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BRIEF COMMUNICATIONS

Effect of Water Deficit on Biomass Production and Accumulation of Secondary Metabolites in Roots of *Glycyrrhiza uralensis*[1]

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Abstract—Two-year-old seedlings of licorice plant (*Glycyrrhiza uralensis* Fisch) were exposed to three degrees of water deficit, namely weak (60–70%), moderate (40–50%), and strong (20–30%) relative water content in soil, whereas control plants were grown in soil with 80–90% water content. Moderate and strong water deficit decreased the net photosynthetic rate, stomatal conductance, and biomass production. Water use efficiency and the root-to-shoot ratio increased significantly in response to water deficit, indicating a high tolerance to drought. Weak water deficit did not decrease root biomass production, but significantly increased the production of glycyrrhizic acid (by 89%) and liquiritin (by 125%) in the roots. Therefore, a weak water deficit can increase the yield of root medical compounds without negative effect on root growth.

Keywords: Glycyrrhiza uralensis, biomass production, gas exchange, glycyrrhizic acid, liquiritin, water deficit.

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INTRODUCTION

Licorice (*Glycyrrhiza uralensis* Fisch.) is a very popular medicinal plant, which roots contain glycyrrhizic acid and liquiritin mainly accumulated in the root and rhizome tissues [1, 2]. Recently, glycyrrhizic acid has been found to be highly active in inhibiting the replication of the severe acute respiratory syndrome (SARS)-associated virus and has been suggested as a potential therapeutic agent for chronic hepatitis and acquired immunodeficiency syndrome (AIDS) [3]. Licorice plants appear to be highly drought-tolerant, being a favorable plant to restore degraded desert, arid and semiarid ecosystems of northwest China [4]. However, data on physiological processes, such as biomass production and secondary metabolite yield, in response to environmental conditions are lacking [5].

Water deficit usually inhibits plant growth and productivity by affecting gas exchange and especially photosynthesis [6, 7]. Water use efficiency (WUE) can be traditionally defined as the ratio of net photosynthesis to transpiration over a period of seconds or minutes [8]. The higher WUE has been mentioned as a strategy to improve crop performance under water-limited conditions [9]. However, in licorice plants photosynthesis and biomass production as well as WUE in response to water deficit were not studied.

Water deficit can induce the biosynthesis of some secondary metabolites [10–12], resulting in their accumulation in medicinal plants [10, 13, 14]. For example, the concentration of rutin and chlorogenic acid increased with drought severity in tomato plants [14]. Although the responses of the metabolites to drought have been investigated in some medicinal plants [4, 10], no reference concerning the effect of various water deficit levels on their production by licorice roots is available.

The present study aims to determine the effect of water deficit on gas exchange, biomass and secondary metabolites production in licorice plants. It was hypothesized that a suitable water deficit, in addition to saving water, can also increase the amount of root secondary metabolites without negative effect on root growth.

MATERIALS AND METHODS

Plants and experimental design. The experiment was performed in a greenhouse at Beijing University of Chinese Medicine. Seeds were collected from one

[1] This text was submitted by the authors in English.

Abbreviations: WC—water content; WUE—water use efficiency.

Table 1. Net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency in *Glycyrrhiza uralensis* under different soil relative water contents

Soil relative water content, %	Net photosynthetic rate, μmol/(m^2 s)	Stomatal conductance, mol/(m^2 s)	Transpiration rate, mmol/(m^2 s)	Water use efficiency, μmol/mmol
80–90	15.0 ± 0.3^a	0.24 ± 0.01^a	5.8 ± 0.1^a	2.6
60–70	14.3 ± 0.4^a	0.23 ± 0.01^a	3.6 ± 0.1^b	4.0
40–50	12.3 ± 0.5^b	0.22 ± 0.02^a	3.5 ± 0.1^b	3.5
20–30	10.3 ± 0.4^c	0.17 ± 0.01^b	3.4 ± 0.1^b	3.0

