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Increased water supply promotes photosynthesis, C/N ratio, and plantamajoside accumulation in the medicinal plant *Plantago depressa* Willd.

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Abstract

Anthropogenic activities are changing global precipitation regimes and result in many middle latitude arid and semiarid regions experiencing less precipitation and more extreme weather events. However, little is known about the response of active ingredient accumulation in the medicinal herb *Plantago depressa* Willd. Therefore, we carried out a greenhouse experiment in order to study effect of control (CK, normal water supply equal to 309 mm per four months), −30 (−WS) and +30% (+WS) of the control water supply on the photosynthesis (P_N), C/N ratio, and plantamajoside accumulation in *P. depressa*. Our results showed that compared with the –WS and CK treatments, the +WS treatment significantly enhanced biomass, the C/N ratio, plantamajoside concentration, yield in shoots and roots, and P_N, but declined the N concentration in shoots and roots. The plantamajoside concentration was positively correlated with P_N, the soluble sugar content, and the C/N ratio, but negatively correlated with the N concentration. Our results suggested that, under experimental conditions, +WS increased the C/N ratio and promoted the plantamajoside accumulation of *P. depressa*.

Additional key words: climate change; controlled environment; gas exchange; phenolic compounds.

Introduction

Human activities are changing the concentration of carbon dioxide in the atmosphere, and one of the consequences of such change is an altered precipitation regime (Marvel and Bonfils 2013). It is predicted that many middle-latitude arid and semiarid regions will possibly experience less precipitation and more extreme weather events (Stocker *et al.* 2013). In China, precipitation intensity significantly increased, while precipitation frequency decreased (Zhang and Cong 2014). The change in precipitation affects the primary and secondary metabolism of plants (Maricle and Adler 2011, Jamieson *et al.* 2013). Previous studies have shown that altered precipitation regimes can influence rate of photosynthesis (Maricle and Adler 2011), carbon (C) and nitrogen (N) concentrations, and their relative ratios in plants (Lü *et al.* 2012, Ren *et al.* 2015), which in turn affects plant growth, resource allocation, and secondary metabolism (Jamieson *et al.* 2013).

Phenolic compounds are among the most influential and widely distributed secondary products in the plant kingdom, many of them play important physiological and ecological roles, and are involved in resistance to different types of stress (Bettaieb *et al.* 2011). Plantamajoside, which is a bioactive caffeic acid derivative, is a dihydroxyphenethyl glucoside in the group of polyphenolic compounds (Ravn *et al.* 2015), has several defense functions, and is generally induced in response to biotic and abiotic stimuli, such as ultraviolet radiation, elevated CO_2, and pesticides (Davey *et al.* 2004, Murai *et al.* 2009, Ravn 2009). Plantamajoside is mainly found in the Plantaginaceae family and in the *Plantago* genus in particular (Ravn *et al.* 2015). Twenty species belonging to the genus *Plantago* contain plantamajoside (Ravn *et al.* 2015), including our study species, *Plantago depressa* Willd., which is a weed widely distributed in China, Afghanistan, Bhutan, India, Kashmir, Kazakhstan, Korea, Kyrgyzstan, Mongolia, Pakistan, and European part of Russia (Li *et al.* 2011). The dried herb of *P. depressa*, 'Plantago Herba', has been used historically as medicine

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Abbreviations: C_i – intercellular CO_2 concentration; Chl – chlorophyll; CK – normal water supply; E – transpiration rate; DM – dry mass; g_s – stomatal conductance; HPLC – high performance liquid chromatography; L_s – stomatal limitation; PAL – phenylalanine ammonia lyase; P_N – net photosynthetic rate; -WS – 30% less water supply; +WS – 30% more water supply.

