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Alterations in non-enzymatic antioxidant components of *Catharanthus roseus* exposed to paclobutrazol, gibberellic acid and *Pseudomonas fluorescens*

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Abstract

The effect of different plat growth regulators like paclobutrazol (PBZ) gibberellic acid and *Pseudomonas fluorescens* treatments on the non-enzymatic antioxidant components of *Catharanthus roseus* was investigated in the present study. The treatments were given to plants by soil drenching on 38, 53, 68 and 83 days after planting (DAP). The plants were taken randomly on 45, 60, 75 and 90 DAP and separated into root, stem and leaves and used for determining antioxidant potentials. The non-enzymatic antioxidant contents like total phenol, ascorbic acid (AA), reduced glutathione (GSH) and α-tocopherol (α-toc), were extracted and assayed from both control and treated plants. It was found that paclobutrazol (PBZ) gibberellic acid and *Pseudomonas fluorescens* treatments have a profound effect on the antioxidant metabolism and caused an enhancement in non-enzymatic antioxidant potentials under treatments in *Catharanthus roseus*. Our results have good significance, as this increase the innate antioxidant potential of this medicinal plant, as this plant being an essential component of traditional as well as modern pharmaceutical systems.

Keywords: *Catharanthus roseus;* paclobutrazol; antioxidants; *Pseudomonas fluorescens;* gibberellic acid

Abbreviations

AA_ascorbic acid; DAP_days after planting; GSH_reduced glutathione; α-toc_α-tocopherol; PBZ _ paclobut-razol; ROS_Reactive oxygen species; MIAs_monoterpenoid indole alkaloids; PGPR_Plant growth promoting rhizobacteria; RH_relative humidity; ANOVA_analysis of variance; DMRT_Duncan's Multiple Range Test; GA_3_ gibberellic acid; DW_dry weight; FW_fresh weight; SD_standard deviation, *Pseudomonas fluoescence* Elicitors (PF-Elicitors)

Introduction

Herbal medicine is still the mainstay of about 75% to 80% of the world population, mainly in the developing countries to promote primary health care with better cultural acceptability, human compatibility and lesser side effects. Although synthetic pharmaceuticals now dominate the drug scene, medicinal plants continue to hold a place in international health care (Jaleel et al., 2006a,b). Awareness of the importance of natural heritage and biodiversity is also growing. India is a gold mine of treasures with traditional and practical knowledge of herbal medicines (Jaleel et al.,

2007a,b). Globally a positive trend has blossomed in favors of traditional and integrative health sciences both in research and practices (Jaleel and Panneerselvam, 2007). Medicinal plants form a large group of important flora. Plants provide basic raw materials for the indigenous Pharmaceutical industries such as pharmaceutical, cosmetic, perfumery and food etc. The medicinal plants are referred to plants that are used for their therapeutic or medicinal values (Jaleel et al., 2008a,b).

Oxidative stress caused by an unbalance between pro-oxidants and antioxidants. Reactive oxygen species (ROS) are divided in two main classes consisted of non radical species (H_2O_2) or free radical forms ($O_2^{\cdot-}$, $OH^{\cdot}$, $OH_2^{\cdot}$). Accumulation of high concentrations of ROS is potentially detrimental to plants cells causing damage to valuable biomolecules like DNA, proteins, lipids, chlorophyll, membrane etc (Blokhina et al., 2003). Antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Jaleel et al., 2008c,d). Free radicals can be scavenged through chemoprevention by utilizing natural antioxidant compounds present in foods (Borelli and Izzo, 2000) and medicinal plants (Jaleel et al., 2007c,d,e,f,g,h,i). Some medicinal plants have been shown to have both chemopreventive and/or therapeutic effects on human diseases (Jaleel et al., 2007j,k,l).

Catharanthus roseus (L.) G. Don. (Madagascar periwinkle) is a perennial tropical plant belonging to the family Apocynaceae that produces more than 100 monoterpenoid indole alkaloids (MIAs) including two commercially important cytotoxic dimeric alkaloids used in cancer chemotherapy (Magnotta et al., 2006; Jaleel et al., 2007d,e, 2008e,g,h,i,j,k). Roots of this plant are the main source of an anti-hypertension alkaloid ajmalicine (Jaleel et al., 2006a). *C. roseus* is also a popular ornamental plant. Three distinct varieties based on the flower colour viz., the pink flowered 'rosea', the white flowered 'alba' and the white with a pink or yellow ring in the orifice region 'Ocellata' are found in *C. roseus*. Pink flowered cultivar gives higher yield of foliage and roots and total alkaloids (Jaleel *et al.,* 2006a, 2007a,m,n).

Triazole compounds are systemic fungicides having plant growth regulating properties. Triazole compounds not only protected plants from stress but also induced stress like symptoms. It is reported that abiotic stress (Jaleel et al., 2007o) and triazole compounds have increased the alkaloid content in *C. roseus* (Jaleel *et al.,* 2006a). More over the triazole compounds has increased the root production in *Catharanthus* (Jaleel et al., 2006b) and *Withania somnifera* (Jaleel et al., 2008f,l) where the alkaloids accumulate in larger quantity than in the shoot. Paclobutrazol (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 plant growth regulating properties. The growth regulating properties of paclobutrazol are mediated by changes in the balance of important plant hormones including the Gibberellins, ABA and cytokinins (Hajihashemi et al., 2007). Paclobutrazol has been proved as an agent in stress amelioration in medicinal plants (Jaleel et al., 2007b, 2008d,e) and crop plants (Sankar et al., 2007; Kishorekumar et al., 2006,2007,2008; Manivannan et al., 2007,2008a,b).

