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PHYSIOLOGICAL RESPONSE OF *APOCYNUM VENETUM* L. SEEDLINGS UNDER OSMOTIC STRESS

HUA ZHAO AND HUIPING DAI[1,2*]

College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, Shaanxi 723001, P. R. China

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Abstract

Effects of drought stress were induced by polyethylene glycol (PEG-6000) (10, 20, and 30%) for 2, 4, 6 and 8 days in *Apocynum venetum* L. seedlings with the potting method. The results showed that PEG significantly increased the activities of glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (APX), and the contents of electrolyte leakage rates, MDA, proline and soluble sugar were increased from 30% PEG. Thus, it was indicated that the application of exogenous 30% PEG induced oxidative damage by enhancing antioxidant defense systems.

Introduction

Drought is perhaps one of the most common abiotic stresses which limits crop productivity (Akhter *et al.* 2007, Dai *et al*. 2012a, Dinler and Aksoy 2014). Physiological studies suggested that drought resistance in plant depends mainly on the capacity for osmotic adjustment, which allows the plant to maintain turgor, protect meristems from desiccation, have the ability to control and reduce water loss (Pinheiro *et al*. 2004, Khanna-Chopra and Selote 2007, Naz and Bano 2013). However, different plants use different strategies to counteract osmotic stresses (Wang *et al*. 2013). For example, mechanism of stress tolerance has been reported to be associated with hyper-accumulation of compatible solutes such as glycine betaine and proline, exclusion of toxic ions, increasing accumulation of total soluble carbohydrates and total soluble proteins (Dai *et al*. 2011a, b, c). It is now well evident that these solutes contribute to prevent dehydration and cellular damage by osmotic adjustment (Al-Khayri 2002, Ashraf *et al*. 2007, Dai *et al*. 2011c, Dai *et al*. 2012 a, b, Jia *et al*. 2008) .

Oxidative processes and free radicals are induced by a wide range of stresses, including dehydration (Dai *et al*. 2015). Reactive oxygen species (ROS) cause lipid peroxidation, protein oxidation and DNA damage, all of which contribute to cell death (Mittler 2002). It has been proposed that desiccation tolerance is associated with a capacity to effectively scavenge ROS because it involves with increased antioxidant enzyme activities (Dai *et al*. 2012 a, b), such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). Antioxidant defense systems accumulate in orthodox seed when desiccation tolerance is acquired, and they degrade when desiccation tolerance is lost (Jin *et al*. 2010, Li *et al*. 2010, Modesto and Martinez 2010). The relationship between changes in ROS content and the acquisition and loss of seed desiccation tolerance has been reported in corn (Jia *et al*. 2008).

*Author for correspondence: <daihp72@aliyun.com> or <zhaohuahz@126.com >. [1]Shanxi Key Laboratory of Bio-resources,Shanxi University of Technology,Hanzhong 723001, P. R. China. [2]School of Life Science, Lanzhou University, Lanzhou 730000,China

However, these studies were only involved in individual development or germination process, or in diverse species with different genetic background such as recalcitrant or orthodox seeds, which make understanding the mechanism of desiccation tolerance difficult.

Apocynum venetum L. (Apocynaceae), known as "Luobuma" in Chinese, is a wild plant that grows widely in the middle to north-western regions of China. Its leaves are commonly used to make tea (Kazuaki *et al*. 2008, Kaoru *et al*. 2009). They have also been used to treat cardiac disease, hypertension, nephritis, neurasthenia, and other diseases. Although studies regarding the mode of medicinal chemistry in *A. venetum* have been conducted, none has been reported for drought tolerance. Hence, additional information on understanding of possible responses, adaptations and the physiological mechanisms of *A. venetum* to changes of soil water levels under prolonged and increasing drought stress conditions is needed.

Materials and Methods

The experiment was carried out in an orchard located at north-west A&F University, Yangling District (lat 34°20′ N, long 108°24′ E), China. Plantlets used in this study was *Apocynum venetum* L. Plants were cultivated for three weeks in a plant growth room (21°C, from 50 to 60% relative air humidity, 16 hrs of light per day, and 150 µmol photons/m^2/s photosynthetically active radiation (PAR). In this experiment, four treatments were cultured with different concentrations of PEG6000 (control, 10, 20, and 30%), respectively and monitored for 2, 4, 6 and 8 (T_4) days. pH was adjusted to 6.5. Selected plants were harvested and used to control PEG levels.

