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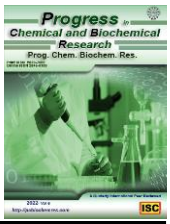

Original Research Article

The Effect of Drought Stress on Physiological Traits in Lines and Cultivars of Bread Wheat (*Triticum aestivum* L.)

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ABSTRACT

All around the globe, drought is considered one of the critical threats putting agricultural industries at stake. With such impediments existing, challenges of feeding the population arise. This study evaluated and selected appropriate wheat cultivars with desirable traits. The objective was achievable by accurately assessing quality traits and resistance characteristics under simulated drought-stress conditions. From 2018 till 2020, six cultivars were evaluated using a split-plot design with three repetitions. The study consists of a control plot (regular irrigation throughout the growing season; zero drought stress), moderate drought stress (irrigation halted before booting stage), and complete drought stress. According to the results, the intensity of drought stress has statistically significant impacts on quality traits; in other words, the content of soluble sugars, protein, and proline were affected. Furthermore, alternations in enzyme functions, such as peroxidase and polyphenol oxidase, were observed. The leaf protein content declined under moderate and full drought treatments (-55% and 71%, respectively). However, a significant increase was detected in total soluble sugar (4 and 10%, respectively) and proline content (26 and 45%, respectively), along with intensified enzymatic functions for peroxidase (10 and 22%, respectively) and polyphenol oxidase (4 and 10%, respectively). According to the results, certain biochemical traits should be taken into account when selecting drought-tolerant wheat varieties. The most durable cultivar was N-93-17, with the highest yield potentials, followed by Tirgan in terms of water deficiency. Rank sum analysis identified the most drought-tolerant cultivars as ‘N-93-17’ and ‘Aftab’. Results of this investigation would be of great importance in selecting desirable parents for the breeding program to develop wheat cultivars resistant to drought stress conditions

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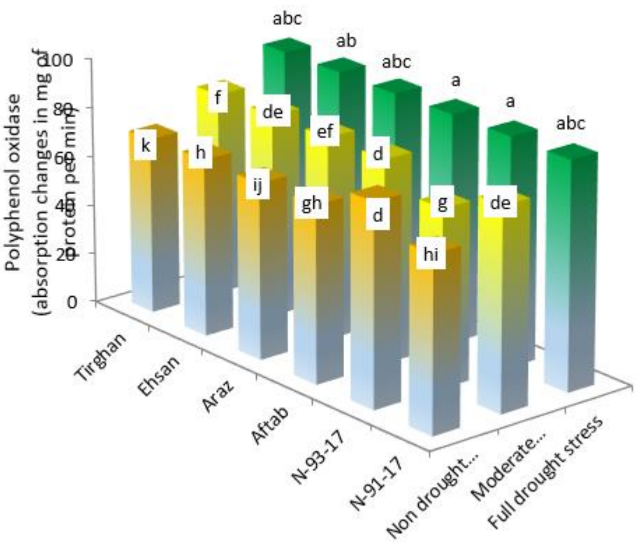
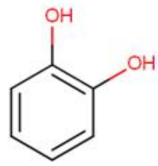
GRAPHICAL ABSTRACT





Introduction

Most parts of Asia, mainly Middle Eastern territories, are prone to recurrent drought episodes [1,2,3]. As a relatively arid region, Iran is also at risk of crops suffering drought stress. This undesirable condition disturbs principal crop productivity and declines the overall quality. Wheat is the most crucial grain and the staple food of Middle Eastern countries, Iran included. According to official statements, Iran's estimated annual wheat yield is about 5. 5 tons.ha^{-1}[4]. Although, given the increasing domestic demand for primary grains due to population growth, agricultural forces encounter deficiency problems when the drought stress intensifies. Major factors including the upsurge of urbanization, duplication of agro-processors, and augmented household incomes would also affect demand fluctuations. Taking the geographical characteristics of Iran into account, implementing cultivation management programs at low altitude lands to prevent productivity decline would be helpful to overcome drought-related obstacles. According to Borlaug and Dowswell [5], wheat production could be enhanced through land area expansion or increased yield per cultivated land unit. However, the first choice might be out of the question due to technical constraints and potential conflicts with industrial applications. Given this fact, the importance of selective breeding for drought-tolerant varieties comes to light. In this regard, developing modified wheat cultivars and obtaining required technologies would be an appropriate strategy.

