3	1.650	1.600	0.625	0.600	1.025	1.000	0.610	0.805
L4	1.050	0.775	0.425	0.100	0.625	0.675	0.680	0.214
L5	1.475	0.850	0.600	0.325	0.875	0.525	0.686	0.517
L6	1.225	0.775	0.500	0.475	0.725	0.300	0.690	0.754
L7	0.852	0.950	0.352	0.375	0.500	0.575	0.703	0.713
L8	1.100	1.100	0.450	0.375	0.650	0.725	0.692	0.655
L9	1.925	1.825	0.700	0.625	1.225	1.200	0.571	0.774
L10	1.573	1.125	0.573	0.425	1.000	0.700	0.573	0.638
Mean	1.203	0.960	0.463	0.353	0.740	0.608	0.607	0.583
F test	**	**	**	**	**	**	**	**
LSD 0.05	0.70	0.67	0.36	0.26	0.34	0.28	2.70	0.85

Where *, ** significant at 0.05 and 0.01 levels of probability, respectively

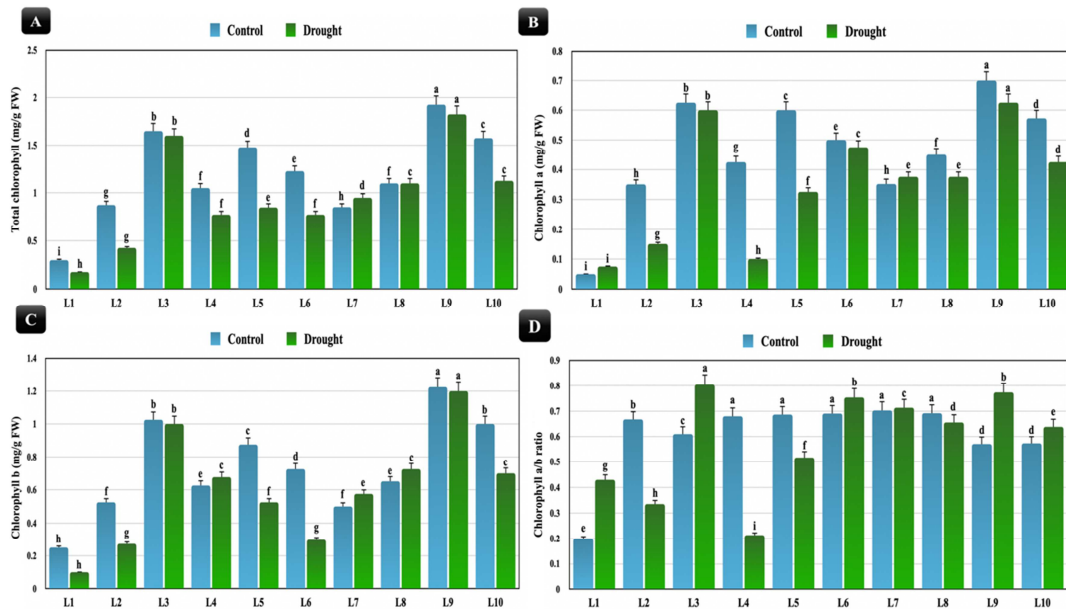


Fig. 2. Chlorophyll concentration variations in the ten wheat genotypes under drought stress conditions. (A) total chlorophyll. (B) chlorophyll a. (C) chlorophyll b. (D) chlorophyll a/b ratio. The different letters mean significant differences among treatments and genotypes range, $p < 0.05$

3.2.2. Free proline

Proline, an amino acid, plays a favorably useful role in plants exposed to several stress environments. In addition to acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule [41]. In former studies, the free proline found in the shoot and root was directly related to drought stress according to synthesis it in root and accumulate in shoots. In Table (4) significant difference was found between the 10 wheat genotypes. The data in Figure (3), shows the effect of drought stress on the shoot and the root of the ten genotypes in normal and stressed conditions.

The L3,L7 and L10 gives higher shoot and root proline accumulation as good performance as tolerant genotypes, where L4,L5, L8 and L9 more sensitive genotypes to drought stress showed low values of proline accumulation. The L1, L2 and L6 genotypes are moderate in adaptation with drought stress by adequate increase in proline level in shoot and root. [42, 43] stated that proline accumulated in wheat plant bigger magnitude than the other osmoregulatory in water limited condition While, [44, 45] found that the most collective attributes in elite of cereals is the existence of proline under drought stress.

Table 4

Mean performance LSD and F-test of free proline trait for the shoot and root of the ten wheat genotypes studied under control and drought stress conditions

Genotype	Free Proline in shoot ($\mu\text{mol g}^{-1}\text{DW}$)		Free Proline in root ($\mu\text{mol g}^{-1}\text{DW}$)	
	Control	Drought	Control	Drought
L1	7.27	7.08	2.83	3.94
L2	13.68	10.67	4.52	3.80
L3	8.00	19.07	3.53	18.50
L4	11.86	5.46	4.47	4.09
L5	9.96	5.56	3.26	6.30
L6	10.20	9.73	7.81	4.17
L7	8.64	14.72	5.20	7.10
L8	11.85	4.58	8.64	1.62
L9	5.52	5.82	9.08	7.84
L10	3.68	9.20	4.17	3.64
Mean	7.27	7.08	2.83	3.94
F test	**	*	**	**
LSD 0.05	2.22	0.73	0.72	0.97

Where *, ** significant at 0.05 and 0.01 levels of probability, respectively

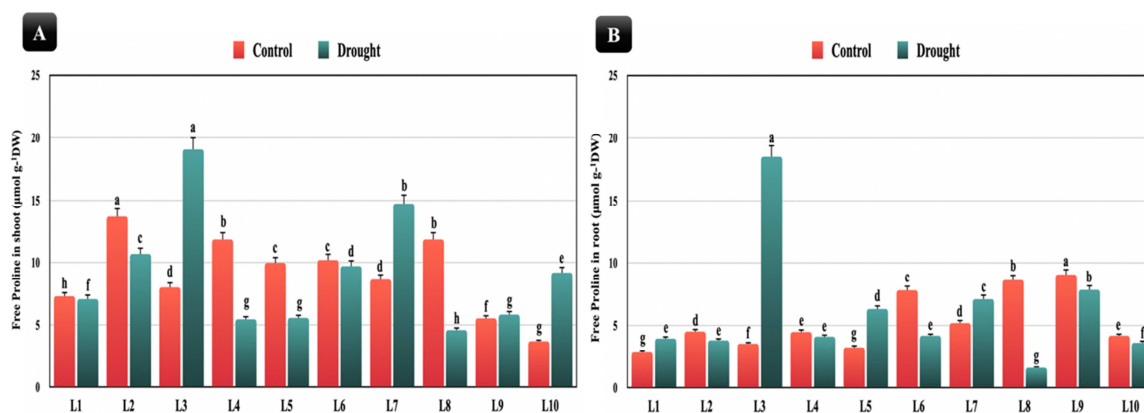


Fig. 3. Free proline variations in the shoot and root of the ten landrace genotypes under drought stress conditions. (A) free proline in shoot (B) free proline in root. The different letters mean significant differences among treatments and genotypes range, $p < 0.05$

