Egypt. J. Bot. **Vol. 63,** No. 3, pp. 1141-1154 (2023)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Correlation between Genetic- physiological Traits of Egyptian Lupin (*Lupinus termis*) Induced by Drought Stress

Hoida Zaki#, Noha A. El-Tayeh, Fayrouz Abo El Hagag, Nora H. Youssef
Botany and Microbiology Department, Faculty of Science, South Valley University, 83523, Qena, Egypt.

THIS study applied SRAP (sequence-related amplified polymorphisms) markers, alterations in some growth, and physiological parameters to study how the *Lupinus termis* plant responded to water deficit. Under drought stress, lupin plants showed decreased growth criterion, photosynthetic pigments, and various elements, including Ca^{2+}, $Mg^{2+,}$ and K^{+}. While raised levels of total free amino acids, soluble carbohydrates, soluble proteins, malondialdehyde, proline, antioxidant enzymes, and Na^{+} amount in the experimental plants. Using 12 SRAP primers, lupin under drought stress appeared 13.44% polymorphic, 75.63% monomorphic, and 10.92% unique bands. The band's number ranged from four to fifteen bands. The UPMGA dendrogram of SRAP separated the lupin plants under drought into two main clusters. The first one contained two sub-clusters; the first included plants exposed to 40% of field capacity, while the second sub-cluster included control and plants exposed to 80% of field capacity. The second cluster included plants exposed to 60% of field capacity. In this sense, these findings can enhance drought monitoring and assessment by establishing accurate correlations between genetic-physiological features and the degree of the drought.

Keywords: Antioxidant enzymes, Drought, *Lupinus*, SRAP marker.

Introduction

Lupin, a plant with high levels of protein and fiber, has been discovered to contain a variety of phytochemicals, particularly bioactive compounds. Due to the substantial amounts of many phytochemicals, lupin is a potential nutritional ingredient, especially for baked products. (Khan et al., 2015). For their primary nutrition during the ancient age, the entire human population depended on the three main crops wheat, rice, and maize (Khan et al., 2015). Scientists are under pressure to discover new and sustainable forms of nourishment due to the widespread prevalence of hunger and poor health throughout the world, caused by factors such as the world's expanding population, the effects of climate change, and the decline in the availability of fertile land for development (Small, 2012). Lupin can considerably increase the number of available food sources because it can thrive on marginal agricultural sites and in various environmental settings (Nelson & Hawthorne, 2000).

Abiotic stress refers to environmental factors that prevent plants from growing and yielding at their maximum potential (Skirycz & Inze, 2010). More than 70 percent of the world's freshwater is used in agriculture, and this sector is facing a severe water supply crisis due to the ongoing effects of climate change and the rapid increase in the world's population (FAO, 2020). Water is a vital constituent of many physiological processes, including many parts of plant metabolism and development, and makes up between 80 and 95 percent of the fresh weight of the plant (Abbasi & Abbasi, 2010; Brodersen et al., 2019). Under drought stress, various ecological, morphological, physiological, biochemical, and molecular features and plant processes are affected (Ortiz et al., 2015; Badran, 2022; Gaafar et al., 2022; Loutfy et al., 2022).

Plants may adapt to different levels of water deficiency despite its adverse impact on their performance. Since cell division is less influenced by water deficits than cell expansion, having a water supply is directly correlated to plant development

#Corresponding author email: hoaida.zaki@sci.svu.edu.eg Orcid: https://orcid.org/0000-0002-0718-7838
Received 03/03/ 2023; Accepted 11/08/ 2023
DOI: 10.21608/ejbo.2023.191189.2245
Edited by: Prof. Dr. Abdelfattah Badr, Faculty of Science, Helwan University, Cairo, Egypt.

(Humplík et al., 2017). Due to the reduction of cell wall elongation and turgor under these situations, plant development is impeded (Seleiman et al., 2019). Depending on the surrounding environment, stress drought can occur at any stage of plant growth. Certain genotypes may be tolerant of dryness during germination or seedling but highly vulnerable during flowering, or vice versa, hence testing for drought resistance at crucial and often divergent growth phases is possible (Sallam et al., 2019; Moursi et al., 2020). One way to classify plants as drought-tolerant is to locate a characteristic that can quantify how they respond to drought stress.

Molecular marker techniques based on sequencing technology have replaced previous marking technologies. Sequence-related amplified polymorphisms (SRAP) are a type of PCR-based marker with high repeatability, simplicity, straightforward separation and sequencing, and straightforward cloning of target fragments. SRAP markers are frequently employed in plant genetic diversity identification and genetic map development **(**Khaled et al., 2019a; El-Sherbeny et al., 2020; Qiao et al., 2022). Genetic diversity using SRAP markers was used among lupin genotypes (El-harty et al., 2016) and many other crop plants like soybean genotypes (Sun et al., 2013; El-Sherbeny et al., 2020), Wheat (Khaled et al., 2019b), Sorghum (Khaled et al., 2019a), and Cucurbita plant (Ferriol et al., 2003; Inan et al., 2012). This study attempts to look into how the *Lupinus termis* plant responds physiologically and molecularly to drought stress using SRAP marker, growth criteria, and some physiological parameters.

Materials and Methods

Preparation of plant for experimentation

Seeds of the *Lupinus termis* plant (cultivar Giza-1) were obtained from Agricultural Research Center, Giza, Egypt. Seeds that were considered healthy underwent a 5min surface sterilization process with 70% ethyl alcohol, followed by multiple washings in distilled water. The next step was soaking some lupin seeds in pure water for three hours. At the experimental farm of the Botany & Microbiology Department, Faculty of Science at Qena, South Valley University, the seeds of the experimental plant were grown in plastic pots filled with air-dried soil (sand/clay =2/1 by V/V; 3kg/pot). Used soil was tested to see how much of an increase in field capacity they could expect from oven-dry soil to field conditions, and they saw roughly 13%. Six to eight seeds were planted in each container, and daily irrigation with running water ensured full germination, yielding a maximum of five plants per container. After three weeks, the pots were grown under different water irrigation conditions (control, 80%, 60%, and 40% of field capacity). Plants were harvested at 50 days old, the experimental plants' yield was harvested at 87 days, and the yielded seeds were analyzed. The experimentation was carried out during the vegetative stage and the yielded seeds, and after plant harvesting, the following parameters were estimated:

Growth criteria

At the end of the experimental period, the shoot and root were separated. Fresh and dry matter yields of different organs (root and shoot) were estimated. The fresh root and fresh shoot were dried in an aerated oven at 70°C for 24h until the weight remained constant to calculate the dry matter yield. Also, in this study, the water content of different parts was calculated.

Photosynthetic pigments

The photosynthetic pigments in fresh leaves (carotenoids, chlorophyll a, and chlorophyll b) were measured spectrophotometrically (Lichtenthaler & Wellburn, 1983). For the measurements of carotenoids, chlorophyll a, and chlorophyll b concentrations, pigment extraction was contrasted with a blank sample of 80% acetone at 452.5, 663, and 644nm, respectively.

Physiological parameters

The adapted method by Badour (1959) using anthrone sulfuric acid was used to estimate soluble sugar content (SS). Using Lowery's technique (1951), we estimated soluble proteins (SP) in the ground tissue. The Moore & Stein method (1948) was used to extract total free amino acids (TAA) from plant tissues and determine their concentrations. According to Bates et al. (1973), proline content was determined. Malondialdehyde (MDA) content was utilized as a marker of lipid peroxidation using the Heath & Paker method (1968).

