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BIOCHEMICAL CHANGES OF MEDITERRANEAN BARLEY AS AFFECTED BY DROUGHT STRESS

Farid Hellal[*1], Saied El-Sayed[1], Zeinab K.Taha[2], S.F. Desouky[2] and Chedly Abdelly[3]

[1]Plant Nutrition Department, National Research Centre, Dokki, El- Behouth St., Cairo 12622, Egypt,
[2]Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Egypt
[3]Centre of Biotechnology, Borj Cedria, Tunisia
*Corresponding author E-mail : hellalaf@yahoo.com

Abstract

Field experiment carried out in the Experimental Research Station of Nubaria, National Research Centre, Egypt to study the influence of drought stress on physiological and morphological structures of the Mediterranean barley (Egyptian, Tunisian, Algerian and Morocco) varieties focusing on chlorophyll, proline content, leaf area and free amino acids. The drip irrigation system applied through two types of in line emitter according to its discharge (4 and 2 liter), which fulfill 75 and 40 % of water holding capacity as normal and stress, respectively. The Egyptian barley varieties Giza 130 and Giza 123 registered the highest SPAD Chlorophyll at stem elongation and Giza 131 and Giza 127 scored the highest proline content. The Tunisian barley variety Sidi-Bou recorded the highest Chlorophyll content whereas Lemsi and Kairouan attained the lowest chlorophyll content at stem elongation stages. The highest proline content observed at Manel, Kebili-3 and Sidi–Bou due to drought stress condition. The anatomical studies appeared Kibili-3 variety was the highest Fiber number and Aleurone layer while, Temacine variety was higher in compressed layer percentage and Giza127 variety gave the highest endosperm ratio. The most productive barely varieties regarding the studied biochemical parameters were Giza 123, 130, 131, 2000 (Egypt), Kibili-1, Tozeur- 2 and Sidi-Bou (Tunis), Saida, Nâilia, Temacine (Algeria), Oussama, Laanaceur and Amira (Morocco) with drought. These varieties registered the lowest reduction percentage in the studied biochemical parameters under drought stress and it could be suggested to be grown under arid and semi-arid climatic conditions.

Keywords: water stress, chlorophyll, proline, amino acids, relative water content, Aleurone.

Introduction

Drought at a certain level, it damages the physiological processes in plant, like photosynthesis is more sensitivity to drought, decline of stomatal conductance and increased the level of chloroplast damages under drought (Boyer et al., 1997 and Lawlor 2002). Pan et al. (2011) stated that the majority of water in plants is consumed through leaf transpiration, so environments impact can affect the exterior structure, activities of physiological and internal structure in leaf.

The mechanical tissue development was better around the vascular bundle in drought-resistant varieties, and lignification degree of thick-walled cells was also higher (Zhu et al., 2010). Chen et al. (2011) they concluded that the chloroplast victimization varied in varieties with different drought resistance in barley. The Egyptian barley is principally become under rainfed conditions in the northern waterfront locales and under water system in the newly reclaimed soil, and in saline soils where water system water isn't adequate. The complete territory of barley development in the soil of Egypt fluctuates relying upon the sum and circulation of yearly precipitation. Deficit of water is a worldwide problem particularly in arid and semiarid climate as a result of not only to climate change, but also rapid expansion in domestic and agricultural use (Borras et al., 2011). Effective management of water in the agricultural production in water scarcity regions needs the use of sustainable approaches.
Moreover, the higher increases in prices of food crops, and some production countries used these crops production; the extension in national cultivation of these crops especially those lesser in water requirements considered one from the important and necessary targets (Rulli et al., 2013). Barley (Hordeum vulgare L.) is a typical crop dry land areas of North African countries and Mediterranean districts extraordinarily in the Northern seaside zones which precipitation fluctuated from year to year and in most years the barley developed territory, as in Egypt, that suffer from water shortage in the season of sowing and at the later growth stages which require supplemental irrigation. Water deficit can also affect the mineral concentration and content in cereal plants (Hussein et al., 2006).
Barley in Egypt is grown under wide range of environmental conditions. It is grown in areas where water supply is low and where crop generation depends predominantly upon rainfed condition. Barley cultivated mainly in Northern coastal region and in the marginal areas of Nile Valley and Delta and in the newly reclaimed soils in order to its tolerance to salinity and drought than wheat.
The aim of this research work was to investigate the differences of drought stress resistance of barley varieties based on chlorophyll, proline content, Leaf area and total amino acids and provide references for drought resistance indexing of the Mediterranean barley varieties.