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in China for the treatment of clearing heat, diuresis, eliminating phlegm, cooling blood, and detoxification. Plantamajoside concentration is used as a test and evaluation index of *P. depressa* (Chinese Pharmacopoeia Committee 2010). Previous studies have shown that the plantamajoside concentration is affected by age and organs of plants (Fons *et al.* 1998, Zubair *et al.* 2011), and also depends on growth conditions (Ravn *et al.* 2015). However, little is known about plantamajoside concentration responses to changes in precipitation regimes.

Predicted by the carbon-nutrient balance hypothesis, under nutrient resource limitation, especially N, which restricts growth to a greater extent than photosynthesis, plants usually show an increase in the C/N ratio and this signifies an increased production of secondary metabolites (Bryant *et al.* 1983). Previous research had shown that a high production of secondary metabolite compounds was elicited by a high C/N ratio and low contents of plant nitrogen under conditions of elevated CO_2 (Lindroth 2010, Royer *et al.* 2013). However, it is unknown whether this hypothesis can be confirmed when precipitation patterns change.

In order to examine impacts of a change in precipitation regimes on the photosynthesis, C/N ratio, and plantamajoside accumulation of the medicinal plant *P. depressa*, we carried out a pot experiment with simulated precipitation change by different water supply. The main objectives of our study were to examine: (*1*) whether altered precipitation regimes induce changes of net photosynthetic rate (P_N) and C/N ratio in *P. depressa*, and (*2*) if so, whether the changes influence plantamajoside (carbon-based secondary metabolites) accumulation in *P. depressa*. We hypothesized that, compared with normal water supply, increased water supply may lead to higher C/N ratios due to dilution by accumulating carbohydrates (Lindroth 2010). Since the increased plant C/N ratios promote the synthesis of carbon-based secondary metabolites (Bryant *et al.* 1983, Royer *et al.* 2013), we also hypothesized that increased water supply would enhance plantamajoside accumulation compared with normal water supply.

Materials and methods

Plant materials and growth conditions: The pot experiment was performed in the greenhouse of the Institute of Botany of the Chinese Academy of Sciences (40°N, 116°28′E, 74 m a. s. l.), Beijing, China from March to July 2014. Seeds of *P. depressa* were collected from the Duolun County (42°02′N, 116°17′E, 1,324 m a. s. l.), a semiarid area in the Inner Mongolia, China in September 2013. The soil used in this experiment was collected at a depth of 0–20 cm below the ground of the Beijing Botanical Garden, Institute of Botany Botanical Garden, Chinese Academy of Sciences. The sieved soil (<2 mm) was homogenized, air dried, and mixed with clean sand (soil:sand = 1:1, v/v). The organic matter content of the mixed soil was 0.82%, total N was 0.39 g kg^{-1}, available N was 18.91 mg kg^{-1}, available P was 9.41 mg kg^{-1}, and pH value was 7.1 (in H_2O). Eight four-month-old *P. depressa* seedlings, as morphologically identical as possible, which were raised in the nursery, were transplanted in a 7-L pot.

Water treatments: Three water treatments were performed in this study: control (CK, normal water supply), −30% (−W), and +30% (+W) of normal water supply. The experiment lasted for four months and the water supply quantities of CK treatment were scheduled on the monthly mean precipitation in June, July, August, and September during 1979–2000 in the seed collection area. The amount of water in −W and +W treatments was 70% and 130% of the CK treatment. The monthly precipitation [mm] of each level (*see* table below) was converted into an irrigation amount [ml]. The experiment started on 24 March 2014 and a completely randomized design was employed, with six replications. The frequency of experimental watering was four times per month. All the treatments were watered at the same time every week.

Average monthly precipitation from 1979 to 2000 in the Duolun County. −W – minus 30% water supply; CK – normal water supply; +W – plus 30% water supply.

