

Fate of biochemical components of *Catharanthus roseus* after treatment with different plant growth regulators

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Abstract

The effect of different plant growth regulators like paclobutrazol (PBZ) gibberellic acid and *Pseudomonas fluorescens* treatments on the biochemical 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 DAP. The plants were taken randomly on 45, 60, 75 and 90 DAP and separated into root, stem and leaves and used for determining biochemicals. Proline, amino acid and glycine betaine were extracted and assayed from both control and treated plants. It was found that plant growth regulators have a profound effect on the biochemicals and caused an enhancement in *Catharanthus roseus*. Our results have good significance, as this alters the biochemical status of this medicinal plant, as this plant being an essential component of traditional as well as modern pharmaceutical systems.

Keywords: *Catharanthus roseus*; Paclobutrazol; Proline, amino acid; glycine betaine; *Pseudomonas fluorescens*; gibberellic acid

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 and Panneerselvam 2007). 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., 2006a,b, 2007a, 2008a). Globally a positive trend has blossomed in favor of traditional and integrative health sciences both in research and practices. 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., 2007b-f).

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 (Jaleel et al., 2007b-e, 2008b-f). Roots of this plant are the main source of an anti-hypertension alkaloid ajmalicine (Jaleel et al., 2006). *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., 2006, 2007a). Triazole compounds are systemic fungicides having plant growth regulating properties. The plant growth

Table 1. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on proline content in roots (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|---------------------------|---------------------------|----------------------------|---------------------------|
| 45 | 0.51 ± 0.018 ^a | 0.62 ± 0.020 ^b | 0.53 ± 0.019 ^d | 0.54 ± 0.019 ^c |
| 60 | 0.52 ± 0.019 ^a | 0.72 ± 0.027 ^b | 0.54 ± 0.0120 ^d | 0.56 ± 0.020 ^c |
| 75 | 0.67 ± 0.022 ^a | 0.75 ± 0.023 ^b | 0.68 ± 0.022 ^a | 0.71 ± 0.024 ^c |
| 90 | 0.69 ± 0.023 ^a | 0.78 ± 0.028 ^b | 0.73 ± 0.027 ^a | 0.71 ± 0.028 ^c |

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).

regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones including Gibberellic acid, ABA and Cytokinins (Kamounsis and Chronopoulou-Sereli, 1999). Triazoles induce a variety of morphological and biochemical responses in plants, including inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in ABA, as well as conferring protection from various environmental stresses (Jaleel et al., 2007f). Protection of plants from apparently unrelated stress by triazole is also mediated by a reduction in free radical damage and increases in the antioxidant potential and has an efficient free-radical scavenging system that enables them to detoxify active oxygen (Kopyra and Gwozdz, 2003). Paclobutrazol (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-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. Paclobutrazol has been proved as an agent in stress amelioration in medicinal plants (Jaleel et al., 2007b, 2008d,e) and crop plants.

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 siderophores that solubilize and sequester iron, or production of plant growth regulators (hormones) (Kloepper 1997). Some bacteria support plant growth indirectly, by improving growth-restricting conditions either via production of antagonistic substances or by inducing resistance against plant pathogens. Since associative interactions of plant and microorganisms must have come into existence as a result of co-evolution, the use of latter group as bioinoculants must be pre-adapted, so that it form into a long-term sustainable agricultural system. A number of bacterial species associated with the plant rhizosphere belonging to

genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium* *Pseudomonas*, *Rhizobium* and *Serratia* are able to exert beneficial effects on plant growth.

The strong and rapidly stimulating effect of elicitor on plant secondary metabolism in medicinal plants attracts considerable attentions and research efforts (Zhao et al., 2005). 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. 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 (Han and Lee 2005). The objectives of the present study are to understand the effect of plant growth regulators such as paclobutrazol, gibberellic acid and *Pseudomonas fluorescens* elicitors on the biochemical contents of *Catharanthus roseus* plants under field conditions.