Material and Method

1. Experimental design

Field tests were directed amid two winter successive seasons (2016/17; 2017/18) to study the biochemical change in Mediterranean barley (Hordeum vulgare L.) varieties grown under drought condition at the Experimental Research Farm of National Research Centre, Nubaria region, Egypt (latitude 30.87 N, and longitude 31.17 E, and mean elevate 21 m above sea level). The experimental area was classified as parched locale with cool winter and sweltering dry summer winning in the experimental area. There was no effective rainfall (low intensity) that can be taken into consideration throughout the two growing seasons. The soil of experimental site is assorted as sandy soil. A randomized complete block design was used with 3 replications.

Drip irrigation regime was applied through two types of in line emitter according to its discharge (4 and 2 liter), which fulfill 75 and 40 % of (WHC) named as Normal and Stress condition, respectively. Thirty five Mediterranean barley varieties were used for test experiment field are Egyptian varieties (1-Giza 123, 2-Giza 125, 3-Giza 126, 4-Giza 127, 5-Giza 130, 6-Giza 131, 7-Giza 2000, 8-El-Arich and 9-Ksar), Tunisian Varieties (10-Kebili 1, 11-Tozeur-2, 12-Kebili 3, 13-

Kairouan, 14-Manel, 15-Raihane, 16-Sidi-Bou, 17-Sabra, 18-Tombari, 19-Lemsi), Algerian Varieties (20-Temacine, 21-Ksar-Megrine, 22-Techedrett, 23-Saida, 24-Sedi Mahdi, 25-Ras El-Mouche, 26-Naïlia) and Morocco Varieties (27-Adrar, 28-Oussama, 29-Amalou, 30-Massine, 31-Taffa, 32-Firdaws, 33-Amira, 34-Tamellalet, 35-Laanaceur).The seeds of the selected varieties obtained from National gene bank of Tunisia. Sowing dates were November 25th 2016 and 2017 season. The soil was prepared as ordinarily, done in traditional cultivation.

2. Estimation at heading stage

Chlorophyll content

Leaf greenness present in a plant was determined with the Minolta-SPAD Chlorophyll Meter (Minolta Camera Co., Osaka, Japan). The SPAD-502 chlorophyll meter measures the chlorophyll absorbance in the red and near-infrared regions and calculates a numeric SPAD value which is proportional to the amount of chlorophyll in the leaf Minolta (1989).

Relative water content

Leaf relative water content (RWC) was evaluated by Castillo (1996) for every dry spell period. For these ten fully matured leaves of five plants / plot (2 leaves per plant) were selected from the same heights and their fresh weight (FW) was recorded. The leaves were drenched into refined water under low lighting conditions for 24 h to gauge their immersed weight. After recording turgescence weight (TW), leaves were dried at 75 °C for 48 h and their dry weights (D.W) were measured.

RWC was calculated by using formula: RWC = (FW-DW)/(TW-DW) × 100

Determination of proline

First, 0.4 g of fresh plant material was homogenized in 1.5 ml of distilled water and then incubated in water bath at 100 °C for 30 min. Then, the samples were cooled to room temperature (22 °C) and centrifuged for 10 min at 4 000 rpm.

Next, 1 ml of a 1% solution of Ninhydrin in 60% acetic acid was added to 0.5 ml of the supernatant and incubated at 100 °C for 20 min. After cooling to 22 °C, 3 ml of toluene was added and the samples were shaken and left in the dark for 24 h for phase separation. One ml of proline extract was introduced to a cuvette and the absorbance was measured by spectrophotometer at a wavelength of ? = 520 nm according to (Bates et al. 1973; Maria et al. 2014).

Determination of total Amino acid

The Determination of total Amino acid was done according to Millipore cooperative (1987) liquid chromatographic analysis of amino acids in barley grains using a modification of the Pico-Tag methods.

3. Anatomical studies

Micro technique practices were carried out at the laboratory of Agric. Bot. Dept. Faculty of Agric., Cairo University, during the second season. Materials were killed and fixed for at least 48 hrs. In F.A.A. (10 ml formalin, 5ml glacial acetic acid, 85 ml ethyl alcohol 70%) and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax melting point 56 C° (Sass, 1951). Sections which were cut on a rotary microtome at a thickness of 15-20 microns were stained with crystal violet/ erythrosine before mounting in Canada balsam according to (Nassar and El–Sahhar, 1998). Slides were examined microscopically and photo micrographic. The following parameters were recorded: (Fiber layer, compressed layer, Aleurone layer and Endosperm)

4. Statistical analysis

Data of two growing seasons were measurably broke down as a Randomized Complete Block Design (RCBD) utilizing examination of variance (ANOVA) and the means of varieties included in this trial compared using fisher test run by (LSD) at (P = 0.05) according to Gomez and Gomez (1984).

Results and Discussion

Pigment content

Some physiological and biochemical processes get affected by the drought, such as photosynthesis. Figure (1) is representing the SPAD chlorophyll reading of the investigated barley varieties as affected by normal (75% WHC) and drought conditions (40% WHC) of water regime. Data revealed that the highest values of SPAD Chlorophyll reading at stem elongation stage and ear emergence recorded for the Egyptian barley varieties El-Arich followed by cultivar Giza 125 for stem elongation and Ksar variety at ear emergence stage while, the lowest chlorophyll content observed for barley variety Giza 131 under water treatment normal and stress.