Antioxidant enzyme activities

The method of Aebi (1984) was used to measure the activity of Catalase (CAT; EC 1.11.1.6). According to the procedure outlined by Chance & Maehly (1955), peroxidase (POD; EC 1.11.1.7)

activity was estimated. The method of Chen & Asada (1992) was used to evaluate the activity of ascorbate peroxidase (APX; EC 1.11.1.11).

Determination of water-soluble ions

According to Williams & Twine (1960), flame photometry was used to determine the amounts of sodium and potassium (410CORNING flame photometer was used). As reported by Estefan et al. (2013), the versine titration method was used to determine the volumetric amounts of calcium and magnesium.

Molecular marker technique

SRAP-PCR reactions

Following the CTAB technique (Murray & Thompson, 1980) DNA extraction from young leaves of treated plants, the total DNA was purified, measured spectrophotometrically using a Nanodrop 2000 (Thermo Scientific), diluted to 25ng/lL, and kept at -20°C. A set of twelve SRAP primers was used in the polymorphism detection (Table 1). The reaction of amplification was performed in 25 µl reaction volume containing 2.5µL template DNA (10ng), 1.5µL forward primer, 1.5µL reverse primer (10pcmol), 12.5µL Master Mix (0), and seven µL dH_2O.

An amplification cycle of 3 minutes at 94 degrees Celsius for denaturation and five cycles at 94 degrees Celsius for 40 seconds, 38 degrees Celsius for 50 seconds, and 72 degrees Celsius for 1min for denaturing, annealing, and extension were carried out using a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) thermal cycler. It was denaturing at 94 degrees Celsius for 1 minute, annealing at 50 degrees Celsius for 1 minute, and stretching at 72 degrees Celsius for 1.5 minutes throughout 35 cycles. The final cycle included a primer extension phase that lasted 7 minutes at 72 degrees Celsius. Electrophoresis on a 1.5% agarose gel stained with ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts separated the amplification products. Using a Gel Documentation System, PCR products were viewed under UV light and photographed (BIO-RAD 2000).

Data analysis

To compare the average of the analyzed traits, one-way ANOVA was used. When comparing treatments, differences were highlighted using the Duncan multiple range test as a post hoc analysis. The data was analyzed using the statistical program "SPSS for Windows version 20". The banding patterns produced by SRAP marker analyses were contrasted to evaluate how genetically related the study samples were. Amplification products that were clear and distinct received a score of "1" for the presence of bands and "0" for the absence of bands. Bands with the same mobility received the same score. The UPGMA formula was used to determine the sample-to-sample coefficients of Dice›s similarity matrix. With this matrix and the PAST ver. 1.91 program, the Euclidean similarity index was calculated and produced a dendrogram. (Hammer et al., 2001).

Results

Effect of drought stress on growth criteria and physiological traits

The data in Fig. 1 demonstrated that plants' shoot and root lengths decreased significantly by increasing field capacity percentages compared with the full field capacity (100%). This reduction became impressive at a higher level. The data in Fig. 2 revealed that by decreasing the percentage of water field capacity fresh, dry weight, and water content in shoots and roots of *Lupinus terms* plants reduced markedly, principally under the highest drought stress (60% and 40%) of field capacity as compared to the control plant.

The results in Fig. 3 showed that (Chl. a, Chl. b, carotenoids, and total Chlorophyll) were retarded markedly under the higher treatments (60% and 40%) as compared with the unstressed control lupin plant. The highest mean value of Chl. a, Chl. b, and carotenoid reached 0.165, 0.189, and 0.149 mg g^{-1} f. wt at 80 % field capacity, respectively. In contrast, the lowest mean value of Chl. a, Chl. b, and carotenoid were 0.107, 0.079, and 0.137mg g^{-1} f. wt at 40% field capacity, respectively.

Shoot soluble sugars (SS), soluble protein (SP), and total free amino acid (TAA) levels were shown to increase dramatically (Fig. 4) across all drought stress treatments compared to control plants. While in the root, soluble sugars increased up to a level of 80% of field capacity (185.249mg g^{-1} d.wt) and then tended to decrease highly significantly under percentages (60% and 40%) as compared with the reference control (155.236mg g^{-1} d.wt). Soluble protein content in the root increased under various levels of drought stress as compared with the reference control. In the case of TAA in the root, there was increasing content with increasing water deficiency.

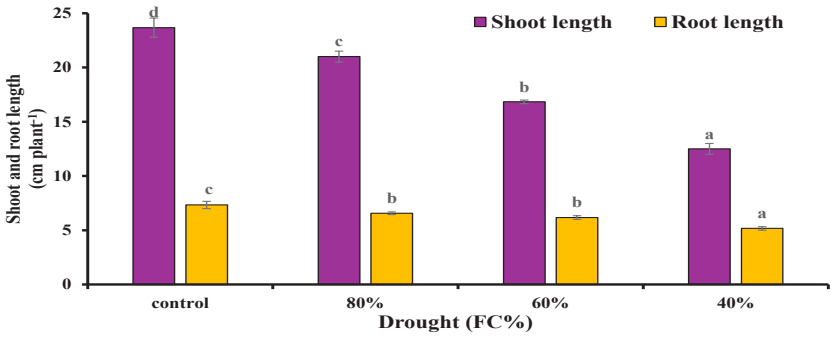

Fig. 1. Shoot length and root length (cm plant^{-1}) of *Lupinus termis* treated with 0, 80%, 60%, and 40% field capacity (FC) [Values are the mean of three replicates. (n= 3) ± Standard error (SE). In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

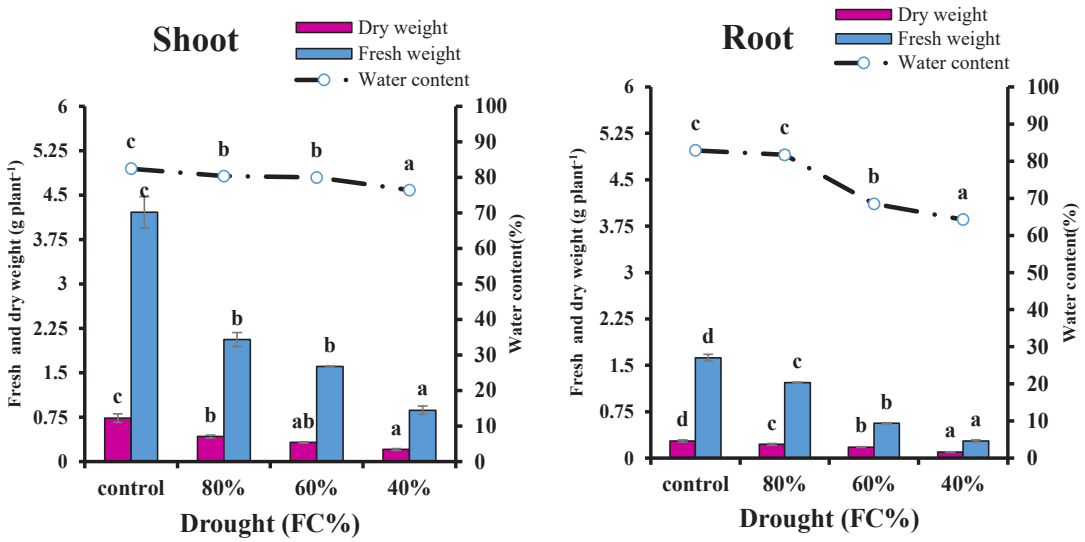

Fig. 2. Fresh and Dry weight (g plant^{-1}), and Water content (%) in the shoots and the roots of *Lupinus termis* treated with 0, 80%, 60%, and 40% field capacity (FC) [Values are the mean of three replicates. (n = 3) ± Standard error (SE). In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

