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Changes in the photosynthetic characteristics of *Catharanthus roseus* L. as a result of exogenous growth regulators

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Abstract

In the present investigation, different plant growth regulators and retardants were used to analyse their effects on photosynthetic characteristics of common periwinkle (*Catharanthus roseus* (L.) G. Don., Family: Apocynaceae). The plant growth retardant used was paclobutrazol. The synthetic growth regulator used was gibberellic acid. The exogenously applied non-traditional growth regulator was an elicitor named *Pseudomonas fluorescens*. From the results of this investigation, it can be concluded that these growth retardant and regulators altered the photosynthetic characteristics of C. *roseus* to a great extent. These findings might have a great role in the ayurvedic or natural medicine, as more and more people became interested in traditional medicine, due to the secondary hazardous effects of most of the modern synthetic medicines.

Keywords: periwinkle; photosynthesis; growth regulators; growth retardants.

Introduction

The plant hormones are organic substances in low concentrations regulates the growth and development. These substances belongs to different classes have different physiological role in plants to modify, regulate and development. The naturally occurring plant growth substances include auxins, gibberellins, cytokinins, abscissic acid and ethylene (Kakimoto, 2003). The role of growth hormones in regulation of indole alkaloids has been extensively studied (Moreno et al., 1995). The term plant growth regulators are not only restricted to synthetic compounds but also include the naturally occurring hormones (Werner et al., 2001). Hence, the plant growth regulator can be defined as either natural or synthetic compounds that modify the plant growth and development pattern exerting profound influence on many physiological processes (Akazawa et al., 1990; Jaleel et al., 2006). Plant growth regulators are found to have many practical applications in controlling vegetative and reproductive growth and physiological activities of plant (Jaleel et al., 2007a). Phytohormones play a crucial role in the regulation and coordination of plant growth, morphogenesis and metabolism. It is thus postulated that they also play a role in the biosynthesis of alkaloids (Jaleel et al., 2007b).

The plant hormones gibberellin (GA) and abscisic acid (ABA) exert profound effects on fundamental processes of plant growth and development (Jaleel et al., 2009). Moreover, recent advances in understanding GA and ABA signaling point to the existence of multiple, non-linear cell and compartment-specific pathways that regulate genomic and non-genomic responses to these phytohormones. GA is widely regarded as a growth-promoting compound that positively regulates processes such as seed germination, stem elongation, leaf expansion, pollen-tube growth, flower and fruit development and floral transition

(Swain and Singh, 2005). ABA, by comparison, has historically and possibly unduly been considered to function as a growth inhibitor (Mahouachi et al., 2005). ABA regulates processes such as embryo maturation, seed development and germination, cell division and elongation, stomatal opening, root development, floral transition and tolerance to abiotic and biotic stress (Divya et al., 2009). In several of the above mentioned processes, including seed germination, floral transition and fruit development, GA and ABA have antagonistic effects, normally with GA promoting and ABA inhibiting these specific processes (Xie et al., 2006).

The gibberellins are a large family of tetracyclic diterpenoid plant growth substances. Some are biologically active others are inactive. The gibberellins are associated with various plant growth and development processes such as seed germination, stem and hypocotyl elongation, leaf expansion, floral initiation, floral organ development and induction of some hydrolytic enzymes in the aleurone of cereal grains (Gomi and Matsuoka, 2003). GAs plays a key role in control of shoot growth (Jaleel et al., 2007c). There are many recently identified factors in the GA signalling pathway (Gomi and Matsuoka, 2003).

The large-scale cultivation of medicinal plants is gaining importance nowadays and several agronomic practices are tried to enhance the production and also to increase the biochemical constituents (active principles). Use of microorganisms in the cultivation of field crop is well documented. However, their effects on medicinal plants have not been thoroughly studied (Karthikeyan et al., 2009). Moreover the literature on the role of microorganisms on the growth enhancement is very limited. Hence literature on the role of plant growth promoting rhizobacteria like *Azospirillum*, *Azotobacter Pseudomonas* and phosphate solubilising bacteria available on medicinal plant and in certain horticultural and agricultural crops are reviewed.