Concerning the Tunisian varieties, data on hand revealed that the highest chlorophyll content recorded for Sidi-Bou variety and Kairouan and Lemsi scored the lowest one at stem elongation under water treatment normal and stress. The sensitive Tunisian varieties to drought stress which attain high reduction percentage were Raihane 21.8% and Sabra 21% in same sequence mentioned above.

According to the SPAD Chlorophyll reading at ear emergence, data in Figure (1) showed the highest reduction resulted from imposing barley plant to drought stress as compared with normal condition. The Tunisian barley variety registered the highest content under normal and drought conditions and it could be arranged in descending orders as follows: Kebili-3> Tozeur-2> Kebili-1, while Lemsi scored the lowest chlorophyll content. Also, data notice that the highest reduction percentage for SPAD Chlorophyll content at ear emergence growth stage was attained to Sabra (22%) and the lowest one (more tolerant) was Kebili-3 variety. The SPAD chlorophyll reading of Algerian barley varieties observed that Ras El-Mouche recorded the highest contents under normal and drought stress condition at stem elongation stage. Whereas, the lowest contents of SPAD Chlorophyll reading observed for Saida varieties at ear emergence growth stage. Also, it is clear to mention that, the morocco barley varieties Amalou, Firdaws and Laanaceur scored the highest SPAD Chlorophyll reading under both normal and drought stress condition at stem elongation growth stages. While the smallest ones observed at Massine, and Amira (stem elongation), Oussama and Tamellalet at ear emergence stages under normal and drought stress conditions.

Relative water content

The values of relative water (RWC) of Egyptian barley varieties at ear emergence growth stage as affected by normal irrigation and water deficit represented in Table (1). Resulted data revealed that the elevate values of relative water content at ear emergence recorded for barley cultivar Giza 127 under normal and water deficit condition. However, the minimum values of RWC recorded for Egyptian barley varieties Kasr and El-Arish at ear emergence growth stage.

Concerning the RWC at ear emergence stage of Tunisian barley varieties, data in Table (1) showed that Sabra and Kebili-3 scored the raise values under normal condition and Kebili-3 gained the raise value under stress one. However, Tozeur-2 was more tolerant (4.3%) and Sabra was more sensitive one relative to the reduction percentage values. Also, data noticed that the most tolerant Algerian barley varieties were Tchedrett and Temacine with reduction percentage values 4.5%. While the highly sensitive ones were Sedi Mahdi (18.9%) and Ras El-Mouche (23.9%).

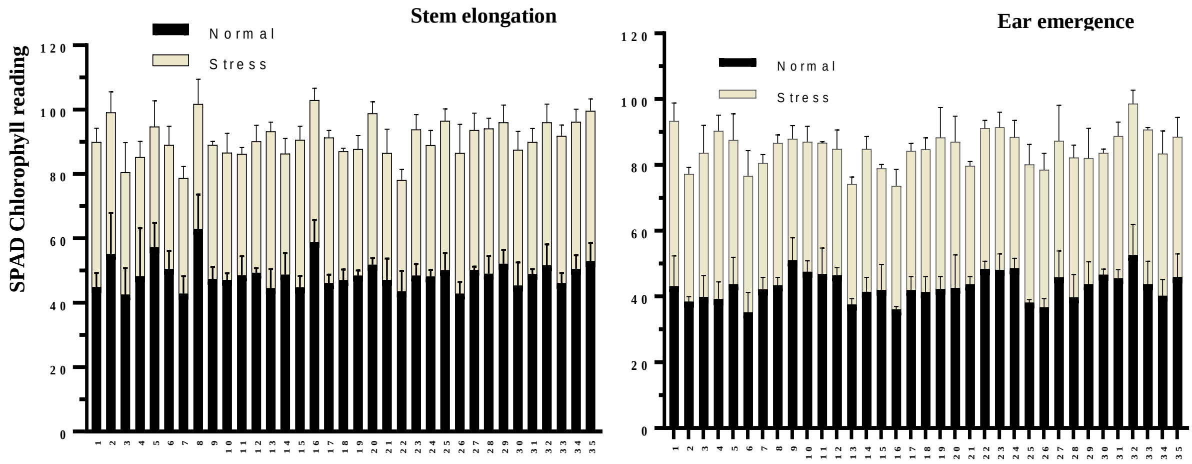

Figure (1): SPAD Chlorophyll reading of barley varieties as affected by water stress