Modification for drought-resistance traits has been recognized globally as an efficient solution to such difficulties. However, few cultivars have displayed proper resistance features under adverse environmental conditions. Degrees of drought tolerance depends on a complex polygenic trait that is affected by environmental factors. Moreover, the unpredictability of drought manifestation and intensity complicates the identification process of resistance-wise superior cultivars. Genetic diversity results in different coping mechanisms towards environmental complications.

Furthermore, identifying drought-tolerant cultivars under various degrees of moisture is also challenging. A standard method of drought resistance evaluation is the field-based empirical selection [6]. According to the growth stages of plants, the intensity of drought stress affecting total yield and quality would vary on many levels. As of yet, accurate timing for drought stress execution in experiments has not been compromised. Drought-induced stress adversely affects plant development, followed by dramatic decreases in overall efficiency [7].

Consequently, such impediments would cause extreme hinders in agriculture and the economics of the involved country [8]. Irrigation deficiency is a major stressing factor in drought-prone countries. Therefore, crop productivity in arid regions is generally at risk [9].

Mechanisms of plants are drastically affected by water stress [10, 11]. Environmental factors are unpredictably fluctuant. Therefore, numerous biotic and abiotic agents would alter overall morphologic and physiologic aspects, along with possible metabolism disorders [12,13]. Responses to varying degrees of drought stress are different among plants and species [10,11]. The innate coping mechanisms of certain plants provided by certain physiologic and biochemical features will allow survival adaptation to stress-inducing conditions [11,14]. Various factors, including the stress phase intensity and extent, plant development stage, genetic traits, and interventions, determine the general response to water depletion [15,16]. Drought resistance in plants has certain thresholds; excessive stress intensity would cause severe and irreversible crop damage [12].

With refining management and selection for resistance, drought tolerance traits could be manipulated and enhanced. This would be an efficiently strategic means by which the adverse impacts on crop yield can be alleviated [16, 17]. So far, modification techniques have made it possible to obtain wheat cultivars with efficient resistance traits [18]. Nevertheless, these approaches are costly, time-consuming, and risk other necessary traits getting lost from the host gene pool during the process [19]. In this regard, principal producers should acquire safer approaches. Selective breeding of currently available wheat cultivars with more acceptable resistance characteristics would prevent unwanted trait loss [20, 21]. Physiological, biochemical, and morphological alternations could enhance adaptation responses to drought stress. Modifiable factors, including growth rate, antioxidant defenses, stomatal conductance, and tissue osmotic potential, could boost resistance mechanisms [22-24]. Generally, drought stress prompts biosynthesis rate in comparison to normal optimum conditions, causing an upsurge in proline and total soluble sugar quantity [25]. Islam *et al*. [26] and Manuchehri *et al*. [27] observed a significant increase in soluble sugar and proline content, with intensified enzymatic free radical scavenging function with imposed drought stress. This mechanism is implemented to prevent the accumulation of reactive oxygen species. Producing defensive biochemical agents in response to water scarcity allows higher yield for tolerant cultivars and partly ensures food safety [14].

The present study investigated various impacts of drought stress on biotic parameters in wheat cultivars. According to the results, tolerant wheat varieties would be identified and selected to prevent drought-induced productivity loss.

Materials and Methods

Location, Duration, Treatments, and Design

The study was carried out during 2018-2019 and 2019-2020. Two field experiments were executed at the Agricultural and Natural Resources Research and Education Center of Ardabil, AREEO, Moghan, Iran. Three conducted treatments consisted of i) regular irrigation through the whole growth season (control treatment, no drought stress); ii) irrigation up to the booting stage (moderate drought stress), and iii) full drought stress. The experiment was performed on six wheat cultivars, including N-93-17, Tirghan, N-92-9, N-91-17, Ehsan, and Aftab. The study was designed as a split plot in randomized complete blocks with three repetitions. Irrigation regimes were accounted for the main plot, while sub-plots were concerned with wheat genotypes. Seed and Plant Improvement Institute (SPII) obtained the genetic features of respective wheat cultivars.

Experimentation

On November 15th the cultivation was performed manually in 2 m long rows with 0.2 m inter-row spacing with a seed rate of 150 kg.ha^{-1}. Each cultivar was sown in specified plots within four rows. NPK fertilizer (consisting of nitrogen, phosphorus, and potassium) was applied by 121 kg.ha^{-1}. Every cultivation practice was performed according to standard regulations set for wheat. Harvesting was carried out after the first indications of maturity were observed. Post-harvest operations were also implemented regarding standard procedures.

