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Impact of licorice application on drought tolerance in maize (*zea mays* L.)

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ABSTRACT

Water deficit stress triggers complex physiological and biochemical retorts in plants. Different plant species have also involved numerous morphological, physiological, biochemical, cellular, and molecular mechanisms to overcome drought stress conditions. This experiment was conducted at the College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, Kurdistan region, Iraq, during April 2021 to study the effects of four treatments of licorice [T1: control with no licorice powder and extract; T2: licorice powder with soil; T3: licorice extract sprayed at 2 weeks after emergence and twice a week thereafter; and T4: licorice powder with soil, and licorice extract sprayed at 2 weeks after emergence and twice a week thereafter] on the vegetative parameters of six maize genotypes under water stress conditions. A factorial completely randomized design (CRD) with 3 replications was applied in this research. The results obtained indicated that, there was genetic variation among the genotypes in the response to water stress. The maximum shoot length, shoot dry weight, leaf area index, proline content, soluble sugar content, and total phenolic content were exhibited by genotype (PR36 BO8) with 49.462 cm, 5.244 g, 0.941, 2844.166 µg g^{-1}, 248.055 µg g^{-1}, and 174.681 µg g^{-1} respectively, while minimum shoot length, shoot fresh weight, shoot dry weight, root length, and root fresh weight were shown by genotype (ZP 434 XA) with 37.05 cm, 29.511 g, 3.357 g, 54.104 cm, and 17.493 g, respectively. This means, genotype (PR36 BO8) is more tolerant to water stress conditions compared to the genotype (ZP 434 XA) that is more susceptible. The second treatment (T2) had a more significant effect on most of the studied criteria compared to other treatments.

تأثير تطبيق عرق السوس على تحمل الجفاف في الذرة

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الخلاصة:
يؤدي الإجهاد الناجم عن نقص المياه إلى معوقات فسيولوجية وكيميائية حيوية معقدة في النباتات. كما طورت أنواع نباتية مختلفة العديد من الآليات المورفولوجية والفسيولوجية والكيميائية الحيوية والخلوية والجزيئية للتغلب على ظروف الإجهاد الناتج عن الجفاف.. أجريت هذه التجربة في كلية علوم الهندسة الزراعية ، جامعة السليمانية ، السليمانية ، إقليم كردستان العراق ، خلال نيسان 2021 لدراسة آثار أربعة معالجات لعرق السوس [(T1) : التحكم مع عدم وجود مسحوق عرق السوس ومستخلصه ؛ (T2) : اضافة مسحوق عرق السوس للتربة ؛ (T3) : مستخلص عرق السوس يرش بعد أسبوعين من ظهوره ومرتين في الأسبوع بعد ذلك ؛ و (T4) : اضافة مسحوق عرق السوس للتربة، وخلاصة عرق السوس رشها بعد أسبوعين من ظهورها ومرتين في الأسبوع بعد ذلك] على البارامترات الخضرية لستة طرز وراثية من الذرة تحت ظروف الإجهاد المائي. تم في هذا البحث تطبيق التصميم العاملي العشوائي الكامل (CRD) بثلاث مكررات. أشارت النتائج التي تم الحصول عليها إلى وجود تباين وراثي بين الطرز في الاستجابة للإجهاد المائي. تم عرض أقصى طول للنبتة ، الوزن الجاف للنبتة ، مؤشر مساحة الورقة ، محتوى البرولين ، محتوى السكر القابل للذوبان ، والمحتوى الفينولي الكلي عن طريق التركيب الوراثي (PR36 BO8) مع 49.462 سم ، 5.244 جم ، 0.941 ، 2844.166 ميكروجرام جم $^{-1}$ ، 248.055 ميكروجرام جم$^{-1}$ ، و 174.681 ميكروغرام جم$^{-1}$ على التوالي ، بينما تم توضيح الحد الأدنى لطول الجذع ، والوزن الطازج للنبتة ، والوزن الجاف للنبات ، وطول الجذر ، ووزن الجذر الطازج بواسطة التركيب الوراثي (ZP 434 XA) مع 37.05 سم ، 29.511 جم ، 3.357 جم ، 54.104 سم و 17.493 جم على التوالي. هذا يعني أن النمط الجيني (PR36 BO8) أكثر تحملاً لظروف الإجهاد المائي مقارنة بالنمط الجيني (ZP 434 XA)الأكثر حساسية. المعاملة الثانية (T2) كان لها تأثير معنوي أكبر على معظم المعايير المدروسة مقارنة بالمعالجات الأخرى.

الكلمات المفتاحية: الإجهاد المائي ؛ حبوب الذرة؛ نمو؛ معالجات كيميائية حيوية.

INTRODUCTION

Maize (*Zea mays* L.) or corn, is one of the most important crops in the world due to its variety, high adaptability, and excellent nutritional value, and is considered to be the third most important grain after wheat and rice. Around the world, maize accounts for 4.8% of the total acreage and is attributed to 3.5% of the world's crop value in agricultural production (Ahmed *et al.*, 2011; Deryng *et al.*, 2014). It is a high-yield crop with the highest rate of photosynthesis among all food crops, and as a C4 plant, it can accumulate dry matter faster than rice, wheat, or other grains (BAD, 2015).

Plants are constantly exposed to various environmental conditions, some of which may be abiotic stress factors, such as lack of available water, salt, excessive light, extreme heat or cold, and nutritional imbalance. These conditions may work at the same time or separately, and can have a significant impact on plant health (Verslues *et al.*, 2006). Plasticity and plant adaptability are related to the plant's potential to respond to abiotic stress (Chaves *et al.*, 2011). Drought (or lack of water) pressure is the fundamental constraint on agricultural production in many arid and semi-arid countries and has been studied in depth (Kabiri *et al.*, 2014). Insufficient precipitation, lower groundwater levels, or water retention by soil particles can cause water shortages for plants

(Salehi-Lisar and Bakhshayeshan-Agdam, 2016). When plants experience water stress, they adapt by changing their morphological, physiological, and biochemical characteristics (Chaves *et al.*, 2011).

Global climate change has significant implications for the environment and socio-economic development. The basic elements of agriculture (soil moisture, heat, sunlight) are affected by climate change as they lead to the occurrence of extreme climate events such as temperature fluctuations, rainfall fluctuations, and droughts (Xu *et al.*, 2017). Drought is one of the most serious natural disasters in the world, and its frequency and severity can be exacerbated in the coming years due to global warming (Ortega-Gómez, Pérez-Martín and Estrela, 2018). Drought is the most important factor limiting plant production in the world's agricultural sector (Sabadin *et al.*, 2012).

Licorice (*Glycyrrhiza glabra*) is one of the representative legumes, grown in many countries around the world, and contains over 100 different compounds. The most important of these are glycyrrhizin and phenolic compounds (Shabani *et al.*, 2009). In addition, it contains many minerals such as iron, potassium, and phosphorus, as well as sugars that are absorbed by the leaves during spraying and increase growth activity and, as a result, play an important role in increasing leaf growth (Laroche *et al.*, 2001). In addition, it contains magnesium, which has great effects on increasing cell division, leaf growth, and some biological plant activity (Moses *et al.*, 2002). It has excellent adaptability in desert areas and grows well in water-scarce environments. Therefore, many species in this family are used for the restoration and management of degraded salty soils (Kushiev *et al.*, 2017). The present study was undertaken to investigate the effect of licorice extract on the growth of maize under drought conditions.