Month	Precipitation [mm]		
	−W	CK	+W
June	45	64	83
July	72	102	133
August	69	98	128
September	31	44	58
Total	216	309	402

Gas exchange was measured from 09:00 to 11:00 h in clear and sunny weather using a portable photosynthesis system (*LI-6400*, *Li-Cor*, Lincoln, NE, USA) in the last month of the study. The vapor pressure deficit based on leaf temperature was maintained at 2.2 kPa and leaf temperature was at 27°C in the leaf chamber. The flow rate was set at 500 μmol s^{-1}, and CO_2 concentration in the leaf chamber was maintained at 380 μmol mol^{-1}. The leaf was illuminated with 1,000 μmol(photon) m^{-2} s^{-1} PAR (light-saturated) from an internal light source in the leaf chamber. As the conditions for gas-exchange measurements became stable, net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were simultaneously recorded. Apparent mesophyll conductance (the conductance of CO_2 from the stomatal cavity to the chloroplast) was calculated from the ratio of P_N to C_i (P_N/C_i) according to Fischer *et al.* (1998), which can be used to estimate the relative importance of the mesophyll limitation to P_N. Stomatal limitation (L_s) was defined as follows: $L_s = 1 - C_i/C_a$ (Li *et al.* 2015).

Plant growth and dry mass (DM): Following pot culture for four months, the plants were sampled randomly from each treatment. Leaf number was counted. Leaf area was analyzed with *WinFOLIA Pro 2006a* software (*Regent Instruments*, Quebec, Canada). Specific leaf area was calculated using the following formula: specific leaf area = leaf area/leaf DM. For DM measurement, whole plants were dried with tissues and separated into shoots and roots, then oven-dried at 60°C to constant mass to record the biomass. For measurements of a soluble sugar content, N concentration, C/N ratio, and plantamajoside concentration, dry shoot and root were ground to pass through a 0.5-mm sieve. For the determination of plantamajoside, the freeze-drying might be the best way. However, in the actual production, traditional Chinese medicine drying methods include sun drying, hot air and microwave drying *etc*. Drying air temperatures of hot air drying between 50 and 60°C appear to be feasible for drying large mass of medicinal plants. And freeze-drying method is less used in actual production. So, in this experiment, the samples used for the determination of plantamajoside were oven-dried at 60°C, and the results were closer to the actual production. To be sure, the values of plantamajoside were used only for comparison between water treatments in this experiment.

Chlorophyll (Chl) content: Dried leaf samples were finely ground in order to pass through a 0.5-mm sieve. Samples of approximately 10 mg were extracted with 5 mL of 95% ethanol in the shade for 48 h in a test tube, which was shaken at intervals until the leaves completely lost their green coloration. The supernatant in the test tube was used for the pigment assay. The absorbance of the extract was recorded at 665 and 649 nm with a UV/visible spectrophotometer *(SmartSpec Plus*, *Bio-Rad Laboratories*, California, USA). The pigment concentrations were expressed in [mg m^{-2}] and calculated using extinction coefficients and equations according to Lichtenthaler (1987).

Soluble sugar content: Soluble sugars were extracted by 80% (v/v) ethanol, with heating at 60°C for 30 min. The homogenate was centrifuged at 3,500 × *g* for 10 min. The supernatant was thoroughly mixed with anthrone reagent (Plummer 1978, Khan *et al.* 2000). Absorbance was read at 630 nm (Guitman *et al.* 1991) with a UV/visible spectrophotometer (*SmartSpec Plus*, *Bio-Rad Laboratories*, California, USA). The soluble sugar content was calculated as [mg g^{-1}(DM)].

N concentration and C/N ratio: N concentration in plant tissue was determined by the standard macroKjeldahl procedure (*Nitrogen Analysis System*, Foss, Denmark), thus, the N concentration was just the total concentration of organic nitrogen and ammonia and did not include nitrogen in NO_3-N form. Carbon concentration was determined with Walkley-Black method (Chen 1983). The C/N ratio was calculated as carbon concentration to N concentration.

