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Role of Paclobutrazol and ABA in Drought Stress Amelioration in *Sesamum indicum* L.

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Abstract: In this study, paclobutrazol, a triazole growth regulator and abscisic acid (ABA), a traditional growth regulator were used to analyse their role in water stress amelioration in drought stressed *Sesamum indicum* L. plants. The main aspects studied were the antioxidant enzyme profile changes in different parts of treated, drought stressed as well as control plants. The enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POX) were analysed. It is found that, these growth regulators can be used as stress ameliorating agents in this crop plant, as they increase the antioxidant enzyme activities and there by providing stress tolerance ability to the plant.

Key words: Superoxide dismutase · Ascorbate peroxidase · Peroxidase · Drought stress · *Sesamum indicum*

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops and has been cultivated in Korea since ancient times for use as a traditional health food. The sesame crop is usually grown under rain-fed conditions, where because of low and irregular precipitation is regularly subjected to mild to severe water-deficit stress [1]. Although sesame has good drought tolerance compared with many other crops, it is particularly susceptible to drought damage during both the seedling and early flowering stage [2].

Water is one of the most important ecological factors determining crop growth and development; water deficit plays a very important role in inhibiting the yields of crops [3-5]. Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation [6]. 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 [7-9]. 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 [10-12]. The biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanism to water limited condition in higher plants [8-11]. For the last few decades, several scales of physiological works have been conducted under drought stress in crop plants [4-16].

Triazole compounds are the chemicals belongs to a class of compounds known as ergosterol biosynthesis inhibitors and are used as fungicides as wells as plant growth regulators [17-19]. Triazoles have been called 'plant multiprotectants' because of their ability to induce tolerance in plants to environmental and chemical stresses [20-23]. The inhibition of gibberellin biosynthesis is the main reason behind the PGR properties of triazoles. Growth substance like ethylene, cytokinin and ABA are also affected by the triazoles [16-20]. Protection of plants from apparently unrelated stress by triazole is also mediated by a reduction in free radical damage and increase in the antioxidant potential and have an efficient free-radical scavenging system that enables them to detoxify active oxygen [18-24].

Exogenous application of ABA was able to increase plant adaptive response to various environmental conditions. The resurrection plant, *Craterostigma plantaginem* can tolerate extreme dehydration. However, in vitro propagated callus derived from this plant has a strict requirement for exogenously

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applied ABA in order to survive severe dehydration [25]. The plant hormone abscisic acid as a stress signal increased as a result of water stress and play important role in the regulation of plant responses from the whole plant level and the cellular level.

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 Antioxidant enzymes.

MATERIALS AND METHODS

The seeds of *Sesamum indicum* L. 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 µM ABA were used for treatment to determine the optimum concentration. Among the treatments, 5 mg L^{-1} paclobutrazol and 10 µM 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 µM 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.

Superoxide Dismutase (SOD, EC: 1.15.1.1): Crude enzyme extract was prepared, for the assay of Superoxide dismutase by the method of Hwang *et al.* [26].

Extraction: One gram of fresh tissue was homogenized with 10 ml of ice-cold 50 mM sodium phosphate buffer containing 1 mM PMSF. The extract was filtered through a double-layered cheesecloth. The extract was centrifuged at 12,500 rpm for 20 minutes at 4 °C. The supernatant was saved and made up to 10 ml with extraction buffer and used for estimation of the SOD enzyme activity. The enzyme protein was determined by Bradford [27] method.

Estimation: Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich [28]. The reaction medium was prepared and to 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} potassium cyanide and 5.6×10^{-5} M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes. Illumination started to initiate the reaction at 30 °C for one hour. Those without illumination saved as blank and kept in dark. The absorbance was read at 560 nm in the spectrophotometer against blank. Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per milligram protein under the assay condition [29].

Ascorbate Peroxidase (APX, EC: 1.11.1.11): Ascorbate peroxidase was extracted and estimated by the method of Nakano Asada [30].

