

Physiology / Physiologie

Endogenous hormonal and enzymatic responses of *Catharanthus roseus* with triadimefon application under water deficits

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Abstract

The effect of triadimefon was investigated in a medicinal plant, *Catharanthus roseus* subjected to water deficit stress. The abscisic acid (ABA) level, DNA and RNA contents and activities of ATPase and protease were found varying in different parts of the plants under treatment. Drought treatment increased the ABA level more than twofold in all parts of the plants. TDM treatment to the drought stressed plants showed highest contents. In roots, stem and leaves, drought stress caused a decrease in the DNA and RNA contents when compared with control and other treatments. TDM treatment with drought increased the nucleic acid contents to the level of the control roots. The activity of ATPase and protease were increased under drought treatment and lowered due to TDM applications. This information could be useful in the field of soil water deficits reclamation efforts by using plant growth regulators. **To cite this article:** C.A. Jaleel et al., *C. R. Biologies 331 (2008)*.

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1. Introduction

Abiotic stress conditions cause extensive losses to agricultural production worldwide and these have been the subjects of intense research [1]. Water deficits are

one of the major abiotic stresses, which adversely affect the crop growth and yield. Coping with water deficit stress is a global issue to ensure survival of agricultural crops and sustainable food production. Conventional plant breeding attempts have changed over to use physiological selection criteria since they are time consuming and rely on present genetic variability [2]. Currently, protection of plant through application of plant growth regulators (PGR) attracts more attention. PGR play an

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important role in crop production and are being increasingly used to manipulate plant growth and yield [3].

Triazoles form the active ingredient of fungicides (propiconazole, penconazole, epoxiconazole) and growth regulators (paclobutrazol, triadimefon) commonly used within the horticultural, agricultural and forestry industries. Triazole compounds are new synthetic plant growth regulators that act as antigibberellins and known to inhibit shoot growth in plants [4]. Triazoles have been called plant multiprotectants because of their ability to induce tolerance to environmental and chemical stresses in plants [5]. Protection of plants from apparently unrelated stress by triazole is mediated by a reduction in free radical damage and increase in antioxidant potential [6,7]. Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels [8]. Triazole treated plants have a more efficient free-radical scavenging system that enables them to detoxify active oxygen [9]. We have reported previously some of the morphological and physiological changes associated with triazole treatment in various plants, include the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, increased antioxidant potentials and an enhancement in alkaloid production [3,10–13].

For the past several years, several scales of physiology have been applied to study responses to water deficit stress tolerance mechanisms and methods to overcome drought and salt stresses [14–16]. However, little information is gained about the physiological basis in terms of ABA accumulation and nucleic acid contents under drought stress and amelioration of drought by the application of PGR in medicinal plants. It seems necessary to do research related to the correlation between medicinal plants and drought stress for the increasing need of medicinal plants. So it seems valuable, to test the important medicinal plants for their drought tolerance capacity. *Catharanthus roseus* (L.) G. Don. (Family: Apocynaceae) is one of the highly exploited and studied medicinal plants. This plant contains alkaloids, which are valuable source of antitumour agents like vinblastine and vincristine used in chemotherapy of leukemia and in the treatment of Hodgkin's disease, and also a popular ornamental plant [13,17]. *C. roseus* is classed as a glycophyte. Despite the relative great number of reports on the medicinal aspects of *C. roseus* plants [11,17–25] there are only a few attempts to explain the physiological basis of stress effects [25–29]. To the best of our knowledge, no information on the

physiological response in terms of growth hormones and nucleic acid contents of *C. roseus* to drought and TDM application is available. The purpose of this study was to provide additional information in the variations in the hormone levels, nucleic acid content and ATPase and protease activities of *C. roseus* under individual and combined drought and TDM treatments.

2. Materials and methods

2.1. Plant cultivation, drought stress induction and TDM treatment

Seeds of *Catharanthus roseus* (L.) G. Don. were collected from Department of Horticulture, Annamalai University, Tamil Nadu, India. The experiments were conducted at the Botanical Garden and Stress Physiology Laboratory, Department of Botany, Annamalai University.

