



Physiology / Physiologie

Osmoregulation and antioxidant metabolism in drought-stressed *Helianthus annuus* under triadimefon drenching

Paramasivam Manivannan, Cheruth Abdul Jaleel *, Ramamurthy Somasundaram,
Rajaram Panneerselvam

Stress Physiology Lab, Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India

Received 5 December 2007; accepted after revision 20 March 2008

Available online 21 April 2008

Presented by Philippe Morat

Abstract

A pot-culture experiment was conducted to estimate the ameliorating effect of triadimefon (TDM) on drought stress in sunflower (*Helianthus annuus* L.) plants. The plants were subjected to 3-, 6-, and 9-day-interval drought (DID) stress and drought stress with TDM @ 15 mg l⁻¹ and 15 mg l⁻¹ TDM alone from the 30th day after sowing (DAS). One-day-interval irrigation was kept as control. The plant samples were collected on and separated into root, stem and leaf for estimating the amino acid (AA), proline (PRO) and glycine betaine (GB) contents and the activities of antioxidant enzymes. Individual and combined drought stress and TDM treatments increased AA, PRO and GB contents, superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and polyphenol oxidase (PPO) activities when compared to control. From the results of this investigation, it can be concluded that the application of TDM caused a partial amelioration of the adverse effects of drought stress by its influence on quaternary ammonium compounds and antioxidant potentials in *H. annuus* plants. **To cite this article: P. Manivannan et al., C. R. Biologies 331 (2008).**

© 2008 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Antioxidants; Drought; Triadimefon; *Helianthus annuus*

1. Introduction

Plants are subjected to several environmental stresses that adversely affect growth, metabolism and yield. Water is one of the most important environmental factors

that regulate plant growth and development. Drought is the major limiting factor in many parts of the world, which seriously affects plant growth and yield [1]. The lack of adequate moisture leading to water stress is common occurrence in rain-fed areas, brought about by infrequent rains and poor irrigation [2]. 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 [3]. Plants experience drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high [4].

Abbreviations: AA, amino acid; APX, ascorbate peroxidase; CAT, catalase; DAS, days after sowing; DID, days interval drought; GB, glycine betaine; PRO, proline; PPO, polyphenol oxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TDM, triadimefon.

* Corresponding author.

E-mail addresses: maniphd78@rediffmail.com (P. Manivannan), abdul79jaleel@rediffmail.com (C.A. Jaleel).

Among the diverse consequences of the drought effect on plant development, restricted nutrient and water acquisition are commonly recognized [5]. Increasing evidences suggest that drought induces oxidative stress in various plants, in which reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$), hydroxy radical ($\cdot OH$), hydrogen peroxide (H_2O_2) and alkoxy radical ($RO\cdot$) are produced [6]. The toxic superoxide radical has a half-life of less than 1 s and is usually rapidly dismutated by superoxide dismutase (SOD) to H_2O_2 , a product that is relatively stable and can be detoxified by catalase and peroxidases. These metalloenzymes constitute an important primary defence of cells against superoxide free radicals generated under stress conditions; thereby, increased SOD activity is known to confer oxidative stress tolerance [7].

Triazole compounds such as TDM, hexaconazole, uniconazole and paclobutrazol, etc., are widely used as fungicides and they also possess varying degrees of plant-growth regulating properties, mediated by their interference with the isoprenoid pathway and subsequent shift in the balance of important plant hormones, including GA, ABA and cytokinins [8]. They inhibit gibberellin and ergosterol biosynthesis in plants and fungi, respectively [9]. Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels [10]. Triazole-treated plants have a more efficient free-radical scavenging system, which enables them to detoxify active oxygen [11]. Some of the previous works carried out in our lab revealed the morphological and physiological changes associated with triazole treatment in various plants, including inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root-to-shoot ratio, increased antioxidant potentials, and enhancement of alkaloid production [12–16].

Comparatively, little work has been devoted to water stress problems and methods to overcome drought stress injuries in this plant. The drought stress amelioration by triazole compounds is of major research interest, because these compounds have innate potentiality for increasing antioxidant enzymes and molecules in oxidative-stressed plants [1,11]. So, in the present investigation, an attempt has been made to evaluate the drought stress ameliorating ability of TDM [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone] [$C_{14}H_{16}ClN_3O_2$], $M_w = 293.75$, a triazole fungicide, with special emphasis to osmoregulators, like AA, PRO and GB, and to antioxidant enzymes,

like APX, PPO, CAT and SOD, in drought-stressed *Helianthus annuus* L. plants in pot culture.

