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Assessment of Morphological Traits and Yield Potentiality of some Egyptian Lentil (*Lens culinaris* Medik) Cultivars under normal and Water Deficit Conditions

Abo-Hegazy, S. R. E.[1*]; Hend M. Farag[2] and I. H. Yacoub[1]

[1]Agron. Dept., Fac. of Agric., Cairo Univ., Giza, 12613, Egypt.
[2]Agric. Botany Dept., Fac. of Agric., Cairo Univ., Giza, 12613, Egypt.

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ABSTRACT

Two field trials were conducted during 2018/2019 and 2019/2020 seasons at the Research Station, Faculty of Agriculture, Cairo University. To evaluate the performance of five Egyptian lentil cultivars (Giza-9, Giza-29, Giza-51, Giza-370 and Sinai-1) under two levels of water treatments; normal and drought (60% and 30% FC). Each trial was conducted as a randomized complete block design (RCBD) in a split-plot arrangement with three replications. Main plots were assigned to the two water treatments, sub-plots were assigned to the five cultivars. Combined analysis of variance exhibited highly significant differences ($p \leq 0.01$) for both water treatments and cultivars for all traits. Giza-51 possessed highest seed yield/plant under normal conditions. Sinai-1 showed a slight decrease in seed yield/plant and seed proline exceed under drought conditions. Six drought tolerance indices {percentage of reduction (ROS %), stress tolerance (TOL), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean (HARM) and stress susceptibility index (SSI)} were used to detect drought tolerance of these cultivars. Seed yield in stress conditions negatively correlated with ROS%, TOL and SSI. Therefore, those indices are relevant factors to identify cultivars with low yield and tolerance to drought. Number of stomata showed significant differences in all cultivars. Also, stomatal width was more affected than length due to stomatal closure as a way to reduce water loss under drought. Overall, Giza-51 and Sinai-1 were more drought tolerance than other cultivars. Thus, drought indices, proline content and no. stomata should be given emphasis for future lentil yield improvement programs under drought conditions.

Keywords: Drought, *Lens culinaris*, cultivars, Proline and Stomata.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the most important cool-season legume crops grown worldwide due to seed richness in protein. Its plant growth is considered a delicate habit, which dramatically affects the fluctuation of yield potentiality between seasons and locations, especially in arid and semi-arid regions. These climatic zones possessa several constraints such as drought stress (Donat *et al.*, 2016). That kind of stress is being a major environmental factor for decline growth, fertility and causes mechanical changes of lentil crop species (Mishra *et al.*, 2014, Sarker*et al.*, 2009 and Kumar *et al.*, 2015).Also, biochemical pathways led to a decrease in starch and increase in osmotic solutes such as soluble sugars in lentil leaves and seed protein contents during drought (Bandeoglu *et al.*, 2004 and Gunes *et al.*, 2008). Likewise, drought stress reduces respiration by stomatal closure then less uptake and transportation of nutrients. However, some attributes of lentil genotypes adapted or responded to enhance the growth and survival rate during water stress, and subsequent recovery into their grown locations (Karim *et al.*, 2004). Yusuf *et al.* (1979) concluded that lentil genotypes generally adapted by two strategies are avoidance and tolerance. Avoidance is related to maintaining high tissue water potential and consists of mechanisms that reduce water loss from plants. It is due to stomatal control of transpiration *via* stomatal closure, which negatively affects CO_2 uptake, photosynthesis, transpiration cooling, and water and nutrient uptake. Therefore, it is crucial to close the stomata only when the benefit of water retention outweighs the adverse effects. Several signaling pathways and mechanisms lead to stomatal closure during unfavorable environments. These pathways can be divided into hydro-passive and hydro-active stomatal closure (Luan, 2002).

Proline plays a pivotal role for characterizing drought tolerance/resistance as an osmo-protectant under stress conditions in lentil. It is expressed widely in higher plants and typically gets accumulated exceeded quantities to defenses for environmental stresses as a reaction (Ankita *et al.*, 2017). Increasing leaf proline content with deficit water supply explained that an efficient mechanism for osmotic regulation, stabilizing sub-cellular structures and cellular adaptation to water stress was observed in the lentil (Mishra *et al.*, 2014). Furthermore, several studies explained that the relationships among plant traits involved shoot traits were associated with drought tolerance. Meanwhile, root characteristics and other functions determine and meet the transpiration demands of the plant (Passioura, 1982). One of these relations may be calculated for seed yield productivity by drought stress indices, several indices used to evaluate tolerance genotype against different stresses (Naveed *et al.*, 2019).

GRAPHICAL ABSTRACT

The investigated study was summarized by simplifying the schematic chart (Fig.1) for all possible experiment procedures to elucidate performance of five

[*] Corresponding author.
E-mail address: samirrabie@agr.cu.edu.eg
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Egyptian lentil cultivars for enhancement yield productivity under different two water treatments duringtwo field trials, with assessments of sixteen studied traitsduring plant growth.

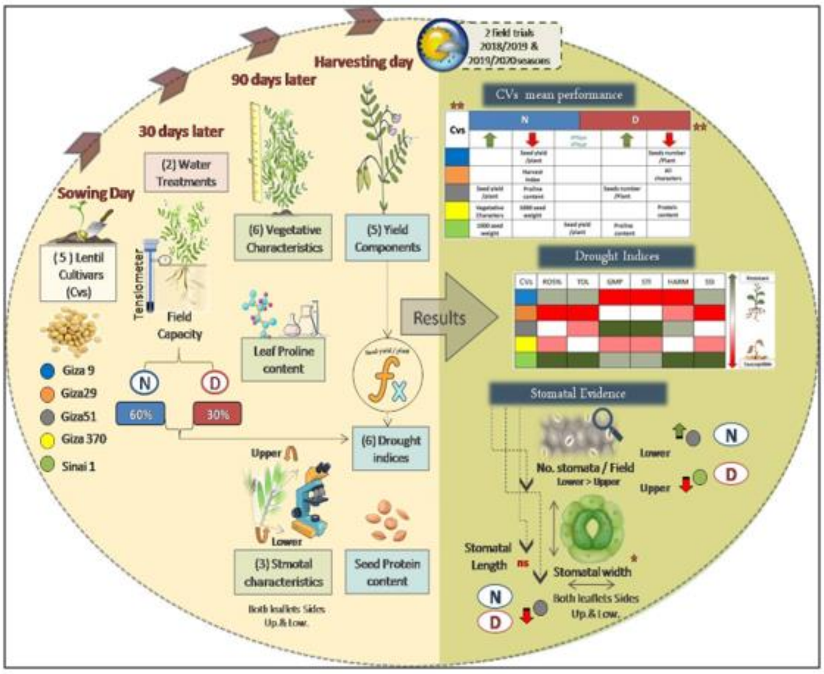

Fig.1. Scheme illustrating the methodology and actual results of investigated study along with plant growth for evaluating performances of 5 lentil cultivars under two water treatments (N); normal and (D); drought conditions.

ns, indicated non-significant; *and **significant and highly significant effects at 5 and 1 percentage level of probability, respectively. : exceeded, reduction and ≈ :approximately equivalent impacts.
Percentage of reduction (ROS %), stress tolerance (TOL), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean (HARM) and stress susceptibility index (SSI).

