Research Journal of

Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Environmental Sciences 4 (3): 288-293, 2010
ISSN 1819-3412

Effect of Water Stress and Recovery on the Water Status and Osmotic Adjustment of Miniature Rose Meshkinjan

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Abstract: We evaluated drought response of miniature rose Meshkinjan under open door environment during a period of water deficit (21 days) and subsequent rewatering. After 21 days of drought, stem water potential, relative water content, turgid weight to dry weight ratio and starch concentration decreased significantly compared with control. While, the amount of proline and potassium increased significantly that could indicate ability of osmotic adjustment in this species. Also the increase in water potential and relative water content values after rewatering period indicate the good recovery of this plant.

Key words: Miniature roses, osmotic adjustment, plant water status

INTRODUCTION

Water deficit, taken with high solar radiation and high temperatures during the summer has been considered the main limiting factor for plant growth in Mediterranean-type ecosystems (Castri, 1981). This stress affects the establishment, survival, growth and performance of shrubs and trees in urban and suburban landscape environments (Fernandez *et al*., 2006) so, under water restriction conditions one strategy to improve the landscape may be the selection of drought-tolerant plants (Niu *et al*., 2008). Some species actively accumulate solutes during water stress and decrease osmotic potential (Zayed and Zeid, 1998). Decrease osmotic potential under water stress that has been referred to as osmotic adjustment, has been considered to be a selection criterion for plants in drought-prone regions (Liu and Stutzel, 2002). Osmotic adjustment through accumulation of compatible solutes has been reported in many crops. Accumulation of sugars (Wang and Stutte, 1992), proline (Sarker *et al*., 2005), sodium and potassium ions (Handa *et al*., 1983) and organic acid (Turner, 1979) are common metabolic responses of higher plants to several stresses, namely water deficit.

Miniatur rose *Rosa chinensis* Jacq. Var. *minima* Rehd hybrids is a small deciduous plants that is commonly used as an ornamental plant in landscapes, parks, homes, gardens etc. in many parts of Iran (Salehi Najaf-Abadi, 1995). Meshkinjan is a beautiful cultivar of miniature rose. This study was undertaken to quantify leaf water relations and osmotic adjustment of miniature rose cv. Meshkinjan under drought stress and recovery period.

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MATERIALS AND METHODS

Plant Materials and Growth Conditions

Own-rooted miniature were grown outdoors (the maximum and minimum air temperature was 40 and 11°C, respectively) in 5 L pots, containing sand, leafmould and loamy soil (1:1:1, v/v/v), at the experimental site of Shiraz university, Iran in July to August, 2008.

Starting from 50 days of sowing, two watering treatments were applied: one group of plants, involving 20 pots were provided with optimal irrigation (control) and the second group, involving 20 pots was subjected to water stress treatment by withholding irrigation. Measurements were made after 21 days. At that time the stressed plants began to show visual signs of sever water deficiency. Recovery of water stressed plants was carried out by re-watering of pots daily for 5 days.

Plant Water Status

Stem Water Potential (SWP) was measured after 21 days and after recovery period using pressure bomb (PMS Instr. Co., Corvallis, OR, USA). For these purpose fully expanded leaves were selected. The leaves at least 1 h before measurement were enclosed in a cellophane bag covered with aluminum foil. This enabled water potential in the xylem of the leaf to come to equilibrium with the potential in the xylem of the stem at the point of attachment of the petiole (Garnier and Berger, 1985).

The Relative Water Content (RWC) was determined as:

$$\text{Relative Water Content (RWC)} = \left(\frac{\text{FW- DW}}{\text{TW- DW}}\right)\times 100$$

where, FW is the fresh weight, DW is the dry weight after oven-drying the leaves at 80°C for 24 h and TW is the turgid weight after re-hydrating the leaves at 4°C. The TW and DW were used to determine the TW/DW ratio.

Proline Measurement

Fully expanded and young leaves were collected. Free proline was extracted from 0.5 g of leaf samples with sulfusalicilic acid 3% and estimated by using ninhydrin reagent according to the protocol described by Bates *et al*. (1973). The absorbance of fraction with toluene aspired from liquid phase was determined using spectrophotometer at 520 nm (Model UV-120-20, Japan).

Water Soluble Carbohydrates and Starch Measurement

To measuring soluble carbohydrate, 150 mg of dried leaf samples was extracted two times with ethanol. Sample were centrifuged at 3500 for 10 min, the volume of upper phase was reached to 25 mL. Soluble carbohydrate was measured according to the method of Dubios *et al*. (1956) and modified by Buysee and Mercks (1993), the absorption was recorded using spectrophotometer at 490 nm (Model UV-120-20, Japan).

Starch was measured by use of anthron reagent (McCready *et al*., 1950). For this purpose 5 mL of cold water and 6.5 mL perchloric acid (52%) were added onto the residual material used for sugar analysis and mixed for 15 min. Then about 20 mL of water were added and were centrifuged. The supernatant was separated and the same procedure was repeated with residue and obtained supernatants were combined and left for 30 min at 0°C. After filtration the volumes of supernatants were adjusted to 100 mL. About 2.5 mL of cold anthron solution (2%) were added to the samples. Then samples were heated at 100°C for 7.5 min and immediately transferred into an ice bath and cooled to room temperature. Absorption of the samples was recorded using spectrophotometer at 630 nm (Model UV-120-20, Japan).

K^+ and Na^+ Measurement

One gram of leaf samples was ashed in a muffle afurnace at 550°C for 5 h. Then the ash was dissolved in 10 mL 2 N HCl and reached volume to 100 mL with distilled water. Potassium and sodium were determined using flame photometer (Model PFP7, Jenway, England).