Extraction: Five hundred milligrams of fresh plant tissue was ground in a pestle and mortar under liquid nitrogen and 10 ml of 50mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 per cent PVP and 1 mM ascorbic acid. The homogenate was filtered through a double-layered cheesecloth and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was used as source of enzymes.

Estimation: One ml of reaction mixture 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 µg per gram dry weight.

Peroxidase (POX, EC 1.11.1.7): Peroxidase was assayed by the method of Kumar and Khan [31]. Assay mixture of Peroxidase contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of

purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at zero time. The activity was expressed in unit mg^{-1} protein. One unit is defined as the change in the absorbance by 0.1 min^{-1} mg^{-1} protein.

RESULTS

Superoxide Dismutase (Fig. 1)

Root: The activity of SOD increased in the root by water deficit in *Sesamum indicum*. It was nearly 198.57 per cent over control on 6 DID. Treatment with paclobutrazol and ABA resulted in an enhancement of SOD activity under drought stress, which was 205.7 and 236.66 percent over control respectively on 6 DID.

Stem: SOD activity was found increased under water deficit condition in stem of *Sesamum indicum*. The SOD activity increased greatly in paclobutrazol treated stem, which was 223.8 and 269.38 percent over control in individual and drought stressed respectively. ABA caused an increased in SOD activity and it was 293.87 percent over control under drought stress on 6 DID.

Leaf: SOD activity increased in the leaves of *Sesamum indicum* under drought condition. Paclobutrazol also resulted in increased SOD activity in leaves of *Sesamum indicum*. Paclobutrazol in combination with drought caused again enhancement in SOD activity when compared to stress and well-watered control plants. It was nearly 464.74 per cent over control on 6 DID. ABA treatment alone increased (163.30 per cent over control) the SOD activity significantly in leaves when compared to all other treatments.

Ascorbate Peroxidase (Fig. 2)

Root: APX activity increased in roots of *Sesamum indicum* under drought condition. Paclobutrazol also resulted in increased APX activity in leaves of *Sesamum indicum*. Paclobutrazol in combination with drought caused again enhancement in APX activity when compared to stress and well-watered control plants. It was nearly 318.75 per cent over control on 6 DID. ABA treatment alone increased (133.69 per cent over control) the APX activity significantly in roots when compared to all other treatments.