In Egypt, lentil harvested area and seed production decreased sharply from 1380 to 411 (ha) and 2178 to 891 (tons), respectively, at the last decade (FAO, 2021) with more than 95% self-insufficiency. Considering this increased demand cited in this region, regarding unstoppable local climate effects on water stress for long or short periods. Moreover, there is a narrow genetic background of Egyptian lentil cultivars which bounded improved newly cultivars to overcome water deficit (Hamdi *et al.*, 2004).Besides that, lentil variability among genotypes in drought-prone zones has not been adequately exploited (Erskine and Saxena, 1993).Thus, accessing the genotype tolerant to drought stress through understanding the mechanisms of plant resistance can play an essential role in its adaptation under drought conditions (Srivastava and Vasishtha, 2012).

This investigation was carried out to evaluate five lentil cultivars under drought conditions by studying some morphological, biochemical and yield characteristics, which are considered an initial stage to determine the extent of the cultivar's response to drought. In addition to, using some drought indices as indicator for distinguish between drought resistance and susceptible cultivar(s).

MATERIALS AND METHODS

1- Experimental procedures and plant materials

The present investigation was carried out at the Agricultural Experiment and Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt (30°01'03" N 31°12'25" E), through two field trials in 2018/2019 and 2019/2020 seasons. Representative soil samples were analyzed from soil surface of experimental site at the depth of 0 to 30 cm before planting. According to Klute (1986) and Page *et al.*, (1982), physical and chemical soil analyses were conducted. Table (1) shows the mechanical and some chemical properties of the experimental soil site during the two studied seasons, where it classified soil texture as clay loam in both seasons with a recorded average of field capacity (FC) 60 % (determined gravimetrically).

Table 1. Mechanical and chemical properties of experimental site (30 cm depth) in two seasons

Character	Season	
	2018	**2019**
	Mechanical analysis	
Coarse sand (%)	6	8
Fine sand (%)	34	30
Silt (%)	20	23
Clay (%)	40	39
Soil type	Clay loam	Clay loam
	Chemical analysis	
Organic matter (%)	1.9	1.45
pH	7.5	7.3
EC (m/mohs/cm)	0.8	1.01

Tensiometer is used to measure field capacity (FC) at point of the soil moisture content in field technique, thus it was estimated by irrigating practices at experimental site until the soil profile is saturated to a depth of about one meter as well as the moisture content reached to 60 % FC it is considered well-watered treatment or normal. However, the drought stress treatment was conducted at 30% FC. The soil moisture of experimental plots was measured each 24 hours until the changes reached for two water treatments. Generally, this stress treatment were applied at 30 days after sowing date and continued up to maturity stage. The experimental plot area was 9.6 m2,established by comprised of 4 ridges; each ridge was 4 meters long and 60 cm apart, and seeds were drilled at both sides. All other cultural practices were applied according to the recommendations of lentil production in Giza.

Five Egyptian lentil cultivars (Giza-9, Giza-29, Giza-51, Giza-370 and Sinai-1) were obtained from Food Legume Crops Section, Field Crops Research Institute, ARC, Giza. Whereas features of these cultivars were identified into Microsperma seed type except the seeds of last cultivar Sinai1 regarded a Macrosperma type and characterized by early maturity and promising established in new reclaimed lands. The pedigree of the studied cultivars are presented in Table (2).

Table 2. Pedigree of the studied Egyptian lentil cultivars

No.	Cultivar	Pedigree
1	Giza-9	Wide spread cultivar
2	Giza-29	Land race
3	Giza-51	Selection from hybrid family
4	Giza-370	Wide spread cultivar
5	Sinai-1	Selection from Argentinian cultivar "Preccoz"

Source: Food Legume Crops Res. Dep., FCRI, ARC, Egypt

2- Plant samples and assessment

During the vegetative growth, some measurements were recorded at 90 days from sowing date including; plant height (cm), number of internodes of the main stem, number of branches per plant, number of compound leaves per plant, plant fresh and dry weights (g). At the similar growth stage, biochemical trait as Proline content was determined by taking 0.5g leaf samples homogenized with 10mL of 3% sulfosalicylic acid and filtered using a Whatman No. 2 filter paper. Proline concentrations in the extract were spectrophotometrically determined as reported by Bates *et al.* (1973). As well as,stomatal characteristics were recorded by ten leaflets which collected from plants grown under normal and drought stress conditions for each cultivar. the epidermal impression was prepared by spread a thin layer of nail polish on each surface upper side and lower side (adaxial and abaxial) of the leaflets, peel off the dried layer of nail polish by using clear stick tape and then placed the tape with leaflet impression onto a clean slide (Brewer, 1992) and observed under light microscope (Leica, Wetzlar, Germany) with digital camera, at 200x magnification. The number of stomata was counted in entire field of view (FOV). Stomatal length and width were measured with a micrometer at scale bar 100µm.

At harvest time, a sample included 10 guarded plants were harvested manually from the central ridge of each plot to record the individual plant traits, and yield components were recorded as follows: pods number per plant, seed number per plant, weight of 1000 seed (g), seed yield per plant (g), and harvest index % (percentage of seed yield per plant to plant dry weight at harvest). Besides that, seed protein content was estimated by using the Kjeldahl method described by AOAC (2000) in dry seeds (%) and this procedure was carried out in Cairo University Research Park (CURP).