Egyptian varieties: (1-Giza 123, **2**-Giza 125, **3**-Giza 126, **4**-Giza 127, **5**-Giza 130, **6**-Giza 131, **7**-Giza 2000, **8**-El-Arich and 9-Ksar), **Tunisian varieties:** (**10**-Kebili 1, **11**-Tozeur-2, **12**-Kebili 3, **13**-Kairouan, **14**-Manel, **15**-Raihane, **16**-Sidi-Bou, **17**-Sabra, **18**-Tombari, **19**-Lemsi), **Algerian varieties:** (**20**-Temacine, **21**-Ksar-Megrine, **22**-Techedrett, **23**-Saida, **24**-Sedi Mahdi, **25**-Ras El-Mouche, **26**-Naïlia) and **Morocco varieties**: (**27**-Adrar, **28**-Oussama, **29**-Amalou, **30**-Massine, **31**-Taffa, **32**-Firdaws, **33**-Amira, **34**-Tamellalet, **35**-Laanaceur).

Regarding to the resistivity and sensitivity to drought stress, estimated reduction percentage relative to the drought effect, one can notice that the sensitive varieties were Adrar (ear emergence) and Tamellalet (Leaf area) whereas the highly tolerant cultivar were Adrar-Amira and Massine in same sequence.

Leaf area

From Table (1) revealed that elevated values of leaf area (LA) at ear emergence growth stage recorded for barley cultivar Giza 131, under drought stress condition as compare to the remaining varieties. However, the lowest leaf area recorded for Egyptian barley Giza 126 and Giza 125 at normal and drought condition, respectively. Regardless stress effect, the barley varieties Giza 131 and Giza 125 scored the ultimate and least values of leaf area, respectively. The highest and lowest LA values were recorded at Tunisian barley varieties Kebili-3 and Lemsi under normal and drought conditions, respectively. Whereas, the highest reduction values (sensitive) were attained at Tombari and Lemsi varieties and the lowest reduction value (Tolerant) was scored at Kebili-3. The Algerian barley varieties Nâilia and Techedrett scored the elevate values of LA and Ras El-Mouche gained the lowest ones under both examined condition whereas, Techedrett showed a high tolerance and the opposite was true in case of Ras El-Mouche which was more sensitive than the others with reduction values 35% comparing drought stress with normal condition. There was a dramatic reduction in LA as a result of exposing the Morocco barley varieties to water stress. The highest and lowest LA values were registered for Morocco barley varieties Amira, Oussama and Amalou under normal and drought stress conditions, respectively. Regarding the change percentage of LA as affected by water stress, the highest reduction percentage (sensitive) attained for Tamellalet (25 %), Firdaws (23%) followed by Oussama = Adrar and the lowest reduction percentage registered for (tolerant) Massine (14%), Amira (15%) and Laanaceur (16%).

Proline content

Data in table (1) indicated that the Egyptian barley varieties Giza 131 and Giza 127 recorded high proline content under studied water regime for examined barley varieties. Whereas, Ksar cultivar was low proline content under different growth stages, except at ear emergence under stress one, whereas barley varieties Giza 126 and Giza 123 registered the lowest ones. The highest and lowest increase percentage of proline content attained for the following barley varieties: Giza 131 and Ksar and Giza 125 under stress condition. Regardless stress effect, barley varieties Giza 131 scored raise value and Ksar and Giza 123 gained the lowest one in same sequence, respectively. The highest and lowest proline values under examined growth stages observed at Tunisian barley varieties (Kebili-3 and Sidi–Bou under normal and drought stress condition, respectively. Whereas the lowest proline values attained at Tombari (ear emergence).

Also, it is obvious that the highest and smallest increase percentage in proline content were observed at Raihane Kebili-1, Kebili-3 and Tozeur-2 respectively.

Amino Acids content

The plants experiencing drought stress sprove changes in some of their biochemical and morphological features. Data from in Table (2) showed the grain amino acids content of barely varieties under both normal irrigation and drought stress condition.

Table 1: Relative water, leaf area and proline content and of barley as affected by drought stress