3.2.3 Mineral elements:

In this study potassium, calcium, sodium, and magnesium are estimated, highly significant difference was found between the 10 wheat genotypes these were the shoot and root trend similarly presented in Table (5) and the results as the following:

Table 5

Mean performance, LSD and F-test for mineral elements (potassium, calcium, sodium, and magnesium) studied under control and drought stress conditions for the shoot and root of the ten wheat genotypes

Genotype	Potassium (K) mg/g DW				Calcium (Ca) mg/g DW			
	Shoot		Root		Shoot		Root	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
L1	49.27	35.77	10.03	5.73	3.34	2.51	1.47	0.67
L2	46.22	38.66	6.18	6.79	3.67	3.34	1.27	1.67
L3	41.84	43.31	7.40	14.32	4.01	2.67	1.74	3.01
L4	40.59	34.09	7.50	6.69	3.51	3.67	1.67	1.27
L5	36.05	45.90	6.45	6.15	3.17	3.84	2.00	1.00
L6	49.42	47.73	7.70	11.69	4.68	2.67	1.34	1.40
L7	45.04	34.44	9.70	5.37	3.84	3.84	1.74	0.87
L8	44.65	37.10	6.82	9.53	4.01	3.84	1.40	1.27
L9	54.19	42.81	6.69	10.13	2.84	4.18	1.34	1.54
L10	47.23	48.37	7.60	5.98	4.18	3.17	1.40	1.67
Mean	45.45	40.82	7.61	8.24	3.72	3.37	1.54	1.44
F. test	**	**	**	*	**	**	**	**
LSD 0.05	0.78	0.76	1.24	0.75	0.73	0.57	1.12	0.82

Genotype	Sodium (Na) mg/g DW				Magnesium (Mg) mg/g DW			
	Shoot		Root		Shoot		Root	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
L1	3.54	2.83	5.31	4.24	3.25	2.43	1.18	0.97
L2	4.48	5.42	6.72	8.13	2.74	3.40	0.72	0.50
L3	4.95	3.18	7.43	4.77	0.41	2.88	0.98	4.69
L4	4.95	5.42	7.43	8.13	4.74	3.59	1.33	1.10
L5	3.07	4.48	4.60	6.72	1.84	4.29	1.24	0.40
L6	5.19	3.65	7.78	5.48	4.49	4.02	0.72	2.93
L7	4.60	3.89	6.90	5.84	3.51	3.79	1.24	0.99
L8	2.95	2.95	4.42	4.42	2.30	2.82	0.97	1.04
L9	3.42	4.83	5.13	7.25	0.70	4.68	0.67	2.10
L10	3.42	3.89	5.13	5.84	4.03	6.42	2.31	1.37
Mean	4.06	4.06	6.08	5.90	2.80	3.83	1.13	1.61
F. test	**	**	**	**	*	**	**	**
LSD 0.05	0.46	0.48	0.72	0.73	0.62	0.61	0.84	0.83

Where *, ** significant at 0.05 and 0.01 levels of probability, respectively

3.2.3.1 Potassium (K⁺)

Estimates of potassium (K) in the shoot and root indicate that the increase in potassium uptake of the genotypes L3, L5, L6 and L10, revealing that these genotypes is the best tolerant to drought stress, while the genotypes L1, L2 and L4 are sensitive to drought stress because of too low potassium. The L7, L8 and L9 Genotypes considered as moderate adapt to drought stress for the shoots and roots of it Figure (4). These results concur with [46] reported for many physiological manners, potassium is elemental such as translocation, photosynthesis, reducing redundant uptake of ions and enzymes activation. Potassium plays an imperative role in the photosynthesis, protein synthesis, enzyme activity and plant growth in wheat plants. In addition to physiological traits improvement through regulation of the plant turgor pressure, control the stomatal opening and closure, reduces the water possible of the cell, supporting water retention in the plant without interrelation with a normal metabolism [47,48].

3.2.3.2 Calcium (Ca⁺²)

Figure (4) shows the calcium in the root and shoot of the genotypes. The calcium decreased in L1, L3 and

L6 as sensitive genotypes to the stress, while in L7 and L8 genotypes show increase in calcium as tolerance to stress compared by normal conditions. The L7 and L8 genotypes considered as moderate adapt to drought stress for the shoots and roots of it. The results are agreed with [49] informed that calcium is very important to catalase activity, improve chlorophyll, reducing plasma membrane destruction, and it's also sustained osmolytes like antioxidants and proline.

3.2.3.3 Sodium (Na⁺)

Figure (4) shows the sodium in shoot and root of the landrace genotypes. Sodium increased in the roots than shoots under drought stress conditions. The L7 and L8 genotypes considered as moderate adapt to drought stress. [50] stated that plants can reduce their osmotic potential under adverse conditions by absorbing salt ions to improve the root ability to absorb the water the ability of plants to resist the drought may enhanced by absorption and accumulation of salts.

3.2.3.4 Magnesium (Mg⁺²)

The results shown in Figure (4) obtained the effect of drought stress on magnesium of the shoot and root of the ten genotypes comparing by normal conditions.

Genotypes L7 and L10 are resistance to the stress in good performance as tolerance genotypes by increasing the magnesium uptake in shoot and root while L4 and L6 shows little resistance with small increase in magnesium as moderate genotypes. The L1 and L2 are susceptible to drought stress in root and shoot with decrease in magnesium uptake as sensitive genotypes. These results coincide with Asghar and Bashir [51] who reported that in chlorophyll molecule the magnesium has in the center, therefore

has substantial importance. It has numerous roles in dry matter division from dig to cradle. The nitrogen and potassium have positive association with magnesium. They are supportive in stress tolerance. Sufficient magnesium rises their mobility [52]. [53] demonstrated that potassium, calcium, sodium and magnesium nutrients are imperative for the crop plants development. The absorption capability of the roots under drought stress conditions disturbed that leads to shortens nutrient uptake [54].

