

Leaf Anatomical Modifications in *Catharanthus roseus* as Affected by Plant Growth Promoters and Retardants

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Abstract: In the present investigation, different plant growth promoters and retardant were used to determine the anatomical characteristics of *Catharanthus roseus*. The plant growth promoters used were gibberellic acid (GA₃) and *Pseudomonas fluorescens* elicitors (PF Elicitors) and retardant was paclobutrazol (PBZ). The 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 the anatomical characteristics changes. From the results of this investigation it can be concluded that this traditional medicinal plant has positive responses towards plant growth promoters and retardants.

Key words: *Catharanthus roseus*, Apocynaceae, Paclobutrazol, Gibberellic acid, *Pseudomonas fluorescens*, Anatomical characteristics

INTRODUCTION

India is a gold mine of treasures with traditional and practical knowledge of herbal medicines. Globally a positive trend has blossomed in favours of traditional and integrative health sciences both in research and practices. Most of the plant-derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous people and some of these could not be substituted, despite the enormous advancement in synthetic chemistry [1-3]. So in almost all areas, peoples prefer the traditional curing methods of different ailments. Herbal medicine is still the mainstay of about 75 to 80 per cent of the world population, mainly in the developing countries to promote primary health care with better cultural acceptability, human compatibility and lesser side effects [4-6].

Catharanthus roseus (L.) G. Don. (Madagascar periwinkle, Family: Apocynaceae) is a perennial tropical plant that produces many indole alkaloids. All parts of the plant are rich in alkaloids, with maximum concentrations found in the root bark, particularly during flowering [7]. An infusion of the leaves is used to treat menorrhagia. The juice of the leaves is applied externally to relieve

wasp stings. All parts of the plant are credited with hypoglycaemic and antioxidant properties [6-8].

The term plant growth regulators can be defined as either natural or synthetic compounds that modify the plant growth and development pattern exerting profound influence on many physiological processes. Number of natural and synthetic substances like Auxins, Gibberellins, Cytokinin, Abscissic acid, Ethylene, Brassinosteroids, Terpenoids, Aliphatic alcohols, Polyamines and these substances induce specific responses in specific plants [9-11]. The responses of plant growth regulators may vary with plant species, variety, age of plant, environmental conditions, physiological and nutritional status, stage of development and endogenous hormonal balance. Plant growth retardants are widely used to modify canopy structure, yield and stress tolerance in many crop plants [12-14].

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 PBZ are mediated by changes in the balance of important plant hormones including the Gibberellins, ABA and cytokinins [14,15]. Gibberellic Acid (GA₃), which comes from a

naturally occurring growth hormone, is a member of a type of plant hormone called Gibberellins, which regulate the growth rate of plants. Gibberellins are involved in several plant development processes and promote a number of desirable effects including stem elongation, uniform flowering, reduced time to flowering and increased flower number and size [16]. GA₃ can increase the antioxidant metabolism and alkaloid production in *C. roseus* [17].

The strong and rapidly stimulating effect of elicitor on plant secondary metabolism in medicinal plants attracts considerable attentions and research efforts [17,18]. 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 [19]. The objectives of the present study are to understand the effect of plant growth promoters (GA₃ and *Pseudomonas fluorescens* elicitors) and retardant (PBZ) on the anatomical characteristics changes of *C. roseus* plants under field conditions.

MATERIALS AND METHODS

Plant and Chemicals: 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 Giberellic 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 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. 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.

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 growth, anatomical characteristics, mineral composition, pigments and biochemical constituents, antioxidant potentials and alkaloid contents.

Leaf Anatomy: Histological studies were made with leaves of treated plants along with control. The materials were processed and sectioned by rotary microtome adapting the method suggested by Johansen [20].

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

RESULTS

Effect of Growth Regulators on Leaf Anatomy (Table 1, Plate 1)

Thickness of Leaf: The thickness of leaf increased in the treatments considerably when compared to control plants. Among the treatments, paclobutrazol increased the thickness of leaf to a larger extent than gibberellic acid and *P. fluorescens*. The lamina thickness was 246.2 μm in paclobutrazol and 215.21 μm gibberellic acid and 235.41 μm in *P. fluorescens* treatment when compared to 198.3 μm of in control leaf.

Epidermal Thickness: The epidermis and cuticle thickness of the leaves of paclobutrazol, gibberellic acid and *P. fluorescens* treated plants increased significantly when compared to control. The thickness was 12, 9, 11 μm respectively in paclobutrazol, gibberellic acid and *P. fluorescens* treatments when compared to control plants.