Drought indices:

Six indices of drought tolerance were done based on seed yield per plant in different two levels of water treatments; well-watered treatment (Y_N), drought (Y_D) and those mean yields ($\bar{Y}_N$), ($\bar{Y}_D$). Percentage index as a reduction over control (% ROC) was suggested according to Ali *et al.* (2004) whose were defined by the following formula**:**

$$\textbf{(ROC \%)} = \frac{\text{value in control} - \text{value in streesed treatment}}{\text{value in control}} \times 100$$

And other five indices; Tolerance index (TOL), Geometric mean productivity (GMP), Harmonic mean (HARM), Stress susceptibility index (SSI) and stress tolerance index (STI) were calculated by the following equations:

$$\mathbf{TOL} = Y_D - Y_N \quad \text{(Rosiele and Hamblin, 1981)}$$

$$GMP = \sqrt{Y_D \times Y_N} \quad \text{(Fernandez, 1992)}$$

$$\mathbf{HARM} = 2[(Y_D \times Y_N) / (Y_D + Y_N)] \quad \text{(Kristin } et\ al.\text{, 1997)}$$

$$\mathbf{SSI} = [1-(Y_D / Y_N)] / [1-(\bar{Y}_D / \bar{Y}_N)] \quad \text{(Fischer and Maurer, 1978)}$$

$$\mathbf{STI} = [(Y_D + Y_N) / \bar{Y}_N^2] \quad \text{(Fernandez, 1992)}$$

3- Statistical analysis

Statistical procedures of the obtained data pre-tested with normality according to Shapiro and Wilk (1965) test, subsequently finding significance of meaning squares for assumption regular split plot design with randomized complete blocks arrangement in 3 replications (Snedecor and Cochran 1989). Two water treatments occupied the main plots which involve dwell-watered (normal) and drought stress at 60%and 30% FC, respectively. However, five studied lentil cultivars (Giza-9, Giza-29, Giza-51, Giza-370 and Sinai-1) were assigned to sub plots. Combined analysis over seasons was conducted as indicated of normality and homogeneity tests. The homogeneity test based on homogeneity error variances of both seasons for each character was performed according to Hartley's F_{max} test (1950).

Furthermore, estimating differences among means of studied treatments depended on significance level ($p \leq 0.05$) by using Duncan's multiple range tests (Duncan, 1955), which were presented by different superscript letter as a significant difference among treatments. Correlation coefficient by using Spearman's rank-order correlation was explained the interrelationships of all possible pairs for 6 drought indices associated with seed yield per plant under two different watering treatments. On the other hand, multivariate analysis such as visualized clustering analysis by heat maps a graphical method utilizing squared Euclidian distance between groups' averages of interactions among different studied traits and lentil cultivars impacted by two water treatments. That, Impressive color scheme is an essential factor for correct interpretation of that heat map. Thus, it might be chosen between various diverging and sequential color schemes for those criteria noted by Harrower and Brewer (2003).

All data were processed by MSTAT- Cv.2.10 and SPSS v.27 software package program modified by extensions hub with R program V.3.5.Heatmap procedure by clustvis online web site tool for visualizing clustering of multivariate data (BETA) https://biit.cs.ut.ee/clustvis/ created according to Metsalu and Vilo (2015).

RESULTS AND DISCUSSION

Studied traits (vegetative, biochemical and yield components) were diagnosed as normal distribution along plant growth stages with normality tests of hypotheses by Shapiro and Wilk at $p > 0.05$. Subsequently, combined analysis of variance across two seasons was performed after testing homogeneity of error variances.

Significance due to different sources of variation for combined analysis are presented in Table (3) based on the combined analysis; mean squares of seasons (S) were insignificant for all studied traits except no. of compound leaves per plant, seed yield per plant, harvest index and proline content. This finding proved that the attributes of lentil seed yield traits and proline content affected from different seasons and environmental impacts.

Mean squares of water treats (W) recorded highly significantly differences for all the studied traits as it would be expected for the differences between well-watered and drought stress, these variations are represented in Figure (2-A). Most of the studied traits had higher attributes in normal condition than water deficit condition that agree with Motas *et al*. (1988) who observed that seed production and yield contributions of peas were significantly affected by the most minor soil moisture regime at 30%, while the proline content was exceeded by +19.1 % under stress condition, these differences are meaningful by calculating a relative change or differences ratio by following the formula of mathematical operation ([D –N] / N × 100) between two averages from normal to drought water treatments of each study trait (Figure 2-B). That accumulation of proline seemed to be a part of the stress signal influencing adaptive the responses and outstanding plants to survive under stress (Maggio *et al*., 2022).On the other hands, plants grown under drought stress were affected by a decrease in their heights by -22.1% and became shorter compared to normal conditions this result agrees with those reported by Juan *et al*. (1995). Plant height was dwarfed in the drought treatment since cell division or cells enlargement was inhibited caused by effects of stress. Moreover, other traits were expressed to a widely percentage reductions values under stress around 35% of some studied traits as plant fresh and dry weights which decreased by -37.7and -40.0%;respectively. Also, the number of branches per plant had a reduction of -33.7% and the importance yield traits as seed yield per plant was recorded declined of production by -29.4%.Generally, significant effects of drought stress *vs.* well-watered proved that, the performance of lentil yield and attributes affected by water treatments.

Table 3. Significance of mean squares due to sources of variation for combined of studied traits (vegetative, yield components and biochemical) over two seasons.

Sources of Variation	Vegetative traits						Yield components					Biochemical	
	Plant height	**No. internodes /main stem**	**No. branches /plant**	**No. Compound leaves /plant**	**Plant fresh weight**	**Plant dry weight**	**Pods number /plant**	**Seeds number /plant**	**Weight Of 1000 seed**	**Seed yield / plant**	**Harvest index**	**Seed protein cont.**	**Proline cont.**
Seasons	ns	ns	ns	*	ns	ns	ns	ns	ns	*	**	ns	*
Water treats	**	**	**	**	**	**	**	**	**	**	**	**	**
S × W	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	**	*
Cultivars (Cvs)	**	**	**	**	**	**	**	**	**	**	**	**	**
S × Cvs	ns	ns	ns	ns	ns	*	*	**	**	**	ns	**	**
W × Cvs	**	ns	**	ns	**	**	**	**	**	**	**	**	**
S × W × Cvs	ns	ns	ns	ns	**	ns	*	*	*	*	ns	ns	ns

ns , * and ** indicated non –significant, significant at 5% and highly significant at 1 % percentage level of probability, respectively.

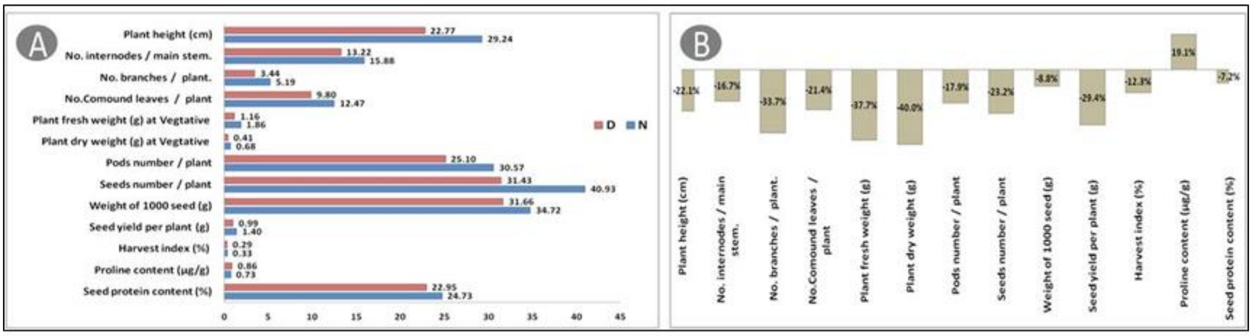

Fig. 2. Bars chart illustrated A) Combined averages of 2 water levels treatments (N: normal & D; drought) effects on each studied character over seasons and cultivars.
B) Relative changes or differences ratio between its two averages from normal to drought water treatments of each study character.

