SILVA FENNICA

Silva Fennica 44(4) research articles

www.metla.fi/silvafennica · ISSN 0037-5330
The Finnish Society of Forest Science · The Finnish Forest Research Institute

Comparison of Growth and Physiological Responses to Severe Drought between Two Altitudinal *Hippophae rhamnoides* Populations

Yongqing Yang, Yinan Yao and Xuejiang Zhang

Yang, Y., Yao, Y. & Zhang, X. 2010. Comparison of growth and physiological responses to severe drought between two altitudinal *Hippophae rhamnoides* populations. Silva Fennica 44(4): 603–614.

Growth and physiological differences in response to drought were compared between two sea buckthorn (*Hippophae rhamnoides* L.) populations inhabited in the southeast of the Qinghai-Tibetan Plateau of China. The experimental design included two water regimes (100% and 25% of field capacity) and two populations from the low and high altitude zone. Our experiments were conducted in a naturally lit greenhouse under semi-controlled environmental conditions for a whole growing season in a dry valley (1800 m above the sea level). We found that drought tolerance is highly related to the plant antioxidant capacity and water use efficiency as well as leaf nutrient status in *H. rhamnoides*. The highland population (HP) experienced a greater inhibition in plant growth and leaf enlargement, lower leaf nitrogen and phosphorus content, lower root nodule biomass and root mass/foliage area ratio, and higher leaf water content loss paralleling with higher enhancement of abscisic acid level in response to drought, as compared with lowland population (LP). Additionally, reduction of leaf lignin content in HP further reduced its drought tolerance. On the contrary, LP showed effective adaptation strategies such as improvement of water economy and maintaining high ascorbic acid content. Therefore, we conclude that LP was more tolerant to drought than HP, and could be selected for reforestation in the dry valleys of upper Minjiang River regions in China.

Keywords sea buckthorn (*Hippophae rhamnoides* L.), physiological response, abscisic acid (ABA), carbon isotope composition
Addresses *Yang*, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, 830011, China & College of Life Sciences, Chongqing Normal University, Chongqing, 400047, China; *Yao*, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, 830011, China; *Zhang*, Institute for Plant Protection and Soil sciences, Hubei Academy of Agricultural Sciences, Wuhan, 430064, China
E-mail yaoya@ms.xjb.ac.cn
Received 2 September 2009 **Revised** 6 May 2010 **Accepted** 24 September 2010
Available at http://www.metla.fi/silvafennica/full/sf44/sf444603.pdf

1 Introduction

The increased frequency and severity of drought caused by climate changes will affect forest primary productivity directly through water depletion and indirectly by reduced nutrient uptake (Saxe et al. 1998). In general, plants possess numerous mechanisms for responding to drought stress, such as changes in plant structure, growth rate and tissue osmotic potential (Larcher 1995). Differences in drought tolerance of species, lines, clones, cultivars or provenances have been attributed to the effects of drought on proline (Heuer 1994, Hare et al. 1999) and abscisic acid (ABA) (Volaire et al. 1998, Li and Wang 2003), since proline is thought of as the most frequent osmoprotectant, and ABA is well-known stress-inducible plant hormone. On the other hand, the carbon isotope composition ($\delta^{13}C$) has been developed as a tool to measure water use efficiency (WUE), since a strong correlation has been found between the carbon isotope ratio ($\delta^{13}C$) and WUE (Farquhar et al. 1989), therefore, measuring $\delta^{13}C$ of plant tissue could provide an integrated measurement of internal plant physiology and external environmental properties influencing photosynthetic gas exchange over the time when the carbon was fixed (Brodribb and Hill 1998). Studies examining the effects of environmental factors on stable isotope composition ($\delta^{13}C$) have been widely used in plant tissues exposed to change of aerial CO_2 content (Polley et al. 1993), nutrient deficit (Livingston et al. 1999) and water stress (Li et al. 2000, Li and Wang 2003). Some previous studies report that drought may also affect the nutritional status in plants at the level of nutrient uptake and long distance transport in xylem and phloem (Hsiao 1973), but the effects of drought on the nutritional status of plants are less well known until now compared with other drought effects on water relations, carbon assimilation and biomass allocation (Peuke and Rennenberg 2004). Additionally, as a major polymer of plant cell walls that confers hydrophobicity and mechanical strength to withstand the negative pressure generated by transpiration, lignin might play an important role in response to water deficit, but its response and adaptation mechanism under severe drought need to be clarified.

In the subalpine areas of southeast of the Qinghai-Tibetan Plateau of China, the dry valleys are widely distributed along upper Minjiang River and its tributaries, which are characterized by a combination of high mountains and low rainfall, paralleled with high solar radiation and high potential evaporation. The environment condition in dry valleys is very fragile and highly prone to desertification (Tang et al. 2004). Various efforts have been conducted on the rehabilitation of degraded ecosystems in these valleys. However, the reforestation objective is still far from being reached. *Hippophae rhamnoides* L. is a thorny, nitrogen-fixing, deciduous perennial shrub, widely distributed in various climate regions, adapted to very diverse ecological conditions (Lu 1995), and plays a very important role in preventing soil water loss, in regulating microclimate and in retaining ecological stability. It has been used as an ideal pioneer species in reforestation of arid and semi-arid regions (Lu 1992), and it was also selected as one of plant species for reforestation of dry valleys in Mingjiang River. Under the contrast environmental condition in the subalpine areas of the Qinghai-Tibetan Plateau, pronounced population differences occurred in this species on their tolerance for environmental stresses such as drought, cold, UV-B, et al. Here we hypothesized whether highland *H. rhamnoides*. population grown and evolved under higher UV radiation and lower tempeature have evolved better tolerance to drought stress, due to cross tolerance to different environmental stresses. In this study, pot-grown seedlings of two altitudinal *H. rhamnoides*. populations were employed to compare their responses in terms of plant growth and physiology to a long-term drought. The objectives were to 1) assess variation in growth traits, leaf nutrients and lignin, carbon isotope composition, proline and ABA accumulation of two selected altitudinal populations as response to drought, and 2) test whether populations of different drought sensitivity could be identified between the different provenances. These differences in drought responses may be used as criteria of genotype selection for reforestation in the dry valleys of upper Minjiang River regions.