Barley varieties	Relative water at Ear emergence			Leaf area (cm^2)			Proline (mg/g fresh weight)		
	Normal	Stress	decrease (%)	Normal	Stress	(%)	Normal	Stress	Increase (%)
Giza 123	93.61	81.11	13.40	8.95	6.82	23.81	0.684	0.843	23.26
Giza 125	91.88	75.32	18.00	8.61	5.68	34.02	0.827	0.904	9.27
Giza 126	98.06	83.42	14.90	8.56	6.11	28.63	0.812	0.843	3.86
Giza 127	97.95	89.07	9.10	9.76	8.12	16.82	1.329	1.604	20.64
Giza 130	95.59	87.28	8.70	9.47	7.66	19.14	0.969	1.216	25.48
Giza 131	92.57	86.74	6.30	10.58	9.03	14.60	1.121	1.559	39.06
Giza 2000	84.97	78.43	7.70	9.23	7.63	17.33	1.009	1.165	15.49
El-Arich	87.02	73.11	16.00	8.79	6.21	29.42	0.845	0.962	13.85
Ksar	84.57	74.41	12.00	9.46	6.92	26.91	0.822	0.91	10.67
Kebili 1	91.24	82.35	9.70	9.89	8.07	18.40	1.004	1.395	39.04
Tozeur-2	85.92	82.20	4.30	8.88	7.19	19.09	1.223	1.611	31.7
Kebili 3	93.32	88.54	5.10	10.71	8.87	17.18	1.483	2.052	38.35
Kairaouan	91.58	77.41	15.50	9.85	7.72	21.62	1.042	1.307	25.4
Manel	89.32	77.61	13.10	9.46	7.63	19.34	1.414	1.659	17.37
Raihane	88.59	77.97	12.00	8.92	6.84	23.32	1.075	1.525	41.93
Sidi-Bou	79.41	70.61	11.10	9.60	7.56	21.24	1.469	2.016	37.29
Sabra	96.75	72.60	25.00	8.49	6.40	24.62	1.015	1.275	25.57
Tombari	74.91	60.10	19.80	8.72	6.22	28.70	0.818	0.944	15.35
Lemsi	87.64	73.20	16.50	8.32	6.02	27.64	0.863	1.114	29.12
Temacine	87.34	83.40	4.50	8.55	7.03	17.78	1.01	1.343	32.97
Ksar-Megrine	92.77	78.74	15.10	8.71	5.82	33.18	0.685	0.755	10.27
Techedrett	94.70	87.03	8.10	9.69	8.11	16.29	1.156	1.545	33.66
Saida	90.40	76.14	15.80	8.33	6.25	24.97	1.148	1.352	17.77
Sedi Mahdi	95.12	77.11	18.90	8.54	6.04	29.27	1.007	1.135	12.73
Ras El-Mouche	80.58	61.33	23.90	8.43	5.44	35.47	0.833	0.922	10.72
Naïlia	96.30	88.40	8.20	9.75	7.88	19.18	1.03	1.434	39.22
Temacine	87.34	83.40	4.50	8.55	7.03	17.78	1.073	1.164	8.46
Adrar	96.55	72.01	25.40	8.77	6.95	20.75	1.264	1.626	28.7
Oussama	87.79	78.22	10.90	11.07	8.75	20.96	1.036	1.211	16.89
Amalou	84.21	75.74	10.00	10.50	8.38	20.18	1.348	1.633	21.16
Massine	81.97	76.56	6.60	10.25	8.83	13.83	1.007	1.176	16.82
Taffa	90.65	73.65	18.80	9.89	8.03	18.81	1.466	1.716	17.1
Firdaws	91.36	73.85	19.20	8.18	6.34	22.51	1.203	1.54	28.05
Amira	87.95	80.53	8.40	11.52	9.81	14.83	0.975	1.094	12.12
Tamellalet	89.49	68.21	23.80	8.36	6.27	25.04	1.617	2.116	30.87
Laanaceur	95.06	87.71	7.70	12.70	10.72	15.60	0.684	0.843	23.26
LSD (0.05)	0.89	0.79		0.19	0.16		0.032	0.038	

One can notice that barley Giza 130 variety was superior relative to its content form determined amino acids, except Arginine; Threonine. Whereas, under stress condition the barley cultivar Giza 127 scored the highest Arginine content, except for serine, Glycine, Histidine, Arginine and Threonine and proline. Generally, the plant response to drought stress mainly depends on severity and period of drought stress and ranges of plant growth. Also, the ratios between stress and normal condition were highly for Aspartic, Glutamic, Serine –Theronine, Alanine, proline, Tyrosine, Valinine and Methonine. According to mean value, mostly barley variety Giza 130 attained the highest contents in all studied amino acids, except Glutamine and Alanine. The values of amino acid content in Tunisian barley verities were high at Kebili-3 than Lemsi. Whereas, the amino acid values under stressed, Kebili-3 improved than normal condition except Glycine and Arginine and the opposite was true in case of Lemsi variety. Also, it is clear to mention that the ratios between stresses to the normal values were highly under Kebili-3 than that of Lemsi variety. The amino acids content of grain, for some Algerian barley varieties (Sedi Mahdi and Nâilia), indicated that exposing barley varieties to drought stress led to increase grain amino acids content with some exception, such as Glycine and Histidine (Nâilia) and Arginine, Proline, Iso-lecrcine, Leucine, Phenylalanine, Lysine and in addition to the previous one at Nâilia cultivar. Also, it is clear to state that Nâilia cultivar was tolerant increases to drought stress and rise values for most determined grain amino acids. The total values for normal, stress and mean values of Nâilia cultivar exceeded that of Sedi Mahdi by about 56, 25 and 37%, respectively. From the point of the view, the ratio between stress and normal conditions for grain amino acids, data pointed out that Sedi Mahdi was superior one, whereas its values ratio was more than Nâilia variety in most studied amino acids.

