

*Full Length Research Paper*

# Effects of soil water deficit on seedlings of different *Medicago falcata* L. populations

He Wang<sup>1</sup>, Li Liu<sup>2</sup> and Daowei Zhou<sup>3\*</sup>

<sup>1</sup>Liaoning Province Key Laboratory of Basin Pollution Control, Liaoning Academy of Environmental Sciences, Shenyang, 110031, China.

<sup>2</sup>Key Laboratory of Vegetation Ecology, Institute of Grassland Science, Northeast Normal University, Changchun, Jilin Province, 130024, China.

<sup>3</sup>Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin Province, 130012, China.

Accepted 6 February, 2012

***Medicago falcata* L. survives in desiccation conditions and is used to provide the genetic material for improved drought survival of this otherwise productive forage species (crosses referred to as *M. media*). Seedlings were grown in one of three soil water contents (80, 50 and 25% of field capacity) to compare physiological and morphological response under soil water stress. After eight weeks, populations differed in drought responses, and populations from Russia displayed superior characteristics such as more survival than others; maintaining stomatal more open, high water use efficiency (WUEi) and photosynthesis rate; less leakage of electrolytes from the plasma membrane, large leaf area, more crown buds, big crown diameter, high root length and relative root length, and an approximately 1.5 fold root: shoot ratio than others, etc. We recommended that the Russian *M. falcata* L. populations with excellent drought tolerance are suitable to grow in arid and semi-arid regions in China, and also a potential selection for breeding new drought tolerant forage.**

**Key words:** *Medicago falcata* L., soil water deficit, photosynthesis, water use efficiency, leaf area, survival, biomass allocation.

## INTRODUCTION

Water deficit is a principal abiotic factor that influence on plant growth in arid and semiarid areas. Insufficient moisture lead to water stress and some negative effects on plants such as reduced plant growth (Ohashi et al., 2000), photosynthesis (Miyashita et al., 2005), and altered biomass allocation (Deblonde and Ledent, 2001), what is more, decreased plant survival (Enright and Lamont, 1992). Differences in drought responses of plants may be a consequence of different physiological and morphological adaptations (Reich et al., 1992; Caspersen and Kobe, 2001). Under soil water deficit, plant will accumulate osmotic solutes like proline in cells;

limit damage of biomembrane structure and photosynthesis rate to assimilate more carbohydrates (Zhang et al., 1996; Miyashita et al., 2005). Besides, plants will show phenotypic plasticity under long-term water deficit (Sultan, 2000), for instance decrease of plant height and increase biomass allocation to root can be helpful to utilize water efficiently. Survival is assumed to begin when stomatal conductance has reached its minimum and plant CO<sub>2</sub> assimilation has essentially stopped (Sinclair, 2000; Spieß et al., 2011). Survival in drought condition is usually not an important corner in production of crops in industrial agriculture since there may be subsistence situations to maintain even a minimum yield as not to lost economic value of crops; what is different, survival is first and foremost for field plants in desiccation regions. And also, it is significant for perennial legume to survive under drought stress

\*Corresponding author. E-mail: [zhoudaowei@neigae.ac.cn](mailto:zhoudaowei@neigae.ac.cn). Tel: 086-024-62835457. Fax: 086-024-62835457.

**Table 1.** Origins of lines.

Lines		Source	
Daviluya	( <i>M.falcata</i> )	Cultivar	Russian Meadow Research Institute, Russia.
Culuskaya	( <i>M.falcata</i> )	Cultivar	Russian Agriculture Research Institute, Russia.
XiMeng	( <i>M.falcata</i> )	Wild	Inner Mongolia, China.
GongNong	( <i>M.sativa</i> )	Cultivar	Jilin Academy of Agricultural Sciences, China.

**Table 2.** Physical and chemical characteristics of soil used in this study (OC: organic contents; CEC: the cation exchange capacity; TN: total N; TP: total P; EN: effective N; EP: effective P).

Type of soil	pH	OC (g kg <sup>-1</sup> )	CEC (cmol kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	TP (g kg <sup>-1</sup> )	EN (mg kg <sup>-1</sup> )	EP (mg kg <sup>-1</sup> )
Chernozem	7.6	20.7	26.8	2.2	0.6	121.5	24.8

because it could lead to increase in the possibility of allowing plants to return to productive growth once water is again supplied.

Attaining information on plant survival, physiological and morphological responses is necessary to provide comprehensive understanding of drought resistance of plants and crucial to identify more adapted genotypes to breed more drought-tolerant lines. Alfalfa (*Medicago sativa* L.) is grown globally as a perennial herbaceous legume on an estimated 32 million hectares (Barnes et al., 1988; Michaud et al., 1988). Alfalfa is highly valued for animal feed because of its high protein content, intake potential and digestibility. Alfalfa is also an important rotational crop which can improve soil structure due to the effects of a large deeply penetrating taproot that contributes to soil aeration and organic matter content (CFIA, 2005). But in northeast China where the climate is cold and semi-arid, seedlings of *M. sativa* usually die from an early season drought or chilly winter. It is urgent to breed a drought and cold resistant leguminous forage in this region. *M. falcata* L., regarded as a more resistant species of *Medicago* is geographically distributed in the cold and dry areas of Russia, Mongolia, Scandinavia, and China (Lesins and Lesins, 1979), and tend to be more drought-winter hardy comparing with *M. sativa* (Barnes et al., 1988; Heinrichs, 1978). Therefore, *M. falcata* is often used as a parent in breeding (Riday et al., 2002). Two Russian populations of *M. falcata* (Daviluya and Culuskaya) in this study were provided by Russian Meadow Research Institute and Russian Agriculture Research Institute respectively. These populations are distributed the region between North Latitudinal 60° to 64°, near the coastwise of Lena River and Vilyuy River in Western Siberian in Russia where annual precipitation is less than 200 to 250 mm. This area is influenced by intense continental climate with annual average temperature of -10.2°C (Ivanov, 2000).