The seeds were surface sterilized with 0.2 percent HgCl₂ solution for 5 min with frequent shaking and then thoroughly washed with deionised water. Seeds were sown in plastic pots (300 mm diameter) filled with 3 kg of soil mixture containing red soil, sand and farmyard manure (FYM) at 1:1:1 ratio. Four seeds were sown per pot and the pots were watered to the field capacity with deionized water up to 20 days after sowing (DAS). After 30 days, the plants were grouped and withhold water supply for 5, 10, 15 and 20 days (days interval drought (DID) [1] for three different growth periods. An equal number of plants, 15 mg l⁻¹ were given TDM to test the ameliorating effect of TDM on drought stress. Plants were uprooted randomly on 26, 31, 36 and 41 DAS and used for estimating the ABA level, DNA and RNA contents.

In the preliminary experiments TDM treatment 5, 10, 15, 20 and 25 mg l⁻¹ concentration were tried in combination with drought of which 15 mg l⁻¹ was found to increase the dry weight more or less the level of control and the higher concentration slightly decreased the dry weight. Hence, 15 mg l⁻¹ of TDM concentration was used to ameliorate the drought stress. The same concentration was used to treat the unstressed plants to determine the effect of TDM on the unstressed *C. roseus* plants.

2.2. Estimation of ABA content

ABA was extracted and purified following the method of Ciha et al. [30] with small modification. Fifty grams of fresh plant material was frozen in liquid nitrogen and

immediately extracted with 80% methanol and the extract was stored at 4 °C for 24 h, later the extract was filtered through two layers of cheese cloth and the filtrate was centrifuged at 1500 g for 20 min. The methanol was removed from the supernatant in vacuum at 35 °C. The supernatant was adjusted to pH 2.5 with 0.1 N HCl and partitioned with hexane (1:1) thrice and the aqueous phase was saved and the pH was adjusted to 9 using 0.1 N KOH and partitioned against methylene chloride (1:1) thrice and the aqueous phase was saved and this was again adjusted to pH 2.5 and partitioned with *n*-butanol thrice and the butanol fraction was saved and evaporated to dryness in a film flash evaporator and the dried fraction was eluted with 2 ml of methanol. The extract was purified using TLC technique and the corresponding region of authentic ABA was eluted with 2 ml of methanol and used for the quantitative analysis of abscisic acid through HPLC.

The methanolic extract was analysed with HPLC (Shimadzu model) using UV detector at 254 nm. The chromatography was done with methanol water (60:40) as solvent with the flow rate of 0–5 ml/min. In a C₁₈ column of 14 cm × 4.6 mm diameter, quantification was done considering specific area of the peak comparing the retention time of the standard abscisic acid sample. The ABA standard was obtained from Sigma, USA. The results were expressed in nano gram⁻¹ fresh weight.

2.3. Estimation of nucleic acid contents

Ribonucleic acid (RNA) and deoxyribo nucleic acid (DNA) were extracted and estimated according to the method of Nieman and Paulson [31]. Five hundred milligrams of fresh material was ground in a mortar and pestle with 5 ml of cold methanol at 4 °C and centrifuged at 800 g for 15 min. The supernatant was discarded and the residue was repeatedly washed with cold methanol to remove pigments and alcohol soluble phosphorus. To the residue, 5 ml of 5% ice cooled TCA was added and the contents were stirred thoroughly with a glass rod and centrifuged at 800 rpm for 15 min. The supernatant thus obtained was discarded. The residue was re-extracted with 5% cold TCA and centrifuged at 800 rpm and the supernatant was discarded to remove acid soluble phosphorus. The resulting residue was washed twice with 10 ml of 90% ethanol, ethanol ether mixture (2:1 v/v) and ether successively centrifuged at 800 rpm and the lipid phosphorus present in the supernatant was discarded for each extraction. The solvent was allowed to boil for 20 min. Then the residue was carefully dried, powdered and used for further extraction of RNA and DNA.

2.3.1. Estimation of RNA

The dried residue was suspended in 5 ml of 0.3 N NaOH and kept at 30 °C for 18 h. The sediment was removed by centrifuging at 5000 rpm and washed once with 5 ml of 0.3 N NaOH and then acidified to pH 1.0 with 15% perchloric acid (PCA) and held at 4 °C for 40 min and then centrifuged again. The supernatant contained RNA fraction. The pellet was then resuspended in 2 ml of water followed by the addition of 2 ml of 1 N PCA. The suspension was held at 4 °C for 20 min, centrifuged and the supernatant was added to the RNA fraction. The pellet was retained. The final RNA fraction was made upto 20 ml with distilled water and the extinction coefficient was measured at 260 nm against distilled water as blank in spectrophotometer. A standard curve was prepared with known amount of yeast RNA for calculation.