Palisade Mesophyll Length: The length of the palisade layer also increased with triazole treatment it was 135, 99 and 128 μm in paclobutrazol, gibberellic acid and *P. fluorescens* treatments respectively when compared to 88 μm of control plants. The number of palisade layer was only 1-2 in the control where as it was 2-3 in treated plants, but there was no change in gibberellic acid treatments. In control the palisade cells were short and narrow while in the paclobutrazol treated plants the palisade cells are broad and oblong.

Spongy Mesophyll Length: The thickness of the spongy mesophyll layer also increased with treatment it was 158, 121, 142 μm in paclobutrazol, gibberellic acid and *P. fluorescens* treatments respectively when compared

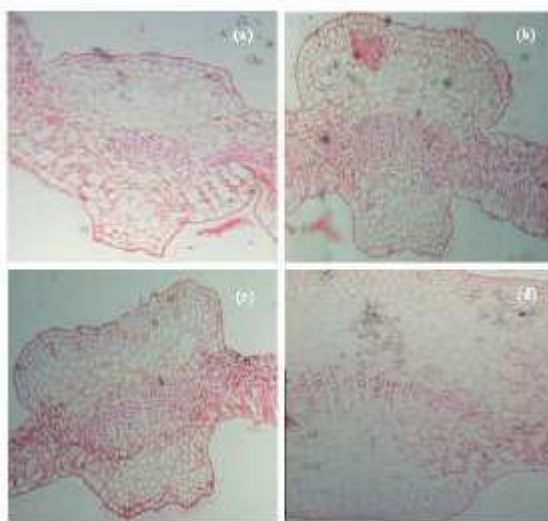


Plate 1: Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on leaf anatomy of *Catharanthus roseus* on 90 DAP. (a) control (b) gibberellic acid (c) paclobutrazol and (d) *Pseudomonas fluorescens*

Table 1: Paclobutrazol, gibberellic acid and *P. fluorescens* induced size variation of different anatomical structures of *Catharanthus roseus* leaf on 90 DAP (expressed in μm)

Sl.No.	Anatomical structures	CON	PBZ	GA	PF
1.	Thickness of leaf (μm)	198.3	246.2	215.21	235.41
2.	Epidermal thickness (μm)	7	12	9	11
3.	Number of palisade layer	1-2 layers	2-3 layers	1-2 layers	2-3 layers
4.	Palisade mesophyll length (μm)	88	135	99	128
5.	Spongy Mesophyll length (μm)	99	158	121	142
6.	Xylem vessel diameter (μm)	28	19	22	19

to 99 μm of control plants. The air space between the spongy cells is reduced in the treated plants when compared to control.

Vascular Bundle: Treated reduced the diameter of xylem vessels significantly when compared to control, however the phloem elements had shown an increased diameter as compared to control in the triazole treated plants. The xylem vessel diameter 19, 22, 19 in paclobutrazol, gibberellic acid and *P. fluorescens* treatments respectively when compared to 28 μm of control plants. The phloem sieve tubes in the paclobutrazol treated plants were significantly larger when compared to control.

DISCUSSION

The treated leaves showed an increased lamina, epidermis and cuticle thickness and number of palisade layer when compared to control. Paclobutrazol treatment increased leaf thickness and exhibited dense spongy mesophyll tissue in potato [21]. Benton and Cobb [22] showed that epoxiconazole caused increase of leaflet thickness and more palisade cells per unit area. The increased lamina thickness was attributed to increase in epidermal cell diameter palisade layer and spongy mesophyll depth. Paclobutrazol increases the thickness of the epicuticular wax layer in rose cultivars [23].

Induction of additional layers of palisade parenchyma was observed with the paclobutrazol treatment in potato [21]. The mesophyll of pecan leaves treated with propiconazole was composed of more closely packed spongy cells with less intercellular space [24]. The treatments reduced the diameter of xylem vessels significantly when compared to control, however the phloem elements had shown as increased diameter in triazole treated plants as compared to control. Paclobutrazol reduced the proportion of xylem and increased that of phloem and cortex and increased xylem density [25]. Tekalign *et al.* [21] found that paclobutrazol suppressed cell wall thickening in the phloem fiber decreased the width of xylem ring.

The leaf anatomical characteristics of *C. roseus* leaves were highly altered by GA treatment when compared to control. Leaf shape and plant stature can also be affected by altered levels of GAs and the application of exogenous GA₃ [25]. Since GA₁ is thought to stimulate cell elongation, thus higher GA₁ content in tissues may also be associated with the stem elongation and subsequent bolting [17]. Stem swelling and alterations in leaf anatomy with GA applications were

reported in mustard [26]. There seems to be no report in the literature on the effect of *Pseudomonas fluorescens* on leaf anatomy of higher plants.

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