Notes: Measurements were made at a sunny day on July, 2007 after more than two-month-long treatment. Water use efficiency was calculated as the ratio of net photosynthetic rate to transpiration rate. Means followed by different letters indicate significant differences at $P < 0.05$. $n = 4$.

population in Hangjinqi, Inner Mongolia. Seeds were soaked in concentrated H_2SO_4 for 20 min, washed several times with tap water, and then sown immediately in small plastic pots filled with 450 ml of sand mixture on May 6, 2006. After germination, four seedlings per pot were selected and cultivated for a year. On April 15, 2007, the seedlings were transplanted to bigger plastic pots filled with sandy soil with pH 7.06, containing nitrogen (223 mg/kg), organic mass (3.1 g/kg), and the available P and K contents of 13.7 and 28.0 mg/kg, respectively. Each pot contained four seedlings.

Water treatments were carried out from May 15 until the end of October in 2007. Four levels of soil relative water content (WC), 80–90, 60–70, 40–50, and 20–30%, represented control plants, weak, moderate, and strong water deficit, respectively. Each pot was weighed and water was added to reach the target level at 6:00 p.m. every day. There were four replications per treatment arranged in a completely randomized block design.

Leaf gas exchange. The newly developed leaves from the middle part of the shoot were chosen for gas exchange measurement using a Li-6400 portable pho-

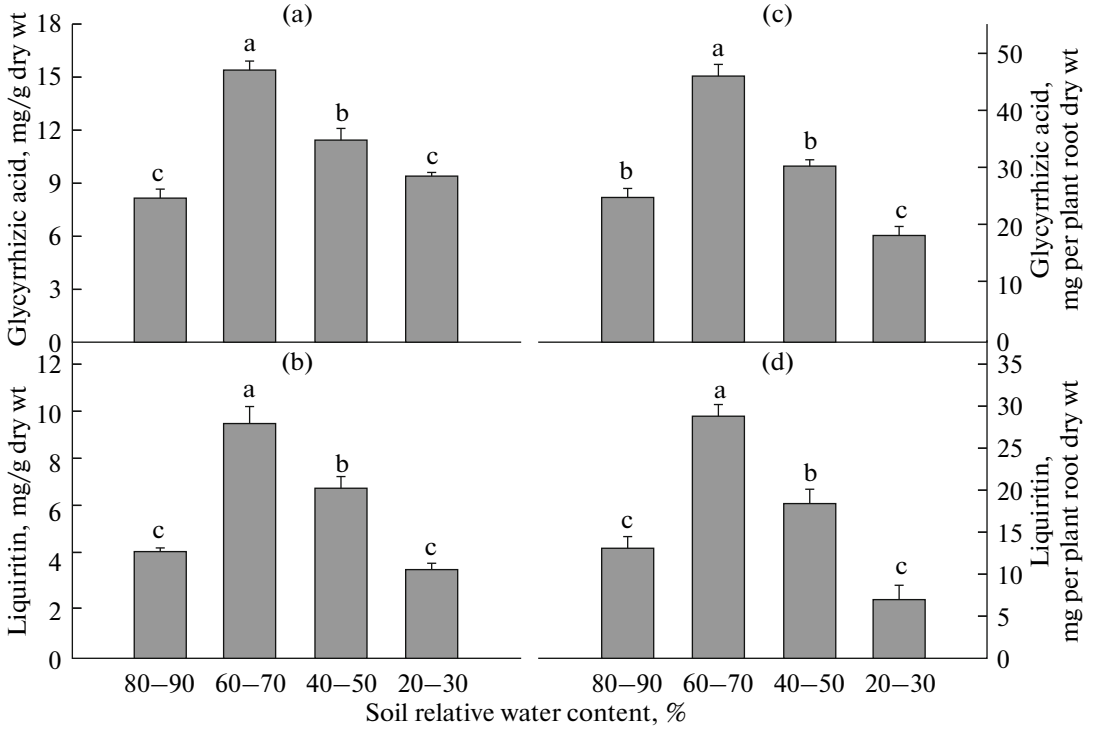

Fig. 1. Contents of glycyrrhizic acid and liquiritin in the roots of *Glycyrrhiza uralensis* expressed per gram dry weight (a, b) and per plant roots (c, d) under different soil relative water contents on the end of October in 2007 after more than five-month-long treatment. Different letters indicate significant differences at $P < 0.05$. $n = 4$.