2. Materials and methods

2.1. Plant material and drought-stress induction

The triazole compound named triadimefon (TDM) was obtained as 25% WP Bayleton-registered trademark, from Bayer, India Ltd., Mumbai. *Helianthus annuus* L. seeds are surface sterilized with a 0.2% $HgCl_2$ solution for 5 min, with frequent shaking, and thoroughly washed many times with deionized water to remove $HgCl_2$. Plastic pots of 40-cm diameter and 45-cm height size were used for the study. The pots were filled with 10 kg of soil mixture containing red soil; sand and farm yard manure at 1:1:1 ratio and 240 pots were arranged in completely randomized block design (CRBD). One set of pots was kept as control and three other sets of pots were used for drought-stress and TDM treatments. Seeds were sown and the seedlings were thinned to 1 per pot on the 10th day after sowing (DAS). The plants were allowed to grow up to 30 DAS. From the 30th to the 50th day, all the potted plants were grown under a polyhouse. Control irrigation was made on alternative days, mild-stress irrigation once in three day from the 30th to the 50th day, medium-stress irrigation once in six days from the 30th to the 50th day, severe-stress irrigation once in nine days from the 30th to the 50th day, with 3-, 6-, and 9-day-interval drought (DID) with 15 mg l^{-1} TDM treatment and 15 mg l^{-1} TDM alone on the 30th DAS. After the drought period, all the pots were irrigated at 1-day intervals until harvest. Plants were uprooted randomly on the 51st DAS, washed carefully and separated into root, stem and leaves for estimating antioxidant contents and antioxidant enzyme activities.

2.2. Osmolyte concentration

2.2.1. Amino acid (AA) content

Extraction and estimation of the AA content was followed by the method of Moore and Stein [17]. 0.5 g of plant material was taken in a pestle and mortar and homogenized with 10 ml of 80% boiling ethanol. The extract was centrifuged at 800 g for 15 min and the supernatant was made up to 10 ml with 80% ethanol and used for the estimation of free AAs. 1 ml of ethanol extract was taken in a 25-ml test tube and neutralized with 0.1 N sodium hydroxide using methyl red indicator, to which 1 ml ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 min, then 5 ml of diluted reagent was added, cooled and diluted

to 25 ml with distilled water. The absorbance was read at 570 nm in a spectrophotometer. The standard graph was prepared by using glycine. The AA content was calculated using the standard graph. The results were expressed in milligrams per gram of dry weight.

2.2.2. Proline (PRO) concentration

The PRO content was estimated by the method of Bates et al. [18]. The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. Supernatant was used for the estimation of the PRO content. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100 °C for 1 h. After termination of the reaction in an ice bath, the reaction mixture was extracted with 4 ml of toluene; absorbance was read at 520 nm.

2.2.3. Glycine betaine (GB) concentration

The amount of GB was estimated according to the method of Grieve and Grattan [19]. The plant tissue was finely ground, mechanically shaken with 20 ml of deionised water for 24 h at 25 °C. The samples were then filtered and filtrates were diluted to 1:1 with 2 N H₂SO₄. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI–I₂ reagent was added, and the reactants were gently stirred with a vortex mixture. The tubes were stored at 4 °C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0 °C. The supernatant was carefully aspirated with a fine glass tube. The periodide crystals were dissolved in 9 ml of 1,2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as a standard and expressed in mg g⁻¹ DW.

2.3. Enzyme extractions and assays

2.3.1. Ascorbate peroxidase (APX) (EC 1.11.1.1)

Ascorbate peroxidase (APX) (EC 1.11.1.1) activity was determined according to Asada and Takahashi [20]. The reaction mixture (1 ml) contained 50 mM of potassium phosphate buffer (pH 7.0), 0.5 mM of ascorbic acid, 0.1 mM of H₂O₂, and 200 µl of enzyme extract. The absorbance was read as the decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H₂O₂ (extinction coefficient: 2.9 mM⁻¹ cm⁻¹). The enzyme activity was expressed in U mg⁻¹ protein (U = change in 0.1 absorbance min⁻¹ of protein).