The paper present here is on the assumption that populations from this cold and drought region will be more resistant to drought conditions such as in agro-pastoral region of northeast China. If the drought survival

capability of *M. sativa* seedlings can be improved by breeding and crossing with *M. falcata* populations then this vast area could be opened for extensive planting of this elite forage plant. The primary objectives of this study were to: (1) compare drought resistance of different populations using plant survival and several physiological, morphological traits and (2) develop an understanding of the strategies underpinning drought resistance in these populations. We compared four populations: two Russian cultivars of *M. falcata*, a wild *M. falcata* of northeast of China and a drought-tolerant cultivar of *M. sativa* selected in China. The experiments were performed in late autumn of 2005.

## MATERIALS AND METHODS

### Plant materials

Origins of the four populations are given in Table 1. Seeds were sown in breeding tubes (1.5 × 1.5 × 3 cm) and placed in a greenhouse held at a temperature of 29/21°C (day/night) and relative humidity of 70%, nature light. Seedlings were thinned to one per tube. At four weeks, similar sized seedlings were transferred into plastic pots (30 × 30 × 28 cm) containing 4.5 kg of soil [mixture of chernozem (N: 0.07%), sand and perlite (2:1:1)]. Each pot contained 30 seedlings.

### Soil characteristics

Soil used in this experiment was the topsoil of infield in western of Jilin Province in China (Table 2).

### Drought stress

The pots were placed on benches in a rain shed outside. Pots containing ten seedlings were subject to one of three soil water levels; 80% (control), 50% (moderate stress, M), and 25% (severe stress, S) of field capacity of water (determined by pre-experiment). The water content of each level was obtained by transplanting plant into pots when soil water content was 80%, then maintained with no water added until the corresponding water content was reached. These soil water levels were maintained by weighing pots at 1800 h and daily adding water to bring the pots to the desired weight. Each

**Table 3.** Comparison between lines in three soil water status (control: CK; moderate soil water stress: M; severe soil water stress: S), showing effect on proline content (PC), membrane permeability (MP); stomatal conductance ( $g_s$ ) and instantaneous water use efficiency (WUEi) at light intensity of  $1400 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (mean  $\pm$  SEM).

	Treatments	Darviluya	Culuskaya	XiMeng	Gong Nong
PC ( $\text{mg g}^{-1}$ )	CK	11.20 ( $\pm 3.57^c$ )	14.27 ( $\pm 3.48^c$ )	6.24 ( $\pm 1.54^c$ )	6.04 ( $\pm 0.92^c$ )
	M	24.47 ( $\pm 1.88^b$ )	17.76 ( $\pm 3.30^c$ )	28.84 ( $\pm 1.52^b$ )	14.81 ( $\pm 1.24^c$ )
	S	35.62 ( $\pm 1.29^{ab}$ )	26.61 ( $\pm 3.28^b$ )	37.07 ( $\pm 0.45^a$ )	34.05 ( $\pm 1.80^{ab}$ )
MP (%)	CK	2.95 ( $\pm 0.22^f$ )	5.72 ( $\pm 1.18^{ef}$ )	1.32 ( $\pm 0.24^f$ )	7.19 ( $\pm 0.97^{ef}$ )
	M	11.45 ( $\pm 3.27^{def}$ )	6.85 ( $\pm 1.15^{ef}$ )	20.92 ( $\pm 5.91^{cde}$ )	50.97 ( $\pm 7.64^a$ )
	S	13.79 ( $\pm 2.87^{cdef}$ )	28.92 ( $\pm 5.15^{bc}$ )	25.47 ( $\pm 1.88^{bcd}$ )	56.33 ( $\pm 8.47^a$ )
$g_s$ ( $\text{mmol m}^{-2} \text{ S}^{-1}$ )	CK	7.30 ( $\pm 1.02^b$ )	5.87 ( $\pm 1.10^{bcd}$ )	4.74 ( $\pm 0.87^{cd}$ )	9.81 ( $\pm 1.76^a$ )
	M	6.57 ( $\pm 0.49^b$ )	6.96 ( $\pm 1.39^b$ )	3.73 ( $\pm 0.35^{de}$ )	5.20 ( $\pm 1.54^{bcd}$ )
	S	3.74 ( $\pm 0.42^{de}$ )	4.95 ( $\pm 1.08^{bcd}$ )	1.81 ( $\pm 0.09^e$ )	1.49 ( $\pm 0.28^e$ )
WUEi ( $10^{-3}$ )	CK	4.52 ( $\pm 0.28^b$ )	4.37 ( $\pm 0.30^b$ )	4.31 ( $\pm 0.45^b$ )	5.47 ( $\pm 0.42^a$ )
	M	4.56 ( $\pm 0.78^b$ )	4.25 ( $\pm 0.31^b$ )	4.29 ( $\pm 0.78^b$ )	2.99 ( $\pm 0.56^c$ )
	S	1.96 ( $\pm 0.26^d$ )	3.76 ( $\pm 0.38^c$ )	0.99 ( $\pm 0.23^e$ )	0.40 ( $\pm 0.62^e$ )

Different letters within each variable indicate significant difference at  $P < 0.01$  level.

treatment was replicated 4 times. The experimental design was a randomized complete block design with 4 populations and 3 water levels arranged randomly.

