Zagazig J. Agric. Res., Vol. 46 No. (3) 2019 595-607

Plant Production Science

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

EFFECT OF DROUGHTAND COMBINED SALT AND HEAT STRESSES ON GERMINATION AND SEEDLING GROWTH PATTERNS OF LENTIL

Ahmed H.I. Hamdi[1*], Rasha Y.S. Abd El-Khalek[2] and Hania A.M. Eraky[2]

1. Food Legumes Res. Section, Field Crops Res. Inst. (FCRI), Agric. Res. Cent. (ARC), Giza Egypt

2. Seed Technol. Res. Section, FCRI, ARC, Giza Egypt

Received: 26/03/2019 ; Accepted: 15/05/2019

ABSTRACT: The present study aimed to investigate the effect of combined salt (three salinity levels of 0.0M, 0.1M and 0.2M NaCl), heat (20 and 30°C) and drought (without drought and drought stress by polyethylene glycol (PEG) at 8000 ppm) stresses on seed germination, seed and seedlings vigor traits of three lentil cultivars (Giza 9, Giza 51 and Sinai 1). Increasing temperature from 20°C to 30°C significantly decreased most studied characters. Both salinity levels significantly decreased all traits comparing with the control treatment (0 salt NaCl). All traits were more sensitive to 0.2M.than 0.1M.NaCl. Effects of drought were insignificant on shoot length, root length, seedling vigor index and root/shoot ratio. Drought significantly decreased seed germination by 5.6% and seed vigor by 4.87%. Cultivar Giza 9 was more tolerant to drought than the other two cultivars. Gel electrophoresis (SDS-PAGE) analysis for lentil cultivars under salt stress and drought stress at two different temperatures produced new protein heat shock protein (HSP) presented under 30°C and was absent under control treatment. The applied laboratory technique is valid to be used for screening large number of lentil accessions for their performance under studied stresses as a rapid technique before screening them for such stresses under field condition.

Key words: Lentil (*Lens culinaris* subsp. *Culinaris* Medikus), salinity stress, drought stress, germination, seedling characters, SDS protein electrophoresis, lentil.

INTRODUCTION

Lentil (*Lens culinaris* subsp. *Culinaris* Medikus) is an important food legume crop in Egypt. It has a high nutritional value, as its seed is a rich source of protein, minerals (K, P, Fe, and Zn) and vitamins for human nutrition **(Grusak, 2010)**. It also reduces the dependence of cropping system on nitrogenous fertilizers because of their ability to fix atmospheric nitrogen in association with *Rhizobia* (**Saxena, 1988)**. In addition, Lentil straw is also a valued animal feed **(Erskine *et al.*, 1990)**.

The national average seed yield of lentil in Egypt reached 5.36 ardab/faddan in 2017 season, which equals 2.04 t/hectare (**Anonymous, 2017)**. This productivity in Egypt is high globally, and it ranks the third after Australia and China and exceeded the world average by 97.5% in 2017 **(FAO, 2017)**. However, lentil and other crops could be negatively affected if the environmental conditions in Egypt change due to the expected climatic change.

Climatic change will increase in coming decades. Greenhouse gas emissions are running ahead of the worst-case scenario of the Intergovernmental Panel on Climate Change (IPCC), so temperature will increase more rapidly than expected **(Kennel, 2010)**. The CWANA region is one of the most vulnerable areas to climate change. The available information indicates several impacts that would affect agricultural activities. Increased temperature can reduce crop productivity and limit biodiversity. Sea water rise could result in salinization of agricultural lands that would reduce crop

***Corresponding author: Tel. :** +201224770216
E-mail address: ahihamdi@yahoo.com

productivity. Lack of water will have a further impact on agricultural and rural development activities **(Abou Hadid, 2010)**. Therefore, focused research is urgently required to develop crop species and verities tolerant to drought, heat, salinity and other stresses related to the future climate in targeted growing regions **(Solh, 2010)**. In this regard, several drought-tolerant varieties have been developed and disseminated for rainfed conditions in the northern coast in Egypt. These include the lentil cultivar Sinai 1, the bread wheat cultivar Sahel 1, and the barley cultivars Giza 125, 126 and 2000 **(Hamdi *et al.*, 2010)**.

In general, legumes are relatively sensitive to salt, and lentil is comparatively more sensitive than field pea and faba bean and similar to chickpea **(Saxena *et al.*, 1993)**. Lentil yield and its biology get reduced by 90-100% at an electrical conductivity (ECe) of 3.1 **(Katerji *et al.*, 2003)**. Variation in tolerance of lentil to $MgSO_4$, Na_2SO_4 and $MgCl_2$ has been identified **(Jana and Slinkard, 1979)** but most research has focused on tolerance to NaCl. NaCl tolerant lentil accessions have been identified based on seedling symptoms **(Rai *et al.*, 1985; Maher *et al.*, 2003).**

The present study aimed to investigate the response of three lentil cultivars to combined salt, heat and drought stresses on seed germination and seedling growth patterns, and in addition to evaluate the used laboratory processing to determine the possibility to use such method as a rapid technique to evaluate a large number of lentil accessions.