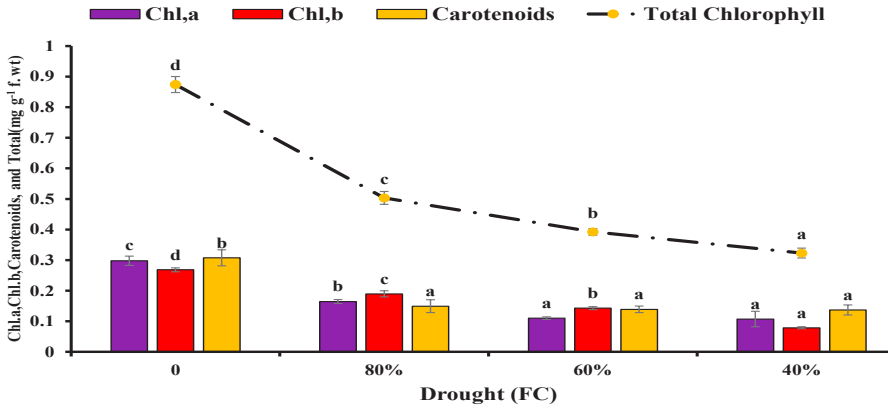

Fig. 3. Concentration of Chlorophyl (Chl) a, Chlorophyl b, Carotenoids, and Total chlorophyl content (mg g^{-1}d. wt) of *Lupinus termis* treated with 0, 80%, 60%, and 40% field capacity (FC) [Values are the mean of three replicates. (n = 3) ± Standard error (SE). In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

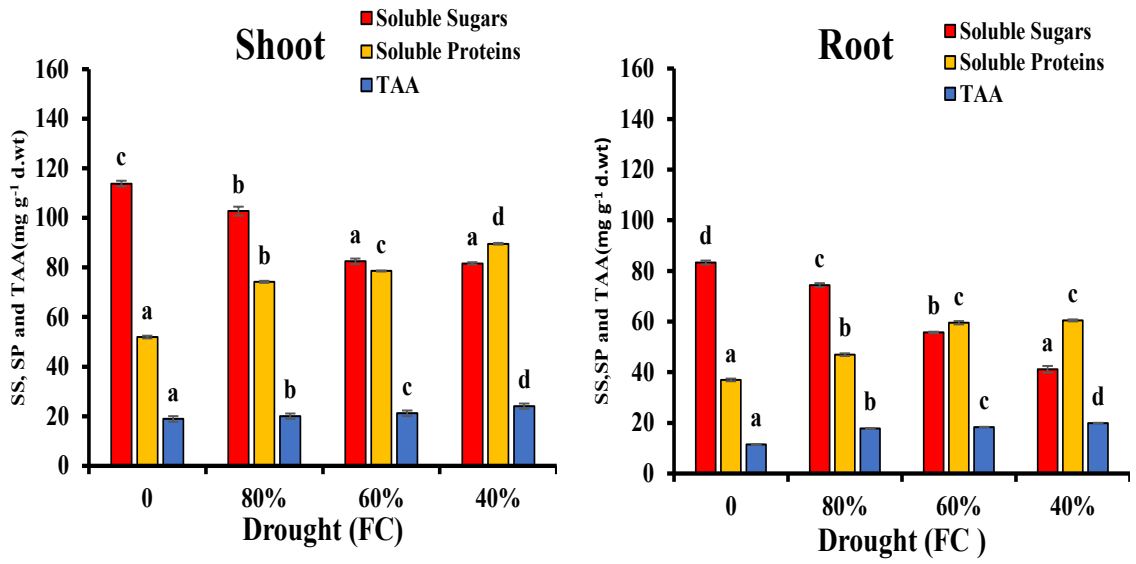

Fig. 4. Soluble sugars (SS), Soluble protein (SP), and Total free amino acids (TAA) contents in the shoots and the roots of *Lupinus termis* treated with 0, 80%, 60%, and 40% field capacity (FC) [Values are mean of three replicates. In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

Proline and malondialdehyde (MDA)

The data in Fig. 5 revealed that the proline content accumulated significantly under different levels of water deficit, whether in each shoot or root; moreover, this accumulation was more than twice that of the absolute control plant at the level of 80% F.C. in the shoot. Also, MDA was significantly increased by increasing drought stress in the shoot and root compared with the absolute control plant. The highest mean value of proline content reached 4.89mg g^{-1} dry matter in 40% of field capacity for shoot compared with absolute control 1.072mg g^{-1} dry matter. The highest mean value of MDA was 0.915mg g^{-1} f. wt for root in 40% of field capacity compared with absolute control 0.398mg g^{-1} f. wt.

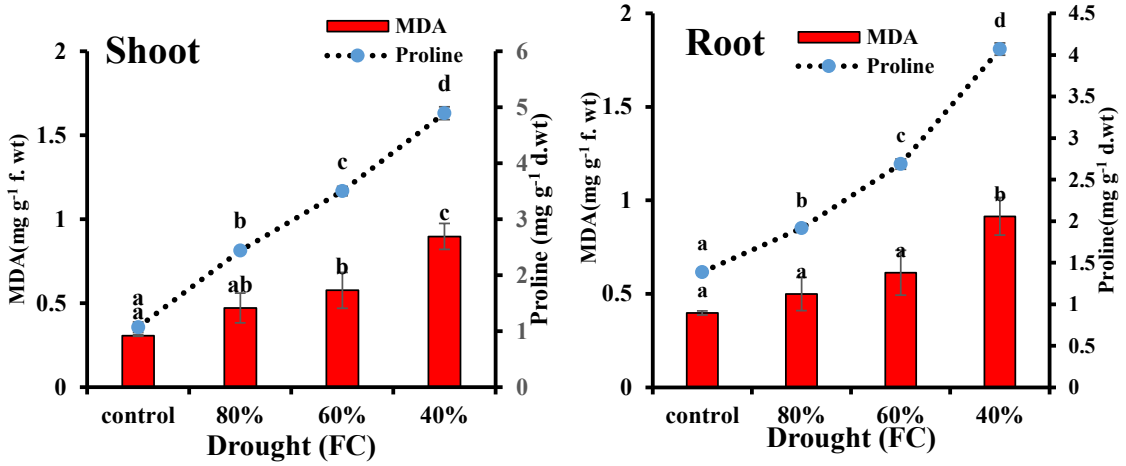

Fig. 5. Contents of Proline (Pr.) in the shoots and the roots of *Lupinus termis* plants (mg g^{-1} d.wt) and malondialdehyde (MDA) in the shoots (mg g^{-1} f.wt) of *Lupinus termis* plants treated with 0, 80%, 60%, and 40% field capacity (FC) stress [Values are the mean of three replicates. (n= 3) ± Standard error (SE). In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

Antioxidant enzymes activity

Figure 6 data illustrate that the activity of the CAT enzyme increased by increasing the water deficit compared to the reference control. Where the highest mean value of CAT enzyme reached 2.63-unit $min^{-1}gm^{-1}$ fresh matter at 40% of field capacity, and the lowest mean value of CAT enzyme was 1.567-unit $min^{-1}gm^{-1}$ fresh matter at 80% of field capacity as compared with absolute control 0.933unit $min^{-1}gm^{-1}$ fresh matter.

