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Licorice (*Glycyrrhiza glabra*)—Growth and Phytochemical Compound Secretion in Degraded Lands under Drought Stress

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Abstract: Water deficiency restricts plant productivity, while excessive soil moisture may also have an adverse impact. In light of this background, field trials were conducted in secondary saline soil (EC 6.5 dS m^{-1}) at the experimental station of Tashkent State Agrarian University (TSAU), Uzbekistan to determine drought tolerance of licorice (*Glycyrrhiza glabra*) by exposure to four levels of water deficit, namely control (70–80%), moderate (50–60%), strong (30–40%) and intense (10–20%) relative water content (WC) in the soil. The moderate drought stress exhibited positive effects on the morphological and physiological parameters of licorice, and was considered to be the most suitable water regime for licorice cultivation. Plant growth under the 50–60% WC treatment was slightly higher as compared to 70–80% WC treatment, exhibiting weak water deficit promotes licorice growth, root yield and secondary metabolite production. In particular, secondary metabolites i.e., ash, glycyrrhizic acid, extractive compounds and flavonoids, tended to increase under moderate water deficit, however further drought intensification brought a sharp decline of these values. These results contribute to the development of licorice cultivation technologies in arid regions and the most important consideration is the restoration of ecological and economical functions of the dryland agricultural system.

Keywords: licorice; drought stress; irrigation regime; biomass; root yield; metabolites

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1. Introduction

The predominance of cotton monoculture in Uzbekistan eventually has led to severe soil degradation by salinization, erosion, and desertification, affecting more than 50% of irrigated crop fields [1]. Increasing water shortage further exacerbated these environmental problems. At the current state, a rehabilitation of these fields requires science-based approaches and strategies [2]. The phytoremediation technique is an opportunity to rehabilitate degraded lands by specific plants and thereby return these lands to their normal functioning in terms of crop production [3].

Licorice (*Glycyrrhiza glabra*) is one of the Fabaceae family representatives, having an excellent adaptation potential in desert areas and growing well under a water deficit environment. Many species of this family are therefore used for the recovery and management of degraded saline soils. Licorice is a salt and drought-tolerant native crop in arid regions that naturally spreads over approximately 2000 hectares across the Zarafshan, Sirdarya and Amudarya deltas and the Chirchiq and Angren areas of Uzbekistan. A total root dry mass in these areas accounted for almost 18.5 million tonnes [4]. However, over the last few years, due to the extensive exploitation of the raw root mass, the natural licorice growing areas have decreased [5]. The use area of this plant is very wide, i.e., exploiting as a raw substance for the pharmaceutical industry. Hayashi et al. (2003) highly valued this plant, specifying high contents of crude drugs, i.e., glycyrrhizin, flavonoids, vitamins and

medicinal ingredient accumulation in the roots and stolons [6]. The above-ground parts are characterized with high concentrations of triterpenoids, flavonoids, polysaccharides (monosaccharide, disaccharide), cellulose, organic acids, chlorophyll, and many other micro and macro-elements [7,8].

The majority of plant species under water deficiency condition primarily retards growth and gradually deteriorates morphological and physiological properties. Nevertheless, licorice may thrive and even generate significant biomass in incredibly harsh conditions [9,10]. Since the native licorice population is declining sharply in recent years, studying production technology is prudent to exclude supply shortages. Given the fact that licorice is a drought-tolerant plant [11], comparatively few studies have been performed on the successful cultivation of licorice in deteriorated soils under water deficit condition by using of a proper irrigation regime. The cultivation of this climate-resilient crop is considered a preventive measure for combating wide-spread land degradation in the region.

A hypothesis of this research is if licorice could grow well under water deficit conditions, then it might contribute to enhance crop productivity and soil quality, thereby may rejuvenate the dryland cropping system. Therefore, this study focused on evaluating the potential of licorice cultivation under different water deficit conditions (control 70–80%, moderate 50–60%, strong 30–40% and intense 10–20% relative water WC in the soil), thereby, contributing to the economic and environmental sustainability of degraded lands in arid zones.