### Measurements

After eight weeks stress, proline content (of leaves) was quantified according to the method of Bates and Waldren (1973). Membrane permeability was measured as follows: five leaves were placed in a test tube with 13 ml of ultrapure water prepared by Water Purification System (UPHW-IV-90T, Shanghai, China) and oscillated at 100 rpm in a shaker at a temperature of  $20^\circ\text{C}$  for 2 h. Electrical conductivity of the liquid ( $\text{EC}_1$ ) were measured with a conductivity meter (Orion 150, ORION Co.USA). Test tubes were then placed in boiling water for 10 min to disrupt cell membranes and the electrical conductivities of the liquid were again measured ( $\text{EC}_2$ ). Membrane permeability was determined as the ratio of  $\text{EC}_1/\text{EC}_2$ . The five leaves were randomly selected from plants in each treatment and there were twenty replicates of five leaves for each treatment. On the other hand, stomatal conductance, net photosynthesis rate and transpiration rate of each treatment were measured under eleven light intensities (0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and  $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) at a  $\text{CO}_2$  concentration of  $350 \pm 5 \mu\text{mol mol}^{-1}$  ( $\text{CO}_2$  level were obtained from the 12 g  $\text{CO}_2$  cartridges and automatically controlled by the LI-COR 6400  $\text{CO}_2$  injector device [6400-01  $\text{CO}_2$  injector, LI-COR, USA]). Gas exchange was measured with a LI-COR 6400 portable photosynthesis system (LI-COR Inc. Lincoln, NE). Water use efficiency was calculated by dividing photosynthetic rate by the transpiration rate. Ten seedlings from each treatment and three terminal fully-open leaflets of each seedling were randomly selected for measurement.

Seedling survival rate (Sr) was calculated as the difference in living individuals between the final and the initial sampling date:

$$\text{Sr} = \frac{N_i}{N_0} \times 100\%$$

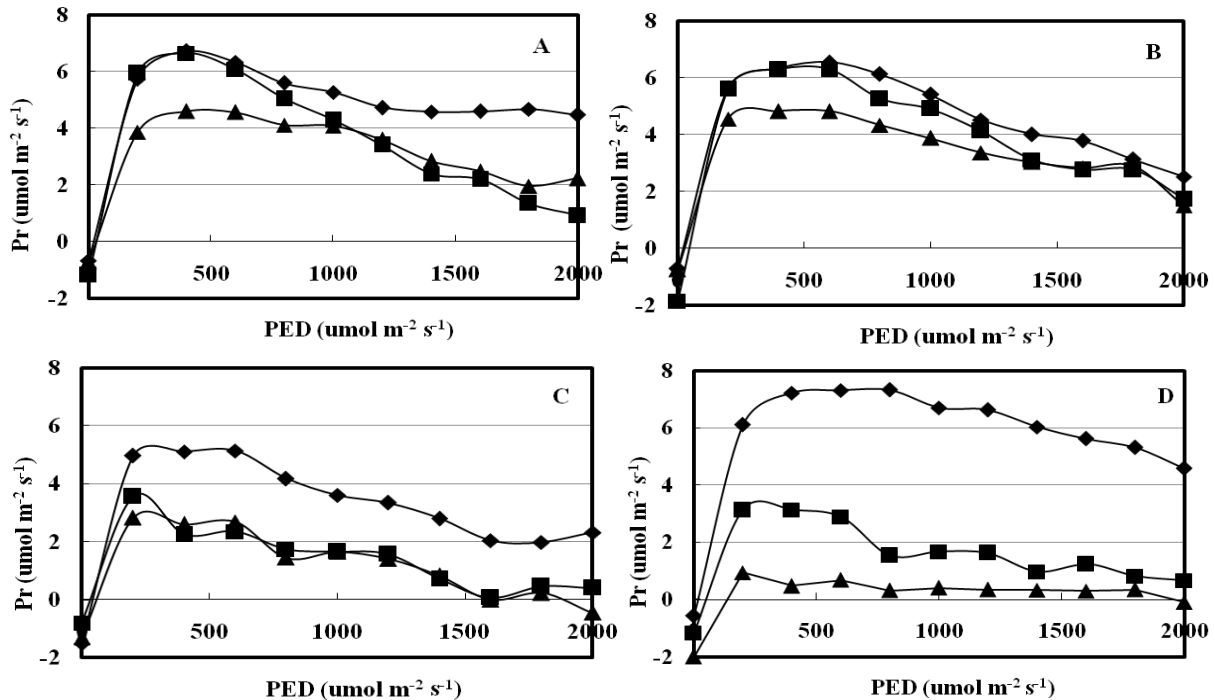
Where  $N_i$  was the final number of living seedlings,  $N_0$  was the initial number of seedlings before treatment.

All seedlings were harvested and roots were carefully washed after ten weeks of drought stress. Plant heights, length of central root, relative root length, and the crown diameter, and crown buds were measured. Length of central root was measured using tapeline with the minimum of 0.1 cm. Relative root length was the ratio of the root length to plant height which was calculated by dividing root length by plant height. Leaf area was calculated using the THSCSA ImageTool (IT Version 2.0) software. Plants were separated into leaves, stems and roots, weighted for fresh weight, then oven dried at  $70^\circ\text{C}$  for 48 h and weighed. Specific leaf area was the leaf area per unit leaf weight; stem: leaf DM ratio and root: shoot DM ratio were calculated. Analyses of variance (ANOVA) and least significant differences (LSD) test or Duncan multiple range tests were performed to detect differences between populations and soil water levels treatments. The SPSS Version 11.0 for Windows statistical software package was used.

## RESULTS

### Physiological effects on seedlings under soil water deficit

The proline content (PC) of all populations increased under soil water deficit (Table 3), for example, Darviluya and XiMeng, PC in M and S stress was significantly more than that in CK, while for Culuskaya and GongNong, the increment was not significant until under severe stress. Membrane permeability (MP) of Darviluya under stress had no significant increase but in Culuskaya under severe stress it is significantly higher than those of the control and M treatment (Table 3). The MP of XiMeng and GongNong increased 16.5- and 6-fold under stress respectively. Data (Table 3 and Figure 1) shows the



**Figure 1.** Relationship between photosynthetic rate and light illumination at no (◆), moderate (■) and severe (▲) water stress for lines (A) Darviluya, (B) Culuskaya, (C) XiMeng and (D) GongNong.

effects of withholding water on net photosynthetic rate (Pr), instantaneous water use efficiency (WUE<sub>i</sub>) and stomatal conductance ( $g_s$ ) of leaves in seedlings. The behaviors of stomatal conductance under stress were varied in different populations: soil water deficit did not influence the  $g_s$  of Darviluya; for Culuskaya and XiMeng it did not significantly decrease under M stress, and when under S stress it sharply decreased; for GongNong it decreased significantly along the stress grade (Table 3). Also in WUE<sub>i</sub>, cultivars demonstrated different changes: for three populations of *M. falcata* it did have significant decrement under M stress, and when in S stress it decreased significantly in Darviluya and XiMeng; for GongNong, it sharply decreased, approximately ½ and 1% of that of CK. However, there were statistically significant differences in these traits among soil water levels within each cultivar as shown by the significant interaction between cultivar and soil water level (Table 6).