The results of PODs enzyme activity revealed a drought stress-induced increase in the PODs enzyme activity. Where the highest mean value of PODs enzyme reached (2.9-unit $min^{-1}gm^{-1}$ fresh matter) at 40% of field capacity, and the lowest mean value of PODs enzyme was 1.767-unit $min^{-1}gm^{-1}$ fresh matter at 80% of field capacity as compared with absolute control 1.2-unit $min^{-1}gm^{-1}$ fresh matter. Also, the behavior of the APX enzyme was the same as the last enzyme activity. In contrast, there was a drought stress-induced sharp increase in the APX enzyme activity, Where the highest mean value of APX enzyme reached 3.267-unit $min^{-1}gm^{-1}$ fresh matter at 40% of field capacity, and the lowest mean value of APX enzyme was 1.867-unit $min^{-1}gm^{-1}$ fresh matter at 80% of field capacity as compared with absolute control 1.167-unit $min^{-1}gm^{-1}$ fresh matter.

Mineral composition

Considering the role of the mineral elements in plants' metabolism and growth activities, it was intended to examine the relationship between salinity and water shortage on the concentrations of four different minerals (potassium K^+, sodium Na^+, calcium Ca^{2+}, and magnesium Mg^{2+}). The data in Fig. 7 illustrates that the sodium concentration increased with the rise of drought levels. The shoot had a larger boost than the root did. While the potassium content of the two main organs of lupin was retarded significantly with the rise of drought levels. Data from Fig. 8 concerning calcium and magnesium contents in shoots and roots revealed that by increasing the level of drought, the contents decreased significantly, especially at the highest levels (60% and 40%).

Molecular marker results

Different profiles were obtained when 12 primers of SRAP were used for genomic DNA amplification of the Lupinus termis cultivar (Giza 1) (Fig. 9). Using these primers, lupin under drought resulted in the appearance of 16 polymorphic out of 119 fragments (13.44%), 90 monomorphic (75.63%), and 13 unique bands (10.92%) (Table 1). The bands number ranged from four using SRAP 4 to fifteen bands using SRAP 7. The highest number of bands was detected when SRAP 7 primer was used. In addition, a unique band was detected when some primers were used. On the other side, unique bands were not detected when several primers were used, such as SRAP 4, SRAP 7, SRAP 9, SRAP10, and SRAP 11. Furthermore, only one primer SRAP 3 expressed five unique bands. One primer showed 88 % polymorphism; it was SRAP 3. The percentage of polymorphism ranged from 0 % when SRAP 1 and SRAP 2 were used to 88 % using SRAP 3. Primer SRAP 8 and primer SRAP 12 gave 38 % polymorphism percentage. The size of the obtained fragment ranged from 150 to 1700 bp. The largest amplified fragment was obtained when SRAP 9 primer was used. On the other hand, the smallest amplified fragment was obtained when the SRAP 4 primer was applied. The polymorphism average of all used primers was 25 % (Table 1). Cluster analysis using the UPGMA method was used to examine the relationship between genetic similarity coefficients and drought stress in *Lupinus termis* plants. The dendrogram of SRAP (Fig. 10) indicated that Lupin plants under drought expressed different patterns with two main clusters. The 1st cluster contained two sub-clusters; the first one included plants exposed to 40% of field capacity, but the second sub-cluster included control and plants exposed to 80% of field capacity. The second cluster included plants exposed to 60% of field capacity.

Discussion

This study assessed the physiological indices chlorophyll, FWT, DWT, SS, SP, TAA, proline, MDA, antioxidant enzymes, and certain minerals to understand physiological changes that occur in lupin plants in response to drought stress. All of these physiological parameters are directly correlated with drought stress; according to earlier research Abid et al. (2018) employed some of these variables to investigate the drought stress on wheat cultivars.

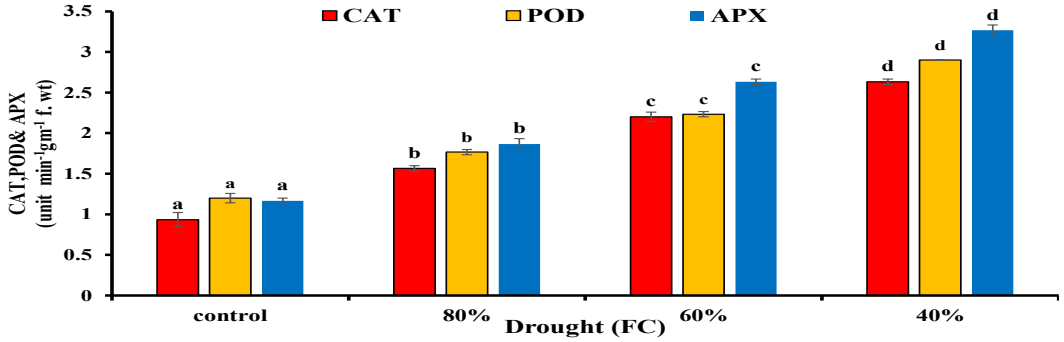

Fig. 6. Antioxidant (catalase (CAT), peroxidase (PODs), and ascorbate peroxidase (APX)) enzymes (unit min^{-1} gm^{-1} f.wt) content of shoots of *Lupinus termis* treated with 0, 80%, 60%, and 40% field capacity (FC) tress [Values are the mean of three replicates. (n = 3) ± Standard error (SE). In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

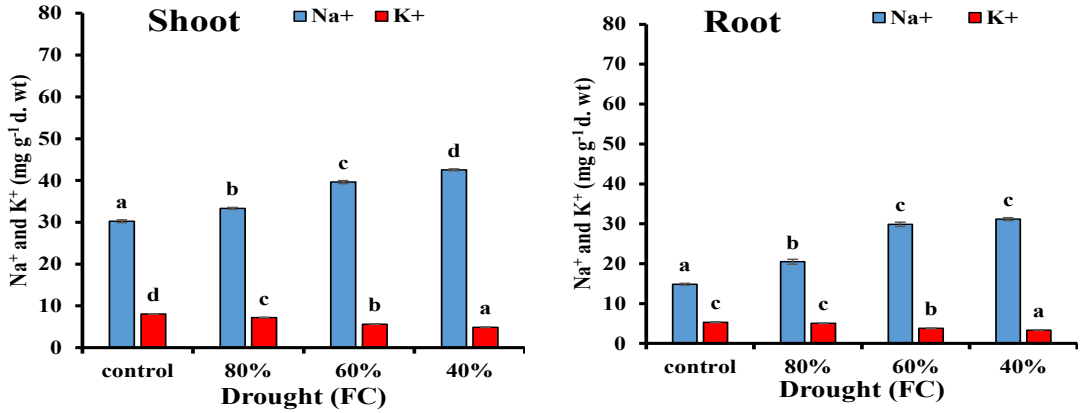

Fig. 7. Sodium (Na^+) and potassium (K^+) content (mg g^{-1}d.wt) in the shoots and the roots of *Lupinus termis* treated with 0, 80%, 60%, and 40% field capacity (FC) stress [Values are the mean of three replicates. (n = 3) ± Standard error (SE). In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