2. Materials and Methods

2.1. The Study Area

The research was carried out at the experimental station of Tashkent State Agrarian University, Uzbekistan (41.4° N, 64.6° E) during the consecutive 2017–2018 growing seasons. The region lies in an arid area with a continental climate where annual rainfall does not exceed 220–240 mm. The main part (90%) of this precipitation falls between October and May after harvesting crops. The rainfall quantity and temperature magnitude were in accordance with the long-term average values during the experiment years. The winter is characterized with cold weather, averaging monthly air temperature 0 °C in January and the highest average temperature reaches up to 37 °C in July. There were 200–210 total frost-free days at this location. Table 1 presents the weather data covering the experimental period.

Before arranging this trial, the soil was heavily exhausted due to the cotton-wheat cropping system for many years. The soil in the experimental field is defined as calcisol (silt loam serozem). Secondary salination already affected this region and the land is characterized as moderate saline soil (EC 6.5 dS m^{-1}). The soil constitutes very low organic matters, containing 1.010% and 0.998% of humus contents in the surface and subsoil, respectively. A bulk density ranges between 1.44–1.54 $g.cm^{-3}$ in soil horizon; pH is equal 6.8–6.9, total organic carbon 0.09–0.87%, total N 1.010–0.998%, total P 0.164–0.095%, total K 1.850–1.000%. The exchangeable forms of macro-elements were also found at a low level and exchangeable phosphorous 25.2–12.4 mg/kg, potassium 240–200 mg/kg at 0–30 and 30–50 cm depths, respectively. Whereas, HCO_3 0.038–0.039%, Cl 0.003–0.004%, SO_4 0.015–0.012%, CO_3 0.010–0.005% at 0–30 and 30–50 cm depths, respectively (Tables 2 and 3).

Table 1. Weather data on air temperature, rainfall and relative humidity of the study area, Tashkent State Agrarian University, Tashkent, Uzbekistan (2017 and 2018 growing years and long-term data).

Year	Month of the Year											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Air temperature (°C)												
2017	4.8	7.3	9.5	14.4	21	26.2	27	26	21.3	12.9	11.5	3.8
2018	1.3	0	11.9	15.3	19.5	26.7	28.8	25.2	22.2	15.3	8	5.5
Long-term average												
	0	2.1	7.6	14.7	20	25.3	27.2	25.3	19.9	13.6	7.1	2.5
Rainfall (mm)												
2017	56.8	118.7	158.5	114.1	66	63.7	-	0	0	14.5	114.3	53.4
2018	12	44.2	13.2	15.1	9.6	1.3	0.6	1	0.3	3.3	62	56.8
Long-term average												
	58.4	60.6	84.8	23.9	39.7	11.1	4.3	2.5	5.1	33.2	52.2	67.3
Relative humidity (%)												
2017	76	76	75	66	64	50	42	52	55	58	61	69
2018	75	67	75	62	60	47	45	46	50	53	60	67
Long-term average												
	76	68	71	60	53	42	42	46	47	50	63	67

Source: Meteorological Station of Tashkent Region, Tashkent, Uzbekistan.

Table 2. Chemical analysis of soil in the experimental field.

Soil Layer (cm)	Total Forms, %				Exchangeable Forms, mg/kg		
	Humus	**N**	**P**	**K**	**N-NO_3**	**P_2O_5**	**K_2O**
0–30	1.010	0.100	0.164	1.850	17.5	25.2	240
30–50	0.998	0.098	0.095	1.000	11.3	12.4	200

Table 3. Soil salinity characteristics, %.

