Acta Pharmaceutica Sciencia
51: 157- 162 (2009)

Pseudomonas fluorescens as a physiological modulator in the enhancement of medicinally important alkaloids of *Catharanthus roseus*

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Abstract

In the present investigation, changes in individual alkaloid profiles were studied in *Catharanthus roseus* (L.) G. Don. plants under treatment with *Pseudomonas fluorescens*. The *Pseudomonas fluorescens* treatments were given by soil drenching on 38, 53, 68 and 83 days after planting (DAP) by soil drenching. The plants were taken randomly on 45, 60, 75 and 90 DAP and used for estimating individual alkaloids like Ajmalicine, Catharanthine, Tabersonine, Serpentine and Vindoline contents. It was found that, *Pseudomonas fluorescens* has a profound effect and it caused a significant enhancement in the production of individual alkaloids when compared to untreated control plants.

Key words: *Catharanthus roseus*, *Pseudomonas fluorescens*, Ajmalicine, Catharanthine, Tabersonine, Serpentine, Vindoline.

Introduction

The strong and rapidly stimulating effect of fungal elicitor on plant secondary metabolism in medicinal plants attracts considerable attentions and research efforts (Jaleel et al. 2007a). The reasons responsible for the diverse stimulating effects of fungal elicitors are complicated and could be related to the interactions between fungal elicitors and plant cells, elicitor signal transduction, and plant defense responses (Karthikeyan et al. 2008).

Catharanthus roseus (L.) G. Don. (Madagascar periwinkle) is one of the highly exploited and studied medicinal plants belong to the family Apocynaceae. *C. roseus* is a perennial tropical plant that produces more than 100 monoterpenoid indole alkaloids (MIAs) including two commercially important cytotoxic dimeric alkaloids used in cancer chemotherapy (Magnotta et al. 2006). *C. roseus* is a good source of non-enzymatic and enzymatic antioxidants (Jaleel et al. 2006, Jaleel and Panneerselvam 2007). Lot of works have already been carried out in this plant in its medicinal importance (Jaleel et al. 2007a-k, 2008a-j, 2009a, b), but the *Pseudomonas fluorescens* effects on this medicinal plant attracted a little attention. To the best of our knowledge, no information on the effect of *Pseudomonas fluorescens* on alkaloid production in this medicinal plant is available. This investigation was aimed for finding out the extent of changes in Ajmalicine, Catharanthine, Tabersonine, Serpentine and Vindoline production in *C. roseus* under *Pseudomonas fluorescens* treatment.

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Materials and Methods

Plant materials and cultivation methods

The seeds of *Catharanthus roseus* (L.) G. Don. were collected from J. P. Laboratories, Rajapalayam, Tamil Nadu, India. In an attempt to remove germination inhibitors, the seeds were leached with distilled water for 5 days before the experiment. Seeds were then surface sterilized in aqueous solution of 0.1 per cent $HgCl_2$ for 60 seconds to prevent fungal attack and rinsed in several changes of sterile water.

The seeds were sown separately in raised seed beds 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 four plots prior to transplantation. Two plots for each variety were prepared, forty plants per plot were planted for both the varieties at a distance of 30 × 45 cm and irrigated immediately for better establishment. Subsequent irrigation was done two times in a week to keep the optimum moisture level in the soil.

One plot for each variety was subjected to triadimefon treatment and another one was kept as control. *Pseudomonas fluorescens* @ 1 mg was given to each plant by soil drenching and another set of plants were subjected to foliar spray. The *Pseudomonas fluorescens* treatments were given by soil drenching on 38, 53, 68 and 83 days after planting (DAP) by soil drenching. The plants were taken randomly on 45, 60, 75 and 90 DAP and used for estimating individual alkaloids like Ajmalicine, Catharanthine, Tabersonine, Serpentine and Vindoline contents.

Extraction and estimation of alkaloids

Extraction of alkaloids ajmalicine, catharanthine, serpentine, tabersonine and vindoline were carried out by following the standard method of Tikhomiroff and Jolicoeur (2002). The quantity of alkaloids was expressed in µg/g FW (El-Sayed and Verpoorte 2004).

