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Superoxide Dismutase and Ascorbate Perxidase Profile Changes with Triazole Applications in *Manihot esculenta* Crantz

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Abstract: In the present study, the effect of Triadimefon and Hexaconazole on the enzymatic antioxidans like Superoxide dismutase and ascorbate perxidase of *Manihot esculenta* Crantz during the growth and maturation period was analysed. One litre of 20 mg L^{-1} triadimefon and 15mg L^{-1} hexaconazole solution per plant was used for the treatment and control was treated with one litre of irrigation water. The treatment was given on 25,45,65 and 100 days after planting (DAP) by soil drenching. Plants were harvested randomly on 40, 80, 120, 160, 200 and 240 DAP and separated into stem,leaf,root and tuber was used for determining Superoxide dismutase and ascorbate perxidase activities. There found significant enhancement of these enzyme activities with triadimefon and hexaconzole treatments in *Manihot esculenta*.

Key words: Superoxide dismutase, Ascorbate perxidase, Triadimefon, Hexaconzole, *Manihot esculenta*

INTRODUCTION

Triazole acts as plant growth regulator also influence hormonal balance, photosynthetic rate, enzyme activities, lipid peroxidation and yield components in various crop plants [1-4]. Triazole inhibits cytochrome P – 450 mediated oxidative demethylation reaction including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in gibberellin biosynthesis pathway. [5-7]. Triazole compounds increased the translocation of photosynthates from shoot to root and have altered mineral uptake and plant nutrition [8,9]. Triazole inhibits the shoot growth and increase the root growth. Triazole induced the tuber initiation and enlargement of tubers [10-13]. Triazoles affect the activities of several enzymes, especially those related to detoxification of active oxygen species and antioxidant metabolism [2-8].

In developing storage organs such as seeds, fruits, tubers and stems translocated photo-assimilates are converted into carbon and nitrogen reserves such as starch, fructans, oils and storage proteins, of which starch in addition to its role as an important energy and carbon reserves in plants, it also serves as a rich source of nutrition for human and animals and as commercial feed stock for many industrial application [14,15].

Tapioca (*Manihot esculenta* Crantz) also known as Cassava, Mandioca and Yucca is a bushy shrub belongs to the family Euphorbiaceae is an important food crop grown throughout the tropics for its enlarged tuberous roots. The tubers are used for sago industry for starch extraction and grown in rainfed areas where a number of sago and starch mills exist. In addition the boiled tubers are consumed as staple food. The leaves of Tapioca are rich in protein serving as an excellent cattle feed. It is an important daily source of starch for 300-600 million of the people around the world. The tuber contains 30 to 35 % starch and appreciable amount of calcium and vitamin-C [16].

The enhancement of yield in tuber crops like tapioca will be beneficial to the farmers if right type of triazole compound and concentration is determined by experiments. A lot of work has been done on the effect of triazole compounds on stress protection in various plants [14]. However, work on increasing the growth, yield and modification of antioxidant potential using triazole

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compounds in tuber crops like tapioca is scanty. Hence, a study becomes essential to evaluate the effect of triazole compounds on the growth and metabolism of Tapioca. The main objectives of this study are to asses the effect of Triadimefon and Hexaconazole on antioxidant enzyme activities of *Manihot esculenta* Crantz during the growth and maturation period.

MATERIALS AND METHODS

The land was prepared by ploughing thoroughly five times to a depth of 35 cm and the soil was sandy loam without any stones and pebbles. The Farm yard manure (FYM) was applied at the rate of 10 tonnes per hectare. The stem cuttings of *Manihot esculenta* Crantz. (Tapioca) CV-H-226 was obtained from Tamil Nadu Agricultural University (TNAU) India. The stem cuttings of uniform thickness having three nodes were used for planting. The stem cuttings are dipped for 10 minutes in 1% Bavestin before planting to avoid fungal infections. Each stem cuttings was planted in a plot of 1.5 × 1.5 to a depth of 5 cm inside the soil and Completely Randomized Block Design (CRBD) was used for this experiment.

No inorganic fertilizer was used throughout the experiment and no systemic pesticide or fungicide was used during the experiment. Only ground water was used for irrigation. In preliminary experiments, 5, 10, 15, 20, 25 and 30 mgL^{-1} triadimefon and hexaconazole were used for treatment to determine the optimum concentration of triadimefon and hexaconazole.