The Morocco barley varieties, Massine was more negatively affected by drought condition where the values of amino acids were more under normal irrigation over water condition and the obverse was true in case of Amira variety. Also, it is clear that total amino acids values of Amira were more than Massine variety under both studied conditions.

Anatomical studies

It is evident that the grain outline is oval the typical monocotyledonous grain features. Data in Table (3) and

Figure (2) explain the comparison held between the barley variety Giza 127, Kebili-3, Temacine and Amira grain due to water stress. The most common one was the average grain diameter. Generally, Giza 127 transverse in the thickness of fiber layer was 60.18 µm, compressed layer is 29.92 µm, the two aleuronat layer of elongated cells are 49.91 µm and endosperm is 1378.52 µm. While Transverse section of Kebili-3 the thickness fiber layer was 75.96 µm, compressed layer was 32.79 µm, three aleurone layer of elongated cells were 76.50 µm and endosperm was 1199 µm.

Table 2: Grain amino acids content of barley varieties as affected by drought stress

Amino acids	Egyptian varieties				Tunisian varieties			
	Giza 127		Giza 130		Kebili 3		Lemsi	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Aspartic acid	0.88	1.67	1.64	0.90	1.14	1.82	1.04	0.86
Glutamic acid	1.71	3.96	2.06	1.32	1.45	2.33	1.94	1.25
Serine	4.00	4.91	10.12	6.70	6.89	9.43	8.27	5.09
Glycine	9.57	1.25	9.36	6.32	6.29	5.57	7.68	3.68
Histidine	7.68	3.06	11.93	11.28	9.27	15.58	9.84	10.62
Arginine	23.68	20.68	14.49	30.46	32.21	16.96	8.90	11.31
Threonine	2.79	2.99	2.78	3.12	3.32	5.13	2.31	1.96
Alanine	5.82	15.61	6.71	8.69	9.02	14.21	6.10	3.94
Proline	0.36	0.37	0.71	0.59	0.68	1.02	0.65	0.25
Tyrosine	2.62	2.66	3.98	2.22	2.45	6.40	3.62	2.25
Valine	1.69	1.74	2.57	1.44	1.90	4.14	2.34	1.46
Methionine	2.15	2.21	3.27	1.83	2.42	5.27	2.98	1.86
Cysteine	1.25	0.69	1.54	0.63	0.85	1.49	1.03	0.66
Isoleucine	4.38	2.42	5.40	2.19	2.96	4.17	3.60	2.31
Leucine	4.38	2.42	5.40	2.19	2.96	4.17	3.60	2.31
Phenylalanine	5.51	3.05	6.80	2.76	3.73	5.25	4.53	2.90
Lysine	4.88	2.70	6.07	2.44	3.30	4.65	4.01	2.57
Total	83.35	72.39	94.83	85.08	90.84	107.59	72.44	55.28
Amino acids	Algerian varieties				Morocco varieties			
	Sedi Mahdi		Naïlia		Massine		Amira	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Aspartic acid	0.57	2.45	0.93	1.77	0.96	1.01	1.19	1.48
Glutamic acid	1.15	5.94	1.35	5.28	1.37	1.32	1.74	3.73
Serine	4.64	5.40	6.86	7.34	6.90	5.37	6.88	4.12
Glycine	3.78	1.48	6.47	3.95	5.40	4.29	5.03	2.85
Histidine	6.94	2.79	11.55	2.55	5.77	9.87	11.49	2.70
Arginine	17.67	18.06	31.20	26.95	10.30	21.77	17.19	19.66
Threonine	2.10	4.43	3.20	5.10	3.15	3.52	3.59	4.06
Alanine	6.20	22.75	8.90	31.06	7.36	9.67	8.09	27.22
Proline	0.24	1.46	0.61	0.29	0.64	1.34	0.81	0.37
Tyrosine	1.47	4.04	2.28	5.65	3.39	2.82	3.66	4.96
Valine	0.95	2.61	1.47	3.65	2.19	1.82	2.37	3.21
Methionine	1.21	3.33	1.87	4.65	2.79	2.32	3.02	4.08
Cysteine	0.55	0.71	0.64	0.64	0.74	0.60	0.99	0.44
Isoleucine	1.93	2.47	2.24	2.02	2.57	2.09	3.47	1.53
Leucine	1.93	2.47	2.24	2.02	2.57	2.09	3.47	1.53
Phenylalanine	2.43	3.10	2.83	2.54	3.24	2.63	4.36	1.93
Lysine	2.15	2.75	2.50	2.25	2.86	2.33	3.86	1.71
Total	55.91	86.24	87.14	107.71	62.20	74.86	81.21	85.58