Soil and climatic parameters

Climatic parameters were recorded at a weather station adjacent to the research location monthly. The recording was carried out for two years (Fig. 1). Initially, the field soil was collected from treatment plots and analyzed. The results are presented in Table 1.

Data collection

Evaluation of biochemical traits

Proline determination

The proline content of leaves was measured according to the method used by Bates *et al.* using ninhydrin acid reagent [28]. Leaf samples were analyzed at the flowering stage after imposing drought stress to measure proline fluctuations in response to water deficit. Fresh leaf samples (0.5g each) were homogenized by 10ml of 3% aqueous sulfosalicylic acid. The resulting homogenate was filtered through No. 2 Whatman paper filters. The filtrate underwent analysis procedures for proline content determination. In a test tube, about 2 ml mL of the filtrate was mixed with ninhydrin acid (2 mL) and glacial acetic acid (2 mL). The mixture was placed in the water bath at 100 °C for one hour to allow reactions.

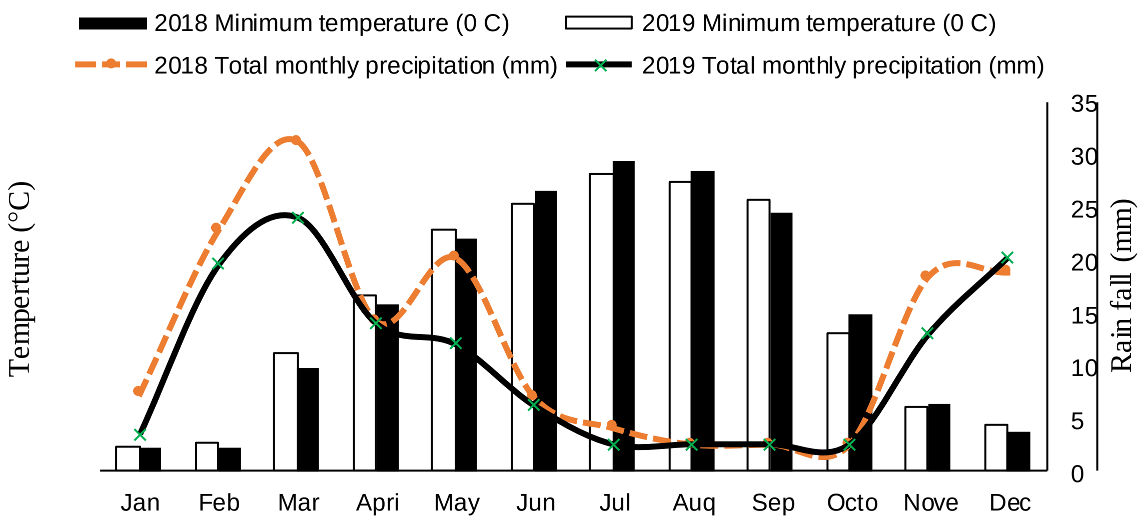


Fig. 1. Climate conditions (temperature and precipitation) during the growing season 2018 and 2019.

Table 1. Physical and chemical properties of soil in the experimental field (mean values)

Texture	Sand	Silt (%)	Clay	K (ppm)	P (ppm)	N (%)	OC (%)	pH	EC (dS/m)	Depth (cm)
SC	14	40	46	626.6	12.72	0.12	1.20	7.9	1.08	0-20

The resulting mixture was given time to be cooled, followed by adding toluene (4ml) and consistent mixing for 15-20 s to form separate layers. The topmost toluene layer (chromophore) comprising proline-ninhydrin reactions was separated from the aqueous medium and transferred to another tube to warm at room temperature. The absorbance rate was measured by a spectrophotometer at 520 nm.

Total soluble sugar

The total soluble sugar content of leaf samples was measured using the methods of DuBois *et al*. [29] (with slight modifications) [30]. For this analysis, a 5% phenol solution (5g phenol solved in 95 of double-distilled water) and a 90% ethanol solution (90 mL of ethanol mixed with 10 mL ofmL double distilled water) were used as chemical reagents. Fresh sample material weighing 0.25 g was set in 10 mL of 90% ethanol at 60 °C inside a water bath for one hour. The resulting extract was filtered and transferred into a volumetric flask, followed by re-extraction of the residue. Adequate amounts of 90% ethanol were added to bring the volume to 25mL. 1 mL of the extract was transferred to a test tube and mixed with 1ml of 5% phenol solution. Some reaction time was allowed, and 5 mL of 95% sulfuric acid was mixed in by vertical agitation. The test tube was cooled at room temperature for an exothermic reaction. The absorbance rate was recorded on a spectrophotometer at 490 nm. Total soluble sugar content was calculated regarding the standard glucose graph.