Mean squares of the interaction (S x W) in a split-plot analysis existed for only two traits of yield components: seed yield per plant and harvest index. Also, biochemical traits, exhibited significant differences for S x W interaction these results reflected the differences in water treats prevailing during the two growing seasons.

Cultivars (Cvs) effects have the same trend with water treats and were highly significant for all studied traits. Therefore, the comparison between genotypic means is valid. The combined analysis of variance exhibited significant differences of interactions among cultivars and seasons (S x Cvs) for all studied traits except plant height, number of internodes/main stem, number of branches, number of compound leaves/plant and harvest index. This result proved that outcomes varied from one season to another for yield and its components, despite the effectiveness of lentil cultivars. In other words, these traits with significant S x Cvs varied under the influence of dominated environmental conditions and different cultivars. The water treatments (W) x Cultivars (Cvs) interactions were exhibited significant variances for all studied traits. Internodes/main stem and no. compound leaves/plant proved that despite the behavior of lentil cultivars varied from well-watered *vs.* drought stress conditions. In other word, these traits with significant W x Cvs varied under the influence of dominated environmental conditions and lentil cultivars (Cvs). Finally, the order interaction (S x W x Cvs) varies significantly for only the actual value of lentil plant fresh weight, pods number/plant, seeds number /plant, weight of 1000 seeds, and harvest index whiles, the studied traits differed insignificantly. The results indicated that lentil cultivars responded differently to the different environments. Therefore, more studies needed to identify the best genetic make up for a particular environment and cultivars affected by drought stress. Similar results were obtained by Hamdi *et al*. (2004), Bayoumi (2008), Abo-Hegazy *et al.* (2013), Mishra *et al*. (2014) and Ankita *et al.* (2017).

Mean performance of five lentil cultivars for studied traits as an average over two levels of water treatments and across two seasons are shown in Table (4). There are higher differences among the cultivars for all studied traits. Despite, Giza-370 cultivar was showed the highest values for all vegetative traits i.e. Plant height (31.49 cm), number of internodes/main stem (15.67), number of branches/plant (6.17), number of compound leaves/plant (11.88), Plant fresh weight (2.30 g), Plant dry weight (0.76 g) and some yield components such as number of pods/plant (32.33) and number of Seeds/plant (41.17). It was declined for 1000 seed weight by recorded the lowest value (about 28.13 g) compared to other cultivars and this reduction reflected finally for harvest index exhibited insignificant differences of the two cultivars Giza-29 and Giza-51. The harvest index can be referred as been the physiological efficiency and crop attributes for converting the plant dry matter into economic yield (Sharifi *et al.,* 2009). In another mean, proper cultivar holds a great promise harvest index enhanced. However, Sinai 1 exhibited the highest yield attributes traits for seed index 40.34g and harvest index 42.3 %. In addition to, chemical traits involved proline and protein content were recorded 0.95 µg/g and 24.62%, respectively. Accordingly, that result indicated a similar attributes for both harvest index and increasing proline percentage of Sinia-1. On the other hand, Giza-9 showed as inferior attributes for most traits, where it showed for all vegetative characters except number of internodes per main stem and number of compound leaves per plant, similarly both of pods and seed numbers per plant, which it was exhibited lowers recorded values for most yield components. These results are similar to those obtained by Hassan *et al*. (2021) when evaluating Giza-9 as a check variety with other genotypes in Upper Egypt.

Another reports outlined that differences were shown with narrow gaps values observed of the reduction between water treatments for some cultivars of studied traits, depending on genotype variability for differed resistance stress. For example, infection by disease as one of biotic stress especially roots diseases caused by practices of irregular irrigation or heavy rain (flooding) that increasing soil moisture indicators for water logging and reducing the yield productivity; even with a short time of plant growth exposure it can cause the crop to die easily (Brennan *et al.,* 2011), However. Nem*a et al.* (1984) reported that the best result from irrigation was a single application at the pre-flowering stage. At harvested plants Ankita *et al.* (2017) reported that seed yield was significantly higher in irrigated than in rain fed conditions, it means that, some released genotypes responses for watering regime or alleviate drought stress effects.

Lentil cultivars attributes under the effects of two water treatments for various traits are displayed in Table (5). Mean performance of cultivars under well-watered (normal irrigation) and drought conditions: Almost all traits were observed under drought stress compared to well-watered conditions except for proline content for all studied cultivars. In general, proline content (µg/g) was higher under drought stress conditions than well-watered for all the studied cultivars. It conformed to the results reported by Mishra *et al*. (2016) and Morgil *et al.* (2017).

Increased proline content under water deficit conditions noted that it can serve as an essential parameter for selecting stress resistance genotypes and maintain cell structure and osmotic balances in cells. It is also uniformed in maintaining the water holding capacity of plants, thus protecting the plant tissue from being injured under stress (Liu *et al*., 2003). Our findings agree with those obtained by Tawfik (2008) who suggested that water deficit caused an increase in the concentration of proline in mung bean. In addition, Raheleh *et al*. (2012) reported high proline content in plants under water stress.

Moreover, seed yield per plant was reduced significantly under drought stress conditions than well-watered conditions. Under drought, the reduction in seed yield/plant was exceeded for all cultivars, while both Giza-51 and Sinai-1 showed a limited reduction, which recorded 1.11 and 1.20 g, respectively. Similar results were also reported by Sharaan *et al.* (2003), Bayoumi (2008) and Salehi *et al*. (2008). That could be regarded from their seeds types as Macrosperm of Sinai-1 which recorded the highest value of speed index (1000 seed weight) under two watering treatments, but the increasing seeds number of plant referred by Giza-51 under drought condition. Where, Giza-29 exhibited reducing ability for productivity under drought stress for most studied traits

Accordingly of vegetative traits, both of plant fresh weight and dry weight were reduced significantly sharply under drought stress compared to well-watered conditions, due to less assimilates production in the plant which caused by inhibited photosynthesis. Similar findings were also reported by Kusmenoglu and Muehlbauer (1998) and Mishra *et al.* (2014).