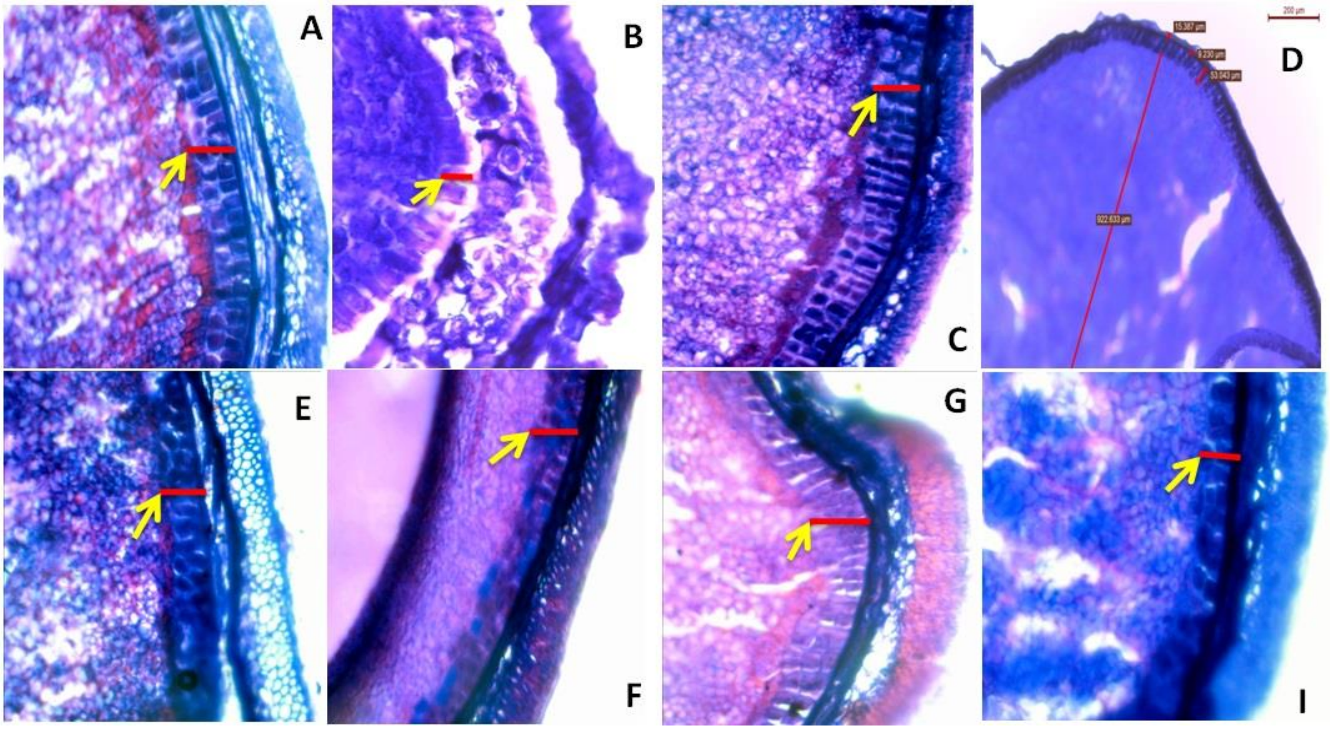

Figure 2: Effect of drought stress on aleurone layer of Barley grains using Image analysis microscope software.

A, B: Aleurone layer of Giza 127 cultivar (70, 40% WHC) (two layer of elongated cell) thickness. **C, D:** Aleurone layer of Kebilie cultivar (70, 40% WHC) (two layer of elongated cell) thickness. **E, F:** Aleurone layer of Amira cultivar (70, 40%WHC) (two layer of elongated cell) thickness. **G, I:** Aleurone layer of Temasine cultivar (70, 40% WHC) (two layer of elongated cell) thickness.

Table 3: Measurements of aleurone layer in barley grain under normal and drought conditions

Grain Layer	Giza 127		Keibilie-3		Amira		Temasine	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Fiber layer	60.18	33.52	75.96	15.38	80.25	68.48	70.69	40.32
Compressed layer	29.92	12.56	32.79	9.23	13.57	10.24	34.31	27.52
Aleurone layer	49.91	30.11	76.51	53.04	76.93	35.29	66.18	50.26
Endosperm	1378.5	967.1	1199	922.63	1069	738.5	817.11	750.5

But in Transverse section of Amira the thickness of fiber layer was 80.25 µm, compressed layer is 13.57 µm, the two aleurone layer of globular cell were 76.93 µm and endosperm was 1069 µm and the transverse section of Amira the thickness of fiber layer was 70.69 µm, compressed layer was 34.30 µm, the two aleurone layer of elongated cell were 66.18 µm and endosperm was 817.01 µm.

