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Influence of Brassinosteroids (BRs) on the vincristine content of *Catharanthus roseus* (L.) G. Don

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ABSTRACT

Catharanthus roseus (L). G. Don is an important anticancerous plant belonging to the family Apocynaceae. It contains so many alkaloids like Vinblastine and vincristine are mainly present in aerial parts of C. roseus, which are used in treatment of various human cancers, so it is considered as mile stone in cancer chemotherapy. Vincristine is also used to cure cancer including acute leukemia, Hodgkin's and non- Hodgkin's lymphoma, neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma, Wilms' tumor, multiple myeloma, chronic leukemias, thyroid cancer, brain tumors and blood disorders. In our study the effect of BRs on vincristine content of Catharanthus roseus was quantified. BRswas applied as foliar spray at different concentrations viz 5, 10, 15, and 20 ppm for 30days regularly. The vincristine content was quantified by HPLC method. The vincristine content was found to increase at all the concentration when compared to the control. The maximum concentration of vincristine was found to be around 15 and 20 ppm of BRs. Hence it is concluded that regular application of BRs to Catharanthus roseus can be recommended for attaining better biomass production and vincristine content.

Key words: Alkaloid, Foliar spray, Vincristine, BRs, Vinblastine.

INTRODUCTION

Catharanthus roseus (L.) G. Don (Apocynaceae) derives its economic importance from its highly valued anticancer alkaloids vincristine and vinblastine, and its antihypertensive root alkaloid ajmalcine[1]. All parts of the plants are rich in alkaloids, with maximum concentrations found in the root All parts of the plant are credited with hypoglycaemic properties and are used to treat diabetes[2]. Thus it is over all a miracle plant which is having the more number of alkaloids used in pharmaceutical industries. Secondary metabolites are present only incidentally and of significance to plant life. Plant produce an array of natural products, the so called secondary metabolites or pharmaceuticals, flavors, dyes, oils and resins which are not essential for plant growth and are normally produced in small amounts. These compounds usually have very complicated structures. Numerous plant secondary metabolites such as alkaloids, anthocyanins, flavanoids quinines, lignin, steroids and terpenoids have found commercial application as drugs, dye, flavor, fragrance, insecticides etc., and many of these compounds are valued for their potential pharmacological activities, industrial or agricultural properties which can be exploited to increase the commercial value of crops. [3].

Secondary metabolites production from plant has not always been satisfactory because several intrinsic and extrinsic factors affect growth, development and secondary metabolites biosynthesis[4-5]. Phytohormone is one of the main factors which influence plant growth and their primary and secondary metabolites pool. Plant growth regulators

have crucial impact on primary and secondary metabolism of plants. Brassinosteroids (BRs) are a new group of phytohormones with significant growth promoting nature[6-7]. BRs are considered as growth regulators with pleiotropic effects, as they influence diverse physiological processes like growth, germination of seeds, rhizogenesis, senescence and also confer resistance to plants against biotic and abiotic stresses[8]. The importance of the brassinosteroids in improving the growth and yield of crop plants is well documented[9].

In the present study, is going to be carried out whether the BRs will increase or decrease the production of anticancer alkaloids such as vincristine and vinblastine.

MATERIALS AND METHODS

Cultivation of Plants

Healthy and uniform seeds of *Catharanthus roseus* were sown in pots containing mixture of red soil, black soil, and sand in the ratio of 2:2:1. Seeds were sown in the pots and they were kept in dark for overnight. Soon after seedling emergence, the pots were shifted to daylight conditions. Different concentrations of BRs were prepared (5, 10, 15, and 20ppm). After 3 months the leaves were sprayed with different concentration of BRs (5, 10, 15, and 20ppm) using sprayer for a continuous period of 30 days. Care was taken to wet both the surfaces completely to ensure maximum application. The control plants were sprayed with distilled water. The growth parameters and alkaloid contend were analysed after 30 days of treatment of various concentration of BRs.

Solvent extraction

Crude plant extract was prepared by soxlet extraction method. About 1 gm of powdered plant material was extracted with 250ml of different solvents separately. Solvents used were methanol, ethanol, and acetone. The process of extraction continues for 24 hours or till the solvent in siphon tube extract was filtered and concentrated to dryness under hot air oven at 550C. The residue appeared as a dark brown powder.