Bacteria associated with plants can be harmful and beneficial. Plant growth promoting (PGP) bacteria may promote growth directly, e.g. by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderosphores that solubilize and sequester iron, or production of plant growth regulators (hormones) (Karthikeyan et al., 2008a). The strong and rapidly stimulating effect of elicitor on plant secondary metabolism in medicinal plants attracts considerable attentions and research efforts (Karthikeyan et al., 2007). The reasons responsible for the diverse stimulating effects of elicitors are complicated and could be related to the interactions between elicitors and plant cells, elicitor signal transduction, and plant defense responses (Karthikeyan et al., 2008b). In plants, certain secondary metabolite pathways are induced by infection with microorganisms. It was reported that, arbuscular mycorrhizal symbiosis maintained more normal water relations in plants.

The objectives of the present study are to understand the effect of plant growth regulators such as paclobutrazol, gibberellic acid and *Pseudomonas fluorescence* elicitors on the non-enzymatic antioxidant contents of *Catharanthus roseus* plants under field conditions.

Materials and methods

Plant materials and treatments

Medicinally important plant species, *Catharanthus roseus* (L.) G. Don. (Family: Apocynaceae) was selected for the present investigation. 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

Table 1. Effect of different growth regulators on total phenol contents (mg/g FW) of *Catharanthus roseus*. Values are given as mean ± SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). Values in parenthesis indicate percentage over control.

Growth Stages (DAP)	Control	Paclobutrazol	Gibberellic acid	PF Elicitors
Root				
45	2.39 ± 0.082^{a}	3.95 ± 0.140^{b} (165.27)	3.12 ± 0.142^{c} (130.54)	2.66 ± 0.092^{d} (111.24)
60	5.78 ± 0.193^{a}	7.50 ± 0.259^{b} (129.75)	6.66 ± 0.243^{c} (115.22)	6.20 ± 0.242^{a} (107.26)
75	6.73 ± 0.240^{a}	9.92 ± 0.333^{b} (147.39)	8.58 ± 0.244^{c} (127.49)	8.00 ± 0.253^{d} (118.87)
90	11.38 ± 0.438^{a}	13.56 ± 0.484^{b} (119.16)	12.52 ± 0.458^{c} (110.02)	12.00 ± 0.453^{a} (105.44)
Stem				
45	2.19 ± 0.080^{a}	3.52 ± 0.143^{b} (160.73)	3.00 ± 0.143^{c} (136.99)	2.92 ± 0.082^{d} (133.33)
60	2.20 ± 0.081^{a}	3.65 ± 0.144^{b} (165.90)	2.86 ± 0.080^{c} (130.00)	2.52 ± 0.083^{d} (114.55)
75	3.98 ± 0.144^{a}	5.55 ± 0.194^{b} (139.45)	5.20 ± 0.195^{c} (130.65)	4.80 ± 0.190^{d} (120.60)
90	4.20 ± 0.193^{a}	6.00 ± 0.003^{b} (142.86)	5.58 ± 0.196^{c} (132.86)	5.20 ± 0.193^{d} (123.81)
Leaf				
45	2.08 ± 0.004^{a}	3.52 ± 0.005^{b} (169.23)	2.50 ± 0.004^{c} (120.19)	2.44 ± 0.004^{c} (117.31)
60	2.98 ± 0.005^{a}	4.56 ± 0.005^{b} (153.14)	3.62 ± 0.005^{c} (121.48)	3.49 ± 0.005^{c} (117.11)
75	3.00 ± 0.004^{a}	5.08 ± 0.005^{b} (169.33)	4.06 ± 0.004^{c} (135.33)	3.56 ± 0.007^{d} (118.64)
90	4.65 ± 0.005^{a}	6.52 ± 0.004^{b} (140.22)	5.95 ± 0.005^{c} (127.96)	5.00 ± 0.005^{a} (107.53)

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 of Department of Botany, Annamalai University. The seeds were sown separately in raised seedbeds by broadcasting method and covered with fine soil to ensure proper germination. The nursery beds were watered twice a day and weeded regularly in order to ensure healthy growth of the seedlings.

Growth regulator treatments

In the preliminary experiments, 5, 10, 15 and 20 mg L^{-1} paclobutrazol was used for treatment to determine the optimum concentration of paclobutrazol. Among the treatments, 10 mg L^{-1} paclobutrazol concentration increased the dry weight significantly and higher concentration slightly decreased the growth and dry weight. In the lower concentrations, there was no change in weight and growth. Hence 10 mg L^{-1} paclobutrazol concentration was used to study the effect of paclobutrazol on the *C. roseus* plant. Similarly 5 µM GA_3 and 1 mg *Pseudomonas fluorescens* concentrations were also determined and used for the treatments.

One plot each was subjected to growth regulator treatments and one was kept as control. The treatments were given on 38, 53, 68 and 83 DAP by soil drenching. The plants were taken randomly on 45, 60, 75 and 90 DAP and separated into root, stem, leaves and used for determining antioxidant potentials.

Non-enzymatic antioxidant estimations

Total Phenols

Total phenol was estimated by the method of Malick and Singh (1980). 500 mg of fresh plant tissue was ground in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved with 5 ml of distilled water and used as extract. To 2 ml of the extract, 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% $Na_2 CO_3$ solution was mixed thoroughly. The mixture was kept in boiling water for exactly one min. and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentration of gallic acid.

