INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0197/2019/22–3–1285–1292
DOI: 10.17957/IJAB/15.1200
http://www.fspublishers.org

Full Length Article

Physiological Mechanisms of Salt and Drought Induced Stress Effects on Root Biomass and Secondary Metabolites in *Stellaria dichotoma*

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Abstract

Stellaria dichotoma L. *var. Lanceolata Bge*, a well-known medicinal plant, has more than 20 effective components, and flavonoids and saponins are major two of important effective components in *S. dichotoma*. A pot experiment was conducted to study the interactive effect of NaCl and drought stresses on root biomass, effective components accumulation and physiological - biochemical parameters of two-year-old cultivated *S. dichotoma* by a pot experiment. The result showed that root yield significantly increased with decrease in soil water content under sample soil (S_0), while decreased with decrease in soil water content under salt stressed soil (S_1). The result also showed that drought stress remarkably affected the accumulation of flavonoids and saponins, and the concentration of endogenous hormone and chlorophylls; generally, this effect was different depending on soil salinity level and on growth stage. The root yield was significantly positive correlated with shoot dry weight, while significantly negative correlated with ABA concentration in leaves. Moreover, the total flavonoids accumulative amount was significantly positive correlated with root biomass, GA_3 and ABA concentrations in leaves. In addition, the total saponins accumulative amount was significantly correlated with root biomass and ABA concentration in leaves. These results suggest that moderate drought stress could enhance secondary metabolites accumulation by changing GA_3 and ABA concentrations and growth of *S. dichotoma* under sample soil condition (S_0). However, drought stress aggravates adverse effects of salt stress on secondary metabolites accumulation by changing GA_3 and ABA concentrations and growth under salt stress condition (S_1).

Keywords *Stellaria dichotoma* L. var. *Lanceolata Bge*; Salt combined drought stress; Root yield; Total saponins; Total flavonoids; Endogenous hormone

Introduction

Stellaria dichotoma L. *var. Lanceolata Bge*, a well-known medicinal plant. Their roots have been extensively used as traditional Chinese medicines due rich in abundant secondary metabolites (Ye *et al.*, 2012). Wild *S. dichotoma* is distribute to the desert and semidesert of northwest China, and possessed well ability of drought and salt tolerance (Bao *et al.*, 2006). Nevertheless, in recent years, disorder over-consumption resulted in severe declines of wild *S. dichotoma*. Therefore, cultivated *S. dichotoma* has become the main source of market supply. Unfortunately, research has shown that the contents of secondary metabolites in cultivated *S. dichotoma* roots was significantly lower than in wild *S. dichotoma* roots (Bao *et al.*, 2006). Therefore, improving secondary metabolites in cultivated *S. dichotoma* would be a meaningful research topic. Previous studies on *S. dichotoma* mainly focused on chemical component (Morita *et al*., 2005), pharmacological action (Sun *et al*., 2004) and amongst others. However, there is sparse information available about effects of soil salinity and drought stress on yield and quality of *S. dichotoma*. Contrarily, in cultivated practice of *S. dichotoma*, soil salinity and drought stress usually reduce growth of *S. dichotoma* in arid and semiarid area of northwest in China. Based on this background, recently, researchers found that mild drought stress or salt stress alone can promote growth and accumulation of active ingredients of cultivated *S. dichotoma* by adjusting some physiological - biochemical indexes (Zhou *et al*., 2015). Actually, combination of salt and drought stress is regarded as universal factor that affects plant growth simultaneously in the field.

To cite this paper: Zhou, L., D. Lang, W. Zhang, J. Wang, X. Gao, X. Wu, X. Fu, X. Zhang, 2019. Physiological mechanisms of salt and drought induced stress effects on root biomass and secondary metabolites in *Stellaria dichotoma*. *Intl. J. Agric. Biol*., 22: 1285–1292

Soil salinity and drought are two of the most vital and universal abiotic stress factors that influence plant growth and development (Bartels and Sunkar, 2005; Zhang *et al*., 2018), and limit crops productivity in cultivated areas worldwide; moreover, this adverse effect is to worsening at present (Chaves *et al*., 2009; Sun *et al*., 2015). Interestingly, many studies found that plants subjected to salt or drought stress could accumulate higher contents of secondary metabolites than those grown under normal condition (Adachi *et al*., 2010; Azhar *et al*., 2011; Ballhorn *et al*., 2011; Chen *et al*., 2011; Forouzandeh *et al*., 2012; Jaafar *et al*., 2012), which indicate that medicinal plants grown under unfavorable conditions usually produce higher concentrations of active substances than same species grown under well-favorable condition (Kleinwächter and Selmar, 2015). Up to now, many studies have been devoted to assess the physiological response of plants in a single stress of salinity or drought. However, there was few researches focus on the combined effects of drought and salt stress on plant (Erdei *et al*., 1990; Wu *et al*., 2011; Ahmed *et al*., 2013), especially in medicinal plants.