2 Materials and Methods

2.1 Plant Material and Experimental Design

A lowland population (LP) (33°16′N, 104°14′E, 1800 m, above the sea level) and a highland population (HP) (30°59′N, 101°07′E, 3300 m, above the sea level) of *Hippophae rhamnoides* L. were selected for this study. The mean annual precipitation is about 520 mm and 650 mm, and the mean annual temperature is 12 °C and 7.5 °C for LP and HP, respectively. The seeds in each population were collected from 20 fruit-riping plants whose pairwise distance was more than 20 metres. In the autumn of 2005, the seeds from 20 plants in each population were separately sowed in 20 pots and grown in growth chamber (kept 22 °C in the day and 18 °C in the night, a 14/10 h light–dark cycle, and 220 μmol m^{-2} s^{-1} PPFD). In March of 2006, healthy seedlings of a uniform height (35–40 cm) were transplanted to 5.0 L plastic pots filled with homogenized soil, one seedling per one pot. In each population, one replicate were set up by 5 pots, totally 8 replicates for each population. The soils in all the pots were brown soil, which were sieved from topsoil and homogenized, with 9.50% organic matter, 2.80g kg^{-1} available N, 0.09 g kg^{-1} available P and 0.36 g kg^{-1} available K, with pH 6.70. A total of 2.5g slow release fertilizer (13% N, 10% P and 14% K) was added to each pot at the transplanting time. Those pots were grown in a naturally lit greenhouse under semi-controlled environmental conditions in Maoxian ecological station (31°41′N, 103°53′E, 1800 m above the sea level) located in dry valley of upper Mingjiang River. During the growing seasons, the seedling were grown in a day temperature range of 12–28 °C, a night temperature range of 9–15 °C and the relative humidity range of 35–85%. Experimental treatments started 30 d after the seedlings were transplanted.

A completely randomized design with two factors (two populations and two watering regimes) was employed. For drought treatment, half of replicates in each population were maintained at 25% of field capacity (generally severe drought condition in the summer in the dry valleys) by watering every four days, four replicates in each population. For the well-watered treatment (as control), another half of replicates in each population were watered to 100% of field capacity by supplying an amount of tap water equal to transpiration losses every four days. Evaporation from the soil surface was prevented by enclosing the pots in plastic bags, which were tied to the stems of the plants. Transpiration water loss was measured gravimetrically by weighing all pots every four days. The experiment treatment lasted for 100 days. At the end of experiment, fully expanded leaves in the middle part of plants of each replicate were randomly sampled to make physiological assays.

2.2 Measurements of Growth Traits and Leaf Water Content

Ten seedlings of each treatment were randomly tagged and chosen to measure seedlings' height every ten days during the experimental period (totally 100 days). At the end of the experiment, ten plants in each treatment were harvested for plant growth characteristic measurements. After leaf area for each seedling was determined by CI-202-scanning planimeter (CID Inc., Camas, WA), plants were divided into leaf, stem, root and root nodule, dried (80 °C, 48 h) to constant mass and weighed for biomass determination. Average leaf size, biomass partition, specific leaf mass (leaf dry mass divided by leaf area, SLM), root mass/foliage area ratio and root/total biomass ratio were then calculated. Leaf water content was also determined by formula as follows: leaf water content (%) = (1 – dry mass / fresh mass) × 100. The relative growth rate (RGR, g g^{-1} day^{-1}) was calculated as: $RGR = (\ln W_2 / \ln W_1) / t$, where W_1 and W_2 are the initial and final total dry mass and t is experiment duration (days). WUE (water use efficiency) was calculated for each plant as the ratio of biomass production to water transpired during the experiment.

2.3 Leaf Pigments Analyses

Total photosynthetic pigments were extracted with dimethyl sulfoxide (DMSO). Fresh leaf tissue (0.1 g, randomly selected from the middle parts of plants in each replicate as mentioned above) was placed in a test tube containing 10 ml

DMSO and left in the dark for 48 h. The absorbance of the extract was measured spectrophotometrically at 480, 665, 649 nm, and the turbidity of the extract was checked at 750 nm to be sure that it was always less than 0.01. The amounts of chlorophyll and carotenoids were calculated using the equations as described by Wellburn (1994) and expressed upon per unit dry mass (DM) basis.

2.4 Proline Content and Ascorbic Acid Content Determination

0.5 g leaves was extracted for determination of free proline content according to the method of Bates et al. (1973). The reaction mixture consisted of 2 ml leaf tissue extract, 2 ml acid ninhydrin and 2 ml of glacial acetic acid, and was boiled at 100 °C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and the absorbance was read at 520 nm, the free proline content was expressed as μg g^{-1} DM. 0.4 g leaves was extracted to estimate the total ascorbic acid content (Asa) using the method as described by Jain et al. (2003), and the Asa concentration was expressed as mg g^{-1} DM.