2.3.2. Polyphenol oxidase (PPO) (EC 1.10.3.1)

Polyphenol oxidase (PPO) activity was assayed by the method of Kumar and Khan [21]. The assay mixture for PPO contained 2 ml of 0.1 M phosphate buffer (pH 6.0), 1 ml of 0.1 M catechol and 0.5 ml of enzyme extract. This was incubated for 5 min at 25 °C, after which the reaction was stopped by adding 1 ml of 2.5 N H₂SO₄. The absorbance of the purpurogallin formed was read at 495 nm. 2.5 N H₂SO₄ was added to the blank at the zero time of the same assay mixture. PPO activity is expressed in U mg⁻¹ protein (U = change in 0.1 absorbance min⁻¹ mg⁻¹ of protein).

2.3.3. Catalase (CAT) (EC 1.11.1.6)

Catalase (CAT) was measured according to Chandless and Scandalios [22], with modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H₂O₂ and 0.04 ml of enzyme extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U mg⁻¹ protein (U = 1 mM of H₂O₂ reduction min⁻¹ mg⁻¹ of protein).

2.3.4. Superoxide dismutase (SOD) assay

The crude enzyme extract was prepared for assay of SOD by the method of Hwang et al. [23]. The enzyme protein was determined according to Bradford [24] for all the three enzymes for expressing the specific activity of enzymes. SOD (EC 1.15.1.1) activity was assayed according to Beauchamp and Fridovich [25]. The reaction mixture contained 1.17 × 10⁻⁶ M of riboflavin, 0.1 M of methionine, 2 × 10⁻⁵ M of potassium cyanide (KCN) and 5.6 × 10⁻⁵ M of nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). Three millilitres of the reaction medium were added to 1 ml of 5-enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Phillips 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 was expressed in units (U mg⁻¹ protein). One U is defined as the amount of change in the absorbance per 0.1 h⁻¹ mg⁻¹ of protein.

2.4. Statistical analysis

The pot culture was carried out in completely randomized design (CRD). The data are expressed as mean ± SD for seven samples in each group.

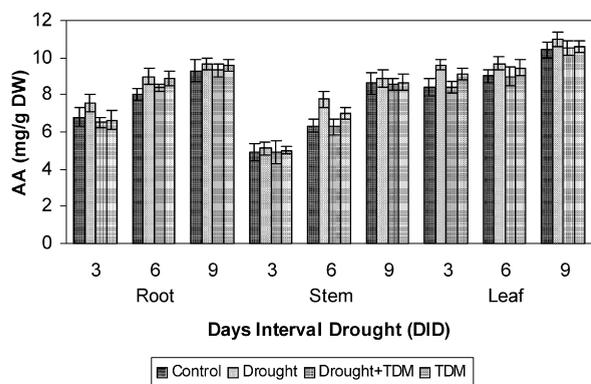


Fig. 1. Effects of drought, TDM and their combination on the amino acid (AA) content of *H. annuus* plants. Values are mean \pm SE of seven replicates.

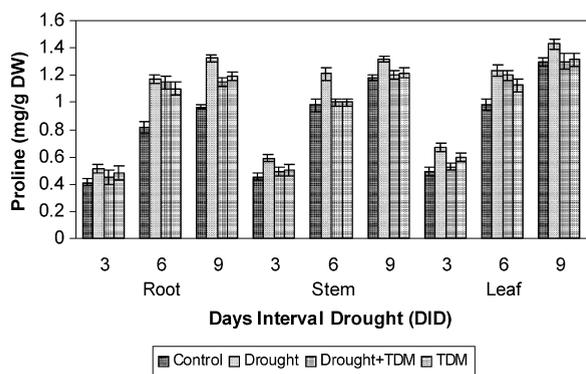


Fig. 2. Effects of drought, TDM and their combination on the proline content of *H. annuus* plants. Values are mean \pm SE of seven replicates.