MATERIALS AND METHODS

The experiment was carried out on April 17th, 2021 at the College of Agricultural Engineering Sciences—University of Sulaimani (latitude 35o 33" N, 45o 27" E, altitude 884.8 masl) to investigate the effect of licorice powder and extract on maize crop growth and drought tolerance. Six maize genotypes (TALAR, Medium 791, MSI XB, PR36 BO8, NK Cobalt/NX 34476, and ZP 434 XA) were cultivated in pots (diameter 30 cm, height 40 cm) filled with an equal amount of soil. Four treatments of licorice were implemented under stress and non- stress conditions. The first treatment of licorice (T1) was control (with no licorice powder and extract);

the second one (T2) was licorice powder with the soil; the third treatment (T3) was licorice extract sprayed at 2 weeks after emergence and twice a week thereafter; and the last one (T4) was licorice powder with soil, and licorice extract sprayed at 2 weeks after emergence and twice a week thereafter. The water deficit was arranged by a soil moisture monitor with time display equipment. There was no uniformity in the irrigation intervals due to crop requirement and air temperature that directly affected soil moisture content and ETo. The experiment was carried out by a factorial, completely randomized design (CRD) with 3 replications. Cultural practices were conducted normally, including phosphorus fertilizer, as triple super phosphate, was applied before sowing time at a rate of 200 kg ha^{-1} and nitrogen fertilizer (200 kg ha^{-1}), as urea 46% N, was applied at the seedling stage. The data were put through an analysis of variance (ANOVA), and the Duncan multiple range test at 0.05 was used to compare the means of the data. The XLSTAT program version 16 was used.

The Studied Criteria: After 40 days after seeding (DAS) on May 28th, 2021 the following criteria were measured:

1. Shoot length (cm).
2. Shoot fresh weight (g).
3. Shoot dry weight (g).
4. Root length (cm).
5. Root fresh weight (g).
6. Root dry weight (g).
7. No. of leaves $plant^{-1}$.
8. Leaf area index (LAI): It was measured by the following equation (Sanderson *et al.*, 1981).

$$LA = Max\ Length \times Max\ Width \times 0.75$$

$$LA/\ plant = LA \times Number\ of\ leaf$$

$$LAI = (LA/\ plant)\ /\ area\ of\ the\ pot \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

9. Relative water content (%): Using the method described by Galle,Haldimann & Feller (2007) and calculated as:

$$LRWC = [(FW-DW)/(TW-DW)] *100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where FW is the fresh weight of a single wholly expanded leaf per plant and DW is dry weight of a single wholly expanded leaf per plant. After the FW of the fully expanded leaves had been recorded, the samples were immediately dipped in distilled water, in the dark, at 4 °C. After 24 h,

leaves were weighed to get turgor weight (TW), and then dried in an oven for 24 h at 70 °C to determine their DW.

10. Total chlorophyll content (mg/g LFW): It was measured by using a SPAD-meter for last apical entire leaf.

11. Proline content (µg g^{-1} LFW): Proline content in leaf samples is well-defined following the method of (Lateef *et al.*, 2021). Fresh leaf (0.1 g) powder was homogenized in 3 mL of 3% (w/v) sulphosalicylic acid and centrifuged at 4000 rpm for 30 min at 4°C. A 2ml of supernatant was mixed with 2 mL of acid ninhydrin reagent (1.25 g ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid with agitation until dissolved) and 2 mL of glacial acetic acid. The samples were subsequently incubated at 100°C for 60 min. The sample leaves were cooled in an ice bath prior to adding 4 mL of toluene to each sample. The toluene layer was read at 520 nm against the blank containing toluene with a UV-visible spectrophotometer (UV-365, SHIMADZU, Japan). To compare the proline content of the samples to a reference, a stock solution of 1 mg/mL was prepared by dissolving 10 mg in 10 mL of 20% ethanol and then the proline concentration series (0.0, 5, 10, 15, 20, 25, 30, 35 µg ml^{-1}) was applied to the stopper tubes. A linear regression between the absorbance values at 520 nm and the L-proline content was detected. The proline content of the leaf sample has been determined from this typical curve. Values are the results of three replicates and are exemplified as µg g^{-1} of fresh leaf. The formula for the determination of the proline content was:

$$PC\ (\mu g\ g^{-1}\ FM) = VW \times C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (3)$$

Where V is the volume of extract (mL), W is the fresh weight of the leaf sample (g), and C is the concentration of proline determined from the standard curve.

12. Total sugar content (µg g^{-1} LFW): Soluble sugar content was determined following the method defined by (Lateef *et al.*, 2021). A stock solution of standard compound (glucose) was prepared by adding 10 mL of deionized water to 10 mg of glucose to get a final concentration of 1 mg ml^{-1}. A series of dilutions of glucose (0, 4, 10, 20, 30, 50, 80, 160, 320, 640 µg) was prepared. Linear regression was demonstrated between the absorbance values at 620 nm and the glucose concentrations. Fresh leaf samples (0.1 g) were soaked in 800 µL deionized water. The solution mixture was boiled at 100°C for 30 min. to extract soluble sugar then, it cooled and centrifuged for 10 min at 4000 rpm. The extracts were decanted and the residue was re-extracted for two more times with deionized water. In all, 0.1 ml extracts and 3 mL of anthrone reagent (0.15 g anthrone

in 84 mL of sulphuric acid and 16 mL deionized water) were mixed. The mixture was heated at 100 ºC for 5 min. After cooling, the absorbance of the mixture was recorded at 620 nm. The content of soluble sugar was calculated from the standard curve of glucose at 620 nm using a UV-visible spectrophotometer (UV-365, SHIMADZU, Japan). The formula for calculating the soluble sugar content was:

$$SSC\ (\mu g\ g^{-1}\ FM) = VW \times C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (4)$$

Where V is the volume of extract (mL), W is the fresh weight of the leaf sample (g), and C is the concentration of glucose determined from the standard curve.

13. Total phenol content (µg g^{-1} LFW): The fresh unstressed and stressed leaf samples were ground in a mortar with a pestle with liquid nitrogen. Fresh powder (0.1 g) was extracted with 0.7 mL of 60% (v/v), acidic methanol (methanol + HCl in a ratio of 99: 1) and incubated at 10°C for 16 h. The mixture was centrifuged for 20 min at 14000 rpm at 4°C. The supernatant solution (extract) was used to determine the total phenolic content (TPC). The content of total phenolic compounds in each extract was measured according to (Lateef *et al.*, 2021) using the Folin–Ciocalteu method with some modifications. An aliquot of 25 µL of each extract (Sample) or deionized water (Blank) was mixed with 2 mL of 10:90 Folin–Ciocalteu phenol reagent: water (v/v) and allowed to react for 7 min. Then,1600 µL of 10% saturated Na2CO3 solution was added and allowed to stand for 50 min in the dark at 40°C. The absorbance of the reaction mixture was read at 750 nm against the blank using a UV-visible spectrophotometer (UV-365, SHIMADZU, Japan). A gallic acid standard curve was obtained for the calculation of phenolic content by dissolving 9 mg of gallic acid in 9 mL of methanol to get a final concentration of 1 mg/ml. A sequence of dilutions of gallic acid (0, 50, 100, 150, 200, 250, 300 µg ml^{-1}) had been used to produce a standard curve and linear association between the absorbance values at 750 nm and the gallic acid content was observed. The total polyphenol content (TPC) of each extract was expressed as the equivalent of µg gallic acid (GAE) per gram of fresh leaf extracts by the formula:

$$TPC\ (\mu g\ GAE\ g^{-1}\ FM) = VW \times C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (5)$$

Where V is the volume of extract (mL), W is the fresh weight of the leaf sample (g), and C is the concentration of gallic acid collected from the standard curve. Each value reflects the mean of three measurements.