Table 2. Dry weights of whole plant, roots, and shoots; decreases in the root and shoot dry weights; and root-to-shoot ratio in *Glycyrrhiza uralensis* under different soil relative water contents

Soil relative water content, %	Whole plant dry wt, g	Root dry wt, g	Root dry wt decrease, %	Shoot dry wt, g	Shoot dry wt decrease, %	Root-to-shoot ratio
80–90	5.6 ± 0.2^{a}	3.1 ± 0.1^{a}	0	2.5 ± 0.2^{a}	0	1.26 ± 0.10
60–70	5.2 ± 0.2^{a}	3.0 ± 0.2^{ab}	–2.3	2.2 ± 0.2^{ab}	–11.9	1.40 ± 0.12
40–50	4.6 ± 0.2^{b}	2.7 ± 0.1^{b}	–13.1	1.9 ± 0.2^{b}	–24.6	1.48 ± 0.15
20–30	3.3 ± 0.2^{c}	1.9 ± 0.1^{c}	–37.6	1.4 ± 0.2^{c}	–46.0	1.53 ± 0.18

Note: Measurements were made at the end of October in 2007 after more than five-month-long treatment. Dry weight of root or shoot decrease percent (%) = (treatment – control)/control × 100%. Means followed by different letters indicate significant differences at $P < 0.05$. $n = 10$.

tosynthesis system (Li-Cor, United States) at a sunny day on July, 2007. The measurements were made from 8:00 a.m. to 9:30 a.m., under approximate photosynthetic photon flux density of 1200–1400 μmol/(m^2 s), and ambient CO_2 concentration of 380 μmol/mol. The net photosynthetic rate, stomatal conductance, and transpiration rate were simultaneously measured. Water use efficiency (WUE) was calculated as the ratio of the net photosynthetic rate to transpiration rate.

Biomass determination. Biomass determination was carried out by the end of October. Licorice plants were separated into the roots and shoots. Dry weights were determined after drying for 72 h at 50°C in an oven. Root-to-shoot ratio = root dry weight: shoot dry weight. Then the dried roots were used for glycyrrhizic acid and liquiritin analyses.

Glycyrrhizic acid and liquiritin analyses. Glycyrrhizic acid and liquiritin were extracted as described in [15]. Dry roots were extracted with a tenfold volume of 0.3% ammonia for 30 min under ultrasonication (250 W, 20 KHz). Glycyrrhizic acid and liquiritin concentrations were determined with a HP1100 high performance liquid chromatography system (Agilent Technologies, United States) consisting of a G1311A pump, a G1379A degasser, and a G1313A autoinjector connected to a G1315B diode array detector (DAD). The separation was performed on a DIKMA Diamonsil^{TM}-C_{18} column (250 mm × 4.6 mm × 5 μm) with a mobile phase consisting of 0.1% H_3PO_4 (solvent A) and acetonitrile (solvent B). The sample (10 μl) was eluted with a gradient profile, and the column was maintained at 25°C [5, 15].

Statistical analysis. Statistical treatment was performed using a SPSS statistical package (version 13, SPSS, Chicago, United States). The difference between the mean values of each treatment was determined using Duncan's multiple range test and considered significant at $P < 0.05$.