MATERIALS AND METHODS

Three lentil cultivars *i.e.* Giza 9, Giza 51 and Sinai 1 were used in the present study. The cultivars are varied in their pedigree and characteristics. Cultivar Giza 9 is a selection from Egyptian landrace, high yield, and widely adapted to Egyptian conditions. The cultivar Giza 51 is a selection from an exotic genotype introduced from the International Center for Agricultural Research in the Dry Areas (ICARDA) named FLIP 84-51L, which was derived from crossing between ILL 883 (from Iran) x ILL 470 (from Syria). The cultivar Sinai 1 is also a selection from an Argentinean variety called Precoz. To obtain fresh seeds, the three cultivars were grown within lentil breeding program, Food Legumes Res. Dept., Field Crops Research Institute (FCRI), Agricultural Res. Center (ARC), in advanced evaluation yield trial at Gemmeiza Research Station at Gharbia Governorate in the Delta in North Egypt in 2017/2018 winter season. The laboratory work was conducted in Seed Technology Research Department, FCRI, ARC at Giza during 2018.

Two separate laboratory experiments were conducted at Seed Technology Research Department, FCRI, ARC at Giza. The first experiment studied the effect of combined salt (two levels of salinity at 0.1, 0.2M NaCl) and heat (at 20ºC and 30ºC) stresses on seed germination, seed and seedling vigor traits (shoot length, root length, seedling fresh weight, seedling dry weight, seedling vigor index, seed vigor index, and root length/shoot length ratio) of three lentil cultivars. Four replicates of 50 seed for each cultivar were germinated in covered, sterilized, disposable Petri dishes containing Whatman filter paper moistened with either distilled water with zero salinity (control), 0.1 M and 0.2 M NaCl were incubated in germinator at 20º C and 30ºC (heat stress) for 10 days. The Petri dishes were laid in factorial Completely Randomized Design (CRD) with three replicates.

The second experiment studied the effect of combined heat and drought stresses on seed germination, seed and seedling vigor as well as SDS protein electrophoresis. Two levels of drought were used, 1- control without drought (where 50 lentil seeds of each cultivar were immersed in distilled water for 6 hr., before germination) and 2- drought stress (which was induced by immersing 50 seed in polyethylene glycol (PEG) 8000ppm for 6 hours before germination, giving -1.4 MPa osmotic potential) as reported by **Michel and Kautmann (1973) and Cochrane (1994)**. Seeds were germinated in covered; sterilized, disposable Petri dishes containing Whitman filter paper moistened with distilled water in germinator at 20º C and 30ºC. At ten days germinated seeds were counted and the seedling growth patterns were measured.

For germination test, four replicates (dishes) each with 25 seeds were used for each treatment and placed in an incubator in the dark at 20ºC and 30ºC after ten days. Seeds were observed daily and considered germinated following radical emergence. Germinated seeds were counted and removed from the Petri dishes.

Seed vigor index: was calculated using the following formula **(Copeland 1976)**:

Seed vigor index = Number of seeds germinated (1^{st} count) ÷ Number of days to first count + Number of seeds germinated (last count) ÷ Number of days to last count

Normal seedlings obtained from standard germination test were used to measure seedling characters according to the rules of the Association of Official Seed Analysis **(AOSA 1991).** Twenty-five seedlings from each Petri dish were randomly selected after 10 days of germination time and seedling shoot and root lengths, root/shoot ratio and seedling vigor index for individual seedling were measured. In some treatments the germination (%) don't reach to 25%, so available seedlings were taken for measuring. Seedling shoots and roots were also dried at 70°C for 72 hr., for dryness. Seedling vigor index was calculated using data recorded on germination percentage and seedling growth according to **ISTA (1985)** by the following formula :

Seedling vigor index = seedling length (cm) × germination percentage

SDS- Protein Electrophoresis

Soluble proteins were extracted from seeds and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique was conducted according to the protocol described by **Laemmli (1970)** and modified by **Studier (1973)**.

Statistical analysis of variance was made for each experiment in a factorial split-split design (with three factors) followed by DMRT (Duncan's multiple range test) at p= 0.05, post hoc test. According to **Gomez and Gomez (1984)**, where in the first experiment, temperature levels were devoted randomly to the main plots and salinity treatments to the sub-plots and lentil cultivars in sub-sub plots. Also, in the second experiment temperature levels were devoted randomly to the main plots and drought treatments to the sub-plots and lentil cultivars in sub-sub plots.

The combined analysis was made for the main source of variance of temperature, salinity, drought and cultivars for all studied traits.

RESULTS AND DISCUSSION

Effect of Combined Salt and Heat Stresses and Varietal Differences on Germination, Seed and Seedling Vigor Traits of Lentil Cultivars

The combined analysis of variance (Table 1) showed highly significant differences among the main sources of variance: temperature levels, salinity levels and cultivars for all studied traits, except root length and seedling fresh weight for temperature, and root/shoot ratio for cultivar. Also, significant differences were occurred among the second sources of variance: temperature (T) × salinity (S), temperature × cultivar (C), salinity × cultivar and T × S × C interactions for all studied traits, except root length and seedling fresh weight for T × C interaction, root length for T × S × C interaction and root/shoot ratio of cultivar and T × S × C interaction.

Means of the studied traits as affected by the lentil cultivars, salinity and temperature levels are given in Table 2. Cultivar Giza 51 gave significant higher values of all traits than Giza 9 and Sinai 1, except seedling fresh and dry weight traits. Cultivar Giza 51 also performed higher values of seedling growth characters than Sinai 1, as reported by **Hamdi *et al.* (2017)**, which agreed with the results obtained in the present study.

Within the range of applied salt stress conditions, the results in Table 2 show that both salinity levels 0.1 M and 0.2 M NaCl were significantly decreased all traits comparing with the control treatment. All traits were more sensitive to 0.2 M NaCl treatment than 0.1M NaCl treatment except root/shoot ratio. For example, the shoot length reduced from 2.36 cm under 0.1M NaCl to 0.39 cm under 0.2M NaCl. The decrease percentages of all traits under 0.1 M NaCl comparing with 0 NaCl ranged from 6.49% for seed germination to 64.22% for root length. While, the corresponding percentages of all traits under 0.2M NaCl comparing with 0 NaCl were higher and ranged from (%) 5.32% for root/shoot ratio to 93.86% for seedling vigor.