The reduction in the number of branches/plant, number of pods/plant, and seed number/plant were also confirmed the earlier findings of Hamdi and Erskine (1996), Sharaan *et al.* (2003) and Abo-Hegazy *et al.* (2013). A similar pattern of reduction in seed protein content% was also reported by Sharaan *et al.* (2003) and El Haddad *et al*. (2022). From the preceding discussion, it may be concluded that water stress had significant effects on the different traits under investigation. Also, it was clear that tolerant and susceptible cultivars responded differently for different studied traits under water-stress conditions (Table 5).

The *per se* performance of lentil cultivars revealed a substantial variability among the cultivars for all the studied traits except the number of internodes/main stem and number of compound leaves/plant under well-watered and drought stress conditions that were insignificant.

Release promising cultivar(s) that identifying consequently synchronous achieve the gain of increasing grain production and saving water as mentioned by Yang and Zhang (2010).

Table 4. Mean Performance of five lentil cultivars across two levels of water treatments for studied traits combined over two seasons.

Cultivars	Vegetative traits						Yield components					Biochemical	
	Plant height (cm)	No. internodes / main stem	No. branches / plant	No. Compound leaves /plant	Plant fresh weight (g)	Plant dry weight (g)	Pods number /plant.	Seeds number / plant.	Weight of 1000 seed (g)	Seed yield / plant (g)	Harvest index (%)	Seed protein cont. (%)	Proline cont. (µg/g).
Giza-9	23.83^{c}	15.17^{ab}	3.96^{b}	12.04^{a}	1.28^{b}	0.47^{c}	22.04^{d}	26.67^{d}	34.78^{b}	0.93^{d}	32.60^{b}	23.73^{b}	0.78^{b}
Giza-29	27.85^{b}	14.98^{ab}	3.54^{b}	10.92^{ab}	1.12^{c}	0.37^{d}	25.83^{b}	37.33^{b}	31.90^{c}	1.21^{bc}	22.60^{c}	23.26^{c}	0.96^{a}
Giza-51	24.96^{c}	14.38^{b}	4.02^{b}	10.33^{b}	1.44^{b}	0.45^{c}	35.50^{a}	43.75^{a}	30.80^{d}	1.36^{a}	31.60^{b}	24.57^{a}	0.62^{d}
Giza-370	31.49^{a}	15.67^{a}	6.17^{a}	11.88^{a}	2.30^{a}	0.76^{a}	32.33^{a}	41.17^{a}	28.13^{e}	1.17^{c}	27.8^{0b}	23.02^{c}	0.66^{c}
Sinai-1	21.88^{d}	12.58^{c}	3.90^{b}	10.50^{b}	1.41^{b}	0.69^{b}	23.47^{c}	32.00^{c}	40.34^{a}	1.29^{ab}	42.30^{a}	24.62^{a}	0.95^{a}

Means of column (different cultivars performance of each study trait) followed by the same letters are not significantly different at 0.05 level of significance.

Table 5. Mean performance of the interaction between five studied lentil cultivars and two water treatments for vegetative, yield components and biochemical traits, combined over two seasons.

Traits	Well-watered (Normal)					Drought stress				
	Giza-9	Giza-29	Giza-51	Giza-370	Sinai-1	Giza-9	Giza-29	Giza-51	Giza-370	Sinai-1
Plant height (cm)	27.00^{d}	32.00^{b}	28.92^{c}	34.27^{a}	24.00^{e}	20.67^{f}	23.70^{e}	21.00^{f}	28.72^{c}	19.75^{f}
No. internodes / main stem.	17.25ns	15.83ns	15.75ns	16.50ns	14.08ns	13.08ns	14.12ns	13.00ns	14.83ns	11.08ns
No. branches / plant.	4.42^{bc}	4.00^{cd}	5.08^{b}	7.75^{a}	4.72b^{c}	3.50^{de}	3.08^{e}	2.96^{e}	4.58^{bc}	3.08^{e}
No. Compound leaves / plant.	13.25ns	11.67ns	12.50ns	13.25ns	11.67ns	10.83ns	10.17ns	8.17ns	10.50ns	9.33ns
Plant fresh weight (g).	1.49^{c}	1.41^{c}	1.87^{b}	2.83^{a}	1.72^{b}	1.07^{d}	0.84^{e}	1.00^{de}	1.78^{b}	1.10^{d}
Plant dry weight (g).	0.55^{cd}	0.48^{d}	0.61^{c}	0.97^{a}	0.80^{b}	0.38^{e}	0.26^{f}	0.28^{f}	0.55^{cd}	0.58^{cd}
Pods number / plant.	23.25^{de}	27.83^{c}	37.50^{a}	38.67^{a}	25.61^{cd}	20.83^{e}	23.83^{de}	33.50^{b}	26.00^{cd}	21.33^{e}
Seeds number / plant.	29.83^{e}	44.67^{b}	49.33^{a}	47.67^{ab}	33.17^{de}	23.50^{f}	30.00^{e}	38.17^{c}	34.67^{cd}	30.83^{de}
Weight of 1000 seed (g).	35.75^{c}	34.17^{d}	32.58^{e}	29.25^{f}	41.85^{a}	33.80^{d}	29.63^{f}	29.02^{f}	27.00^{g}	38.83^{b}
Seed yield / plant (g).	1.07^{cd}	1.53^{ab}	1.60^{a}	1.40^{b}	1.39^{b}	0.80^{e}	0.89^{e}	1.11^{c}	0.94^{de}	1.20^{c}
Harvest index (%)	38.00^{b}	26.00^{de}	34.00^{bc}	31.00^{cd}	45.00^{a}	28.00^{cd}	20.00^{e}	30.00^{cd}	25.00^{de}	40.00^{ab}
Seed protein content (%)	24.43^{cd}	24.83^{bc}	25.33^{a}	24.00^{de}	25.03^{ab}	23.02^{f}	21.69^{g}	23.82^{e}	22.03^{g}	24.20^{de}
Proline content (µg/g)	0.65^{ef}	0.92^{c}	0.61^{f}	0.64^{ef}	0.81^{d}	0.92^{c}	1.01^{b}	0.64^{ef}	0.67^{e}	1.08^{a}

Means of row (different cultivars performance of each studied traits) followed by the same letters are not significantly different at 0.05 level of significant; ns: indicate non-significant differences.

Effect of water stress on stomatal characteristics

Significances of studied factors (water treatments and cultivars) and the interaction between them for each three stomatal character of leaflets' upper and lower surfaces are shown in Figure (3). Stomatal characters for both sides of leaflets along five studied cultivars in two levels of water treatments showed significant effects except stomatal length for interaction among them and stomatal width of upper leaflet surface.