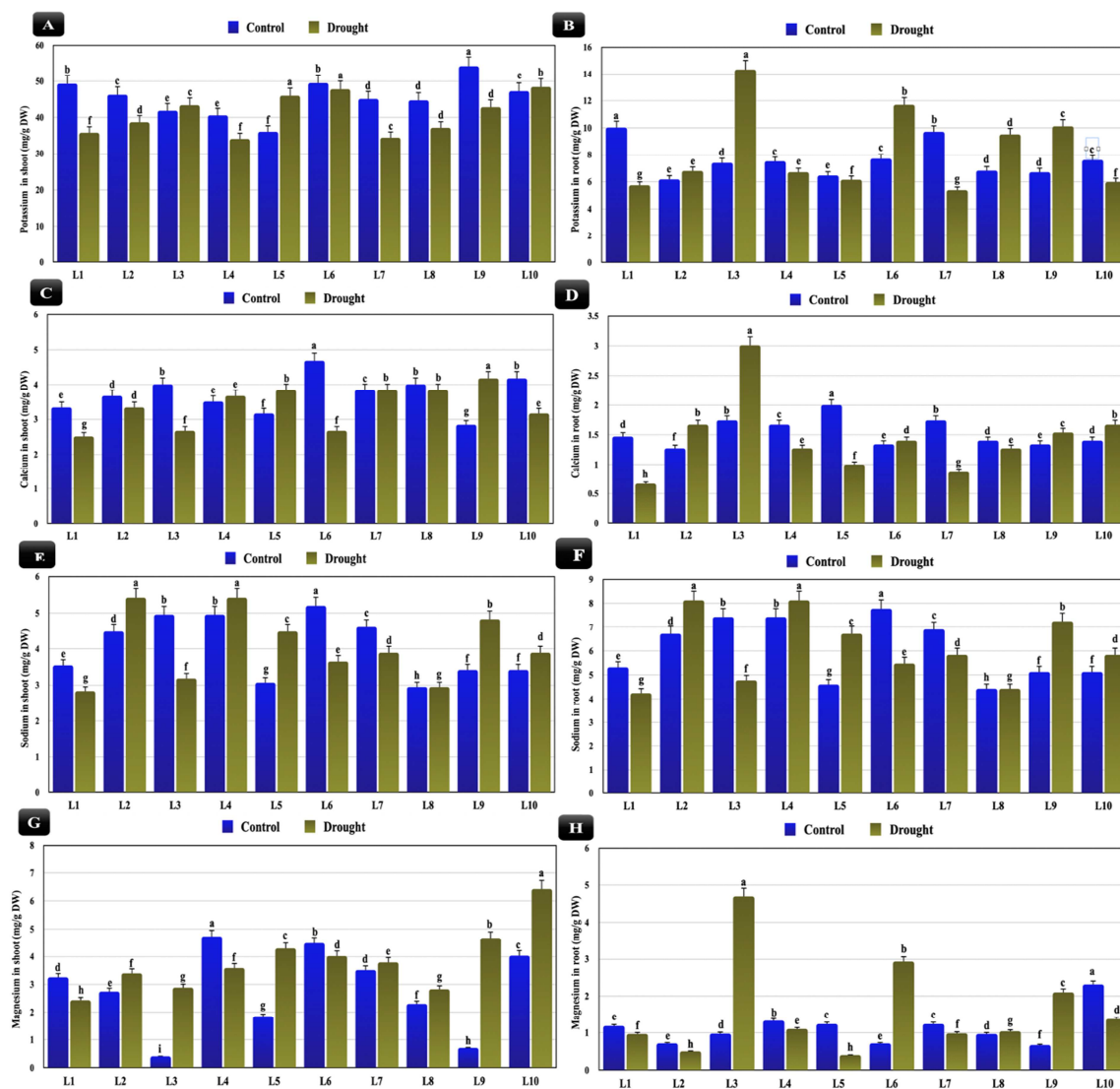


Fig. 4. Mineral elements variations in the shoot and root of the ten wheat genotypes under control and drought stress conditions. (A) Potassium in shoots. (B) Potassium in root. (C) Calcium in shoots. (D) Calcium in root. (E) Sodium in shoot. (F) Sodium in root. (G) Magnesium in shoot. (H) Magnesium in root. The different letters mean significant differences among treatments and genotypes according to range, $p < 0.05$

3.2.4 Total sugars

As shown in Table (6) the total sugars in the shoot and root of the wheat plant genotypes were significantly affected by drought stress conditions. In Figure (5) the highest total sugars were found in L7, L8 and L9 as tolerance genotypes for the stress, while

the lowest was recorded in L3 as sensitive one to the drought as compared to the normal conditions. L4, L5 and L6 shows little resistance as moderate genotypes. These results are in accordance with [55, 56] stated that the sugars distribution and its accumulation in distinctive parts of the plants could

be an effective trait to classify cultivars of altered tolerance to drought stress.

Total sugars one of the osmotic factors. Pierre and Savoure [57] who informed that one of the most important plant defense mechanisms is osmotic adjustment which improves drought tolerance of the plants. It permits plant growth and cell expansion with drought stress, osmolyte accumulation permits

the cell to accomplish their dehydration and membrane structural integrity to provide tolerance against cellular dehydration and drought [58].

Table 6

Mean performance, LSD and F-test for total sugars trait studied under control and drought stress conditions for the shoot and root of the ten wheat genotypes

Genotype	Total sugars in shoot (mg/g DW)		Total sugars in root (mg/g DW)	
	Control	Drought	Control	Drought
L1	24.5	27.0	36.0	30.0
L2	28.6	34.4	30.7	34.0
L3	28.1	32.1	33.9	19.1
L4	21.7	31.2	32.8	33.4
L5	30.5	34.5	34.5	28.9
L6	22.4	33.4	29.6	33.2
L7	32.1	35.4	31.0	36.1
L8	30.4	47.9	25.4	36.4
L9	25.4	40.1	24.3	27.4
L10	28.1	33.4	33.2	35.0
Mean	27.18	34.94	31.14	31.35
F. test	**	**	**	**
LSD 0.05	1.09	1.10	1.06	2.99

Where *, ** significant at 0.05 and 0.01 levels of probability, respectively

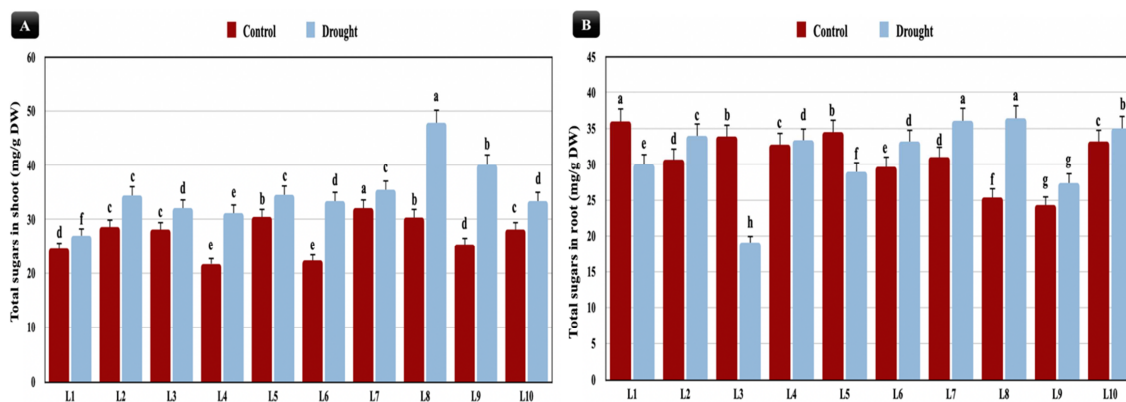


Fig. 5. Total sugars variations in the ten wheat landrace genotypes under control and drought stress conditions. (A) Total sugars of the shoot (B) Total sugars of the root. The different letters mean significant differences among treatments and genotypes range, $p < 0.05$