14. Soil moisture holding capacity: The available water for each soil was determined after estimating the soil water content at -33 and -1500kpa for the soil from the models proposed by Karim (1999):

$$F.C = 13.28 + 0.397 \times (clay\ \%) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (6)$$

$$P.W.P = 4.57 + 0.35 \times (clay\ \%) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (7)$$

Where F.C=soil water content at (-33kpa), P.W. P=soil water content at (-1500kpa).

Soil bulk density was determined by core sampler method (Blake, 1965). Available water (AW) in the soil was computed as the differences between the water content at the field capacity (F.C) (33kpa) and the permanent wilting point (W.P) (1500kpa) (Bowles, 1970).

$$A.W = F.C - P.W.P \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (8)$$

15. Soil analysis: The soil analysis data were summarized in table 1.

Table 1: Physical and chemical properties of soil

Physicochemical properties		Value
Particles size distribution g kg^{-1}	**Sand**	87
	Silt	435
	Clay	458
	Textural Name	SiC
PH		7.59
ECe (micro Siemens cm^{-1}) or (µS cm^{-1})		490
O.M. (g kg^{-1})		22.4
CaCO3 (g kg^{-1})		304.3

RESULTS AND DISCUSSION

The data in table 2, reveal the significant differences in growth characteristics among maize genotypes. genotype exhibited maximum values for root length (RL), and root dry weight (RDW) with 60.531 cm and 10.594 g respectively. MSI XB genotype exceeded the other genotypes significantly in shoot fresh weight (SFW), root fresh weight (RFW), and number of leaves, reaching 44.366 g, 29.047 g, and 8.083 respectively. The PR36 BO8 genotype achieved the highest values for shoot length (SL), shoot dry weight (SDW), and leaf area index (LAI) with 49.462 cm, 5.244 g, and 0.941, respectively. While ZP 434 XA genotype recorded the minimum value for all growth characteristics except root dry weight (RDW), No. of leaves and leaf area index (LAI), it was recorded by NK Cobalt/NX 34476 genotype.

Table 2: Impact of maize genotypes treated with licorice powder with soil, licorice root extract, and both licorice application on the morpho-physiological traits

Genotypes	SL (cm)	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)	No. of leaves	L A I
TALAR	44.315abc	36.431ab	4.460ab	60.531a	28.659a	10.594a	8.000a	0.785ab
Medium 791	40.775bc	34.049ab	3.622ab	60.395a	24.372ab	6.467b	7.125b	0.692b
MSI XB	48.283ab	44.366a	4.949ab	60.437a	29.047a	8.807ab	8.083a	0.926ab
PR36 BO8	49.462a	44.273a	5.244a	60.25a	26.005ab	8.520ab	7.249b	0.941a
NK Cobalt / NX 34476	44.345abc	33.588ab	3.939ab	56.233ab	18.959ab	5.491b	6.416c	0.718ab
ZP 434 XA	37.05c	29.511b	3.357b	54.104b	17.493b	5.564b	7.166b	0.822ab

SL: Shoot Length, SFW: Shoot Fresh Weight, SDW: Shoot Dry Weight, RL: Root Length, RFW: Root Fresh Weight, RDW: Root Dry Weight, No. of leaves: Number of leaves, LAI: Leaf Area Index, Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

It is obvious from tables 3 that significantly exceeding the PR36 BO8 genotype in most physio-biochemical attributes resulted in the higher rate of proline content (PC), soluble sugar content (SSC), and total phenolic content (TPC) reaching 2844.166 µg g^{-1}, 248.055 µg g^{-1}, and 174.681 µg g^{-1} respectively, with a significant raise from the other genotypes. Although, there was no significant effect between all genotypes in relative water content (RWC%), the medium 791 genotype recorded the highest value. There were significant differences in total chlorophyll content by TALAR, PR36 BO8, and NK Cobalt/NX 34476 genotypes.

Table 3: Impact of maize genotypes treated with licorice powder with soil, licorice root extract, and both licorice application on the physio-biochemical attributes

Genotypes	PC (µg g^{-1})	SSC (µg g^{-1})	TPC (µg g^{-1})	RWC (%)	TCC (mg g^{-1})
TALAR	1468.829c	171.828e	134.383c	61.890a	13.662a
Medium 791	1050.897f	175.164e	130.018d	67.709a	11.6ab
MSI XB	1248.493e	187.484d	125.820e	59.544a	10.433b
PR36 BO8	2844.166a	248.055a	174.681a	62.483a	13.241a
NK Cobalt / NX 34476	2084.871b	230.668b	135.699b	58.661a	13.133a
ZP 434 XA	1364.038d	198.904c	130.346d	64.376a	9.766b

PC: Proline Content, SSC: Soluble Sugar Content, TPC: Total Phenolic Content, RWC: Relative Water Content, TCC: Total Chlorophyll Content. Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

Fig. 1 illustrates the response of genotypes to stress conditions through physio-biochemical attributes. Under stress conditions, the PR36 BO8 genotype had maximum proline content (PC) and total phenolic content (TPC) of 4924.29 µg g^{-1} and 240.79 µg g^{-1} respectively. The minimum value under stress conditions for soluble sugar content (SSC) and relative water content (RWC) was exhibited by the TALAR genotype with 150.28 µg g^{-1} and 48.24%, respectively. Also under the same condition, the ZP 434 XA genotype recorded a minimum total chlorophyll content (TCC) of 8.43 mg g^{-1}. However, when the PR36 BO8 genotype was grown in normal conditions, it had

the highest amount of soluble sugar content (SSC) with 271.62 µg g^{-1} in it. Under non-stress conditions, the Medium 791 genotype achieved the highest percentage for relative water content (RWC) and the lowest value for total phenolic content (TPC), reaching 81.38% and 99.49 µg g^{-1} respectively. genotype had the maximum total chlorophyll content (TCC) of 14.75 mg g^{-1} under non-stress conditions. Under normal conditions, the MSI XB genotype has the least amount of proline in it with 606.35 µg g^{-1}.