Table 1. Sources of variance (SV), degrees of freedom (df) and mean square values of tested lentil characters in combined analysis of variance.

SV	df	SL	RL	SFW	SDW	Germ	SVI	Seed VI	RL/SL
Temperature (T)	**1**	16.05**	0.052	0.00	0.00**	3143**	162312**	34.2**	1.0**
Salinity (S)	**2**	55.96**	46.54**	0.68**	0.126**	14258**	1610759**	140**	0.83**
T × S	**2**	2.68**	0.22*	0.02**	0.002**	2161**	10484**	10.2**	0.30**
Cultivar (C)	**2**	2.38**	2.77**	0.02**	0.007**	2863**	152555**	20.4**	0.03
T × C	**2**	0.80**	0.11	0.001	0.00**	2301**	44819**	11.7**	0.16**
S × C	**4**	0.16*	2.04**	0.029**	0.006**	476*	39591**	8.3**	0.41**
T × S × C	**4**	0.25**	0.03	0.009**	0.002**	391*	10357**	3.3**	0.05
Error	**36**	0.06	0.06	0.001	0.00	143	718.77	0.87	0.24
CV (%)		10.75	15.02	12.26	1.44	17.78	8.37	13.41	19.62

SL: Shoot length, RL: Root length, SFW: Seedling fresh weight, SDW: Seedling dry weight, Germ: Seed germination, SVI: Seedling vigor index, Seed VI: seed vigor index, RL/SL: Root length/ Shoot length ratio. *,** Significant at 0.05 and 0.01levels of probability, respectively.

Table 2. Means of the tested lentil characters as affected by cultivars, salinity and temperature levels

Treatment	Character							
	SL (cm)	RL (cm)	FW(gm)	DW(gm)	Germ%	SVI	SeedVI	RL/SL
				Cultivar				
Giza 9	2.02	1.76	0.310	0.134	67.4	324	7.04	0.83
Giza51	2.64	1.95	0.296	0.133	79.9	411	7.99	0.76
Sinai 1	1.99	1.19	0.246	0.099	54.7	227	5.87	0.77
F-test	**	**	**	**	**	**	**	
LSD at 5%	0.11	0.12	0.017	0.0002	5.7	12.8	0.45	NS
				Salinity level				
0 NaCl	3.90	3.41	0.473	0.204	86.3	635	9.03	0.94
0.1 M	2.36	1.22	0.293	0.125	80.7	287	8.07	0.54
0.2 M	0.39	0.27	0.085	0.037	35.0	39	3.80	0.89
F-test	**	**	**	**	**	**	**	**
LSD at 5%	0.11	0.12	0.02	0.06	5.7	12.8	0.45	0.08
				Temperature level				
20°C	2.76	1.60	0.285	0.123	75.0	375	7.76	0.65
30°C	1.67	1.66	0.283	0.121	59.7	266	6.17	0.92
F-test	**	NS	NS	**	**	**	**	**
LSD at 5%	0.093	NS	NS	0.0	4.66	10.4	18.55	0.06

SL: Shoot length, RL: Root length, FW: Seedling fresh weight, DW: Seedling dry weight, Germ: Seed germination, SVI: Seedling vigor index, Seed VI: seed vigor index, RL/SL: Root length/Shoot length ratio. ** Significant at 0.01levels of probability. NS: not significant.

Increasing temperature from 20°C to 30°C significantly decreased shoot length, seedling dry weight, seed germination, seedling vigor and seed vigor traits, while it increased root/shoot ratio from 0.65 to 0.92. Increased temperature did not significantly affect root length and seedling fresh weight.

The effect of combined temperature and salinity on seedling growth characters of the three lentil cultivars are presented in Table 3. The results showed that shoots length of the three cultivars were decreased by increasing temperature from 20ºC to 30ºC, for example, shoot length of Giza 9 cultivar decreased from 4.60 cm under 20ºC and zero treatment NaCl to 2.85 cm under 30ºC and zero treatment NaCl, and similar performance was occurred by the other two cultivars Giza 51 and Sinai 1. Interestingly, different performance of the three cultivars was observed for root length and seedling fresh weight, where increasing temperature from 20ºC to 30ºC increased the average of these two traits in the three cultivars. For example average root lengths of Giza 9, Giza 51 and Sinai 1 under 20ºC and zero treatment NaCl were 4.13, 3.37 and 2.35 cm, respectively, while the corresponding values under 30ºC and zero treatment NaCl were 4.35, 3.68 and 2.57, respectively. Similar performance of the three cultivars was observed in root/shoot trait.

Whereas the averages of all the six seedling growth traits for Giza 9 and Giza 51 were decreased by increasing temperature from 20ºC to 30ºC and zero treatment NaCl, except the average of seedling dry weight of Giza 9, which increased from 0.204 g under 20ºC and zero treatment NaCl to 0.246 g under 30ºC and zero treatment NaCl. In contrast, cultivar Sinai 1 had varied performance, where its averages in the four traits, seed vigor index, germination (%), seedling vigor index and root/shoot ratio under 30ºC and zero treatment NaCl were higher than those under 20ºC and zero treatment NaCl, in addition its averages were equale in both seedling dry weight and seedling fresh weight under 20ºC and zero treatment NaCl and 30ºC and zero treatment NaCl indicating that it did not affected by increasing temperature.

