Global Journal of Molecular Sciences 4 (2): 49-55, 2009
ISSN 1990-9241

Induction of Drought Stress Tolerance by Paclobutrazol and Abscisic Acid in Gingelly (*Sesamum indicum* L.)

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Abstract: For the past several years, several techniques of physiology have been applied to overcome the water deficit and drought stress in field crops. However little information is gained on the response to PBZ and ABA treatments under drought stress and their ameliorative actions on *Sesamum*. So a study was carried out to understand the effect of PBZ and ABA on drought stress amelioration in *Sesamum indicum* L. The main aspects studied were the non-enzymatic antioxidant changes in different parts of treated, drought stressed as well as control plants. The non-enzymatic antioxidants (ascorbic acid and tocopherol) were extracted and estimated. The non-enzymatic antioxidant molecules like ascorbate and α-tocopherol showed significant increase under drought condition in *Sesamum indicum*. PBZ caused significant enhancement in these antioxidant enzymes under drought stress and also in well-watered conditions. It is not so with ABA treatment. ABA slightly reduced the non-enzymatic antioxidant contents. From the results, it can be concluded that, these growth regulators are better suited as stress ameliorating agents in gingelly.

Key words: Non-enzymatic antioxidants · Ascorbic acid · Tocopherol · Drought stress · *Sesamum indicum*

INTRODUCTION

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 plants will experience increasing water deficit stress in their natural communities [1-4]. Water stress tolerance is seen in almost all plant species but its extent varies from species to species [2-3]. 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 [5]. The numerous physiological responses of plant to water deficits generally vary with the severity as well as the duration of water stress [6].

Water stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange. Desiccation is a much more extensive loss of water which can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme catalyzed reaction [7,8]. Water stress is characterized by reduction of water content, turgor, total water potential, wilting, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in arrest of photosynthesis, disturbance of metabolism and finally dying [6-9].

Tolerance to abiotic stresses is very complex, due to the complexity of interactions between stress factor and various molecular, biochemical and physiological phenomena affecting plant growth and

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development [10-12]. High yield potential is the target of most crop breeding, not superior drought resistance and in many cases high yield potential can contribute to yield in moderate stress environment [7-9].

PBZ (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-trizol-1-yl)-pentan-3-ol] is a triazolic group of fungicide which have PGR properties. The growth regulating properties of PBZ are mediated by changes in the balance of important plant hormones including the Gibberellins, ABA and cytokinins [13-15]. The application of PBZ have no risk to human health and showed increased yield of crops. PBZ has been proved as an agent in stress amelioration in medicinal plants [16-19]. In spite of the plants growth regulating properties, it is reported that PBZ have some negative side effects on soil microorganisms. Anyhow, there are reports on microorganisms, which are capable of PBZ degradation on soil.

Paclobutrazol has been proved as an agent in stress amelioration in medicinal plants. Paclobutrazol increased the diameter and length of fibrous roots and enhanced the lateral root formation [14-16]. Gingelly (*Sesamum indicum* L.) is a member of the Pedaliaceae family. Gingelly is one of the ancient oil seed crop cultivated for its superior quality oil and seed, hence it is regarded as "Queen of the oil seeds". This species is native to Africa. It is cultivated extensively in USA, India, Burma, Indo-china and Japan. India is the largest producer of sesame in the world. Tamil Nadu is one of the major sesame growing state in India. Sesame is used in traditional systems of medicine for curing many diseases. It has a good composition of amino acids, protein, niacin, folic acid, vitamin E, calcium and Phosphorus.

The objectives of the present study were to understand the effect of paclobutrazol and ABA in drought stress amelioration in *Sesamum indicum* L. through their effects on the plant's non-enzymatic antioxidant potential under drought stress conditions.

MATERIALS AND METHODS

The seeds of *Sesamum indicum* L. were obtained from Department of Agronomy, Faculty of Agriculture, Annamalai University. The triazole compound paclobutrazol was obtained from Syngenta, India Ltd., Mumbai. ABA was purchased from Himedia India Ltd., Mumbai.

During the study, average temperature was 32/26°C (maximum/minimum) and relative humidity (RH) varied between 60-75 per cent. The experimental part of this work was carried out in Botanical Garden and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu. The methodologies adopted are described below.

The plants were raised in Botanical Garden, during the months of February – May, 2006. The seeds were surface sterilized with 0.2% Mercuric chloride solution for five minutes with frequent shaking and thoroughly washed with tap water. The experiments were carried out in polythene bags (27x16 cm). The pots were filled with 3 kg uniform soil mixture containing red soil: sand: farm yard manure (FYM) in 1:1:1 ratio. The experiment was laid out in a Completely Randomized Block Design (CRBD).