3. Results and discussion

3.1. Effect of drought, TDM and their combination on osmolyte concentration

Drought stress increased the AA (Fig. 1), PRO (Fig. 2) and GB (Fig. 3) contents in all the parts of the sunflower when compared to control. Drought stress with TDM treatment leads to an enhancement in biochemical contents when compared to drought-stressed and control plants. AA content has been shown to increase under drought condition in *Vigna* [1]. Similar results were obtained in *Abelmoschus* [26] and *Vigna unguiculata* [3]. The accumulation of AA may be due to protein hydrolysis and also may occur in response to the change in the osmotic adjustment of their cellular contents [26]. Free AA accumulation is a more important account for most of the changes in osmotic potential. The accumulation of AA under stress at all the growth stages indicates the possibility of their involvement in osmotic adjustment [27]. Osmotic adjust-

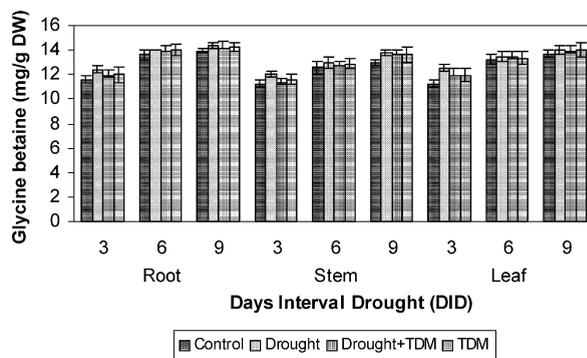


Fig. 3. Effects of drought, TDM and their combination on glycine betaine content of *H. annuus* plants. Values are mean \pm SE of seven replicates.

ment is one of the important mechanisms that alleviate some of the detrimental effects of water stress [26]. The extent of increase was higher in leaf, followed by root and stem. The GB content increased under salt stress in *Catharanthus* [28]. Aliphatic quaternary ammonium compounds (QAC) such as GB, stachydrine, homostachydrine, trigonelline have been found to accumulate in a large number of plants exposed to salt and water stress. Increased proline accumulation was reported in *Catharanthus* under different abiotic stress situations [29–31]. Increased proline in stressed plants may be an adaptation to overcome the stress conditions. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate the stress. Proline accumulation in plants might be a scavenger and acting as an osmolyte. The reduced proline oxidase may be the reason for increasing proline accumulation [32–34].

TDM increased the AA content in unstressed plants. Similar results were observed in *Catharanthus* [14,30,35]. Propiconazole and paclobutrazol induced a moderate increase in AA in higher plants [36–38]. Treatment with TDM significantly increased the free PRO content in *Catharanthus roseus* [11]. Triazole induced a transient raise in ABA content [8] and this raise in ABA induced by TDM might have caused the increase in AA and proline content in sunflower.

3.2. Effect of drought, TDM and their combination on APX activity

APX activity (Fig. 4) increases in all the drought treatments when compared to control. Drought stress with TDM treatment decreased APX activity in drought-stressed plants, and increased it in control plants. TDM treatment increased APX activity when compared to

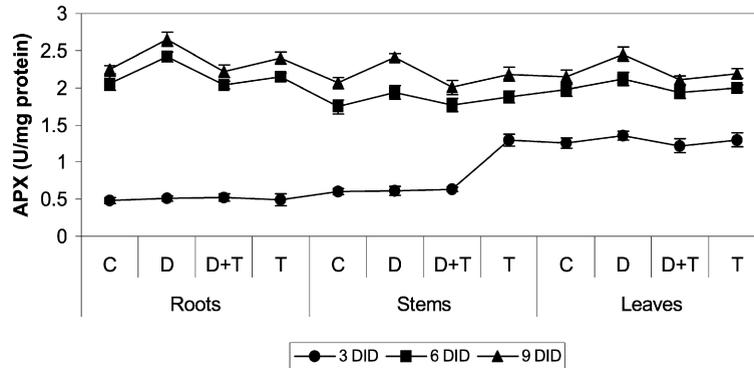


Fig. 4. Effects of drought, TDM and their combination on ascorbate peroxidase (APX) activity of *H. annuus* plants. Values are mean \pm SE of seven replicates.

