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Comparative effects of different triazole compounds on antioxidant metabolism of *Solenostemon rotundifolius*

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Abstract

The effect of different triazole compounds, viz., triadimefon (TDM) and hexaconazole (HEX) treatments on the antioxidant metabolism of *Solenostemon rotundifolius* Poir., Morton plants was investigated in the present study under pot culture. Plants were treated with TDM at 15 mg l^{-1} and HEX at 10 mg l^{-1} separately by soil drenching on 80, 110 and 140 days after planting (DAP). The plants were harvested randomly on 90, 120 and 150 DAP for determining the effect of both the triazoles on non-enzymatic antioxidant contents like ascorbic acid (AA), reduced glutathione (GSH) and α-tocopherol (α-toc), activities of antioxidant enzymes like superoxide dismutase (SOD) and ascorbate peroxidase (APX). All the analyses were made in leaf, stem and tubers of both control and treated plants. It was found that both these triazole compounds have profound effects on the antioxidant metabolism and caused an enhancement in both non-enzymatic and enzymatic antioxidant potentials under treatments. These results suggest that, the application of triazoles may be a useful tool to increase the antioxidant production in *S. rotundifolius* and thereby make it an economical food crop.

Keywords: *Solenostemon rotundifolius*; Triadimefon; Hexaconazole; Antioxidants; Antioxidant enzymes

1. Introduction

Triazoles are a group of compounds, which have both fungitoxic and plant growth-regulating properties [1]. In addition, they can also protect plants against various environmental stresses. Triazoles affect the isoprenoid pathway, and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution, and increasing cytokinin levels [2]. Triazoles inhibit cytochrome P_450 mediated oxidative demethylation reactions, which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellin biosynthetic pathway which can affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels [3]. Triazole compounds have been shown to improve the yield of many root crops such as carrot, radish, sugarbeet and potato [1].

Some of the previous works carried out in our lab revealed the morphological and physiological changes associated with triazole treatment in various plants, include the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, alkaloid production and enhancement in carbohydrate metabolism [4–14]. Triadimefon (TDM) and hexaconazole (HEX) are triazole group of fungicides, having plant growth regulator (PGR) properties, are reported to inhibit gibberellic acid biosynthesis and increase in abscisic acid and cytokinin contents [1].

Chinese potato (*Solenostemon rotundifolius* Poir., Morton) is one of the minor seasonal tuber crops cultivated for its edible tubers in India, Sri Lanka, South Asia and parts of tropical

Abbreviations: TDM, triadimefon; HEX, hexaconazole; DAP, days after planting; AA, ascorbic acid; GSH, reduced glutathione; α-toc, α-tocopherol; SOD, superoxide dismutase; APX, ascorbate peroxidase; PGR, plant growth regulator.

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Africa. Its tubers have an aromatic flavour on cooking and have delicious taste [15]. In India, it is extensively cultivated in Kerala, Karnataka and Southern districts of Tamil Nadu [16]. Chinese potato is also medicinally used for the treatment of eye diseases, dysentery due to indigestion, foot itching and ulcer. Stem cuttings commonly propagate it. Previous reports revealed the changes in photosynthetic characteristics and carbohydrate metabolism in this plant under TDM and HEX applications [8]. But little attention has been drawn to the antioxidant properties of this food cum medicinal plant. It therefore seems important to test the methods to increase the innate antioxidant potential of this food crop, in order to satisfy the needs of antioxidants in diet and thereby make it as an economically important food cum medicinal crop. Hence this study aims to evaluate the ability of TDM and HEX to enhance the antioxidative potentials, with special emphasis to both non-enzymatic and enzymatic antioxidant constituents.

2. Materials and methods

2.1. Plant materials and triazole treatments

Tubers of *S. rotundifolius* were obtained from Central Tuber Crop Research Institute, Kerala and planted in the Botanical Garden of Annamalai University. The plants were treated with different concentrations (5, 10, 15 and 20 mg l^{-1}) of triadimefon and hexaconazole to determine the optimum concentration. Among these concentration 15 mg l^{-1} of TDM and 10 mg l^{-1} HEX increased the dry weight significantly and higher concentration slightly decreased the growth and dry weight. Each plant was treated separately with 1 l of aqueous solution containing 15 mg TDM and 10 mg HEX on vegetative stages like 80, 110 and 140 days after planting (DAP). The treatments were given by soil drenching. The plants were uprooted on 90, 120 and 150 DAP and separated into shoot and tubers for analyses.

2.2. Non-enzymatic antioxidant estimations

2.2.1. Ascorbic acid content

Ascorbic acid (AA) content was assayed as described by Omay et al. [17]. 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 upto 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 mg g^{-1} dry weight (DW).

2.2.2. Reduced glutathione

The reduced glutathione (GSH) content was assayed as described by Griffith and Meister [18]. 200 mg fresh material was ground with 2 ml of 2% metaphosphoric acid and centrifuged at 17,000 rpm for 10 min. The supernatant was neutralized by adding 0.6 ml 10% sodium citrate. 1 ml 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, and the absorbance was read at 412 nm in spectrophotometer and the GSH contents were expressed in μg g^{-1} fresh weight (FW).

2.2.3. α-Tocopherol content

α-Tocopherol (α-toc) activity was assayed as described by Backer et al. [19]. 500 mg 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. Superoxide dismutase (SOD, EC 1.15.1.1)

The activity of SOD was assayed as described by Beauchamp and Fridovich [20]. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} M KCN and 5.6×10^{-5} M nitroblue tetrazolium (NBT) salt dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). 3 ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30 °C for 1 h. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity is expressed in U mg^{-1} protein (U = change in 0.1 absorbance h^{-1} mg^{-1} protein).

2.3.2. Ascorbate peroxidase (APX, EC 1.11.1.1)

The activity of APX was determined by the method of Asada and Takahashi [21]. The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 μl of enzyme extract. The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient 2.9 mM^{-1} cm^{-1}). The enzyme activity was expressed in U mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein). For both enzyme assays, enzyme protein was determined by the method of Bradford [22].

2.4. Statistics

The experiment was carried out in completely randomized design (CRD). Each treatment was analyzed with at least three replicates and a standard deviation (S.D.) was calculated. The data were expressed in mean ± S.D. of three replicates.