Enzymatic Antioxidant Activities

Antioxidant enzyme extraction was performed through the methods of Sunohara and Matsumoto [31] with modifications. In this procedure, 200 mg of fresh leaves were chopped and homogenized in 26 mM K-P buffer (10ml; pH 7.8) containing one mM AsA, 0.4 mM EDTA, and 2% PVPP. The process was carried out for one minute under 4°C. The homogenate was centrifuged by 15000 g at 4 °C for 20 min, and the supernatant was separated via the No. 1 Whatman paper filter. The filtrate was collected for peroxidase enzyme activity determination (EC 1.11.1) [32]. The peroxidase functionality was surveyed through methods proposed by Chance and Maehly [33]. An alcoholic solution of the tissue extract (100 µL) was added to assay solution (3 mL) containing 3 ml of the reaction mixture (13 mM guaiacol, 5mM H_2O_2 mixed with 50 mM sodium Na-phosphate (pH 6.5)). The spectrophotometer detected an increase of optical density at 470 nm for 1 min at 25 °C. The polyphenol oxidase activity (PPO, EC 1.10.3.1) was also surveyed through methods presented by Kumar and Khan [34].

Protein Content

Methods of Bradford were used for protein content determination [35]. In this procedure, bovine albumin serum (BSA, Sigma chemical) was considered the standard. 50 mg of fresh leaf material was incubated in 5ml of extraction buffer (Tris-HCL at 25 mM (pH 7.6)). The solution was centrifuged at 2000 g for 15 min. After ELISA (Power Wave XS, BioTek, USA) was carried out, total soluble protein content was detected at 595.

Data Analysis

The physiological, morphological, and biochemical data were subjected to two-way analysis of variance (ANOVA) using the Statistical Analysis System (SAS) version of 9.0 (SAS Institute Inc., Cary NC, USA). To quantify the effects of cultivars, environmental factors, and irrigation regimes. The cultivars and various irrigation treatments were separated by Fisher's unprotected least significant difference (LSD) at a 0.05 significance level. The bivariate correlations among biomass and agronomic traits were analyzed using the Pearson correlation procedure via Statistical Software for Social Science [36]. Multivariate associations were analyzed using the principal component and bi-plot analyses in GenStat® version 18.2.0, VSN, International [37]. The graphs were plotted using Microsoft Excel 2010 (Microsoft® Corp., Redmond, Washington, USA).

Results and Discussion

Analysis of variance (ANOVA) revealed that there were significant differences ($p < 0.01$) among the wheat cultivars in terms of the studied traits (Table 2). Results further indicated that there were significant interaction effects between cultivars and water stress for all the studied traits (Table 2). Only the significant interaction effects were presented and discussed.

The influence of water scarcity was highly significant ($p < 0.001$) on the production of proline, total soluble sugar, polyphenol oxidase, peroxidase, and protein content in wheat cultivars (Table 2). The results revealed that the highest amount of proline (11.264 µg/g FW) was recorded in N-93-17 cultivar under full drought stress conditions, and the lowest amount of proline (3.806 µg/g FW) in Ehsan cultivar under non-stress conditions (Figure 2). Implies that the tested cultivars produced significantly higher amounts of proline under the water stress condition as a mitigation strategy to cope with the drought. Accumulation of proline in plants tissues is a clear indication of their inherent ability for tolerance against environmental stress, particularly in plants under drought stress. Proline accumulation may also be part of the stress signal influencing adaptive responses [26, 38]. Therefore, the more the proline, the better the ability of plants to withstand water stress, which is one of the most common compatible osmolytes in drought-stressed plants. Proline is a compatible solute, playing a significant role as an enzyme-stabilizing agent. It has the ability of osmotic balance in the cytoplasm and vacuole to stabilize the subcellular structure and protect membrane and proteins from oxidative damage during water stress [39-41]. Proline is one of the osmoprotectants accumulated in many crops in response to different kinds of stresses, including drought [42]. Plants with higher proline content in their body develop resistance to cope with water stress and result in better yield than other plants with lower proline content [43].