3.2.5 Total protein

To allow plants to survive with the stress, the drought stress stimulated proteins permit plants to create structural and biochemical regulations these were trend similarly as shown in Table (7) the total protein in the shoot and root of the wheat genotypes were significantly affected by drought stress conditions. The results shown in Figure (6) indicated that the in-drought stress levels are significant advanced decrease in total protein matter in the shoot and root of the plant. Increase the level of total protein in landrace genotypes L2, L5, L7 and L9 as tolerant to drought stress, while decrease in protein levels observed in L3 and L6 landraces as sensitive to

drought stress. The L1 and L10 are moderate tolerant to the drought stress conditions. So, these results contrast with [59,60,61] who stated that the fundamental compounds necessary for all the functions in the cell are the proteins. [62] stated that the drought has negative effect on synthesis of protein. In this perspective, nitrogen concentration was significantly dropped in flag leaves of wheat drought-stressed plants [63]. The most totally induced processes under environmental stresses is the protein synthesis as it reduces the activity of nitrate reductase and retard the uptake of nitrate.

Table 7

Mean performance, LSD and F-test for total protein trait studied under control and drought stress conditions for the shoot and root of the ten wheat genotypes.

Genotype	Total Protein in shoot (mg/g DW)		Total Protein in root (mg/g DW)	
	Control	Drought	Control	Drought
L1	78.76	63.00	14.18	11.50
L2	78.76	120.76	12.60	17.64
L3	105.00	85.04	11.55	19.95
L4	99.76	107.64	10.50	6.30
L5	89.24	105.00	12.60	23.10
L6	94.52	99.76	12.18	15.75
L7	86.64	103.44	14.91	18.90
L8	99.76	94.52	13.97	21.00
L9	99.76	112.88	13.65	22.05
L10	105.00	73.52	12.60	12.60
Mean	93.72	96.56	12.87	16.88
F. test	**	**	**	**
LSD 0.05	6.61	7.60	2.59	3.76

Where *, ** significant at 0.05 and 0.01 levels of probability, respectively

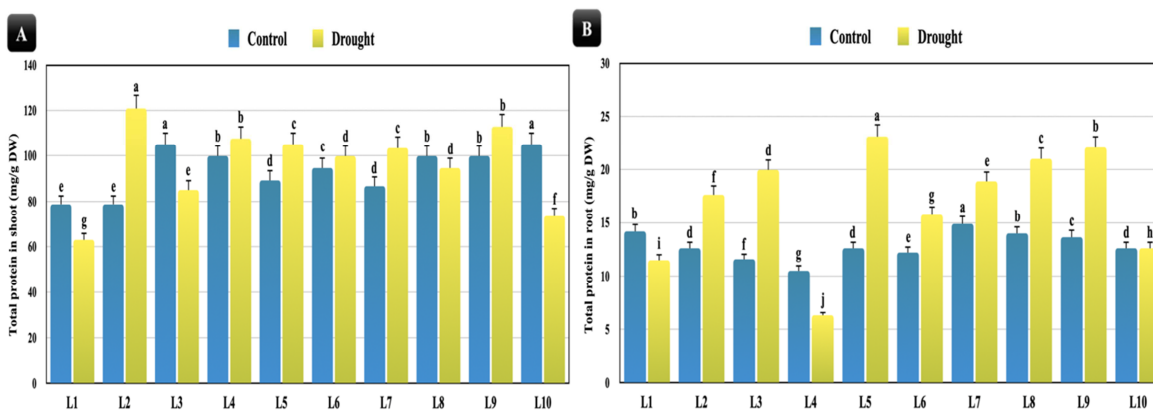


Fig.6. Total Protein variations in the ten wheat landrace genotypes under control and drought stress conditions. (A) Total Protein of the shoot. (B) Total Protein of the root. The different letters mean significant differences among treatments and genotypes range, $p < 0.05$

3.2.6 Antioxidant Properties

As shown in Table (8) the antioxidant properties studied (total phenols and peroxidase enzyme) in the shoot and root of the wheat genotypes were

significantly affected by drought stress conditions compared to the normal conditions and these were trend similarly from root to shoot as the following:

Table 8

Mean performance, LSD and F-test for antioxidant properties (total phenols and peroxidase enzyme) trait studied under control and drought stress conditions for the shoot and root of the ten wheat genotypes

Genotype	Total phenols ($\mu\text{g}/\text{mg FW}$)				Peroxidase enzyme ($\mu\text{mol.g}^{-1}\text{FW}$)			
	Shoot		Root		Shoot		Root	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
L1	12.1	10	2.1	1.2	43.9	10.7	23.4	2.0
L2	24.3	20.4	3.4	2.7	14.6	16.5	15.7	19.6
L3	16.2	39.3	2.4	7.2	23.7	13.1	19.6	20.9
L4	20.1	12.5	3.1	3.5	17.6	16.1	17.6	13.8
L5	19.1	12.4	2.1	4.8	11.7	7.9	12.0	14.7
L6	20.1	20.8	4.1	3.5	20.2	17.6	37.0	23.0
L7	12.2	29.4	3.5	1.8	21.2	23.1	16.5	13.7
L8	20.7	8.4	3.9	1.1	27.4	9.8	17.6	12.1
L9	8.4	12.4	4.3	5.1	36.7	17.6	18.5	27.4
L10	5.2	17	3.5	2.4	9.5	12.5	21.3	13.8
Mean	15.85	18.25	3.24	3.33	22.65	14.49	19.92	16.1
F. test	**	**	**	**	**	**	**	**
LSD 0.05	0.88	0.67	0.55	0.74	1.04	0.88	1.05	0.93

Where *, ** significant at 0.05 and 0.01 levels of probability, respectively

3.2.6.1. Phenolic compounds

The results in Figure (7) for total phenols as non-enzymatic antioxidants affected by drought stress showed highly increase in phenols in drought conditions for L3, L6 and L7 genotypes that indicates their ability to adapt and tolerate the stress compared with normal conditions. While decrease in the phenol's compounds in L1, L4 and L9 as indicator for the sensitivity of that genotypes to the drought stress. The L2, L5 and L10 are moderate tolerant landrace genotypes to the stress. The results in

agreement with [5] who stated that, various studies in wheat have revealed that there is variation in the activity of the antioxidant resistance system in plant to regulator the oxidative stress prompted by several environmental aspects like drought. Due to the antioxidant activities of phenolic compounds, the phenolics play a role in adaptation with abiotic stress, under drought stress conditions the stress sensitive wheat genotype accumulated low leaf total phenolic compared to that in stress tolerant one [64].