The results in Table 3 also show that increasing salinity decreased the average of all tested traits for the three lentil cultivars. In fact, The negative effects of salinity have been attributed to increase in Na+ and Cl¯ ions in plants hence these ions produce critical conditions for plant survival by intercepting different plant mechanisms. Although both Na+ and Cl¯ are major ions, which produce many physiological disorders in plants, Cl¯ is the most dangerous **(Tavakkoli *et al.*, 2010)**. An increase of NaCl concentration in soil resulted in increasing uptake of Na and Cl, and reduces dry weight, K concentration and K: Na ratio of lentil **(Turan *et al.*, 2007)**. Studies of seed germination under salinity stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevate salinity at the germination and seedling phases of development **(Ghoulam and Fares, 2001)**. Among the environmental factors that influence seed germination, water is one of the most important. The greater or smaller seed water absorption depends on the gradient of the water potential between the soil and the seed **(Ribeiro *et al.*, 2001).**

Effect of Combined Drought and Heat Stresses on Germination and Seedling Vigor Traits of Lentil Cultivars

The combined analysis of variance in Table 4 show significant differences among the main sources of variance: temperature, drought and cultivar for all studied traits, except seedling fresh weight for temperature stress, shoot length, root length, seedling vigor and root/shoot ratio for drought stress. Also significant differences were occurred among the second sources of variance: temperature (T) × drought (D), temperature × cultivar (C), drought × cultivar and T × D × C interactions for all studied traits, except shoot length and root/shoot ratio for T × D, and seedling vigor for T × S × C interaction.

Increasing temperature from 20°C to 30°C was significantly decreased shoot length, root length, seed germination, seedling vigor, and seed vigor (Table 5). While, increasing temperature from 20°C to 30°C significantly increased seedling dry weight from 0.148 to 0.170 g and root/shoot ratio from 1.162 to 1.295. Increased temperature did not significantly affect seedling fresh weight.

Table 3. Means of shoot length, root length and seedling fresh weight, seed vigor, seedling dry weigh, germination (%), seedling vigor and root/shoot ratio for the three lentil cultivars as affected by combined temperature (Tem.) and salinity

Treatment		Shoot length (cm)			Root length (cm)			Seedling fresh weight (g)			Seed vigor index		
		Cultivar			Cultivar			Cultivar			Cultivar		
Tem. °C	Salin. M	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1
20	**0**	4.60	5.40	4.48	4.13	3.37	2.35	0.493	0.392	0.488	9.47	9.67	8.40
20	**0.1 M**	2.57	3.80	2.37	0.81	1.78	0.89	0.279	0.319	0.225	8.67	9.60	7.80
20	**0.2 M**	0.74	0.85	0.05	0.50	0.54	0.05	0.186	0.157	0.023	6.73	8.53	1.00
30	**0**	2.85	3.19	2.88	4.35	3.68	2.57	0.581	0.397	0.488	8.33	8.87	9.47
30	**0.1 M**	1.37	1.95	2.09	0.75	1.87	1.23	0.309	0.401	0.225	6.73	8.47	7.13
30	**0.2 M**	0.02	0.62	0.07	0.02	0.45	0.07	0.009	0.111	0.026	2.33	2.80	1.40
F-test			**			**			**			**	
LSD at 5%			0.278			0.286			0.041			1.09	
Treatment		**Seedling dry weight (g)**			**Germination (%)**			**Seedling vigor index**			**Root/shoot ratio**		
		Cultivar			**Cultivar**			**Cultivar**			**Cultivar**		
Tem. °C	**Salin.**	**Giza 9**	**Giza 51**	**Sinai 1**	**Giza 9**	**Giza 51**	**Sinai 1**	**Giza 9**	**Giza 51**	**Sinai 1**	**Giza 9**	**Giza 51**	**Sinai 1**
20	**0**	0.204	0.182	0.211	94.7	96.7	60.0	825	847	412	0.90	0.62	0.52
20	**0.1 M**	0.137	0.149	0.078	86.7	96.0	78.0	294	536	260	0.32	0.46	0.37
20	**0.2 M**	0.075	0.064	0.011	67.3	85.3	10.0	84	118	115	0.69	0.64	1.33
30	**0**	0.246	0.169	0.211	83.3	88.7	94.7	600	609	515	1.53	1.15	0.89
30	**0.1 M**	0.136	0.176	0.077	67.3	84.7	71.3	141	323	170	0.55	0.96	0.58
30	**0.2 M**	0.007	0.058	0.007	15.3	28.0	14.0	19	30	183	1.00	0.72	0.93
F-test			**			**			**			**	
LSD at 5%			0.001			13.97			31.28			0.18	

** Significant at 0.01levels of probability. NS: not significant.

Table 4. Sources of variance (S.V), degrees of freedom (df) and mean square values of tested lentil characters in combined analysis of variance

SV	df	SL	RL	FW	DW	Germ	SVI	Seed VI	RL/SL
Temperature (T)	**1**	9.68**	8.13**	0.002	0.004**	676**	566957**	5.76**	0.160*
Drought (D)	**1**	0.01	0.60	0.051**	0.049**	256**	2410	1.96**	0.046
T × D	**1**	0.07	2.83**	0.010**	0.183**	75.1**	16177*	0.444**	0.044
Cultivar (C)	**2**	0.99**	1.81**	0.011**	0.007**	24.8**	23256**	0.254*	0.249**
T × C	**2**	0.41**	3.13**	0.016**	0.075**	19.0*	40039**	0.250*	0.304**
D × C	**2**	3.18**	6.25**	0.018**	0.022**	14.3*	105295**	0.323**	1.102**
T × D × C	**2**	0.64**	3.60**	0.015**	0.046**	53.4**	6509	0.848**	0.896**
Error	**24**	0.052	0.188	0.002	0.001	4.33	3196	0.57	0.024
CV (%)		6.35	10.43	8.69	1.21	2.27	7.94	2.58	12.74

SL: Shoot length, RL: Root length, SFW: Seedling fresh weight, SDW: Seedling dry weight, Germ: Seed germination, SVI: Seedling vigor index , Seed VI: seed vigor index, RL/SL: Root length/ Shoot length ratio. *, ** Significant at 0.05 and 0.01levels of probability, respectively.