Materials and methods

Medicinally important plant species, *Catharanthus roseus* (L.) G. Don. (Family: Apocynaceae) was selected for the present investigation. The seeds were obtained from Herbal Folklore Research Centre, Tirupati, Andhra Pradesh, India. The triazole compound paclobutrazol was obtained from Syngenta, India Ltd., Mumbai. The plant growth regulator Gibberellic acid (GA₃) was purchased from Himedia India Ltd., Mumbai. The elicitor, *Pseudomonas fluorescens* was obtained from Krishi Care Bioinputs, Chennai, India. During the study, average temperature was 32/26°C (maximum/minimum) and relative humidity (RH) varied between 60-75 per cent. The experimental part of this work was carried out in Botanical Garden and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu. The methodo-logies adopted are described below.

Table 2. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on proline content in stem (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| 45 | 0.51 ± 0.018 ^a | 0.61 ± 0.023 ^b | 0.54 ± 0.018 ^d | 0.58 ± 0.023 ^c |
| 60 | 0.65 ± 0.021 ^a | 0.76 ± 0.023 ^b | 0.68 ± 0.021 ^d | 0.72 ± 0.022 ^c |
| 75 | 0.71 ± 0.028 ^a | 0.81 ± 0.035 ^b | 0.75 ± 0.027 ^c | 0.77 ± 0.029 ^a |
| 90 | 0.82 ± 0.027 ^a | 0.91 ± 0.029 ^b | 0.85 ± 0.027 ^a | 0.87 ± 0.028 ^a |

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).

Cultivation methods

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. The land was repeatedly ploughed and brought to fine tilth and divided into 28 plots prior to transplantation. 60 plants per plot were planted. The seedlings were transplanted at a distance of 30 × 45 cm in plots. Irrigation was done twice in a week to keep the optimum moisture level required in the soil.

Treatments

Determination of optimum concentration of paclobutrazol

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 dry weight and growth. Hence 10 mg L⁻¹ paclobutrazol concentration was used to study the effect of paclobutrazol on the *C. roseus* plant.

Determination of optimum concentration of ga₃

In the preliminary experiments, 1, 2, 3, 4, 5 and 6 μM GA₃ was used for treatment to determine the optimum concentration of GA₃. Among the treatments, 5 μM GA₃ 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 dry weight and growth. Hence 5 μM GA₃ concentration was used to study the effect of GA₃ on the *C. roseus* plant.

Determination of optimum concentration of *P. fluorescens*

In the preliminary experiments 0.5, 1, 2, and 3 mg *P. fluorescens* was used for treatment to determine the optimum concentration of *P. fluorescens*. Among the treatments, 1 mg *P. fluorescens* concentration increased the dry weight significantly and higher concentration slightly decreased the growth and dry weight. In the lower and higher concentrations, there was no change in dry weight and growth. Hence 1 mg *P. fluorescens* concentration was used to study the effect of *P. fluorescens* on the *C. roseus* plant.

Treatments and samplings

Seven plots were selected by randomized block design (RBD). 10 mg L⁻¹ paclobutrazol, 5 μM gibberellic acid and 1 mg *Pseudomonas fluorescens* concentrations were used for the treatments and control plants were irrigated with well water. 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 flowers and used for determining biochemical constituents.

Statistics

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 seven samples in each group. P values ≤ 0.05 were considered as significant.

Table 3. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on proline content in leaf (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| 45 | 0.48 ± 0.017 ^a | 0.58 ± 0.018 ^a | 0.51 ± 0.017 ^a | 0.51 ± 0.018 ^a |
| 60 | 0.58 ± 0.023 ^a | 0.67 ± 0.023 ^a | 0.60 ± 0.005 ^a | 0.62 ± 0.023 ^a |
| 75 | 0.62 ± 0.023 ^a | 0.72 ± 0.025 ^a | 0.63 ± 0.024 ^a | 0.64 ± 0.025 ^a |
| 90 | 0.71 ± 0.028 ^a | 0.78 ± 0.035 ^a | 0.73 ± 0.031 ^a | 0.74 ± 0.030 ^a |

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).

Biochemical analysis

Proline

Proline content was estimated following the method of Bates et al. (1973).

Extraction

Five hundred mg of plant material was taken in a pestle and mortar and homogenized with 10 ml of 3 per cent aqueous sulfosalicylic acid. Then the homogenate was filtered through whatman No. 2 filter paper. The residue was re-extracted two times with 3 per cent sulfosalicylic acid and pooled. The filtrates were made upto 20 ml with 3 per cent sulfosalicylic acid and used for the estimation of proline.