2.3.2. Estimation of DNA

The residual powder after RNA extraction containing DNA was suspended in 3 ml of 0.5 N PCA and then heated at 70 °C for 15 min, then centrifuged at 800 rpm at 2 °C and the residue was washed twice with 0.5 N PCA and the supernatant were pooled. Then final DNA extract was made up to 5 ml with 0.5 N PCA. The absorbance of the colourless solution was measured at 260 nm using a spectrophotometer against 0.5 N PCA as blank. A standard curve was prepared with calf thymus DNA for calculating the DNA content.

2.4. Determination of ATPase (Adenosine Triphosphatase, EC 3.6.1.3) activity

ATPase activity was assayed by the method of Evans [32]. Fresh plant material of one gram was placed in a precooled mortar and pestle and ground with 5 ml of 0.1 M Tris HCl buffer at pH 7.5 at 4 °C. The extract was passed through cheesecloth and centrifuged at 17,000 rpm for 10 min. The final volume of the supernatant was made upto 10 ml with 0.1 M Tris HCl buffer and used for assay. The incubation mixture in a total volume of 2.0 ml contained 1.0 ml of 0.1 M Tris HCl buffer (pH 7.5), 0.1 ml of 0.0075 M ATP solution, 0.1 ml of each 0.1 MgCl₂ 0.1 M Drought, 0.1 M KCl, 0.1 M CaCl₂ and 0.1 ml of extract. The incubation was carried out at 37 °C for 20 min and reaction was terminated by the addition of 1.0 ml of 10% TCA. Control tubes received 1.0 ml of 10% TCA solution before the addition of the enzyme extract. The suspension was centrifuged and the inorganic phosphorus content of the supernatant was assayed as described by Jaffe and Galston [33]. The enzyme activity (one unit) was

defined as the amount of enzyme liberating one μ mole $\text{Pi min}^{-1} \text{mg}^{-1}$ protein.

2.5. Assay of protease

Protease was extracted and assayed using the method of Prisco et al. [34]. One gram of fresh plant tissue was macerated 10 ml of 0.1 M sodium phosphate buffer (pH 7.6) containing 1% NaCl and 7% PVP. The homogenate was centrifuged. The supernatant was made up to 10 ml with the extraction buffer and used as the enzyme source. One milliliter of enzyme extract was incubated with 5 ml of 1% casein solution in 0.1 M sodium phosphate buffer (pH 6.0) at 30 °C. The reaction was stopped after 30 min by adding 1 ml of 40% TCA. The reaction mixture was centrifuged at 4000 rpm for min. The supernatant was saved to estimate the TCA soluble amino nitrogen released, by the activity of protease. Lee and Takahashi [35] method was followed for estimation of the ammonical nitrogen. Five milliliters of ninhydrin reagent was added to 0.5 ml of supernatant and the tubes were heated in boiling water bath for 12 min with vigorous shaking and they were cooled and measured at 570 nm in a spectrophotometer against a blank prepared with 40% TCA. A standard curve was prepared by using glycine as standard. The enzyme activity was expressed in terms of enzyme units. One enzyme unit is equal to micrograms NH_2 released $\text{hour}^{-1} \text{mg}^{-1}$ protein. For all the enzymatic calculations protein was determined by the method of Bradford [36], using bovine serum albumin (BSA, Sigma, USA) as the standard.

2.6. 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 \pm SD for six samples in each group. P values ≤ 0.05 were considered as significant.