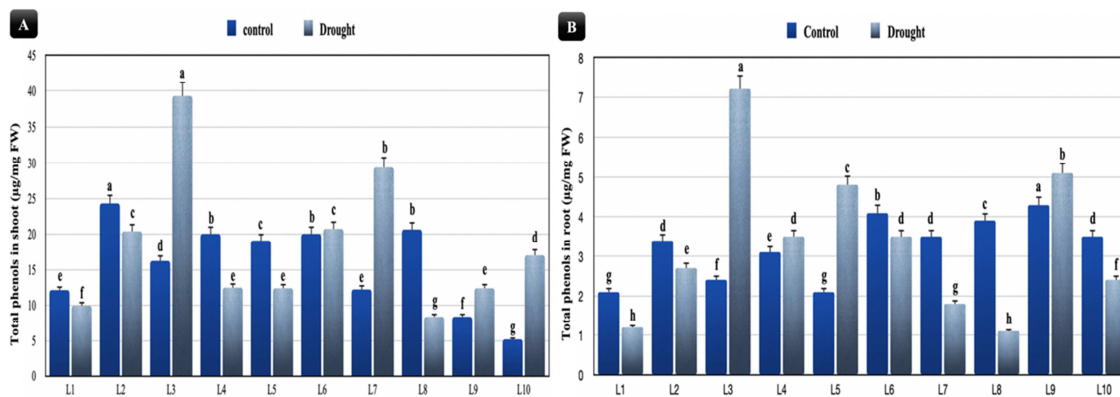


Fig. 7. Antioxidant properties variations in the ten wheat landrace genotypes under control and drought stress conditions. (A) Total Phenols of the shoot. (B) Total Phenols of the root. The different letters mean significant differences among treatments and genotypes multiple range, $p < 0.05$

3.2.6.2 Peroxidase enzyme activity (POD)

In Figure (8) the highest peroxidase activity as enzymatic antioxidants in the shoot and root of the wheat genotypes recorded and the shoot and root were trend similarly in L4, L6 and L7 were tolerant genotypes compared to the normal condition followed by L2, L9 and L10 genotypes as moderate

tolerance, while the lowest activity of peroxidase showed in L3, L5 and L8 as sensitive one to drought stress conditions. These results are in agreement with [65] reported to detoxify the toxic levels of ROS, there is initiation of both non enzymatic and enzymatic system which is destructive as result of water stress to plant constructed.

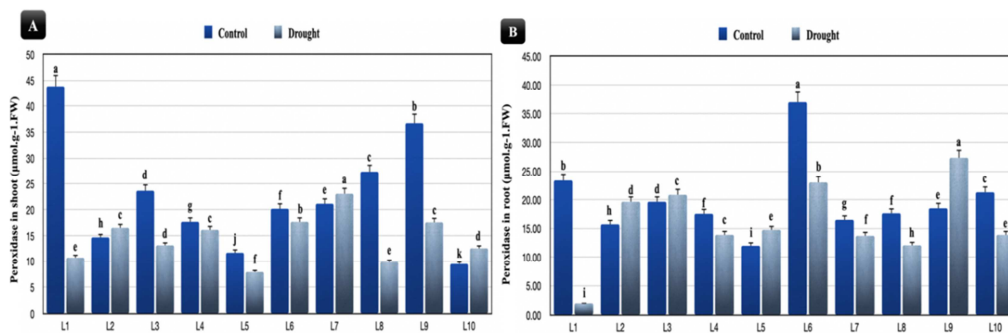


Fig. 8. Antioxidant properties variations in the wheat ten landrace genotypes under control and drought stress conditions. (A) Peroxidase enzyme activity in the shoot. (B) Peroxidase enzyme activity in the root. The different letters mean significant differences among treatments and genotypes range, $p < 0.05$

3.3 Trait means, Coefficient of Variation (C.V %) of means, Reduction percentage (R%) and correlation among studied traits

Trait means, coefficient of variation and ranges under control and drought conditions as well as reduction percentage due to drought compared to control are presented in Table (9). Coefficients of variation increased by drought stress, the highest C.V% estimates were found for total phenols, free proline and total chlorophyll under control and drought. Whereas thousand grain weight, Potassium and total sugars revealed the lowest estimates of C.V% under previous conditions, respectively. In addition, maximum R% under drought was observed for magnesium (-44.46%) followed by total sugars trait (-28.55%), while total protein (-3.03%) exhibited one of the minimum R%. Pearson correlation coefficients were calculated among the studied (morphological, biochemical and physiological) traits under control and drought stress conditions and presented in Figure (9). Under control conditions grain yield positive and highly significant correlated with thousand grain weight (0.68), positive significant correlated with total sugars (0.42) and negative significant correlated with total phenols, total chlorophyll, while under drought conditions grain yield positive and highly significant correlated with thousand grain weight (0.75) and plant height (0.74), positive significant with magnesium (0.53) and negative significant correlated with free proline (-0.02) and total phenols (-0.02). In control and drought conditions total phenols positive and highly significant correlated with free proline. Under drought condition total protein was positively correlated. with sodium and calcium, while under normal condition low positive correlation were detected. Under drought conditions, the total chlorophyll positive highly correlated with fresh weight (0.85), dry weight (0.85) and flag leaf area (0.77). The results are agreed with [65] who stated that during water deficiency conditions, photosynthesis reduction occurs, resulting from reduction the productivity of biochemical activities, which led to destroying vegetative growth and dry matter production. Due to inhibition of biochemical and physiological process, the biomass, straw and grain yields were decreased. The grain yield decreased by 14% and 41% under moderate and severe drought, respectively, compared with normal conditions. Drought tolerant plants to tolerate drought stress adapt various mechanisms, such as increasing of water uptake by developing deep and large root systems, decrease in water loss by raising stomatal resistance and osmolytes accumulation. The accumulated osmolytes include sugars and proline. They play an important role in enzyme inactivation and preventing membrane disintegration in drought stress environment [67]. [68] reported that the reduction in leaf area and shoots dry weight are the most studied effect of the early vegetative water deficiency. Inhibiting cell expansion can restrict leaf expansion and internode elongation.

