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Antioxidant potentials and ajmalicine accumulation in *Catharanthus roseus* after treatment with giberellic acid

C. Abdul Jaleel, R. Gopi *, P. Manivannan, B. Sankar, A. Kishorekumar, R. Panneerselvam

Stress Physiology Lab, Department of Botany, Annamalai University, Annamalainagar 608002, Tamil Nadu, India

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Abstract

Changes in antioxidant potentials and indole alkaloid, ajmalicine, production were studied in *Catharanthus roseus* (L.) G. Don. plants under treatment with gibberellic acid (GA_3). The GA_3 treatments were given in two ways, foliar spray and soil drenching methods on 30, 45, 60 and 75 days after planting (DAP). The plants were uprooted randomly on 90 DAP and separated into root, stem and leaves and used for analyses. The antioxidant potential was studied in terms of non-enzymatc antioxidant molecules like ascorbic acid (AA), α-tocopherol (α-toc) and reduced glutathione (GSH) and activities of antioxidant enzyme, viz., superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). The alkaloid ajmalicine was extracted and estimated from roots of both control and treated plants. It was found that, GA_3 has a profound effect upon the antioxidant potentials and it caused a significant enhancement in the production of ajmalicine when compared to untreated control as well as foliar-sprayed plants. There was no significant enhancement in GSH and ajmalicine content under GA_3 foliar spray in *C. roseus*. These preliminary results suggest that, the application of GA_3 may be a useful tool to increase the antioxidant potential and alkaloid production in medicinal plants like *C. roseus*.

Keywords: *Catharanthus roseus*; Gibberellic acid; Ascorbic acid; α-Tocopherol; Reduced glutathione; Ascorbate peroxidase; Superoxide dismutase; Catalase; Ajmalicine

1. Introduction

Application of plant growth hormones is a promising benefit to enhance yields of vegetative parts, which produce secondary metabolites. Gibberellins are involved in several plant development processes and promote a number of desirable effects including stem elongation, uniform flowering, reduced time to flowering, increased flower number and size [1]. The most common methods of application of growth regulators are foliar sprays and media drenches. In practical application of plant hormones, the best practices in stable production of secondary metabolite contents in medicinal plants, is through soil drenching [2]. *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 [3]. All parts of the plant are rich in alkaloids, with maximum concentrations found in the root bark, particularly during flowering. An infusion of the leaves is used to treat menorrhagia. The juice of the leaves is applied externally to relieve wasp stings.

Previously it was reported that there is a continual effect of gibberellic acid (GA_3) on *C. roseus* plant phenotypes [4]. Earlier studies have reported that GA_3 application (at 50, 100, and 500 $g\,m^{-3}$) as foliar spray on transplanted cuttings increased plant height [5]. Continuous availability (for 1 month) of GA_3 at 5.8 and 11.6 μM resulted in elongation of shoots, lowering number of leaves with little effects on alkaloid content on seedlings grown on MS solid medium [6].

C. roseus is a good source of non-enzymatic and enzymatic antioxidants [2,7]. Antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions, such as anaemia, asthma, arthrists,

Abbreviations: AA, ascorbic acid; α-toc, α-tocopherol; GSH, reduced glutathione; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase; GA_3, gibberellic acid; DAP, days after planting

* Corresponding author. Tel.: +91 4144 238248x354; fax: +91 41 44 222265.
E-mail addresses: abdul79jaleel@rediffmail.com (C.A. Jaleel), suriyagopi@yahoo.co.in (R. Gopi).

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inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias [8]. Free radicals can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods [9] and medicinal plants [10]. Ascorbic acid (AA) is a very important reducing substrate for H_2O_2 detoxification in photosynthetic organisms [11]. AA participates in the removal of H_2O_2 as a substrate of ascorbate peroxidase (APX). Reduced glutathione (GSH) is another most important non-enzymatic antioxidant molecule, which functions as an effective ROS detoxifier [12]. α-Tocopherol (α-toc) was consumed predominantly as a radical scavenging antioxidant against lipid peroxidation [13]. The enzymatic antioxidant defense system includes superoxide dismutase (SOD), ascorbate peroxidase (APX), polyphenol oxidase (PPO), catalyase (CAT) and glutathione reductase (GR). SOD is a major scavenger of superoxide anion radical ($O_2^{-\bullet}$) that catalyses the dismutations of $O_2^{-\bullet}$ with great efficiency resulting in the production of H_2O_2 and O_2 [14]. The H_2O_2 scavenging system represented by APX and CAT are most important in imparting tolerance than SOD as reported in oxidative stressed plants [15,7].