3. Results

3.1. Effect of drought, TDM and their combination on ABA level

Drought treatment increased the ABA level to more than twofold in all parts of the plants. TDM treatment to the drought stressed plants showed the highest ABA level when compared with control and all other treatments. TDM treated plants also showed an increased ABA level when compared with control (Fig. 1). However, it was lower than that of other treatments. The leaf

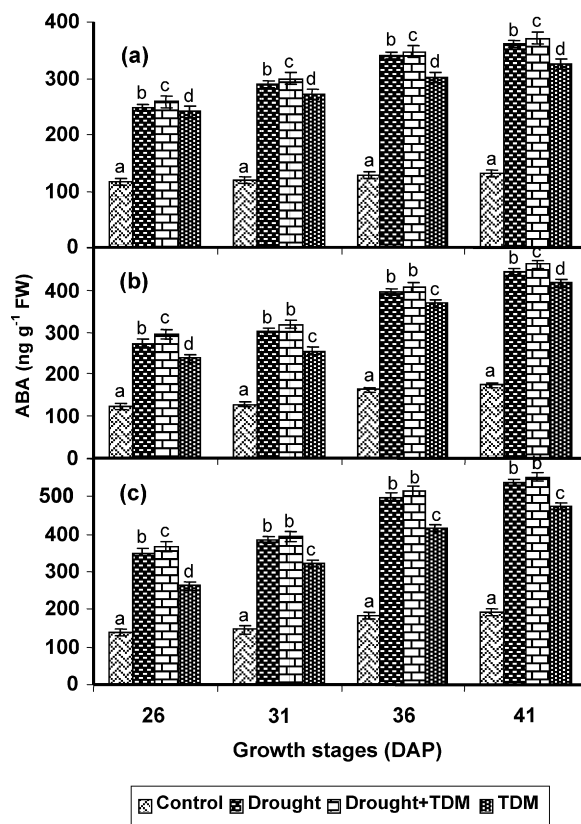


Fig. 1. Effect of drought, TDM and their combination on ABA accumulation in *Catharanthus roseus*. Values are given as mean \pm SD of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $p \leq 0.05$ (DMRT).

showed a higher ABA level, which was followed by root and stem in all treated plants.

3.2. Effect of drought, TDM and their combination on DNA content

In roots, stem and leaves drought stress caused a decrease in the DNA content when compared with control and other treatments (Fig. 2). Treatment with TDM in combination of drought increased the DNA content to the level of control roots. TDM treated plants also showed an increase in the DNA content was higher when compared with control and other treatments.

3.3. Effect of drought, TDM and their combination on RNA content

Drought stress decreased the RNA content of in all parts of the plants as compared to control. Addition to TDM to the drought stressed plants increased the RNA content to a level even above that of control. TDM

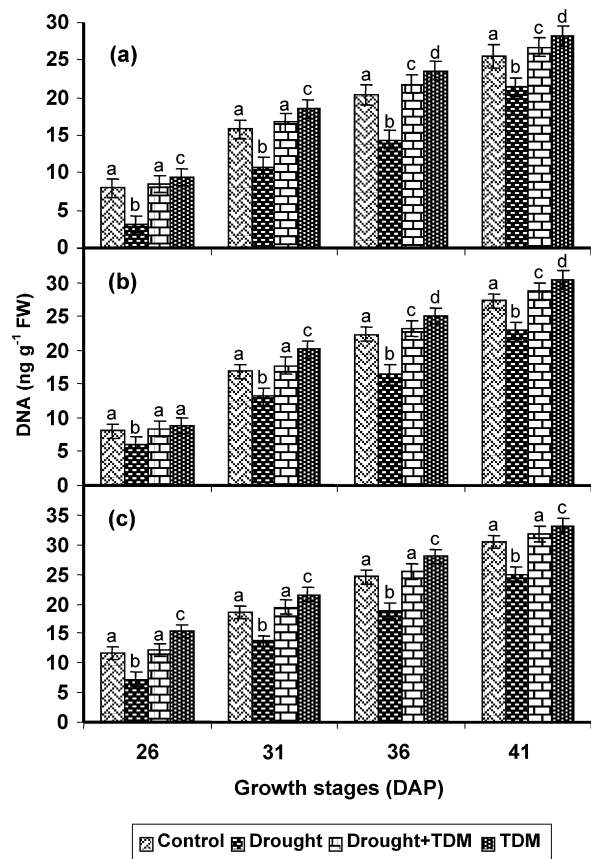


Fig. 2. Effect of drought, TDM and their combination on DNA content in *Catharanthus roseus*. Values are given as mean \pm SD of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $p \leq 0.05$ (DMRT).

treated plants showed an increase in the RNA content of the root when compared to control and other treated plants (Fig. 3).