Results and Discussion

During the early stages of plant growth, the alkaloid contents were less in *C. roseus*. But the content increased with the age of plant in both control and treated plants. The alkaloid content increased in *Pseudomonas fluorescens* treated plants when compared to control. Similar results were obtained in PGR application in *Catharanthus* plants. Increased alkaloid content was also reported in *Catharanthus* plants by the application of 2,4-D, kinetin and IAA (Amit et al. 2005). In cell suspension culture, if the glucose concentration is increased in the medium, the secologanin got increased simultaneously with increase in the terpenoid indole alkaloid production in *Catharanthus* plants (Contin et al. 1998). There are reports on improvement of indole alkaloid production in cell cultures of *C. roseus* treated by various elicitors (Zaho et al. 2005). The content of alkaloids in *C. roseus* have been found influenced by individual factor, such as stage of plant growth and triadimefon, a plant growth regulator, treatment (Jaleel et al. 2006). In conclusion, our results indicated that the exogenous *Pseudomonas fluorescens* application at low concentration could be used as a potential tool to increase defense mechanisms and the level of active principles in medicinal plants.

Karthikeyan (2008) reported that due to PGPR inoculation besides increasing yield also enhanced the alkaloid contents of roots especially ajmalicine in *C. roseus* due to the production of IAA. In the present study, increase in ajmalicine content in the root may be due to the production of growth promoting substances like IAA by the PGPR. Since the PGPR, which normally induces, gibberellins, auxins and thereby by the rhizobacteria enhanced proliferation of root system, which in turn enhanced mineral uptake and consequently increased dry matter production (Karthikeyan et al. 2007)? Continuous availability of growth regulators induced different alkaloids with variable effects among the regulators (El-Syed and Verpoorte 2004).

Our results have good significance, as these increases the secondary metabolites of this traditional medicinal plant.

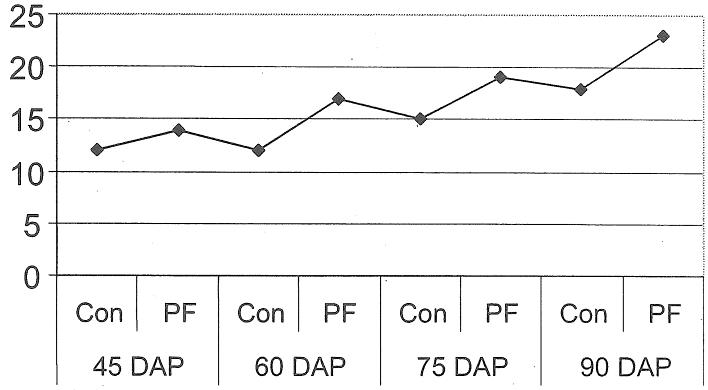

Figure 1. Effect of *Pseudomonas fluorescens* elicitation on alkaloid Ajmailicine contents of *C. roseus* seedlings. (Con- Control; PF- *Pseudomonas fluorescens*; DAP- Days after planting).

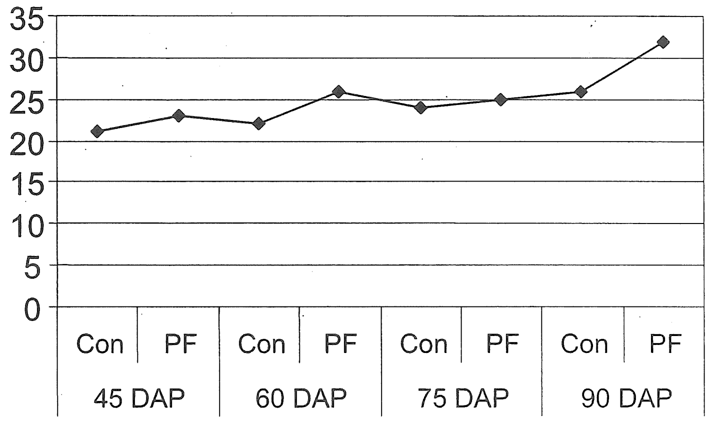

Figure 2. Effect of *Pseudomonas fluorescens* elicitation on alkaloid Serpentine contents of *C. roseus* seedlings. (Con- Control; PF- *Pseudomonas fluorescens*; DAP- Days after planting).