Plantamajoside concentration was determined using a *Waters* high performance liquid chromatography (HPLC) system (*Milford*, MA, USA). The methods and conditions for determination were all according to Sun *et al.* (2010). Specifically, the HPLC was performed on a C_{18} column (*Agela*, *Promosil* C_{18}, 4.6 mm × 250 mm, 5 µm) at 30°C with a sample injection volume of 20 µL. Detection wavelength was 330 nm and the flow rate was 1.0 mL min^{-1}. The mobile phase was acetonitrile–0.1% formic acid (17:83).

Samples for HPLC quantitation were prepared using the methods described by Sun *et al.* (2010). In particular, 0.5 g of powdered shoot and root samples were extracted by sonication for 30 min in 25 mL of 60% methanol solution. The solution was cooled to ambient temperature, weighed, and then adjusted to a volume of 25 mL with 60% methanol solution. The solution was filtered through a 0.22-µm organic membrane filter before injection into the HPLC system.

Separate standard stock solution containing 1,994.4 mg(plantamajoside) L^{-1} was prepared by dissolving the chemicals in 60% methanol solution (Sun *et al.* 2010). We prepared nine concentrations of standard solution, which contained 1196.64, 997.20, 797.76, 598.32, 398.88, 199.44, 99.72, 49.86, and 4.99 mg(plantamajoside) L^{-1}. The HPLC system was calibrated with the plantamajoside standards at an injection volume of 20 µL.

Statistical analysis: All measurements were performed six times, and the means and calculated standard error (SE) were reported. Data were analyzed using one-way analysis of variance (*ANOVA*) of *SPSS* package (*version 21*, *SPSS*, Chicago, USA), and significant differences between the treatments were determined using *Duncan*'s multiple range test at a 0.05 probability level. A regression line was constructed using *Sigmaplot 12.5* (*Systat Software,* San Jose, CA, USA).

Results

Plant growth and DM: Leaf area and leaf number of +W treatment were significantly higher than the other two treatments. No significant difference in leaf area and leaf number was found between CK and −W treatments (Table 1). Similar results were obtained for the total, shoot, and root DM (Table 1). Moreover, DM was significantly higher in shoots compared to roots.

Chl content and gas exchange: Both CK and +W treatments showed comparable Chl contents in leaves, which were significantly higher than that of the −W treatment (Table 2).

Plants in CK and -W treatments showed a comparable P_N and P_N/C_i, whereas those for the +W treatment was significantly higher than the other two (Table 2). E and g_s in the +W treatment were significantly higher than in −W treatment, and were comparable with CK treatment (Table 2). No significant difference in E and g_s were found between CK and −W treatments. Compared with the +W treatment, C_i increased in the −W and CK treatments, whereas the value of L_s decreased, although these differences were all insignificant, which indicated that the decrease in P_N observed in the plants under −W and CK treatments was caused by nonstomatal limiting factors.

Soluble sugar content: Shoot soluble sugar content in the +W treatment was remarkably higher than that in −W treatment, and was comparable with the CK treatment (Fig. 1*A*). No significant difference in shoot soluble sugar content was found between CK and −W treatments. Both CK and -W treatments showed a comparable root soluble sugar content, which was significantly lower than that of the +W treatment. Furthermore, soluble sugar content was significantly higher in roots than in shoots.

N concentration and C/N ratio: Shoot N concentration in the +W treatment was significantly lower than that in CK and −W treatments, which were not different from one another (Fig. 1*B*). N concentration in roots differed significantly from one another in the three water treatments, and decreased in the order of −W > CK > +W. Moreover, the N concentration was significantly higher in shoots than in roots.

The C/N ratio in shoots and roots showed a rising trend with the increase in water supply (Fig. 1*C*). The +W treatment significantly increased the C/N ratio in shoots and roots. Compared with the CK treatment, shoot and root C/N ratio of the +W treatment increased by 44 and 43%, respectively. In addition, compared with the −W treatment, +W treatment enhanced shoot and root C/N ratio by 62% and 73%, respectively. No significant difference in shoot and root C/N ratio was found between CK and −W treatments. In addition, the C/N ratio was significantly lower in shoots compared to roots.