It is noticeable from Figures (3,A & 4) that the lower surface of leaflet in all studied cultivars shows a greater number of stomata per studied field than those found on the upper surface that agree with Patel *et al.* (2021). Under normal conditions, Giza-51 possessed the greatest number, about 55.3 stomata per field, followed by Sinai 1 and Giza-370, while Giza-29 recorded the lowest number of stomata for the lower surface. At the same time, there was a severe decrease in stomata number under water stress, especially in Sinai 1 and Giza-370. The numbers were decreased by almost half from 49.7 to 27.3 and 48.0 to 23.3 stomata per field, respectively. Likewise, Sinai1 recorded the lowest value (2.7) for stomatal number under drought for the upper surface. This reduction may be due to the plant's response to adaptation under stress. Previous studies reported that early response to water deficit reduces leaf area and plant growth, allowing plants to reduce their transpiration (Xu and Zhou, 2005; Monclus *et al*., 2006 and Aguirrezabal *et al.*, 2006). The balance between leaf area and its stomata may be associated with the number of guard cells suggested by (Xu and Zhou, 2008).

On the other hand, stomatal length showed no significant differences in interaction between two levels of water treatments and five lintel cultivars (Fig. 3, B), but Sinai-1 seemed to be the longest one compared to other than studied cultivars, which considered a unique cultivar due to classified into Macrosperma type.

Stomatal width character was more varied than its length in case interaction between studied factors. There was reduction of stomatal width for all cultivars under water stress on lower surface compared to the upper. Decreasing stomatal width results from the stomatal closure as a way to reduce water loss through transpiration. However, Giza-51 showed the lowest value of stomatal width for both the upper and lower leaflet surfaces under two water treatments. Doheny-Adams *et al.* (2012) and Franks *et al.* (2015) indicated that plants exposed to water stress in the short term increase their water use efficiency by reducing stomatal aperture and transpiration rate; however, under conditions of prolonged water deficit plants produce leaves with reduced maximum stomatal conduction resulting from a change in stomatal size. Moreover, Cutler *et al.* (1977), Spence *et al.* (1986) and Martinez *et al.* (2007) showed that water deficit decreased stomatal size (both length and width) these changes in stomatal morphology may increase the plant adaptation to drought stress.

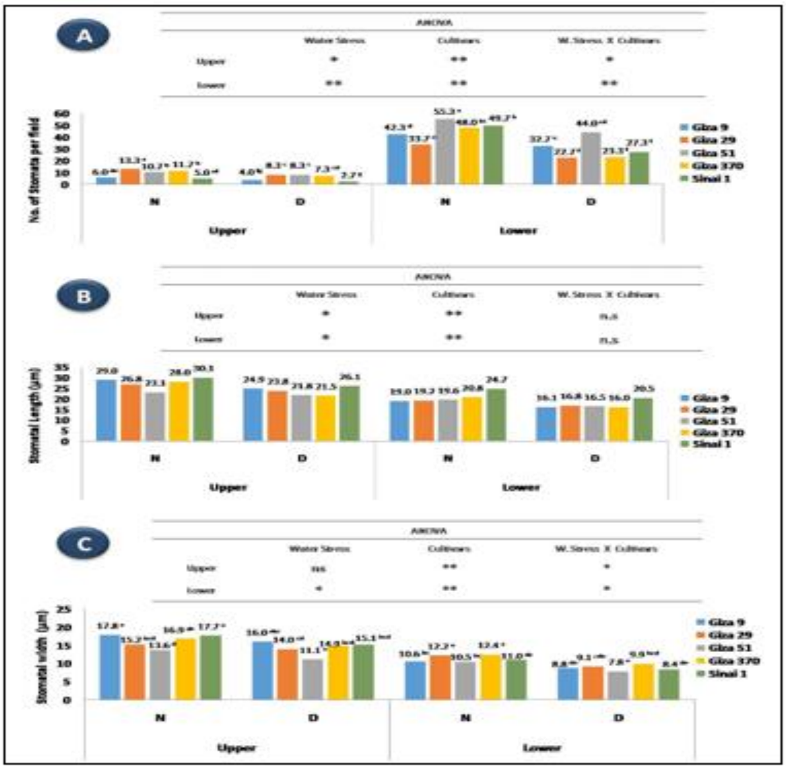

Fig. 3. Illustrating both upper and lower surface patterns of three studied stomatal characters (A; number of stoma taper filed, B; stomatal length, and C; stomatal width, µm). Each character included significance of mean squares tested by ANOVA of two factors; the 1st factor including 2 levels of water treatments (N; normal and D; drought) & the 2nd factor; five lentil cultivars and their interactions.

***, ** and n.s indicated: significant, highly significance and non-significance, respectively.**

Means of cultivars under water treatments have different letters above the bars are significant differences at level 0.05 of probability.

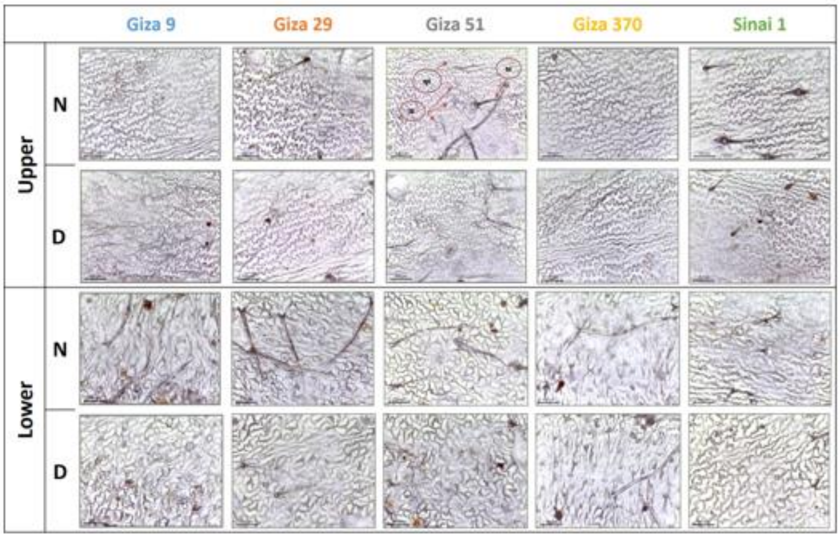

Fig.4. Epidermal impressions of upper and lower leaflet surfaces for five lentil cultivars at 90 days to water testaments: (N); normal and (D); drought conditions at scale bar 100µm. epi; epidermal cells, st; stomata and tri; trichomes.