In the present study, to better understand the adaptability of cultivated *S. dichotoma* to soil salinity and drought stresses. According to the recent studies (Zhou *et al*., 2015), two levels of soil salinity and three levels of water supply were designed to assess root biomass production, secondary metabolites and physiological characteristics, aimed to explicit the effect on salt combined drought on root yield and secondary metabolites and its physiological mechanism, and further to confirm the optimum soil salinity and water requirement for cultivation of *S. dichotoma*.

Materials and Methods

Plant Materials and Experimental Design

One-year-old cultivated *Stellaria dichotoma* seedlings were collected from Shennong garden herbs Co., Ltd., in Guyuan city, Ningxia province, China.

Thirty days after transplanting the *S. dichotoma*, the seedlings were thinned to four per pot and exposed to salt and drought stress treatment. Salt stress (soil salt content / soil dry weight) by NaCl solution configuration of different gradient irrigation to pots with the plants, set up two levels, that is sample soil (containing 0.2 g soluble salt per kg dry soil, S_0) and salt stressed soil (sample soil added 0.2 g NaCl per kg dry soil, S_1) respectively. Seven days after NaCl treatment, moisture stress was imposed. Specifically, drought stress using soil field capacity design method, includes three soil moisture levels, (i) 60–70% soil field capacity (D_0), represent as the normal supply water; (ii) 50–60% soil field capacity (D_1), represent as the mild drought stress; (iii) 40–50% soil field capacity (D_2), represent as the severe drought stress. The water supplies were maintained by weighing the pots. The experiment was consisted of 6 (2×3) salt-drought treatments, specifically, S_0D_0 (as control, CK), S_0D_1, S_0D_2, S_1D_0, S_1D_1, S_1D_2. Each treatment was repeated 8 pots and designed by randomized block arrangement. After drought stress treatment for 60 d and 90 d (Sep and Oct), the *S. dichotoma* samples were respectively harvested to determine growth, biomass accumulation and physiological characteristics.

Root Yield Assay

Plants were washed by running water and separated into roots and shoots, then oven dried at 60°C for 48 h for dry weight.

Content of Total Flavanones and Total Saponins Assay

Total flavanones and saponins were extracted as described in State Pharmacopoeia Committee (2015). Generally, for estimation of total flavonoid content (TFC), 0.059 g powdered plant (root or shoot) material was added with 25 mL methanol (50% pure) and then extracted by ultrasonic at room temperature for 1.5 h, filtered, diluted to 25 mL volumetric flask and set aside. Colorimetric method was used for TFC determination (Ul–Haq *et al.*, 2012). TFC was quantified according to the corresponding rutin standard curve. As to total saponins content (TSC) determination, colorimetric method was used. TSC was quantified according to the corresponding ginsenosides standard curve of. Absorption of extracts for determination of TFC and TSC were taken at 530 nm and 537 nm, respectively, using an SP-752PC spectrophotometer (Shanghai Spectrum Instruments Inc., Shanghai, P. R. China). The relative content and accumulative amount of total flavanones and total saponins were calculated as following:

Relative content (mg g^{-1})= TFC or TSC weight (mg) / plant material dry weight (g)

Accumulative amount (mg pot^{-1})= relative content (mg g^{-1})× root or shoot dry weight (g pot^{-1}).

Endogenous Hormone Concentration Assay

Two plants per pot were selected randomly and the similar, healthy and mature leaves of that plant was cut and immediately frozen in liquid nitrogen for hormone extraction (Yang *et al*., 2001). Extraction and purification of phytohormones (ABA, GA_3 and IAA) according to method described by Yang *et al*. (2001) and Haver *et al*. (2003). Assays of ABA, GA_3 and IAA were conducted using an HPLC system based on the methods with modifications (Iqbal *et al*., 2006).