2.5 Measurements of ABA Content and Carbon Isotope Composition

Fully expanded leaves were used to measure ABA content. The samples were weighed immediately after harvest in the morning, then frozen in liquid nitrogen and stored at –80 °C until analyzed. ABA was analyzed as described by Li et al. (2002) and expressed as ng g^{-1} DM.

The abundances of stable isotopes of carbon in leaf samples were determined, as described by Li et al. (2000). The samples were oven-dried for 48 h at 80 °C and homogenized by grinding in a ball mill, and the relative abundance of ^{13}C and ^{12}C was determined with an isotope rationing mass spectrometer (Finnegan MAT Delta-E). The overall precision in δ-values was higher than 0.1 ‰, as determined from repeated samples.

2.6 Chemical Analyses

At last, the remaining leaves in the middle parts of plants in each replicate were pooled and dried to constant mass. Dried leaves were milled to pass through a 1 mm sieve and a 0.4 g subsample was assayed for total P (molybdenum blue colorimetry) and N (Kjeldahl digestion and titration). Leaf carbon (C) content was analyzed by the wet oxidation method (Moore and Chapman 1986). The analysis procedure for acetyl bromide lignin determination was according to Iiyama and Wallis (1990) and Hatfield et al. (1999). Individual samples were placed in a 15-ml glass reaction tube with a solution of 25% (w/w) AcBr in acetic acid (2.5 ml), and the tubes were capped immediately and heated in dry blocks set at 70 °C for 30 min. The tubes were shaken at 10-min intervals to promote dissolution of the samples. After heating, the samples were quantitatively transferred, with the aid of acetic acid, to 50 ml volumetric flasks that contained 10 ml of 2 M NaOH and 12 ml of acetic acid. Hydroxylamine (1.75 ml, 0.5 M) was added to each flask, and samples were diluted to 50 ml with acetic acid. The absorption at 280 nm was determined. All measurements were run in five times for each treatment.

2.7 Statistical Analysis

Analyses were performed with the software Statistical Package for the Social Science (SPSS) version 11.0 (SPSS Inc., Chicago, IL, USA). Data were log-transformed if necessary to ensure assumptions of normality and homogeneity of variances. Main factor effects (Population and Water regimes) and their interaction were tested using two-way ANOVA. Among the all treatments, the means were compared using the LSD (least significance difference) test. For height growth analysis, a three-way ANOVA was used to test the effects of population, water regime and duration. Pearson's correlation coefficients were calculated to determine the relationships between variables for different populations.

3 Results

3.1 Effects of Drought on Plant Morphological Traits

The drought significantly depressed height growth of *H. rhamnoides* throughout the whole experiment in both populations (Fig. 1). The main effects analysis show that water regimes, population and duration had significant impacts on height increment of both populations, and interactions between these three factors were also significant. LP had a higher height increment in both well watered and drought conditions. Drought also significantly decreased relative growth rate (RGR), nodule biomass (Fig. 2ac), and leaf size (Table 1), while significantly increased root/total biomass ratio (Fig. 2b), specific leaf mass (SLM) and root mass/foliage area ratio in both populations (Table 1). On the other hand, LP had higher values in RGR (Fig. 2a) and leaf size at the control

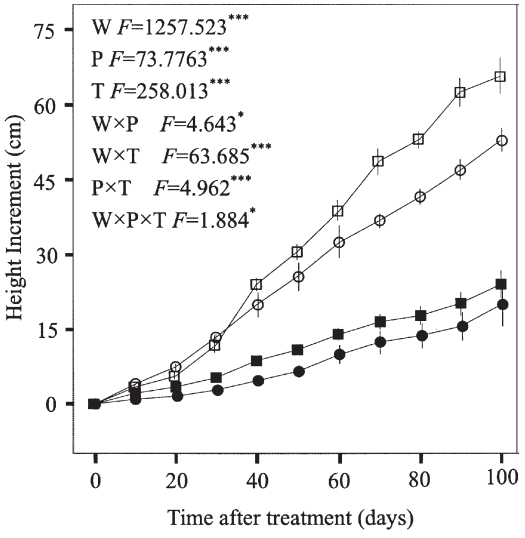

Fig. 1. Height growth in *H. rhamnoides* L. seedlings (□, ○ denote the lowland and highland population, respectively) in response to drought stress (open, control, black, drought, respectively). Values are means ± SE of 8 replicates. Symbols (*, ** and ***) indicate significant *P*-values at 0.05, 0.01 and 0.001 levels, respectively. W, water regime, P, population, T, time.

Table 1. The effects of drought on morphological and physiological properties in two altitudinal *H. rhamnoides* L. populations.