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

In roots, stem and leaves drought stress caused an increase in the ATPase activity when compared with control and other treatments. Treatment with TDM in combination of drought decreased the ATPase activity to the level of control roots. TDM treated plants also showed decreased ATPase activity when compared with control (Fig. 4).

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

In roots, stem and leaves drought stress caused an increase in the protease activity when compared with

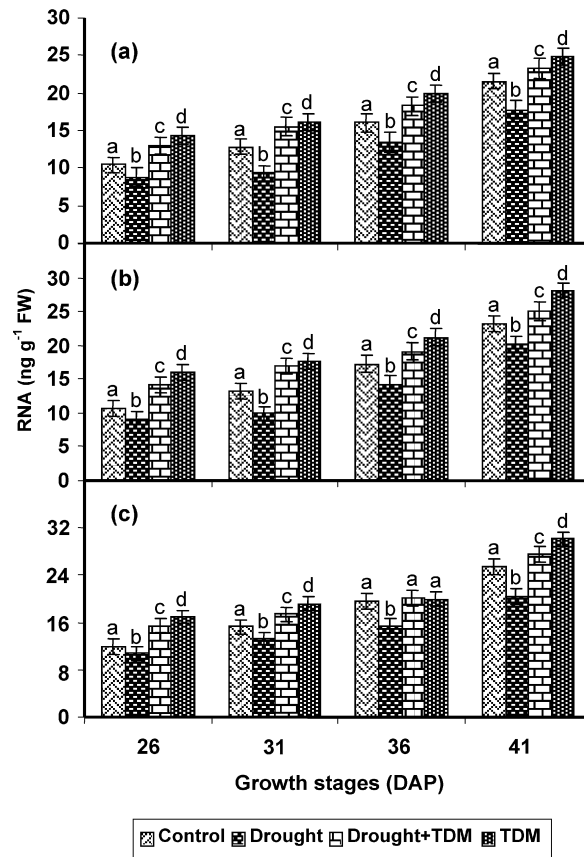


Fig. 3. Effect of drought, TDM and their combination on RNA content in *Catharanthus roseus*. Values are given as mean \pm SD of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $p \leq 0.05$ (DMRT).

control and other treatments. Treatment with TDM in combination of drought inhibited the protease activity to the level of control roots. TDM treated plants also showed a decrease in the protease activity when compared with control (Fig. 5).

4. Discussion

Drought treatment increased the ABA level to more than two folds in all parts of the plants TDM treatment to the drought stressed plants showed the highest. Increased ABA level was observed in *C. roseus* plants under drought stress and the increase is 2.5 times higher than that of control. Drought and cold stress resulted in elevated endogenous ABA levels [37]. ABA has been shown to mediate various responses such as proline accumulation, stomatal closure and shoot growth inhibition [38]. ABA might act as modulator of the response to salt stress mostly through its involvement in the osmotic process [39]. It has been suggested to play a role

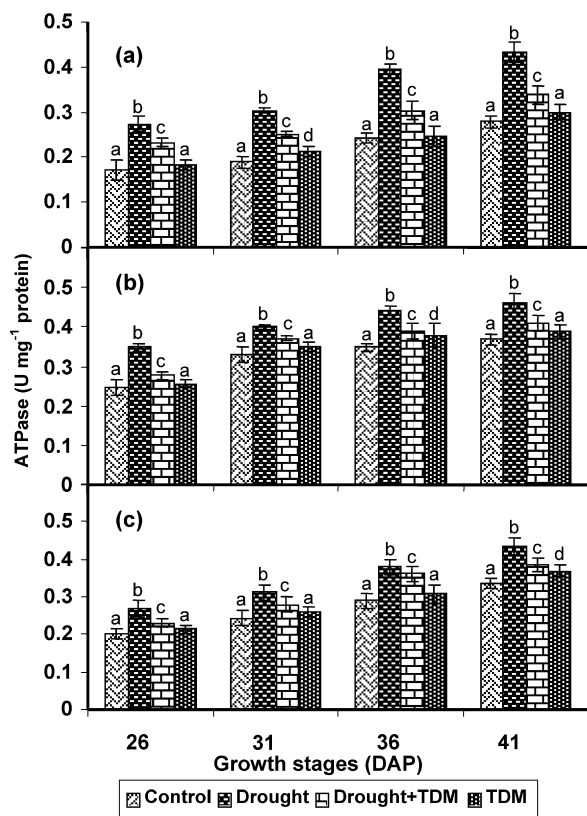


Fig. 4. Effect of drought, TDM and their combination on ATPase activity in *Catharanthus roseus*. Values are given as mean \pm SD of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $p \leq 0.05$ (DMRT).