Statistical Analysis

Statistical differences between measurements were analyzed following the analysis of variance ANOVA using SPSS statistical computer package. Differences were considered significant at a probability level of $p<0.05$.

RESULTS

Plant Water Status

The average Stem Water Potential (SWP) of the control plants were -2.05 MPa. At the end of the 21 day drought treatment, SWP decreased to -3.14 MPa in drought-stressed plants. Five days after re-irrigation, SWP of the stressed plants recovered to the level of the control plants (Table 1). Plants were exposed to soil water deficit, which resulted in a significant reduction of RWC, which decreased to 64.5% after 21 days of stress. The RWC values recovered after re-irrigation attaining values near to those observed in control plants (Table 1). The TW/DW ratio increased over time under both water treatments. However, TW/DW ratio in the stressed plants increased more sharply and had not significant difference with control plants at the end of the drying cycle (21 days after the imposition of stress) (Table 1).

Solute Accumulation

Examining the proline, we found that proline was at a low level in young leaves of control plants. During water deficit period it greatly increased in young leaves. After 5 days of rewatering, proline was maintained at a high level and did not recover to it's initial value. During water deficit, there were no marked differences in soluble carbohydrate in the leaves. Upon re-watering, there was a tendency for an increase in soluble carbohydrate in young leaves (Table 2). Starch concentration decreased significantly in stressed plants. After re-watering, a tendency was observed to re-establish the initial starch values. In respect to inorganic ion content, K^+ concentration increased significantly with water deficit. Re-watering led to a K^+ increase in leaves. In the stressed plants the Na^+ content decreased however 5 days after re-watering started, the differences in Na^+ content of stressed plants and control plants were not significant at $p = 0.05$ (Table 2).

Table 1: Water relation parameters in control and stressed plants

	Water deficit period		Recovery period	
Parameters	Control plants	Drought-stressed plants	Control plants	Drought-stressed plants
Stem water potential (MPa)	-2.05b*	-3.14a	-1.99b	-2.03b
Relative water content	83.50a	64.50c	81.96ab	77.16b
Turgid weight/dry weight	5.58a	4.78b	5.84a	5.64a

*Different letter(s) within the same raw indicates significant differences between treatments at p = 0.05

Table 2: Solute concentration in leaves of control and stressed plants expressed on the basis of mg g^{-1} dry weight

	Water deficit period		Recovery period	
Parameters	Control plants	Drought-stressed plants	Control plants	Drought-stressed plants
Proline	1.56b*	2.90a	2.16ab	2.48a
Soluble carbohydrate	625.70b	617.30b	675.20ab	716.90a
K^+	10.48c	13.64b	18.00a	18.60a
Na^+	0.72b	0.55b	1.04a	1.07a
Starch	459.40a	335.40b	478.00a	494.90a

*Different letter(s) within the same raw indicates significant differences between treatments at p = 0.05

DISCUSSION

A significant reduction of SWP and RWC was observed after 21 days of soil water deficit. Some species avoid drought and don't experience low water potential in their tissue (Tripathy *et al*., 2000). However, in drought tolerant plants with low water potential metabolic process continue even though tissue water potential decreases (Nilsen and Orcutt, 1996). A significant decrease of SWP and then rapid recovery after re-watering period suggests that there was a tolerance mechanism in the miniature rose Meshkinjan. A high TW/DW ratio may be associated with low drought resistance (Jensen *et al*., 1996). Drought resistant species might be expected to adapted to large losses of water without loss of turgor and the leaf cells would be small and thick walled causing low TW/DW ratio (Liu and Stutzel, 2002).

The decrease in total soluble carbohydrate concentration in stressed plants could be attributed to the reduction in the photosynthetic rate, as the latter is considered to be the main source of accumulation of organic solutes under water stress conditions (Kameli and Losel, 1995). Thus, the consumption of assimilates seems to exceed their production by photosynthesis, leading to a reduction in carbohydrate concentrations in stressed plants (Patakas *et al*., 2002). Starch concentration decreased under water stress condition, but despite the significant starch depletion, carbohydrate concentration was not increased by drought. This indicates that in miniature rose, starch might not be an important source of sugar accumulation (Patakas *et al*., 2002) and also it seems that photosynthesis was so severely inhibited under water stress condition that less sugar was produced and or sucrose was transferred from leaves to growing roots and shoot apex, where it may contribute to osmotic adjustment (Wang *et al*., 1995). The recovery of soluble carbohydrates concentration upon re-watering suggests that the photosynthetic capacity was only temporarily affected during water deficit period, confirming the remarkable resistance of miniature rose photosynthetic apparatus to dehydration (Pinherio *et al*., 2004). Proline contributes in solute accumulation and its accumulation is effective in dehydration tolerance since it may also protect protein and membrane structure and act as a scavenger of Reactive Oxygen Species (ROS) under stress condition (Verslues *et al*., 2006). The data of this study showed that Na^+ almost had not any role in increasing solute concentration and thereby decreasing osmotic potential. Nevertheless K^+ increased significantly by increasing drought severity and had great role in decreasing osmotic potential. An alternative to producing organic osmotica is for plants to accumulate a sufficiently high content of ions from the soil. By using this alternative mechanism, plants save energy, which enable them to grow in less favorable conditions (Patakas *et al*., 2002).

CONCLUSION

In conclusion, present results indicate that the relative tolerance to water stress in miniature rose cv. Meshkinjan enable it to withstand under watering restriction conditions.

ACKNOWLEDGMENT

We are grateful to the Mrs. Marjan Vaezpour for technical assistance.

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