As the comparison held between the Giza 127, Kebili-3, Temacine and Amira grain many differences were found. The most common one is the average grain diameter. Transverse in section Giza 127 the thickness of fiber layer was 33.52 µm, compressed layer was 15.56 µm, three aleurone layer of elongated cells were 30.11 µm and endosperm was 967.1 µm while transverse in section Kebili-3 the thickness of fiber layer is 15.38 µm, compressed layer is 9.230 µm, three aleurone layer of elongated cells were 53.04 µm and endosperm was 922.63 µm. But in transverse section Amira the thickness of fiber layer was 10.24 µm, the two aleurone layer of elongated cell were 35.29 µm and endosperm was 738.5 µm.

Finely The transverse in section Teamicne the thickness of fiber layer was 40.32 µm, compressed layer was 27.52 µm, the two aleurone layer of elongated cell were 50.26 µm and endosperm was 750.5 µm. Briggs (1978) concluded that aleurone tissue is a protein-rich cells layer found under the nucellus and testa. Aleurone grains are storage granules containing protein, phytic acid and hydrolytic enzymes.

Discussion

Some physiological and biochemical processes may alter by the water stress, such as photosynthesis. The rise values of SPAD Chlorophyll reading at stem elongation and ear emergence growth stages recorded for Egyptian barley varieties El-Arich followed by cultivar Giza 125 for stem elongation and Ksar cultivar at the ear emergence growth stage. Sãnchez-Díaz et al. (2002) stated that barley seems to be one the main temperate cereals best adapted to water stress. The Tunisian variety Sidi-Bou registered more chlorophyll content at stem elongation stage under normal and stress condition of irrigation and Lemsi and Kebili-3 were more tolerant varieties. With respect to the Algerian barley variety (Ras El-Mouche) and Morocco varieties (Amalou, Firdaws and Laanaceur) recorded the highest Chlorophyll values under normal and drought stress condition at stem elongation. Verma et al. (2004) indicated that increasing the chlorophyll content is associated with increased yield production and evapotranspiration efficiency under drought stress conditions in sorghum, maize and wheat. The highest increase percentage of proline content under stress condition were attained at Giza 131 and Ksar.

Tunisian barley varieties (Kebili-3 and Sidi–Bou) under drought stress gained the highest proline content and the lowest proline value was attained by Tombari (at ear emergence). Relative water content (RWC %) at ear emergence stage were attained at Giza 127, Sabra and Kebili-3 (Tunisian varieties), however, Tozeur-2 was more tolerant under drought stress condition. Whereas, the most tolerant Algerian barley varieties (Tchedrett and Temacine) and the highly tolerant Morocco varieties were Adrar-Amira. Ramos et al., (2003) concluded that the relative water of bean leaves under water deficit was significantly lower than control. Lower-molecular-weight osmolytes, as proline, glycine, betaine and organic acids, and polyols are crucial to maintain cellular functions under the drought stress condition (Farooq et al. 2009).The plants imposed to drought stress as barley Giza 130 (Egyptian variety) was superior relative to its content form most determined amino acids, while Giza 127 scored the highest Arginine content, except for serine, Glycine, Histidine, Arginine and Threonine and proline under stress condition and Giza 130 gained elevate content in all studied amino acids. The amino acid content in Tunisian barley verities was high at Kebili-3 than Lemsi.

Whereas, Kebili-3 improved its content than normal condition. Baker (1989) noticed increased in the total free amino acids under water stressed cereal leaves, with proline being the most pronounced. Algerian barley varieties (Sedi-Mahdi and Nâilia) scored highly amino acids content of grain, exposing barley varieties to drought stress led to increase grain amino acids content with some exception. The ratio of grain amino acid between stress and normal conditions, proved Sedi Mahdi was superior one. Amino acids act as bio stimulant which has important role the root growth, yield production and significantly correct the injuries appeared by drought stress (Azimi et al. 2013).

Conclusion

The elevated values of SPAD Chlorophyll at stem elongation stage and ear emergence growth stages recorded in Egyptian barley varieties. Sidi-Bou (Tunisian varieties) superior at stem elongation under normal and drought stress condition of irrigation and Lemsi and Kebili-3 (Tunisian) were more tolerant varieties.

Barely Giza 130 (Egyptian variety) was superior relative to its content form most determined amino acids, while Giza 127 gained a highest content, which related mainly to the riskiness and period of stress and stage of plant growth. The amino acid content in Tunisian barley verities was high at Kebili-3 than Lemsi, whereas, Kebili-3 improved its content than normal condition.

The most productive of biochemical parameters were Giza 123, 130, 131, 2000 (Egypt), Kibili-1, Tozeur- 2 and Sidi-Bou (Tunis), Saida, Nâilia, Temacine (Algeria), Oussama, Laanaceur and Amira (Morocco) under water stress. These varieties enhance the petter change percentage in the studied biochemical items under drought stressand it could be suggested to be grown under aridic conditions.

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