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

Materials and methods

The seeds of *Catharanthus roseus* 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 four plots prior to transplantation. 60 plants per plot were designed for both the varieties. The seedlings were transplanted at a distance of 30 × 45 cm in plots and irrigated immediately for better establishment. Subsequent irrigation was done twice in a week to keep the optimum moisture level required in the soil. In the preliminary experiments, 5, 10, 15 and 20 mg L^{-1} PBZ was used for treatment to determine the optimum concentration of PBZ. Among the treatments, 10 mg L^{-1} PBZ 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} PBZ concentration was used to study the effect of PBZ on the *C. roseus* plant. In the preliminary experiments, 1, 2, 3, 4, 5 and 6 µM GA_3 was used for treatment to determine the optimum concentration of GA_3. Among the treatments, 5 µM GA_3 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 GA_3 concentration was used to study the effect of GA_3 on the *C. roseus* plant.

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.

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 air dried in shade and used for estimation of photosynthetic pigments.

Estimation of chlorophyll and carotenoid contents

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949). Five hundred milligrams of fresh leaf material was ground with 10 ml of 80 per cent acetone at 4°C and centrifuged at 2500 rpm for 10 minutes at 4°C. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80 per cent acetone and assayed immediately.

Table 1. Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on total chlorophyll contents (mg/g FW) of *Catharanthus roseus* on different growth stages.

Growth Stages (DAP)	Control	Paclobutrazol	Gibberellic acid	*P. fluorescens*
45	0.060 ± 0.004^a	0.095 ± 0.005^a	0.079 ± 0.004^a	0.069 ± 0.004^a
60	0.063 ± 0.005^a	0.099 ± 0.005^a	0.084 ± 0.005^a	0.076 ± 0.005^a
75	0.097 ± 0.004^a	0.109 ± 0.005^a	0.100 ± 0.004^a	0.105 ± 0.007^a
90	0.088 ± 0.005^a	0.095 ± 0.004^a	0.092 ± 0.005^a	0.096 ± 0.005^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).

Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80 per cent acetone as blank. Chlorophyll content was calculated using the formula of Arnon.

Total chlorophyll (mg/ml) = (0.0202) × (A.645) + (0.00802) × (A.663)

Chlorophyll 'a' (mg/ml) = (0.0127) × (A.663) – (0.00269) × (A.645)

Chlorophyll 'b' (mg/ml) = (0.0229) × (A.645) – (0.00468) × (A.663)

and expressed in milligram per gram fresh weight.

Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight.

Carotenoid = A.480 + (0.114 × A.663 – 0.638 × A.645).

Anthocyanin

Anthocyanin was extracted and estimated from the flowers by the method of Beggs and Wellmann (1985).

Five hundred milligrams of flowers was cut into small pieces and immersed in 10 ml of HCl : methanol (1 : 100 v/v) and kept in darkness at 5°C for 24 hours. The absorbance of decanted extract was measured at 525nm in spectrophotometer (U-2001-Hitachi) against HCl : methanol (1:100 v/v) as blank. The results were expressed in milligrams per gram fresh weight.

Xanthophyll

Xanthophyll was extracted and estimated from the flowers by the method of Neogy *et al.* (2001).

Five hundred milligrams of fresh petels was ground with 10 ml of 80 per cent ethanol. The extract was centrifuged at 800 rpm for 15 minutes. The supernatant was collected and made up to 10 ml with 80 per cent ethanol. The absorbance was read at 450 nm against 80 per cent ethanol as blank. The results were expressed in milligrams per gram fresh weight.

