Biological Forum – An International Journal **7**(1): 1811-1815(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Nutritional and Biochemical Response of Water-stressed Valerian Plants to Foliar Application of Spermidine

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(Received 07 May, 2015, Accepted 17 June, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The objective of this study was to determine the effect of foliar spermidine (Spd) application on biochemical and nutritional response of valerian grown under different water stress (100, 70, 50 and 30% Available water content). The study was conducted in a pot experiment under greenhouse conditions. Water - stressed valerian seedlings were treated with foliar Spd application at different concentrations (0, 0.5 and 1 mM).The results showed that water stress significantly affected most biochemical and physiological characteristics as well as nutritional status of valerian plants. Leaf relative water content, chlorophyll a and b contents decreased while carotenoids and electrolyte leakage increased with the increase of water stress. Nutritional imbalance was also observed in water-stressed plants. For the ameliorating the adverse effect of water stress, among the investigated defense traits, plants increased proline levels and catalase and ascorbate peroxidase activities. Foliar application of Spd provoked the antioxidant enzymes activity and potassium accumulation that resulted in membrane damages alleviation. Therefore, exogenously applied Spd increased photosynthetic pigments content which supply energy for plant growth and production. Results also revealed that in most cases, effectiveness of Spd concentration had reverse trend with water shortage increasing, so, under moderate water stress, 1mM Spd was better than 0.5 mM.

Keywords: Antioxidant enzyme, mineral content, osmotic adjustment, photosynthetic pigment, water deficit

INTRODUCTION

Drought is one of the major constraints limiting crop production worldwide which decreases crop productivity more than any other environmental stress (Lambers *et al.* 2008). Under drought stress, growth and function suppression in plants involves many morphological, physiological and molecular changes including oxidative stress, metabolic disturbance (Li *et al.* 2013). Plants respond to adverse conditions by altering their morphology, physiology, and biochemistry, however, biochemical events mainly take place earlier than others.

Polyamines, including putrescine (Put), spermidine (Spd), and spermine (Spm), are low molecular weight natural compounds with aliphatic nitrogen structure, and exist in almost all organisms from bacteria to animals and plants (Hussain*et al.*2011). For plant growth and development, polyamines are widely implicated in cell division and differentiation, root elongation, leaf senescence, programmed cell death, DNA synthesis and protein translation (Shi and Chan 2014). Polyamines, being cationic in nature, can associate with anionic components of the membrane such as phospholipids thereby stabilizing the bilayer surface and retarding membrane deterioration (Shi and Chan, 2014). It has been shown that PAs in chloroplasts can covalently bound to chlorophyll protein complexes in thylakoids and Rubisco in the stroma (Alcázar*et al.*2011). These ionic interactions are considered to increase stabilization of the subcellular compounds and membranes under stress (Hussain *et al.* 2011). PAs can also act as free radical scavengers, so protect the membranes from oxidative damages (Shi and Chan 2014).Recently several reports showed that exogenous PAs application also improves tolerance against several abiotic stresses (Shi *et al.* 2013; Shi and Chan 2014). Nayyar *et al.* (2005) found that exogenous application of Put and Spd substantially improved the drought tolerance in soybean. Although, they induce tolerance against several abiotic stresses in plants (Nayyar *et al.*2005; Loka *et al.* 2015), mechanisms of their action during exogenous application in modulating physiological phenomena and improving drought stress tolerance are not fully understood. To the best of our knowledge, no study has been conducted concerning biochemical changes in water-stressed valerian plants to exogenously applied Spd. So, the present work was conducted and analyzed at biochemical and physiological levels to determine the effects of these factors on goal plants.

MATERIALS AND METHODS

A pot experiment was conducted in 2014 at the University of Maragheh, Maragheh, Iran. We filled 8 L pots with 12 kg soil and clean sand (2:1, v/v).

The soil had been passed through a mesh number 10. 20 days-old *Valeriana officinalis* seedlings were obtained from medicinal plants institute, Tehran, Iran. Uniform seedlings were collected and then transplanted into each pot. The pots were placed under a rain-shelter in a completely randomized block design. During 30 days of transplanted, plants were irrigated with tap water, and then divided into four lots subjected to different water levels.Drought stress levels were measured using control plants with 100% water availability (L1), 70% (L2), 50% (L3) and 30% (L4) of available water.The pots were weighed daily and water was added to maintain soil moisture content. Spermidine was sprayed 2 times at 0.5 and 1 mM concentrations. Untreated plants were sprayed with distilled water. The first spray was made 35 days after transplanting and repeated following 15-day. The plants were sprayed with a manual pressure pump at an average of 10 cc.The measurements were carried out at three days after spraying.