Net photosynthetic rates (Pr) differed between populations and soil water levels (Figure 1). For GongNong, Pr declined sharply with the decreasing of soil water level whereas for the other populations, there was no significant difference between the two soil water treatments except at low light illuminations.

#### Morphological effects on seedlings under soil water deficit

After water deficit, leaves of some seedlings were drying

and wilting. The most sensitive seedlings was GongNong (*M. sativa*), with leaves almost gone which could also be shown by stem/leaves ratio of biomass indirectly (Table 5). There was a gradual loss of living seedlings during 8 weeks water stress. One-way ANOVA of survival revealed significant differences among populations under stress. Survival was significantly lower in GongNong which was 50% of M stress and 20% of S stress (Table 4). The survival of different populations followed the decreased order: Culuskaya, Darviluya, XiMeng and GongNong. Seedlings differed significantly in their morphological properties, for example plant height (PH), leaf area (LA), and root: shoot ratio (RS) etc. under three watering regimes (Tables 4 and 5). The watering regimes significantly affected all growth traits, and the watering  $\times$  line effect was also significant in these traits except for root length, leaf biomass and root: shoot ratio (Table 6). Soil water deficit had significant negative effects on plant height (PH), leaf area (LA), and crown buds (CB), while drought stress increased specific leaf area (SLA) (Table 4). Darviluya and XiMeng did not show any difference in SLA between moderate and severe stress but showed a significant increase in SLA when compared with control (Table 4). The increase in SLA of Culuskaya was significant only when it was under severe stress.

For GongNong, both M and S stress significantly increased its SLA which was significantly higher than the other three populations. Soil water deficit affected root morphology, including root length (RL), relative root length (RRL) and root crown diameter (RCD) (Table 4).

**Table 4.** Comparison between lines in three soil water status (control: CK; moderate soil water stress: M; severe soil water stress: S), showing effect on survival (Sr), plant height (PH); leaf area (LA), special leaf area (SLA), and crown buds (CB) (mean  $\pm$  SEM).

	Treatments	Darviluya	Culuskaya	XiMeng	Gong Nong
Sr (%)	CK	95.83 ( $\pm 3.54^a$ )	97.5 ( $\pm 2.34^a$ )	98.33 ( $\pm 4.67^a$ )	99.1 ( $\pm 7.00^a$ )
	M	83.33 ( $\pm 4.99^b$ )	87.83 ( $\pm 4.41^b$ )	70.83 ( $\pm 3.22^{bc}$ )	50 ( $\pm 1.98^d$ )
	S	60 ( $\pm 6.78^c$ )	66.67 ( $\pm 2.15^c$ )	55 ( $\pm 2.09^d$ )	20 ( $\pm 1.03^e$ )
PH (cm)	CK	17.35 ( $\pm 0.98^d$ )	24.49 ( $\pm 1.39^c$ )	36.35 ( $\pm 1.51^a$ )	32.92 ( $\pm 1.04^{ab}$ )
	M	12.2 ( $\pm 0.80^e$ )	16.86 ( $\pm 0.87^d$ )	26.65 ( $\pm 1.15^c$ )	31.06 ( $\pm 0.97^b$ )
	S	12.01 ( $\pm 0.76^e$ )	14.35 ( $\pm 0.53^{de}$ )	26.28 ( $\pm 1.29^c$ )	25.46 ( $\pm 0.89^c$ )
LA (mm <sup>2</sup> )	CK	152.87 ( $\pm 6.99^{ab}$ )	163.59 ( $\pm 8.69^a$ )	108.62 ( $\pm 3.17^{de}$ )	167.42 ( $\pm 8.67^a$ )
	M	126.62 ( $\pm 5.02^{cd}$ )	136.84 ( $\pm 6.29^{bc}$ )	94.07 ( $\pm 3.57^{ef}$ )	129.56 ( $\pm 10.58^{bcd}$ )
	S	111.6 ( $\pm 6.44^{cde}$ )	127.41 ( $\pm 8.96^{bcd}$ )	75.72 ( $\pm 3.38^f$ )	104.09 ( $\pm 7.58^{de}$ )
SLA (mm <sup>2</sup> mg <sup>-1</sup> )	CK	7.76 ( $\pm 0.36^f$ )	12.67 ( $\pm 0.58^c$ )	9.45 ( $\pm 0.28^{ef}$ )	9.73 ( $\pm 0.50^{def}$ )
	M	12.03 ( $\pm 0.70^{cde}$ )	16.3 ( $\pm 0.79^b$ )	12.22 ( $\pm 0.46^{cd}$ )	12.7 ( $\pm 1.04^c$ )
	S	13.47 ( $\pm 0.53^c$ )	9.45 ( $\pm 0.28^{ef}$ )	12.21 ( $\pm 0.55^{cd}$ )	21.68 ( $\pm 1.58^a$ )
CB (No. plant <sup>-1</sup> )	CK	4.03 ( $\pm 0.31^a$ )	4 ( $\pm 0.25^a$ )	2.27 ( $\pm 0.20^d$ )	3.07 ( $\pm 0.14^{bc}$ )
	M	3.3 ( $\pm 0.18^b$ )	4.23 ( $\pm 0.22^a$ )	1.87 ( $\pm 0.12^{de}$ )	2.03 ( $\pm 0.15^d$ )
	S	3.07 ( $\pm 0.19^{bc}$ )	2.47 ( $\pm 0.12^{cd}$ )	1.33 ( $\pm 0.09^e$ )	2.07 ( $\pm 0.14^d$ )

Different letters within each variable indicate significant difference at P<0.01 level.