Morphological and physiological properties	Lowland population		Highland population		*F*-values		
	Control (100%)	Drought (25%)	Control (100%)	Drought (25%)	W	P	W × P
Leaf size (cm^2)	2.41±0.10a	2.30±0.05ab	1.99±0.10b	0.78±0.03c	47.96^{***}	103.28^{***}	32.54^{***}
SLM ($g\ m^{-2}$)	46.43±1.29d	61.59±0.41b	56.10±0.70c	79.72±1.48a	208.79^{***}	107.33^{***}	9.93^{**}
Root mass/Foliage area ($g\ m^{-2}$)	15.88±1.16d	64.5±8.32b	26.92±1.44c	77.38±2.57a	240.97^{***}	14.03^{***}	0.08^{NS}
Leaf water content (%)	67.13±0.19b	63.14±0.36c	69.78±0.36a	63.97±0.39c	209.25^{***}	26.28^{***}	7.27^{*}
Chlorophyll content ($\mu g g^{-1}$dm)	847.00±13.70b	770.79±13.43c	971.73±8.44a	871.92±31.83ab	8.88^{**}	27.40^{***}	0.44^{NS}
Total carotenoids ($\mu g g^{-1}$ dm)	148.22±2.24b	145.66±2.41b	168.95±2.31a	167.54±0.35a	0.02^{NS}	25.49^{***}	0.32^{NS}
Carotenoids/Chlorophyll	0.175±0.001c	0.189±0.0006a	0.175±0.001c	0.186±0.001b	186.97^{***}	4.71^{*}	2.25^{NS}
Proline content ($\mu g\ g^{-1}$dm)	64.95±2.04b	72.44±3.64ab	67.08±1.22b	77.42±2.92a	9.98^{**}	3.36^{NS}	1.95^{NS}
ABA content ($ng\ g^{-1}$dm)	325.52±149.33bc	591.48±132.50b	187.51±36.32c	1451.57±57.71a	45.27^{***}	10.08^{*}	19.26^{**}
Asa content ($mg\ g^{-1}$dm)	345.87±9.39b	330.84±7.58b	426.55±22.87a	234.57±9.43c	56.56^{***}	0.32^{NS}	41.32^{***}
WUE($g\ kg^{-1}$)	3.46±0.15b	5.62±0.35a	3.36±0.08b	3.64±0.34b	43.75^{***}	3.20^{NS}	11.76^{*}
$\delta^{13}C$	–28.6±0.25b	–24.64±0.54a	–29.18±0.10b	–25.53±0.17a	119.366^{***}	4.394^{NS}	0.198^{NS}

Values shown are mean ±SE of at least six replicates. Values within a row followed by the same letter do not differ significantly at $P<0.05$ by LSD pairwise comparisons. dm, dry matter; SLM, specific leaf mass; W, watering effect; P, population effect; W × P, watering × population interaction.*, **, *** indicate significant treatment main effects at $P<0.05$, $P<0.01$, $P<0.001$, respectively.

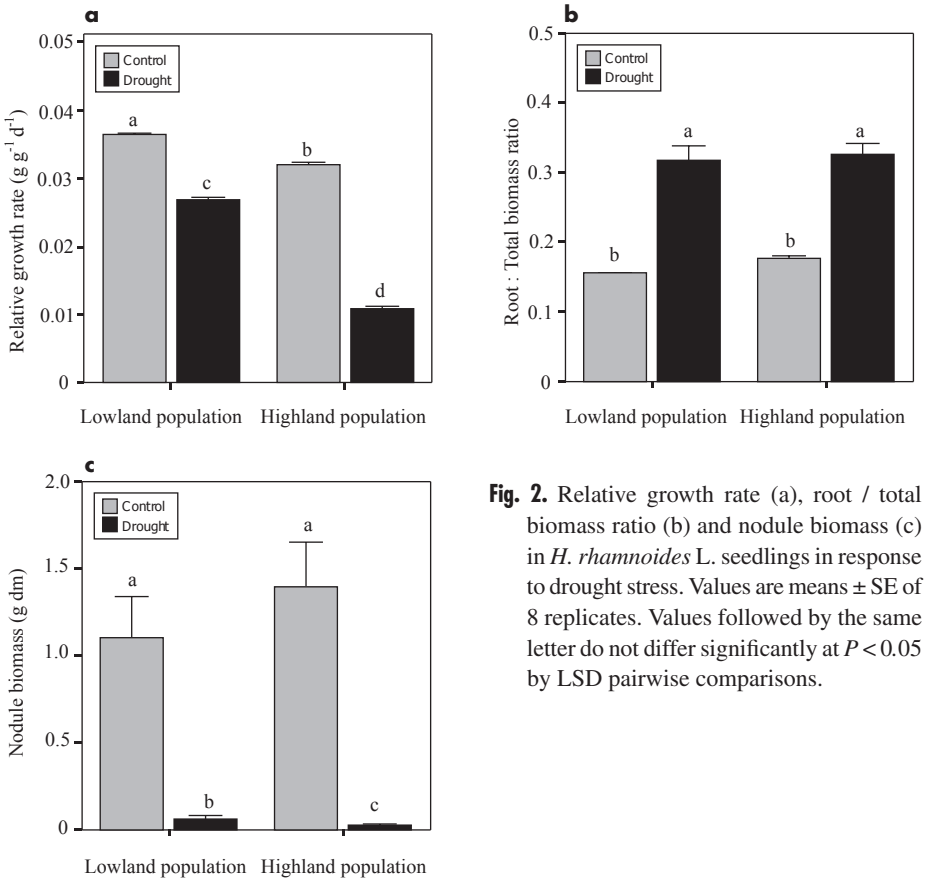

Fig. 2. Relative growth rate (a), root / total biomass ratio (b) and nodule biomass (c) in *H. rhamnoides* L. seedlings in response to drought stress. Values are means ± SE of 8 replicates. Values followed by the same letter do not differ significantly at $P < 0.05$ by LSD pairwise comparisons.

Table 2. Statistical significance of the *F* values (ANOVA) for the effects of water regime (W), population (P) and their interaction (W × P) on different parameters in two contrasting *H. rhamnoides* populations.