Measurement of Chlorophylls Concentration

Chlorophyll a and b of the fully expanded leaf was collected for quantitatively measured in 80% acetone mixed with the same volume of anhydrous ethanol extract according to Ouzounidou *et al*. (2008).

Statistical Analysis

All experimental data were subjected to analysis of variance (ANOVA) using S.P.S.S. software and the treatment means were tested using least significant differences (LSD) at $P < 0.05$ level.

Result

Effects on Root Dry Weight

Drought stress solely increased root dry weight compared with control during the experiment period, and this increase effect was increase with increase of drought stress level. However, NaCl combined drought significantly decreased root dry weight at 90 d after drought treatment, and with a greater degree of inhibition caused by a higher drought stress level (Fig. 1).

Effects on the Relative Contents and Accumulative Amount of Total Flavonoids

At 60 d after drought treatment, drought, NaCl or NaCl combined drought had no significant effect on the total flavonoids relative content in root and shoot; however, at 90 d after drought treatment, drought, NaCl or NaCl combined drought increased the total flavonoids relative content in root compared with control, but there was no significant difference except for the NaCl combined severe drought stress treatment (S_1D_2) (Fig. 2A and C).

During the experiment period, drought stress sole had no significant effect on the total flavonoids accumulation in root and shoot, except for the NaCl combined severe drought stress treatment (S_1D_2) on the total flavonoids accumulation in shoot. However, NaCl solely or NaCl combined drought all significantly decreased the total flavonoid accumulation (Fig. 2B and D).

Effects on the Relative Contents and Accumulation of Total Saponins

At 60 d after drought treatment, drought stress solely significant increased the relative content of total saponins in root and shoot compared with control, while NaCl solely or NaCl combined drought had no significant effect on the relative content of total saponins. However, at 90 d after drought treatment, drought and NaCl solely or drought combined NaCl all significantly decreased the relative content of total saponins in root compared with control; also, NaCl solely or NaCl combined drought significantly decreased relative content of total saponins in shoot (Fig. 3A and C).

At 60 d after drought treatment, NaCl solely significantly decreased the total flavonoids accumulation in root, while drought and NaCl solely, or NaCl combined drought all significantly decreased the total flavonoids accumulation in shoot. However, at 90 d after treatment,

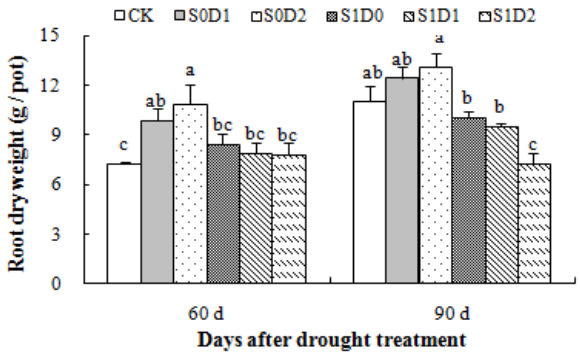

Fig. 1: Effect of NaCl combined drought stress on root dry weight of *S. dichotoma* at 60 d and 90 d respectively after treatment. Vertical bars indicate ± standard error. Values followed by the same letter are not significantly different at $P \leq 0.05$ (Student's t test), within the same growth stage

Note: C. control; S0D1. sample soil with 50-60% soil field capacity; S0D2. sample soil with 40-50% soil field capacity; S1D0, sample soil added 0.2 g NaCl per kg dry soil with 60-70% soil field capacity; S1D1. sample soil added 0.2 g NaCl per kg dry soil with 50-60% soil field capacity; S1D2. sample soil added 0.2 g NaCl per kg dry soil with 40-50% soil field capacity

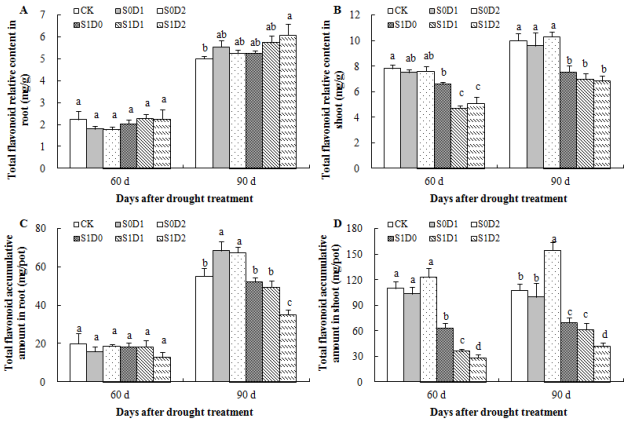

Fig. 2: Effect of NaCl combined drought stress on relative contents and accumulative amount of total flavonoids in *S. dichotoma* at 60 d and 90 d respectively after treatment. Vertical bars indicate ± standard error. Values followed by the same letter are not significantly different at $P \leq 0.05$ (Student's t test), within the same growth stage