Estimation of AA content

AA content was assayed as described by Omaye et al. (1979). The extract was prepared by grinding 1 g of fresh material with 5 ml of 10% TCA, centrifuged at 3500 rpm for 20 min, reextracted twice and supernatant made upto 10 ml and used for assay. To 0.5 ml of extract, 1 ml of DTC reagent (2,4-dinitrophenyl hydrazine-thiourea-$CuSO_4$ reagent) was added, incubated at 37 °C for 3 h and 0.75 ml of ice-cold 65% H_2SO_4 was added, allowed to stand at 30 °C for 30 min, resulting colour was read at 520 nm in spectrophotometer (U-2001-Hitachi). The AA content was determined using a standard curve prepared with AA and the results were expressed in mg g^{-1} dry weight (DW).

Estimation of GSH content

The GSH content was assayed as described by Griffith and Meister (1979). 200 mg fresh material was ground with 2 ml of 2% metaphosphoric acid and centrifuged at 17000 rpm for 10 min. Adding 0.6 ml 10% sodium citrate neutralized the supernatant. 1 ml of assay mixture was prepared by adding 100 µl extract, 100 µl distilled water, 100 µl 5,5-dithio-bis-(2-nitrobenzoic acid) and 700 µl NADPH. The mixture was stabilized at 25 °C for 3-4 min. Then 10 µl of glutathione reductase was added, read the absorbance at 412 nm in spectrophotometer and the GSH contents were expressed in µg g^{-1} fresh weight (FW).

Estimation of α-Toc content

α-Toc content was assayed as described by Backer et al. (1980). 500 mg 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 min and the supernatant was used for estimation of α-toc. To one ml of extract, 0.2 ml of 2% 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 min. 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-toc content was calculated using a standard graph made with known amount of α-toc.

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for six samples in each group. p values ≤ 0.05 were considered as significant.

Results

Total phenol

The total phenol contents of different parts (root, stem and leaf) of the plant increased with the age in control and growth regulator treated *Catharanthus roseus* plants. The increase was highly significant in PBZ treatments and a highest increase in total phenol content in root tissue was observed on 45 DAP (165.27 percent over control). Under Gibberellic acid and PF Elicitors the increase was significant but lower than PBZ treatments (Table 1). In the case of stem, the total phenol contents varied with growth regulator treatments. The increase was highly significant in PBZ treatments and a highest increase in total phenol content was observed on 60 DAP (165.90 percent over control), in the same day samplings the increase was only 114.55 in the case of PF Elicitors (Table 2). Under gibberellic acid treatments, although the increase in total phenol content was significant, but it was lower that PBZ treated plants. The total phenol contents varied with growth regulator treatments in leaf tissue. All the growth regulators significantly increased the total phenol content in leaf of *Catharanthus roseus*. In PBZ treatments, a highest increase in total phenol content was observed on 75 DAP (169.33 percent over control), and the lowest level of increase was observed in samplings of 90 DAP, upto 114.55 in the case of PF Elicitors (Table 2).

Table 2. Effect of different growth regulators on ascorbic acid contents (mg/g FW) of *Catharanthus roseus*. Values are given as mean ± SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). Values in parenthesis indicate percentage over control.

Growth Stages (DAP)	Control	Paclobutrazol	Gibberellic acid	PF Elicitors
Root				
45	3.00 ± 0.101^{a}	4.21 ± 0.145^{b} (140.33)	3.88 ± 0.117^{c} (129.33)	3.61 ± 0.122^{d} (120.33)
60	3.39 ± 0.117^{a}	4.99 ± 0.172^{b} (147.20)	4.20 ± 0.147^{c} (123.89)	3.99 ± 0.122^{d} (117.69)
75	4.23 ± 0.141^{a}	5.98 ± 0.173^{b} (141.37)	5.39 ± 0.179^{c} (127.42)	4.96 ± 0.173^{d} (117.25)
90	6.87 ± 0.229^{a}	8.99 ± 0.272^{b} (130.86)	7.95 ± 0.283^{c} (115.72)	7.70 ± 0.283^{c} (112.08)
Stem				
45	2.12 ± 0.073^{a}	3.56 ± 0.119^{b} (167.92)	2.98 ± 0.083^{c} (140.56)	2.25 ± 0.072^{a} (106.13)
60	2.61 ± 0.074^{a}	4.21 ± 0.144^{b} (161.30)	3.24 ± 0.113^{c} (124.14)	2.86 ± 0.082^{a} (109.58)
75	3.52 ± 0.118^{a}	5.66 ± 0.174^{b} (160.79)	4.32 ± 0.144^{c} (122.72)	3.82 ± 0.144^{a} (108.52)
90	5.12 ± 0.173^{a}	7.26 ± 0.283^{b} (141.79)	6.00 ± 0.223^{c} (117.19)	5.34 ± 0.173^{a} (104.29)
Leaf				
45	1.98 ± 0.068^{a}	2.96 ± 0.085^{b} (149.49)	2.52 ± 0.084^{c} (127.27)	2.40 ± 0.084^{d} (121.01)
60	2.31 ± 0.085^{a}	4.02 ± 0.105^{b} (174.03)	3.00 ± 0.115^{c} (129.87)	2.56 ± 0.085^{d} (110.82)
75	3.00 ± 0.114^{a}	4.26 ± 0.115^{b} (142.00)	3.52 ± 0.124^{c} (117.33)	3.42 ± 0.127^{d} (114.00)
90	4.11 ± 0.145^{a}	6.67 ± 0.204^{b} (162.29)	5.60 ± 0.185^{c} (136.25)	4.58 ± 0.125^{d} (111.43)

Gibberellic acid treatments also increased the total phenol content in leaves of *Catharanthus roseus* plants.