	RGR	R/TB	NB	Carbon	Nitrogen	Phosphorus	Lignin
W	1691.995***	190.932***	39.346***	29.931***	1.568 NS	78.64***	24.96***
P	745.713 ***	1.851 NS	0.425 NS	1.066 NS	29.439***	2.029NS	15.899***
W×P	245.907***	0.269 NS	0.714 NS	7.643*	0.484 NS	25.083***	1.644 NS

RGR, relative growth rate; R/TB, root/total biomass ratio; NB, nodule biomass.
NS, not significant. *, **, *** indicate significant treatment main effects at $P<0.05$, $P<0.01$, $P<0.001$, respectively.

condition than those of HP (Table 1). In addition, significant drought × population interactions were observed in RGR (Table 2, Fig. 2a), leaf size and SLM (Table 1), which made changes of these parameters caused by water stress in HP much higher than the other population.

3.2 Effects of Drought on Physiological Traits

Drought significantly decreased leaf water content, total chlorophyll content, and Asa content, but increased carotenoids/chlorophyll ratio, pro-

line content, ABA content and $\delta^{13}C$ value in both populations. Furthermore, population differences were also detected. HP had higher values in leaf water content, total chlorophyll content, total carotenoids content, carotenoids/chlorophyll and Asa content in the control condition, but had lower ABA content. Significant increase of WUE value was only seen in LP under drought condition. In addition, interactions of drought × population demonstrated that HP experienced greater reductions in water content and Asa content, and higher increment of ABA content response to drought compared with LP. The accumulation of ABA content under drought was as high as eightfold compared with the control in HP (Table 1).

3.3 Effects of Drought on Leaf Carbon, Nitrogen, Phosphorus and Lignin

Drought significantly decreased leaf carbon (C), phosphorus (P) and lignin content, but had little effect on nitrogen in both populations (Fig. 3a–c). However, LP had a higher nitrogen value than HP ($P < 0.1$, Fig. 3a). The drought × population interaction showed that drought-caused decrease in phosphorus and lignin were much higher in HP (Table 2, Fig. 3cd).

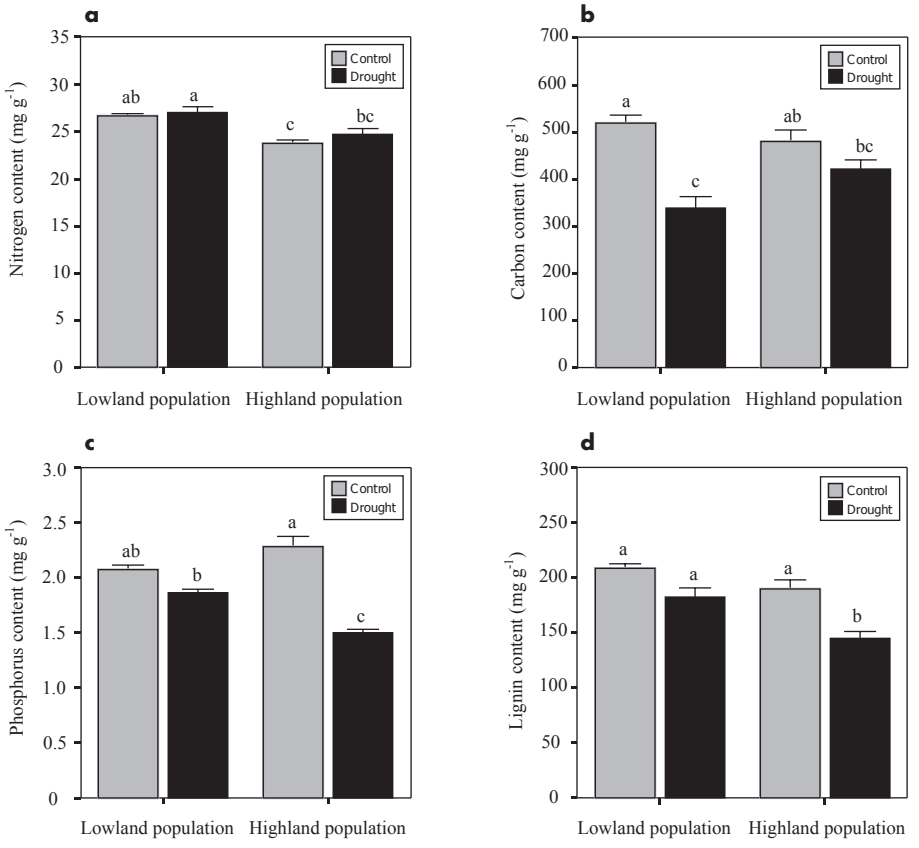

Fig. 3 Carbon content (a), Nitrogen content (b), Phosphorus content(c) and Lignin content(d)) in *H. rhamnoides* L. leaves in response to drought stress. Values are means ± SE of at least 4 replicates. Values followed by the same letter do not differ significantly at $P < 0.05$ by LSD pairwise comparisons.

Table 3. Correlation coefficients for some morphological and physiological properties of the contrasting *H. rhamnoides* populations. LP (upper triangle) and HP (lower triangle).