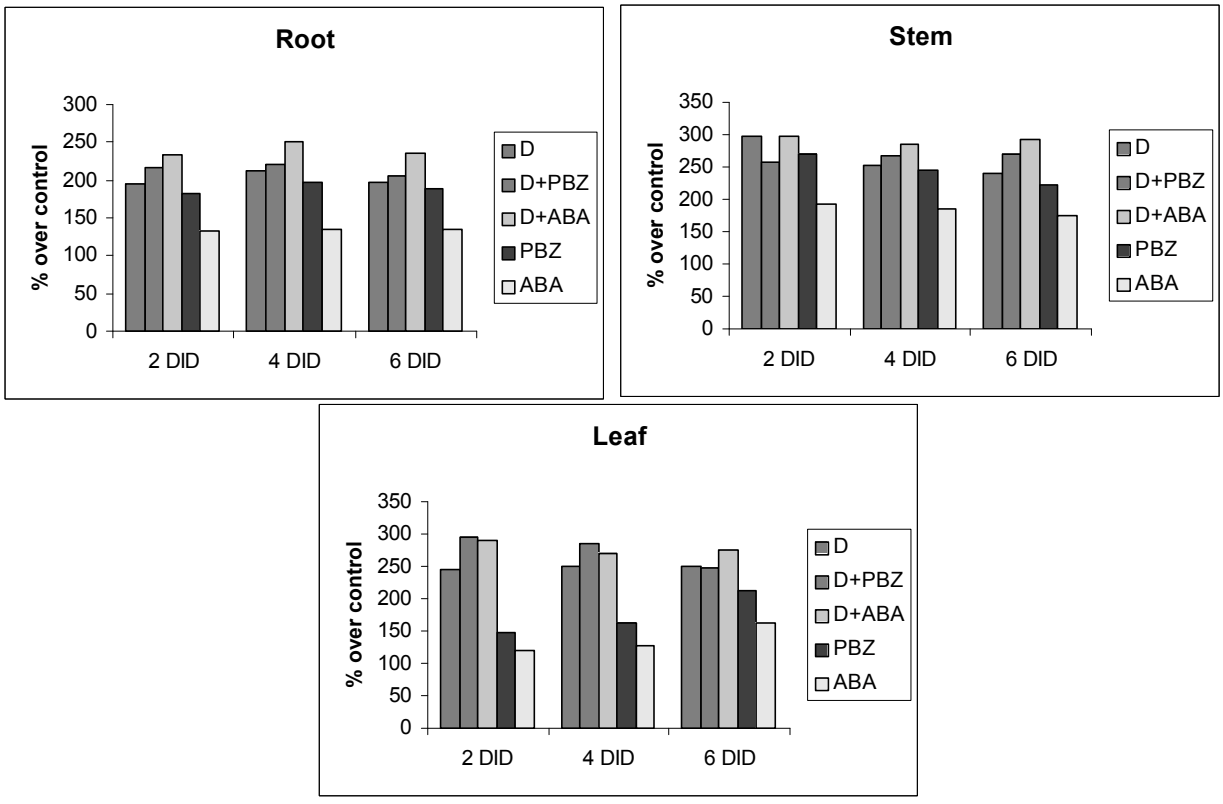

Fig. 1: Individual and combined effects of drought, PBZ, ABA on SOD activity o f *Sesamum indicum*. [D-Drought, PBZ-Paclobutrazol, ABA- Abscisic acid]