Ascorbic acid

The total ascorbic acid content of the plant increased with the age in control and growth regulator treated *Catharanthus roseus* plants. The increases were significant in all treatments. Ascorbic acid content under Gibberellic acid and PF Elicitors were significant but lower than PBZ treatments. In root tissue, a maximum of increase was noted on 60 DAP in PBZ treatments and it was nearly 147.20 percent over control. A least increase was recorded in 90 DAP samples under PF Elicitors treatments and which was upto 112.08 percent over control (Table 2). In stem tissues, the increases were significant in all treatments. A maximum of increase was noted on 45 DAP in PBZ treatments and it was nearly 167.92 percent over control. A least increase was recorded in 90 DAP samples under PF Elicitors treatments and which was upto 104.29 percent over control (Table 2). The increase was in the order PBZ> gibberellic acid >PF Elicitors.

In leaf tissues, the ascorbic acid content under Gibberellic acid and PF Elicitors were significant but lower than PBZ treatments. A maximum of increase was noted on 60 DAP in PBZ treatments and it was nearly 174.03 percent over control. A least increase was recorded in the same day samplings under PF Elicitors treatments and which was upto 110.82 percent over control (Table 2). The increase was in the order PBZ> gibberellic acid >PF Elicitors.

Table 3. Effect of different growth regulators on reduced glutathione contents (mg/g FW) of *Catharanthus roseus*. Values are given as mean ± SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). Values in parenthesis indicate percentage over control.

Growth Stages (DAP)	Control	Paclobutrazol	Gibberellic acid	PF Elicitors
Root				
45	2.04 ± 0.078[a]	3.47 ± 0.082[b] (170.71)	3.42 ± 0.002[c] (168.06)	2.84± 0.074[d] (139.55)
60	2.56± 0.088[a]	4.66 ± 0.152[b] (182.84)	3.99 ± 0.003[c] (155.86)	2.99 ± 0.075[d] (116.79)
75	4.54 ± 0.151[a]	6.66 ± 0.183[b] (146.69)	5.45 ± 0.004[c] (120.04)	5.09 ± 0.143[d] (112.11)
90	7.53 ± 0.252[a]	9.21 ± 0.292[b] (122.31)	8.78 ± 0.289[c] (116.60)	8.24 ± 0.278[a] (109.32)
Stem				
45	1.08 ± 0.038[a]	2.00 ± 0.082[b] (185.19)	1.98 ± 0.043[c] (183.33)	1.54± 0.002[d] (142.90)
60	1.23 ± 0.045[a]	2.18 ± 0.084[b] (177.23)	2.04 ± 0.085[c] (165.85)	1.86 ± 0.042[d] (151.22)
75	4.40± 0.163[a]	5.67 ± 0.194[b] (128.86)	5.40 ± 0.184[c] (122.73)	5.01 ± 0.174[d] (113.86)
90	6.02± 0.208[a]	7.89 ± 0.223[b] (131.03)	7.38 ± 0.213[c] (122.59)	7.29 ± 0.203[d] (121.03)
Leaf				
45	2.02 ± 0.004[a]	3.60 ± 0.005[b] (178.22)	2.84 ± 0.004[c] (140.59)	3.00 ± 0.004[c] (148.51)
60	2.20 ± 0.005[a]	4.01 ± 0.005[b] (182.27)	3.18 ± 0.005[c] (144.55)	3.04 ± 0.005[c] (138.18)
75	3.44 ± 0.004[a]	4.98 ± 0.005[b] (144.77)	4.66 ± 0.004[c] (135.46)	4.25 ± 0.007[d] (123.55)
90	6.72 ± 0.005[a]	8.21 ± 0.004[b] (122.75)	7.81 ± 0.005[c] (116.22)	7.36 ± 0.005[a] (109.53)

Reduced glutathione

The reduced glutathione contents of the plant increased with the age in control and growth regulator treated *Catharanthus roseus* plants. All the three growth regulators increased the reduced glutathione content significantly when compared to control. In root tissue A maximum of increase was noted on 60 DAP in PBZ treatments and it was nearly 182.84 percent over control. The increase was in the order PBZ> gibberellic acid >PF Elicitors. A least increase was recorded in 90 DAP samples under PF Elicitors treatments and which was upto 109.32 percent over control (Table 3).

All the three growth regulators increased the reduced glutathione content significantly in stem tissue also when compared to control. A maximum of increase was noted on 45 DAP in PBZ treatments and it was nearly 185.19 percent over control. The increase was in the order PBZ> gibberellic acid >PF Elicitors. A least increase was recorded in 75 DAP samples under PF Elicitors treatments and which was up to 113.86 percent over control (Table 3).

In leaf tissue also, all the three growth regulators increased the reduced glutathione content significantly when compared to control. A maximum of increase was noted on 60 DAP in PBZ treatments and it was nearly 182.27 percent over control. The increase was in the order PBZ> gibberellic acid >PF Elicitors. A least increase was recorded in 90 DAP samples under PF Elicitors treatments and which was up to 109.53 percent over control (Table 3).

Table 4. Effect of different growth regulators on α – tocopherol contents (mg/g FW) of *Catharanthus roseus*. Values are given as mean ± SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). Values in parenthesis indicate percentage over control.