Assessment of lentil cultivars by drought stress tolerant indices

Various drought resistance/tolerance indices were calculated based on seed yield/plant of five genotypes under irrigated (Yield N) and drought-stressed (Yield D) conditions (Table 6). The lowest value for TOL was recorded in Sinai 1 cultivar, obviously, TOL only pointed out the cultivars with the lowest seed yield in normal conditions. The results showed that the greater value of ROS, TOL and SSI, the larger yield's reduction under stress conditions, and the higher drought sensitivity. Lower values of ROS, TOL and SSI showed more yield in stress than normal irrigated conditions. The ranks of the genotypes for GMP, STI, and HAM were almost identical (Saba *et al*., 2001 and Tigkas *et al*., 2013 and 2019). Geometric mean productivity seed yield (GMP) and stress tolerance index (STI) were recorded in cultivar Giza-51 (GMP = 1.33 g/pl and STI =1.39 g/pl), (Table 5). Based on GMP and STI values, in this case, the cultivar Giza-51 could be considered relatively drought tolerant.

Table 6. Ascending of ranks means of five cultivars seed yield/plant under two water treatments through d six different drought indices.

Cultivars	Yield N	Yield D	ROS†	TOL†	GMP§	STI§	HARM§	SSI†
Giza-9	1.07 (1)	0.80 (1)	25.58% (2)	0.27 (2)	0.92 (1)	0.95 (1)	2.39 (1)	0.87 (2)
Giza-29	1.53 (4)	0.89 (2)	41.77% (5)	0.64 (5)	1.17 (3)	1.24 (3)	2.67 (2)	1.42 (5)
Giza-51	1.60 (5)	1.11(4)	30.80% (3)	0.49 (4)	1.33 (5)	1.39 (5)	3.32 (4)	1.05 (3)
Giza-370	1.40 (3)	0.94 (3)	32.90% (4)	0.46 (3)	1.15 (2)	1.20 (2)	2.81 (3)	1.12 (4)
Sinai-1	1.39 (2)	1.20 (5)	13.69% (1)	0.19 (1)	1.29 (4)	1.33 (4)	3.59 (5)	0.47 (1)

(†) and (§), low and high index values showed more tolerant cultivars for each index, respectively (Yield N: normal, Yield D: droughts tress) Percentage of yield reduction (ROS %), stress tolerance (TOL), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean (HARM) and stress susceptibility index (SSI). Numbers Between the brackets of each Column indicated its index's position or rank.

The colorful correlation matrix illustrated the genotypic correlation coefficient (rg) between Yield D, Yield N, and other quantitative drought tolerance indices to determine the most desirable drought tolerance criteria (Fig. 5). The yield N under normal irrigated conditions has a very weak association with stress conditions (Yield D) characterize that high yield potential under the best available conditions does not anticipate superior yield under drought conditions. Therefore, indirect selection for drought environments based on the performance of irrigated conditions would not be effective. These findings agree with those obtained by Gholipouri et al. (2009) and Javed et al. (2011). Seed yield under normal irrigated conditions (Yield N) was positively and significantly associated with TOL (0.71), GMP (0.88) and STI (0.92). Also, a positive and significant correlation has observed between seed yield under Yield D and GMP (0.88), STI (0.84) and completed with HARM (1.00), and GMP showed positive and significant associated between seed yield and Yield D (0.88), Yield N (0.92), HARM (0.88) and STI (1.00), so they were the better predictor of potential yield D, Yield N, and GMP than ROS, TOL, HARM. These findings agree with those obtained by Rad et al., (2009) and Javed et al. (2011). In stress conditions, seed yield showed a negative correlation with ROS (-0.48), TOL (-0.18), and SSI (-0.47). Therefore, ROS, TOL, and SSI indices are relevant factors to identify wheat genotypes with low yield and tolerance to drought stress because under stress conditions yield decreased with increasing SSI. There was no significant correlation of TOL with Yield D, HARM, STI, and GMP. However, it had a positive and significant correlation with SSI (0.94) and Yield N (0.71). Therefore, it gave the impression that SSI and TOL had the same capability in performing tolerance against drought stress.

Interrelationships of studied traits assessments for lentil cultivars

Two dimensional dendrograms were presented (Figure 6) in order to conclude the multivariate analyses of detected varied patterns of all studied traits, yield components, vegetative, biochemical, and stomatal characters, at different periods of plant growth.

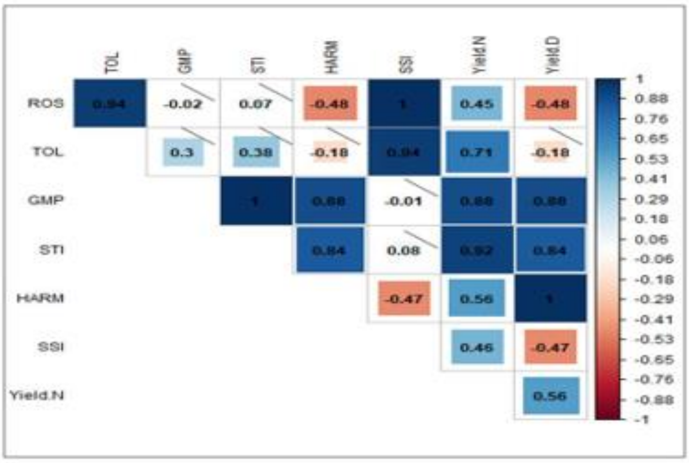

Fig. 5. Illustrated the colorful correlation matrix for relationships among seed yield per plant under normal: Yield N, drought stress: Yield D conditions, and six drought tolerance indices over 2 seasons (Blue, Red indicated positive; negative relationships; respectively).

The first dimension, tracks placed at the top of the matrix, can be configured and annotated to interpret them in conjunction with the second dimension, clustering tree. Meanwhile, five lentil cultivars were classified under two effects of water treatments to detect similarity performances and find their relationships through all studied traits. Generally, heatmap simplified all possible effects, whereas it was presented two major groups of lentil cultivars performances classified at top matrix, the first group involved three cultivars Giza-370, Giza-29 and Giza-51, and another group consisted by rest cultivars (Giza-9 and Sinai-1). Although both Giza-9 and Sinai1 were genetically divergent due to differences in their seed types (Microsperma and Macrosperma), they had taken a similar performance trend and clustered into one group, branches and nodes from its created trees two watering treatments for each cultivar.

Fill colored cell indicated significant at 5 % level of significance (p <0.05); Blank cell with dashed (⁄) value its Indicated non-significant (ns) relationship.