Table 2. Combined analysis of variance for traits in six wheat genotypes with three irrigation treatments

Sources of variation	df	Mean square				
		Proline	Total soluble sugar	Polyphenol oxidase	Peroxidase	Protein content
Year (Y)	1	0.835^{ns}	4.480*	0.009^{ns}	2.159*	0.013^{ns}
Year/replication	4	0.400^{ns}	0.980^{ns}	0.092^{ns}	0.029^{ns}	2.561^{ns}
Irrigation	2	244.490**	383.590**	4.686**	30.374**	204.556**
Y× I	2	2.005^{ns}	1.370^{ns}	0.088^{ns}	0.046^{ns}	0.731^{ns}
Error (a)	8	2.347	0.720	0.083	0.118	2.837
Cultivar (C)	5	72.578**	54.750**	1.991**	1.969**	13.887**
Y× C	5	0.286^{ns}	5.930**	0.162*	0.630**	7.340**
I× C	10	9.882**	9.250**	0.325**	0.840**	20.089**
Y× I× C	10	0.210^{ns}	3.510**	0.078^{ns}	0.250^{ns}	0.387^{ns}
Error (b)	60	0.641	1.010	0.052	0.183	0.284
CV(%)	-	13.097	9.177	11.223	13.304	9.521

^{ns},* and **: non-significant and significant at 5% and 1 % probability levels, respectively

Similarly, the results revealed that the highest soluble sugar (77.74 mg/g FW) was obtained for the first year × full drought stress × N-93-17 cultivar, and the lowest soluble sugar (64.750 mg/g FW) was recorded in the second year × non-stress condition × cultivar Ehsan (Figure 3). It also implies that the tested cultivars under water stress treatment produced a significantly higher amount of soluble sugar in response to the water scarcity as a stress-escaping strategy. Leaf osmotic adjustment through synthesizing soluble sugars has been a well-known mechanism to withstand water stress in many plants [44, 45]. Synthesis of soluble sugars plays a great role in osmotic adjustment for water scarcity. Osmotic cell potential in plants can be adjusted by increasing the concentration of soluble sugar, which can decrease the water potential of cells without inhibiting the enzyme's function and does not reduce the turgidity of the cell. Increased concentration of sugar because of water scarcity helps maintain the membrane's stability, which in turn prevents and protects the membrane fusion. It keeps protein to stay functional [45, 46]. According to Zhang *et al*. [24], solutes accumulated during stress serve as an aid to cope with the existing condition. Current result is following Hou *et al*. [47], who reported that the sugar content in the leaves of the plant could increase under drought conditions.

The results revealed that the highest polyphenol oxidase (92.687 changes in adsorption per microgram of protein per minute) was obtained under full drought stress for N-93-17 cultivar, and the lowest polyphenol oxidase (71.032 absorption changes in micrograms of protein per minute) was obtained under non-stress condition for Ehsan cultivar (Figure 4). Results also revealed that the highest peroxidase (78.484 absorption changes in mg of protein per minute) was obtained under full drought stress for N-93-17 cultivar, and the lowest peroxidase (57.208 absorption changes in mg of protein per minute) was obtained under non-stress condition for Ehsan cultivar (Figure 5). The results indicate that the highest leaf protein (14.85%) was obtained under non-stress conditions for the cultivar Ehsan, and the lowest leaf protein (6.63%) was obtained under full drought stress for the cultivar N-93-17 (Figure 6).

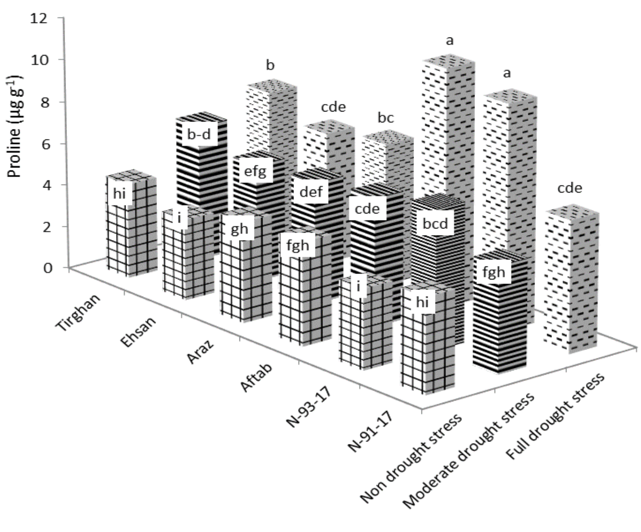


Fig. 2. Proline content in the leaves of six wheat lines and cultivars subjected to drought stress treatments