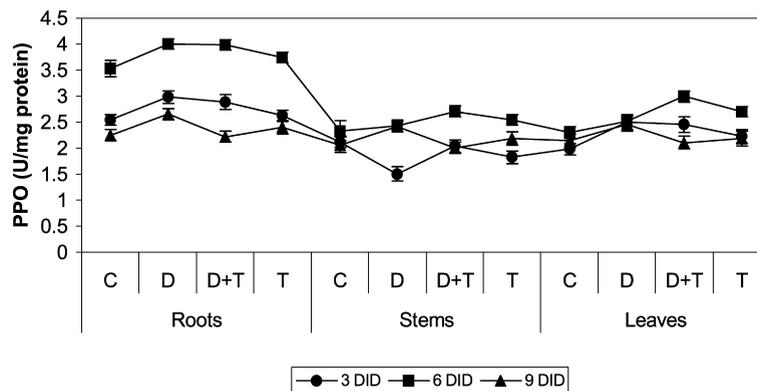


Fig. 5. Effects of drought, TDM and their combination on polyphenol oxidase (PPO) activity of *H. annuus* plants. Values are mean \pm SE of seven replicates.

the case of control and drought-stressed plants. Similar results were obtained by many workers in many higher plants under drought stress [1,2,6,11,39–41]. APX found in organelles is believed to scavenge H_2O_2 produced from the organelles, whereas the function of cytosolic APX is probably to eliminate H_2O_2 that is produced in the cytosol or apoplast and that has diffused from organelles. In the chloroplast, H_2O_2 can be detoxified by the ASA–GSH–NAPDH system, which has been catalyzed by APX [10,12–14].

3.3. Effect of drought, TDM and their combination on PPO activity

PPO activity increased in drought treatment when compared to control (Fig. 5). TDM treatment resulted in an enhancement of the PPO activity when compared to drought-stressed and control plants. TDM treatment in combination with drought increased PPO activity when compared to control plants. ROS scavenging is important in imparting tolerance against oxidative stress. It

may be presumed that enhancement of the antioxidative system favours water-stress resistance [42,43].

3.4. Effect of drought, TDM and their combination on CAT activity

CAT activity (Fig. 6) was increased in all parts of drought-stressed cowpea plants and of those undergoing TDM treatment when compared to control. TDM treatment decreased catalase activity when compared to drought stress and TDM treatment, and increased it in controls. This result is in accordance with the findings in *Catharanthus roseus* [10,43]. The combined action of CAT and SOD converts the toxic $O_2^{\cdot-}$, H_2O_2 into water and molecular oxygen, averting the cellular damage under unfavourable conditions like water stress [1].

3.5. Effect of drought, TDM and their combination on SOD activity

SOD activity increased in all the DID stress and with TDM treatments when compared to control (Fig. 7).

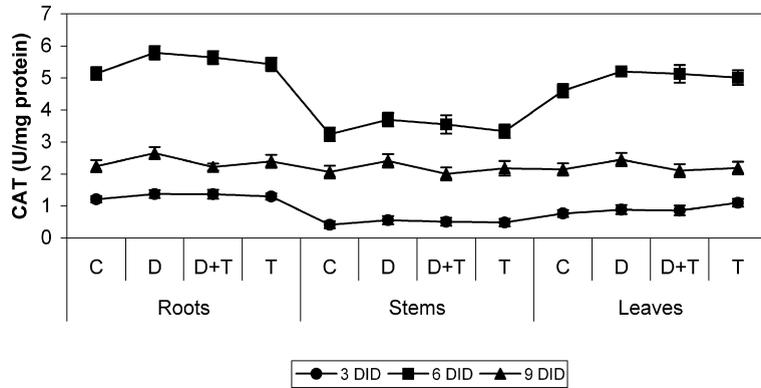


Fig. 6. Effects of drought, TDM and their combination on catalase (CAT) activity of *H. annuus* plants. Values are mean ± SE of seven replicates.