Soil Layer (cm)	Dry Remnant	HCO_3	CO_3	Cl	SO_4
0–30	0.064	0.038	0.010	0.003	0.015
30–50	0.063	0.039	0.005	0.004	0.012

2.2. Experiment Design

The seeds of licorice genotype named "Autumn gift" were provided by Botanika Scientific Research Institute, Tashkent, Uzbekistan. These seeds were planted on 20 of April 2015 in 1–2 cm soil depth at the experimental station of TSAU and the emerged seedlings cultivated for two years allowing to establish a strong root system. The seed sowing rate was 15–16 kg ha^{-1} when grown as a perennial crop. Thereafter, the three-year-old plants were subjected to various drought stress according to the experiment design. The trials on licorice performance under various water stress regimes were arranged in a split-plot design with three replications. The following four regimes of surface furrow irrigation were used: 1-3-2 (6 times irrigated) for 70–80% WC as a control treatment and three deficit irrigated conditions 1-2-1 (4 times irrigated) for 50–60% WC, 0-2-0 (2 times irrigated) for 30–40% WC and irrigation not used to keep soil moisture 10–20% during the vegetation period. In this experiment, the used watering regimes (1-3-2; 1-2-1 and 0-2-0) defining a number of irrigation at the leaf forming, flowering, and fruiting stages, respectively. The water supply for each irrigation phase was 750–800 m^3 per hectare.

The plot dimensions were 4.8 m (width) by 20 m (length), equaling 96 m^2 in three replications total 288 m^2. Distance between rows was 0.6 m, consisting 8 rows per plot. 4 m borderline between replications and 3 m borderline between variables were spared to exclude water leakage effect. Chemical fertilizers ammonia nitrate (N 34%), superphosphate (P 17–18%) and muriate of potash (K 60%) were applied at rates of 70, 90 and 110 kg ha^{-1} the same amount for all plots. The main part of these chemical fertilizers was applied as a plough sole placement. The remained part was split into two portions and applied 20 and 60 days after germination. All plots were processed with the same agronomic operations such as weeding and other plant protection measures according to local agronomic practices.

2.3. Soil Analyses and Plant Samples

Soil samples were collected at 0–30 and 30–50 cm soil layers in sealable plastic bags at the beginning and the end of this field trial. These soil samples were air-dried at room temperature for two weeks and prepared for chemical analysis after grounding and sieving through a 2-mm mesh. EC and pH meters were employed to determine soil EC and pH values following the preparation of a 1:5 ratio of soil and distilled water. The Tyurin and Lancaster (NIAST 2000) methods were used to extract organic matters and available phosphate in the soil samples [12]. The standard methods discovered by Ryan, Estefan, and Rashid (2001) were used to analyse the chemical contents of these soil samples [13]. Water productivity was determined by dividing the root yield of the crop per unit of water used in the field.

Plant samples i.e., shoots, residues and roots, were taken from three points (50 × 50 cm; ten plants per plot and 30 plants per treatment) of each plot at 0–30 and 30–50 cm soil layer according to the standard method and washed thoroughly. The cleaned samples were weighted for fresh weight analysis and converted to 1 ha. The dried plant samples at 65 °C for 72 h were grounded and sieved through 2 mm mesh for conducting a chemical content analysis. 1 mL of concentrated acid and 10 mL of 50% perchloric acid were added in a tube containing 0.5 g of plant samples. After thoroughly shaking, the mixtures were subjected to the decomposition process by heating on a hot plate. Kjeldahl distillation, Vanadate methods and inductively coupled plasma spectrophotometer were employed to determine total N, P_2O_5, and K_2O concentrations, respectively [12].

2.4. Phytochemical Analysis

Available protein in the plant samples were determined via the Kjeldahl method; oil and cellulose were analysed by the methods described in NIAST (2000) [12].

The total ash was determined by the following standard procedures: The plant samples consisting of an amount of 2 g were incinerated in an tarred silica crucible at 450 °C temperature for 4 h, allowing complete carbon removal. After cooling and weighing procedures of the obtained ash, the percentage of total ash was detected by dividing the before and end values, accordingly.