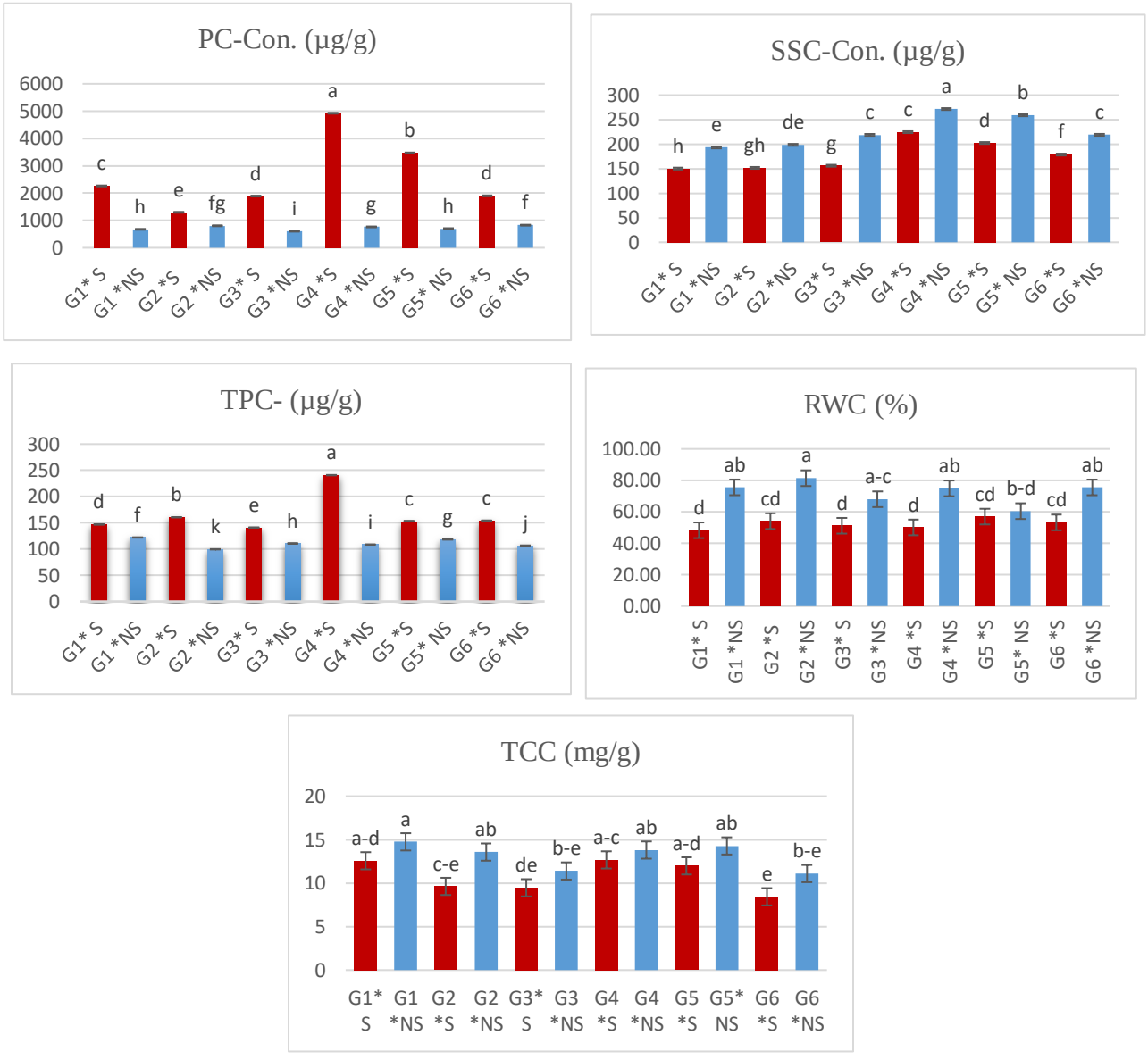

Figure 1: Response of genotypes to stress condition through physio-biochemical attributes.

The results of the analysis in table 4 showed that all growth characteristics except root dry weight (RDW) increased significantly with the treatment of licorice powder added to the soil (T2) among other treatments, which reached 45.086 cm, 49.165 g, 60.533 g, 30.842 g, 7.583, 1.101 respectively. Shoot length (SL), shoot fresh weight (SFW), shoot dry weight (SDW), and number of leaves were higher in the control treatment with no licorice powder and extract added (T1) than in the other treatments with 43.088 cm, 28.053 g, 3.532 g, and 7.111 respectively.

Table 4: Influence of Licorice powder with soil, licorice root extract, and both licorice application on the morpho-physiological traits

Treatments	SL (cm)	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)	No. of Leaves	LA I
T1	43.088a	28.053b	3.532b	58.786ab	21.763bc	7.630ab	7.111a	0.783b
T2	45.086a	49.165a	5.465a	60.533a	30.842a	7.673ab	7.583a	1.101a
T3	43.930a	34.273b	3.946b	60.416a	26.219ab	9.832a	7.361a	0.742b
T4	44.049a	36.654b	4.104b	54.898b	17.533c	5.161b	7.305a	0.630b

T1: control (with no licorice powder and extract adds); T2: was licorice powder adds with the soil; T3: was licorice extract sprayed at 2 weeks after emergence and twice a week thereafter; T4: was licorice powder adds with soil, plus licorice extract sprayed at 2 weeks after emergence and twice a week thereafter, SL: Shoot Length, SFW: Shoot Fresh Weight, SDW: Shoot Dry Weight, RL: Root Length, RFW: Root Fresh Weight, RDW: Root Dry Weight, No. of Leaves: Number of Leaves, LAI: Leaf Area Index . Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

Data in table 5 illustrated that the proline content (PC), soluble sugar content (SSC), and relative water content (RWC) were improved significantly with the treatment of licorice powder added with soil (T2), reaching 2528.247 µg g^{-1}, 212.990 µg g^{-1}, and 64.446 % respectively. The treatment of control (T1) with no licorice powder and extract added exhibited the highest value of total phenolic content (TPC) with 169.875 µg g^{-1}, while the treatment of licorice powder added with soil plus licorice extract sprayed at 2 weeks after emergence and twice a week thereafter (T4) had the maximum total chlorophyll content (TCC) reached 14.997 mg g^{-1}. The minimum value for soluble sugar content (SSC), relative water content (RWC), and total chlorophyll content (TCC) exhibited by the treatment of control with no licorice powder and extract added (T1).

Table 5: Influence of Licorice powder with soil, licorice root extract, and both licorice application on the physio-biochemical attributes

Treatments	PC (µg g^{-1})	SSC (µg g^{-1})	TPC (µg g^{-1})	RWC (%)	TCC (mg g^{-1})
T1	1518.205c	187.698c	169.875a	59.619a	9.441c
T2	2528.247a	212.990a	131.506b	64.446a	13.027b
T3	1050.790d	195.260b	124.588d	61.892a	10.425c
T4	1610.287b	212.120a	127.997c	63.818a	14.997a

T1: control (with no licorice powder and extract adds); T2: was licorice powder adds with the soil; T3: was licorice extract sprayed at 2 weeks after emergence and twice a week thereafter; T4: was licorice powder adds with soil, plus licorice extract sprayed at 2 weeks after emergence and twice a week thereafter, PC: Proline Content, SSC: Soluble Sugar Content, TPC: Total Phenolic Content, RWC: Relative Water Content, TCC: Total Chlorophyll Content. Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

The obtained data concerning the water deficit impacts on the growth characteristics were plotted in table 6, manifesting reduction in all growth characteristics except root length (RL) at water deficit condition.