Plantamajoside concentration and yield: Plantamajoside concentration in shoots and roots of the +W treatment was significantly higher than the other two; no significant difference in shoot and root plantamajoside concentration was found between CK and −W treatments (Fig. 2*A*). Shoot plantamajoside concentrations were 84 and 41% higher in the +W treatment compared to CK and −W treatments, respectively. Similarly, the +W treatment increased root plantamajoside concentration by 42 and 39%, compared to CK and −W treatments, respectively. In addition, plantamajoside concentration was significantly higher in shoots than in roots. Similar results were observed for plantamajoside yield in shoots and roots (Fig. 2*B*).

Table 1. Effects of the water treatments on growth indicators and shoot, root, and total dry biomass in *Plantago depressa*. −W – minus 30% water supply; CK – normal water supply; +W – plus 30% water supply. Data are means ± SE ($n = 6$). Different *lowercase letters* within a row indicate significant differences ($P<0.05$). DM – dry mass.

Treatment	Leaf area [cm^2 per plant]	Leaf number [No. per plant]	Total DM [g per plant]	Shoot DM [g per plant]	Root DM [g per plant]
−W	279.92 ± 14.01^{b}	21.83 ± 0.65^{b}	1.84 ± 0.11^{b}	1.52 ± 0.07^{b}	0.32 ± 0.04^{b}
CK	349.14 ± 48.14^{b}	23.50 ± 2.25^{b}	2.02 ± 0.18^{b}	1.67 ± 0.16^{b}	0.35 ± 0.03^{b}
+W	580.80 ± 102.80^{a}	31.67 ± 3.01^{a}	4.24 ± 0.70^{a}	3.48 ± 0.60^{a}	0.77 ± 0.12^{a}

Table 2. Effects of water treatments on chlorophyll content and photosynthetic parameters in the leaves of *Plantago depressa*. −W – minus 30% water supply; CK – normal water supply; +W – plus 30% water supply. Data are means ± SE ($n = 6$). Different *lowercase letters* within a row indicate significant differences ($P<0.05$). C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; g_s – stomatal conductance; L_s – stomatal limitation; P_N – net photosynthetic rate.

Treatment	Chl content [$mg\ m^{-2}$]	P_N [$\mu mol(CO_2)\ m^{-2}\ s^{-1}$]	g_s [$mol(H_2O)\ m^{-2}\ s^{-1}$]	E [$mmol(H_2O)\ m^{-2}\ s^{-1}$]	C_i [$\mu mol(CO_2)\ mol^{-1}$]	L_s	P_N/C_i
−W	192.23 ± 15.07^{b}	4.09 ± 0.60^{b}	0.13 ± 0.016^{b}	2.71 ± 0.33^{b}	312.12 ± 7.36^{a}	0.18 ± 0.02^{a}	0.014 ± 0.002^{b}
CK	261.02 ± 19.42^{a}	5.01 ± 0.89^{b}	0.15 ± 0.025^{ab}	3.00 ± 0.49^{ab}	311.55 ± 3.91^{a}	0.18 ± 0.01^{a}	0.016 ± 0.003^{b}
+W	272.65 ± 15.76^{a}	7.38 ± 0.48^{a}	0.19 ± 0.016^{a}	4.09 ± 0.29^{a}	301.52 ± 3.86^{a}	0.20 ± 0.01^{a}	0.025 ± 0.002^{a}

Correlation analysis: Plantamajoside concentration was positively related to P_N, g_s, E, soluble sugar content, and C/N ratio (Fig. 3*A*−*E*), whereas a significant negative linear relationship was found with the N concentration (Fig. 3*F*).

Discussion

Chl content and gas exchange: Chl plays an important role in capturing sun light and converting it into chemical energy; changes in photosynthesis therefore occur due to any disturbance in the Chl content (Miao *et al.* 2015). The present study demonstrated that −W treatment resulted in a marked decrease in the Chl content compared with the CK and +W treatments (Table 2), which might occur due to the accelerated breakdown of Chl or inhibited Chl synthesis caused by stress (Harpaz-Saad *et al.* 2007, Kaewsuksaeng 2011).

