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Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary metabolite accumulation

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Abstract

A pot culture experiment was conducted to estimate the drought stress mitigating effect of ketoconazole (KCZ), a fungicide cum plant growth regulator, in *Catharanthus roseus* plants. The plants under pot culture were subjected to drought stress and drought stress with KCZ from 30 days after sowing (DAS) and regular irrigation was kept as control. Antioxidant contents and activities of antioxidant enzymes were estimated from root, stem and leaf of both control and treated plants. The alkaloid ajmalicine was extracted and estimated from the roots of control, drought stressed and KCZ treated plants. Individual and combined drought stress and KCZ treatments increased ascorbic acid, α-tocopherol contents, superoxide dismutase, ascorbate peroxidase, catalase and polyphenol oxidase activities when compared to control. There was a significant enhancement in ajmalicine production under KCZ treated plants under drought stress when compared to well watered control as well as drought stressed plants. The KCZ treatment resulted in partial mitigation of drought stress by increasing the antioxidant potentials in *C. roseus* plants.

Keywords: Ajmalicine; Antioxidants; *Catharanthus roseus*; Medicinal plant; Ketoconazole; Water deficit

1. Introduction

Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle [1]. The lack of adequate moisture leading to water stress is common occurrence in rainfed areas, brought about by infrequent rains and poor irrigation [2]. Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. These two conditions often coincide under arid and semiarid climates. Among the diverse consequences of drought effect on plant development, restricted nutrient and water acquisition are commonly recognized [3]. Water stress tolerance is seen in almost all plant species but its extent varies from species to species [4].

Drought induces oxidative stress in plants, in which reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), hydroxy radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) and alkoxy radical ($RO^{\bullet}$) are produced [5]. The toxic superoxide radical has a half-life of less than one second and is usually rapidly dismutated by superoxide dismutase (SOD) to H_2O_2, a product that is relatively stable and can be detoxified by catalase and peroxidases. These metalloenzymes constitute an important primary defense of cells against superoxide free radicals generated under stress conditions and thereby increased SOD activity is known to confer oxidative stress tolerance [6]. Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant metabolisms [7]. These mechanisms include β-carotenes, ascorbic acid (AA), α-tocopherol (α-toc), reduced glutathione (GSH) and enzymes including SOD, peroxidase (POX), ascorbate peroxidase (APX),

Abbreviations: DID, days interval drought; KCZ, ketoconazole; DAS, days after sowing; ROS, reactive oxygen species; SOD, superoxide dismutase; AA, ascorbic acid; α-toc, α-tocopherol; GSH, reduced glutathione; POX, peroxidase; APX, ascorbate peroxidase; CAT, catalase; PPO, polyphenol oxidase

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catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR) [8]. There is an intimate relationship between enhanced antioxidant enzyme activities and increased resistance to environmental stresses [9]. α-Toc and AA are two important antioxidants in higher plants. They are concentrated in the chloroplasts and the cytosol and protect the photosynthetic apparatus under stress by scavenging excess ROS [10].

Triazole compounds such as triadimefon (TDM), ketoconazole (KCZ), paclobutrazol (PBZ), propiconazole (PCZ), etc. are widely used as fungicides and they also posses varying degrees of plant growth regulating properties [11–14]. Triazoles have been called plant multi-protectants because of their ability to induce tolerance in plants to environmental and chemical stresses. Protection of plants from apparently unrelated stress by triazole is mediated by a reduction in free radical damage and increase in antioxidant potential [15,16]. Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels [17]. Triazole treated plants have a more efficient free-radical scavenging system that enables them to detoxify active oxygen [18]. One of the previous works carried out in our lab revealed the increased antioxidant potentials and an enhancement in alkaloid production under TDM application in *Catharanthus roseus* [19].

Exploitation of medicinal plants became more and more popular with increasing realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics [20]. For the past several years, several scales of physiology have been applied to study responses to drought stress tolerance mechanisms and methods to overcome drought stress in field crops [16,21–23]. However, little information is gained about the physiological basis in terms of antioxidant metabolism under drought stress in medicinal plants. Comparatively a little work has been reported on water stress problems and methods to overcome drought stress injuries in this plant. The drought stress amelioration by triazole compounds is of major research interest, because, these compounds have innate potentiality for increasing antioxidant enzymes and molecules in oxidative stressed plants [11]. It seems necessary to do research related to the correlation between medicinal plants and drought stress for the increasing need of medicinal plants. So it seems valuable, to test the important medicinal plants for their abiotic stress tolerance capacity and methods to overcome stress through the application of plant growth regulators and stress protectants.

C. roseus (L.) G. Don. (family: Apocynaceae) is one of the highly exploited and studied medicinal plants. This plant contains alkaloids which are valuable source of antitumour agents like vinblastine and vincristine used in chemotherapy of leukemia and in the treatment of Hodgkin's disease, and also a popular ornamental plant [24]. Despite the relative great number of reports on the medicinal aspects and growth regulator effects on *C. roseus* plants [12,21,25–27], there are only a few attempts to explain the physiological basis of drought effects and osmoregulation [28–30]. To the best of our knowledge, no information on the physiological response interms of antioxidant metabolism of *C. roseus* to KCZ treatment in water deficit stress is available. The objectives of this study were to provide additional information on the osmolyte concentration non-enzymatic (AA and α-toc) and antioxidant enzyme activities (SOD, APX, CAT and PPO) and alkaloid, ajmalicine accumulation in *C. roseus* under KCZ treatment in different water regimes.

2. Materials and methods

2.1. Plant material and drought stress applications

The seeds of *C. roseus* (L.) G. Don. (family: Apocynaceae) were collected from the Department of Horticulture, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. The triazole compound KCZ was obtained from United Phosphorus Ltd., Gujarat, India. Seeds were surface sterilized with 0.2% $HgCl_2$ solution for 5 min with frequent shaking and thoroughly washed many times with deionized water to remove $HgCl_2$. The experiments were carried out in plastic pots. Six seeds were sown in each pot of 30 cm × 30 cm containing 3 kg of soil mixture composed of red soil, sand and the farmyard manure at 1:1:1 ratio. All the pots were watered to the field capacity with ground water up to 30 days after sowing (DAS). The seedlings were thinned to 2 pot^{-1} on 20 DAS. Pots were irrigated with ground water 1-day interval as a control and other treatment 10, 15 and 20 days interval drought (DID) from 30 DAS and drought with 15 mg l^{-1} KCZ treatment and 15 mg l^{-1} KCZ alone. Plants were uprooted randomly on 41, 46 and 51 DAS, washed carefully and separated into root, stem and leaf for analyses. The roots of control, drought stressed and KCZ treated plants were then shade dried, finely powdered in an electronic blender and kept in separate containers for ajmalicine extraction.

2.2. Non-enzymatic antioxidants

2.2.1. Ascorbic acid (AA) content

The AA content was assayed as described by Omaye et al. [31]. 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 and 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 mg g^{-1} dry weight (DW).

2.2.2. α-Tocopherol (α-toc) content

The α-toc content was assayed as described by Backer et al. [32]. 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 one 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