A method developed by Tian et al. (2008) was used to extract glycyrrhizic acid [14]. The licorice root's powder was deliquesced with the solvent mixture of acetone and diluted in nitric acid for 2 h. Following filtration of the content, 20 mL of acetone was added to the marc and warmed gently. The supernatant was filtered, and a sufficient volume of dilute ammonia solution was added until precipitation of ammonium glycyrrhizinate is completed. The precipitate was collected and washed with 5 mL of acetone, dried and collected.

A traditional extraction method with some modification by Sharma et al. (2010) was used to determine the total flavonoids content in licorice roots [15]. Root extract at an amount of 1 g was mixed with 10 mL methanol for preparation stock solution. Then, a separate mixture was prepared with 5 mL stock solution by adding 1 mL water and 10 mL ethyl acetate. From the mixture, 100 µL were pipetted into a test tube and added 90 µL phosphate buffer. The test tube was filled in with 3 mL two-fold dilution of Folin-Ciocalteu

Reagent FCR and 4 mL Na_2CO_3 following incubation in dark for 45 min. A 100 mL tube was used to dissolve 20 µg Quercitin in distilled water. Absorption of the mixture solution was detected on a spectrophotometer (SP-1901, Shanghai Spectrum Instruments Co., Shanghai, China) at 765 nm.

2.5. Data Analysis and Statistics

The effect of soil relative water contents on growth, root yield, metabolite production and its impact on soil quality in two growth seasons (2017 and 2018) were statistically analysed with the one-way ANOVA (CropStat) program. The significance of differences between mean values was estimated using Tukey's least significant difference (LSD) test.

3. Results

3.1. Plant Growth Characteristics

The effect of water regime on plant growth, i.e., shoots height, diameter, leaf number, was more pronounced at the 50–60% soil relative WC treatment than that of other variables (Table 4). All the estimated growth parameters decreased with reducing water content in the soil, although no significant difference was observed between 70–80% and 50–60% WC treatments in most cases. The lowest values were detected in the 10–20% WC treatment with more than a 2-fold decrease of the estimated growth parameters.

Table 4. Growth parameters of *Glycyrrhiza glabra* (at the end of vegetation period).

Soil Relative Water Content (%)	2017			2018			Plant Density (Thousand per Hectare)	
	Shoot Height (cm)	Root Diam. (mm)	Leaf Number	Shoot Height (cm)	Root Diam. (mm)	Leaf NUMBEr	2017	2018
70–80	95.6 a	6.9 a	18.1 a	115.8 a	7.4 a	24.0 a	722.0 b	1222.0 b
50–60	88.5 ab	6.5 a	17.7 a	104.1 a	7.2 b	23.4 b	735.0 a	1235.0 a
30–40	70.5 b	5.8 b	14.0 b	93.1 b	5.9 c	16.1 c	720.0 b	1220.0 b
10–20	51.4 c	3.4 c	7.8 c	54.2 c	3.8 d	10.8 d	711.3 c	1145.6 c

Means marked with different letters (a to d) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

Data presented in Table 5 shows that the green mass, straw and root yield traits considerably reduced with increasing water deficit stress. The highest values of the green mass, straw and root yield were found at 50–60% WC, which was exerted by 6.6%, 10% and 7.9%, respectively compared to the well-watered control treatment. Whereas, the lowest values were detected at 10–20% WC, decreasing the green mass, straw and root yield traits by more than three-fold than that of the control values. A reverse trend was observed in water productivity (WP) that enhancing water deficiency increased WP. The highest WP was detected at 30–40% WC, followed by 50–60% and 70–80% WC treatments. Compared to the 70–80% WC treatment, the highest plant productivity features were achieved at 50–60% WC.