Photosynthesis is a crucial phenomenon, which contributes considerably to the plant growth and development (Ashraf and Harris 2013). Drought stress can depress gas exchange parameters to a different degree, thereby influencing overall photosynthetic capacity of most plants. Stomatal or nonstomatal limitation were generally considered as an explanation for the phenomenon of reduced photosynthesis under water-deficit conditions (Wilson *et al.* 2000). In the present study, compared with +W treatment, P_N and P_N/C_i significantly decreased, and C_i increased, whereas L_s decreased in the −W and CK treatments, which indicated that the reduction in the P_N in CK and -W treatments was primarily due to nonstomatal limitations and mainly resulted from the low efficiency of mesophyll cells taking advantage of the available CO_2 (Dias and Brüggemann 2010). In addition, the +W treatment resulted in higher E and g_s than that of the −W treatment (Table 2). Previous studies have suggested that drought treatment significantly reduced E and g_s of plants than water treatments in greenhouses (Miyashita *et al.* 2005, Miao *et al.* 2015). Nevertheless, Rodgers *et al.* (2012) found that a 50% increased rainfall treatment did not cause a significant shift in E of *P. lanceolata* compared to a 50% reduced rainfall treatment.

Soluble sugar content: Various reports showed that soluble sugar accumulated when plants were under environmental stresses (Moustakas *et al.* 2011). In the present study, the +W treatment significantly raised the shoot soluble sugar content compared with -W treatment and root soluble sugar content compared with CK and −W treatments (Fig. 1*A*). Soluble sugar accumulation in plants might be due to the dilution of plant tissue N during enhanced plant growth under increased water supply, especially, when N is limited. This could reduce the sink size of the plant, hence, reducing the translocation of carbohydrates to other plant parts (de Souza *et al.* 2008).

N concentration and C/N ratio: Many previous studies have been done on the effect of water changes on plant N concentrations. However, empirical studies have shown various results. While some studies have reported negative effects of water addition on plant N concentrations (Huang *et al.* 2009), others found no effects or even positive effects (Lü and Han 2010, Lü *et al.* 2012, Ren *et al.* 2015). These inconsistencies may be attributed to soil N availability (Huang *et al.* 2009, Lü *et al.* 2012). In the present study, increased water supply significantly decreased N concentration in shoots and roots compared with the control and reduced water supply (Fig. 1*B*). A possible reason for the decreased N concentration might be related to the increase of P_N which led to improvement of plant growth and primary productivity, consequently, dilute plant N concentration under experimental conditions (the content of soil available N was low in the experiment).