Lot of works have already been carried out in this plant in its medicinal importance [2], but the GA_3 effects on this medicinal plant attracted a little attention. Previous works revealed the influences of triadimefon on the antioxidant metabolism and ajmalicine production [2], paclobutrazol mediated growth regulation [16], salinity problems [17], salt stress protection by paclobutrazol [5], drought stress [18], and methods to overcome drought stress [19,20] in *C. roseus*. To the best of our knowledge, no information on the effect of GA_3 on antioxidant metabolism and ajmalicine production in this medicinal plant is available. This investigation was aimed for finding out the extent of changes in AA, α-toc and GSH and antioxidant enzyme activities, viz., SOD, APX and CAT, and ajmalicine production in *C. roseus* under GA_3 treatment.

2. Materials and methods

2.1. Plant materials and cultivation methods

The seeds of *C. 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% $HgCl_2$ for 60 s 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, 40 plants per plot were planted for both the varieties at a distance of 30 cm × 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. GA_3 @ 5 mM was given to each plant by soil drenching and another set of plants were subjected to foliar spray. The treatment was given on 30, 45, 60 and 75 days after planting (DAP). The plants were uprooted randomly on 90 DAP and separated into root, stem and leaves and used for estimating the AA, α-toc and GSH and antioxidant enzyme activities, viz., SOD, APX and CAT, and ajmalicine content.

2.2. Non-enzymatic antioxidant estimations

2.2.1. Estimation of AA content

AA content was assayed as described by Omay et al. [21]. The extract was prepared by grinding 1 g of fresh material with 5 ml of 10% TCA, centrifuged at 3500 rpm for 20 min, reextracted twice and supernatant made up to 10 ml and used for assay. To 0.5 ml of extract, 1 ml of DTC reagent (2,4-dinitrophenyl hydrazine-thiourea–$CuSO_4$ reagent) was added, incubated at 37 °C for 3 h and 0.75 ml of ice-cold 65% H_2SO_4 was added, allowed to stand at 30 °C for 30 min, resulting colour was read at 520 nm in spectrophotometer (U-2001-Hitachi). The AA content was determined using a standard curve prepared with AA and the results were expressed in milligram per gram dry weight (DW).

2.2.2. Estimation of GSH content

The GSH content was assayed as described by Griffith and Meister [22]. Two hundred milligrams of fresh material was ground with 2 ml of 2% metaphosphoric acid and centrifuged at 17,000 rpm for 10 min. Adding 0.6 ml 10% sodium citrate neutralized the supernatant. One milliliter of assay mixture was prepared by adding 100 μl extract, 100 μl distilled water, 100 μl 5,5-dithio-bis-(2-nitrobenzoic acid) and 700 μl NADPH. The mixture was stabilized at 25 °C for 3–4 min. Then 10 μl of glutathione reductase was added, read the absorbance at 412 nm in spectrophotometer and the GSH contents were expressed in microgram per gram fresh weight (FW).

2.2.3. Estimation of α-toc content

α-Toc content was assayed as described by Backer et al. [23]. Five hundred milligrams of fresh tissue was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6, v/v) and the extract was centrifuged at 10,000 rpm for 20 min and the supernatant was used for estimation of α-toc. To 1 ml of extract, 0.2 ml of 2% 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 min. The resulting red colour was diluted with 4 ml of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α-toc content was calculated using a standard graph made with known amount of α-toc.

2.3. Antioxidant enzyme extractions and assays

2.3.1. Assay of SOD (EC 1.15.1.1) activity

The activity of SOD was assayed as described by Beauchamp and Fridovich [24]. The reaction mixture con-