Quantification of Alkaloids

The alkaloids were separated by recording the absorption maxima in the 200-300nm wave band at room temperature. The alkaloids were separated on a TLC plate and eluted using 95% ethanol. To confirm the presence of vincristine, standard vincristine purchased from-Aldrich (U.S.A.) was allowed to co-run with the samples.

Preparation of TLC plates[10]

The glass plates to be used in TLC were cleaned carefully with ethanol in order to remove grease. Slurry of silica gel G in water (20g of silica gel per plate) was shaken vigorously for 90 seconds and coated on the plates to a thickness of 0.5mm using a commercial spreader. Then the plates were activated at 105^0C for 30 minutes and then used. After the spothing , the TLC plates were kept in a chamber which contains the solvent. The chromatogram was developed by ascending technique. The running solvent used was a tertiary mixiture of chloroform, methanol and benzene mixed in the ratio of 4:1:5.

Identification

The TLC plate was viewed under a background of white light blended with UV-A light. The corresponding bands were scrapped and eluted in 100% ethanol solvent.

Spectral analysis by HPLC

The ethanollic extract of pure vincristine and vincristine obtained from BRs treated plants were injected into C18 column 7µm (4×250mm) with a flow rate 0.7ml /min-1. The elution of the alkaloid was done using ethanol. Determination of vincristine was carried out with mobile phase composed of ethanol. The optimum separation of HPLC was achieved at 30^0C and monitored at 254nm.

RESULTS AND DISCUSSION

Growth promoting substances have obviously been used owing to their beneficial effects on growth, and yield of plants. The crude extract of alkaloids was separated by TLC and subjected to HPLC analysis.

HPLC analysis

To confirm the presence of vincristine or any other alkaloids in *catharanthus* leaf, the eluted alkaloid spot from the TLC plate was subjected to HPLC analysis. The eluted spot was re-extracted with 10ml ethanol and a low spin was made to clear off the silica gel. The standard vincristine was run through C_{18} column with ethanol as carrier solvent. Pure vincristine showed a major peak with retention time (RT) of 4.420 min and a few secondary peaks with varying RT. Different concentration of BRs (5, 10, 10 and 20ppm) treated samples showed similar peaks with marginal

differences in RT. The differences in RT could be due to the moisture traces or minor contamination in the samples. In the plant samples a major peak with a minor shoulder appeared in the spectrum which could be alkaloids or any other secondary metabolites absorbing at 254nm. The HPLC spectra of plant samples are shown in. Among the samples, 20ppm was found to induce more amount of vincristine when compared to other samples. The amount of vincristine content in 50, 10, 10 and 20ppm treated plant was 102, 153, 239 and 297µg/gLDW respectively and the control showed 142µg of vincristine only (Fig. 1).

Alkaloid contents in the leaves of *Balanites aegyptiaca* plants were increased significantly by using different concentrations of BRs as compared with the control[11]. Phytoregulators might modulate the expression of secondary compounds of a terpenic[12] and alkaloid[13]. High concentration of BRs has brought about induction of alkaloids. Than the control. Since, there was a differential role of BRs on the popular leaf alkaloid of *C. roseus*. Foliar application of BRs has influenced the biosynthetic pathway of vincristine.

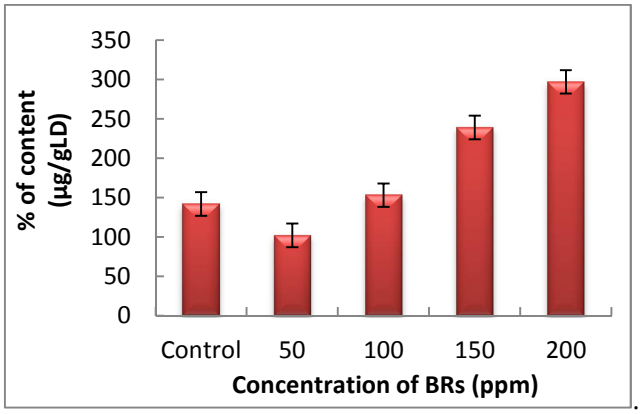

Fig. 1 The effect of different concentrations of IAA (5, 10, 15 and 20ppm) on vincristine content of *Catharanthus roseus*. Calculated by HPLC analysis

CONCLUSION

BRs has a good potential in improving accumulation of alkaloid composition. The concentrations of 20ppm of BRs give the best results. So, the farmers may be advised to make up of IAA for improving biomass and alkaloid content in *Catharanthus roseus*.

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