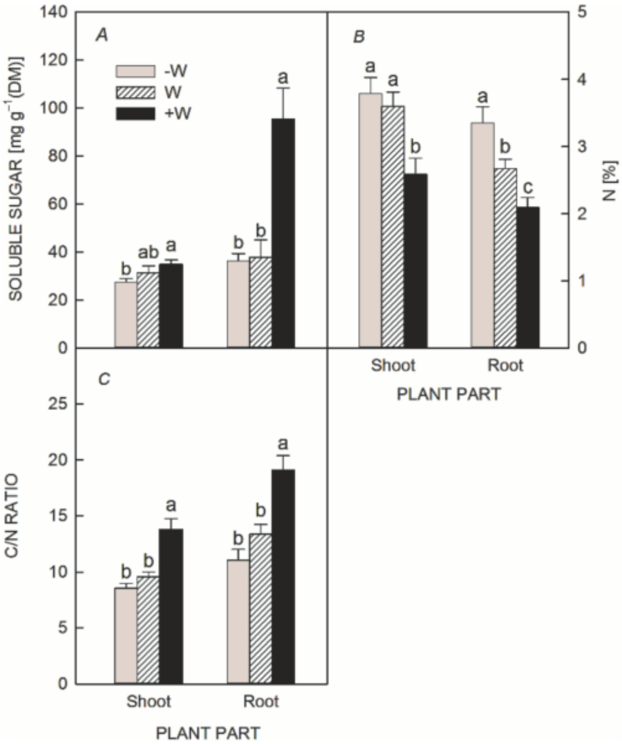

Fig. 1. Soluble sugar content (*A*), N concentration (*B*), and C/N ratio (*C*) in shoots and roots of *Plantago depressa* under different water treatments. −W – minus 30% water supply; CK – normal water supply; +W – plus 30% water supply. Values are means ± SE (n = 6). Different *lowercase letters* indicate significant differences (P<0.05) between the three water treatments.

The C/N ratio is often considered as an indicator of plant long-term N-use efficiency and might be an important signal to regulate gene expression in plants (Yang *et al.* 2013). The present results showed that the C/N ratio in shoots and roots significantly increased with increased water supply (Fig. 1*C*). Similar results have been reported by Patterson *et al.* (1997) in black and white spruce. Increased water supply had no significant effect on C concentration in shoots and roots (data not shown), but significantly reduced the N concentration in shoots and roots (Fig. 1*B*). Therefore, the increase of the C/N ratio observed under increased water supply conditions could result from decline in N concentration.

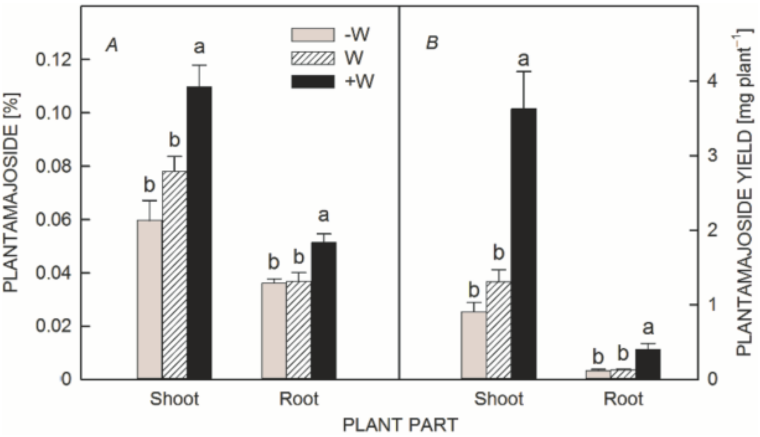

Fig. 2. Plantamajoside concentration (*A*) and yield (*B*) in shoot and root of *Plantago depressa* under different water treatments. −W – minus 30% water supply; CK – normal water supply; +W – plus 30% water supply. Values are means ± SE ($n = 6$). Different *lowercase letters* indicate significant differences ($P<0.05$) between the three water treatments.