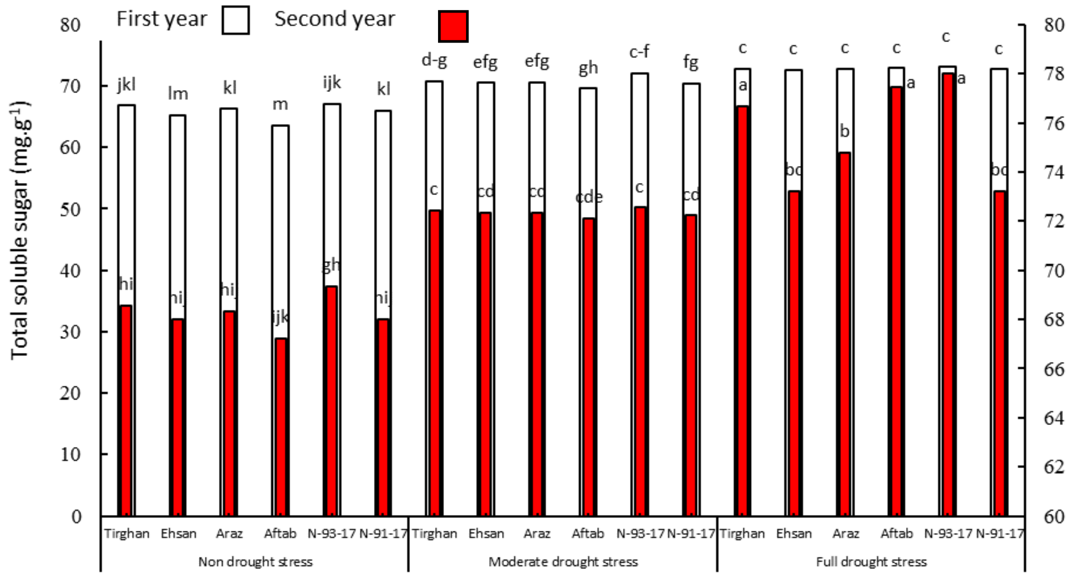


Fig. 3. Total soluble sugar content in the leaves of six wheat lines and cultivars subjected to drought stress treatments

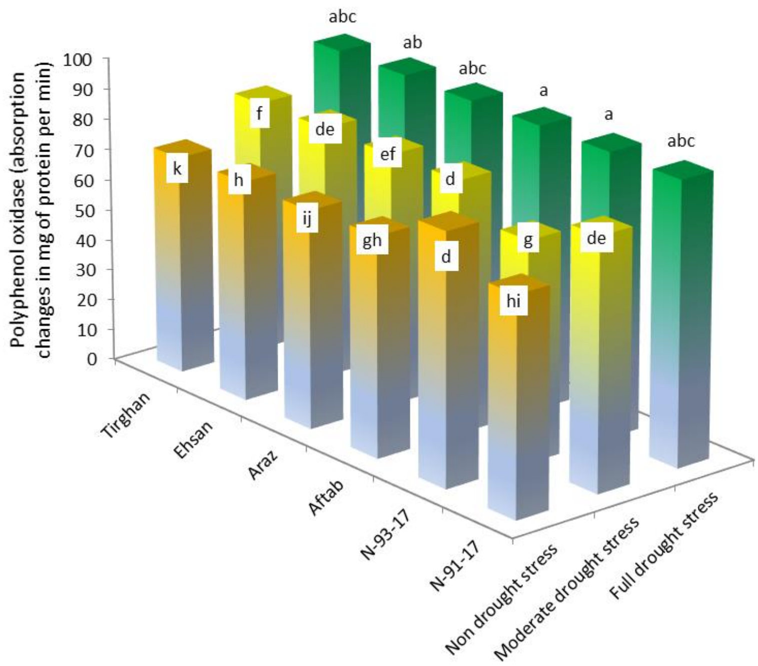


Fig. 4. Polyphenol oxidase enzyme activity in the leaves of six wheat lines and cultivars subjected to drought stress treatments