Soluble sugar and proline contents were determined according to the method of Jia *et al.* (2008). Oxidative damage to lipids was estimated as the content of the total 2-thiobarbituric acid (TBA) reactive substance and expressed as equivalents of malondialdehyde (MDA) as described by the method of Dai *et al*. (2012 a, b). In order to estimate the amount of electrolytes released during each 15 min interval, only the newly released electrolytes taken into account and expressed in relative terms. The activity of SOD (EC 1.15.1.1) was determined according to Morina *et al*. (2010). One unit of SOD was defined as the amount of enzyme that caused a 50% decrease in the SOD-inhibited nitrobluetetrazolium reduction at 550 nm. The activity of APX (EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) after the method of Dai *et al*. (2011c) were determined.

Results and Discussion

The effects of PEG stress and electrolyte leakage rate in *A. venetum* leaves are shown in Table 1. For 4 and 8 days of treatment, the electrolyte leakage rate increased 1.3 and 1.8-fold, respectively, compared to the control. The electrolyte leakage rate incased significantly was considerably higher in 30% >20% >10%. Fig. 1b showed the accumulation of MDA in leaves under PEG stress. After 2, 4, 6 and 8 days, four levels of PEG stress led to increment in MDA content induced by PEG stress. These results indicated that different levels of PEG stress could significantly inhibite the accumulation of electrolyte leakage and MDA contents in leaves of *A. venetum* under water stress.

Table 1 showed that the PEG stress affected soluble sugars and proline contents within the 4 d of treatment. Four levels of PEG stress led to increments in soluble sugars and proline contents within the 4 days of treatment. The soluble sugars and proline contents were considerably higher in 30% >20% >10%.

Components of ROS scavenging system (i.e. enzymatic antioxidants; SOD, APX and GR) were studied in *A. venetum* plants treated with increasing PEG concentration (Fig.1A, B, C). After 8 days of 30% PEG stress, SOD, APX and GR which are involved in H_2O_2 removal, generally

exhibited increases in activities compared with controls regardless of the different time. There were significantly increase in the activities of SOD, APX and GR which were observed after exposure to high levels of PEG (20%) treatments. And enzymes activities were increased in different time, respectively ($p < 0.01$).

Table 1. Changes of electrolyte leakage rate, MDA, proline content and soluble suger content in *Apocynum venetum* l leaves under PEG stress.

Time (d)		PEG treatment			
		Control	10%	20%	30%
Electrolyte leakage rate (mg/g)	0	5.8±0.3*	6.1±0.5*	6.0±0.8*	6.0±0.4*
	2	5.9±0.6*	6.0±0.8*	5.8±0.3*	7.2±0.5**
	4	5.8±0.5*	6.4±0.6*	7.7±0.9*	8.6±0.6**
	6	6.0±0.7*	6.6±0.8*	8.1±0.9*	9.2±0.8*
	8	6.1±0.5*	6.8±0. 3*	8.6±0.8*	9.8±0.4*
MDA content (μmol/g)	0	2.4±0.6	2.5±0.4	2.6±0.3*	2.8±0.2*
	2	2.6±0.8	2.7±0.04	2.8±0.4*	2.9±0.3*
	4	3.2±0.5*	3.9±0.3*	4.9±0.5*	6.6±0.7*
	6	4.6±0.8*	6.6±0.5*	7.9±0.6**	10.5±0.9*
	8	5.2±0.7*	10.1±0.5*	12.7±0.8*	15.3±0.4*
Proline content (mg/g)	0	2.0±0.3	2.1±0.5	2.6±0.8*	3.0±0.4*
	2	2.0±0.8*	2.3±0.6*	3.8±0.6*	4.8±0.5**
	4	2.0±0.6*	2.7±0.5*	4.6±0.9*	6.6±0.7**
	6	2.1±0.8*	3.7±0.9*	6.2±0.9**	9.9±0.9*
	8	2.2±0.5*	5.1±0.8*	7.9±0.7*	12.8±0.4*
Soluble sugar content (mg/g)	0	1.5±0.3	1.6±0.5	1.7±0.8*	1.9±0.4*
	2	1.5±0.8	1.7±0.6	1.8±0.6*	2.2±0.5*
	4	1.5±0.6	1.8±0.5*	2.1±0.9*	2.6±0.7*
	6	1.5±0.8	2.0±0.9*	2.3±0.9*	3.0±0.9*
	8	1.5±0.5*	2.3±0.8*	2.9±0.7*	3.8±0.4*

	Primary effects		Interactions
	PEG concentration	Time	P×T
ANOVA p values			
Electrolyte leakage rate	0.000	0.016	0.009
MDA content	0.000	0.003	0.011
Proline content	0.000	0.000	0.028
Soluble sugar content	0.000	0.015	0.039

Means ± SE from at least six independent measurements. * and ** significantly differ from the control at $p < 0.05$ and $p < 0.01$, respectively.