On the other side, the hieratical dendrogram illustrated the relation with different traits attributes, where this relation seemed to be identical between number of branches and plant fresh weight per plant. At the same time, it was closely related between stomatal width in lower surface leaflet and plant height, both seeds numbers and pods numbers per plant were showed similarity too, and stomatal width associated with its length in upper leaflet. However, the obtained data cleared and figure visualized by color key, which seems to distinguish different effects by various number of variables for studied traits. Whereas, red color remarkably positively effects, the blue color indicated as negatively associated .Thus, the results showed that Giza-370 under normal condition closely related and assertive with number branches and plant fresh weight during vegetative growth plant, and stomatal characters such as stomatal length in lower side related positively with sinai1 in normal condition. According to biochemical traits proline content showed increasing of Sinai1 that it may be indicator for resistance stress while protein contents decreased for Giza-29 under drought stress.

The determination of narrow stomatal width in the upper leaflet could be regarding for inhibited of the plant transpiration and that mechanism helpful for plant protect under drought condition, it was observed of Giza-51 as an unique negatively effects that Generally, the relation within groups of different studied traits for the stomatal length of lower leaflet and harvest index % showed that closely related between them. In addition to, numbers of stomatal of lower surface leaflet were matched with seed yield per plant and seed protein content. That indicates different stomatal structures related to other characteristics for yield components and vegetative characters of studied lentil cultivars under different watering treatments.

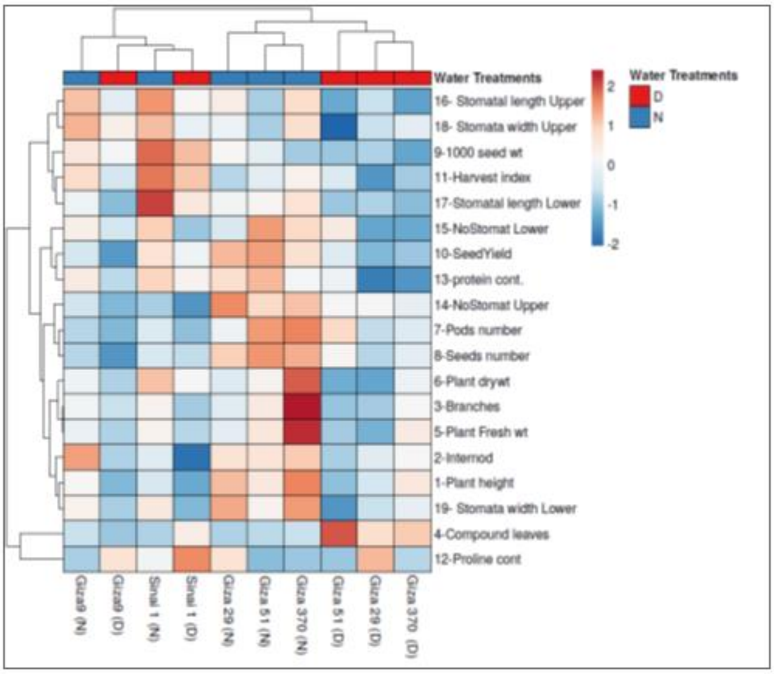

Fig.6. Visualized heat map based on Euclidean distance elucidate different effects and interrelationships of all studied traits by mean performances of 5 lentil cultivars under two water levels in 2nd season.

CONCLUSION

The drought indices are relevant factors to identify lentil cultivars with low yield and tolerance to drought stress. Number of stomata showed significant differences of all studied cultivars and stomatal width was more affected than length due to stomatal closure as a way to reduce water loss under drought. Giza-51 and Sinai 1 were more drought tolerance than other cultivars according to tolerance indices and proline content.

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تقييم الصفات المورفولوجية والقدرة المحصولية لبعض أصناف العدس المصري تحت ظروف الري العادي والإجهاد المائي

سمير ربيع السيد أبوحجازي[1]، هند محمد عبد الغنى فرج[2] و ابراهيم حسن يعقوب[1]
[1]قسم المحاصيل – كلية الزراعة – جامعة القاهرة – الجيزة – مصر
[2]قسم النبات الزراعى- كلية الزراعة - جامعة القاهرة– الجيزة – مصر

أجريت تجربتان حقليتان خلال موسمي 19/2018 و2020/2019 بمحطة التجارب والبحوث الزراعية، كلية الزراعة، جامعة القاهرة. لتقييم أداء خمسة أصناف مصرية من العدس (جيزة 9، جيزة 29، جيزة 51، جيزة 370 وسيناء1) تحت ظروف مستويين من المعاملات المائية (العادية والجفاف) (60 و30 ٪ من السعة الحقلية). ولقد نفذت كل تجربة بإستخدام تصميم القطع المنشقة مرة واحدة تحت نظام القطاعات الكاملة العشوائية فى ثلاثة مكررات، حيث تم توزيع معاملات الري عشوائياً فى القطع الرئيسية، والأصناف فى القطع المنشقة. أظهرت نتائج تحليل التباين التجميعي للموسمين تباينات عالية المعنوية لكل من معاملات الري والأصناف لجميع الصفات. أظهر الصنف جيزة 51 أعلى قيمة لمحصول البذور/النبات فى الظروف العادية، بينما أظهر سيناء 1 إنخفاضاً طفيفاً فى محصول البذور/النبات مع زيادة برولين البذور تحت ظروف الإجهاد، والذى قد يكون مؤشراً على تحمل الجفاف. واستخدمت الدراسة ستة من أدلة تحمل الجفاف "النسبة المئوية لنقص المحصول، دليل تحمل الجفاف، المتوسط الهندسي للإنتاجية، معامل تحمل الجفاف، المتوسط التوافقى، ودليل الحساسية للجفاف" للكشف عن استجابات الأصناف لتأثير الجفاف. وأظهرت صفة محصول البذور إرتباطاً وراثياً سالباً لكل من نسبة انخفاض المحصول، دليل تحمل الجفاف ودليل الحساسية للجفاف تحت ظروف الإجهاد، لذلك فإن هذه الأدلة مناسبة لتحديد الأصناف ذات المحصول المنخفض وتتحمل الإجهاد المائي. كما أظهرت صفة عدد الثغور فروق معنوية لجميع الأصناف. أيضًا اظهرت صفة عرض الثغور اختلافات معنوية مقارنة بصغة الطول بسبب إغلاقها كوسيلة لتقليل فقد الماء في ظل الجفاف. وبشكل عام كان الصنفين جيزة 51 وسيناء1 الأكثر تحملاً للجفاف. وبالتالى يمكن لمربي النبات الإنتخاب المباشر لمحصول البذور المرتفع تحت ظروف نقص المياة بالإعتماد على أدلة الجفاف، محتوى البرولين وعدد الثغور.

الكلمات الداله: الجفاف - العدس - أصناف - برولين - الثغور