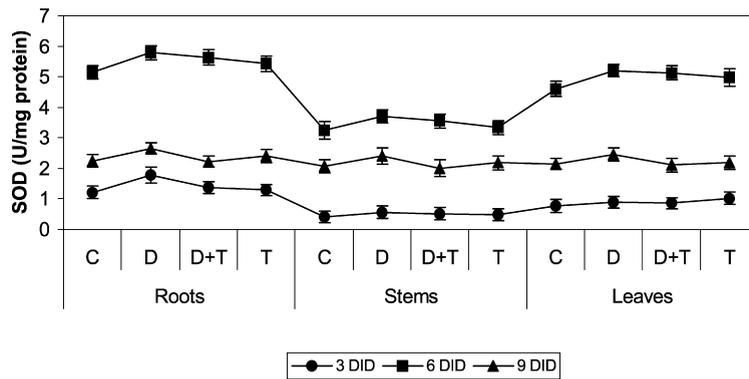


Fig. 7. Effects of drought, TDM and their combination on superoxide dismutase (SOD) activity of *H. annuus* plants. Values are mean ± SE of seven replicates.

TDM treatment decreased SOD activity when compared to drought-stress and TDM treatment, and increased it in the control. Triazoles increased the antioxidant potential in oxidative stressed plants under treatment when compared to control [12–14,44,45]. It was reported that SOD enhances water-stress tolerance in plants. The cytosolic Cu/Zn-SOD was induced strongly by stress, while Cu/Zn-SOD remained largely unaffected [46,47].

4. Conclusion

Thus, from these results, it is clear that plants are highly regulated by TDM, in terms of enhanced components of osmoprotectants under drought stress. Drought-stressed plants under TDM treatment maintain a balance between formation and detoxification of activated oxygen species, leading to partial improvement of their response to drought-induced oxidative stress.

References

[1] P. Manivannan, C.A. Jaleel, A. Kishorekumar, B. Sankar, R. Somasundaram, R. Sridharan, R. Panneerselvam, Changes in an-

tiioxidant metabolism of *Vigna unguiculata* (L.) Walp. by propiconazole under water deficit stress, *Colloids Surf. B: Biointerfaces* 57 (2007) 69–74.
 [2] P. Manivannan, C.A. Jaleel, B. Sankar, A. Kishorekumar, R. Somasundaram, G.M.A. Lakshmanan, R. Panneerselvam, Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress, *Colloids Surf. B: Biointerfaces* 59 (2007) 141–149.
 [3] P. Manivannan, C.A. Jaleel, A. Kishorekumar, B. Sankar, R. Somasundaram, R. Panneerselvam, Protection of *Vigna unguiculata* (L.) Walp. plants from salt stress by paclobutrazol, *Colloids Surf. B: Biointerfaces* 61 (2008) 315–318.
 [4] P. Manivannan, C.A. Jaleel, A. Kishorekumar, B. Sankar, R. Somasundaram, R. Sridharan, R. Panneerselvam, Drought stress induced changes in the biochemical parameters and photosynthetic pigments of cotton (*Gossypium hirsutum* L.), *Indian J. Appl. Pure Biol.* 22 (2007) 369–372.
 [5] P. Manivannan, C.A. Jaleel, A. Kishorekumar, B. Sankar, R. Somasundaram, R. Sridharan, R. Panneerselvam, Changes in antioxidant metabolism under drought stress in *Vigna unguiculata* (L.) Walp., *Indian J. Plant Physiol.* 12 (2007) 133–137.
 [6] C.A. Jaleel, P. Manivannan, A. Kishorekumar, B. Sankar, R. Gopi, R. Somasundaram, R. Panneerselvam, Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit, *Colloids Surf. B: Biointerfaces* 59 (2007) 150–157.