Table 5. Biomass, forage and root yield production of *Glycyrrhiza glabra.*

Soil Relative Water Content (%)	Root Dry Weight, g		Green Mass (Mg/ha)	Straw Yield (Mg/ha)	Root Yield (Mg/ha)	Water Productivity (kg/m^3)
	0–30	30–50				
70–80	260.7 b	392.5 b	32.35 b	12.10 b	3.04 b	0.68 c
50–60	272.8 a	418.4 a	34.47 a	13.31 a	3.28 a	1.09 b
30–40	176.5 c	261.2 c	26.25 c	8.68 c	2.23 b	1.92 a
10–20	61.3 d	145.4	11.3 d	4.5 d	1.03 d	–

Means marked with different letters (a to d) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

3.2. Nutrients and Phytochemical Parameters

The licorice straw's nutrient content changed in connection with the soil WC treatments at both 2017–2018 growing seasons (Table 6). Substantial increases of N, P, and K contents in the plant straw was observed in the second year, showing a similar trend as in the case of plant growth parameters. As compared to the control (70–80% WC) treatment, significant increases of N, P and K contents by 10%, 8.3% and 6.25%, respectively, were recorded under the 50–60% WC treatment. The lowest nutrient content was detected at the 10–20 WC treatment, showing a substantial decrease of N, P and K by 12%, 10.6% and 17.1%, respectively, compared to the well-irrigated control treatment.

Table 6. N, P and K contents in the forage of *Glycyrrhiza glabra*.

Soil Relative Water Content (%)	2017			2018		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
70–80	0.690 b	0.460 b	1.050 b	1.100 b	0.480 b	0.900 b
50–60	0.785 a	0.480 a	1.080 a	1.210 a	0.520 a	0.960 a
30–40	0.670 c	0.440 c	1.000 c	1.080 c	0.470 c	0.820 c
10–20	0.569 d	0.378 d	0.963 d	0.982 d	0.368 d	0.812 d

Means marked with different letters (a to d) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

Water deficit affected the forage nutritional quality of licorice, causing considerable decreases of protein, oil, cellulose, and calcium, especially at the 10–20% WC treatment (Table 7). In both years, the highest values were recorded at the 50–60% WC treatment, followed by 70–80% WC treatment and the lowest parameters were found at the 10–20% WC treatment. Under the 50–60% WC treatment, the readings were as follows, protein 19.1%, oil 18.0%, cellulose 28.5%, ash 8.0% and calcium 2.1%. A slight decrease of these parameters was observed under the 70–80% WC treatment, but the difference did not reach to a significant level in most cases. However, significant differences were observed in all measured parameters at the 10–20% WC treatment, as compared to the control treatment values, decreasing the contents of protein 8.5%, oil 11.8%, cellulose 14.9%, ash 14.3% and calcium 16.7%.

Table 7. Forage nutritional quality of *Glycyrrhiza glabra*, (%).

Soil Relative Water Content, %	2017					2018				
	Protein	Oil	Cellulose	Ash	Ca	Protein	Oil	Cellulose	Ash	Ca
70–80	16.8 b	16.0 b	26.0 b	7.50 ab	2.00 ab	18.4 b	17.3 ab	26.5 b	7.08 b	1.95 b
50–60	18.5 a	17.2 a	27.5 a	8.93 a	2.20 a	19.1 a	18.0 a	28.5 a	8.00 a	2.10 a
30–40	11.0 c	12.8 c	22.9 c	7.00 b	1.78 b	17.6 c	16.1 b	24.8 c	7.00 b	1.80 b
10–20	9.8 d	8.7 d	20.1 d	6.03 c	1.12 c	10.2 d	9.4 c	19.8 d	6.08 c	1.03 c

Means marked with different letters (a to d) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

Data presented in Table 8 shows changes of the phytochemical compounds in the licorice roots in association with soil water content. At 50–60% WC, the amounts of ash, glycyrrhizic acid, extractive compounds and flavonoids were the highest among the tested water content treatments, exhibiting increases by 5.1%, 5.5%, 3.1% and 8.7%, respectively as compared to the control values, some values did not reach to a significant level between these treatments. At the lowest WC treatment, these parameters were significantly decreased by 19.2%, 21.9%, 25.4% and 22.8%, respectively than those of the control values.