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 and discussion

The total chlorophyll contents of the leaves increased with the age in control and treated *Catharanthus roseus*. But it slightly decreased on 90 DAP (Table 1). The highest increase was recorded in PBZ treatments when compared to other treatments. The carotenoid content (Fig. 1) increased with the age in control and treated plants on all sampling days. The highest increase was observed under PBZ treatments on 45 DAP. Gibberellic acid and *P. fluorescens* followed this. Among the treatments, gibberellic acid slightly increased the carotenoid content on 60 DAP.

The anthocyanin contents (Fig. 1) of the flowers increased with the age in control and treated *Catharanthus roseus*, but the increase was very high in PBZ treatments when compared to other treatments. Under gibberellic acid and *P. fluorescens* treatments, the increase in anthocyanin content was not significant ($P \leq 0.05$). But PBZ treatment significantly increased anthocyanin content on 45 DAP.

The xanthophyll content (Fig. 1) increased with the age in control and treated *Catharanthus roseus* plants. There was only slight increase in xanthophyll content under Gibberellic acid and *P. fluorescens* treatments but significantly increased under PBZ treatments when compared to control on 90 DAP. A least increase was recorded in 90 DAP samples under *P. fluorescens* treatments.

Treatments with paclobutrazol, GA and *P. fluorescens* significantly increased the total

chlorophyll contents in the leaves of *C. roseus* plants. Paclobutrazol treated barley seedlings (Sunitha *et al.* 2004) and tomato (Still and Pill 2003), retained two times more chlorophyll than control. Paclobutrazol treated leaves were dark green due to high chlorophyll content in potato (Jaleel *et al.* 2007d). This increased chlorophyll content was attributed to more densely packed chloroplast in a small leaf area as reported in maize (Khalil and Rahman 1995). It was also been reported that triadimefon has a cytokinin like activity with antisenescence properties. An increase in cytokinin caused by triazole treatment could lead to enhance chloroplast size and chlorophyll levels. Gibberellic acid increased the vegetative growth and pigment concentration in maize (Kaya *et al.* 2006). In *Lotus tenuis* low photosynthetic photon flux density induced an ortotropic growth of stems with greater supply of GA_1 and GA_3. Foliar application of GA_3 improved the chlorophyll levels in salinity stressed maize plants (Tuna *et al.* 2008).

Salamone et al. (2001) *P. fluorescens* produced highest amount of cytokinins viz., isopentenyl adenosine (IPA) trans–zeatinribose (ZR) and dihydrozeatinribose (DHZR) during stationary phase. Photosynthetic rate in all treatments containing PGPR was increased (Audenaert *et al.* 2002). This is understandable since the reduction of plant growth is the result of the alteration of many physiological activities in the plant, such as photosynthetic activity, mineral uptake and antioxidant activity. Chlorophyll content was also increased significantly in all the PGPR strain treatments in soybean (Salamone et al., 2001).

In the leaves of *C. roseus,* the carotenoid content increased in all treatments when compared to control. Increased carotenoid content was reported in *Withania somnifera* under triadimefon treatment (Jaleel *et al.* 2008b). Carotenoid are involved in the protection of the photosynthetic apparatus against photoinhibitory damage by singlet oxygen ($^1O_2$), which is produced by excited triplet stage of chlorophyll thus indirectly reducing the formation of reactive oxygen species in wheat (Foyer and Harbinson 1994). Increased level of cytokinin particularly transzeatin and its riboside has been reported in sunflower cell suspension, rice, soybean and rape seedlings after uniconazole treatment and thus increased zeatin might be responsible for the increased synthesis of carotenoid in these plants.

An increase in carotenoid content was reported in Maize plants (Kaya *et al.* 2006). Plant growth of wheat decreased with increasing salinity levels, but was increased by seed treatment with GA_3, which accompanied increased photosynthetic pigment contents. There seems to be no report in the literature on the effect of *Pseudomonas fluorescens* on carotenoid content of higher plants.