Relative water content (RWC) andElectrolyte leakage was calculated by the method of Shi*et al.* (2006).Chl-a, chl-b, and carotenoid were determined according to the method of Arnon (1949). Proline content of leaf tissues was estimated spectrophotometrically following the ninhydrin method described by Bates et al. (1973). The activities of guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT) were assayed by using the method of (Nakano and Asada 1981). Potassium and sodium concentrations were determined in the roots and shoots after digesting 100 mg powder of the oven-dried tissues in a mixture of concentrated nitric acid and perchloric acid (3:1; v/v) at 175ºC. The potassium and sodium contents of the digested extracts were quantified using a flame photometer (model Jenway PFP7, UK). Zn and Fe contents were measured using atomic absorption spectrophotometer (AAS) (Faithfull. 2002). Analysis of variance appropriate to the experimental design was conducted, using SPSS and MSTATC software. Means of each trait were compared according to Duncan multiple range test at $P<0.05$. Excel software was used to draw figures.

RESULTS AND DISCUSSION

Data presented in Table 1 indicated that RWC was significantly decreased with increasing the irrigation water at 100 to 30% AWC. At the levels of 70%, 50% and 30% F.C the percent of reduction in RWC was 14.5, 26 and 28 respectively in compared with the well watered plants. All Spd foliar application, failed to revert the decrease in RWC caused by the water shortage treatments.These results are in agreement with previous finding of Kubis *et al.* (2014) who showed that plant treatment with Spd practically did not modify the pattern of the water-stress evoked RWC changes.

Cell membrane stability is considered to be one of the best physiological indicators of drought stress tolerance (Blum and Ebercon 1981). Results showed that water stress induced a marked increase in membrane permeability, so, the injury index of leaves increased with increasing the water shortage (Table 1).El level increased from 21 to 42%, when AWC dropped from 100 to 30%. Plants treated with Spd showed a significant reduction of stress-induced electrolyte leakage depending on its concentration and water stress level. Under moderate water stress (70% AWC), application of 1 mMSpd was better than 0.5 mM, but at severe water stress (50% AWC), it was reverse (Fig. 1a). Regardless of its concentration, exogenously applied Spd mainly was effective under moderate water stress and with increasing the stress levels, its positive effect was decreased. Similar results were also reported by Shaddad *et al.* (2011) who reported that at very severe water stress condition, application of polyamines did not alleviate the adverse effect of water stress. Results reported in the present paper showed that exogenous Spd could effectively alleviate membrane damage induced by water shortage up to 50% AWC (Fig. 2). The observation is in harmony with the results of Chattopadhayay *et al.* (2002) on rice plants.

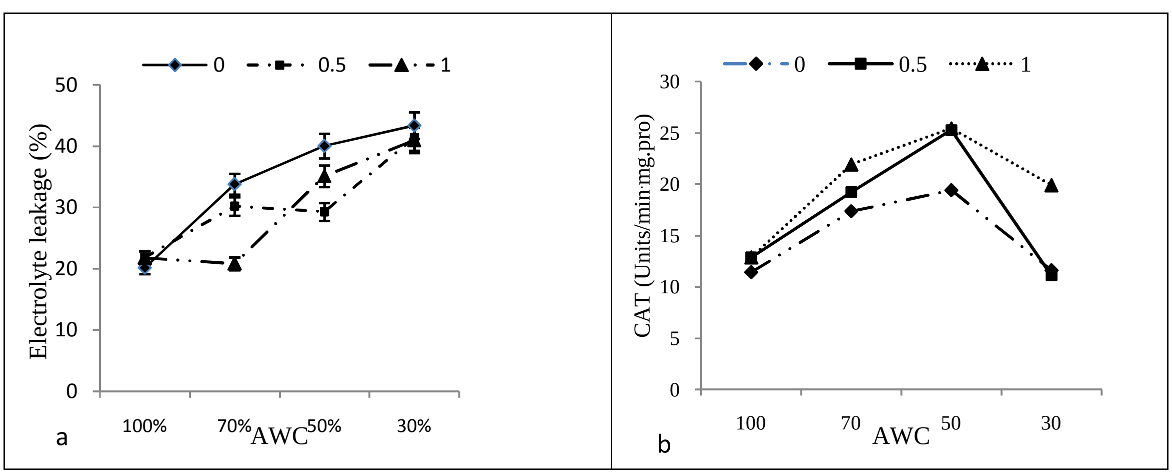

Fig. 1. Effect of foliar Spd treatments on electrolyte leakage (a) and CAT activity (b) in leaves of valerian under water stress.