Table 8. Phytochemical compounds of the roots of *Glycyrrhiza glabra*.

Soil Relative Water Content, %	Ash (%)	Glycyrrhizic Acid (%)	Extractive Compounds (%)	Flavonoids (%)
70–80	4.90 ab	7.40 b	38.98 a	2.05 b
50–60	5.15 a	7.81 a	40.15 a	2.23 a
30–40	4.76 b	6.95 c	35.95 b	1.91 c
10–20	4.11 c	6.07 d	31.08 c	1.67 d

Means marked with different letters (a to d) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

3.3. Soil Quality Attributes

Results of the experiment demonstrate that the production of licorice is crucial to reclaiming of drought-affected depleted lands and improving of soil fertility. Before beginning the experiment, soil humus content varied from 1.010% to 0.998% respectively at 0–30 and 30–50 cm soil layer (Table 9). These figures significantly enhanced at the end of the experiment, exhibiting increases by 8.5%, 10.9% and 6.5% at the 70–80%, 50–60% and 30–40% WC treatments, respectively. Similar patterns were observed for N and P concentrations in the soil, showing the highest values at the 70–80% WC treatment in both cases. Whereas, the highest values for K content in the soil were recorded under 30–40% WC treatment. On the other hand, exchangeable nitrate forms were higher at the 50–60% WC treatment than those of other treatments.

Table 9. Effect of *Glycyrrhiza glabra* cultivation on soil quality.

Soil Relative Water Content, %	Humus (%)		N (%)		P (%)		K (%)		Exchangeable Forms (NO_3), mg/kg	
	0–30	30–50	0–30	30–50	0–30	30–50	0–30	30–50	0–30	30–50
Before the experiment										
	1.010 c	0.998 c	0.100 c	0.098 b	0.164 d	0.095 b	1.85 a	1.000 a	20.8 e	11.3 d
At the end of the experiment										
70–80	1.096 a	0.115 a	0.118 a	0.109 a	0.178 b	0.100 a	1.78 b	0.900 b	29.1 b	13.8 a
50–60	1.120 a	1.010 b	0.119 a	0.110 a	0.188 a	0.100 a	1.75 c	0.890 b	30.1 a	13.9 a
30–40	1.076 b	1.010 b	0.117 b	0.109 a	0.172 c	0.099 a	1.73 c	1.000 a	28.1 c	13.1 b
10–20	1.011 c	1.002 c	0.103 c	0.999 b	0.165 d	0.096 b	1.69 d	0.820 c	27.5 d	12.8 c

Means marked with different letters (a to e) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

Licorice residue decomposition could be the main reason for the decreased soil bulk density while improving soil physical properties (Table 10). After straw and root residues combined, the total amount of soil residue was 11.09 Mg ha^{-1} under the 30–40% WC treatment. As soil WC increases, root residues enhanced concomitantly, although no significant difference was recorded between 70–80% WC and 50–60% WC treatments.

Table 10. Residues of licorice in the soil (Mg/ha).

Soil Relative Water Content, %	Residues	Roots		Total Residues
		0–30cm	30–50 cm	
70–80	3.51 ab	6.01 a	2.72 a	12.24 a
50–60	3.59 a	5.98 a	2.68 ab	12.25 a
30–40	3.01 b	5.41 b	2.67 b	11.09 b
10–20	2.88 c	4.86 c	2.14 c	9.88 c

Means marked with different letters (a to c) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

Whereas, the increased residues matter in the soil, especially at the higher WC treatment reflected on the improvement of soil chemical and possibly biological functions

(Table 11). For example, the licorice straw residues significantly increased soil nutrients such as N, P, and K values depending the soil WC treatments. The 50–60% WC treatments exerted additive effects on the macronutrient concentrations, presenting the increases of total values by 16.9% for N, 17.2% for P, and 13.1% for K over the 30–40 WC treatment. As predicted, substantial nutrients depletion was detected in the lowest WC plot.