**Table 5.** Comparison between lines in three soil water status (control: CK; moderate soil water stress: M; severe soil water stress: S), showing effect on root length (RL), relative root length (RRL); crown diameter (CD), shoot: root ratio (S: R) and stem: leaf ratio (S: L) (mean  $\pm$  SEM).

	Treatment	Darviluya	Culuskaya	XiMeng	Gong Nong
RL (cm)	CK	31.43 ( $\pm 0.96^{ab}$ )	33.68 ( $\pm 1.05^a$ )	29.09 ( $\pm 1.01^{bc}$ )	27.04 ( $\pm 0.84^{cde}$ )
	M	28.85 ( $\pm 1.01^{bcd}$ )	30.43 ( $\pm 0.97^{ab}$ )	27.07 ( $\pm 0.75^{cde}$ )	25.48 ( $\pm 0.83^{de}$ )
	S	26.96 ( $\pm 0.98^{cde}$ )	29.77 ( $\pm 0.90^{bc}$ )	27.95 ( $\pm 1.00^{cd}$ )	24.11 ( $\pm 0.73^e$ )
RRL	CK	1.97 ( $\pm 0.12^b$ )	1.54 ( $\pm 0.11^c$ )	0.85 ( $\pm 0.05^d$ )	0.83 ( $\pm 0.04^d$ )
	M	2.7 ( $\pm 0.21^a$ )	1.88 ( $\pm 0.13^{bc}$ )	1.01 ( $\pm 0.03^d$ )	0.77 ( $\pm 0.03^d$ )
	S	2.42 ( $\pm 0.19^{ab}$ )	2.27 ( $\pm 0.14^b$ )	1.10 ( $\pm 0.06^d$ )	0.96 ( $\pm 0.05^d$ )
CD (mm)	CK	2.32 ( $\pm 0.08^c$ )	2.59 ( $\pm 0.07^b$ )	2.17 ( $\pm 0.06^{cd}$ )	3.15 ( $\pm 0.07^a$ )
	M	2.14 ( $\pm 0.05^{cd}$ )	2.34 ( $\pm 0.04^c$ )	1.87 ( $\pm 0.12^{de}$ )	1.96 ( $\pm 0.04^{de}$ )
	S	1.83 ( $\pm 0.05^{ef}$ )	1.96 ( $\pm 0.06^{de}$ )	1.33 ( $\pm 0.09^e$ )	1.71 ( $\pm 0.05^f$ )
R: S	CK	1.13 ( $\pm 0.06^{bc}$ )	1.27 ( $\pm 0.03^b$ )	0.62 ( $\pm 0.03^{de}$ )	1.08 ( $\pm 0.05^{bc}$ )
	M	1.38 ( $\pm 0.09^{ab}$ )	1.47 ( $\pm 0.01^a$ )	0.66 ( $\pm 0.05^d$ )	0.99 ( $\pm 0.02^{bc}$ )
	S	0.93 ( $\pm 0.07^c$ )	0.97 ( $\pm 0.02^c$ )	0.56 ( $\pm 0.02^{de}$ )	0.81 ( $\pm 0.07^{cd}$ )
S: L	CK	0.91 ( $\pm 0.06^c$ )	1.01 ( $\pm 0.06^c$ )	1.52 ( $\pm 0.06^c$ )	1.47 ( $\pm 0.06^c$ )
	M	1.38 ( $\pm 0.06^c$ )	1.63 ( $\pm 0.16^c$ )	2.11 ( $\pm 0.03^{bc}$ )	2.93 ( $\pm 0.21^{bc}$ )
	S	1.82 ( $\pm 0.32^{bc}$ )	2.48 ( $\pm 0.29^{bc}$ )	3.87 ( $\pm 0.35^b$ )	7.10 ( $\pm 1.79^a$ )

Different letters within each variable indicate significant difference at P<0.01 level.

**Table 6.** Statistical analysis of values of *M. falcata* seedlings under different treatments.

		MP	PC	$g_s$	WUEi	PH	LA	SLA	CB	Sr	RL	RRL	CD	R:S	S:L
W	F	41.32	110.26	20.78	44.734	72.81	34.89	73.91	35.56	825.26	12.3	12.85	129.01	32.49	22.86
	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	F	28.01	4.95	8.32	4.43	194.43	29.54	16.22	62.09	162.82	19.95	122.68	94.3	49.24	11.63
	P	0	0.008	0	0.002	0	0	0	0	0	0	0	0	0	0
W×L	F	6.86	5.07	11.03	6.91	3.83	2.45	9.8	4.84	51.49	0.8	3.32	4.87	2.08	4.03
	P	0	0.002	0	0	0.001	0.027	0	0	0	0.572	0.003	0	0.093	0
n		15	15	15	15	30	30	30	30	30	30	30	30	30	30

RL of XiMeng and GongNong was not affected by soil water deficit, while the difference only appeared between CK and S treatment for the two Russia populations. RRLs of XiMeng and GongNong did not show any response to drought while those of the two Russia populations increased under soil water deficit. Crown diameters of Darviluya and XiMeng decreased under S treatment and this index of Culuskaya and GongNong decreased significantly whatever from CK to M and from M to S treatments. Soil water deficit changed the biomass allocation of *Medicago* (Table 4). The root: shoot ratio of *M. falcata* increased under M stress, and that in GongNong was decreased though did not reach statistical significant level (Table 5). Soil water deficit significantly increased the stem: leaf ratio of the all cultivars.