In the presentstudy, in response to water stress, proline concentration was gradually increased and then reached a peak value at 50% AWC and then commenced to reduce (Table 1). Proline accumulation is with the fact that many higher plants accumulate free proline in response to water stress (Salehi-Shanjani*et al.* 2014).Severalpossible roles have been attributed to this accumulation of proline: osmoregulation, detoxification of free radicals, conservation of nitrogen and energy for the post-stress period andregulating the stress protective proteins (Khedr*et al.* 2003). Exogenously applied Spd had no effect on proline accumulation (Table 1). Our results are accordance with Anjum (2011).Results revealed that total soluble sugars concentration was not significantly affected by water stress and foliar application of Spd (Table 1).In general, the inability of Spd in reducing water loss (Table1) suggests that osmotic adjustment in valerian leaves cannot be regulated by Spd under drought conditions.

The activity of catalase (CAT) and Ascorbateperoxidase (APX) were increased with the increase of water stress from control (100% AWC) to 50% AWC. However, further increase in water shortage shapely reduced CAT activity, but maximum value of APX activity was achieved under 30% AWC. In contrast to CAT and APX, activity of Guaiacolperoxidase (GPX) tended to decrease by water deficit increasing. Liu et al. (2011) reported that catalase activity under mild drought stress was higher than severe drought stress and well-watered treatments. In current study, exogenous Spd significantly promoted activities of CAT, APX relative to untreated plants, but was not effective on altering GPX activity. Maximum CAT (20 Units/min.mg.pro) and APX (1.58 Units/min.mg.pro) were recorded from 1 mMSpd treatment followed by 0.5 mMSpd (Table 1). Water stress × Spd application interaction on CAT activity was significant. According to figure 1b, both of the Spd concentrations positively influenced CAT activity up to 50% AWC, but when water shortage reached to 30% AWC, 0.5 mMSpd had no effect on CAT activity. It suggests that Spd is able to influence oxidative stress intensity through activating some activities of scavenging system enzymes under water stress. The same result was obtained in study of Li *et al* (2014) who showed that exogenous Spd significantly promoted activities of antioxidant enzymes under water stress.

Table 1: Effect of foliar Spd treatments on some physiological and biochemical traits of valerian under water stress.

	RWC (%)	El (%)	Chl.a (mg/g FW)	Chl.b (mg/g FW)	Car (mg/g FW)	CAT	APX	GPX	Proline (µmol/g FW)	Carbohydrate (mmol/g DW)
						Units/min·mg.pro				
100	77. 9a	21.27d	40.7a	35.7a	1.27b	12.3c	1.14c	10.67a	0.35d	101.5a
70	67. 4b	28.30c	39.2a	33.2ab	1.35a	19.5b	1.48b	10.24a	1.58c	104.4a
50	59.1c	34.84b	32.3b	32.7b	1.40a	23.3a	1.79a	7.74b	2.41a	101.1a
30	57.5c	41.92a	18.5c	26.5b	1.21c	14.2c	1.82a	6.74c	1.90b	82.55a
	**	**	**	**	**	**	**	**	**	ns
Control	63.0a	34.36a	30.1b	30.5b	1.30a	14.9c	1.52b	8.74a	1.57a	93.58a
Spd (0.5)	67.9a	30.70b	34.8a	31.0b	1.32a	17.1b	1.57a	8.94a	1.62a	98.41a
Spd (1)	65.6a	29.69b	33.1a	34.6a	1.32a	20.0a	1.58a	8.86a	1.53a	100.25a
	ns	**	**	*	ns	**	**	ns	ns	ns
A*B	ns	**	**	ns	ns	*	ns	ns	ns	ns

Numbers with the same letters in the same column are not statistically different ($P<0.05$).