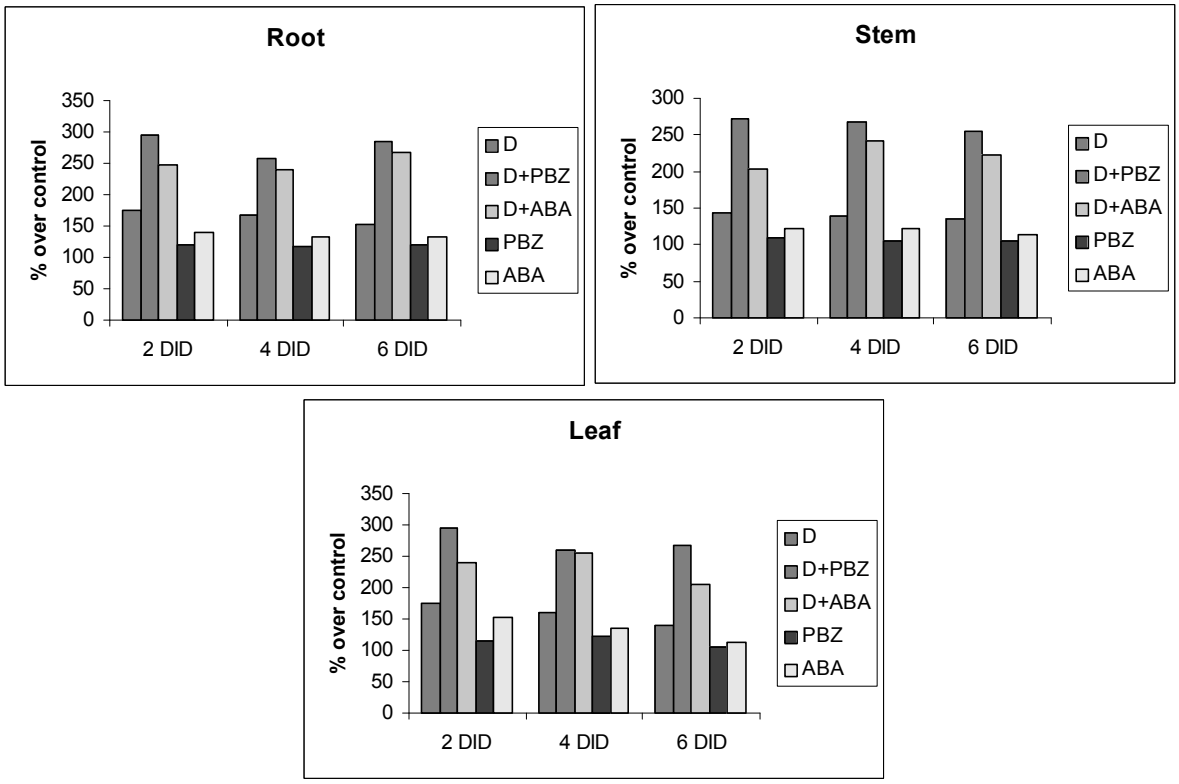

Fig. 2: Individual and combined effects of drought, PBZ, ABA on APX activity of *Sesamum indicum*. [D-Drought, PBZ- Paclobutrazol, ABA- Abscisic acid]

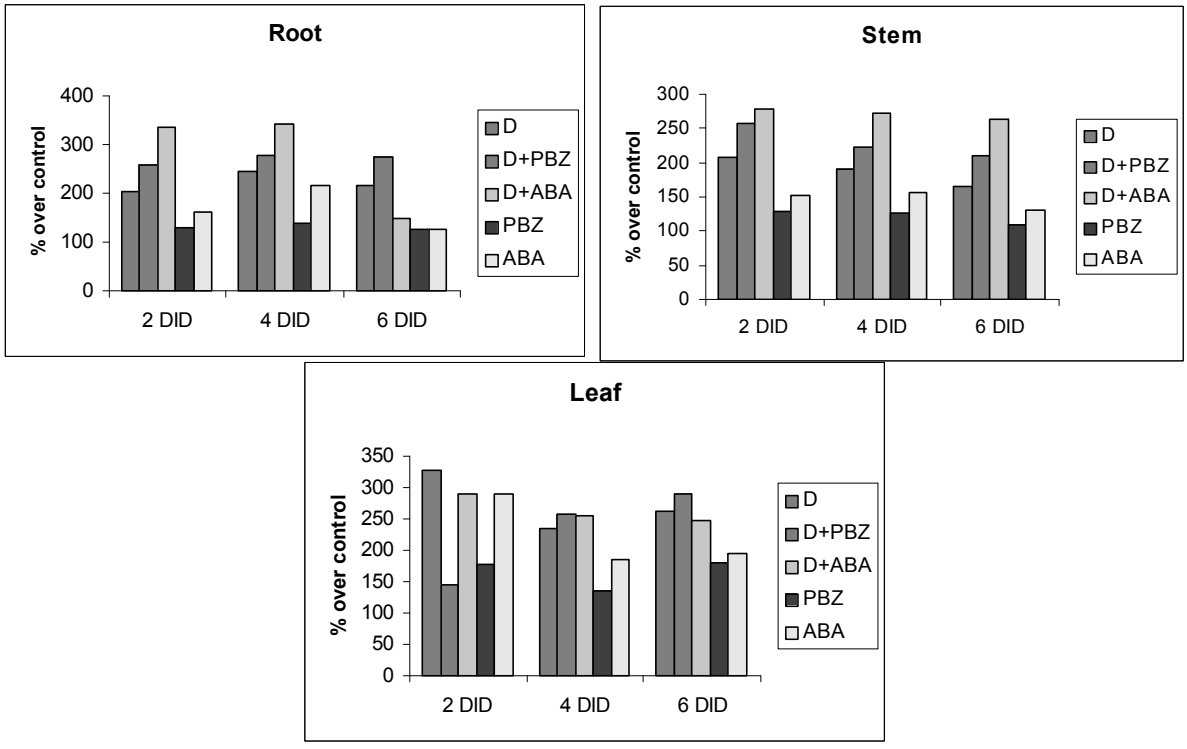

Fig. 3: Individual and combined effects of drought, PBZ, ABA on POX activity o f *Sesamum indicum*. [D-Drought, PBZ- Paclobutrazol, ABA- Abscisic acid]

Stem: APX activity increased in stem of drought stressed *Sesamum indicum* plants. The APX activity increased greatly in paclobutrazol treated stem, which was 106.79 and 284.57 percent over control in individual and drought stressed respectively. ABA caused an increased in APX activity and it was 223.82 percent over control in stem under drought stress on 6 DID.