Estimation

2 ml of extract was taken in a test tube and 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to it. The mixture was incubated for one hour at 100 °C in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then, to each test tube 4 ml of toluene was added and mixed vigorously using a test tube and stirred for 10-20 seconds. The toluene containing the chromophore was separated from the aqueous phase with the help of separating funnel and the absorbance was measured at 520 nm in a spectrophotometer using an appropriate blank. The proline content was determined from a standard curve prepared with proline and the results were expressed in mg per g dry weight.

Aminoacid

Extraction and estimation of the amino acid content was followed by the method of Moore and Stein (1948).

Extraction

0.5 gram of plant material taken in a pestle and mortar and homogenized with 10 ml of 80 per cent boiling ethanol. The extract was centrifuged at 800 g for 15 minutes and the supernatant was made upto 10 ml with 80 per cent ethanol and used for the estimation of free amino acids.

Estimation

1 ml of ethanol extract was taken in a 25 ml test tube and neutralized with 0.1 N sodium hydroxide using methyl red indicators. To which, 1 ml ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes then 5 ml of diluting 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 amino acid content was calculated using the standard graph. The results were expressed in milligrams per gram dry weight.

Glycine Betaine

Extraction

The samples were extracted and estimated following the method of Grieve and Grattan (1983). Five hundred milligrams of finely ground dry plant sample were mechanically shaken with 20ml of distilled water for 24 hours at 25°C. Time required for this step was determined by extracting the plant samples for 4, 8, 16, 24 and 48 hours. The samples were then filtered through Whatmann No.1 filter paper and the filtrates were made upto 20ml with deionized water and used for estimation immediately.

Table 4. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on aminoacid content in root (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| 45 | 6.30 ± 0.217 ^a | 8.10 ± 0.300 ^b | 7.20 ± 0.252 ^c | 7.41 ± 0.252 ^d |
| 60 | 10.46 ± 0.374 ^a | 12.08 ± 0.352 ^b | 11.24 ± 0.377 ^a | 11.51 ± 0.373 ^c |
| 75 | 14.31 ± 0.493 ^a | 16.01 ± 0.529 ^b | 15.20 ± 0.483 ^a | 15.30 ± 0.494 ^a |
| 90 | 13.20 ± 0.440 ^a | 17.12 ± 0.502 ^b | 15.12 ± 0.499 ^c | 14.01 ± 0.499 ^a |

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).

Estimation

One millilitre of the extract was diluted with one millilitre of 2N H₂SO₄ and 0.5ml of this acidified extract was cooled in ice water for 1 hour. Later 0.2ml of cold potassium tri iodide solution was added and mixed gently with a vortex mixture and the tubes were stored at 4°C for 15 minutes and then centrifuged at 10,000 g for 15 minutes at 0°C. The supernatant was aspirated with a fine tipped glass tube. The per iodide crystals were dissolved in 9 ml of 1,2-dichloroethane with vigorous vortexing. After 2.5 hours the absorbance was measured at 365 nm in a Spectrophotometer. Reference standard of glycine betaine was prepared in 1 N H₂SO₄ and used for estimating the glycine betaine content and the results were expressed in µg/g dry weight.

Results

Proline Content

Root (Table 1)

In roots, the proline content increased with the age in control and treated *Catharanthus roseus* plants in all growth stages. There was slight increase in proline content under gibberellic acid treatments when compared to control. Higher proline content was noted on 60 DAP in PBZ treatments and it was nearly 138.46 percent over control. Among the treatments Gibberellic acid showed lower proline contents on 75 DAP when compared to control.

Stem (Table 2)

In stem the proline contents increased with the age in control and treated *Catharanthus roseus* plants in all stages. In stem tissue also, there was only a marginal increase in proline content under Gibberellic acid treatments when compared to control. A maximum of increase was noted on 45 DAP in PBZ treatments and it was 119.61 percent over control.

Leaf (Table 3)

The proline content of the leaf tissue increased with the age in control and treated *Catharanthus roseus* plants in all stages. There was only a marginal increase in proline content under gibberellic acid treatments when compared to control. A least increase was recorded in 75 DAP samples under gibberellic acid treatments and which was upto 101.61 percent over control.

