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Physiological Basis of Defense Mechanisms in *Withania somnifera* under Water Deficit Stress

[1,2]*Cheruth Abdul Jaleel*

[1]Department of Botany, Stress Physiology Labortary,
Annamalai University, Annamalainagar 608 002, Tamilnadu, India
[2]DMJM International (AECOM Middle East Ltd.), Consultant of Gardens Sector Projects,
Alain Municipality and Eastern Emirates, P.O. Box 1419, Al-Ain, Abu Dhabi, United Arab Emirates

Abstract: In the present investigation, alterations in defense mechanisms in terms of antioxidant metabolism of Withania somnifera plants were studied 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 enzymatic antioxidant potentials. Antioxidant enzymes like superoxide dismutase, ascobate peroxidase and catalase 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 enzymes and altered overall metabolism in this plant.

Abbreviations: DID, days interval drought; SOD, Superoxide dismutase; APX, Ascobate peroxidase; CAT, Catalase

Key words: *Withania somnifera* · Water stress · Antioxidant enzymes · Superoxide dismutase · Ascobate peroxidase · Catalase

INTRODUCTION

Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rate and plant productivity. Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle [1-3]. Water deficit is one of the major abiotic stresses, which adversely affect the crop growth and yield. Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation [3-5]. Water stress tolerance is seen in almost all plant species but its extent varies from species to species. Water deficit stress is a global issue to ensure survival of agricultural crops and sustainable food production [5-7]. Conventional plant breeding attempts have changed over to use physiological selection criteria since they are time consuming and rely on present genetic variability [4-6]. Currently, protection of plants from abiotic stresses through application of plant growth regulators (PGR) attracts more attention [2-7].

Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation [8-11]. One way to ensure future food needs of the increasing world populations should involve a better use of water by the development of crop varieties which needs less amount of water and more tolerant to drought. The biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanism to water limited condition in higher plants [10-14]. For the last few decades, several scales of physiological works have been conducted under drought stress in crop plants [12-14]. Soil water availability represents a major environmental constraint under Mediterranean conditions and predictions suggest that the decline in total rainfall in the Mediterranean area will be drastic. Under such conditions, it is likely that

Corresponding Author: Dr. C.A. Jaleel, DMJM International (AECOM Middle East Ltd.),
Consultant of Gardens Sector Projects, Alain Municipality and Eastern Emirates,
P.O. Box 1419, Al-Ain, Abu Dhabi, United Arab Emirates

plants will experience increasing water deficit stress in their natural communities. Water stress tolerance is seen in almost all plant species but its extent varies from species to species. A better understanding of the physiological strategy adopted by a drought resistant variety to cope with water deficit requires through study of the relationship between water use efficiency and transpiration. In crops like wheat, the detrimental effects of water deficits on the harvest index also minimizes the impact of the water limitation on crop productivity and increase the efficiency of water use. The numerous physiological responses of plant to water deficits generally vary with the severity as well as the duration of water stress [8-12].

Withania somnifera Dunal, known as ashwagandha, has been an important herb in the Ayurvedic and indigenous medical systems for centuries in India [15]. In view of its varied therapeutic potential, it is the subject of considerable modern scientific attention [16]. 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 enzymatic (Superoxide dismutase, Ascobate peroxidase, Catalase) activities in *Withania somnifera*.

MATERIALS AND METHODS

Plant Materials and Drought Stress Induction: 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 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.

Antioxidant Enzyme Extractions and Assays

Assay of SOD (EC 1.15.1.1) activity: The activity of SOD was assayed as described by Beauchamp and Fridovich [17]. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} M KCN and 5.6×10^{-5} M nitroblue tetrazolium (NBT) salt dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). Three ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30 °C for 1 h. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity is expressed in U mg^{-1} protein (U = Change in 0.1 absorbance $hour^{-1}$ mg^{-1} protein).

Assay of APX (EC 1.11.1.1) activity: The activity of APX was determined by the method of Asada and Takahashi [18]. The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 µl of enzyme extract. The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient 2.9 $mM^{-1}cm^{-1}$). The enzyme activity was expressed in units mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

Assay of CAT (EC 1.11.1.6) activity: The activity of CAT was measured according the method of Chandlee and Scandalios [19] with small modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein). For all the enzymatic calculations protein was determined by the method of Bradford [27], using bovine serum albumin (BSA, Sigma, USA) as the standard.

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.

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

	SOD		APX		CAT	
Treatments	Root	Leaf	Root	Leaf	Root	Leaf
Control	7.475	8.078	3.472	3.618	3.505	2.074
10 DID	8.336	8.431	4.420	4.714	4.057	3.289
Control	7.362	8.157	3.509	4.153	2.063	1.363
15 DID	8.765	9.936	4.733	5.714	3.077	1.698
Control	8.176	8.103	4.316	5.002	2.486	2.431
20 DID	9.412	10.556	6.259	7.553	3.832	3.355

RESULTS AND DISCUSSION

Antioxidant Enzymes (Table 1): The activity of SOD increased in the root by water deficit in *Withania somnifera*. SOD activity increased in the leaves of *Withania somnifera* under drought condition. Several studies have reported enhanced stress tolerance related to over production of chloroplastic SOD [20]. GR and DHAR were exclusively localized in mesophyll cells whereas most of the SOD and APX were localized in mesophyll and bundle sheath cells. Increased SOD activity was reported in plants under water deficit stress, which varied in three different genotypes [21-23]. Stress has significant effect in the enhancement of SOD activity in seedlings [22]. The increase in SOD activity under drought stress was about 25% in plants [21-24]. Double the amount of SOD activity was noted in water stressed citrus plants when compared to well watered control plants during seedling stage [22-25].

The catalase activity showed an increase in the root and leaves of *Withania somnifera* under drought conditions. Plants produce H_2O_2 in metabolic process and cause damage of cell oxidation function, while CAT eliminates H_2O_2 and plays a key role in the elimination O2_ [2-4]. APX activity increased in roots of *Withania somnifera* under drought condition. APX activity increased in the leaves of *Withania somnifera* under drought condition. Ascorbate peroxidase is one of the most important antioxidant enzymes of plants that detoxify hydrogen peroxide using ascorbate for reduction. APX reduces H_2O_2 to water by ascorbate as specific electron donor [26]. In trifoliate orange an increased APX activity was observed under water stress, but at mild water deficit no significant variation in APX activity was recorded [27-29].

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