Leaf: APX activity increased in the leaves of *Sesamum indicum* under drought condition. Paclobutrazol also resulted in increased APX activity in leaves of *Sesamum indicum*. Paclobutrazol in combination with drought caused again enhancement in APX activity when compared to stress and well-watered control plants. It was nearly 267.49 per cent over control on 6 DID. ABA treatment alone increased (113.18 per cent over control) the APX activity significantly in leaves when compared to all other treatments.

Peroxidase (Fig. 3)

Root: The peroxidase activity was showed an increase the root of *Sesamum indicum* under drought conditions. Treatment with paclobutrazol and ABA increased the peroxidase activity and it was 125.83 per cent and 166.72 per cent over control respectively on 37 DAP. Paclobutrazol and ABA in combination with drought increased the peroxidase activity and it was 275.27 per cent and 150 per cent over control respectively on 37 DAP.

Stem: Peroxidase activity increased in the stem of *Sesamum indicum* under drought condition. Paclobutrazol also resulted in increased peroxidase activity in leaves of *Sesamum indicum*. Paclobutrazol in combination with drought caused again enhancement in peroxidase activity when compared to stress and well-watered control plants. It was nearly 209.02 per cent over control on 6 DID. ABA treatment alone increased (131.14 per cent over control) the peroxidase activity significantly in stem when compared to all other treatments.

Leaf: The peroxidase activity was increased in leaves of *Sesamum indicum* under drought condition. The peroxidase activity increased greatly in paclobutrazol treated leaf, which was 179.91 and 359.08 percent over control in individual and drought stressed respectively.

DISCUSSION

The activity of SOD increased in the root by water deficit in *Sesamum indicum*. Treatment with paclobutrazol and ABA resulted in an enhancement of SOD activity under drought stress. The SOD activity increase under drought in *Catharanthus roseus* [6,9]. An increase in SOD activity was reported in *Helianthus annuus* plants under water deficit stress and triazole application [13]. Water stress increased the SOD activity in *Abelmoschus esculentus* plants [12]. SOD activity increased under drought stressed higher plants [5,6]. Antioxidant potentials increased in *Dioscorea rotundata* Poir. following paclobutrazol drenching [23].

APX activity increased in *Sesamum indicum* under drought condition. Paclobutrazol also resulted in increased APX activity in leaves of *Sesamum indicum*. Paclobutrazol in combination with drought caused again enhancement in APX activity when compared to stress and well-watered control plants. Exogenous application of triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal [32] and in *Catharanthus roseus* [33]. Drought stress induced generation of active oxygen species is well recognized at the cellular level and is tightly controlled at both the production and consumption levels through increased antioxidant systems [4-6]. Water stress and salt stress increased the APX activity in *Catharanthus* plants [8]. SOD activity increased under drought stressed *Arachis hypogaea* plants [5]. Paclobutrazol treatment [34] and propiconazole [35] increased the APX activity in *Catharanthus* plants under salt stress.

The peroxidase activity was showed an increase the root of *Sesamum indicum* under drought conditions. Treatment with paclobutrazol and ABA increased the peroxidase activity. Wheat plants under water deficit stress showed an enhancement in POX activity irrespective of different genotypes [16]. Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary metabolite accumulation [20]. Water deficit stress increased the POX activity in *Catharanthus roseus* [10].

REFERENCES

1. Ucan, K., F. Killi, C. Gencoglan and H. Merdun, 2007. Effect of irrigation frequency and amount on water use efficiency andyield of sesame (*Sesamum indicum* L.) under field conditions. Field Crops Res., 101: 249-258.
2. Orruno, E., M.R.A. 2007. Morgan Purification and characterisation of the 7S globulin storage protein from sesame (*Sesamum indicum* L.). Food Chem., 100: 926-934.