Table 6: Effect of water stress condition on growth characteristics compared to normal condition.

Condition	SL (cm)	SFW (g)	SDW (g)	RL cm	RFW (g)	RDW (g)	No. of Leaves	L A I
S	39.745b	28.746b	3.483b	61.180a	19.937b	5.834b	6.819b	0.764a
NS	48.331a	45.326a	5.041a	56.137b	28.242a	9.313a	7.861a	0.864a

S: Stress Condition, NS: Non-Stress Condition, SL: Shoot Length, SFW: Shoot Fresh Weight, SDW: Shoot Dry Weight, RL: Root Length, RFW: Root Fresh Weight, RDW: Root Dry Weight, No. of Leaves: Number of Leaves, LAI: Leaf Area Index. Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

There were significant differences between effect of water stress condition on physio-biochemical attributes table 7. At drought stress condition, proline content (PC) and total phenolic content (TPC) were increased significantly with 2624.289 µg g^{-1} and 166.081 µg g^{-1} respectively. In contrast, at normal condition (non-stress condition) there were significant variation in soluble sugar content (SSC), relative water content (RWC), and total chlorophyll content (TCC) reached 226.767 µg g^{-1}, 72.620 %, and 13.152 mg g^{-1} respectively.

Table 7: Effect of water stress condition on physio-biochemical attributes compared to normal condition.

Condition	PC (µg g^{-1})	SSC (µg g^{-1})	TPC (µg g^{-1})	RWC (%)	TCC (mg g^{-1})
S	2624.289a	177.267b	166.081a	52.268b	10.793b
NS	729.476b	226.767a	110.901b	72.620a	13.152a

S: Stress Condition, NS: Non-Stress Condition, PC: Proline Content, SSC: Soluble Sugar Content, TPC: Total Phenolic Content, RWC: Relative Water Content, TCC: Total Chlorophyll Content. Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

Fig. 2 demonstrated significant variation in growth characteristics when licorice powder and extract were combined with a stress condition. Root length exhibited under water stress conditions interacted with the treatment of licorice extract sprayed at 2 weeks after emergence and twice a week thereafter (T3) significantly. Under non-stress conditions, the treatment of licorice powder added to the soil (T2) significantly exceeded others for root fresh weight (RFW), shoot length (SL), shoot fresh weight (SFW), shoot dry weight (SDW), no. of leaves, and leaf area index (LAI) reaching 38.222 g, 50.822 cm, 64.272 g, 6.839 g, 7.889, and 1.229 respectively.

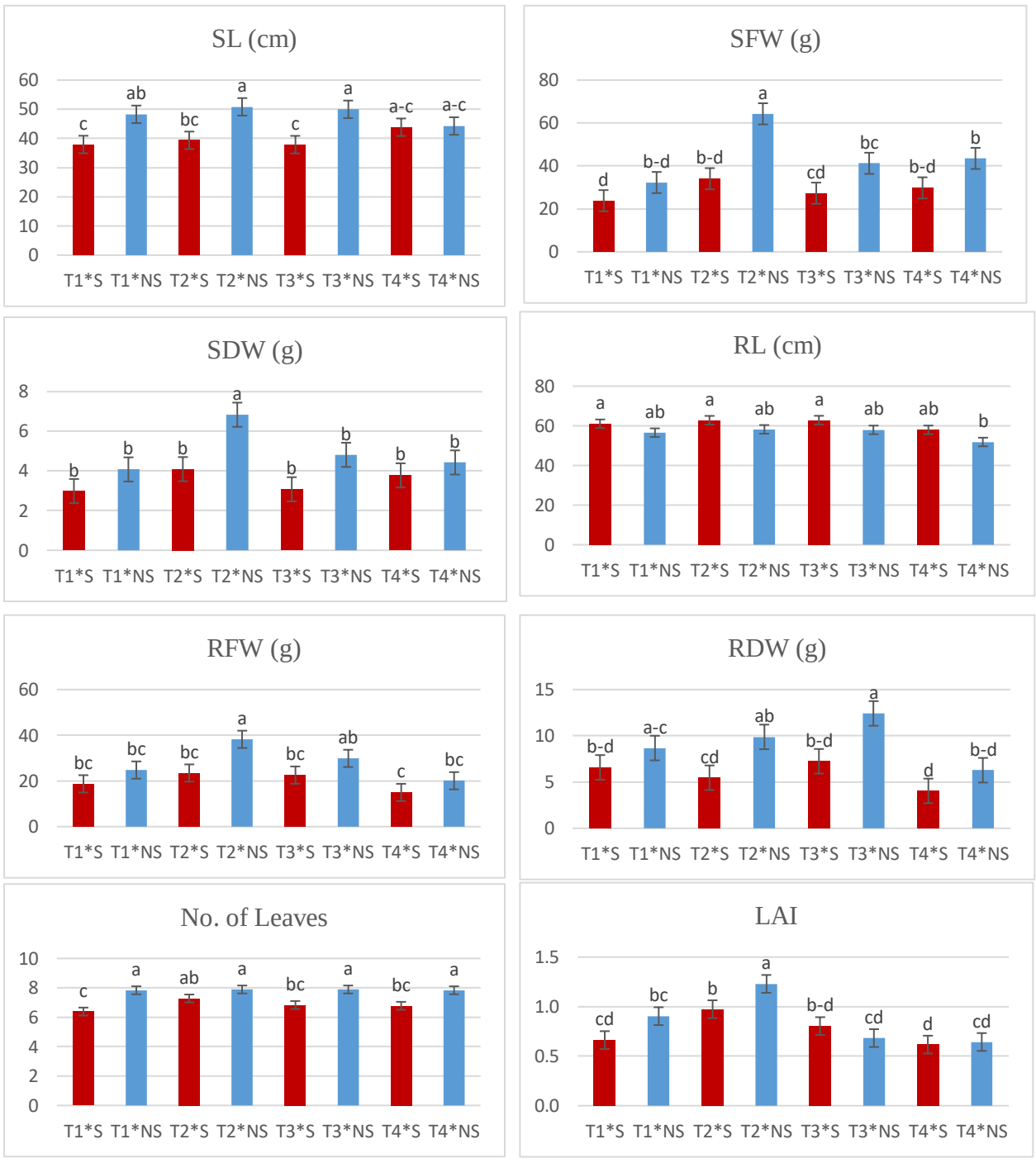

Figure 2: Effect of licorice application in improving drought stress through growth characteristics.

In fig. 3, under stress condition, the treatment of licorice powder added with soil (T2) significantly exceeded others for proline content (PC) with 4302.821µg g^{-1}, while the treatment of control (T1) with no licorice powder and extract added exhibited the highest value of total phenolic content (TPC) with 217.628 µg g^{-1}. Under non-stress conditions, the treatment of licorice powder added to soil (T2) recorded the maximum values of soluble sugar content (SSC) and relative water content (RWC) of 247.353 µg g^{-1} and 80.556 %, respectively. Under non-stress conditions, the highest value of total chlorophyll content (TCC) was shown by the treatment of licorice powder

added with soil, plus licorice extract sprayed 2 weeks after emergence and twice a week thereafter (T4) with 16.611 µg mg^{-1}.