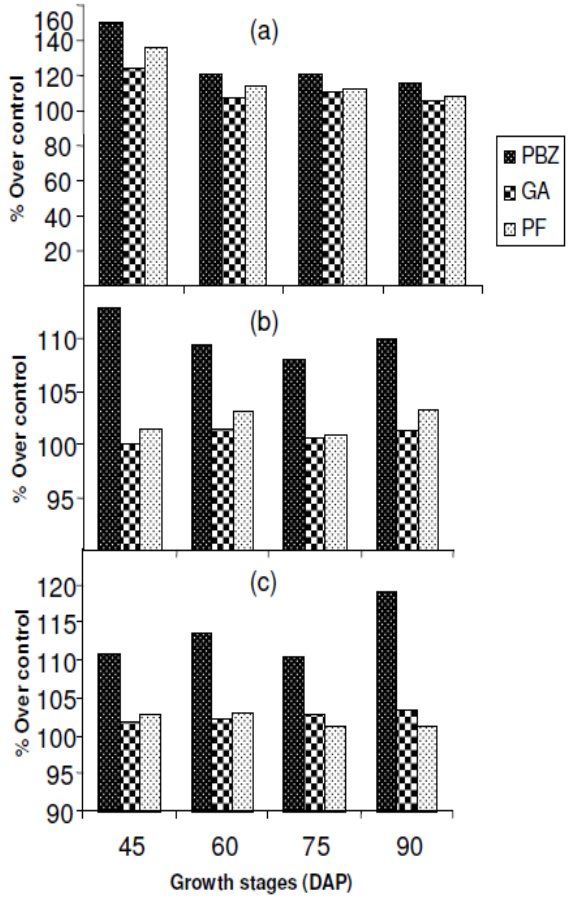

Fig 1. Effect of paclobutrazol (PBZ), gibberellic acid (GA) and *P. fluorescens* (PF) on (a) carotenoid (b) anthocyanin and (c) xanthophyll contents of *Catharanthus roseus* different growth stages. Bar values are representing the percentage increase or decrease from control values.

Paclobutrazol treatment increased the anthocyanin content in *Catharanthus* plants, but GA and *Pseudomonas fluorescens* have no significant effect upon this. Increased anthocyanin content with triazole treatment has been reported in *Catharanthus* plants (Jaleel et al., 2007d). Triadimefon increased the chlorophyll and anthocyanin content in radish cotyledons and Triazoles greatly increase anthocyanin accumulation in carrot tissue cultures (Ilan and Dougall 1992). Triazole induced a transient raise in abscisic acid content. This increased ABA content induced by triazole

might be the cause for the increased anthocyanin content.

In *C. roseus* plants the increase in anthocyanin content under GA_3 treatments was not so significant. An increase in anthocyanin content was reported in chick pea under gibberellic acid treatments (Kaur *et al.* 1998). The growth and flowering was controlled by the application of GA, by its effects on pigment composition. There seems to be no report in the literature on the effect of *P. fluorescens* on anthocyanin content of higher plants.

Paclobutrazol treatment increased the xanthophyll content in *Catharanthus* plants, but GA and *P. fluorescens* have no significant effects. Triadimefon treatment increased the chlorophyll, carotenoid and xanthophyll content in the leaves, they by protects the photosynthetic apparatus. The unsaturated C_{40} hydrocarbons not only give color to fruits and flowers but also have multiple functions in photosynthesis. They participate in light harvesting in photosynthetic membranes and protect the photosynthetic apparatus from excessive light energy by quenching triplet chlorophylls and singlet oxygen. Epoxiconazole increased the chlorophyll content to a larger extent when compared to the control in Gallium aparine plants (Benton and Cobb 1995). From the results of this investigation, it can be concluded that these growth retardant and regulators altered the photosynthetic characteristics of C. *roseus* to a great extent. These findings might have a great role in the ayurvedic or natural medicine, as more and more people became interested in traditional medicine, due to the secondary hazardous effects of most of the modern synthetic medicines.

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