The expansion of arid zones on our planet and the growing population of world will have a direct impact on water resources and water availability (Jia *et al*. 2008, Dai *et al*. 2011c, Dai *et al*. 2012). Water scarcity and the concurrent high temperatures will significantly limit crop productivity (Dai *et al*. 2011c). Thus results showing the changes in metabolites in response to drought may reflect changes in cell damage. Responses of crops to a soil water deficit involve the

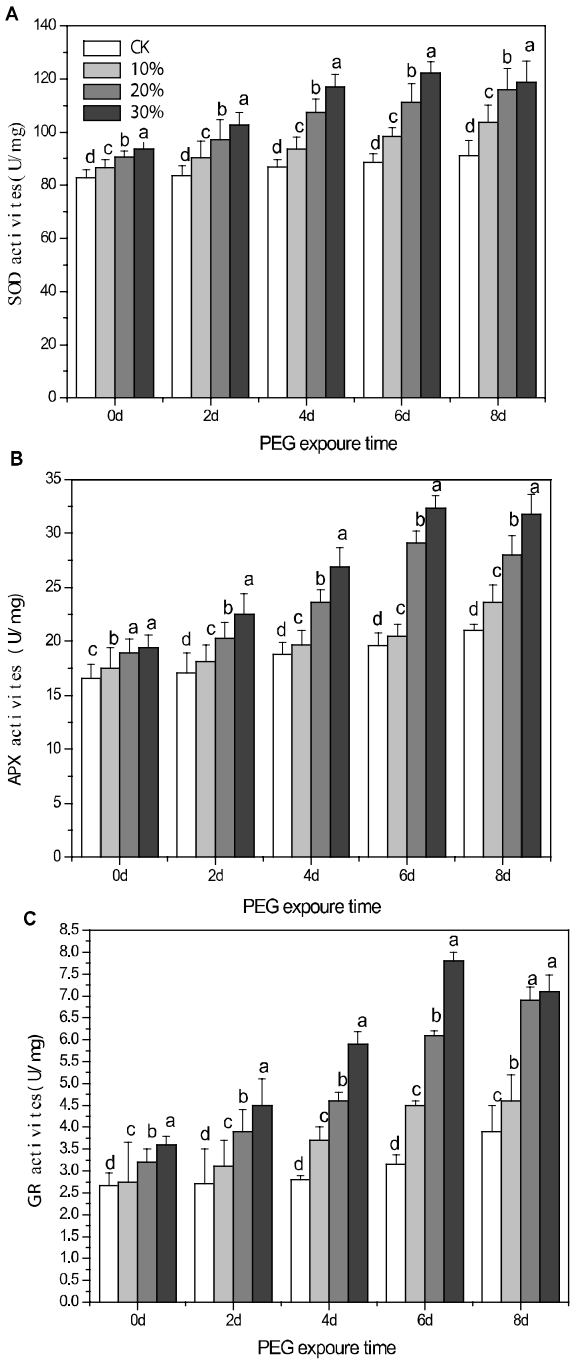

Fig. 1. Changes of SOD, APX and GR activities in *Apocynum venetum* leaves under PEG stress. Values are means of six replicates ± standard deviation. Different small alphabets are representing the significant difference at $p < 0.05$.

implementation of a variety of mechanisms, including those for avoiding water loss (Jia *et al*. 2008, Dai *et al*. 2012 a, b, Li and Wang 2002), protecting cellular components, and repairing cell damage (i.e., via scavengers of toxic oxygen species). Plants could protect themselves from active oxygen species by a complex antioxidant system. Results of the present investigation indicated that electrolyte leakage, MDA, soluble sugars and proline contents gradually increased under PEG stress.

Thus, it can be concluded that the plants under drought stress are highly regulated by components of the antioxidative system and secondary metabolite contents. The results indicated that the cultivation of medicinal plants like *A. venetum* in water deficit areas would increase its antioxidant metabolism and the level of active principles. Furthermore, the data presented here reflect the importance of a physiological analysis of plant response, which must accompany with field experiments and evaluation. Further investigations are required to ascertain this conclusion.

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