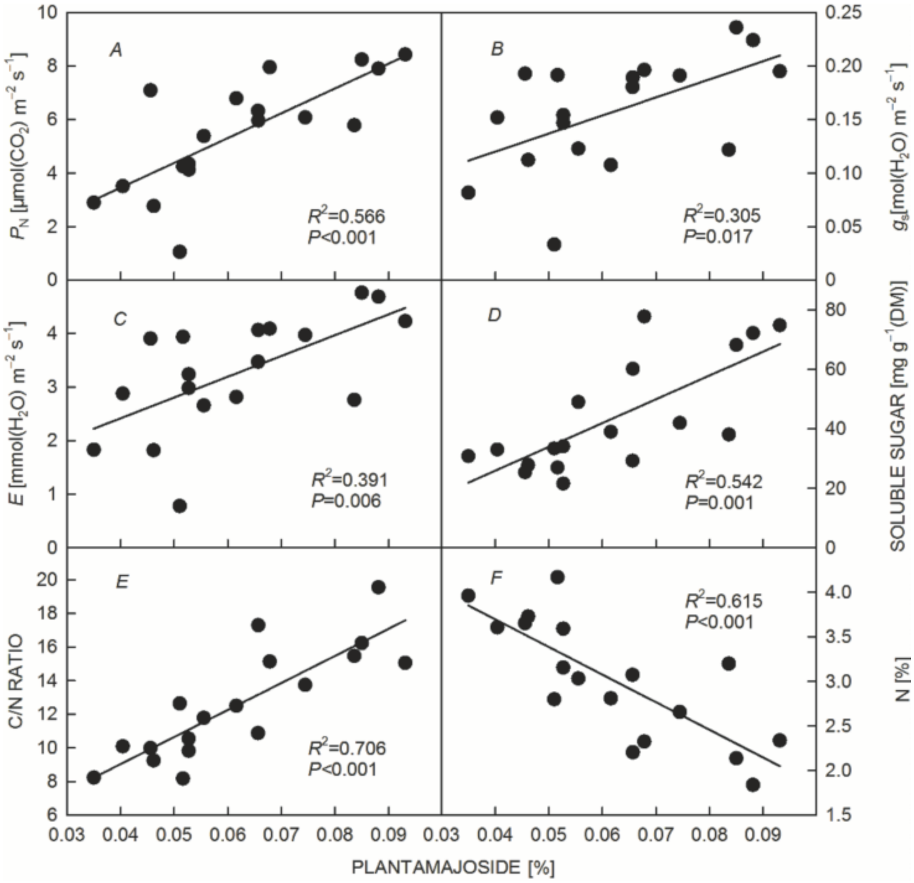

Fig. 3. Correlations between plantamajoside concentration and net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), transpiration rate (*E*, *C*), soluble sugar content (*D*), C/N ratio (*E*), and N concentration (*F*). Plantamajoside concentration, soluble sugar content, C/N ratio, and N concentration data are the average values in shoots and roots.

Plantamajoside concentration: In the present study, increased water supply significantly increased plantamajoside concentration and yield in shoots and roots. A similar phenomenon has been observed with elevated CO_2 environments (Davey *et al.* 2004). A possible explanation for the increase in plantamajoside concentration, when *P. depressa* is grown under conditions of increased water supply, is the carbon nutrient balance hypothesis (Bryant *et al.* 1983), which suggests that if plants increase photosynthesis and carbon gain under increased water supply, the “excess” carbon is allocated to carbon-based secondary metabolites. Although this prediction cannot explain all carbon-based secondary metabolites, it does seem to apply to products of the shikimate pathway, such as phenolics (Koricheva *et al.* 1998). The results of the present study showed that the increase in photosynthesis, soluble sugar content, and C/N ratio could stimulate the concentration of plantamajoside, as shown by the positive correlation coefficient between photosynthesis, soluble sugar content, C/N ratio, and plantamajoside concentration (Fig. 3*A,D,E*). Moreover, under low nitrogen conditions, increased water supply significantly decreased the plant N concentration (Fig. 1*B*). Increased activity of phenylalanine ammonia lyase (PAL) is usually observed in N-deficient plants (Kováčik and Bačkor 2007), and it has been suggested that N-deficient plants increase the availability of ammonia by enhancing PAL activity, leading to polyphenolic compound accumulation (Margna 1977).

Conclusion: We demonstrated that plus 30% water supply enhanced the net photosynthetic rate and reduced N concentration, leading to the increased C/N ratio and, consequently, increased plantamajoside concentration in *P. depressa* under experimental (low N) conditions. We believe that under dry environmental conditions with N limitation, an increase in precipitation would increase phenolic compound synthesis in plants. Besides, in practical production, we may be able to select N-poor soil to cultivate medicinal plants, and by increasing the soil water supply to a certain extent, both the yield and quality of medicinal plants could be improved. In the present experiment, we studied the effect of different water supply on the effective ingredient of *P. depressa*. However, mechanisms of active ingredient accumulation were not studied in our experiment. Therefore the study of this aspect needs to be strengthened in the future.

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