Growth Stages (DAP)	Control	Paclobutrazol	Gibberellic acid	PF Elicitors
Root				
45	3.92 ± 0.141^{a}	4.52 ± 0.152^{b} (115.31)	4.45 ± 0.150^{c} (113.52)	4.30 ± 0.149^{a} (109.69)
60	4.20 ± 0.142^{a}	6.22 ± 0.201^{b} (148.09)	5.89 ± 0.193^{c} (140.26)	4.86 ± 0.182^{d} (115.71)
75	8.68 ± 0.292^{a}	9.92 ± 0.323^{b} (114.29)	9.71 ± 0.314^{c} (111.87)	9.00 ± 0.303^{a} (103.69)
90	12.90 ± 0.478^{a}	14.51 ± 0.502^{b} (112.48)	14.34 ± 0.503^{c} (111.66)	14.12 ± 0.493^{a} (109.45)
Stem				
45	2.62 ± 0.102^{a}	4.66 ± 0.153^{b} (177.86)	3.18 ± 0.143^{c} (121.37)	2.89 ± 0.122^{d} (110.30)
60	2.91 ± 0.132^{a}	5.21 ± 0.194^{b} (179.03)	4.85 ± 0.183^{c} (166.67)	3.42 ± 0.142^{d} (117.53)
75	7.62 ± 0.254^{a}	9.82 ± 0.324^{b} (128.87)	8.98 ± 0.294^{c} (117.85)	8.21 ± 0.304^{a} (107.74)
90	11.94 ± 0.463^{a}	13.51 ± 0.493^{b} (113.14)	12.88 ± 0.473^{a} (107.87)	12.51 ± 0.463^{a} (104.85)
Leaf				
45	2.89 ± 0.134^{a}	4.55 ± 0.185^{b} (157.43)	3.66 ± 0.145^{c} (126.67)	3.22 ± 0.144^{d} (111.42)
60	3.20 ± 0.145^{a}	5.01 ± 0.005^{b} (156.56)	4.52 ± 0.185^{c} (141.25)	3.86 ± 0.155^{d} (120.63)
75	7.99 ± 0.004^{a}	9.98 ± 0.325^{b} (124.91)	8.81 ± 0.314^{c} (110.26)	8.61 ± 0.307^{a} (107.74)
90	12.75 ± 0.485^{a}	14.10 ± 0.494^{b} (117.55)	13.78 ± 0.385^{c} (110.55)	13.32 ± 0.395^{a} (104.53)

∝-tocopherol

In *Catharanthus roseus* plants, the ∝-tocopherol contents of the different plant parts increased with the age in control and growth regulator treated. The increase was highly significant in all the treatments. A maximum of increase was noted on 60 DAP in PBZ treatments and it was nearly 148.09 percent over control in root tissue. A least increase was recorded in 75 DAP samples under PF Elicitors treatments and which was up to 103.69 percent over control (Table 4).

In stem, the ∝-tocopherol contents of the different plant parts increased with the age in control and growth regulator treatments. The increase was highly significant in all the treatments. A maximum of increase was noted on 60 DAP in PBZ treatments and it was nearly 179.03 percent over control in root tissue. A least increase was recorded in 90 DAP samples under PF Elicitors treatments and which was up to 104.85 percent over control (Table 4). Gibberellic acid also increased this antioxidant content significantly when compare to control plants.

In leaf, the ∝-tocopherol contents varied greatly with growth regulator treatments. The increase was highly significant in all the treatments. A maximum of increase was noted on 45 DAP in PBZ treatments and it was nearly 157.43 percent over control in root tissue. A least increase was recorded in 90 DAP samples under PF Elicitors treatments and which was upto 104.53 percent over control (Table 4). Gibberellic acid also increased this antioxidant content significantly when compare to control plants.

Discussion

All the growth regulators significantly increased the total phenol contents in all the parts of *Catharanthus roseus* plants on all sampling days when compared to control plants. Increased total phenol content was previously reported in triazole treated radish (Gopi et al., 2007). It has been suggested that peroxidase could act as efficient H_2O_2 scavenging system in plant vacuoles in the presence of phenolics and reduced ascorbate (Jaleel et al., 2008n). Jaleel et al., (2008n) hypothesized a cycle where H_2O_2 is scavenged by phenolic compounds. Phenolics are oxidized to phenoxyl radicals. This phenoxyl radical reduces the ascorbic acid into monodehydro ascorbate. Increased phenol content was previously reported in *Coleus* plants under hexaconazole treatments (Lakshmanan et al., 2007).

Protection as a result of microbial antagonism was excluded because the inducing rhizobacteria and the challenging pathogens were inoculated at, and remained confined to, spatially separated parts on the same plants. Upon infection with a challenging pathogen this enhanced defensive capacity is manifested as a reduction in the rate of disease development, resulting in fewer diseased plants or in lesser disease severity. The induced resistance is also evident locally and sometimes does not extend systemically (Karthikeyan et al., 2007).

All the three growth regulators significantly increased the ascorbic acid content in the leaves, stem and roots of *C. roseus.* Ascorbic acid has been proposed to have roles on regulation of photosynthesis (Shao et al., 2008a). Ascorbic acid is readily oxidised to monodehydro ascorbic acid as part of its antioxidant function (Gomathinayagam et al., 2007; Shao et al., 2008b). Ascorbic acid prevents or reducing oxidative damage was reported in ketoconazole treated drought stressed *C. roseus* (Jaleel et al., 2007p).