Note: C. control; S0D1. sample soil with 50-60% soil field capacity; S0D2. sample soil with 40-50% soil field capacity; S1D0, sample soil added 0.2 g NaCl per kg dry soil with 60-70% soil field capacity; S1D1. sample soil added 0.2 g NaCl per kg dry soil with 50-60% soil field capacity; S1D2. sample soil added 0.2 g NaCl per kg dry soil with 40-50% soil field capacity

drought solely significantly increased the total flavonoids accumulation in shoot, while NaCl solely or NaCl combined drought all significantly decreased the total flavonoids accumulation in shoot compared with control (Fig. 3B and D).

Effects on Endogenous Hormones in Leaves

Drought stress solely decreased GA_3 and ABA concentration in leaves of *S. dichotoma* compared with

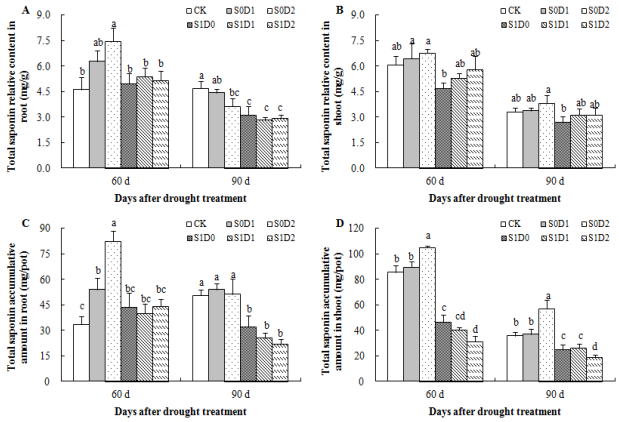

Fig. 3: Effect of NaCl combined drought stress on relative contents and accumulative amount of total saponins in *S. dichotoma* at 60 d and 90 d respectively after treatment. Vertical bars indicate ± standard error. Values followed by the same letter are not significantly different at $P \leq 0.05$ (Student's t test), within the same growth stage

Note: C. control; S0D1. sample soil with 50-60% soil field capacity; S0D2. sample soil with 40-50% soil field capacity; S1D0, sample soil added 0.2 g NaCl per kg dry soil with 60-70% soil field capacity; S1D1. sample soil added 0.2 g NaCl per kg dry soil with 50-60% soil field capacity; S1D2. sample soil added 0.2 g NaCl per kg dry soil with 40-50% soil field capacity

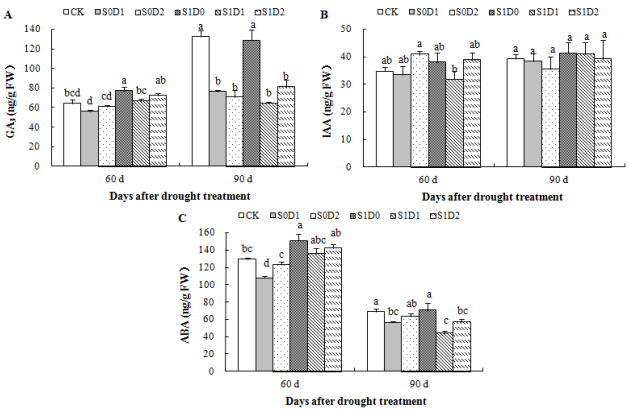

Fig. 4: Effect of NaCl combined drought stress on endogenous hormones in *S. dichotoma* leaves on at 60 d and 90 d respectively after treatment. Vertical bars indicate ± standard error. Values followed by the same letter are not significantly different at $P \leq 0.05$ (Student's t test), within the same growth stage

Note: C. control; S0D1. sample soil with 50-60% soil field capacity; S0D2. sample soil with 40-50% soil field capacity; S1D0, sample soil added 0.2 g NaCl per kg dry soil with 60-70% soil field capacity; S1D1. sample soil added 0.2 g NaCl per kg dry soil with 50-60% soil field capacity; S1D2. sample soil added 0.2 g NaCl per kg dry soil with 40-50% soil field capacity

control during the experiment period; however, NaCl solely or NaCl combined drought all increased GA_3 concentration at 60 d after drought treatment and ABA concentration during the experiment period, while NaCl combined drought significantly decreased GA_3 concentration at 90 d after drought treatment compared with control (Fig. 4A and C). Drought and NaCl solely or NaCl combined drought had no significant effect on IAA concentration in leaves of *S. dichotoma* compared with control (Fig. 4B).