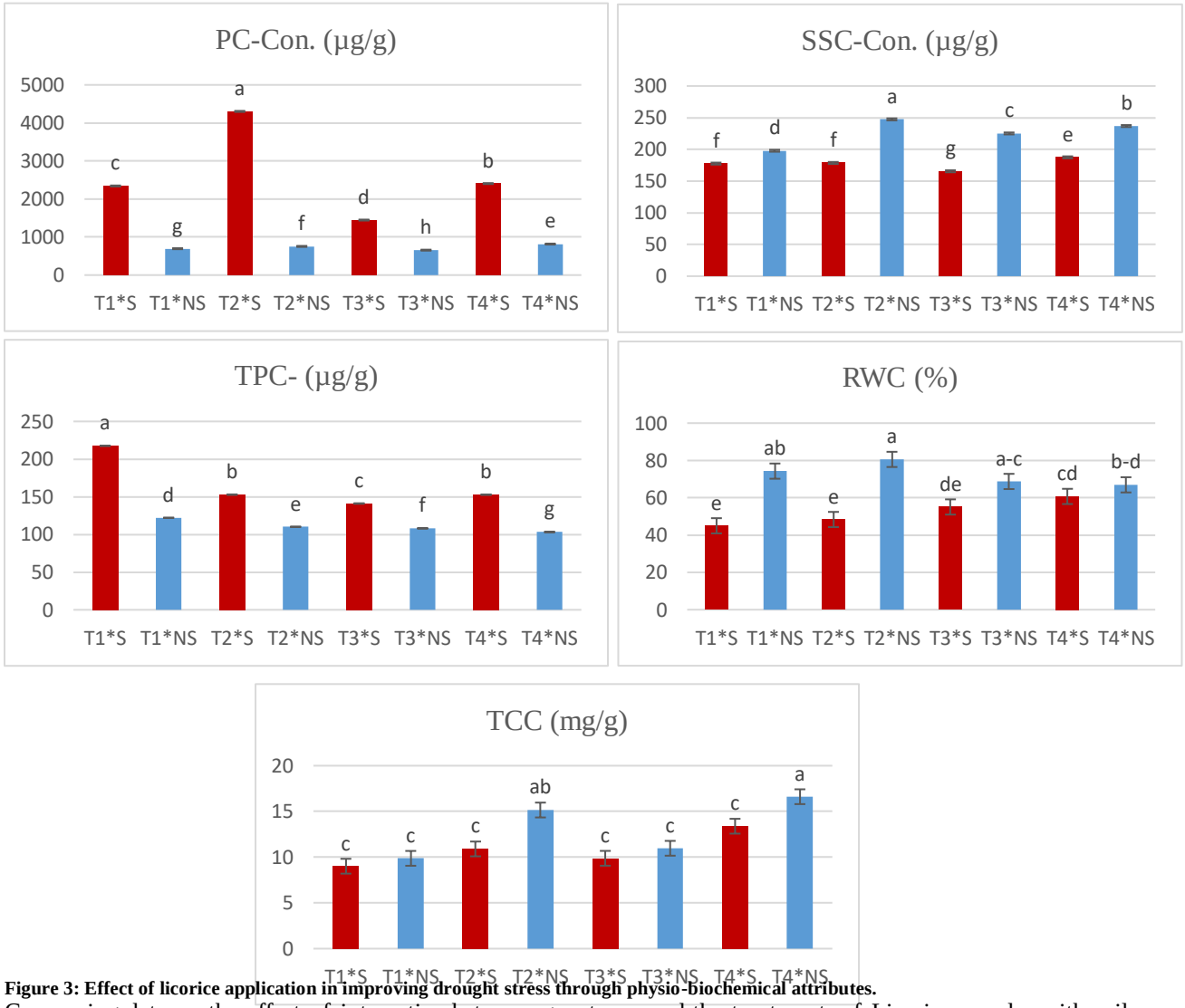

Figure 3: Effect of licorice application in improving drought stress through physio-biochemical attributes.

Concerning data on the effect of interaction between genotypes and the treatments of Licorice powder with soil, licorice root extract, and both licorice application on the morpho-physiological attributes under stress and normal conditions, which represented in table (8). The maximum value of number of leaves was shown under the interaction between genotype Medium 791 with T4 at normal condition with 10 leaves, while minimum number of leaves revealed by genotype ZP 434 XA interacted with T1 under stress condition with 5 leaves.

The interaction between the effect of the genotypes and treatments of Licorice powder with soil, licorice root extract, and both licorice application on the physio-biochemical attributes under stress and normal conditions was illustrated in table (9). The genotype PR36 BO8 interacted with the T2 displayed significant maximum value of proline content (PC) and soluble sugar content (SSC) under stress and normal condition (NS) with 9649.62 µg g^{-1}, and 314.36 µg g^{-1} respectively. While minimum value of proline content (PC) recorded by MSI XB genotype interacted with T2 under normal condition (NS) with 325.26 µg g^{-1}. The minimum soluble sugar content (SSC) and total phenolic content (TPC) exhibited by genotype Medium 791 interacted with T2 under stress and normal condition with 129.18 µg g^{-1},79.24 µg g^{-1} respectively. While the interaction between genotype PR36 BO8 and T1 under stress condition has highest total phenolic content (TPC) with 331.80 µg g^{-1}.

Table (8): Effect of the interaction between genotypes and treatments of Licorice powder with soil, licorice root extract, and both licorice application on the morpho-physiological attributes under stress and normal conditions.