Most studies on mechanisms for plant growth promotion by PGPR have focused on bacterial traits without examining the host plant's physiological responses (Karthikeyan et al., 2007). The PGPR could have mineralized nutrients, making them more available to plants. In one study, some bacilli PGPR strains promoted growth of maize seedlings through production of extracellular phytase, which degrades phytate (myo-inositol hexakisphosphate) under the conditions of limited phosphate availability.

Growth regulator treatments resulted in an increase in reduced glutathione content in *Catharanthus roseus*. Triazoles increased the reduced glutathione content in carrot (Gopi *et al.,* 2007). A reduction in oxidative damage symptoms was reported in paclobutrazol treated wheat seedlings indicating that there might be an efficient active oxygen scavenging system (Gopi et al., 2008; Lakshmanan et al. 2007). Triadimefon (Jaleel et al., 2006a) and Paclobutrazol treatment increased the reduced glutathione content in *Catharanthus* (Jaleel et al., 2007b).

Growth regulators like GA_3 treatments increased the reduced glutathione content in *C. roseus*, which was previously tested in soil drench and foliar application methods (Jaleel et al., 2007c). The increase in reduced glutathione can be correlated with its ability to scavenge single oxygen, peroxides and hydroxyl radicals and is involved in recycling of AA in the ascrobate-glutathione pathway in chloroplasts (Zhao et al., 2008). GA up to 1.2µM induced changes in glutathione metabolism, which was associated with anthocyanin content in *C. roseus* cell cultures (Jaleel et al., 2007h).

PGPR can affect plant growth directly or indirectly. Indirect promotion of plant growth occurs when PGPR antagonize or prevent the effects of phytopathogens or deleterious microorganisms (Karthikeyan et al., 2007). The fact that no significant growth promotion was found in response to *P. polymyxa* inoculation under gnotobiotic conditions, in the absence of pathogens or deleterious microorganisms, supports the idea that indirect growth-promoting mechanisms might be involved. Most mechanisms proposed to explain indirect growth promotion suggest that the active principle may be a secondary bacterial metabolite that antagonizes pathogens.

Growth regulator treatments resulted in an increase in α-tocopherol content in *Catharanthus roseus*. It was observed that the α-tocopherol was consumed predominantly as a radical scavenging antioxidant against the lipid peroxidation in the soybean membrane. The active oxygen species formed at the membrane of wheat leaves under water stress was efficiently removed upon rehydration with increase in the content of α-tocopherol and β-carotene (Jaleel et al., 2007m,n,o). The increase in α-tocopherol level in the triazole treated *Catharanthus* plants can increase the antioxidant potential in the plants (Jaleel et al., 2006a).

References

Backer, H., O. Frank, B. De Angells and S. Feingold. (1980) Plasma tocopherol in man at

various times after ingesting free or ocetylaned tocopherol. Nutr. Rep. Int. 21: 531-536.

Blokhina O, Virolainen E, Gagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91: 179-194

Gomathinayagam M., Jaleel CA,G. M. A. Lakshmanan, R. Panneerselvam (2007) Changes in carbohydrate metabolism by triazole growth regulators in cassava (*Manihot esculenta* Crantz); effects on tuber production and quality. Comptes Rendus Biologies. 330: 644-655.

Gopi R., Jaleel CA,R. Panneerselvam (2008) Leaf anatomical responses of *Amorphophallus campanulatus* to triazoles fungicides. EurAsian Journal of Biosciences. 2: 46-52.

Gopi R., Jaleel CA,R. Sairam, G. M. A. Lakshmanan, M. Gomathinayagam, R. Panneerselvam (2007) Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of *Daucus carota* L. Colloids and Surfaces B: Biointerfaces. 60: 180-186. IMPACT FACTOR: 2.109

Griffith O.W., A. Meister (1979) Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (s-n-butylhomocysteine sulfoximine), J.Biol. Chem. 254: 7558-7560.

Hajihashemi, S., K. Kiarostami, A. Saboora, S. Enteshari (2007) Exogenously applied paclo-butrazol modulates growth in salt-stressed wheat plants. Plant Growth Regul. 53: 117–128.

Jaleel CA, A. Kishorekumar, P. Manivannan, B. Sankar, M. Gomathinayagam, Gopi R, R. Somasundaram and R. Panneerselvam (2007m) 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.

Jaleel CA, B. Sankar, P.V. Murali, M. Gomathinayagam, G.M.A. Lakshmanan, R. Panneerselvam (2008c) Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation, Colloids and Surfaces B: Biointerfaces 62(1)105-111.

Jaleel CA, B. Sankar, R. Sridharan, R. Panneerselvam. (2008j) Soil salinity alters growth, chlorophyll contents and secondary metabolite accumulation in *Catharanthus roseus*. Turkish Journal of Biology. 32: 79-83.

Jaleel CA, G.M.A. Lakshmanan, M. Gomathina-yagam, R. Panneerselvam(2008f) Triad ime- fon induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system. South African Journal of Botany. 74(1): 126-132.

Jaleel CA, P. Manivannan, A. Kishorekumar, B. Sankar, Gopi R, R. Somasundaram and R. Panneerselvam (2007b) Alterations in osmo-regulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. Colloids and Surfaces B: Biointerfaces, 59: 150–157.

Jaleel CA, P. Manivannan, A. Kishorekumar, B. Sankar, R. Panneerselvam (2007c) Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in Catharanthus roseus. Comptes Rendus Biologies. 330(9): 674-683.