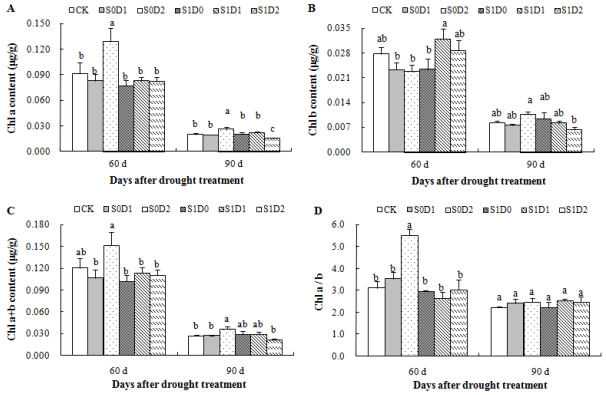

Fig. 5: Effect of NaCl combined drought stress on chlorophyll concentration in *S. dichotoma* leaves at 60 d and 90 d respectively after treatment. Vertical bars indicate ± standard error. Values followed by the same letter are not significantly different at $P \leq 0.05$ (Student's t test), within the same growth stage

Note: C. control; S0D1. sample soil with 50-60% soil field capacity; S0D2. sample soil with 40-50% soil field capacity; S1D0, sample soil added 0.2 g NaCl per kg dry soil with 60-70% soil field capacity; S1D1. sample soil added 0.2 g NaCl per kg dry soil with 50-60% soil field capacity; S1D2. sample soil added 0.2 g NaCl per kg dry soil with 40-50% soil field capacity

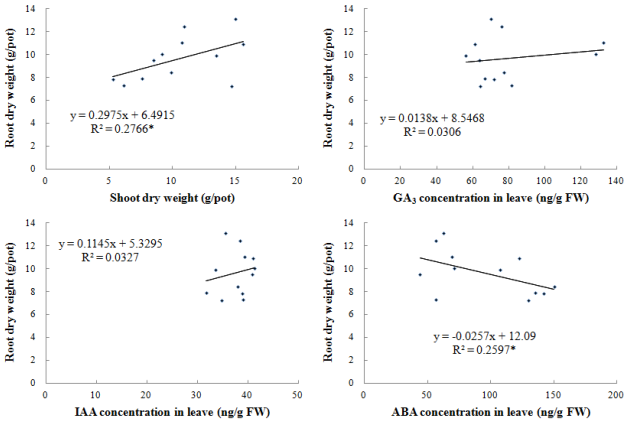

Fig. 6: Correlation between biomass, hormone concentrations and root dry weight. R=correlation coefficient, *=statistically significant ($P < 0.05$), **=statistically significant ($P < 0.01$)

Effects on Chlorophyll Content in Leaves

At 60 d after drought treatment, severe drought (S_0D_2) solely significantly increased chlorophyll a content and chl a/b ratio. However, at 90 d after drought treatment, severe drought (S_0D_2) solely significantly increased chlorophyll a and chlorophyll a+b contents (Fig. 5).

Relationship between Biomass and Hormone Concentrations, Root Yield and the Relative Content and Accumulative amount of Total Flavonoids and total Saponins in Root

As shown in Fig. 6, there was significant positive correlation between shoot dry weight and the root yield of *S. dichotoma*, and the linear regression was

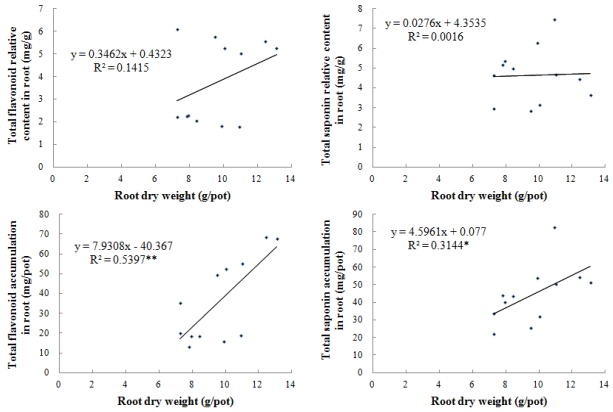

Fig. 7: Correlation between root yield and the relative content and accumulative amount of total flavonoids and total saponins in root. R=correlation coefficient, *=statistically significant (P < 0.05), **=statistically significant (P < 0.01)