G*T	RL (cm)		RFW (g)		RDW (g)		SL (cm)		SFW (g)		SDW (g)		No. of Leaves		LAI	
	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
G1*T1	58.2a-e	62.0a-d	24.2a-f	24.9a-f	11.1b-e	7.2c-e	36.3a-d	52.8a-c	21.6d-f	20.0ef	2.7c-e	3.0b-e	6.7d-h	8.0a-f	0.7d-h	0.8c-h
G1*T2	71.2a	65.5a-c	22.6a-f	50.6a	5.9c-e	13.2b-d	32.6a-d	40.3a-d	23.9c-f	54.4a-f	3.3b-e	6.6a-d	7.3b-g	8.0a-f	0.7d-h	0.9c-h
G1*T3	59.7a-d	59.0a-e	22.4a-f	39.9a-e	9.3b-e	23.9a	43.8a-d	50.0a-c	30.0c-f	54.8a-f	4.5b-e	7.0a-c	7.3b-g	9.3ab	0.9b-h	1.1a-g
G1*T4	48.6b-f	60.2a-d	18.4a-f	26.3a-f	6.5c-e	7.7c-e	43.1a-d	55.5ab	33.2f	53.4c-f	4.2b-e	4.5b-e	8.0a-f	9.3ab	0.7d-h	0.6e-h
G2*T1	58.5a-e	55.0a-e	10.7d-f	33.6a-f	3.5c-e	9.6b-e	31.0b-d	54.0a-c	15.6c-f	38.7b-f	1.3e	4.3b-e	6.3e-h	7.0c-h	0.6d-h	0.9c-h
G2*T2	65.2a-c	59.3a-e	21.2a-f	28.2a-f	4.5c-e	4.6c-e	31.7a-d	54.0a-c	24.8c-f	53.3a-f	2.6c-e	5.1a-e	6.0f-h	6.0f-h	0.6e-h	0.7d-h
G2*T3	66.3a-c	60.7a-d	30.4a-f	32.6a-f	8.6b-e	10.1b-e	22.3d	47.7a-d	24.7c-f	39.3b-f	3.0b-e	4.8a-e	6.7d-h	8.0a-f	0.9b-h	0.5e-h
G2*T4	62.8a-d	55.3a-e	6.2f	32.1a-f	1.8de	8.9b-e	33.0a-d	52.5a-c	12.8f	63.2a-d	1.6de	6.4a-e	7.0c-h	10.0a	0.5f-h	0.8c-h
G3*T1	69.7ab	61.0a-d	35.6a-f	21.2a-f	10.7b-e	7.7c-e	46.3a-d	45.0a-d	34.8c-f	30.5c-f	4.6b-e	3.6b-e	7.3b-g	9.0a-c	0.9c-h	0.6d-h
G3*T2	55.7a-e	64.3a-c	26.9a-f	49.5ab	6.1c-e	11.6b-e	44.7a-d	57.2ab	49.0b-f	79.4ab	6.4a-e	8.1ab	8.3a-e	8.0a-f	1.4a-d	1.4a-d
G3*T3	62.8a-d	57.3a-e	19.8a-f	48.3a-c	6.8c-e	19.6ab	34.4a-d	57.3a	26.7c-f	65.3a-c	2.9b-e	5.4a-e	7.0c-h	8.7a-d	0.7d-h	1.1a-g
G3*T4	58.7a-e	54.0a-e	21.6a-f	9.4d-f	5.9c-e	2.1de	54.7ab	46.7a-d	35.0c-f	34.1c-f	4.4b-e	4.3b-e	8.3a-e	8.0a-f	0.8c-h	0.5f-h
G4*T1	64.0a-c	51.8b-e	25.8a-f	32.1a-f	8.7b-e	14.4a-c	45.7a-d	48.9a-c	39.7b-f	33.6c-f	4.7a-e	5.1a-e	7.3b-g	8.3a-e	1.0b-h	1.3a-e
G4*T2	58.0a-e	60.7a-d	15.9c-f	42.2a-d	4.0c-e	11.2b-e	44.5a-d	56.2ab	32.8c-f	90.8a	3.5b-e	9.8a	7.3b-g	9.0a-c	0.9c-h	1.8a
G4*T3	67.7ab	59.0a-e	23.6a-f	23.2a-f	7.2c-e	9.0b-e	42.7a-d	52.0a-c	26.5c-f	29.6c-f	2.9b-e	3.6b-e	7.3b-g	7.0c-h	0.8c-h	0.6e-h
G4*T4	56.7a-e	64.2a-c	15.3d-f	30.1a-f	4.4c-e	9.2b-e	55.0ab	50.8a-c	38.4b-f	62.7a-e	6.1a-e	6.3a-e	5.7gh	6.0f-h	0.6e-h	0.6d-h
G5*T1	67.5ab	53.9a-e	5.4f	18.2a-f	1.5e	6.8c-e	39.7a-d	45.5a-d	15.9f	24.3c-f	2.3c-e	3.2b-e	5.7gh	6.0f-h	0.4gh	0.7d-h
G5*T2	65.7a-c	40.8ef	26.4a-f	26.7a-f	4.8c-e	6.2c-e	37.7a-d	55.0ab	35.9c-f	60.3a-e	4.2b-e	5.5a-e	6.0f-h	8.0a-f	0.7d-h	1.2a-f
G5*T3	62.0a-d	56.5a-e	25.1a-f	17.5b-f	7.8c-e	6.1c-e	38.7a-d	54.7ab	28.2c-f	33.7c-f	2.9b-e	5.8a-e	5.7gh	6.3e-h	0.7d-h	0.3gh
G5*T4	58.7a-e	44.7d-f	18.0a-f	14.2d-f	3.1c-e	7.7c-e	46.0a-d	37.5a-d	39.4b-f	31.2c-f	4.0b-e	3.7b-e	5.3gh	8.3a-e	0.6d-h	1.1a-f
G6*T1	48.3c-f	55.5a-e	10.7d-f	18.6a-f	4.0c-e	6.3c-e	28.5cd	43.3a-d	15.3f	46.6b-f	2.4c-e	5.4a-e	5.0h	8.7a-d	0.5f-h	1.0a-g
G6*T2	61.3a-d	58.7a-e	27.7a-f	32.2a-f	7.4c-e	12.4b-e	45.0a-d	42.3a-d	38.0b-f	47.4b-f	4.7a-e	5.9a-e	8.7a-d	8.3a-e	1.6ab	1.6a-c
G6*T3	58.8a-e	55.2a-e	14.1d-f	17.7b-f	3.8c-e	5.8c-e	45.3a-d	38.2a-d	27.7c-f	24.8c-f	2.4c-e	2.3c-e	7.0c-h	8.0a-f	0.7d-h	0.4f-h
G6*T4	62.3a-d	32.7f	10.4d-f	8.5ef	2.6de	2.1de	31.2a-d	22.6d	19.9ef	16.5f	2.4c-e	1.4de	6.3e-h	5.3gh	0.5f-h	0.2h
Pr > F	0.05		0.41		0.20		0.84		0.36		0.79		0.01		0.28	
F	1.76		1.05		1.34		0.63		1.11		0.69		2.16		1.21	
Significant	No		No		No		No		No		No		Yes		No	

Table (9): Effect of the interaction between hybrids and treatments of Licorice powder with soil, licorice root extract, and both licorice application on the physio-biochemical attributes under stress and normal conditions.