	SLW	R/TB	RGR	WUE	ABA	$\delta^{13}C$	C	P	lignin	Chl	proline
SLW		0.932**	–0.937**	0.949***	0.575	0.906**	–0.808*	–0.811*	–0.812*	–0.816*	0.597
R/TB	0.972***		–0.995***	0.995***	0.506	0.982***	–0.944***	–0.866**	–0.922**	–0.872**	0.752*
RGR	–0.980***	–0.999***		–0.997***	–0.514	–0.972***	0.920***	0.852**	0.918**	0.863**	–0.765*
WUE	0.397	0.386	–0.370		0.508	0.975***	–0.917**	–0.850**	–0.918**	–0.868**	0.771*
ABA	0.959***	0.997***	–0.994***	0.355		0.451	–0.301	–0.584	–0.321	–0.521	–0.002
$\delta^{13}C$	0.963***	0.987***	–0.990***	0.268	0.984***		–0.952***	–0.910**	–0.954***	–0.904**	0.734*
C	–0.629	–0.620	0.637	–0.418	–0.592	–0.636		0.791*	0.880**	0.880**	–0.759*
P	–0.947***	–0.953***	0.953***	–0.307	–0.944***	–0.949***	0.442		0.910**	0.779*	–0.465
lignin	–0.936**	–0.964***	0.955***	–0.414	–0.961***	–0.938**	0.430	0.978***		0.764*	–0.769*
Chl	–0.810*	–0.910**	0.892**	–0.336	–0.923**	–0.875**	0.385	0.891**	0.932**		–0.614
proline	0.928**	0.920**	–0.927**	0.148	0.920**	0.926**	–0.455	–0.941**	–0.914**	–0.854**	

SLW, specific leaf mass; R/TB, root/total biomass ratio; RGR, relative growth rate; WUE, water use efficiency; ABA, abscisic acid; $\delta^{13}C$, carbon isotope composition; C, leaf carbon content; P, leaf phosphorus content; Chl, total leaf chlorophyll content.
*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

3.4 Relationships among the Morphological and Physiological Responses of Leaves

The relationships among SLW, R/TB, RGR, WUE, ABA, $\delta^{13}C$, C, P, lignin, Chl and proline are shown in Table 3. In both populations, RGR were positively correlated with P, lignin and Chl, but negatively correlated with $\delta^{13}C$ and proline, while some morphological traits such as SLW and R/TB were positively correlated with $\delta^{13}C$, negatively correlated with P, lignin and Chl. For physiological traits, $\delta^{13}C$, lignin and proline were significantly correlated with most of the tested indices. However, population differences could also be observed, WUE and C were significantly correlated with most physiological parameters in LP, while correlations between ABA and other parameters was found mostly in HP population.

4 Discussion

Drought significantly decreased plant height, RGR and leaf size, while increased root/total biomass ratio, SLM and root mass/foliage area ratio in both tested sea buckthorn populations. All these responses in plant structure and growth pattern are often considered as an important acclimation strategy to severe water deficit over long time scales (Larcher 1995, Li et al 2000). Furthermore, in agreement with a recent research conducted by Marino et al (2007), which reported that drought stress affected rhizobial growth, formation, longevity and nodule functions, in our study, the nodule production of sea buckthorn populations was also markedly decreased by drought. On the other hand, much greater reductions in RGR and leaf enlargement (leaf size) paralleled with thicker leaves (indicated by higher SLM) were detected in HP in comparison with LP. As biomass accumulation (indicated by RGR) is the integrated consequence of morphological, physiological and biochemical responses to environmental condition, the greater reduction of RGR in HP implies that this population is more sensitive to drought. Additionally, compared with LP, smaller increment of root mass/foliage area ratio in HP may further reduce its capacity to uptake soil water and nutrients under drought condition.

It is well documented that ABA is a sensitive indicator of leaf water deficit and the change of soil water availability (Peuke et al. 2002, Li et al. 2002). In the well watered condition, HP possessed higher leaf water content and lower ABA content than LP, but drought treatment caused greater reduction in leaf water content and pronouncedly higher increment of ABA content in HP. The two parameters showed that HP was physiologically more affected by drought. Also, the free proline, a well-known indicator for physiological dryness (Patel and Vora 1985),

was significantly enhanced in HP but not in LP. The different physiological dryness may lead to population-specific responses in ascorbic acid synthesis. In our result, Asa content was significantly decreased in HP but remained unaffected in LP under drought condition. It was reported in previous studies that ascorbate synthesis could be decreased due to limitation of carbon fixation under severe drought condition (Smirnoff 1996, Bartoli et al. 1999), as is detected in HP in our study, which would significantly limit plant protective capacity against high levels of oxidative pressure under severe drought conditions. In addition, the different physiological dryness would cause population-specific changes in plant water use efficiency (WUE). In the present study, WUE was increased only in LP under drought condition, and this result demonstrated that LP could accumulate more assimilated substance and survive severe drought better than HP in the water-limited conditions. As a tool to measure long-term WUE (Farquhar et al. 1989), carbon isotope composition ($\delta^{13}C$) in our present study was increased by drought, however, there existed little difference between the two populations and that was not consistent with WUE. Many previous studies also validated that $\delta^{13}C$ does not always correlated with WUE (Ebdon et al. 1998).

As far as photosynthetic pigments are concerned, similar changes occurred in the two tested populations under drought condition. The chlorophyll content was significantly decreased as observed in other studies (Moran et al. 1994, Duan et al. 2005), which could be due to either slow synthesis or fast breakdown of chlorophyll pigments (Ashraf 2003). Since the carotenoid content remained at a high level, the significant increase of carotenoids/chlorophyll ratio could help protect chloroplast from photooxidative destruction under drought condition.