Y=0.2975X+6.4916 (R^2=0.2766, P < 0.05). There was significant negative correlation between ABA concentration and the root yield of *S. dichotoma*, and the linear regression was Y=-0.0257X+12.09 (R^2=0.2597, P < 0.05). However, there was no significant correlation between IAA concentration, GA_3 concentration and the root yield of *S. dichotoma.*

Relationship between Root Yield and the Relative Content and Accumulative Amount of Total Flavonoids and Total Saponins in Root, Endogenous Hormones and the Relative Content and Accumulative Amount of Total Flavonoids in Root, Endogenous Hormones and the Relative Content and Accumulative Amount of Total Saponins in Root

There were no significant correlations between root yield and the relative contents of total flavonoids and total saponins in root respectively. However, there were significant positive correlation between root yield and the accumulative amount of total flavonoids and total saponins in root, and the linear regressions were Y=7.9308X-40.367 (R^2=0.5397, P<0.01) and Y=4.5961X+0.077 (R^2=0.3144, P < 0.05) (Fig. 7).

There was significant positive correlation between GA_3 concentration and the relative content and accumulative amount of total flavonoids in root, and the linear regressions were Y=0.0339X+1.0846 (R^2=0.5397, P < 0.05) and Y=0.3891X+5.2848 (R^2=0.209, P < 0.05). There was highly significant negative correlation between ABA concentration and the relative content and accumulative amount of total flavonoids in root, and the linear regressions were Y=-0.0436X+7.9375 (R^2=0.8862, P < 0.01) and Y=-0.4662X+80.631 (R^2=0.7359, P < 0.01) (Fig. 8). There was highly significant positive correlation between ABA concentration and the relative content of total saponins in root, and the linear regressions were Y=0.0239X+2.3366 (R^2=0.459, P < 0.01) (Fig. 9).

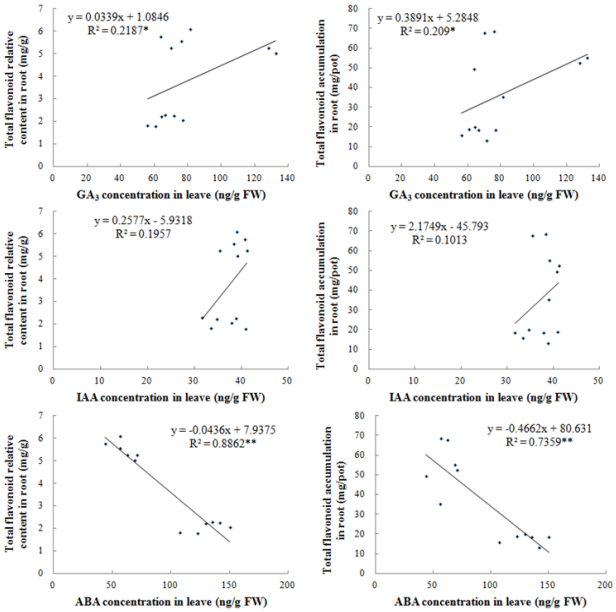

Fig. 8: Correlation between endogenous hormones and the relative content and accumulative amount of total flavonoids in root. R=correlation coefficient, *=statistically significant (P < 0.05), **=statistically significant (P < 0.01)