The results revealed that Chlorophyll "a, b" contents decreased by decreasing the soil moisture content. Drought stress up to 70% F.C induced a slight effect in the chl-a, chl-b of valerian leaf, then it reduced markedly by the further increase in the level of drought stress. Severe(50% AWC) and very severe drought stress (30% AWC) decreased chl-a as much as 21 and 55% compared to the control, respectively. Contrary to these results, plants carotenoid valuesincreased by water stress increasing up to 50% and then commenced to reduction (Table 1). Our results about chl-aandchl-b changes in response to drought stress agree with reports of Liu *et al.* (2011). Reduction in chlorophyll concentration is identified as a drought response mechanism in order to minimize the light absorption by chloroplasts (Pastenese*et al.* 2005). Since carotenoid plays an important role in photo-protection, the increased carotenoid content under drought conditions, indicate a higher need ofphoto-protection by carotenoid (Elsheery and Cao 2008).A marked and progressive increase in the photosynthetic pigments concentration was exhibited when the drought stressed plants sprayed with Spd.

Chla content also was significantly affected by water stress × Spd interaction. Under moderate water stress, high concentration of Spd (1 Mm) was better than low concentration (0.5 Mm), but when it sprayed on severe water-stressed plants showed toxic effect. All Spd treatments had not significant effect on carotenoid content. In connection with these results, Chattopadhayay *et al.* (2002) found that the exogenous application of polyaminesenhanced the total chlorophyll level of salt-stressed rice plants.This enhancement effect of Spd may be attributed to increased stability of thylakoids membranes(Chattopadhayay *et al.* 2002). Thus polyamines could bind to the negatively charged phospholipid head groups on membranes, thereby influencing stability and permeability characteristics of these membranes.

The results in Table 2 showed that potassium content in leaves was gradually increased by decreasing water depletion from 100 to 70% (moderate drought stress) but further increasing drought stress caused low K content compared with control. Na content in leaves was gradually decreased due to increasing drought stress, however, differences were not statistically significant. These findings are generally in line with those previously reported by Mohsenzadeh *et al.* (2006). Shaheen *et al.* (2011) also reported that the leaves of olive contained a lower content of K when these plants grown under severe water stress. Kuchenbuch *et al.* (1986) showed that low levels of soil moisture reduced root growth and the rate of potassium inflow in onion plants in terms of both per unit of root weight and per unit of root length. Data presented in Table 2 indicated that exogenous application of Spd improved accumulation of K in leaves of valerian, while had no effect on Na accumulation. Therefore, spermidine foliar application enhanced the K^+/Na^+ ratio of water-stressed plants and this is in accordance with the findings of Chattopadhayay *et al.* (2002).

Data presented in Table 2 indicated that zinc and iron contents in leaves were decreased by decreasing water depletion from 100 to 30% AWC. However, negative effect of moderate water stress on Zn content was not significant compared with well watered. Similar results were also reported by (Sanchez-Rodriguez *et al.*2010). Since the transport of micronutrients to the plant roots occurs via diffusion, low soil moisture content will reduce micronutrient uptake. However, since plants require much smaller quantities of micronutrients, the effects of drought stress on micronutrient uptake are not as great as that on P and K uptake. Treatment with Spd did not affect Zn content, but increased Fe accumulation. When water-stressed plants were sprayed with Spd, Fecontent showed complex response. 0.5 mMSpd application under water stress gave the higher values for this parameter than 1 Mm (Table 2).

In conclusion, the data obtained from the present study suggest that foliar Spd applications can ameliorate the deleterious effects of water stress by increasing antioxidant enzymes activity and improving the nutritional status, thus inducing drought tolerance in valerian plants. Results also revealed that in most cases, effectiveness of Spd concentration had reverse trend with water shortage increasing, so, under moderate water stress, 1mM Spd was better than 0.5 mM.

Table 2: Effect of foliar Spd treatments on mineral contents in leaves of valerian under water stress.

	Na+ (mg/g DW)	**K+ (mg/g DW)**	**Zn (ppm)**	**Fe(ppm)**
100	5.33a	5.45b	0.37a	14.49a
70	5.24a	6.02a	0.35a	12.11b
50	5.30a	4.62c	0.31b	11.44b
30	5.18a	3.44d	0.25c	9.29c
	ns	**	**	**
Control	5.42a	4.66b	0.31a	10.01c
Spd (0.5)	5.15a	4.94a	0.33a	13.53a
Spd (1)	5.21a	5.05a	0.32a	11.96b
	ns	**	ns	**
A*B	ns	ns	ns	ns

Numbers with the same letters in the same column are not statistically different (P<0.05).

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