In the preliminary experiments, 2, 5, 10, 15 and 20 mg L^{-1} paclobutrazol and 5, 10 and 15 ìM ABA were used for treatment to determine the optimum concentration. Among the treatments, 5 mg L^{-1} paclobutrazol and 10 ìM ABA concentration increased the growth and dry weight significantly and higher concentration slightly decreased the growth and dry weight when compared to drought stressed plants. In the lower concentrations, there was no change in weight and growth. Hence 5 mg L^{-1} paclobutrazol concentration was used to study the effect of paclobutrazol and 10 ìM ABA on the drought stress amelioration of *Sesamum indicum.*

Drought Treatment intervals were from 30 DAS 2, 4 and 6 days interval drought (DID). The treatments were given as foliar spray for ABA and soil drenching for PBZ on 32, 34 and 36 days after sowing (DAS).

The plants were taken randomly on 33 (2 DID), 35 (4 DID) and 37 (6 DID) DAS and separated into roots, stems and leaves and used for determining antioxidant potentials.

Antioxidants

Ascorbic Acid: Ascorbic acid content was assayed as described by Omaye *et al.* [20].

Extraction: One gram of fresh material was ground in a pestle and mortar with 5 ml of 10 per cent TCA, the extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10 percent TCA and supernatant was made to 10 ml and used for estimation.

Estimation: To 0.5 ml of extract, 1 ml of DTC reagent (2,4-Dinitrophenyl hydrazine-Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 37 °C for 3 hours and to this 0.75 ml of

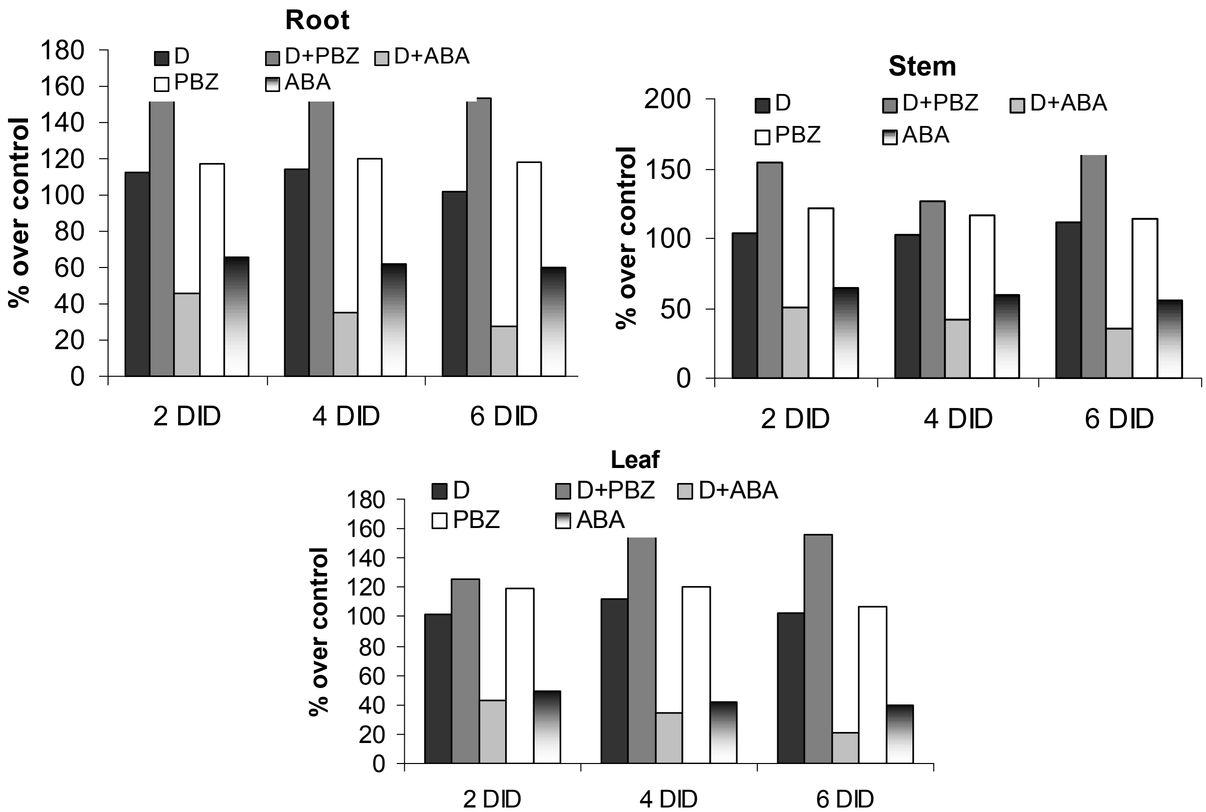

Fig. 1: Individual and combined effects of drought, PBZ, ABA on ascorbic acid contents o f Sesamum indicum