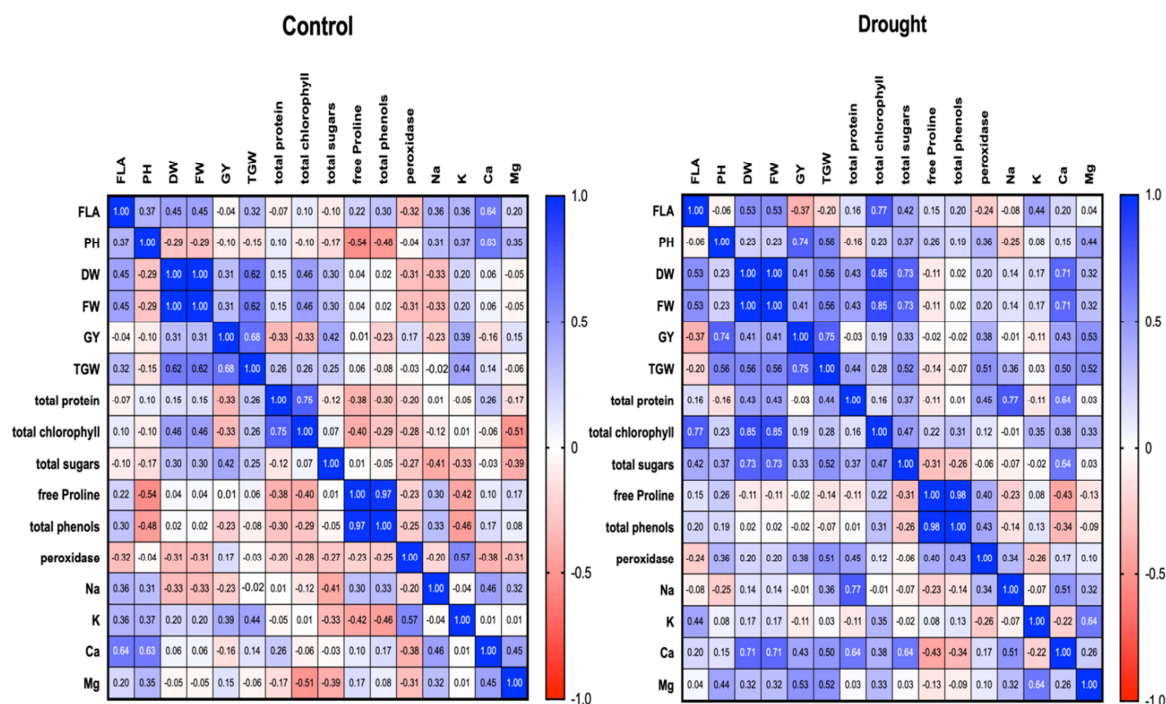


Fig. 9. Pearson correlation coefficients among studied traits under control (Left) and drought conditions (right). Where, FLA is Flag Leaf Area (cm²), PH is plant height (cm), DW is dry weight for whole plant (g), FW is fresh weight of whole plant (g), GY is grain yield (g/plant), TGW is thousand grain yield (g), Na⁺ is sodium (mg/g DW), K⁺ is potassium (mg/g DW), Ca²⁺ is calcium (mg/g DW), Mg²⁺ magnesium (mg/g DW).

Table 9

Trait means, Coefficient of Variation (C.V%), Standard Deviation (S.D), range and Reduction percentage (R%) under control and drought stress condition

Trait	Control				Drought				
	C.V%	S.D	Mean ± S.E	Range	C.V%	S.D	Mean ± SE	Range	R%
Flag Leaf Area (FLA)	25.96	2.713	10.45 ± 0.857	7.50 - 15.90	29.11	1.908	6.55 ± 0.060	4.10 - 9.78	37.32
Plant Height (PH)	12.73	6.449	50.68 ± 2.039	42.40 - 61.40	28.85	9.828	34.06 ± 3.108	20.50 - 49.80	32.79
Dry Weight (DW)	14.73	1.046	7.10 ± 0.330	5.24 - 8.23	30.68	1.763	5.75 ± 0.557	2.18 - 8.56	19.01
Fresh Weight (FW)	14.73	5.228	35.49 ± 1.653	26.20 - 41.15	30.68	8.814	28.73 ± 2.787	10.90 - 42.80	19.05
Grain Yield (GY)	15.48	2.703	17.46 ± 0.854	13.45 - 22.32	15.48	2.703	17.49 ± 0.854	13.45 - 22.32	-0.17
1000 Grain Weight (TGW)	8.45	3.009	35.62 ± 0.951	30.82 - 39.17	7.01	2.161	30.79 ± 0.683	26.56 - 32.95	13.56
Total protein	10.57	9.901	93.72 ± 3.13	78.70 - 105.0	18.58	17.94	96.56 ± 5.67	63.0 - 120.8	-3.03
Total chlorophyll	39.33	0.468	1.20 ± 0.148	0.302 - 1.925	51.36	0.490	0.96 ± 0.159	0.17 - 1.82	20.00
Total sugars	13.02	3.538	27.18 ± 1.119	21.70 - 32.10	16.10	5.623	34.94 ± 1.778	27.00 - 47.88	-28.55
Free proline	33.91	3.074	9.07 ± 0.972	3.88 - 13.68	50.63	4.653	9.19 ± 1.471	4.58 - 19.07	-1.43
Total phenols	38.69	6.130	15.84 ± 1.938	5.20 - 24.30	53.12	9.699	18.26 ± 3.067	8.40 - 39.30	-15.28
Peroxidase enzyme	47.98	10.860	22.64 ± 3.435	9.46 - 43.88	31.31	4.538	14.49 ± 1.435	7.90 - 23.11	36.00
Sodium (Na ⁺)	21.16	0.860	4.06 ± 0.271	2.95 - 5.192	23.56	0.953	4.05 ± 0.313	2.83 - 5.42	0.25
Potassium (K ⁺)	11.27	5.123	45.45 ± 1.622	30.05 - 54.19	13.44	5.484	40.82 ± 1.733	34.09 - 48.37	10.19
Calcium (Ca ⁺²)	14.38	0.533	3.72 ± 0.172	2.84 - 4.68	17.59	0.601	3.37 ± 0.196	2.51 - 4.18	9.41
Magnesium (Mg ⁺²)	53.40	1.513	2.80 ± 0.471	0.41 - 4.74	23.56	0.963	4.05 ± 0.301	2.83 - 5.42	-44.64

Conclusion

Drought has demonstrated to be one of supreme destructive environmental cause controlling the productivity and growth of most crop plants. The tested wheat genotypes revealed significant differences in measured traits under drought stress conditions in this study. In genotype L7 the two antioxidant systems used to avoid the drought stress, the total phenols and peroxidase enzyme were increased and grain yield increased also, while in genotype L3 the increase only happen in the non-enzymatic antioxidant activity (total phenols). Both genotypes are fighting the drought to survive. In genotype L3 there was a huge increase in free proline but there was huge decrease in dry weight and grain yield so its sensitive genotype according to compare it with other traits and measure the significant correlation between the studied traits. The tested wheat genotypes differed in their behavior from one to the other under drought stress conditions to survive and avoid stress. The results also revealed that each genotype has its own mechanism of coping with stress, and therefore no genotype showed superiority for all the traits studied and it's mentioned in the correlation coefficient between the morphological, physiological and biochemical traits under drought and normal conditions. Therefore, we can find different sources of gene expression responsible for the different traits responsible for drought tolerance that can be exploited in wheat breeding program for drought tolerance.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