## DISCUSSION

Water deficit can be defined as insufficient moisture necessary for a plant to grow normally and complete its lifecycle (Cabuslay et al., 2002). Survival in soil water deficit could inform us the distributions of plants which are primarily restricted by humidity, and directly reflect drought tolerance of plant under stress when survival is the most crucial issue rather than keeping productivity for plant (Turner, 1981a). Poor survival of seedlings under desiccation condition had been noted in lots of studies. Enright and Lamont (1989) showed that the first summer drought was critical in explaining recruitment of *Banksia* species in natural scrub-death and drought stress was the major cause of seedling death. According to Sinclair (2000), there may be a balance between maintaining the least  $g_s$  to limit water loss through stoma and keeping fundamental photosynthesis. If plant cannot conquer this difficulty, it will be very adverse to survival in desiccation condition. In present experiment, the greater mortality of seedlings was GongNong corresponded to much reduction of  $g_s$ , lower water availability and its incapability of drought adaptation which was also demonstrated by

slack growth. Changes in physiological properties modified by environmental stress influence the response and tolerance of plant, also reflected the adaptability of plant to adverse environments. A major strategy for plant to overcome the negative effects of soil water deficit is lowering of the cell osmotic potential by means of solutes accumulation (Wright et al., 1997), of which proline is one with low molecular weight of the most important.

At the end of treatment, S stressed plants accumulated approximate three-, two-, six- and fivefold proline than controls respectively in Darviluya, Culuskaya, XiMeng and GongNong. We also observed that the tolerant populations like Darviluya, Culuskaya were capable of maintaining relatively low values of proline accumulation. The same response had been reported for several species subjected to drought, like alfalfa (Djilianov et al., 1997), barley (Hanson, 1979), sorghum (Premachandra et al., 1995) and cassava (Sundaresan and Sudhakaran, 1995). Membrane permeability, an indication of a damage of membrane system (Hanson, 1979; Heuer and Nadler, 1998; Gazal, 2004) was significantly increased by drought stress, except for Darviluya which had no significant increase under stress. It is widely accepted that ion leakage from plant tissues under stress served as a marker for relative tolerance (Djilianov et al., 1997) and with relative intact plasma membrane will be helpful for seedlings to complete their normal metabolic activity. The relative low photosynthesis of all seedlings observed in this study was due to the fact that the experiment was conducted in autumn when there was low radiation. Even so, the effect of soil water deficit on photosynthesis was quite obvious. Decline of Pr under drought stress would be a consequence of stomatal and nonstomatal inhibitions of photosynthesis under water deficit (Kaiser, 1987). Upon moderate drought conditions, photosynthesis decreased mainly due to stomatal closure. As the stress progresses, biochemical constraints may limit the photosynthetic  $CO_2$  fixation more directly (Lawlor, 1995). Greater reductions in Pr than  $g_s$  have been observed of several species suggesting that nonstomatal factors significantly influence photosynthesis of plants under

water stress (Kubiske and Abrams, 1992).

As we investigated in this study, GongNong with both sharply decrease of  $g_s$  and Pr was speculated suffering the combination of stomatal and nonstomatal inhibitions in soil water deficit. Instantaneous water use efficiency was calculated by dividing photosynthetic rate by transpiration under stress. It is the stabilization of WUE that could ensure the absorption of water and nutrition of plant under stress (Tjus and Moller, 1998; Egert, 2002). Thus improvement of WUE is considered as an important goal of genetic improvement in arid regions (Ray et al., 2004). In previous studies, mild drought stress increased WUE of plant; that is possible because in some cultivars, an increase of WUEi is attained largely by stomatal closure which will decrease transpiration, rather than by maintenance of photosynthetic activity (Kaiser, 1987; Khan et al., 2010). However, some others reported WUE remained unchanged in sorghum (Rosenthal et al., 1987), bermudgrass (Dean-Knox et al., 1998) and vegetable amaranth (Liu and Stützel, 2004). In addition, we should be aware that WUE of a plant is highly affected by the vapour pressure deficit (VPD) of the surrounding air (Sinclair et al., 1984). The different effects of drought on WUE across experiments may be partially due to the different experimental conditions and species used in research. Drought stress limited the growth of seedlings including plant height, root length, and crown buds. Increasingly, as a wild species of *M. falcata*, XiMeng showed the similar properties as GongNong (*M. sativa*) which had a relative large height, small crown buds and short root even without stress. That may be due to nature cross with *M. sativa* populations during long time evolution.

Under drought stress, Daviluya and Culuskaya developed some morphological response to undergo stress such as reducing the plant height to minish the transport course of water, maintaining relative large root absorption surface (relative big crown diameter and root length) and more crown buds than leaves to accomplish photosynthesis by providing enough water and light. A large decrease in leaf area under water stress is disadvantageous to plants because it leads to reduce nutrient uptake and photosynthesis (Pr) which is a consequence of reduced demand and transpiration (O'Toole and De Datta, 1986; Schulze, 1986). This means that crop productivity depends on the development of leaf area (LA) to intercept that radiant energy, and the rate of Pr to convert it into dry matter. Nevertheless, it is helpful for plants under desiccation to reduce leaf area in that it can decrease water loss through transpiration. For crops like *Medicago*, losing too many leaves means decline of production. In this paper, the LA of XiMeng and GongNong decreased 30.3 and 37.8% respectively. The large decreases in production of new leaves, increased leaf shedding and reduced average leaf size, as indicated by the declining number of living leaves per plant and leaf size in treated plants

induced total leaf area and leaf biomass reduced which will contribute to the reduction of yield of plants (Turner, 1981b). The decline of LA indicated that leaf production and leaf expansion growth are very sensitive to changes in water availability (Turner and Begg, 1978; Hsiao and Xu, 2000).