ice cold 65 per cent H_2SO_4 was added. The tubes were then allowed to stand at 30 °C for 30 minutes. The resulting colour was read at 520 nm in spectrophotometer (U-2001-Hitachi). The ascorbic acid content was determined using a-standard curve prepared with ascorbic acid and the results were expressed in milligrams per gram dry weight.

α-Tocopherol: α-Tocopherol activity was assayed as described by Backer *et al.* [21].

Extraction: Five hundred milligrams 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 minutes and the supernatant was used for estimation of α-tocopherol.

Estimation: To one ml of extract, 0.2 ml of 2 per cent 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 minutes. 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-tocopherol content was calculated using a standard graph made with known amount of α-tocopherol.

Statistical Analysis: Each treatment was analysed with at least seven replicates and a standard deviation (SD) was calculated and data are expressed in mean ± SD of seven replicates. RESULTS

Ascorbic Acid (Fig. 1)

Root: In the roots the ascorbic acid content was increased with age in paclobutrazol treated, drought stressed and control plants. ABA decreased the ascorbic acid content in roots at all stages of growth and it was 60.99 per cent and 28.96 per cent over control in well watered and stressed respectively on 6 DID.

Stem: The ascorbic acid content of the stem of *Sesamum indicum* increased with age in the treated and control plants. Paclobutrazol and drought treatments increased the ascorbic acid content in the stem at all stages of growth and it was 114.76 per cent and 99.15 per cent over