in the protection against damages provoked by oxidative stress conditions [40]. TDM treatment to the drought stressed *C. roseus* plants also showed an increased ABA level when compared to control. Similar observation was made in water stressed bean [41]. ABA has been implicated as the initial trigger in the hardening process for various types of plant stresses [37]. TDM treatment to the unstressed plants also increased the ABA content to a large extent when compared with control. An increase in the concentration of ABA was found in the uniconazole treated *Phaseolus vulgaris* [42]. The increased ABA in the stressed and TDM treated plants can be well correlated with the increased stomatal resistance in *C. roseus* plants. ABA also induced protein synthesis and the synthesis of new mRNAs in the wilted vegetative tissues [43]. The increased nucleic acid and protein content can be correlated with the increased ABA content.

In roots, stem and leaves drought stress caused a decrease in the DNA content when compared with control and other treatments. Treatment with TDM in combina-

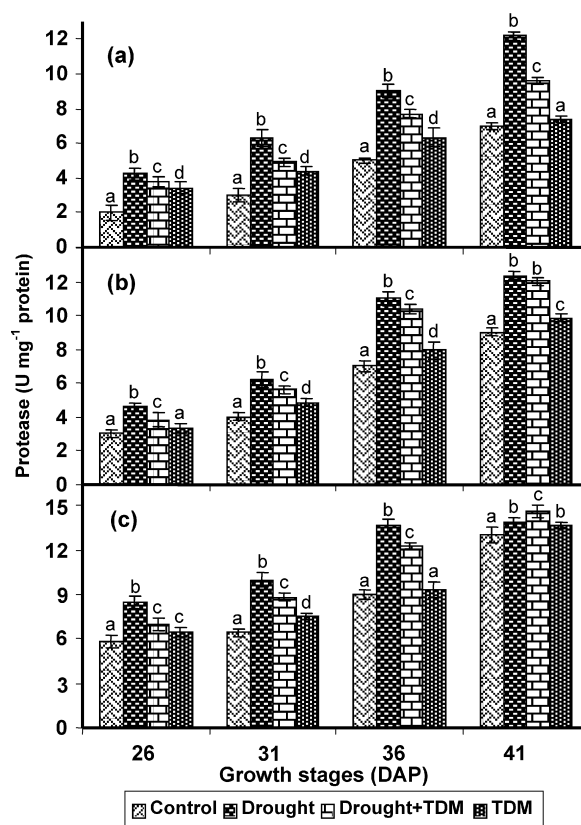


Fig. 5. Effect of drought, TDM and their combination on protease activity in *Catharanthus roseus*. Values are given as mean \pm SD of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $p \leq 0.05$ (DMRT).

tion of drought increased the DNA content to the level of control roots. Drought stress decreased the RNA content of in all parts of the plants as compared to control. Addition to TDM to the drought stressed plants increased the RNA content to a level even above that of control. Drought stress lowered the DNA and RNA contents to a large extent in the *C. roseus* plants when compared to control and other treated plants. Reduction in nucleic acid content was observed in the salt stressed *Vicia faba* [44]. The reduction may be due to the increased activities of DNase and RNase as reported by Tewari and Singh [45]. The decreased DNA and RNA content can be well correlated with the reduction in dry weight of the drought stressed plants and it also can be correlated with the reduction in number of cells per unit area in the leaves of the drought stressed *C. roseus* plants. TDM treatment to the drought stressed and unstressed plants increased the nucleic acid content to a higher level than that of control. TDM and triadimenol treatments increased the nucleic acid content in the barley leaves [46]. The increased dry weight and number

of cells per unit area in the TDM treated stressed and unstressed *C. roseus* plants can also be positively correlated.