- [7] C.A. Jaleel, R. Gopi, P. Manivannan, B. Sankar, A. Kishorekumar, R. Panneerselvam, Antioxidant potentials and ajmalicine accumulation in *Catharanthus roseus* after treatment with gibberellic acid, *Colloids Surf. B: Biointerfaces* 60 (2007) 195–200.
- [8] C.A. Jaleel, A. Kishorekumar, P. Manivannan, B. Sankar, M. Gomathinayagam, R. Gopi, R. Somasundaram, R. Panneerselvam, Alterations in carbohydrate metabolism and enhancement in tuber production in white yam (*Dioscorea rotundata* Poir.) under triadimefon and hexaconazole applications, *Plant Growth Regul.* 53 (2007) 7–16.
- [9] C.A. Jaleel, R. Gopi, P. Manivannan, A. Kishorekumar, M. Gomathinayagam, R. Panneerselvam, Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage, *J. Zhejiang Univ. Sci. B* 8 (2007) 283–288.
- [10] C.A. Jaleel, R. Gopi, G.M. Alagu Lakshmanan, R. Panneerselvam, Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don., *Plant Sci.* 171 (2006) 271–276.
- [11] C.A. Jaleel, R. Gopi, R. Panneerselvam, Alterations in lipid peroxidation, electrolyte leakage, and proline metabolism in *Catharanthus roseus* under treatment with triadimefon, a systemic fungicide, *C. R. Biologies* 330 (2007) 905–912.
- [12] C.A. Jaleel, R. Gopi, P. Manivannan, R. Panneerselvam, Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity, *Acta Physiol. Plant.* 29 (2007) 205–209.
- [13] C.A. Jaleel, R. Gopi, A. Kishorekumar, P. Manivannan, B. Sankar, R. Panneerselvam, Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*, *Acta Physiol. Plant.* (2007), doi:10.1007/s11738-007-0119-1.
- [14] C.A. Jaleel, G.M.A. Lakshmanan, M. Gomathinayagam, R. Panneerselvam, Triadimefon-induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system, *S. Afr. J. Bot.* 74 (2008) 126–132.
- [15] C.A. Jaleel, Molecular profiling of *Catharanthus roseus* (L.) G. Don. ecotypes for its phytoactive components, thesis, Annamalai University, Tamilnadu, India, 2005.
- [16] C.A. Jaleel, R. Gopi, P. Manivannan, A. Kishorekumar, B. Sankar, R. Panneerselvam, Paclobutrazol influences on vegetative growth and floral characteristics of *Catharanthus roseus* (L.) G. Don., *Indian J. Appl. Pure Biol.* 21 (2006) 369–372.
- [17] S. Moore, W.H. Stein, Photometric method for use in the chromatography of amino acids, *J. Biol. Chem.* 175 (1948) 367–388.
- [18] L.S. Bates, R.P. Waldren, I.D. Teare, Rapid determination of free proline for water stress studies, *Plant Soil* 29 (1973) 205–207.
- [19] C.M. Grieve, S.R. Grattan, Rapid assay for determination of water soluble quaternary 10 ammonium compounds, *Plant Soil* 70 (1983) 303–307.
- [20] K. Asada, M. Takahashi, Production and scavenging of active oxygen in photosynthesis, in: D.J. Kyle, B. Osmond, C.J. Arntzen (Eds.), *Photoinhibition*, Elsevier, Amsterdam, 1987, pp. 227–287.
- [21] K.B. Kumar, P.A. Khan, Peroxidase and polyphenol oxidase in excised ragi (*Elusine corocana* cv. PR202) leaves during senescence, *Indian J. Exp. Biol.* 20 (1982) 412–416.
- [22] J.M. Chandlee, J.G. Scandalios, Analysis of variants affecting the catalase development program in maize scutellum, *Theor. Appl. Gen.* 69 (1984) 71–77.
- [23] S.Y. Hwang, H.W. Lin, R.H. Chern, H.F. Lo, L. Li, Reduced susceptibility to water logging together with high light stress is related to increases in superoxide dismutase and catalase activity in sweet potato, *Plant Growth Regul.* 27 (1999) 167–172.
- [24] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding, *Ann. Biochem.* 72 (1976) 248–253.
- [25] C.O. Beauchamp, I. Fridovich, Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels, *Ann. Biochem.* 44 (1971) 276–287.
- [26] B. Sankar, C.A. Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram, R. Panneerselvam, Effect of paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in *Arachis hypogaea* L., *Colloids Surf. B: Biointerfaces* 60 (2007) 229–235.
- [27] B. Sankar, C.A. Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram, R. Panneerselvam, Relative efficacy of water use in five varieties of *Abelmoschus esculentus* (L.) Moench. under water-limited conditions, *Colloids Surf. B: Biointerfaces* 62 (2008) 125–129.
- [28] C.A. Jaleel, P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram, R. Panneerselvam, Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation, *Colloids Surf. B: Biointerfaces* 60 (2007) 110–116.
- [29] C.A. Jaleel, P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram, R. Panneerselvam, Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary metabolite accumulation, *Colloids Surf. B: Biointerfaces* 60 (2007) 201–206.
- [30] C.A. Jaleel, R. Gopi, P. Manivannan, R. Panneerselvam, Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress, *Turk. J. Bot.* 31 (2007) 245–251.
- [31] C.A. Jaleel, P. Manivannan, A. Kishorekumar, B. Sankar, R. Panneerselvam, Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*, *C. R. Biologies* 330 (2007) 674–683.
- [32] C.A. Jaleel, P. Manivannan, G.M.A. Lakshmanan, R. Sridharan, R. Panneerselvam NaCl as a physiological modulator of proline metabolism and antioxidant potential in *Phyllanthus amarus*, *C. R. Biologies* 330 (2007) 806–813.
- [33] C.A. Jaleel, P. Manivannan, M. Gomathinayagam, R. Sridharan, R. Panneerselvam, Responses of antioxidant potentials in *Dioscorea rotundata* Poir. following paclobutrazol drenching, *C. R. Biologies* 330 (2007) 798–805.
- [34] C.A. Jaleel, R. Gopi, R. Panneerselvam, Growth and photosynthetic pigments responses of two varieties of *Catharanthus roseus* to triadimefon treatment, *C. R. Biologies* 331 (2008) 272–277.
- [35] C.A. Jaleel, R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan, R. Panneerselvam, Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress, *S. Afr. J. Bot.* 73 (2007) 190–195.
- [36] C.A. Jaleel, R. Gopi, P. Manivannan, M. Gomathinayagam, P.V. Murali, R. Panneerselvam, Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status, *Pestic. Biochem. Physiol.* 90 (2008) 135–139.
- [37] C.A. Jaleel, P. Manivannan, B. Sankar, A. Kishorekumar, S. Sankari, R. Panneerselvam, Paclobutrazol enhances photosynthesis and ajmalicine production in *Catharanthus roseus*, *Process Biochem.* 42 (2007) 1566–1570.