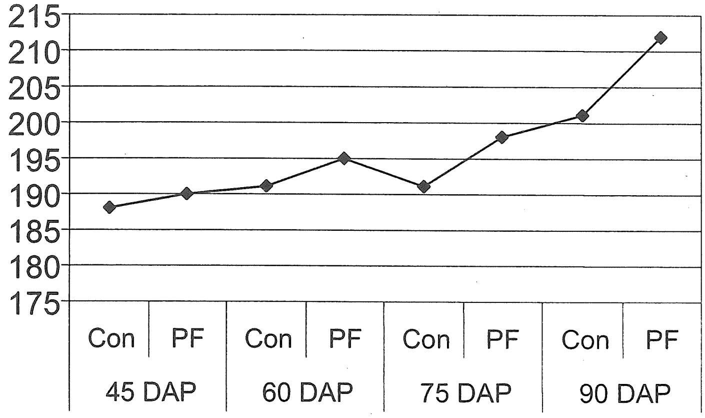

Figure 3. Effect of *Pseudomonas fluorescens* elicitation on alkaloid Catharanthine contents of *C. roseus* seedlings. (Con- Control; PF- *Pseudomonas fluorescens*; DAP- Days after planting).

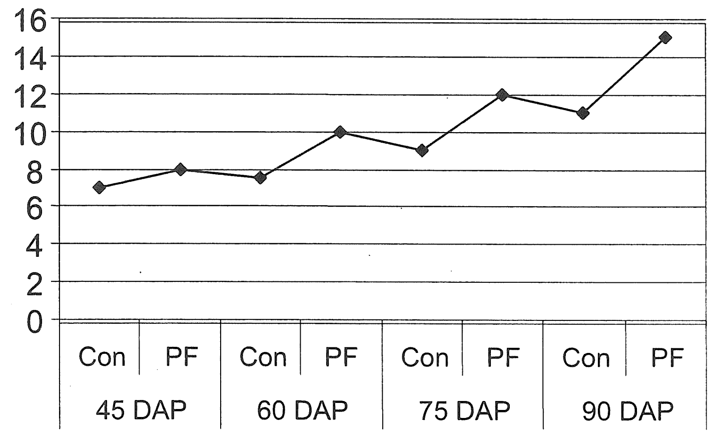

Figure 4. Effect of *Pseudomonas fluorescens* elicitation on alkaloid Tabersonine contents of *C. roseus* seedlings. (Con- Control; PF- *Pseudomonas fluorescens*; DAP- Days after planting).

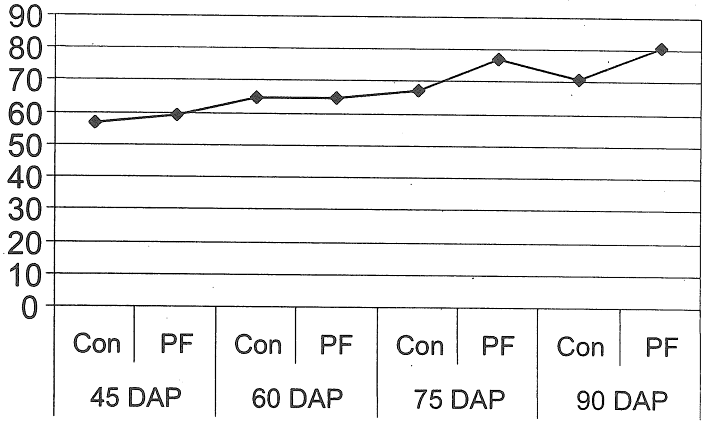

Figure 5. Effect of *Pseudomonas fluorescens* elicitation on alkaloid Vindoline contents of *C. roseus* seedlings. (Con- Control; PF- *Pseudomonas fluorescens*; DAP- Days after planting).

References

El-Sayed, M., Verpoorte, R. (2007). *Catharanthus* terpenoid indole alkaloids: biosynthesis and regulation. *Phytochem. Rev.* 6: 277–305.