RESULTS

Gas Exchange

The net photosynthetic rate, stomatal conductance, and transpiration rate decreased with increasing water deficit (Table 1). Compared to the control, 60–70% WC had no effecton the net photosynthetic rate and stomatal conductance, but at 40–50 and 20–30% WC, photosynthesis and transpiration were significantly reduced. However, water deficit increased WUE and the highest value was observed at the 60–70% WC.

Biomass Production

Dry weight of the plant and its organs decreased with increasing water deficit, but no effect was exerted at 60–70% WC (Table 2). Root dry weight decreased by 2.3, 13.1, and 37.6% with increasing water deficit, while shoot dry weight decreased by 11.9, 24.6, and 46.0%, respectively (Table 2). Root-to-shoot ratio increased as water deficit progressed (Table 2).

Changes in Glycyrrhizic Acid and Liquiritin Contents in the Roots

The gain in the content of glycyrrhizic acid and liquiritin in the roots under 60–70% WC had the highest (89.4 and 124.6%, respectively), followed by the moderate treatment (40.0 and 61.3%, respectively) as compared to the control plants. There was no significant difference between the strong WC treatment and control (Figs. 1a, 1b). At 60–70% WC, the amounts of glycyrrhizic acid and liquiritin in plant roots were the highest among the studied four levels of soil water conditions: the gain increased by 85.0 and 119.4%, respectively (Figs. 1c, 1d). At the lowest WC, glycyrrhizic acid amount in plant roots decreased, although

no difference in liquiritin amount between these and control plants was found (Figs. 1c, 1d).

DISCUSSION

It is well known that water deficit is one of the major factors limiting plant growth and yield [6, 7]. In this study, licorice plants were able to grow and produce biomass even at 20–30% WC (Table 2), suggesting that this plant can acclimate in response to unfavorable environment and exhibit high drought resistance. Water deficit induces partial closing of stomata and both transpiration and photosynthesis decrease, thereby slightly increasing WUE [16], especially at 60–70% WC (Table 1). The increase in the efficiency of water use under drought occurs at the expense of absolute biomass production [16]. In order to diminish metabolism consumption and increase uptake of water under dry conditions, plants often decrease their growth rate and biomass production, and contribute more synthesized biomass to roots, so that they could maintain a higher root-to-shoot ratio (Table 2). Partitioning more assimilate to the underground parts and maintaining the higher root-to-shoot ratio may contribute to enhanced water uptake [16]. Thus, these responses allow licorice plants to survive and even to continue to grow under conditions of water shortage, i.e., to develop drought tolerance.

Water deficit decreases plant photosynthesis and thereby reduces plant growth and biomass production (Tables 1, 2). However, the dry weight of the roots decreased less than that of shoots (Table 2). No significant difference was found between the dry weights of roots and the shoots at 60–70 and 80–90% WC, while other treatments significantly decreased biomass production (Table 2). Our findings indicate that weak water deficit did not affect growth and biomass production of licorice roots.

It has been well documented that water deficit can also affect the production of secondary metabolites in some medicinal plants [10, 12, 13]. Biosynthesis of secondary metabolites is known to be affected by drought, indicating influence by environmental stimuli [10, 11]. For example, the content of several alkaloids increased in response to drought in *Tabernaemontana pachysiphon* [13]. Liu [10] also found that camptothecin concentrations briefly rose when *Camptotheca acuminata* seedlings experienced drought. Glycyrrhizic acid and liquiritin, the major bioactive components of *G. uralensis*, are accumulated in the underground parts of licorice plants [1, 2]. Thus, soil WC plays a key role in their biosynthesis. Weak water deficit significantly increased not only glycyrrhizic acid and liquiritin concentrations per gram dry weight (Figs. 1a, 1b), but also total amount of these compounds in plant roots (Figs. 1c, 1d). The synthesis of secondary metabolites was stimulated under weak drought conditions. Zhu et al. [12] also found that mild water deficit significantly increased saikosaponin a and d contents in *Bupleurum chinense* roots. Our results confirm that weak water deficit can increase secondary metabolite contents and thereby increase the quality of medical row material.

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