Aminoacid Content

Root (Table 4)

The aminoacid content increased with the age in control and treated *Catharanthus roseus* plants in all growth stages. The increase was very higher and significant under PBZ treatments when compared to Gibberellic acid and *P. fluorescens* treatments and it was 129.69 percent over control in root tissue of *Catharanthus roseus* plants on 90 DAP.

Stem (Table 5)

In stem, the aminoacid content increased with the age in control and treatments. There was only a marginal increase in aminoacid content under gibberellic acid treatments when compared to control on 75 DAP. A maximum of increase was noted on 60 DAP with PBZ treatments and it was nearly 125.33 percent over control.

Leaf (Table 6)

The aminoacid contents of the leaf tissue increased with the age in control and treated plants in all growth stages. There was only slight increase in aminoacid content under gibberellic acid treatments when compared to control. A lower aminoacid content was recorded in 60 DAP under gibberellic acid treatments and which was upto 101.76 percent over control. Higher aminoacid content was noted

Table 5. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on aminoacid content in stem (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| 45 | 8.20 ± 0.302 ^a | 9.45 ± 0.293 ^b | 8.38 ± 0.312 ^a | 8.71 ± 0.353 ^a |
| 60 | 9.12 ± 0.292 ^a | 11.43 ± 0.384 ^b | 9.36 ± 0.298 ^a | 10.11 ± 0.373 ^c |
| 75 | 11.31 ± 0.374 ^a | 13.32 ± 0.444 ^b | 11.45 ± 0.387 ^a | 12.45 ± 0.384 ^c |
| 90 | 12.28 ± 0.353 ^a | 14.11 ± 0.493 ^b | 12.71 ± 0.353 ^a | 13.37 ± 0.354 ^a |

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).

Table 6. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on aminoacid content in leaf (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| 45 | 7.01 ± 0.294 ^a | 9.20 ± 0.295 ^b | 7.28 ± 0.296 ^a | 8.04 ± 0.324 ^c |
| 60 | 8.50 ± 0.325 ^a | 10.31 ± 0.375 ^b | 8.65 ± 0.332 ^a | 9.03 ± 0.295 ^a |
| 75 | 10.04 ± 0.374 ^a | 12.20 ± 0.365 ^b | 10.64 ± 0.377 ^a | 11.13 ± 0.384 ^c |
| 90 | 12.12 ± 0.385 ^a | 14.13 ± 0.494 ^b | 13.00 ± 0.445 ^a | 13.12 ± 0.446 ^a |

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).

in 45 DAP samples under PBZ treatments and it was 131.24 percent over control.

Glycine Betaine Content

Root (Table 7)

The glycine betaine content increased in all the parts of plant with the age in control and treatments. There was only a marginal increase in glycine betaine content under gibberellic acid and *P. fluorescens* but significantly increased under PBZ treatments when compared to control. In roots, a maximum of increase was noted on 45 DAP in PBZ treatments and it was 116.35 percent over control. In *P. fluorescens* treatments the increase was upto 101.49 percent over control.

Stem (Table 8)

The glycine betaine content significantly increased under PBZ treatments when compared to control. In stem, a maximum of increase was noted on 90 DAP in PBZ treatments and it was nearly 120.77 percent over control. A least increase was recorded in 75DAP samples under *P. fluorescens* treatments and which was upto 104.09 percent over control.

Leaf (Table 9)

In leaf tissue, the glycine betaine content significantly increased under PBZ treatments when compared to control. A maximum of increase was noted on 90 DAP in PBZ treatments and it was nearly 121.84 percent over control. A least increase was recorded in 60 DAP samples under *P. fluorescens* treatments and which was upto 104.19 percent over control.

Discussion

Paclobutrazol treatment increased the free proline content in *Catharanthus* plants, but GA and *Pseudomonas fluorescens* have no significant effects. Treatment with triadimefon and uniconazole significantly increased the free proline content in mulberry (Sreedhar 1991). Triazole treatment increased the proline content in plants. Triazole induced a transient raise in abscisic acid content (Mackay et al., 1990) and this raise in ABA induced by paclobutrazol might have caused the increase in proline content in *Catharanthus roseus*. Tang et al. (2008) reported that, cultivars variation occurs in levels of free proline and gibberellins and a lower level of ABA, along with higher pollen vigour and pollen germination rate even after prolonged high temperature treatment in rice. Thus they suggested a