The increase of SLA suggesting loss in leaf weight in relation to leaf expansion was 73.6, 45.5, 29.2 and 122.8% respectively among populations under S stress. The response of SLA to soil water deficit had been widely discussed in many studies. Liu and Stützel (2004) observed SLA of vegetable amaranth significantly reduced during 30 days drought stress in a pot experiment (Liu and Stützel, 2004), and the similar result was gained in some other species like *Populus davidiana* (Zhang et al., 2004), groundnut (Nautiyal et al., 2002) and strawberry (Keutgen et al., 2005). Other authors reported the SLA of plant under drought stress increased due to relative greater decrease of leaf biomass than leaf expansion (Anyia and Herzog, 2004). The result of this paper was in accordance with the latter. Overall, translocation and assimilate distribution are affected by water deficits. Increment in root: shoot ratio in *M. falcata* populations under M stress showed a relative larger proportion of assimilates was transferred to the root, which had been reported by other researchers (Van Loo, 1992; Ramos and Gordon, 1999). It is important for perennial plants under water deficit to allocate more production to roots which will result in high strong root system for absorption and reserving energy for possible coming approval environment. However, this allocation pattern was reversed when soil water deficit became severe, indicating that the leaves (source) were incapable of providing enough assimilate products to the root (sink). The restricted development of root resulted in the decrease of the crown diameter rather than root length, consequently strengthening the relative root length in order to extend the area of absorption by the roots (Cabuslay et al., 2002).

GongNong showed different response of root: shoot ratio from others which decreased under soil water deficit. Root: shoot ratio of many crop and pasture species increased under water deficit condition (Leiva and Rocío, 1998; Zhang et al., 2004) which may simply arise from relative greater decrease in shoot biomass. But there is evidence that it presents a greater allocation of limited carbon available to roots rather than shoots as water stress developed (Sharp and Davies, 1979; Turner, 1981b).

## Conclusion

In summary, this study demonstrated differences in the response of *M. falcata* seedlings to soil water deficit. The different drought tolerance of seedlings was expressed by changes in certain traits. Russian populations with their

adaptive mechanisms to water stress showed relative high ability to drought stress. Although a seedling study in a greenhouse may not well-predict the performance of mature plants, observed physiological and morphological responses to water deficit can in some ways represent the tolerance and adaptation of plant. Evidence of an inter-relation between morphology, physiology responses and drought tolerance in these populations suggests that selecting the Russian cultivars maybe beneficial for adaptation in water deficit and superior for breeding as drought-tolerant parents.

## ACKNOWLEDGEMENTS

This research was supported by the National Basic Research Program of China (2011CB403203) and National Special Science and Technology program water pollution control and treatment (2009ZX07526006, 2012ZX07505001). We express our gratitude to the anonymous reviewers of this manuscript for their useful comments and perspectives.