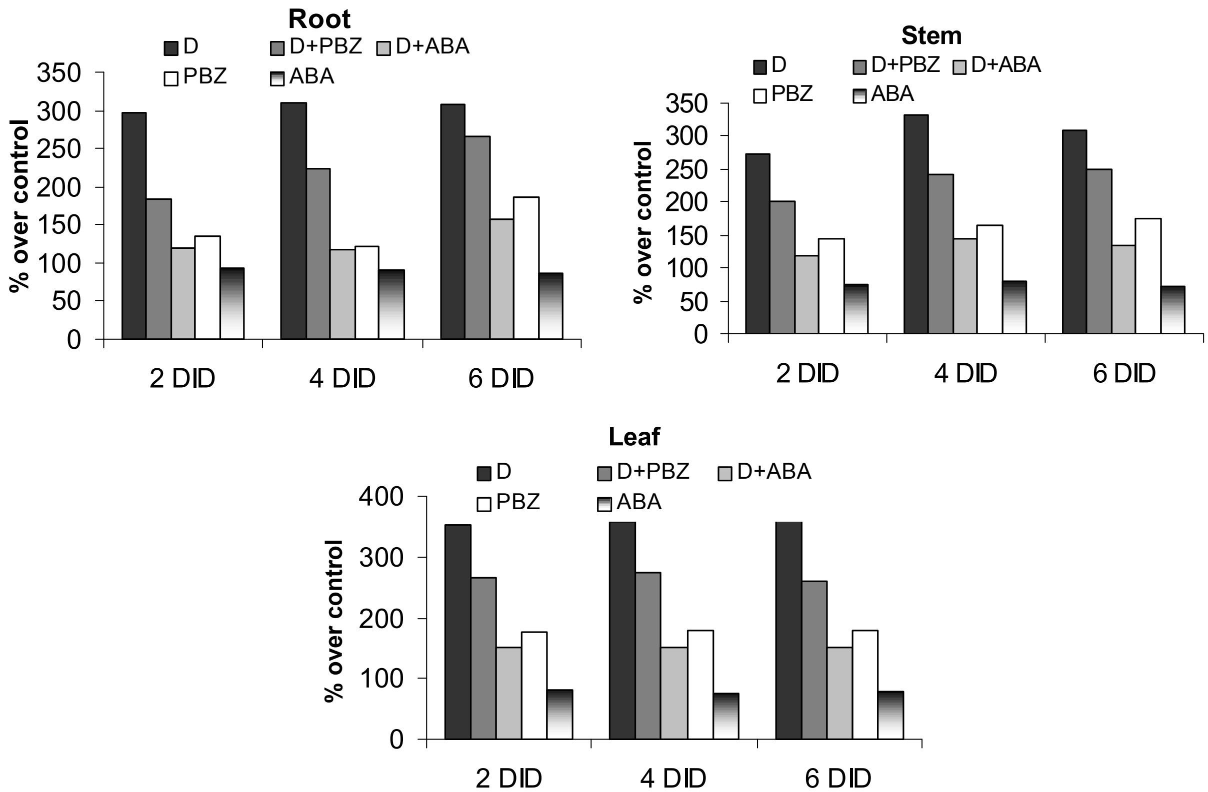

Fig. 2: Individual and combined effects of drought, PBZ, ABA on a-tocopherol contents o f Sesamum indicum

control respectively on 6 DID. ABA resulted a significant reduction (56.16 per cent over control) in ascorbic acid content in stem of *Sesamum indicum* at 6 DID.

Leaf: The ascorbic acid content of the leaves of *Sesamum indicum* increased with age in the treated and control plants. Paclobutrazol and drought treatments increased the ascorbic acid content in the leaves at all stages of growth and it was 107.62 per cent and 93.38 per cent over control respectively on 6 DID. ABA resulted a significant reduction in ascorbic acid content in leaves of *Sesamum indicum* at all sampling days.

α-Tocopherol (Fig. 2)

Root: α-tocopherol of the drought stressed plant roots significantly increased when compared to control plants. The extent of increase was 339.4 per cent over control on 6 DID. Paclobutrazol caused an enhancement in α-tocopherol content of roots under drought as well as well-watered conditions. ABA was an inhibitor of α-tocopherol individually and also under drought stress.

Stem: The α-tocopherol content of the stem of *Sesamum indicum* increased with age in the treated and control plants. Paclobutrazol and drought treatments increased the α-tocopherol content in the stem at all stages of growth and it was 174.40 per cent and 309.02 per cent over control respectively on 6 DID. ABA resulted a significant reduction in α-tocopherol content in stem of *Sesamum indicum* at all sampling days.

Leaf: In the leaves the α-tocopherol content was increased with age in paclobutrazol treated, drought stressed and control plants. ABA decreased the α-tocopherol content in leaves at all stages of growth and it was 78.35 per cent and 150.31 per cent over control in well watered and stressed respectively on 6 DID.

DISCUSSION

Ascorbic Acid: The ascorbic acid content was increased with age in paclobutrazol treated drought stressed and control plants. ABA decreased the ascorbic acid content in roots at all stages of growth. Ascorbate is one of the most extensively studied anti-oxidant and has been detected in the majority of plant cell types, organelles and apoplast [1,22]. Water stress resulted in significant increases in antioxidant AA concentration in *Catharanthus roseus* [23].

Triazole increased the level of antioxidants like ascorbic acid and α-tocopherol like in *Withania somnifera* Dunal. seedlings and protected membrane by preventing or reducing oxidative damage [24]. Increase in ascorbic acid content was reported in the triadimefon treated *Catharanthus roseus* [25] and *Withania somnifera* [26]. A decrease in ascorbic acid was reported in ABA treatment in *Ocimum sanctum* plants [27].

α- Tocopherol: α-tocopherol of the drought stressed plants significantly increased when compared to control plants. Paclobutrazol caused an enhancement in α-tocopherol content of roots under drought as well as well-watered conditions. ABA was an inhibitor of α-tocopherol individually and also under drought stress.

The active oxygen species formed at the membrane of leaves under drought stress was efficiently removed upon rehydration with increase in the α-tocopherol and β-carotene [28-30]. Soil applied propiconazole alleviated the impact of salinity on *Catharanthus roseus* by improving antioxidant status especially α-tocopherol [31]. Alterations in biochemicals including non-enzymatic antioxidants in white yam (*Dioscorea rotundata* Poir.) under triazole fungicides application was previously reported [32].

CONCLUSION

The non-enzymatic antioxidant molecules like ascorbate and α-tocopherol showed significant increase under drought condition in *Sesamum indicum*. PBZ caused significant enhancement in these antioxidant enzymes under drought stress and also in well-watered conditions. It is not so with ABA treatment. ABA slightly reduced the non-enzymatic antioxidant contents. From the results, it can be concluded that, these growth regulators are better suited as stress ameliorating agents in gingelly.

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