- [38] C.A. Jaleel, R. Gopi, P. Manivannan, R. Panneerselvam, Soil salinity alters the morphology in *Catharanthus roseus* and its effects on endogenous mineral constituents, *EurAsian J. BioSci.* 2 (2008) 18–25.
- [39] C.A. Jaleel, B. Sankar, R. Sridharan, R. Panneerselvam, Soil salinity alters growth, chlorophyll contents and secondary metabolite accumulation in *Catharanthus roseus*, *Turk. J. Biol.* 32 (2008) 79–83.
- [40] C.A. Jaleel, R. Gopi, B. Sankar, M. Gomathinayagam, R. Panneerselvam, Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress, *C. R. Biologies* 331 (2008) 42–47.
- [41] C.A. Jaleel, P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram, R. Panneerselvam, *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress, *Colloids Surf. B: Biointerfaces* 60 (2007) 7–11.
- [42] C.A. Jaleel, P. Manivannan, G.M.A. Lakshmanan, M. Gomathinayagam, R. Panneerselvam, Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits, *Colloids Surf. B: Biointerfaces* 61 (2008) 298–303.
- [43] C.A. Jaleel, R. Gopi, P. Manivannan, R. Sridharan, M. Gomathinayagam, R. Panneerselvam, Antioxidant potential and indole alkaloid profile variations with water deficits along different parts of two varieties of *Catharanthus roseus*, *Colloids Surf. B: Biointerfaces* 62 (2008) 312–318.
- [44] B. Sankar, C.A. Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram, R. Panneerselvam, Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench, *Acta Bot. Croat.* 66 (2007) 43–56.
- [45] C.A. Jaleel, B. Sankar, P.V. Murali, M. Gomathinayagam, G.M.A. Lakshmanan, R. Panneerselvam, Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation, *Colloids Surf. B: Biointerfaces* 62 (2008) 105–111.
- [46] C.A. Jaleel, R. Panneerselvam, Variations in the antioxidative and indole alkaloid status in different parts of two varieties of *Catharanthus roseus*, an important folk herb, *Chin. J. Pharmacol. Toxicol.* 21 (2007) 487–494.
- [47] C.A. Jaleel, P. Manivannan, B. Sankar, A. Kishorekumar, R. Panneerselvam, Salt stress mitigation by calcium chloride in *Phyllanthus amarus*, *Acta Bot. Croat.* 67 (2008) 53–62.