The ATPase activity was found to be increased in the drought stressed plants and its activity was very high in the roots when compared to other organs. A similar result was made in the abiotic stresses like salt stress in *Vigna unguiculata* [47]. It has been suggested that H⁺-ATPase play major role in the osmoregulation under saline conditions [48]. The increase in ATPase activity will increase the hydrolysis of ATP and produce more energy to absorb water and other minerals against the concentration gradient through the drought-injured membrane of the root system in the drought stressed plants.

TDM treatment to the drought stressed and unstressed plants decreased the ATPase activity when compared with drought stressed plants. However, it was higher than that of control. Penconazole and Flusilazole inhibited the ATPase activity with increase in concentration of these triazole compounds in *Acerpseudoplatanus* [49]. The TDM treated *Picea glauca* needles showed an inhibited plasma membrane ATPase activity with altered composition of plasma membrane, which led to the maintenance of higher water potential and lower electrolyte leakage under osmotic stress [50]. Maintenance of membrane integrity with TDM treatment might have lead to a lower ATPase activity in the drought stressed *C. roseus*.

An increased activity of the protease was found in all stages of growth in *C. roseus* under drought stress. Increased activity of protease was reported in mulberry plants [51] under stress and our results support the observation of the above authors. The increased protease activity with decreased protein content and increased amino acid and proline content can be well correlated. TDM treatment to the drought stressed and unstressed *C. roseus* plants lowered the protease activity when compared with drought stressed plants. However, it was higher than control.

5. Conclusion

Water stress after planting is generally recognised as a major factor resulting in death of newly transplanted plants. Results of this investigation indicate applications of triazole derivatives, as a soil drench induces a suite of physiological adaptations that confer a greater degree of drought tolerance and aid in the recovery from drought induced damage. It is suggested that triazole induced protection of *C. roseus* from damage caused by drought

stress is mediated by increased hormonal and enzyme activities.