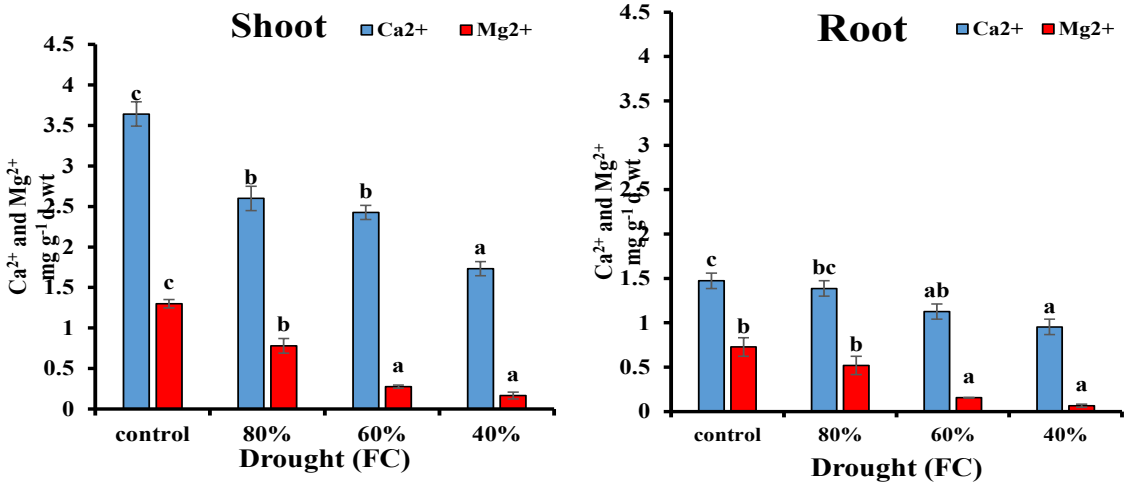

Fig. 8. Calcium and magnesium content (mg g^{-1}d.wt) in the shoots and the roots of *Lupinus termis* treated with 0, 80%, 60%, and 40% field capacity (FC) [Values are the mean of three replicates. (n = 3) ± Standard error (SE). In each panel: for comparison between drought levels, values with different letters are significantly different, (P<0.05) according to Duncan's test]

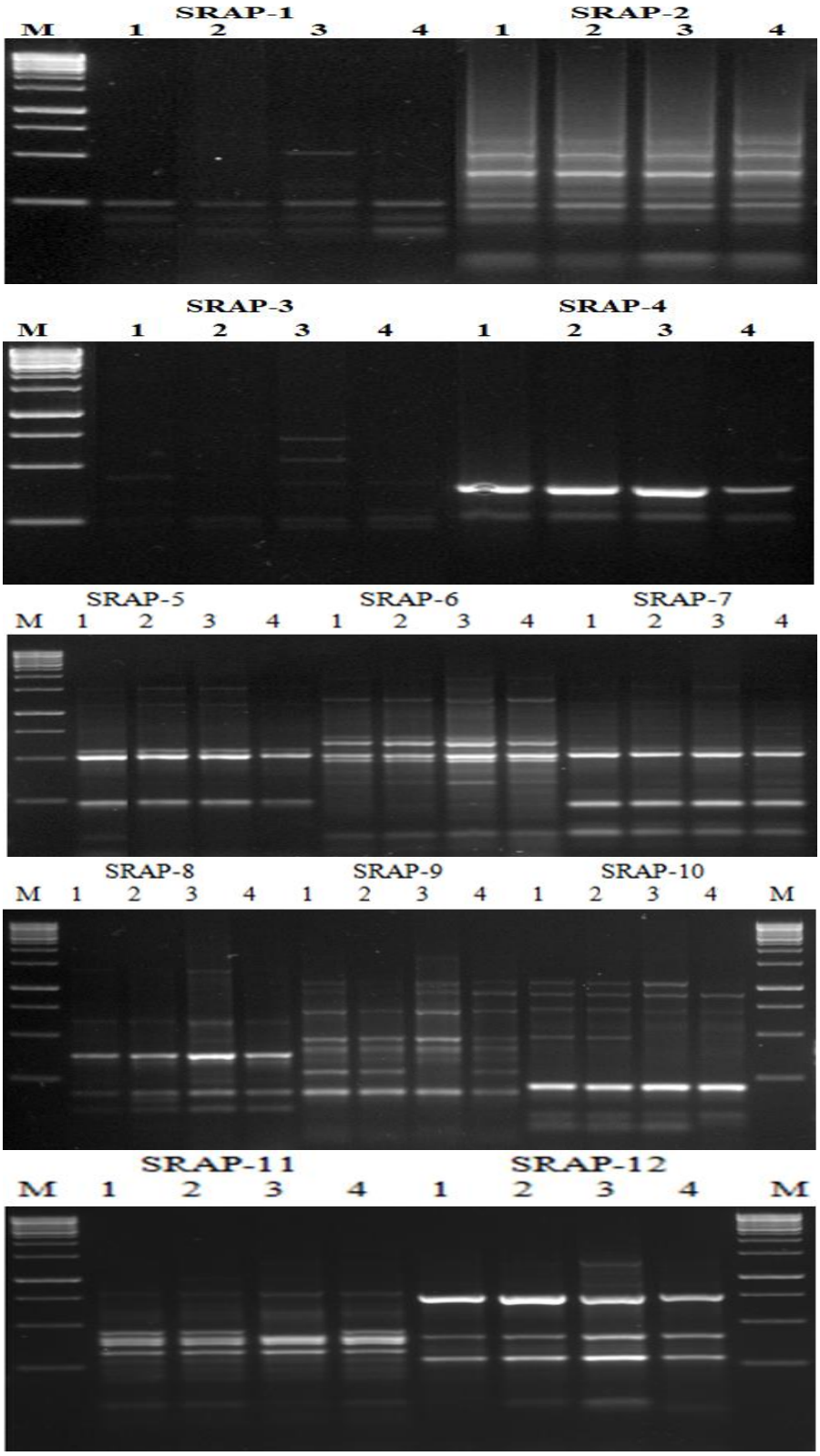

Fig. 9. SRAP profile generated by 12 primers using leaves of *Lupinus termis* plants undergoing drought levels [*Lane M: DNA ladder, lane 1: control, lane 2: L. termis leaves treated with 80% of field capacity, lane 3: L. termis* leaves treated with 60% field capacity (FC), lane 4: *L. termis* leaves treated with 40% FC]

TABLE 1. Twelve SRAP primers, their sequences, size of amplified fragments (bp), the total number of amplified fragments, number of polymorphic bands, and unique bands identified per primer of *Lupinus termis* Giza1 cultivar under the influence of drought stress

Primer	Forward primer	Reverse primer	Polymorphic bands	Monomorphic bands	Unique bands	Total bands	Size range (bp)	Polymorphism (%)
SRAP-1	me1- 5′-TGAGTCCAAACCGGATA-3′	em1- 5′-GACTGCGTACGAATTAAT-3	**0**	**4**	**1**	**5**	200-600	0
SRAP-2	me1- 5′-TGAGTCCAAACCGGATA-3′	em2- 5′-GACTGCGTACGAATTTGC-3′	**0**	**11**	**1**	**12**	160-1000	0
SRAP-3	me1- 5′-TGAGTCCAAACCGGATA-3′	em3- 5′-GACTGCGTACGAATTGAC-3′	**2**	**1**	**5**	**8**	240-750	88
SRAP-4	me1- 5′-TGAGTCCAAACCGGATA-3′	em5- 5′-GACTGCGTACGAATTAAC-3′	**1**	**3**	**0**	**4**	280-400	25
SRAP-5	me2- 5′-TGAGTCCAAACCGGAGC-3′	em1- 5′-GACTGCGTACGAATTAAT-3	**1**	**9**	**1**	**11**	180-1500	18
SRAP-6	me2- 5′-TGAGTCCAAACCGGAGC-3′	em2- 5′-GACTGCGTACGAATTTGC-3′	**1**	**12**	**1**	**14**	170-1600	14
SRAP-7	me2- 5′-TGAGTCCAAACCGGAGC-3′	em5- 5′-GACTGCGTACGAATTAAC-3′	**1**	**14**	**0**	**15**	180-1500	7
SRAP-8	me3- 5′-TGAGTCCAAACCGGAAT-3′	em1- 5′-GACTGCGTACGAATTAAT-3	**1**	**5**	**2**	**8**	180-1400	38
SRAP-9	me3- 5′-TGAGTCCAAACCGGAAT-3′	em2- 5′-GACTGCGTACGAATTTGC-3′	**2**	**11**	**0**	**13**	150-1700	15
SRAP-10	me3- 5′-TGAGTCCAAACCGGAAT-3′	em5- 5′-GACTGCGTACGAATTAAC-3′	**3**	**6**	**0**	**9**	150-1050	33
SRAP-11	me4- 5′-TGAGTCCAAACCGGACC-3′	em1- 5′-GACTGCGTACGAATTAAT-3	**3**	**9**	**0**	**12**	150-800	25
SRAP-12	me4- 5′-TGAGTCCAAACCGGACC-3′	em2- 5′-GACTGCGTACGAATTTGC-3′	**1**	**5**	**2**	**8**	150-1400	38
Total			**16**	**90**	**13**	**119**		25