Leaf quality was also affected by drought in both populations in our study. Higher leaf nitrogen content in LP would be beneficial for photosynthesis and therefore maintaining higher RGR, in particular under severe drought condition. Drought had little effect on leaf nitrogen content in both populations, and it would be a result of plant structure adjustment (i.e. significantly enhanced root mass/foliage area ratio and root/total biomass), which compensated the nitrogen absorbing insufficiency due to destroying of nodules. And this thus helps maintain the functioning of the photosynthetic system and improve water economy (Li et al 2006). On the other hand, drought stress caused greater reduction of nodule biomass in HP, which probably lowered this population's ability for nitrogen uptake and consequently led to lower leaf nitrogen content compared with LP under drought condition. The reduction of carbon accumulation by drought is well reported (e.g. Peuke et al. 2002, Peuke and Rennenberg 2004) and consistent with our results. Compared with HP, there existed a more active "growing sink" in LP for the pronouncedly higher RGR and lower ABA content under drought condition. So the leaf carbon assimilates in the mature leaves were allocated more to other growing organs such as root, shoot and expanding leaves, which resulted in lower level of leaf carbon content in LP in our study. Consistent with previous studies (e.g. Peuke and Rennenberg 2004, Sardans et al. 2008), the leaf P content of sea buckthorn populations was also significantly reduced by drought because of the decreases of soil phosphorus availability (Passioura 2002). In the present study, the marked reduction of leaf phosphorus content in HP may affect many physiological processes related to phosphorus, in particular the conversion and transportation of plant metabolites. Recently, Fernandez et al. (2006) observed that a decrease of leaf P content led to increased stomatal conductance and hence lower WUE, and we also observed that HP with lower leaf P content have low level of WUE under drought condition. Vincent et al. (2005) reported that lignin content in maize leaves was decreased by drought, which also occurred in HP population in our study. Lignin play an important role in preventing leaf water dissipation, and the reduction of lignin content in HP would further aggravate the damaging effect of drought stress. Until now, very little is known about the effects of drought on leaf lignin biosynthesis (Moura et al. 2010). In an early study, Harrak et al (1999) observed the decreases of lignin concentration and transcript of one relative gene (PTGRP) under drought condition. Recently, Alvarez et al. (2008) observed reductions of ferulic acid and anionic peroxidase activity, increases of *p*-coumaric, caffeic acids and cationic peroxidase activity in the

xylem sap of maize under drought condition, and they suggested this as an indication that drought decreased the biosynthesis of lignin in maize. Hu et al (2009) also observed the decreases in leaf lignin concentration and expressions of two key enzymes for lignin synthesis when withholding water availability in drought-sensitive maize cultivars, and concluded that leaf lignin could serve as a useful index for evalution of drought tolerance. In our results, different changes of leaf lignin in two *H. rhamnoides* populations might be important reason for different tolerance to drought.

In conclusion, HP had much lower rates of biomass accumulation, plant growth and leaf enlargement than LP under drought condition, and it also experienced higher loss of leaf water content paralleling with greater enhancement of ABA level. The higher vulnerability of HP to drought was related to its lower ability for uptake of plant nutrition such as nitrogen and phosphorus under drought condition. Just contrary to our previous hypothesis, the present resusts demonstrate that drought tolerance of HP was not necessarily improved by acclimation to low temperature and high UV-B radiation. However, LP exhibited effective adaptation strategies such as improvement of water economy and maintaining high level of Asa content. We suggest that many factors should be taken into account in the genotype selection of *H. rhamnoides* for reforestation of upper Minjiang River regions in China. Not only the plant antioxidant capacity and WUE affect plant drought tolerance, but also nutrient availability does.

Acknowledgments

The research was supported by the National Science Foundation of China (Nos. 30972341 and 30800127) and the Program of "100 Distinguished Young Scientists" in Chinese Academy of Sciences.

References

Alvarez, S., Marsh, E.L., Schroeder, S.G. & Schachtman, D.P. 2008. Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant, Cell and Environment 31: 325–340.

Ashraf, M. 2003. Relationships between leaf gas exchange characteristics and growth of differently adapted populations of blue panicgrass (Panicum antidotale Retz.) under salinity or waterlogging. Trends in Plant Science 165: 69–75 .

Bartoli, C.G., Simontacchi, M., Tambussi, E., Beltrano, J., Montaldi, E. & Puntarulo, S. 1999. Drought and watering-dependent oxidative stress: effect on antioxidant content in Triticum aestivum L. leaves. Journal of Experimental Botany 50: 375–383.

Bates, L.S, Waldren, R.P. & Tear, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205–207.

Brodribb, T. & Hill, R.S. 1998. The photosynthetic drought physiology of a diverse group of southern hemisphere conifer species is correlated with minimum seasonal rainfall. Functional Ecology 12: 465–471.

Duan, B., Lu, Y., Yin, C., Junttila, O. & Li, C. 2005. Physiological responses to drought and shade in two contrasting Picea asperata populations. Physiologia Plantarum 124: 476–484.

Ebdon, J.S., Petrovic, A.M. & Dawson, T.E. 1998. Relationship between carbon isotope discrimination, water use efficiency, and evapo-transpiration in Kentucky bluegrass. Crop Science 38: 157–162.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40: 503–537.

Fernandez, M., Novillo, C. & Pardos, J.A. 2006. Effects of water and nutrient availability in Pinus pinaster ait. Open pollinated families at an early age: growth, gas exchange and water relations. New Forest 31: 321–342.

Hare, P.D., Cress, W.A. & van Staden, J. 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. Journal of Experimental Botany 50: 413–434.

Hatfield, R.D., Grabber, J., Ralph, J. & Brei, K. 1999. Concentrations in Herbaceous Plants: Some Cautionary Notes. Journal of Agricultural and Food Chemistry 47: 628–632.

Heuer, B. 1994. Osmoregulatory role of proline in water- and salt-stressed plants. In: Pessarakli M, ed. Handbook of plant and crop stress. New York, NY, USA: Marcel Dekker Inc. p. 363–381.