Table 11. Nutritional profiles in residues and roots of *Glycyrrhiza glabra*, (%).

Soil Relative Water Content, %	Residue			Root			Total			A Amount of N Remained in 1 ha, kg
	N	P	K	N	P	K	N	P	K	
70–80	0.47 a	0.25 a	0.62 b	1.10 b	0.48 a	0.68 a	1.57 b	0.73 a	1.3 b	31.8 b
50–60	0.48 a	0.26 a	0.68 a	1.18 a	0.49 a	0.70 a	1.66 a	0.75 a	1.38 a	32.9 a
30–40	0.42 b	0.22 b	0.58 c	1.00 c	0.42 b	0.64 b	1.42 c	0.64 b	1.22 c	28.5 c
10–20	0.39 c	0.21 b	0.46 d	0.82 d	0.34 c	0.54 c	1.31 d	0.48 c	1.08 d	25.6 d

Means marked with different letters (a to d) represent significant differences ($p < 0.05$) according to Tukey's LSD test.

Figure 1 shows the increased licorice residues had an additive effect on soil quality depending on the water regimes. Highly positive correlations were revealed between soil humus content and soil N content vs. total residues with Pearson's coefficient values of $R^2 = 0.931$ and $R^2 = 0.927$, respectively.

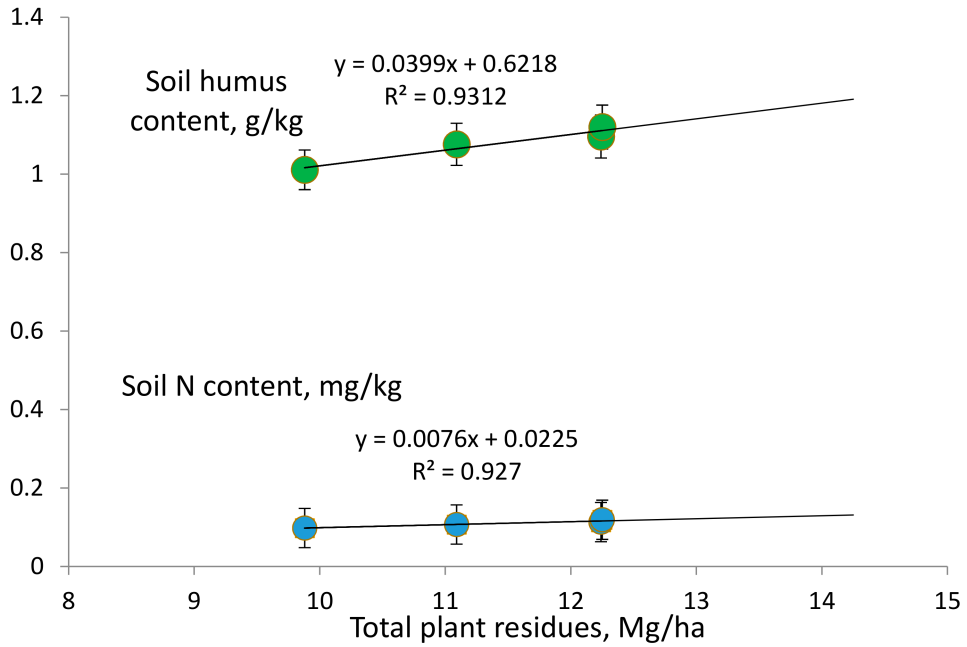

Figure 1. The correlation between total plant residues vs. soil humus content and soil N content determined at the end of the experiment. Blue circles define soil N content and green circles define soil humus content in response to the total licorice residues.