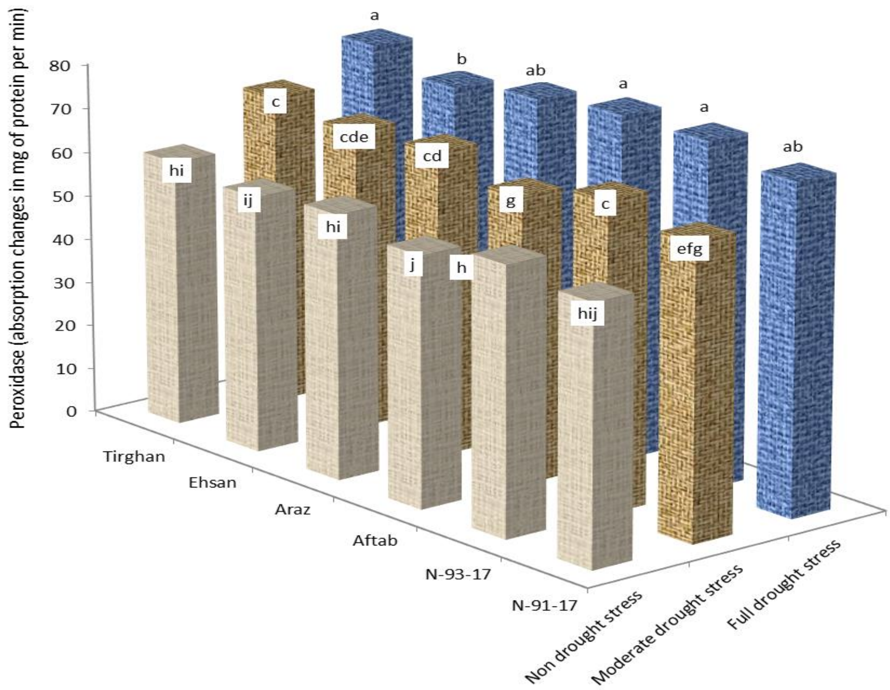


Fig. 5. Peroxidase enzyme activity in the leaves of six wheat lines and cultivars subjected to drought stress treatments

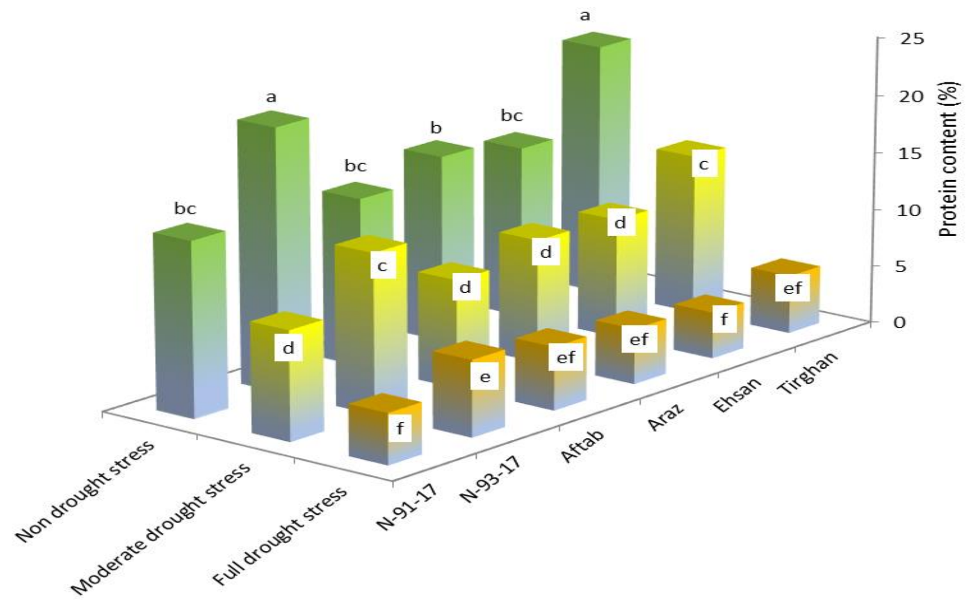


Fig. 6. Protein content in the leaves of six wheat lines and cultivars subjected to drought stress treatments

Cluster Analysis

The treatment (cluster analysis) was classified to decrease the number of experimental treatments and determine the most effective treatment. As seen in Figure 7, treatments were classified into three clusters. Under non-stress (first cluster), wheat cultivars fall into three groups. Thus, N-93-17 and Tirgan cultivars are in the first group, N-92-9 and N-91-17 cultivars are in the second group, and Ehsan and Aftab cultivars are in the third group. In moderate drought stress (second cluster), wheat cultivars are divided into three groups. The N-93-17 and Tirgan cultivars are in the first group, N-92-9 and Ehsan cultivars are in the second group, and N-91-17 and Aftab cultivars are in the third group. Under complete drought stress (third cluster), wheat cultivars fall into three groups, and the N-93-17 and Aftab cultivars are in the first group. Tirgan and N-92-9 cultivars are in the second group, and N-91-17 and Ehsan cultivars are in the third group. A population can show the maximum dispersion whose parents are completely different in drought tolerance (the intersection of the most tolerant and sensitive cultivars). Therefore, grouping cultivars into different groups will help us choose the breed's parents.