Table 5. Means of the tested lentil characters as affected by temperature and drought levels

Treatment	Character							
	SL (cm)	RL (cm)	FW (g)	DW (g)	Germ (%)	SVI	Seed (VI)	RL/SL
				Temperature level				
20ºC	4.09	4.62	0.51	0.148	96.11	837.8	9.61	1.162
30ºC	3.06	3.67	0.50	0.170	87.44	586.7	8.81	1.295
F-test	**	**	NS	**	**	**	**	*
LSD at 5%	0.11	0.21	NS	0.001	1.01	27.5	0.12	0.076
				Drought level				
No drought	3.56	4.02	0.46	0.122	94.44	720.4	9.44	1.193
Drought	3.59	4.28	0.54	0.196	89.11	704.1	8.98	1.264
F-test	NS	NS	**	**	**	NS	**	NS
LSD at 5%	NS	NS	0.02	0.001	1.01	NS	0.12	NS

SL: Shoot length, RL: Root length, SFW: Seedling fresh weight, SDW: Seedling dry weight, Germ: Seed germination, SVI: Seedling vigor index, Seed VI: seed vigor index, RL/SL: Root length/ Shoot length ratio. *'** Significant at 0.05 and 0.01levels of probability. NS: not significant.

Regarding the effect of drought stress, the results in Table 5 show no significant effects of drought on shoot length, root length, seedling vigor and root/shoot ratio. While the traits seedling fresh weight and seedling dry weight were significantly increased under drought stress, the percentage increases of both traits were 17.4 and 60.66%, respectively. On the other hand, drought stress significantly decreased seed germination by 5.6 and seed vigor by 4.87%, thus both traits were more sensitive to drought than other traits.

The effect of combined temperature and drought stress on germination, seed and seedling vigor traits presented in Table 5. Increasing temperature from 20ºC to 30ºC significantly decreased shoot length, root length, seed germination, seedling vigor and seed vigor (Table 5). While increasing temperature from 20ºC to 30ºC significantly increased seedling dry weight from 0.148 to 0.170g and root/shoot ratio from 1.162 to 1.295. Increased temperature did not significantly affect seedling fresh weight. The results showed no significant effects of drought on shoot length, root length, seedling vigor and root/shoot ratio. While the traits seedling fresh weight and seedling dry weight were significantly increased under drought stress. On the other hand, drought stress was significantly decreased seed germination and seed vigor where both traits were more sensitive to drought than other traits.

The effect of combined temperature and drought stresses on seedling growth characters of the three lentil cultivars were presented in Table 6.

The results in Table 6 show that shoot length, seedling dry weight and germination (%) of the three cultivars were decreased by increasing temperature from 20ºC to 30ºC, except seedling dry weight of Sinai 1 under drought. For example, the shoot length of Giza 9 decreased from 3.84 cm in 20ºC with no drought (control) to 2.48 cm at 30ºC without drought, similar performance was occurred by the other two cultivars Giza 51 and Sinai 1. Also under drought the shoot length of Giza 9 decreased from 4.50 cm at 20ºC under drought to 2.95 cm in 30ºC under drought, similar performance was occurred by the other two cultivars Giza 51 and Sinai 1 under drought stress. While the trait seedling vigor was no significant under drought stress. Cultivar Giza 9 gave higher values of shoot length, root length, seedling fresh weight,

Table 6. Means of shoot length, root length and seedling fresh weight, seed vigor index, seedling dry weigh, germination (%), seedling vigor index and root/shot ratio for the three lentil cultivars as affected by combined temperature (Tem.) and drought

Treatment		Shoot length (cm)			Root length (cm)			Seedling fresh weight (g)			Seed vigor index		
		Cultivar			Cultivar			Cultivar			Cultivar		
Tem. °C	Drought	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1
20	Control	3.84	4.73	3.80	4.89	5.45	4.00	0.514	0.440	0.409	9.47	9.93	9.80
20	Drought	4.50	3.83	3.87	6.28	3.53	3.63	0.650	0.467	0.572	9.27	9.87	9.33
30	Control	2.48	4.24	2.28	2.40	2.85	4.55	0.391	0.490	0.541	9.20	9.47	8.80
30	Drought	2.95	2.83	3.57	4.78	4.30	3.18	0.577	0.489	0.480	8.27	8.07	9.07
F-test			**			**			**			**	
LSD at 5%			0.27			0.52			0.05			0.28	

Treatment		Seedling dry weight (g)			Germination (%)			Seedling vigor index			Root/shoot ratio		
		Cultivar			Cultivar			Cultivar			Cultivar		
Tem. °C	Drought	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1	Giza 9	Giza 51	Sinai 1
20	Control	0.075	0.238	0.235	94.67	99.33	98.00	825.9	1011.2	764.2	1.283	1.150	1.050
20	Drought	0.081	0.203	0.058	92.67	98.67	93.33	1000.6	725.4	699.1	1.623	0.923	0.940
30	Control	0.069	0.063	0.053	92.00	94.67	88.00	449.5	670.9	600.8	0.967	0.673	2.033
30	Drought	0.071	0.062	0.090	82.67	80.67	86.67	638.4	575.5	585.4	1.613	1.587	0.897
F-test			**			**			NS			**	
LSD at 5%			0.001			2.48			NS			0.186	

seedling dry weight, seedling vigor and root/shoot ratio under drought at 20ºC and 30ºC comparing with control. These results indicating that cultivar Giza 9 is more tolerant to drought than other two cultivars.