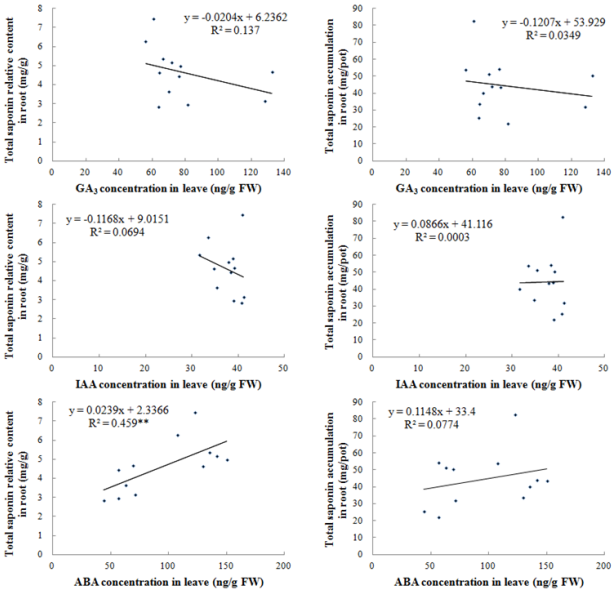

Fig. 9: Correlation between endogenous hormones and the relative content and accumulative amount of total saponins in root. R=correlation coefficient, *=statistically significant (P < 0.05), **=statistically significant (P < 0.01)

Discussion

Biomass is the comprehensive reflection of plants responded to environmental stresses. Actually, salt or drought stress can inhibit plant growth and reduce plant biomass, which resulted in a decrease of crop yield (Yang *et al*., 2006). In this

study, drought stress at moderate level can increase the root yield of *S. dichotoma* under sample soil (S_0), while drought stress reduced the root yield under salinity condition (S_1) (Fig. 1). There are contrary reports about the effect of salt and drought stress on biomass of plants (Rezaei *et al*., 2006). Some studies showed that biomass increased under drought stress (Munns and Weir, 1981); while others reported that biomass decreased (Hanson and Hitz, 1982) or remain unchanged under drought stress (Morgan, 1992) during salt stress conditions. These results suggest that the effect of drought stress on biomass accumulation of various plants grown salinity condition was different depending on the specific-species and on the levels of drought stress. The present study also found that the effect of NaCl stress to root dry weight was more obvious than drought stress, indicating that *S. dichotoma* had stronger tolerance to drought stress than salt stress (Liu *et al*., 2014).

S. dichotoma contains flavonoids and saponins as the major bioactive components, which also is part of plant secondary metabolites. Plant secondary metabolites are important for the plants to adaptation and defense for unfavorable environment (Ramakrishna and Ravishankar, 2011).

It has been widely reported that drought or salt stress could increase the content of specific secondary metabolites in medicinal plants. Specifically, salt stress significantly increased the content of methylxanthines in *Ilex paraguariensis* (Coelho *et al*., 2007), alkaloid content in *Delphinium barbeyi* (Ralphs *et al*., 1998) and aloin content (barbaloin) in *Aloe mutabilis* (Chauser-Volfson and Gutterman, 1998); drought stress also significantly increased the contents of hyperforin in *Hypericum perforatum* leaf tissues (Zobayed *et al*., 2007), ajmalicin in *Catharanthus roseus* roots (Jaleel *et al*., 2008) and total phenolic in desi ajwain (Azhar *et al*., 2011). In present study, on Oct. is the harvest time in practice, under the same salt stress condition, drought stress had no significant effect on relative content of total flavanones both in roots and shoots of *S. dichotoma*. However, under the same drought stress condition, salt stress slightly increased relative content of total flavanones in roots but significantly decreased it in shoots. Similarly, under the same drought stress, salt stress significantly decreased the relative content of total saponins in *S. dichotoma* roots. These results indicated that the effect of salt stress on active components of *S. dichotoma* was stronger than drought stress. This study also showed that, drought stress ($\geqslant$ 40–50% of soil field capacity) significantly increased accumulative amount of active components in *S. dichotoma* under sample soil condition (S_0), while significantly decreased active components of *S. dichotoma* under salt stress condition (S_1).

Plant hormones are naturally synthesized compounds that play a vital role in plant responded to salt or drought stress (Iqbal *et al*., 2014; Fahad *et al*., 2015), by which the plant may try to escape from or survive in the harmful conditions and cause to inhibited growth (Skirycz and Inze, 2010). Soil salinity or droughts usually lead to alterations in growth and biomass distribution, and plant hormones (Eyidogan *et al*., 2012). Many plant could maintain normal growth under mild salinity or drought stress due to the changed hormonal balance (Iqbal *et al*., 2012), others were found that decreased emergence and inhibited growth resulted from decreased levels of endogenous hormones (Jackson, 1997). IAA is considered as a most important hormone for regulating plant growth related processes (Wang *et al*., 2001) and it is also involved in regulating plant responds to salinity in many plants (Iqbal *et al*., 2014). However, in this study, there was no significant effect on IAA concentration in leaves of *S. dichotoma* whether salt or drought stress (Fig. 4B), indicating that *S. dichotoma* responded to NaCl combined drought could not by regulating IAA concentration.