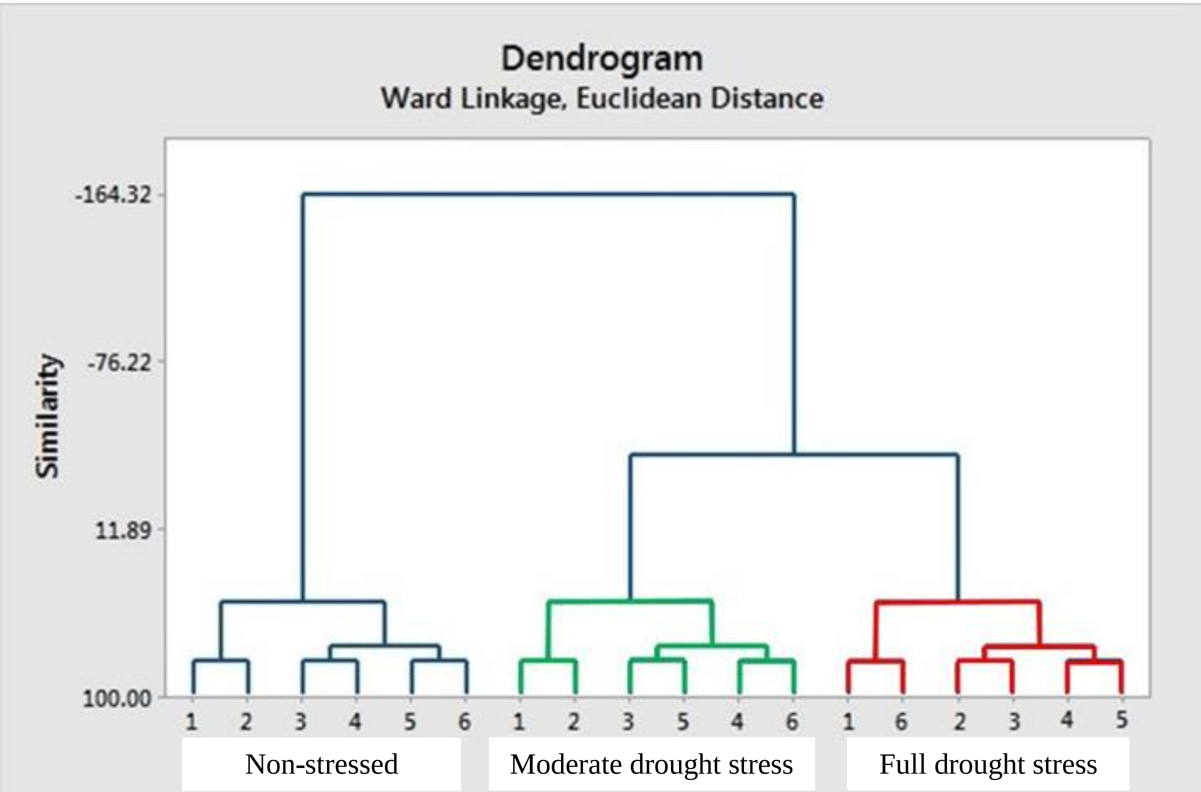


Fig. 7. Dendrogram of cluster analysis of wheat lines and cultivars under drought stress conditions based on Ward method (1: N-93-17, 2: Tirghan, 3: N-92-9, 4: N-91-17, 5: Ehsan, 6: Aftab)

Conclusions

The results revealed that the differences in the synthesis of proline and total soluble sugar with an increase in water scarcity among the selected wheat cultivars showed the diverse ability of the tested cultivars to cope up with drought. N-93-17 and Aftab cultivars among bread wheat were found to have a better capacity to endure water stress by synthesizing more proline and total soluble sugar as mitigation strategies. Overall, the physiological and biochemical parameters could be effective indicators to identify tolerant wheat cultivars in water-limited environments. The dependability of the observed traits for the selection ofselecting tolerant wheat cultivars for specific area, could be recommended to be verified under different environmental conditions to solve problems related to water scarcity. Results of this investigation could also provide usable genetic variability for wheat yield improvement and would be of great importance in selecting appropriate wheat cultivars and selecting desirable parents for the breeding program to develop the wheat cultivars resistant to drought stress conditions.

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