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Non-Enzymatic Antioxidant Changes in *Withania somnifera* with Varying Drought Stress Levels

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Abstract: A study was conducted in *Withania somnifera* plants in order to analyse the alterations in non-enzymatic antioxidant contents under different water stress regimes. Plants were grown with different water regimes like 10 days interval drought (DID), 15 DID and 20 DID water stress. The plants were uprooted randomly after DID induction for determining the effect of water deficit on non-enzymatic and enzymatic antioxidant potentials. The studied non-enzymatic antioxidants were ascorbic acid, α-tocopherol and reduced glutathione. They were assayed from leaf and roots of both control and treated plants. It was found that all the water stress treatments have profound effects on these parameters and resulted in an increase in this medicinal plant.

Key words: *Withania somnifera* · Water stress · Ascorbic acid · α-Tocopherol · Reduced glutathione

INTRODUCTION

Plant growth and productivity is adversely affected by natures' wrath in the form of various abiotic and biotic stress factors. Plants are frequently exposed to many stress conditions such as low temperature, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity. Various anthropogenic activities have accentuated the existing stress factor [1-4]. Water stress may arise as a result of two conditions, either due to excess of water or water deficit [2-5]. The more common water stress encountered is the water deficit stress known as the drought stress [5,6]. The water deficit stress has profound impact on ecological and agricultural systems. The reactions of the plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development [2-8]. Understanding plant responses to drought is of great importance and also a fundamental part for making the crops stress tolerant [6-7].

Withania somnifera Dunal, known as ashwagandha, has been an important herb in the Ayurvedic and indigenous medical systems for centuries in India. In view of its varied therapeutic potential, it is the subject of considerable modern scientific attention [9-10]. Lot of reports are there regarding the medicinal and agronomical aspects of this *Withania somnifera* plant, but little attention has been drawn to the alterations in antioxidant metabolism of this plant under water stress. Hence this study aims to evaluate the effects of water stress on non-enzymatic (Ascorbic acid, α-Tocopherol, Reduced glutathione) and enzymatic (Superoxide dismutase, Ascobate peroxidase, Catalase) activities in *Withania somnifera*.

MATERIALS AND METHODS

The seeds of *Withania somnifera* were surface sterilized with 0.1% $HgCl_2$ for 2 min and sown in pots. Six seeds were sown in each pot of 30 × 30 cm containing 3 kg of soil mixture composed of red soil, sand and the farmyard manure at 1:1:1 ratio. All the pots were watered to the field capacity with ground water upto 30 days after sowing (DAS). The seedlings were thinned to 2 pot^{-1} on 20 DAS. Pots were irrigated with ground water one-day

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interval as a control and other treatments are 10, 15 and 20 days interval drought (DID) from 30 DAS. The pots were covered with a rain out shelter, made up of plastic sheets, whenever rainfall was anticipated and immediately after rain, rain out shelter was pulled back so that, pots received maximum sunlight. Further, the pots were regularly covered with rain out shelter during nighttime. Using this system the pots were protected from rainfall and any external moisture entry. Plants were uprooted randomly on 40, 45 and 50 DAS, washed carefully and separated into root and leaf for analyses.

Estimation of AA Content: AA content was assayed as described by Omaye *et al*. [11]. The extract was prepared by grinding 1 g of fresh material with 5 ml of 10% TCA, centrifuged at 3500 rpm for 20 min, reextracted twice and supernatant made upto 10 ml and used for assay. To 0.5 ml of extract, 1 ml of DTC reagent (2,4-dinitrophenyl hydrazine-thiourea-$CuSO_4$ reagent) was added, incubated at 37°C for 3 h and 0.75 ml of ice-cold 65% H_2SO_4 was added, allowed to stand at 30 °C for 30 min, resulting colour was read at 520 nm in spectrophotometer (U-2001-Hitachi). The AA content was determined using a standard curve prepared with AA and the results were expressed in mg g^{-1} dry weight (DW).

Estimation of GSH Content: The GSH content was assayed as described by Griffith and Meister [12]. 200 mg fresh material was ground with 2 ml of 2% metaphosphoric acid and centrifuged at 17000 rpm for 10 min. Adding 0.6 ml 10% sodium citrate neutralized the supernatant. 1 ml of assay mixture was prepared by adding 100 µl extract, 100 µl distilled water, 100 µl 5,5-dithio-bis-(2-nitrobenzoic acid) and 700 µl NADPH. The mixture was stabilized at 25 °C for 3-4 min. Then 10 µl of glutathione reductase was added, read the absorbance at 412 nm in spectrophotometer and the GSH contents were expressed in µg g^{-1} fresh weight (FW).

Estimation of α-Toc Content: α-Toc content was assayed as described by Backer *et al*. [13]. 500 mg of fresh tissue was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 min and the supernatant was used for estimation of α -toc. To one ml of extract, 0.2 ml of 2% 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 min. The resulting red colour was diluted with 4 ml of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α -toc content was calculated using a standard graph made with known amount of α -toc.

Statistical Analysis: Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for six samples in each group. p values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

Non-Enzymatic Antioxidants (Table 1): In the roots the ascorbic acid content was increased with age in drought stressed and control plants. The ascorbic acid content of the leaves of *Withania somnifera* increased with age in the treated and control plants. In plant cells, the most important reducing substrate for H_2O_2 removal is ascorbic acid [14]. Ascorbate is one of the most extensively studied anti-oxidant and has been detected in the majority of plant cell types, organelles and apoplast [15-17]. Ascorbate is synthesized in the mitochondria and is transported to the other cell components through a proton- electrochemical gradient or through facilitated diffusion. Further, ascorbic acid also has been implicated in regulation of cell elongation [18-19].

α-tocopherol of the drought stressed plant roots significantly increased when compared to control plants. In the leaves the α -tocopherol content was increased with age in drought stressed and control plants. α–tocopherols interact with the polyunsaturated acyl groups of lipids, stabilize membranes, scavenge and quench various reactive oxygen species (ROS) and lipid soluble byproducts of oxidative stress [18-20]. Synthesis of low-molecular-weight antioxidants, such as α-tocopherol, has been reported in drought-stressed plants [21]. Oxidative stress activates the expression of genes responsible for the synthesis of tocopherols in plants [21-29].

Table 1: Water deficit stress induced changes in the non-enzymatic antioxidant contents of *Withania somnifera* under different water regimes. (DID-days interval drought)

	Ascorbic acid		Reduced glutathione		Tochopherol	
Treatments	Root	Leaf	Root	Leaf	Root	Leaf
Control	8.919	8.910	9.938	7.603	11.560	9.109
10 DID	9.082	9.081	10.239	8.754	11.801	11.038
Control	9.625	9.658	10.544	7.223	12.432	9.013
15 DID	9.382	10.012	11.102	9.605	13.86	12.682
Control	8.569	8.546	9.662	8.340	12.851	9.452
20 DID	9.983	9.309	10.948	9.384	14.17	12.79

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