Biochemical Analysis by Protein Electrophoresis

Salt stress under 20ºC and 30ºC temperatures

Electrophoresis analysis was carried out using SDS-PAGE for water soluble protein fraction of the three lentil genotypes under control (zero salinity) and salinity treatment (0.1 and 0.2M NaCl) . This analysis aimed at detects biochemical genetic markers for salt tolerance in lentil under two temperatures 20ºC (normal) and 30ºC. The results showed that a maximum number of 17 bands was detected with molecular weights (MW) ranged from 210 to 12 KDa (Table 7), under salt stress 20ºC. Sinai1 cultivar was stable of number of bands under control (15 bands) and salt stress 0.1M NaCl (14 bands), also Giza 51 had 13 bands in control to 12 bands in treatment 0.1M NaCl, while Giza 9 showed 11 bands under control to 14 bands under treatment 0.1M NaCl. All cultivars under higher salinity (0.2M NaCl) showed lower number of bands, in Sinai 1 ten bands to six bands in Giza 51 and five bands in Giza 9. In contrast Sinai1 had close number of bands in two temperatures, which present stable band number under salt stress.It produced 14 bands in. 1M NaCl at 20ºC, while in .2M NaCl at 30ºCit produced 13 bands.

The results in Table 7 refer to that under 30ºC temperature Giza 51 gave high number of bands (14 bands) under control and Sinai 1 had 11 bands, while Giza 9 had low bands number (4 bands). Band number 6 at molecular weights (MW) 115 bp is considering a marker to 0.1M

Table 7. SDS-PAGE of water-soluble protein extracted from three lentil genotypes grown under zero salt (C) and salt stresses 0.1(T_1) and 0.2 (T_2) M NaCl at 20 and 30^{o}C temperatures (MW: molecular weight, NB: No. of bands)

MW	20^{o}C									30^{o}C						NB
	Sinai 1			Giza 9			Giza 51			Sinai 1		Giza 9		Giza 51		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	C	T_1	C	T_1	
210	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
165	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	2
150	+	+	+	+	+	-	+	+	-	+	+	-	+	-	-	3
140	+	+	-	+	+	-	+	-	-	-	-	-	+	+	-	4
120	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	5
115	+	+	+	+	+	-	+	+	-	-	+	-	+	-	+	6
100	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	7
95	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	8
80	+	+	-	+	+	-	+	+	-	-	+	-	-	+	+	9
75	+	+	-	-	-	-	+	+	-	+	+	-	+	+	-	10
60	+	+	+	+	+	-	-	-	-	+	+	-	+	+	-	11
50	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	12
35	+	+	-	-	+	-	+	+	+	-	-	-	+	+	-	13
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17
Total	15	14	10	11	14	5	13	12	6	11	13	4	12	14	7	

MW= molecular weight, C= control treatment, $\mathbf{T_1}$=.1M NaCl treatment, $\mathbf{T_2}$=.2M NaCl treatment. NB= No. of bands.

NaCl at 30ºC, This band present under treatment and absent under control in the three cultivars, may be this band considered heat shock protein (HSP) marker to salt stress. Combined salt and high temperature shock treatments decreased final germination percentage and rate of highly viable lentil seed proportionally to the imposed stress and to seed vigor degree **(Dell, 1999)**. In wheat and barley seeds, subjected to heat-shock alone, the expression of most polypeptides normally synthesized during germination was reduced **(Dell and Di Turi, 1999)**.

Drought stress under 20ºC and 30ºC temperatures

Electrophoresis analysis was carried out using SDS-PAGE for water soluble protein fraction of three lentil genotypes under control (zero drought) and drought treatment (8000 ppm), by used two temperature 20^{o}C (normal) and 30ºC. The results showed that a maximum number of 20 bands was detected with molecular weights (MW) ranged from 275 to 10 KDa (Table 8).

Under drought stress Sinai 1 was the best cultivar under this study. It had 19 bands in

Table 8. SDS-PAGE of water-soluble protein extracted from three lentil genotypes to drought stress under to different temperatures

MW	**20°C**						**30°C**						**NB**
	Sinai 1		**Giza 9**		**Giza 51**		**Sinai 1**		**Giza 9**		**Giza 51**		
	C	T	C	T	C	T	C	T	C	T	C	T	
275	-	-	-	-	-	-	-	-	-	-	-	+	1
250	+	+	-	-	-	-	-	-	-	-	-	+	2
210	+	-	-	-	-	-	-	-	-	-	-	+	3
200	+	+	-	-	-	-	-	-	-	-	-	+	4
175	+	+	-	-	-	-	-	-	-	-	-	-	5
165	+	+	-	-	-	-	-	-	-	-	-	-	6
155	+	+	+	+	-	-	+	+	+	-	-	+	7
135	+	+	+	-	-	-	+	+	+	-	-	-	8
120	+	+	+	+	+	+	+	+	+	+	+	+	9
105	+	+	-	+	-	-	-	-	+	-	+	+	10
95	+	+	-	+	-	-	-	-	+	-	+	+	11
70	+	+	+	+	+	+	+	+	+	+	+	+	12
55	+	+	-	-	-	+	-	-	-	-	+	+	13
40	+	+	-	-	-	-	-	-	-	-	+	+	14
20	+	+	+	+	+	+	+	+	+	+	+	+	15
18	+	+	+	+	+	-	+	+	+	-	+	+	16
16	+	+	+	+	+	+	+	+	+	-	+	+	17
14	+	+	+	+	+	-	+	+	+	-	+	+	18
12	+	+	+	+	-	-	-	-	+	-	+	+	19
10	+	+	+	+	-	-	-	-	+	-	+	+	20
Total	19	18	10	11	6	5	8	8	12	3	12	17	