3. Hong-Bo Shao, Li-Ye Chu, C. Abdul Jaleel, P. Manivannan, R. Panneerselvam and M.A. Shao, 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Critical Reviews in Biotechnology, 29(2): 131-151.
4. Abdul Jaleel, C., R. Gopi, P. Manivannan, M. Gomathinayagam, Ksouri Riadh, Jallali Inès, Zhao Chang-Xing, Shao Hong-Bo and R. Panneerselvam, 2009. Antioxidant defense responses: Physiological plasticity in higher plants under abiotic constraints. Acta Physiologiae Plantarum, 31(3): 427-436.
5. Sankar, B., C. Abdul Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram and R. Panneerselvam, 2007. Effect of paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in *Arachis hypogaea* L. Colloids and Surfaces B: Biointerfaces, 60: 229-235.
6. Abdul Jaleel, C., R. Gopi, P. Manivannan, M. Gomathinayagam, Shao Hong-Bo, Chang-Xing Zhao and R. Panneerselvam, 2008. Endogenous hormonal and enzymatic responses of *Catharanthus roseus* with triadimefon application under water deficits. Comptes Rendus Biologies, 331: 844-852.
7. Abdul Jaleel, C., P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram and R. Panneerselvam, 2007. *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. Colloids and Surfaces B: Biointerfaces, 60: 7-11.
8. Abdul Jaleel, C., P. Manivannan, G.M.A. Lakshmanan, M. Gomathinayagam and R. Panneerselvam, 2008. Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. Colloids and Surfaces B: Biointerfaces, 61(2): 298-303.
9. Abdul Jaleel, C., B. Sankar, P.V. Murali, M. Gomathinayagam, G.M.A. Lakshmanan and R. Panneerselvam, 2008. Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation, Colloids and Surfaces B: Biointerfaces, 62(1): 105-111.
10. Abdul Jaleel, C., P. Manivannan, P.V. Murali, M. Gomathinayagam and R. Panneerselvam, 2008. Antioxidant potential and indole alkaloid profile variations with water deficits along different parts of two varieties of *Catharanthus roseus*. Colloids and Surfaces B: Biointerfaces, 62: 312-318.
11. Abdul Jaleel, C., R. Gopi, B. Sankar, M. Gomathinayagam and R. Panneerselvam, 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. Comptes Rendus Biologies, 331(1): 42-47.
12. Sankar, B., C. Abdul Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram and R. Panneerselvam, 2008. Relative efficacy of water use in five varieties of *Abelmoschus esculentus* (L.) Moench. under water-limited conditions. Colloids and Surfaces B: Biointerfaces, 62(1): 125-129.
13. Manivannan, P., C. Abdul Jaleel, R. Somasundaram and R. Panneerselvam, 2008. Osmoregulation and antioxidant metabolism in drought stressed *Helianthus annuus* under triadimefon drenching. Comptes Rendus Biologies, 331(6): 418-425.
14. Hong-Bo Shao, Li-Ye Chu, C. Abdul Jaleel and Chang-Xing Zhao, 2008. Water-deficit stress-induced anatomical changes in higher plants. Comptes Rendus Biologies, 331: 215-225.
15. Zhao Chang-Xing, Guo Ling-Yu, C. Abdul Jaleel, Shao Hong-Bo and Yang Hong-Bing, 2008. Prospects for dissecting plant-adaptive molecular mechanisms to improve wheat cultivars in drought environments. Comptes Rendus Biologies, 331: 579-586.
16. Zhao Chang-Xing, MA Dong-Hui, WANG Yue-Fu, LIN Qi, WU Gang, SHAO Hong-Bo and C. Abdul Jaleel, 2008. Effects of nitrogen fertilizer rate and post-anthesis soil water content on yield and quality of high quality strong gluten wheat. Acta Ecologica Sinica, 28(9): 4396-4404.
17. Abdul Jaleel, C., Ragupathi Gopi and Rajaram Panneerselvam, 2007. Alterations in lipid peroxidation, electrolyte leakage and proline metabolism in *Catharanthus roseus* under treatment with triadimefon, a systemic fungicide, Comptes Rendus Biologies, 330(12): 905-912.
18. Abdul Jaleel, C., R. Gopi and R. Panneerselvam, 2008. Growth and photosynthetic pigments responses of two varieties of *Catharanthus roseus* to triadimefon treatment. Comptes Rendus Biologies, 331: 272-277.
19. Gopi, R., B.M. Sujatha, S.N. Rajan, L. Karikalan and R. Panneerselvam, 1999. Effect of triadimefon in the sodium chloride stressed cowpea (*Vigna unguiculata*) seedlings. Indian J. Agri. Sci., 69(10): 743-745.