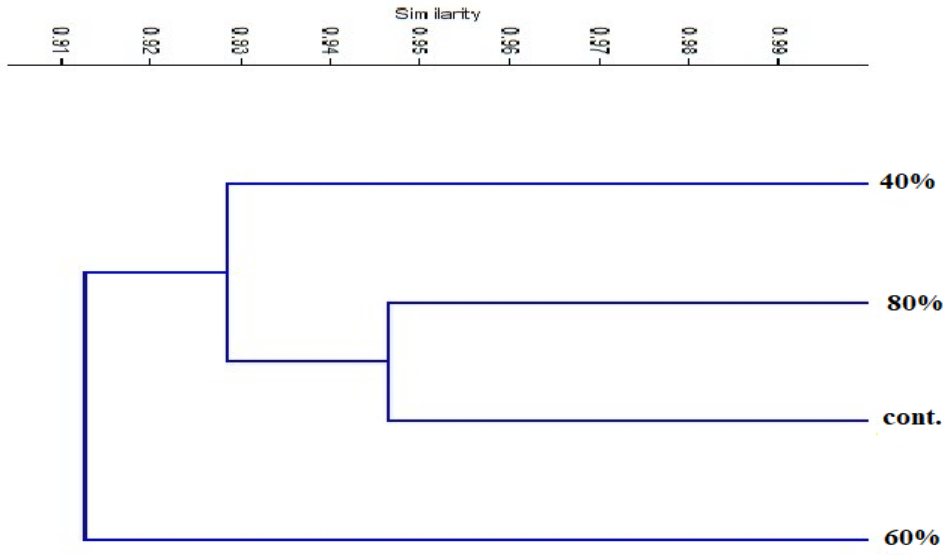

Fig. 10. Dendrogram for *Lupinus termis* plants exposed to field capacity (FC) levels (control, 80%, 60%, and 40% FC) constructed from the SRAPs data using unweighed pair-group arithmetic (UPGMA) and similarity matrices computed according to Dice coefficients

Plants will display various growth and survival mechanisms depending on the amount of evaporation or insufficient water supply (Radwan et al., 2020; Tambussi et al., 2007).

To clarify, legumes suffer when subjected to drought regarding total biomass, fruit number, weight, seed quality, and number, as well as seed yield per plant (Latef & Ahmad, 2015). The yield of soybeans was reduced by nearly 40% due to drought stress (Valentine et al., 2011). It has been said that *Vicia faba* and *Pisum sativum* are both sensitive to dry conditions, while *Lens culinaris* and *Cicer arietinum* were recorded as resistant plants to drought (Toker & Yadav, 2010).

Depending on the species or genotype under study, drought has varying effects on photosynthetic pigments. Reducing photosynthetic pigments is a major physiological aspect of drought stress (Reddy et al., 2004). Closing stomata, which in turn inhibits fixation of carbon dioxide (CO_2) due to damage to the photosynthetic machinery, ultimately, it is due to this that photosynthesis slows down in stressed plants (De Swaef & Steppe, 2010).

The most accurate indicator of a plant's resistance to dehydration is its relative water content (RWC). This shows metabolic activity in plant tissues and evaluates the plant's water status (Sallam et al., 2019). Some species of plants have been seen lowering their relative water content (RWC) in response to drought stress. (Allahverdiyev, 2015).

Under drought, some plants showed increased shoot proteins (Noman et al., 2018). The increased proteins in response to drought stress enable plants to adapt biochemically and structurally to the stress (Al-jebory, 2012). The presence of proline is one of the most prevalent drought-resistant crop features (Gurumurthy et al., 2019). The results given in the present work show that the *Lupinus termis* plant with different levels of field capacity caused an increase significantly in TAA. The increase in stress-related amino acids may result from an adaptive mechanism that aids in osmotic adaptation (Dubey & Singh, 1999).

Some crop plants accumulated more proline than others; in their study, Kaya et al. (2019) discovered that pepper plants' proline content rises in response to drought stress. When a plant undergoes drought, proline content can accumulate due to either inhibited biosynthesis or degradation (Szabados & Savoure, 2010). The drought stress effects on MDA accumulation in the shoots and roots of the experimental plants are consistent with those reported by Zahra et al. (2021) on *Silybum marianum* (L.) Gaertn. ecotypes. Inadequate activation of the antioxidant system is responsible for this trend (Hossain et al., 2013).

Cations (K^+, Ca^{2+}, and Mg^{2+}) crossing by active transport or the membrane's permeability is reduced by drought stress, which decreases the absorption of these elements through the root (Farooq et al., 2012). Due to the decreased transpiration flux, drought stress tends to reduce the calcium concentrations in the plant biomass (Sardans et al., 2008). Wheat (*Triticum sativum*) plants' calcium and potassium levels in their shoots and roots dropped equally when water was limited, as Noman et al. (2018) observed.

One well-known adaptation mechanism in some crop plants has been demonstrated to be the antioxidant enzyme production, such as CAT, POD, and APX, in response to water stress (Sallam et al., 2019). Under drought stress, the genotype and stage of plant development affect the expression pattern of some antioxidant enzymes such as CAT, APX, and SOD in *Hordeum vulgare* (barley). In drought-tolerant wheat, an appreciable change in the expression pattern of the genes encoding some antioxidant enzymes was noticed during drought stress (Sallam et al., 2019). As a result, plants stressed by drought tend to have higher activity levels in one or many antioxidant enzymes, which might work together to protect cells from damage. This increased activity is correlated with higher tolerance to drought.

The findings herein agree with those of El-Sherbeny et al. (2020), they used 15 pairs of SRAP primer combinations among *Glycine max* L. genotypes were grown under drought stress, 49 bands in total were obtained, 24 of which were polymorphic (48.98%). On the other hand, Khaled et al. (2019a) found 39 bands from 82 were polymorphic when using molecular markers (SRAP) for seven *Sorghum bicolor* genotypes. Also, Khaled et al. (2019b) obtained 64 polymorphic bands from a total of 95, with a total number of bands ranging from two to twelve. Their polymorphic bands ranged from 200 bp to 1750 bp, in the same range as the present study, whereas the size of the obtained fragment ranged from 150 to 1700 bp. Using 34 different SRAP primer combinations, Baloch et al. (2010) discovered that the number of polymorphic bands ranged from zero to three.