Jaleel CA, P. Manivannan, B. Sankar, A. Kishorekumar, Gopi R, R. Somasundaram and R. Panneerselvam (2007f) *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. Colloids and Surfaces B: Biointerfaces. 60: 7–11.

Jaleel CA, P. Manivannan, B. Sankar, A. Kishorekumar, Gopi R, R. Somasundaram, R. Panneerselvam (2007g) Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*; effects on oxidative stress, proline metabolism and indole alkaloid accumulation. Colloids and Surfaces B: Biointerfaces. 60: 110–116.

Jaleel CA, P. Manivannan, B. Sankar, A. Kishorekumar, Gopi R, R. Somasundaram, R. Panneerselvam (2007i) Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary metabolite accumula- tion. Colloids and Surfaces B: Biointerfaces. 60(2): 201-206

Jaleel CA, P. Manivannan, B. Sankar, A. Kishorekumar, R. Panneerselvam (2008k) Salt stress mitigation by calcium chloride in *Phyllanthus amarus*. Acta Botanica Croatica. 67 (1):53–62

Jaleel CA, P. Manivannan, B. Sankar, A. Kishorekumar, S. Sankari, R. Panneerselvam (2007a) Paclobutrazol enhances photosynthesis and ajmalicine production in *Catharanthus roseus*. Process Biochemistry. 42: 1566–1570.

Jaleel CA, P. Manivannan, G.M.A. Lakshmanan, M. Gomathinayagam, R. Panneerselvam (2008b) Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits Colloids and Surfaces B: Biointerfaces. 61(2): 298-303.

Jaleel CA, P. Manivannan, G.M.A. Lakshmanan, R. Sridharan, R. Panneerselvam (2007j) NaCl as a physiological modulator of proline metabolism and antioxidant potential in *Phyllanthus amarus*. Comptes Rendus Biologies. 330: 806–813.

Jaleel CA, P. Manivannan, M. Gomathinayagam, R. Sridharan, R. Panneerselvam (2007k) Responses of antioxidant potentials in *Dioscorea rotundata* Poir. following paclobutrazol drenching. Comptes Rendus Biologies. 330: 798–805.

Jaleel CA, P. Manivannan, P.V. Murali, M. Gomathinayagam, R. Panneerselvam (2008d) 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

Jaleel CA, R. Gopi, M. Gomathinayagam, R. Panneerselvam (2008m) Calcium chloride effects on metabolism of *Dioscorea rotundata* exposed to sodium chloride-induced salt stress. Acta Biologica Cracoviensia Series Botanica. 50(1): 63–67

Jaleel CA, R. Gopi, P. Manivannan, M. Gomathinayagam, Shao Hong-Bo, Chang-Xing Zhao, R. Panneerselvam (2008o) Endogenous hormonal and enzymatic responses of *Catharanthus roseus* with triadimefon application under water deficits. Comptes Rendus Biologies. 331: 844-852.

Jaleel CA, R. Gopi, P. Manivannan, R. Panneerselvam (2007o) Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. Turkish Journal of Botany 31: 245-251.

Jaleel CA, Gopi R, P. Manivannan, R. Panneerselvam (2008l) Exogenous application of triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal. Pesticide Biochemistry and Physiology. 91(3): 170-174

Jaleel CA, R. Gopi, P. Manivannan, R. Panneerselvam (2008i) Soil salinity alters the morphology in *Catharanthus roseus*; effects on endogenous mineral constituents. EurAsian Journal of BioSciences 2: 18-25.

Jaleel CA, R. Gopi, R. Panneerselvam (2008n) Biochemical alterations in white yam (*Dioscorea rotundata* Poir.) under triazole fungicides; impacts on tuber quality. Czech Journal of Food Sciences. 26(4): 298–307.

Jaleel CA, Gopi R, A. Kishorekumar, P. Manivannan, B. Sankar, R. Panneerselvam (2008h) Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiologiae Plantarum. 30: 287-292.

Jaleel CA, Gopi R, B. Sankar, M. Gomathinayagam, R. Panneerselvam. (2008e) Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. Comptes Rendus Biologies. 331(1): 42-47.

Jaleel CA, Gopi R, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan and R. Panneerselvam (2007d) Alterations in germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South African Journal of Botany. 73: 190-195.

Jaleel CA, Gopi R, G. M. Alagulakshmanan and R. Panneerselvam (2006a) Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Science. 171: 271-276.

Jaleel CA, Gopi R, P. Manivannan and R. Panneerselvam (2007e) Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. Acta Physiologiae Plantarum. 29: 205-209.

Jaleel CA, Gopi R, P. Manivannan, A. Kishorekumar, B. Sankar. R. Panneerselvam (2006b) Paclobutrazol influences vegetative growth and floral characteristics of Catharanthus roseus (L.) G. Don. Indian Journal of Applied and Pure Biology. 21: 369-372.

Jaleel CA, Gopi R, P. Manivannan, A. Kishorekumar, M. Gomathinayagam and R. Panneerselvam (2007n) Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage. Journal of Zhejiang University Science B. 8(4): 283-288.

Jaleel CA, Gopi R, P. Manivannan, B. Sankar, A. Kishorekumar, R. Panneerselvam (2007h) Antioxidant potentials and ajmalicine accumulation in *Catharanthus roseus* after treatment with giberellic acid. Colloids and Surfaces B: Biointerfaces. 60(2), 195-200.