20. Abdul Jaleel, C., P. Manivannan, B. Sankar, A. Kishorekumar, Ragupathi Gopi, Rajaram Somasundaram and R. Panneerselvam, 2007. Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary metabolite accumulation. Colloids and Surfaces B: Biointerfaces, 60(2): 201-206.
21. Abdul Jaleel, C., R. Gopi, P. Manivannan and R. Panneerselvam, 2007. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. Acta Physiologiae Plantarum, 29: 205-209.
22. Abdul Jaleel, C., P. Manivannan, B. Sankar, A. Kishorekumar, S. Sankari and R. Panneerselvam, 2007. Paclobutrazol enhances photosynthesis and ajmalicine production in *Catharanthus roseus*. Process Biochemistry, 42: 1566-1570.
23. Abdul Jaleel, C., P. Manivannan, M. Gomathinayagam, R. Sridharan and R. Panneerselvam, 2007. Responses of antioxidant potentials in *Dioscorea rotundata* Poir. following paclobutrazol drenching. Comptes Rendus Biologies, 330: 798-805.
24. Abdul Jaleel, C., A. Kishorekumar, P. Manivannan, B. Sankar, M. Gomathinayagam, R. Gopi, R. Somasundaram and R. Panneerselvam, 2007. Alterations in carbohydrate metabolism and enhancement in tuber production in white yam (*Dioscorea rotundata* Poir.) under triadimefon and hexaconazole applications. Plant Growth Regulation, 53: 7-16.
25. Desikan, R., M.K. Cheung, J. Bright, D. Henson, J.T. Hancock and S.J. Neill, 2004. ABA, hydrogen peroxide and nitric oxide signalling instomatal guard cells. J. Exp. Bot., 55: 205-212.
26. Hwang, S.Y., H.W. Lin, R.H. Chern, H.F. LO and L. Li, 1999. Reduced susceptibility to waterlogging together with high – light stress is related to increases in superoxide dismutase and catalase activity in sweet potato. Plant Growth Regul., 27: 167-172.
27. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anals of Biochemistry, 72: 248-253.
28. Beauchamp, C.O. and I. Fridovich. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-287.
29. Cherry, J.H., 1963. Nucleic acid in mitochandria and enzyme changes in cotyledon of peanut seeds during germination. Plant Physiol., 38: 440-446.
30. Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxide in spinach chloroplasts. Plant Cell Physiol., 22: 867-880.
31. Kumar, K.B. and P.A. Khan, 1982. Peroxidase and polyphenol oxidase in excised ragi (*Elusine corocana* cv. PR202) leaves during senescence. Indian J. Exp. Biol., 20: 412-416.
32. Abdul Jaleel, C., R. Gopi, P. Manivannan and R. Panneerselvam, 2008. Exogenous application of triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal. Pesticide Biochemistry and Physiology, 91(3): 170-174.
33. Abdul Jaleel, C., R. Gopi, G.M. Alagulakshmanan and R. Panneerselvam, 2006. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Science, 171: 271-276.
34. Abdul Jaleel, C., R. Gopi, A. Kishorekumar, P. Manivannan, B. Sankar and R. Panneerselvam, 2008. Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiologiae Plantarum, 30: 287-292.
35. Abdul Jaleel, C., R. Gopi, P. Manivannan, M. Gomathinayagam, P.V. Murali and Rajaram Panneerselvam, 2008. Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status. Pesticide Biochemistry and Physiology, 90(2): 135-139.