## REFERENCES

- Anyia AO, Herzog H (2004). Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Eur. J. Agron.* 20: 327-339.
- Barnes DK, Goplen BP, Baylor JE (1988). Highlights in the USA and Canada. In: Hanson AA, Barnes DK, Hill Jr, RR (Eds.), *Alfalfa and Alfalfa improvement*. Am. Soc. Agron. Monogr. 29: 1-24.
- Bates LS, Waldren RP (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207.
- Cabuslay GS, Ito O, Alejar AA (2002). Physiological evaluation of responses of rice to water deficit. *Plant Sci.*, 163: 815-827.
- Canadian Food Inspection Agency (2005). *Biology Document Bio 2005-02: The Biology of Medicago sativa L. (Alfalfa)* Plant Products Directorate Plant Biosafety Office.
- Caspersen JP, Kobe RK (2001). Interspecific variation in sapling mortality in relation to growth and soil moisture. *Oikos* 92:160-168.
- Dean-Knox DE, Devitt DA, Verchick LS, Morris RL (1998). Physiological response of two turfgrass species to varying ratios of soil matric and osmotic potentials. *Crop Sci.*, 38: 175-181.
- Deblonde PMK, Ledent JF (2001). Effects of moderate drought conditions on green leaf number stem height, leaf length and tuber yield of potato cultivars. *Eur. J. Agron.*, 14: 31-41.
- Djilianov D, Dragiiska R, Yordanova R, Doltchinkova V, Yordanov Y, Atanassov A (1997). Physiological changes alfalfa in osmotically stressed detached genotypes selected in vitro. *Plant Sci.*, 129: 147-156.
- Egert M (2002). Influence of drought on some physiological parameters symptomatic for oxidative stress in leave of chives. *Environ. Exp. Bot.*, 48: 43-49.
- Enright NJ, Lamont BB (1989). Seed banks, fire season, safe sites and seedling recruitment in five co-occurring *Banksia* species. *J. Ecol.*, 77: 1111-1122.
- Enright NJ, Lamont BB (1992). Survival, growth and water relations of *Banksia* seedlings on a sand mine rehabilitation site and adjacent scrub-heath sites. *J. Appl. Ecol.*, 29: 663-671.
- Gazal RM (2004). Influence of initial root length on physiological responses of cherry bark oak and Shumard oak seedlings to field drought conditions. *For. Ecol. Manag.*, 189: 295-305.
- Hanson AD (1979). Capacity for proline accumulation during water stress in barely and its implications for drought resistance. *Crop Sci.*, 19: 489-493.
- Heinrichs DH (1978). The future of alfalfa for pasture in dry regions and research requirements. In D. K Barnes, ed. June 1978. USDA-ARS, St. Paul, MN. 4-9.
- Heuer B, Nadler A (1998). Physiological response of potato plants to soil salinity and water deficit. *Plant Sci.*, 137: 43-51.
- Hsiao TC, Xu LK (2000). Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *J. Exp. Bot.*, 51: 1595-1616.
- Ivanov BI (2000). *Lucerne in Yakutia*. (in Russian) Western Siberian Science Publishing Company, Russian Academy of Sciences.
- Kaiser WM (1987). Effects of water deficit on photosynthetic capacity. *Physiol. Plant*, 71: 142-149.
- Keutgen AJ, Noga G, Pawelzik E (2005). Cultivar-specific impairment of strawberry growth, photosynthesis, carbohydrate and nitrogen accumulation by ozone. *Environ. Exp. Bot.*, 53: 271-280.
- Khan HR, Paull JG, Siddique KHM, Stoddard FL (2010). Faba bean breeding for drought-affected environments: A physiological and agronomic perspective. *Field Crops Res.*, 115: 279-286.
- Kubiske ME, Abrams MD (1992). Photosynthesis, water relations and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central Pennsylvania in relation to moisture stress. *Can. J. For. Res.*, 22(9): 1402-1407.
- Lawlor DH (1995). The effects of water deficit on photosynthesis. In: N. Smirnov (Ed.), *Environment and Plant Metabolism-Flexibility and Acclimation*. BIOS Scientific Publishers, Oxford, pp. 129-156.
- Leiva MJ, Rocio Fernández-Alés (1998). Variability in seedling water status during drought within a *Quercus ilex subsp. ballota* population, and its relation to seedling morphology. *For. Ecol. Manag.*, 111: 147-156.
- Lesins K, Lesins I (1979). Genus *Medicago* (Leguminasae): A taxogenetic study. Kluwer, Dordrecht, the Netherlands.
- Liu and Stützel (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Sci. Hortic.*, 102(1): 15-27.
- Michaud R, Lehman WF, Rumbaugh MD (1988). World distribution and historical development, In: Hanson, A.A., Barnes, D.K., Hill Jr., R.R. (Eds.), *Alfalfa and Alfalfa improvement*. Am. Soc. Agron. Monogra., 29: 25-91.
- Miyashita K, Tanakamaru S, Maitani T, Kimura K (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environ. Exp. Bot.*, 53: 205-214.
- Spieß N, Oufir M, Matusikova I, Stierschneider M, Kopecky D, Homolka A, Burg K, Fluch S, Hausman JF, Wilhelm E (2011). Ecophysiological and transcriptomic responses of oak (*Quercus robur*) to long-term drought exposure and rewatering. *Environ. Exp. Bot.*, In Press.
- Nautiyal PC, Rachaputi NR, Joshi YC (2002). Moisture-deficit-induced changes in leaf-water content leaf carbon exchange rate and biomass production in groundnut cultivars differing in specific leaf area. *Field Crop Res.*, 74: 67-79.
- Ohashi Y, Saneoka H, Fujita K (2000). Effect of water stress on growth, photosynthesis, and photoassimilate translocation in soybean and tropical pasture legume siratro. *Soil Sci. Plant Nutr.*, 46: 417-425.
- O'Toole JC, De Datta SK (1986). Drought resistance in rainfed lowland rice, in: *Progress in Rainfed Lowland Rice*, International Rice Research Institute, Makati City, Philippines. pp. 145-158.
- Premachandra GS, Hahn DT, Rhodes D, Joly RJ (1995). Leaf water relations and solute accumulation in two grain sorghum lines exhibiting contrasting drought-tolerance. *J. Exp. Bot.*, 46: 1833-1841.
- Ramos MLG, Gordon AJ (1999). Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean. *Ann. Bot.*, 83: 57-63.
- Ray IM, Segovia-Lerma A, Murray LW (2004). Diallel Analysis of Carbon Isotope Discrimination and Its Association with Forage yield among nine historically recognized alfalfa germplasms. *Crop Sci.*, 44: 1970-1975.
- Reich PB, Walters MB, Ellsworth DS (1992). Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecol. Monogra.*, 62: 365-392.
- Riday H, Brummer EC, Moore KJ (2002). Heterosis of forage quality in alfalfa. *Crop Sci.*, 42: 1088-1093.
- Rosenthal WD, Arkin GF, Shouse PJ, Jordan WR (1987). Water deficit



- effects on transpiration and leaf growth. *Agron. J.*, 79: 1019-1026.
- Sharp RE, Davies WJ (1979). Solute regulation and growth by roots and shoots of waterstressed maize plants. *Planta* 147: 43-49.
- Schulze ED (1986). Whole-plant responses to drought. *Aust. J. Plant Physiol.*, 13: 127-141.
- Sinclair TR, Tanner CB, Bennett JM (1984). Water-use efficiency in crop production. *Biosci.*, 34: 36-40.
- Sinclair TR (2000). Model analysis of plant traits leading to prolonged crop survival during severe drought. *Field Crops Res.*, 68: 211-217.
- Sultan SE (2000). Phenotypic plasticity for plant development, function and life history. *Trends in plant sci.*, 5 (12): 537-542.
- Sundaresan S, Sudhakaran PR (1995). Water stress-induced alterations in the proline metabolism of drought-susceptible and -tolerant cassava (*Manihot esculenta*) cultivars. *Physiol. Plant*, 94: 635-642.
- Tjus SE, Moller HV (1998). Photosystem is an early target of photoinhibition in barely illuminated at chilling temperatures. *Plant Physiol.*, 116: 755-764.
- Turner NC (1981a). Designing crops for dryland Australia: can the deserts help us? *J. Aus. Inst. Agric. Sci.*, 47: 29-34.
- Turner NC (1981b). Plant-water relations and adaption to stress. *Plant and soil*, 58: 97-131.
- Turner NC, Begg JE (1978). Responses of pasture plants to water deficits. In: Wilson, J. R. (ed.), *Plant relations in pastures*, pp. 50-66. CSIRO, Melbourne.
- Van Loo EN (1992). Tillering, leaf expansion and growth of plants of two cultivars of perennial ryegrass growth using hydroponics at two water potentials. *Ann. Bot.*, 70: 511-518.
- Wright PR, Morgan JM, Jessop RS (1997). Turgor maintenance by osmoregulation in *Brassica napus* and *B. juncea* under field conditions. *Ann. Bot.*, 80: 313-319.
- Zhang JW, Marshall JD, Fins L (1996). Correlated population differences in dry matter accumulation, allocation, and water-use efficiency in three sympatric conifer species. *For. Sci.*, 42: 242-249.
- Zhang XL, Zang RG, Li CY (2004). Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. *Plant Sci.*, 166: 791-797.