MW= molecular weight, C= control treatment, T= PEC-8000 ppm treatment, NB= No. of bands.

control treatment and 18 bands under drought treatment of PEG 8000 ppm at 20ºC, while under 30ºC it had 8 bands under control and 8 bands under drought treatment of PEG 8000 ppm. Giza 9 gave 10 bands under control and 11 bands under drought treatment of PEG 8000 ppm at 20ºC, while under 30ºC it had 12 bands under control and 3 bands under drought treatment of PEG 8000 ppm. Cultivar Giza 51 gave 6 bands under control and 5 bands under drought treatment of PEG 8000 ppm at 20ºC, while under 30ºC it performed 12 bands under control and 17 bands under drought treatment of PEG 8000 ppm. Giza 51 produced five marker bands under treatment of PEG 8000 ppm at 30ºC, this band considered HSP at different molecular weights (MW) of 275bp, 250bp, 210bp, 200bp and 155bp. This band presented under treatment, but it was absent under control. In legume seeds, HSP synthesis increases with increasing temperature and a large heterogeneity of HSP classes has been found **(Dell, 2000)**.

Conclusion

The present results indicated that the procedure used in the present study was useful to investigate the performance of tested lentil cultivars and describe their seedling characters under tested stresses. Therefore, this laboratory technique is valid to be used for screening large number of lentil accessions for their performance under studied stresses as a rapid technique before screening them for such stresses under field condition.

REFERENCES

Abou Hadid, A.F. (2010). Climate change information center- a regional perspective. The tenth International Conference on Development of Drylands. Meeting the Challenge of Sustainable Development in Drylands under Changing Climate-Moving from Global to Local, Cairo, Egypt.

Anonymous (2017). Statistical report of lentil crop in Egypt. Minist. Agric., Egypt.

AOSA (1991). Association of Official Seed Analysis. Rules for testing seed. J. Seed Tech., 12 (3): 1-25.

Cochrane, T.T. (1994). A new equation for calculating osmotic potential. Plant Cell. Environ., 17: 427-433.

Copeland, L.O. (1976). Principles of Seed Science and Technology. Burgess Pub. Com., Minneapolis, Minnesota, USA.

Dell, A. (2000). Effect of combined salt and heat treatments on germination and heat shock protein synthesis in lentil seeds. Biologiaplantapum, 43 (4): 591-594.

Dell, A.A. (1999). Interaction effect between salt and high temperature on germination of fresh and aged lentil (*Lens cultinaris* Medik.) seed lots. Plant Varieties Seeds, 12: 91-98.

Dell, A.A. and M. Di Turi (1999). Amplification of aging symptoms in two differently thermal sensitive wheat (*Triticum durum* L.) genotypes by heat-shock relationships between germination response and embryo protein patterns. Seed Sci. Technol., 27: 467-476.

Erskine, W., S. Rihawe and B.S. Capper (1990). Variation in lentil straw quality. Anim. Feed Sci. and Technol., 28:61-69.

FAO (2017). Food and Agriculture Organization. FAOSTAT Statistical Database of the United Nation Food and Agriculture Organization (FAO), Rome.

Ghoulam, C. and K. Fares (2001). Effect of salinity on seed germination and early seedling growth of sugar beet (*Beta vulgaris* L.). Seed Sci. Tech., 29: 353-361 .

Gomez, K.A. and A.A. Gomez (1984). Statistical Procedures for Agricultural Research. 2nd Ed., John Wiley and Sons, New York.

Grusak, M.A. (2010). Nutritional and Health-beneficial Quality. In: Erskine, W., F.J. Muehlbauer, A. Sarker and B. Sharma (eds.), The Lentil, Botany, Prod. and Uses, 368-390. (CABI, UK. (ISBN-13: 978-1-84593-487-3).

Hamdi, A., A.A. Ahmed and T.A. Silim (2017). Variation in seed size between and within lentil cultivars and its effect on seedling growth. Egypt. J. Plant Breed., 21(6): 1013-1022.

Hamdi, A., M.S.E. Sharsher, A. Shehada, F.H. Shalaby and A.M.O. El-Bawab (2010). Development of crop varieties resistant to drought and heat stresses in Egypt. The tenth International Conference on Development of Drylands. Meeting the Challenge of Sustainable Development in Drylands under Changing Climate-Moving from Global to Local, Cairo, Egypt.

ISTA (1985). International Rules for Seed Testing. Seed Sci. and Tech., 24: 155-202.

Jana, M.K. and A.E. Slinkard (1979). Screening for salt tolerance in lentils. LENS Newsletter 6:25-27.

Katerji, N., J.W. Hoorn, A. Hamdy and M. Mastrorilli (2003). Salinity effect on crop development and yield analysis of salt tolerance to several classification methods. Agric. Water Manag., 62 : 37-66.

Kennel, C.F. (2010). Adaptation to the natural and social impacts of regional climate change. The tenth International Conference on Development of Drylands. Meeting the Challenge of Sustainable Development in Drylands under Changing Climate-Moving from Global to Local, Cairo, Egypt.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London), 227: 680 - 685.