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

Jaleel, C.A., Panneerselvam, R. (2007). Variations in the antioxidative and indole alkaloid status in different parts of two varieties of *Catharanthus roseus*, an important folk herb. *Chinese J. Pharmacol. Toxicol.* 21: 487-494.

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

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

Jaleel, C.A., Manivannan, P., Kishorekumar, A., Sankar, B., Gopi, R., Somasundaram, R., and Panneerselvam, R. (2007c). Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. *Colloids Surf. B: Biointerf.* 59: 150–157.

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

Jaleel, C.A., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R., Panneerselvam, R. (2007e). Alterations in germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *S. African J. Bot.* 73: 190-195.

Jaleel, C.A., Gopi, R., Manivannan, P., and Panneerselvam, R. (2007f). Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta Physiol. Plant.* 29: 205-209.

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

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

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

Jaleel, C.A., Gopi, R., Panneerselvam, R. (2007j). Alterations in lipid peroxidation, electrolyte leakage, and proline metabolism in *Catharanthus roseus* under treatment with triadimefon, a systemic fungicide, *Comptes Rendus Biol.* 330: 905-912.

Jaleel, C.A., Gopi, R., Manivannan, P., Panneerselvam, R. (2007k). Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. *Turk. J. Bot.* 31: 245-251.

Jaleel, C.A., Gopi, R., Manivannan, P., Gomathinayagam, M., Murali, P.V., Panneerselvam, R. (2008a). Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status. *Pest. Biochem. Physiol.* 90: 135-139.

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

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

Jaleel, C.A., Manivannan, P., Murali, P.V., Gomathinayagam, M., Panneerselvam, R. (2008d). Antioxidant potential and indole alkaloid profile variations with water deficits along different parts of two varieties of *Catharanthus roseus*. *Colloids Surf. B: Biointerf.* 62: 312–318

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

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

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

Jaleel, C.A., Gopi, R., Manivannan, P., Panneerselvam, R. (2008h). Soil salinity alters the morphology in *Catharanthus roseus*; effects on endogenous mineral constituents. *EurAsia. J. Biosci.* 2: 18-25.

Jaleel, C.A., Sankar, B., Sridharan, R., Panneerselvam, R. (2008i). Soil salinity alters growth, chlorophyll contents and secondary metabolite accumulation in *Catharanthus roseus*. *Turk. J. Biol.* 32: 79-83.

Jaleel, C.A., Gopi, R., Manivannan, P., Gomathinayagam, M., Hong-Bo, S., Zhao, C.X., Panneerselvam, R. (2008j). Endogenous hormonal and enzymatic responses of *Catharanthus roseus* with triadimefon application under water deficits. *Comptes Rendus Biol.* 331: 844-852.

Jaleel, C.A., Gopi, R., Gomathinayagam, M., Panneerselvam, R. (2009a). Traditional and non-traditional plant growth regulators alters phytochemical constituents in *Catharanthus roseus*. *Process Biochem.* 44: 205–209.

Jaleel, C.A., Gopi, R., Panneerselvam, R. (2009b). Alterations in non-enzymatic antioxidant components of *Catharanthus roseus* exposed to paclobutrazol, gibberellic acid and *Pseudomonas fluorescens*. *Plant Omics J.* 2: 30-40.

Karthikeyan, B., Jaleel, C.A., Gopi, R., Deiveekasundaram, M. (2007). Alterations in seedling vigour and antioxidant enzyme activities in *Catharanthus roseus* under seedpriming with native diazotrophs. *J. Zhejiang Univ. Sci. B.* 8: 453-457.

Karthikeyan, B., Jaleel, C.A., Changxing, Z., Joe, M.M., Srimannarayan, J., Deiveekasundaram, M. (2008). AM fungi and phosphorus levels enhances the biomass yield and ajmalicine production in *Catharanthus roseus*. *EurAsia. J. Biosci.* 2: 26-33.

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

Zhao, J., Lawrence, T., Davis, C., Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.* 23: 283–333.

Received: 02.04.2008
Accepted: 05.05.2009