4. Discussion

During the one-year experiment, licorice growth was slow in all plots depending on the soil WC treatments (Table 4), because these seed-grown plants need some to establish. However, substantial growth, i.e., plant height, leaf number, stem diameter, in all watering regimes was observed at the second experiment season. It is highly likely that the plants could have formed a strong root system by this period. The reduction of leaf area and shoot weight eventuates in plants as a typical response mechanism to water stress, and this challenge is alleviated through reducing the transpiration area [16]. Under severe water deficit condition, plant transpiration and photosynthesis processes reduce after partial closing of stomata which usually lead to the increase of WP [17]. Plant biomass

production under drought stress contributes to the increase of the efficiency of consumed water, thereby the plant strives to avoid water loss.

Drought stress induces changes in plant physiology and morphology through decreasing metabolism consumption and shoot growth, thereby partitioning more assimilates to the roots that allow maintaining a higher root-to-shoot ratio [11]. These stress adaptability functions of licorice plants allow them to survive under extreme conditions. i.e., drought, salinity, and heat stresses, and thereby develop proper tolerance mechanisms. In agreement with these phenomena, the results of this experiment also showed a slight reduction in the weight of the dry root compared with that of the shoot mass. More specifically, in the 50–60% WC application, the plant growth parameters were higher than in the 70–80% WC procedure, suggesting a low water deficit promotes growth and biomass development of the licorice plant. This impact is consistent with a depth rooting method and an effective licorice transpiration process that retains growth dynamics over a long vegetative period, also under a deficit irrigated environment.

It is well-known that licorice accumulates less secondary metabolites in the roots under stress environment, defining sufficient WC is essential for normal biosynthesis processes [18]. Glycyrrhizic acid level varies between 5–10% of the weight of the root of the licorice plant depending on various growing conditions. In our experiments, this value ranged between 6.04 to 7.40%, which are in agreement with previous reports [19]. However, this experiment confirms that a low water deficit may induce secondary metabolite biosynthesis in the licorice roots, improving the quality of the raw products. This outcome is consistent with the findings of several researchers who confirmed that low drought-stress plants generally produce higher levels of secondary metabolites [20,21]. It is highly likely that mutualistic association of beneficial microbes in the rhizosphere of licorice contributed to utilising water and nutrients more efficiently and improved soil quality.

Many researchers have noted that crop residues decrease soil bulk density and temperature while maintaining a good plant growth condition [22–25]. Consistent with these findings, licorice residues in this study also had many beneficial effects on the improvement of soil physical structures, chemical compositions and possibly biological functions. It turned out that the enhanced abundance of macronutrients in the soil contributes to the beneficial actions of microbes in the root rhizosphere to secrete organic acids and lower the pH in their area, which could have induced the breakdown of bound phosphates in the soil [26]. It is well documented that the significant effects of soil microorganisms depend on soil moisture [27–30]. According to Shantz et al. (2016), a number of processes have developed in licorice to thrive under exceptionally harsh conditions, including mutualism with beneficial soil microbes that promote nutrient and water absorption and improve drought stress tolerance of plants [31].

5. Conclusions

In this study, licorice plants were able to grow and produce biomass even at 10–20% WC, suggesting that this plant can acclimate in response to unfavorable environment and exhibit high drought resistance. However, the morphological and physiological parameters of licorice progressively decreased with increasing WC. The weak water deficit (50–60% WC) showed the highest values of total root weight, plant biomass, leaf number and promoted the secondary synthesis metabolites—i.e., glycyrrhizic acid, protein, oil—more than other water regimes. Moreover, the correct irrigation regime of licorice had a significant positive influence on soil organic matter, and available N, P, K contents.

Stress adaptability features of licorice, i.e., drought and salt tolerance, allow for this plant's cultivation under extremely harsh environments and might be used as a valuable practice to combat desertification through developing a highly value-added sustainable crop production system in arid regions.

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