Maher, L., R. Armstrong and D. Connor (2003). Salt tolerant lentils-a possibility for the future? In: Solution for Better Environment. Proceedings of the 11th Aust. Agron. Conf., Aust. Soc. Agron., Geelong, Victoria, Australia. Available at: www.regional.org. au/ au/asa/2003/c/1/hobson.htm.

Michel, B.E. and M.R. Kautmann (1973).The osmotic potential of polyethylene glycol 6000. Plant Physiol., 51: 914-916.

Rai, R., S.K.T. Nasar, S.J. Singh and V. Prasad (1985). Interaction between *Rhizobium* strains and lentil (*Lens culinaris*) genotypes under salt stress. J. Agric. Sci. Camb., 104:199-205.

Ribeiro, M.C.C., M.B. Amaro and J.A. Filho (2001). Effect of salinity on seed germination of sunflower cultivar quarter. Revistabrasileira de Sementeslondrina, 23 (1): 281-284.

Saxena, M.C. (1988). Food Legumes in the Mediterranean Type of Environment and ICARDA Efforts in Improving Their Productivity. In: Nitrogen Fixation by Legumes in Mediterranean Agriculture. Beck, DP and LA Materon (eds.), Kluwer Academic Pub. Dordrecht, The Netherlands.

Saxena, N.P., C. Johansen, M.C. Saxena and S.N. Silim (1993). The Challenge of Developing Biotic and Abiotic Stress Resistance in Cool-Season Food Legumes. In: Singh, K.B. and M.C. Saxena (eds.), Breeding for Stress Tolerance in Cool-season Food Legumes. John Wiley and Sons, Chichester, UK.

Solh, M. (2010). How science and technology can help farmers cope with climate change to enhance food security. The tenth International Conference on Development of Drylands. Meeting the Challenge of Sustainable Development in Drylands under Changing Climate-Moving from Global to Local, Cairo, Egypt.

Studier, F.W. (1973). Analysis of bacteriophage T1 early RNAs and proteins of slab gels. J. Mol. Biol., 79: 237 -248.

Tavakkoli, E., P. Rengasamy and G.K.L. McDonald (2010). High concentration of Na^+ and Cl^- ion in soil solution has simultaneous detrimental effects on growth of faba bean under salinity stress. J. Expt. Bot., 61: 4449-4459.

Turan, M.A., N. Turkmen and N. Taban (2007). Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. J. Agron., 6 : 378-381.

تأثيــر الجفـــاف والملـــوحـــة والحـــرارة معـــاً علـــى الإنبـــات وصفـــات نمـــو البـــادرات فـــى العدس

أحمد حمدى إسماعيل حمدى[1]- رشا يوسف سيد عبدالخالق[2]- هنية أحمد محمد عراقي[2]

١- قسم بحوث المحاصيل البقولية - معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية - الجيزة - مصر

٢- قسم بحوث تكنولوجيا البذور- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية - الجيزة- مصر

تهدف هذه الدراسة إلى التعرف على تأثير الملوحة (بدون ملوحة، ٠,١ ، و٠,٢ مول من كلوريد الصوديوم) مع الحرارة (٢٠ و ٣٠ درجة مئوية) والجفاف (بدون جفاف و جفاف تم استحداثه بنقع البذور فى محلول جليكول بولى إيثيلين بتركيز ٨٠٠٠ وحدة فى المليون) على إنبات البذور وسبعة صفات نمو لبادرات ثلاثة أصناف من العدس وهى جيزة ٩، وجيزة ٥١ وسيناء ١، وقد أجريت الدراسة بمعمل بحوث تكنولوجيا البذور بمعهد بحوث المحاصيل الحقلية بمركز البحوث الزراعية خلال عام ٢٠١٨، وأظهرت النتائج أن ارتفاع درجة الحرارة من ٢٠ إلى ٣٠ أدى إلى انخفاض معنوى فى كل صفات البادرات، وأحدثت معاملتى الملوحة ٠,١ ، و٠,٢ مول من كلوريد الصوديوم خفضاً معنوياً فى كل صفات البادرات مقارنة مع معاملة المقارنة (بدون ملوحة)، وكان تأثر صفات البادرات بمعاملة الملوحة ٠,٢ مول بدرجة أكبر من تأثرها بمعاملة ٠,١ مول، ولم يكن هناك تأثيراً معنوياً للجفاف على طول الريشة وطول الجذير وقوة البادرات وصفة نسبة وزن الجذور/وزن الريشة، بينما أدت معاملة الجفاف إلى حدوث نقص معنوى فى نسبة إنبات البذور بحوالى ٥,٦% وفى دليل قوة البذور بنسبة ٤,٨٧%، وقد وضح أن الصنف جيزة ٩ كان أكثر الأصناف تحملاً للجفاف، وقد أظهرت نتائج التفريد الكهربى تحت تأثير الملوحة والجفاف عند درجتى حرارة ٢٠ و٣٠ درجة مئوية تكوين بروتين الصدمة الحرارية (heat shock protein) (HSP) تحت درجة حرارة ٣٠ مئوية، بينما لم يتكون هذا البروتين تحت معاملة المقارنة، وقد أكدت النتائج أن التقنية المعملية المستخدمة تصلح للإستخدام كتقنية سريعة لغربلة عدد كبير من سلالات العدس لتقييم أدائها تحت ظروف إجهادات الملوحة والحرارة والجفاف وذلك قبل تقييمهم تحت ظروف الحقل.

المحكمـــــون:

١- أ.د. نبيل على خليل علي أستاذ المحاصيل المتفرغ – كلية الزراعة – جامعة القاهرة.

٢- أ.د. أحمد عبدالغني علي أستاذ المحاصيل المتفرغ – كلية الزراعة – جامعة الزقازيق.