Conclusion

Our study indicated that the treated plants showed marked and various responses at different levels of drought stress via significant reduction of growth parameters, photosynthetic pigments, soluble carbohydrate content, potassium, calcium, and magnesium. At the same time, there is an increase in the plant's defense system (antioxidant enzymes), total free amino acids, carbohydrates, MDA, as well as the amount of sodium. These physiological changes were correlated with and confirmed using molecular markers (SRAP marker), which confirmed the results and clustered control with the treatment of 80% FC.

Conflict of interests: The authors confirm that there is no conflict of interest to disclose

Authors' contributions: Fayrouz Abo El Hagag and Nora H. Youssef conceived the presented idea and experimented. The analysis, manuscript draft, figure design, and writing were all done by Hoida Zaki and Noha A. El-Tayeh. The final manuscript has been read and approved by all authors.

Ethical approval: Not applicable.

References

Abbasi, T., Abbasi, S.A. (2010) Biomass energy and the environmental impacts associated with its production and utilization. *Renewable and Sustainable Energy Reviews*, **14**, 919–937.

Abid, M., Ali, S., Qi, L.K., Zahoor, R., Tian, Z., Jiang, D., et al. (2018) Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Scientific Reports,* **8**, 4615. https://doi.org/10.1038/s41598-018-21441-7

Aebi, H. (1984) Catalase in vitro. *Methods in Enzymology,* **105**, 121–126

Al-jebory, E.I. (2012) Effect of water stress on carbohydrate metabolism during *Pisum sativum* seedlings growth. *Euphrates Journal of Agricultural Science*, **4**, 1–12.

Allahverdiyev, T.I. (2015) Effect of drought stress on some physiological parameters, yield, yield components of durum (*Triticum durum* desf.) and bread (*Triticum aestivum* L.) wheat genotypes. *EKIN, Journal of Crop Breeding and Genetics*, **1**, 50–62.

Badran, A.E. (2022) Assessment of variation and

stability parameters of five quinoa genotypes under drought stress conditions. *Egyptian Journal of Botany,* **62**(1), 21-30.

Badour, S.S.A. (1959) Analytish-chemische unteruchung des Kaliummangles bei Chlorella im Vergleich mit andern Mangelezustanden *Ph.D. Dissertation,* Gottingen.

Baloch, F.S., Kurt, C., ArIoglu, H., Ozkan, H. (2010) Assaying of diversity among soybean (*Glycin max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) genotypes at DNA level. *Turkish Journal of Agriculture and Forestry,* **34**, 285-301.

Bates, L., Waldren, R., Teare, I. (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*, **39**, 205–207.

Brodersen, C.R., Roddy, A.B., Wason, J.W., McElrone, A.J. (2019) Functional status of xylem through time. *Annual Review of Plant Biology*, **70**, 407–433.

Chance, B., Maehly, A.C. (1955) Assay of catalases and peroxidases. *Methods in Enzymology,* **2**,764–775.

Chen, G.X., Asada, K. (1992) Inactivation of ascorbate peroxidase by thiols requires hydrogen peroxide. *Plant & Cell Physiology*, **33**, 117–123.

De Swaef, T., Steppe, K. (2010) Linking stem diameter variations to sap flow, turgor and water potential in tomato. *Functional Plant Biology*, **37**, 429.

Dubey, R.S., Singh, A.K. (1999) Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants. *Biologia Plantarum*, **42**, 233-239.

El-harty, E., Ashrie, A., Ammar, M., Alghamdi, S. (2016) Genetic Variation Among Egyptian White Lupin (*Lupinus Albus* L.) Genotypes. *Turkish Journal of Field Crops*, **21**(1), 148-155.

El-Sherbeny, G.A.R., Khaled, A.G.A., Morsy, A.R., Abotaleb, M.A.A. (2020) Genetic diversity of some soybean genotypes under drought stress using SRAP markers. *Journal of Sohag Agriscience (JSAS)*, **5**(1), 8-29.

Estefan, G., Sommer, R., Ryan, J. (2013) *"Methods of Soil, Plant, and Water Analysis*", A manual for the West Asia and North Africa Region; International Center for Agricultural Research in the Dry Areas (ICARDA): Aleppo, Syria.

Farooq, M., Hussain, M., Wahid, A., Siddique, K.H.M., (2012) "*Plant Responses to Drought Stress*", Springer: Berlin/Heidelberg, Germany, pp. 1–6.

Ferriol, M., Pico, B., Nuez, F. (2003) Genetic diversity of some accessions of *Cucurbita* maxima from Spain using RAPD and SRAP markers. *Genetic Resources and Crop Evolution,* **50**, 227-238.

FAO (2020) Food and Agriculture Organization of the United Nations. Overcoming water challenges in agriculture. In The State of Food and Agriculture 2020; FAO: Rome, Italy.

Gaafar, R., Seyam, M., El-Shanshory, A.R. (2022) Expression patterns of drought-related miRNAs in Chickpea (*Cicer arietinum* L.) under drought stress. *Egyptian Journal of Botany*, **62**(1), 227-240.

Gurumurthy, S., Sarkar, B., Vanaja, M., Lakshmi, J., Yadav, S.K., Maheswari, M. (2019) Morpho-physiological and biochemical changes in black gram (*Vigna mungo* L. Hepper) genotypes under drought stress at flowering stage. *Acta Physiologiae Plantarum*, **41-42**, 1-14.

Hammer, A.T., David, A.T.H., Paul, D.R. (2001) PAST: Palaeontological statistics software package for education and data analysis. *Palaeontologia Electronica*, **4**, 9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

Heath, R.L., Packer, L. (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives in Biochemistry and Biophysics*, **125**,189–198.

Hossain, M.A., Mostofa, M.G., Fujita, M. (2013) Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings. *Molecular Plant Breeding,* **4**(7), 50-70.

Humplík, J.F., Bergougnoux, V., Van Volkenburgh, E. (2017) To stimulate or inhibit? That is the question for the function of abscisic acid. *Trends in Plant Science*, **22**, 830–841.

Inan, N., Yildiz, M., Sensoy, S., Kafkas, S., Abak, K. (2012) Efficacy of ISSR and SRAP techniques for molecular characterization of some *Cucurbita* genotypes including naked (hull-less) seed

pumpkin. *The Journal of Animal & Plant Sciences*, **22**(1), 126-136.

Kaya, C., Ashraf, M., Wijaya, L., Ahmad, P. (2019) The putative role of endogenous nitric oxide in brassinosteroid-induced antioxidant defense system in pepper (*Capsicum annuum* L.) plants under water stress. *Plant Physiology and Biochemistry*, **143**, 119–128.

Khaled, A., El-Sherbeny, G., Abdelaziz, H. (2019a) SRAP and ISSR molecular markers-trait associations in sorghum genotypes. *Assiut Journal of Agricultural Sciences*, **50**(2), 159-175.

Khaled, A.G.A., El-Sherbeny, G.A.R., Elsayed, H.M.A. (2019b) Three SRAP molecular markers linked to yield component traits in wheat. *Asian Journal of Research and Review in Agriculture*, **1**(1), 25-35.

Khan, M.K., Karnpanit, W., Nasar-Abbas, S.M., Huma, Z.-e. Jayasena, V. (2015) Phytochemical composition and bioactivities of lupin: a review. *International Journal of Food Science & Technology*, **50**, 2004-2012.