- [1] Lesk, C., P. Rowhani and N. Ramankutty (2016). Influence of extreme weather disasters on global crop production. *Nature*, 529: 84-87.
- [2] Geravandi, M., E. Farshadfar and D. Kahrizi (2011). Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. *Russian Journal of Plant Physiology*, 58:69-75.
- [3] Zahoor, A., E. A. Waraich, S. Akhtar, S. Anjum, T. Ahmad, W. Mahboob, O. Abdul Hafeez, T. Tapera, M. Labuschagne and M. Rizwan (2018). Physiological responses of wheat to drought stress and its mitigation approaches. *Acta Physiologiae Plantarum*, 40:80-92.
- [4] Manickavelu, A., K. Kawaura, K. Oishi, T. ShinI, Y. Kohara. and N. Yahiaoui. (2012). Comprehensive functional analyses of expressed sequence tags in common wheat (*Triticum aestivum*). *DNA Res*, 19:165-177.
- [5] Bipin, R., B. Prakash, C. Madhukar, C. Sandesh, K. Sushank, K. Saugat and B.P. Padam (2021). Drought stress impacts on wheat and its resistance mechanisms. *Malaysian Journal of Sustainable Agriculture*, 5(2): 67-76.
- [6] Diego, N. L., M. H. Ixchel, M. Reynolds, K. Sonder, A. Molero, R. D. Robertson, M. S. Lopes, W. Xiong, M. Kropff and S. Asseng (2021). Climate impact and adaptation to heat and drought stress of regional and global wheat

- production. *Environmental Research Letters*, 16 (5):1-17.
- [7] Maisura, M. A., L. I. Chozin and J. Ahmad (2014). Some physiological character responses of rice under drought conditions in a paddy system. *Journal of International Society for Southeast Asian Agricultural Sciences*, 20: 104-114.
- [8] Nadeem, S. M., M. Ahmad, Z. A Zahir, A. Javaid and M. Ashraf (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances*, 32: 429-448.
- [9] Camaille, M., N. Fabre, C. Clément and E. Ait Barka (2021). Advances in wheat physiology in response to drought and the role of plant growth promoting rhizobacteria to trigger drought tolerance. *Microorganisms*, 9(4): 1-22.
- [10] Chen, W., K. Yao, X. Cai and J. Chen. (2011). Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biological Trace Element Research*, 142: 67-76.
- [11] Larkunthod, P., N. Noppawan, L. Jonaliza and T. Theerayut (2018). Physiological responses under drought stress of improved drought tolerant rice lines and their parents. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 46(2): 679-687.
- [12] Ali, H. E. and Z. H. Saad, (2020). The effect of drought on chlorophyll, proline, and chemical composition of three varieties of Egyptian rice. *Journal of Biological Chemistry and Environmental Sciences*, 15(1): 21-30.
- [13] Wasson, A. P., R. A. Richards, R. Chatrath, S. V. Misra, S. V. Prasad, G. J. Rebetzke, J. A. Kirkegaard, J. Christopher and M. Watt, (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, 63: 3485-3498.
- [14] Nawaz, F., M. Y. Ashraf, R. Ahmad, E. A. Waraich and R. N. Shabbir (2014). Selenium (Se) regulates seedling growth in wheat under drought stress. *Advances in Chemistry*, 2014 :1-7.
- [15] Vinocur, B. and A. Altman (2005). Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. *Current Opinion in Biotechnology*, 16 :123-132.
- [16] Sallam, A., A. M. Alqudah, M. F. A. Dawood, P. Stephen and B. A. Börner (2019). Drought stress tolerance in wheat and barley. *Advances in Physiology, Breeding and Genetics Research. International Journal of Molecular Sciences*, 20: 31-37.
- [17] Dawood, M., A. Abeed and E. Aldaby (2019). Titanium dioxide nanoparticles model growth kinetic traits of some wheat cultivars under different water regimes. *Indian Journal of Plant Physiology*, 24 :129-140.
- [18] Bray, E. A. (2002). Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: an analysis using microarray and differential expression data, *Annals of Botany*, 89: 803-811.
- [19] Lichtenthaler, H. K. (1987). Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown *Journal of Plant Physiology*, 13:101-110.
- [20] Bates, L. S., R. P. Waldern and I. D. Teare (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205-207.
- [21] Piper, C.S. (1947) *Soil and Plant Analysis*. The University of Adelaide, Adelaide.
- [22] Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28: 350-356.
- [23] Kjeldahl, J. (1883) A new method for the determination of nitrogen in organic matter. *Zeitschrift für Analytische Chemie*, 22, 366-382.
- [24] Singleton, V. L. and J. A. Rossi (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *The American Journal of Enology and Viticulture*, 16(3):144-158.
- [25] Madhava, R. and T. Sresty (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Science*, 157(1):113-128.
- [26] Gomez, K. A. and A. A. Gomez (1984). *Statistical procedures for agricultural research*. John Wiley and Sons. New York, 680-693.
- [27] Hussain, S., M. Ahmad, S. Ahmad, J. Iqbal, M. N. Subhani, S. M. Nadeem, S. Atta and M. Ibrahim (2013). Improvement of drought