GA_3 plays a vital role in regulating the ability of plants response to the growth environment (Chakrabarti and Mukherji, 2003) and it also a key hormone that can improve the ability of many plants tolerant to stress environment including salinity or drought (Hoque and Haque, 2002). Generally, GA_3 concentration rapidly increased when plants subjected to abiotic stresses (Iqbal *et al*., 2011). In this study, drought stress decreased GA_3 concentration in *S. dichotoma* leaves under normal and NaCl stress conditions during the experiment, and this decreased effect was increase with the increasing of stressed-time. Also, NaCl stress remarkedly increased GA_3 concentration in *S. dichotoma* leaves under the same drought stress condition at 60 d after treatment, while there was no significant different at 90 d after treatment, which suggest that the influenced effect of NaCl stress on GA_3 concentration in *S. dichotoma* leaves was gradually decreased with the increasing of stressed-time.

ABA has been considered as an important hormon in stress response and/or adaptation (Sharma *et al*., 2005; Eyidogan *et al*., 2012; Devinar *et al*., 2013). It is usually up regulated when plant subjected to osmotic stress and involved in altering environment stress-induced gene expression then help plant survive in stressed condition (Keskin *et al*., 2010). Previous studies found that water stress significantly increased ABA concentration in many plants such as rice (Yang *et al*., 2001), cucumber (Pustovoitova *et al*., 2004) and tall fescue (Zhang *et al*., 2009). In addition, ABA synthesis in roots increases and it is transported into leaves that induces stomatal closure as soil moisture decreasing (Da *et al*., 2011). In present study, at 60 d after treatment, NaCl stress solely significantly increased ABA concentration, while drought stress solely slightly decreased ABA concentration in *S. dichotoma* leaves both at sample and NaCl stressed conditions. However, at 90 d after treatment, NaCl stress solely had no significant effect on ABA concentration, while drought stress solely significantly decreased ABA concentration in leaves of *S. dichotoma* at NaCl stressed condition. These results indicate that ABA play an important role for *S. dichotoma* in

responded to both NaCl and drought stress conditions, and the dominant factor affected ABA concentration was changed with the growth and development of *S. dichotoma.*

Chloroplast is organelles of plant photosynthesis, and thylakoid membranes are the sites of light absorption and transformation. Many studies have shown that thylakoid crib stack can increase the ability to capture light and energy transfer rate. The ratio of chlorophyll a/b is a sensitive indicator which reflects the degree of thylakoid membrane crib fold. Chlorophyll is the main material chloroplast light energy transfer and conversion, and closely related to plant photosynthesis and yield formation. A concentration dependent response of salt - drought stress was observed on chlorophyll. The above results also showed that the maximum chlorophyll a, a + b and their ratio (a/b) in *S. dichtoma* in September was under S_0W_3 condition, indicating higher drought stress and lower salt stress caused a marked increase in chlorophyll content. These results different from those of Li *et al*. (2010) who found NaCl stress significantly decreased chlorophyll concentration in alfalfa. These results indicating that variations in chlorophyll content under salt - drought stress was may cause by different species and experimental period. In terms of the overall trend of the content of chlorophyll a, chlorophyll b and chlorophyll a + b decreased with the advancement of the growth process (Fig. 5), which showed that photosynthesis ability decreased with the plant gradually senescence in October. On the other hand, the decrease in the level of chlorophyll may be due to salinity-induced inhibition of chlorophyll biosynthesis that maybe caused by nutrient deficiency induction or imbalance (Li *et al*., 2010).

Conclusion

Present study indicate that moderate drought stress could enhance secondary metabolites accumulation by changing GA_3 and ABA concentrations and growth of *S. dichotoma* under sample soil condition (S_0). However, drought stress aggravate adverse effects of salt stress on secondary metabolites accumulation by changing GA_3 and ABA concentrations and growth under salt stress condition (S_1).

Acknowledgment

The authors are grateful for the financial support provided by the project of the National Natural Science Foundation of China (31860343 and 81202868) and the Key National Research and Development Programs of China (2017YFC1700706).

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[Received 29 Jan 2019; Accepted 18 Jun 2019; Published (online) 22 Dec 2019]