Latef, A.A.H.A., Ahmad, P. (2015) Legumes and breeding under abiotic stress, In: "*Legumes under Environmental Stress*", M.M. Azooz and P. Ahmad (Eds.), pp. 1-20, https://doi.org/10.1002/9781118917091.ch1

Lichtenthaler, H., Wellburn, A. (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, **603**, 591-592.

Loutfy, N., Hassanein, A., Inouhe, M., Salem, J.M. (2022) Biological aspects and proline metabolism genes influenced by polyethylene glycol and salicylic acid in two wheat cultivars. *Egyptian Journal of Botany,* **62**(3), 671-685.

Lowery, O.H., Rasebrough, N.J., Farr, A.L, Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* **193**, 291–297.

Moore, S., Stein, W.W. (1948) Amino acid free photometric ninhydrin method for use in the chromatography of amino acids. *Journal of Biological Chemistry*, **176**, 367–388.

Moursi, Y.S., Thabet, S.G., Amro, A., Dawood, M.F.A., Baenziger, P.S., Sallam, A. (2020) Detailed Genetic Analysis for Identifying QTLs Associated with Drought Tolerance at Seed Germination and Seedling Stages in Barley. *Plants*, **9**, 1425. https://doi.org/10.3390/plants9111425

Murray, M., Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research,* **8**(19), 4321–4326.

Nelson, P., Hawthorne, W.A. (2000) Development of lupins as a crop in Australia. In: *"Linking Research and Marketing Opportunities for Pulses in the 21st Century*", R. Knight (Ed.), pp. 549– 559. Amsterdam, the Netherlands: Springer.

Noman, A., Ali, Q., Naseem, J., Javed, M.T., Kanwal, H., Islam, W., et al. (2018) Sugar beet extract acts as a natural bio-stimulant for physio-biochemical attributes in water stressed wheat (*Triticum aestivum* L.). *Acta Physiologiae Plantarum*, **40**, 110. https://doi.org/10.1007/s11738-018-2681-0.

Ortiz, N., Armada, E., Duque, E., Roldán, A., Azcón, R. (2015) Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. *Journal of Plant Physiology*, **174**, 87–96.

Qiao, Q., Ye, M., Wu, C., Wang, J., Liu, Q., Tao, J., Zhang, L., Feng, Z. (2022) Analysis of leaf morphology variation and genetic diversity via SRAP markers for near-threatened plant *Acer truncatum*. *Global Ecology and Conservation*, **33**, e01980.

Radwan, U., Radwan, T., Abouelkasim, E. (2020) Comparison of ecophysiological responses of *Acacia raddiana* and *Acacia nilotica* during seedling establishment in extreme arid conditions. *Egyptian Journal of Botany*, **60**(2), 593-603.

Reddy, A.R., Chaitanya, K.V., Vivekanandan, M. (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology,* **161**, 1189–1202.

Sallam, A., Alqudah, A.M., Dawood, M.F.A., Baenziger, P.S., Börner, A. (2019) Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. *International Journal of Molecular Sciences*, **20**, 3137. https://doi.org/10.3390/ijms20133137

Sardans, J., Peñuelas, J., Ogaya, R. (2008) Drought's impact on Ca, Fe, Mg, Mo and S concentration and accumulation patterns in the plants and soil of a Mediterranean evergreen Quercus ilex forest. *Biogeochemistry*, **87**, 49–69.

Seleiman, M.F., Refay, Y., Al-Suhaibani, N., Al-Ashkar, I., El-Hendawy, S., Hafez, E.M. (2019) Integrative effects of rice-straw biochar and silicon on oil and seed quality, yield and physiological traits of *Helianthus annuus* L. grown under water deficit stress. *Agronomy*, **9**, 637. https://doi.org/10.3390/agronomy9100637

Skirycz, A., Inze. D. (2010) More from less: plant growth under limited water. *Current Opinion in Biotechnology,* **21**, 197-203.

Small, E. (2012) 38. Lupins – benefit and harm potentials. *Biodiversity*, **13**, 54–64.

Sun, B., Fu, C., Yang, C., Ma, Q., Pan, D., Nian, H. (2013) Genetic diversity of wild soybeans from some regions of Southern China based on SSR and SRAP markers. *American Journal of Plant Sciences*, **4**, 257-268.

Szabados, L., Savoure, A. (2010) Proline: a multifunctional amino acid. *Trends in Plant Science*, **15**(2), 89–97.

Tambussi, E.A., Bort, J., Araus, J.L. (2007) Water use efficiency in C3 cereals under Mediterranean conditions: A review of physiological aspects. *Annals of Applied Biology*, **150**, 307-321.

Toker, C., Yadav, S.S. (2010) Legume cultivars for stress environments, In: "*Climate Change and Management of Cool Season Grain Legume Crops*", Yadav, S.S., McNeil, D.L., Redden, R., Patil, S.A. (Eds.), pp. 351–376. Springer, Dordrecht.

Valentine, A.J., Benedito, V.A., Kang, Y. (2011) Legume nitrogen fixation and soil abiotic stress: From physiology to genomic and beyond. *Annual Plant Reviews,* **42**, 207–248.

Williams, V., Twine, S. (1960) "*Flame Photometric Method for Sodium, Potassium and Calcium*", Springer International Publishing: Cham, Switzerland, Vol. 5, pp. 3–5.

Zahra, N., Wahid, A., Shaukat, K., Hafeez, M. B., Batool, A., Hasanuzzaman, M. (2021) Oxidative stress tolerance potential of milk thistle ecotypes after supplementation of different plant growth-promoting agents under salinity. *Plant Physiology and Biochemistry*, **166**, 53-65.

الارتباط بين الصفات الوراثية الفسيولوجية للترمس المصري الناجم عن إجهاد الجفاف

هويدا زكي، نهي احمد التايه، فيروز ابوالحجاج، نورا حسن يوسف
قسم النبات والميكروبيولوجي- كلية العلوم- جامعة جنوب الوادي- قنا - مصر.

في هذه الدراسة، تم استخدام المعلم الجزيئي SRAP وبعض المعاملات الفسيولوجية لدراسة كيفية استجابة نبات الترمس لإجهاد الجفاف. وتبين انه تحت إجهاد الجفاف، أظهرت نباتات الترمس انخفاضًا في معدل النمو وأصباغ التمثيل الضوئي ومجموعة من العناصر مثل البوتاسيوم والكالسيوم والماغنسيوم. بينما تم زيادة في مستويات الكربوهيدرات والبروتينات الذائبة والأحماض الأمينية الحرة وثنائي الدهيد المالون والبرولين والإنزيمات المضادة للأكسدة وكمية الصوديوم. وباستخدام 12 من البادئاتSRAP، نتج عن الترمس تحت ضغط الجفاف ظهور 13.44٪ متعدد الأشكال، 75.63٪ أحادي الشكل، و10.92٪ حزم فريدة. أظهر تحليل شجرة القرابة UPMGA الخاص بـ SRAP فصل نباتات الترمس تحت الجفاف إلى مجموعتين رئيسيتين. احتوت المجموعة الأولى على مجموعتين فرعيتين، الأولى تضمنت النباتات المعرضة لـ 40٪ من السعة الحقلية، ولكن المجموعة الفرعية الثانية تضمنت الكنترول والنباتات المعرضة لـ 80٪ من السعة الحقلية. المجموعة الثانية تضمنت نباتات معرضة لـ 60٪ من السعة الحقلية. وبهذا، يمكن لهذه النتائج أن تعزز مراقبة الجفاف وتقييمه من خلال إنشاء علاقات ارتباط دقيقة بين السمات الوراثية الفسيولوجية ودرجة الجفاف.