Table 7. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on glycine betaine content in root (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| 45 | 18.78 ± 0.722 ^a | 21.85 ± 0.798 ^b | 20.82 ± 0.685 ^c | 19.91 ± 0.722 ^d |
| 60 | 20.78 ± 0.717 ^a | 22.97 ± 0.801 ^b | 21.99 ± 0.793 ^c | 21.09 ± 0.792 ^d |
| 75 | 21.42 ± 0.793 ^a | 24.64 ± 0.887 ^b | 23.88 ± 0.844 ^c | 22.45 ± 0.803 ^a |
| 90 | 23.64 ± 0.844 ^a | 26.98 ± 0.902 ^b | 24.91 ± 0.883 ^a | 24.08 ± 0.873 ^c |

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).

Table 8. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on glycine betaine content in stem (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| 45 | 20.15 ± 0.892 ^a | 23.74 ± 0.843 ^b | 22.28 ± 0.803 ^c | 21.64 ± 0.792 ^a |
| 60 | 22.67 ± 0.802 ^a | 26.31 ± 0.905 ^b | 24.94 ± 0.883 ^c | 23.69 ± 0.842 ^a |
| 75 | 22.94 ± 0.804 ^a | 26.25 ± 0.904 ^b | 25.12 ± 0.894 ^a | 23.88 ± 0.864 ^a |
| 90 | 23.11 ± 0.843 ^a | 27.91 ± 0.923 ^b | 25.88 ± 0.899 ^c | 24.10 ± 0.883 ^a |

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).

Table 9. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on glycine betaine content in leaf (mg/g DW) of *Catharanthus roseus* on different growth stages.

| Growth Stages (DAP) | Control | Paclobutrazol | Gibberellic acid | <i>P. fluorescens</i> |
|---------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| 45 | 19.18 ± 0.764 ^a | 22.37 ± 0.804 ^b | 21.00 ± 0.004 ^c | 20.22 ± 0.894 ^a |
| 60 | 21.98 ± 0.899 ^a | 25.64 ± 0.895 ^b | 23.99 ± 0.865 ^c | 22.90 ± 0.895 ^a |
| 75 | 23.57 ± 0.844 ^a | 26.37 ± 0.905 ^b | 24.98 ± 0.884 ^a | 24.81 ± 0.897 ^a |
| 90 | 22.61 ± 0.895 ^a | 27.55 ± 0.914 ^b | 25.01 ± 0.895 ^c | 24.98 ± 0.899 ^d |

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).

possible correlation between IAA, GAs, ABA and free proline contents. Gibberellic acid treatments reduced the proline accumulation in maize plants (Kaya et al., 2006). Salt stressed plants accumulated various organic compounds such as proline, glucose and glycine betaine in the cell membrane for osmoregulation to occur to enable growth by protecting enzyme activity under PGPR strains (Han and Lee 2005).

The amino acid content increased a higher extent in all the parts of the *C. roseus* plant with treatments. Among the treatments, paclobutrazol caused higher level of amino acids accumulation in all parts. Triadimefon increased the amino acid content in *Phaseolus vulgaris* (Mackay et al., 1990), penconazole increased amino acid in higher plants (Radice and Pesci, 1991) and LAB-150978 treated sunflower

and mungbean (Saha and Gupta, 1993). Similarly triadimefon treatment increased the amino acid content in *Catharanthus* in all parts at all growth stages when compared to control (Jaleel et al., 2007e-g).

The treatments resulted in an increase in glycine betaine content in *C. roseus*. Paclobutrazol treatments increased the glycine betaine content on all sampling days. Glycine betaine is considered to be one of the most abundant quaternary ammonium compounds produced in higher plants under stressful environment (Yang et al., 2003). Penconazole increased the glycine betaine content in higher plants (Radice and Pesci 1991) and LAB-150978 treated sunflower and mungbean (Saha and Gupta 1993). Similarly triadimefon treatment increased the glycine betaine content in

Catharanthus in all parts at all growth stages when compared to control (Jaleel et al., 2008c-f, 2009a,b).

Salt stressed plants accumulated various organic compounds such as proline, glucose, glycine betaine etc. in the cell membrane for osmoregulation to occur to enable growth by protecting enzyme activity under PGPR strains (Han and Lee 2005).

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