References

- [1] 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.
- [2] 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.
- [3] 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.
- [4] 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.
- [5] 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.
- [6] 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.
- [7] 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 (4) (2007) 283–288.
- [8] 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.* 30 (2008) 287–292.
- [9] C.A. Jaleel, R. Gopi, R. Panneerselvam, Biochemical alterations in white yam (*Dioscorea rotundata* Poir.) under triazole fungicides; impacts on tuber quality, *Czech J. Food Sci.* 26 (2008) 298–307.
- [10] 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.
- [11] 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.
- [12] C.A. Jaleel, R. Gopi, G.M. Alagulakshmanan, R. Panneerselvam, Triadimefon-induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don, *Plant Sci.* 171 (2006) 271–276.
- [13] 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.
- [14] 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 (2) (2007) 201–206.
- [15] 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.
- [16] C.A. Jaleel, R. Gopi, P. Manivannan, R. Panneerselvam, Exogenous application of triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal, *Pestic. Biochem. Physiol.* 91 (2008) 170–174.
- [17] 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.
- [18] 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 (2) (2007) 195–200.
- [19] 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, *South African J. Bot.* 74 (2008) 126–132.
- [20] 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.
- [21] 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.
- [22] C.A. Jaleel, B. Sankar, R. Sridharan, R. Panneerselvam, Soil salinity alters growth, chlorophyll contents and secondary metabolite accumulation in *Catharanthus roseus*, *Turkish J. Biol.* 32 (2008) 79–83.
- [23] C.A. Jaleel, R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan, R. Panneerselvam, Alterations in germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress, *South Afr. J. Bot.* 73 (2007) 190–195.
- [24] 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.
- [25] 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.
- [26] 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.
- [27] C.A. Jaleel, R. Gopi, P. Manivannan, R. Panneerselvam, Soil salinity alters the morphology in *Catharanthus roseus*; effects on endogenous mineral constituents, *EurAsia. J. BioSci.* 2 (2008) 18–25.
- [28] 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.
- [29] 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.
- [30] A.J. Cihra, M.L. Breanner, W.A. Brun, Rapid separation and quantification of abscisic acid from plant tissue using high performance liquid chromatography, *Plant Physiol.* 59 (1977) 821–826.
- [31] R.H. Nieman, L.L. Paulson, Spectrophotometric estimation of nucleic acids of plant leaves, *Plant Physiol.* 38 (1963) 31–35.
- [32] D.J. Evans Jr., Membrane adenosine triphosphatase of *Escherichia coli*. Activation by calcium ion and inhibition by monovalent cations, *J. Bacteriol.* 100 (1969) 914–922.
- [33] M.J. Jaffe, C. Galston, Physiological studies on pea tendril II. The sole of light and ATP in contact coiling, *Plant Physiol.* 141 (1996) 1153–1158.
- [34] J.T. Prisco, I.L. Ainouz, S.C. Melo, Changes in nitrogenous compounds and protease during germination of *Vigna sinensis* seeds, *Physiol. Plant.* 33 (1975) 18–21.
- [35] Y.P. Lee, T. Takahashi, An improved colorimetric determination of aminoacids with the use of ninhydrin, *Anal. Biochem.* 14 (1966) 69–71.
- [36] 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.
- [37] J. Zhang, W. Jia, J. Yang, A.M. Ismail, Role of ABA in integrating plant responses to drought and salt stresses, *Field Crops Res.* 97 (2006) 111–119.
- [38] R.A. Credman, H.S. Manson, R.J. Bensen, J.S. Boyer, J.E. Mallet, Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings, *Plant Physiol.* 92 (1990) 205–214.
- [39] A.G. Cadenzas, F.R. Tadeo, E. Millo, M. Talon, Involvement of abscisic acid and ethylene in the responses of citrus seedlings to drought shock, *Physiol. Plant.* 103 (1998) 475–484.
- [40] R. Desikan, M.K. Cheung, J. Bright, D. Henson, J.T. Hancock, S.J. Neill, ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells, *J. Exp. Bot.* 55 (2004) 205–212.
- [41] N.K. Asare-Boamah, G. Hofstra, R.A. Fletcher, E.B. Dumproff, Triadimefon protects bean plants from water stress through its effects on abscisic acid, *Plant Cell Physiol.* 27 (1986) 383–390.
- [42] C.E. Mackay, J.C. Hall, G. Hofstra, R.A. Fletcher, Uniconazole induced changes in abscisic acid, total amino acids and proline in *Phaseolus vulgaris*, *Pestic. Biochem. Physiol.* 37 (1990) 71–82.
- [43] A. Cohen, E.A. Bray, Characterization of three mRNAs that accumulate in wilted tomato leaves in response to elevated levels of endogenous abscisic acid, *Planta* 182 (1990) 27–33.
- [44] H.M. Abdel-samad, Counteraction of NaCl with CaCl₂ or KCl on pigment saccharide and mineral contents in wheat, *Biol. Plant* 35 (1993) 555–560.
- [45] T.N. Tewari, B.B. Singh, Stress studies in lentil (*Lens Esculenta* Moench.). II. Sodidity-induced changes in chlorophyll, nitrate and nitrite reductase, nucleic acid, proline, yield and yield component in lentil, *Plant Soil* 136 (1991) 225–230.

- [46] H. Forster, Mechanism of action and side effects of triadimefon and triadimenol in barley plants, in: 3rd Int. Con. Plant. Pathol., Munchon, 1978, p. 365.
- [47] F. De Melo, D.Y. Jolivet, A.R. Facanha, E.G. Filho, M.S. Lima, P. Dizengremet, Effect of drought stress on mitochondrial energy metabolism of *Vigna unguiculata* cultivars differing in salt tolerance, *Plant Physiol. Biochem.* 32 (1994) 405–412.
- [48] M. Reuveni, R. Colombo, H.R. Lerner, A. Pradet, M. Poljakoff-Mayber, Osmotically induced proton extrusion from carrot cells in suspension culture, *Plant Physiol.* 85 (1987) 383–388.
- [49] R. Colombo, R. Cerana, P. Lado, Effect of penconazole and flusilazol on the tonoplast of *Acer pseudoplatanus* cells, *Plant Sci.* 76 (1991) 167–174.
- [50] E. Sailerova, J.J. Zwiazek, Effects of triadimefon and osmotic stress on plasma membrane composition and ATPase activity in white spruce (*Picea gluca*) needles, *Physiol. Plant.* 87 (1993) 475–482.
- [51] S. Ramanjulu, K. Veeranjanyulu, C. Sudhakar, Short-term shifts in nitrogen metabolism in mulberry (*Morus alba*) under drought shock, *Phytochem.* 37 (1994) 991–995.