- tolerance in sunflower (*Helianthus annuus* L.) by foliar application of abscisic acid and potassium chloride. Pakistan Journal of Nutrition, 12(4):345- 352.
- [28] Davoud, A. and P. Mohammad (2015). Effect of drought stress on total protein, essential oil content, and physiological traits of *Levisticum Officinale* Koch. Journal of Plant Nutrition, 39(10):1-19.
- [29] Omidi, H. (2010). Changes of proline content and activity of antioxidative enzymes in two canola genotype under drought stress. American Journal of Plant Physiology, 5: 338-349.
- [30] Jongrunklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, T. Kesmala and A. Patanothai. (2008). Identification of peanut genotypes with high water use efficiency under drought stress conditions from peanut germplasm of diverse origins. Asian Journal of Plant Science, 7: 628-638.
- [31] Abdalla, M. M. and N. H. El-Khoshiban (2007). The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticum aestivum* cultivars. Journal of Applied Science, 3(12): 2062-2074.
- [32] Hamada, A. M. (1996). Effect of NaCl, water stress or both on gas exchange and growth of wheat. Biologia Plantarum, 38: 405-412.
- [33] Colom, M. R. and C. Vazzana (2002). Water stress effects on three cultivars of *Eragrostis curvula*. Italy Journal of Agronomy, 6: 127-132.
- [34] Bather, Z. F., M. Mirza, M. Ghorbanli and M. B. Rezai (2002). The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. Flavor and Fragrance Journal, 17: 275-277.
- [35] Poudel, M. R., S. Ghimire, M. P. Pandey, K. H. Dhakal, D. B. Thapa and H. K. Poudel (2020) Evaluation of wheat genotypes under irrigated, heat stress and drought conditions. The Journal of Biology and Today's World, 9(212):1-12.
- [36] Tayeb, M. A. (2006). Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid, peroxidation, organic solutes, catalase, and peroxidase activity. Acta Agronomica Hungarica, 54:25-37.
- [37] Maghsoudi, E. and M. Ashraf (2015). Influence of foliar application of silicon on chlorophyll fluorescence, photosynthetic pigments, and growth in water-stressed wheat cultivars differing in drought tolerance Turkish Journal of Botany, 39 (4): 625-634.
- [38] Tabaeizadeh, Z. (1998). Drought-induced responses in plant cells. International Review of Cytology, 182:193-247.
- [39] Nikolaeva, M. K., S. N. Maevskaya, A. G. Shugaev, and N. G. Bukhov (2008). Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. Russian Journal of Plant Physiology, 57(1): 87-95.
- [40] Ashraf, M. and P. Harris (2013). Photosynthesis under stressful environments: An overview. Photosynthetica, 51 (2): 163-190.
- [41] Shamsul, H., H. Qaiser, N. Mohammed, S. Arif, John, P. and A. Aqil (2012) Role of proline under changing environments, Plant Signaling & Behavior, 7(11): 1456-1466.
- [42] Farshadfar, E., H. Ghasempour and H. Vaezi (2008). Molecular aspects of drought tolerance in bread wheat (*T. aestivum*). Pakistan Journal of Biological Sciences, 11:118-122.
- [43] Endang, P., K. Florentina and F. Eny (2017). Growth, yield and physiological characters of three types of Indonesian rice under limited water supply. Asian Journal of Plant Sciences, 16: 101-108.
- [44] Maralian, H., A. Ebadi, T. R. Didar and B. Haji- Eghrari (2010). Influence of water deficit stress on wheat grain yield and proline accumulation rate. African Journal of Agriculture Research, 5:286-289.
- [45] Marcińska, I., I. Czyczyło-Mysza, E. Skrzypek, M. Filek, S. Grzesiak, T. Grzesiak, F. Janowiak, T. Hura, M. Dziurka, K. Dziurka et al., (2013). Impact of osmotic stress on physiological and biochemical characteristics in drought susceptible and drought resistant wheat genotypes. Acta Physiology Plant, 35:451-461.
- [46] Mengel, K. and E. A. Kirkby (2001). Principles of Plant Nutrition. 5th ed., Kluwer Academic Publishers Dordrecht, 181-242.
- [47] Roy, B. and A. B. Kumar (2009). Abiotic stress tolerance in crop plants: breeding and biotechnology: aluminum toxicity. New India Publishing Agency, 333-380.

- [48] Farooq, M., M. Hussain and K. Siddique (2014). Drought stress in wheat during flowering and grain-filling periods. *CRC Crit. Rev. Plant Sci*, 33:331-349.
- [49] Qiang, L., C. Jianhua, Y. Longjiang, L. Maoteng, L. Jinjing and G. Lu (2012). Effects on physiological characteristics of Honeysuckle (*Lonicera japonica* Thunb) and the role of exogenous calcium under drought stress. *Plant Omics*, 5(1):1-5.
- [50] Huang, G. T., S. L. Ma and L. P. Bai (2012). Signal transduction during cold, salt, and drought stresses in plants. *Molecular Biology Reports*, 39: 969 - 987.
- [51] Asghar, M. G. and A. Bashir (2020). Protagonist of mineral nutrients in drought stress tolerance of field crops. *Abiotic Stress in Plants Intech Open*, 25-39.
- [52] Marschner, H. (1995). *Mineral Nutrition of Higher Plants*. Second edition, Academic Press, San Diego. 417-430.
- [53] Waraich, E. A., R. Ahmad, M. Y. Ashraf and M. Ahmad (2011). Improving agricultural water use efficiency by nutrient management in crop plants. *Acta Agriculture Scandinavica, Section B-Soil & Plant Science*, 61(4):291-304.
- [54] Mottaleb, S., E. Darwish, M. Mostafa and G. Safwat (2017). Phenotypic root system architecture of cotton (*Gossypium barbadense* L.) grown under salinity. *Agriculture (Poľnohospodárstvo)*, 63(4): 142-150.
- [55] Marwa, R. and M. Ahmed (2014). Growth and physiological changes induced by drought and salicylic acid treatment of wheat genotypes (*Triticum aestivum* L.) at vegetative stage. *American-Eurasian Journal of Agriculture & Environmental Sciences*, 14 (12): 1498-1505.
- [56] Ballbrea, M. E., A. M. Rus-Alvarez, M. C. Bolarin, and F. Perez-Alfocea (1997). Fast changes insoluble carbohydrate and proline contents in tomato seedling in response to ionic and non-ionic iso-osmotic stresses. *Journal of Plant Physiology*, 5:221-226.
- [57] Pierre, M. and A. Savoure (1990). Effect of water stress and SO₂ pollution on spruce endopeptides. *Plant Physiology and Biochemistry*, 28(1):95-104.
- [58] Shao, B., Z. Liang, M. Shao and Q. Sun (2005). Dynamic changes of antioxidative enzymes of 10 wheat genotypes at soil water deficits. *Colloids Surf Biointerfaces*, 42:187-195.
- [59] Roy-Macauley, H., F. Zuily, Y. Kidric, M. Pham A. T. Thi and S. J. Viera (1992). Effect of drought stress on proteolytic activities in phaseolus and vigna leaves from sensitive and resistant plants. *Physiologia Plantarum*, 85 (1): 90-96.
- [60] Loutfy, N., M. A. El-Tayeb, A. M. Hassanen, M. M. Moustafa, Y. Sakuma and M. Inouhe (2012). Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum*). *Journal of Plant Research*, 125:173-184.
- [61] Sara, K., H. Abbaspour, J. M. Sinaki and H. Makarian (2012). Effects of water deficit and chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean (*Ricinus communis* L.). *Journal of Stress Physiology and Biochemistry*, 8:160-169.
- [62] Garmendia, I., Y. Gogorcena, I. Aranjuelo and N. Goicoechea (2017). Responsiveness of durum wheat to mycorrhizal inoculation under different environmental scenarios. *Journal of Plant Growth Regulation*, 36:855-867.
- [63] Talaat, N. B. and B. T. Shawky (2014). Protective effects of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) plants exposed to salinity. *Environmental and Experimental Botany*, 98:20-31.
- [64] Liu, H., D. Bruce, M. Sissons, A. Able and J. Able (2018). Genotype dependent changes in the phenolic content of durum under water deficit stress. *Cereal Chemistry*, 95:59-78.
- [65] Caverzan, A., C. Alice and P. S. Brammer (2016). Antioxidant responses of wheat plants under stress. *Genetics and Molecular Biology*. 39(1):1-6.
- [66] Hammad, S. R. and O. M. Ali (2014). Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. *Annals of Agriculture Sciences*, 59(1):133-145.
- [67] Mahajan, S. and N. Tuteja (2005). Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics*, 444:139-158.
- [68] Waraich, E. A. and R. Ahmad (2010). Physiological responses to water stress and nitrogen management in wheat (*Triticum aestivum* L.): evaluation of gas exchange, water relations and water use efficiency. In: Fourteenth International Water Technology Conference (IWTC 14th), 46-51.