G*T	PC-Con. (µg g^{-1})		SSC-Con. (µg g^{-1})		TPC- (µg g^{-1})		RWC (%)		TCC (mg g^{-1})	
	S	NS	S	NS	S	NS	S	NS	S	NS
G1*T1	3849.6d	641.9w	153.2r-u	197.4jkl	229.8c	163.7i	41.7h-l	74.4a-j	10.3e-j	10.0e-j
G1*T2	1940.6ij	607.3w	155.1r-t	235.5fg	120.4q	127.9m-o	58.2c-l	82.3a-e	11.9b-j	16.6a-e
G1*T3	1613.7m	834.2t-v	141.1u	193.5klm	128.1m-o	106.0u	48.0e-l	71.0a-l	12.4b-j	11.2b-j
G1*T4	1648.1lm	615.0w	151.6r-u	146.8stu	108.7t	90.1x	44.8g-l	74.3a-j	15.5a-g	21.1a
G2*T1	1394.4no	982.9q-s	144.1tu	158.8rs	168.7h	101.7vw	55.8c-l	85.4a-c	8.6g-j	12.1b-j
G2*T2	1748.3kl	929.1q-t	129.1v	222.5hi	203.7d	79.2y	40.3i-l	81.7a-e	10.7d-j	13.0b-j
G2*T3	1015.0qr	559.8wx	175.0o-q	217.8i	149.7k	108.5t	48.2d-l	98.0ab	8.1h-j	13.4b-i
G2*T4	1029.1pq	748.3v	158.1rs	195.4j-m	119.9q	108.4t	71.6a-k	60.2c-l	11.0b-j	15.7a-f
G3*T1	1865.0j	882.9s-u	144.8tu	184.1m-o	205.5d	112.5s	38.8kl	52.9c-l	6.3j	7.7ij
G3*T2	1995.7i	325.2z	159.4rs	199.1j-l	127.4no	100.0w	45.8f-l	102.034a	10.6e-j	12.8b-j
G3*T3	840.6t-v	645.7w	148.3stu	203.4jk	117.4r	126.6o	57.1c-l	75.5a-i	8.6g-j	8.6g-j
G3*T4	2861.1f	571.4wx	171.5pq	289.0b	113.5s	103.2v	62.6c-l	41.3h-l	12.3b-j	16.4a-e
G4*T1	3572.6e	475.2xy	221.1hi	236.1fg	331.7a	101.5vw	42.0h-l	76.4a-h	12.7b-j	8.3h-j
G4*T2	9649.6a	902.1st	250.7e	314.3a	180.9f	118.8qr	40.1j-l	78.470a-g	10.8c-j	17.6a-d
G4*T3	1602.1m	795.7uv	163.9qr	269.0cd	154.8j	113.6s	58.9c-l	61.6c-l	12.1b-j	11.4b-j
G4*T4	4872.6c	882.9stu	262.0d	266.8cd	295.4b	100.2w	59.2c-l	83.1a-e	15.1a-h	17.8ab
G5*T1	1618.8m	553.4wx	224.4g-i	206.3j	193.4e	130.3m	46.3f-l	72.7a-k	8.2h-j	10.7e-j
G5*T2	9165.0b	832.9t-v	223.0hi	275.6c	156.2j	111.5s	64.0b-l	70.5a-l	12.6b-j	17.7a-c
G5*T3	1463.7n	456.0y	173.6o-q	262.3d	133.0l	103.9uv	53.8c-l	36.1l	10.9b-j	12.1b-j
G5*T4	1630.3m	958.5q-s	188.4l-n	291.5b	129.9mn	127.0o	63.4b-l	62.2c-l	16.1a-e	16.6a-e
G6*T1	1756.0k	625.2w	177.5n-p	204.0jk	176.3g	122.8p	44.8g-l	83.5a-d	7.8ij	10.3e-j
G6*T2	1317.5o	925.2r-t	154.2r-t	236.7f	128.3m-o	123.3p	41.4h-l	68.1a-l	8.5g-j	13.1b-j
G6*T3	2140.6h	641.9w	190.4lm	204.2jk	163.1i	89.6x	64.1b-l	70.0a-l	7.1ij	9.0f-j
G6*T4	2391.9g	1113.7p	192.7k-m	231.0f-h	148.9k	90.3x	62.4c-l	80.3a-f	10.2b-j	11.9e-j
Pr > F	< 0.0001		< 0.0001		< 0.0001		0.08		0.75	
F	1844.78		46.55		1653.71		1.62		0.73	
Significant	Yes		Yes		Yes		No		No	

T1: control (with no licorice powder and extract adds); T2: was licorice powder adds with the soil; T3: was licorice extract sprayed at 2 weeks after emergence and twice a week thereafter; T4: was licorice powder adds with soil, plus licorice extract sprayed at 2 weeks after emergence and twice a week thereafter, PC: Proline Content, SSC: Soluble Sugar Content, TPC: Total Phenolic Content, RWC: Relative Water Content, TCC: Total Chlorophyll Content, S: stress condition, NS: non-stress condition. Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

Results collected from ANOVA revealed significant variations between maize genotypes for all growth characteristics and physio-biochemical constituents (tables 2 and 3). These results documented the existence of genetic diversity in the estimated genotypes. High proline accumulation was due to low activity of antioxidant enzymes like CAT, APX, and GR. The results are similar to those obtained by previous researchers (Bavei *et al.*, 2011; Al-Obady, 2015). The accumulation of osmolyte compounds (fig. 1) is a common plant response to drought stress, and the extent of their accumulation is often larger in tolerant genotypes when compared to sensitive ones (Caballero *et al.*, 2005). Soil addition of licorice led to increased shoot length, root length, No. of leaves, and leaf area index (table 4). This may be due to the availability of licorice extract in increasing the activity of apical meristem tissue as it is one of the organic fertilizers rich in vitamins, amino acids, and growth-stimulating phytohormones that result in cell division and

elongation. Accumulation of physio-biochemical institutes such as proline and soluble sugar (table 5) might have a physiologically important role in energy supply and osmotic adjustment to sustain leaf water potential and relative water content, and it can decrease cell osmotic potential and upswing stress tolerance (Babaeian *et al.*, 2011). The affirmative effects of licorice may be due to the fact that it contains mevalonic acid, which is the initiator in the synthesis of GA 3 acid in plants. Treating the plant with licorice improves the vegetative growth. These results are in harmony with Shayal Alalam (2009). Water deficit stress in plants occurs when the demand for transpiration exceeds water uptake. The water deficit during the vegetative period (table 6), may have created deficiencies in plant water status that were not matched to cell turgor conditions, which were necessary for cell division and elongation. The results agree with Cakir (2004) and Garcia *et al.*, (2014). There were significant differences between the effects of water stress condition and physio-biochemical attributes (table 7), results were similar to those obtained by previous researchers (Caballero *et al.*, 2005; Nasrollahi *et al.*, 2014). In addition, licorice has many important macro and micronutrients, as well as a number of amino acids that are good antioxidants and osmoprotectants, as well as sugar and gas that help plants grow (Ghaloom and Faraj, 2012; Marie and Al-Allaf, 2012; Elrys and Merwad, 2017). These nutrients help plants grow, as well as a number of other nutrients that help plants grow. Some nutrient elements in licorice as vital constituents improve plant growth under stress conditions that are positively revealed in growth characteristics (fig. 2). These results are in harmony with those of (Yildirim *et al.*, 2009; Bargaz *et al.*, 2016). These nutrients sustain the number of leaves on the plant and stay green leaves to maximize photosynthesis, elevating the sink capacity fulfilled during the supply of photoassimilates from stressed leaves (Thomas and Howarth, 2000). Stressed plants fight stress with LRE bioactive constituents (fig. 3). Application of nutrient-containing LRE maximized the number of photosynthetic active leaves and leaf area while staying green, which maintained chlorophyll at a higher content. The existence of GAs and nutrients in LRE inhibits premature leaf senescence and maintains a higher leaf area, increasing photosynthetic pigments. In addition, Fe found in LRE may be available in plants after treatment to activate many enzymes involved in the pathway of chlorophyll biosynthesis and some antioxidant enzymes such as APOX and GR that scavenge ROS and protect chlorophyll from degradation (Zayed *et al.*, 2011).

CONCLUSION

In conclusion, it could be commended that the application of natural constituents may lead to overcoming the adverse effects of drought stress by regulating osmoprotectant content. In particular, the second treatment (T2: licorice powder added to the soil only) was the best compared to the rest of the other treatments. These natural compounds can be used as an alternative to chemical or synthetic fertilizers and growth regulators to improve the growth of maize crops, which are harmless to health and the environment.

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