Hsiao, T.C. 1973. Plant responses to water stress. Annual Review of Plant Physiology 24: 519–570.

Hu, Y., Li, W.C,. Xu, Y.Q., Li, G.J., Liao, Y. & Fu, F.L. 2009. Differential expression of candidate genes for lignin biosynthesis under drought stress in maize leaves. Journal of Applied Genetics 50: 213–223 .

Iiyama, K. & Wallis, A.F.A. 1990. Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. Journal of the Science of Food and Agriculture 51: 145–161.

Jain, K., Kataria, S. & Guruprasad, K.N. 2003. Changes in antioxidant defenses of cucumber cotyledons in response to UV-B and to the free radical generating compound AAPH. Plant Science 165: 551–557.

Larcher, W. 1995. Physiological Plant Ecology, Springer-Verlag, Berlin.

Li, C. & Wang, K. 2003. Differences in drought responses of three contrasting Eucalyptus microtheca F. Muell. populations. Forest Ecology and Management 179: 377–385.

— , Berninger, F., Koskela, J. & Sonninen, E. 2000. Drought responses of Eucalyptus microtheca provenances depend on seasonality of rainfall in their place of origin. Australian Journal of Plant Physiology 27: 231–238.

— , Puhakainen, T., Welling, A., Viherä-Aarnio A., Ernstsen, A., Junttila, O., Heino, P. & Palva, E.T. 2002. Cold acclimation in silver birch (Betula pendula). Development of freezing tolerance in different tissues and climatic ecotypes. Physiologia Plantarum 116: 478–488.

— , Zhang, X., Liu, X., Luukkanen, O. & Berninger, F. 2006. Leaf morphological and physiological responses of Quercus aquifolioides along an altitudinal gradient. Silva Fennica 40: 5–13.

Livingston, N.J., Guy, R.D., Sun, Z.J. & Ethier, G.J. 1999. The effects of nitrogen stress on the stable carbon isotope composition and water use efficiency of irrigated and dry land white spruce (Picea glauca (Moench) Voss.) seedlings. Plant, Cell and Environment 22: 281–289.

Lu, R. 1992. Seabuckthorn: a multipurpose plant species for fragile mountains. ICIMOD Publication Unit, Katmandu, Nepal.

— 1995. Eco-geographical distribution of seabuckthorn and prospects of international cooperation. In ICRTS, Worldwide Research Development of Seabuckthorn. China Science and Technology Press. p. 11–22.

Marino, D., Frendo, P., Ladrera, R., Zabalza, A., Puppo, A., Arrese-Igor, C. & González, E.M. 2007. Nitrogen fixation control under drought stress localized or systemic? Plant Physiology 143: 1968–1974.

Moore, P.D. & Chapman, S.B. 1986. Methods in Plant Ecology, second ed. Blackwell Scientific Publications, Oxford. p. 301–317.

Moran, J.F., BecLinna, M., Iturbe-Ormaetxe, I., Frechilla, S., Klucas, R.V. & Aparicio-Tejo, P. 1994. Drought induces oxidative stress in pea plants. Planta 194: 346–352.

Moura, J.C.M.S., Bonine, C.A.V., Viana, J.O.F., Dornelas, M.C. & Mazzafera, P 2010. Abiotic and biotic stresses and changes in the lignin content and composition in plants. Journal of Integrative Plant Biology 52: 360–376.

Passioura, J.B. 2002. Soil conditions and plant growth. Plant, Cell and Environment 25: 311–318.

Patel, J.A. & Vora, A.B. 1985. Free proline accumulation in drought-stressed plants. Plant and Soil 84: 427–429.

Peuke, A.D. & Rennenberg, H. 2004. Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (Fagus sylvatica L.): phosphorus most affected by drought. Trees 18: 639–648.

— , Hartung, W., Schraml, C. & Rennenberg, H. 2002. Identification of drought sensitive beech ecotypes by physiological parameters. New Phytologist 154: 373–388.

Polley, H.W., Johnson, H.B., Marino, B.D. & Mayeux, H.S. 1993. Increase in C_3 plant water-use efficiency and glacial to present CO_2 concentrations. Nature 361: 61–64 .

Sardans, J., Peñuelas, J., Prieto, P. & Estiarte, M. 2008. Drought and warming induced changes in P and K concentration and accumulation in plant biomass and soil in a Mediterranean shrubland. Plant and Soil 306: 261–271 .

Saxe, H., Ellsworth, D.S. & Heath, J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. New Phytologist 139: 395–436.

Smirnoff, N. 1996. The function and metabolism of ascorbic acid in plants. Annals of Botany 78: 661–669.

Tang, Y., Xie, J. & Sun, H. 2004. Revisiting sustainable

development of dry valleys in Hengduan mountains region. Journal of Mountain Science 1(1): 38–45.

Vincent, D., Lapierre, C., Pollet, B., Cornic, G., Negroni, L. & Zivy, M. 2005. Water deficits affect caffeate O-methyltransferase, lignification and related enzymes in Maize leaves. A proteomic investigation. Plant Physiology 137: 949–960.

Volaire, F., Thomas, H., Bertagne, N., Bougeois, E., Gautier, M.F. & Lelièvre, F. 1998. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought II. Water status, solute accumulation, abscisic acid concentration and accumulation of dehydrin transcripts in bases of immature leaves. New Phytologist 140: 451–460.

Wellburn, A.R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology 144: 307–3.

Total of 40 references