Jaleel CA, Gopi R, P. Manivannan, M. Gomathinayagam, P.V. Murali, R. Panneerselvam (2008a) Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status. Pesticide Biochemistry and Physiology. 90(2): 135-139.

Jaleel CA, Gopi R, R. Panneerselvam (2007l) 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.

Jaleel CA, Gopi R, R. Panneerselvam (2008g) Growth and photosynthetic pigments responses of two varieties of *Catharanthus roseus* to triadimefon treatment. Comptes Rendus Biologies. 331: 272–277.

Jaleel CA, R. Panneerselvam (2007) Variations in the antioxidative and indole alkaloid status in different parts of two varieties of *Catharanthus roseus*, an important folk herb. Chinese journal of Pharmacology and Toxicology. 21(6): 487-494.

Karthikeyan B., C Abdul Jaleel, Gopi R, M. Deiveekasundaram (2007) Alterations in seedling vigour and antioxidant enzyme activities in *Catharanthus roseus* under seedpriming with native diazotrophs. Journal of Zhejiang University Science B, 8: 453-457.

Karthikeyan B., Jaleel CA,G.M. Alagu Lakshmanan, M. Deiveekasundaram (2008a) Studies on rhizosphere microbial diversity of some commercially important medicinal plants. Colloids and surfaces B: Biointerfaces. 62(1) 143-145.

Karthikeyan B., Jaleel CA,Zhao Changxing, M.M. Joe, J. Srimannarayan, M. Deiveekasundaram. AM (2008b) Fungi and phosphorus levels enhances the biomass yield and ajmalicine production in *Catharanthus roseus*. Eur Asian Journal of Biosciences. 2: 26-33

Kishorekumar A., Jaleel CA,P. Manivannan, B. Sankar, R. Sridharan, R. Somasundaram, R. Panneerselvam (2006) Differential effects of hexaconazole and paclobutrazol on the foliage characteristics of Chinese potato (*Solenostemon rotundifolius* Poir., J.K. Morton), Acta Biologica Szegediensis, 50: 127-129.

Kishorekumar A., Jaleel CA,P. Manivannan, B. Sankar, R. Sridharan, R. Panneerselvam (2007) Comparative effects of different triazole compounds on growth, photosynthetic pigments and carbohydrate metabolism of *Solenostemon rotundifolius*. Colloids and Surfaces B: Biointerfaces. 60:207-212.

Kishorekumar A., Jaleel CA,P. Manivannan, B. Sankar, R. Sridharan, P.V. Murali, R. Panneerselvam (2008) Comparative effects of different triazole compounds on antioxidant metabolism of Solenostemon rotundifolius. Colloids and Surfaces B: Biointerfaces. 62:307–311.

Lakshmanan G. M. A, Jaleel CA,M. Gomathinayagam, R. Panneerselvam (2007) Changes in antioxidant potential and sink organ dry matter with pigment accumulation induced by hexaconazole in *Plectranthus forskholii* Briq. Comptes Rendus Biologies. 330: 814–820.

Magnotta, M., J. Murata, J. Chen, V. De Luca (2006) Identification of a low vindoline accumulating cultivar of *Catharanthus roseus* (L.) G. Don. by alkaloid and enzymatic profiling. Phytochemistry. 67**:** 1758–1764.

Malick, C.P., M.B. Singh (1980) Plant enzymology and histo enzymology, Kalyani Publishers, New Delhi, p. 286.

Manivannan P., Jaleel CA,A. Kishorekumar, B. Sankar, R. Somasundaram, R. Sridharan and R. Panneerselvam (2007) Changes in antioxidant metabolism of *Vigna unguiculata* (L.) Walp. By propiconazole under water deficit stress. Colloids and Surfaces B: Biointerfaces. 57:69–74.

Manivannan P., Jaleel CA,A. Kishorekumar, B. Sankar, R. Somasundaram, R. Panneerselvam (2008a) Protection of *Vigna unguiculata* (L.) Walp. plants from salt stress by paclobutrazol. Colloids and Surfaces B: Biointerfaces. 61(2): 315-318.

Manivannan P., Jaleel CA,B. Sankar, A. Kishorekumar, R. Somasundaram, G.M. Alagu Lakshmanan and R. Panneerselvam (2007) Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. Colloids and surfaces B: Biointerfaces. 59:141–149.

Manivannan P., Jaleel CA,R. Somasundaram, R. Panneerselvam (2008b) Osmoregulation and antioxidant metabolism in drought stressed *Helianthus annuus* under triadimefon drenching. Comptes Rendus Biologies. 331(6): 418-425.

Omaye, S.T., J.D. Turn bull and H.E. Sauberilick (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Method Enzymol. 62: 3-11.

Sankar B., Jaleel CA,P. Manivannan, A. Kishorekumar, R. Somasundaram, 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.

Shao Hong-Bo, Li-Ye Chu, Jaleel CA,Chang-Xing Zhao (2008) Water-deficit stress-induced anatomical changes in higher plants.Comptes Rendus Biologies. 331:215–225.

Shao Hong-bo, Li-ye Chu, Ming-an Shao, Cheruth Abdul Jaleel, Mi Hong-mei (2008) Higher plant antioxidants and redox signaling under environmental stresses.Comptes Rendus Biologies. 331(6):433-441.

Zhao Chang-Xing, Guo Ling-Yu, Jaleel CA,Shao Hong-Bo, Yang Hong-Bing (2008) Prospects for dissecting plant-adaptive molecular mechanisms to improve wheat cultivars in drought environments. Comptes Rendus Biologies. 331:579–586.