Among these treatments, 20mg L^{-1} triadimefon and 15mg L^{-1} hexaconazole concentrations were found to increase the dry weight significantly and in higher concentrations they slightly decreased the growth and dry weight. Hence 20 mg L^{-1} triadimefon and 15 mgL^{-1} hexaconazole concentrations were used to determine the effect of these chemicals on the growth and metabolism of tapioca. One litre of 20 mg L^{-1} triadimefon and 15mg L^{-1} hexaconazole solution per plant was used for the treatment and control was treated with one litre of irrigation water. The treatment was given on 25,45,65 and 100 DAP by soil drenching. The EC of the soil was 0.21 dSm^{-1} and pH was 6.8 after the treatment. The average temperature was 32/26°C (maximum and minimum) and relative humidity (RH) varied between 60-75 percent during the experimental period.

Triadimefon [1- (4- chlorophenoxy) –3, 3- dimethyl –1- (1H-1, 2, 4- triazole –1 –Y1) –2 butanone] [$C_{14} H_{16} Cl N_3 O_2$] M.W. 293.75 has been obtained from Bayer India Ltd., Mumbai and Hexaconazole (2- (2, 4- dichlorophenyl)-1- (2 H-1, 2, 4- triazole-1-Y1) hexan –2-01) [$C_{14} H_{17} Cl_2 N_3 O$] M. W. 314.2 has been obtained from Rallis India Ltd., Mumbai used for this study.

Plants were harvested randomly on 40, 80, 120, 160, 200 and 240 DAP and separated into stem, leaf, root and tuber was used for determining antioxidant enzyme status.

Statistical Analysis: The data was analysed using the analysis of variance (ANOVA) as described by the method outlined by Ridgman [17]. Means were compared between treatments from the error mean square by LSD (Least Significant Difference) at the P = 0.05 and P = 0.01 confidence level using Tuckey's [18] test.

Antioxidant Enzymes

Superoxide Dismutase (EC 1.15.1.1): Crude enzyme extract was prepared for the assay of superoxide dismutase by the method of Hwang *et al*. [19].

Extraction: One gram of plant tissue was homogenized with 10 ml of ice cold buffer (50mM sodium phosphate buffer containing 1 mM PMSF). The homogenate was strained through two layers of cheese cloth and centrifuged at 12,500 g for 20 minutes at 4°C. The supernatant was made upto 10 ml with the same buffer and used as the source of enzyme. The enzyme protein was determined by Bradford [20] method for expressing the specific activity of all the three enzymes.

Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich [21].

Assay: Three millilitres of the reaction medium was added to 1 ml of enzyme extract. The reaction mixture contained 1.17 X 10^{-6} M riboflavin, 0.1 M methionine, 2 × 10^{-5} M potassium cyanide and 5.6 × 10^{-5} M nitroblue tetrazolium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixtures were illuminated in glass test tubes of selected uniform thickness. The illumination was performed by two sets of Philips 40 W fluorescent tubes. The test tubes were arranged in a single row, with a set of tube lights fixed on either side. Illumination started to initiate the reaction at 30°C for 1 hour. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in a Spectrophotometer against the blank.

Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per mg protein under the assay condition [22].

Ascorbate Peroxidase (EC;1:11:1:11): Ascorbate perxidase was extracted and estimated by the method of Nakano and Asada [23].

Extraction: 500 milligrams of fresh plant tissue was ground in a pestle and mortar under liquid Nitrogen in 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1% PVP and 1mM ascorbate peroxidase. The homogenate was filtered through double layered cheese cloth and centrifuged at 15,000 g for 20 minutes at 4°C. The supernatant was used as source of enzyme.

Estimation: 1ml of reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2 and 200µl of enzyme extract. The absorbance was read at 290 nm in spectrophotometer against the blank prepared without enzyme. The correction was done for the low, non-enzymatic oxidation of ascorbate by Hydrogen peroxide (extinction coefficient 2.9 $mM^{-1} cm^{-1}$) at 290nm. The results were expressed in milligram per gram dry weight.

RESULTS AND DISCUSSION

Superoxide Dismutase (SOD)

Leaf (Fig. 1): The activity of antioxidant enzyme SOD increased with the age in the leaves of control and treated tapioca plants. In the triadimefon and hexaconazole treated plants it increased to a larger extent when compared to control and it was 126.04 and 123.15 percent over control on 240 DAP.

Tuber (Fig. 2): The activity of SOD in the tubers of tapioca treated with triazole increased a larger extent when compared to control in all stages of growth. In triadimefon and hexaconazole treatments the SOD activity was 121.26 and 116.55 percent over than control on 240 DAP. The SOD activity was low in the tubers as compared to leaves of the tapioca.

In tapioca triadimefon and hexaconazole increased the activity of superoxide dismutase to a larger extent and this level was very high in the tuber when compared to leaves. Superoxide dismutase is a major scavenger of reactive oxygen species and it catalyses the dismutation

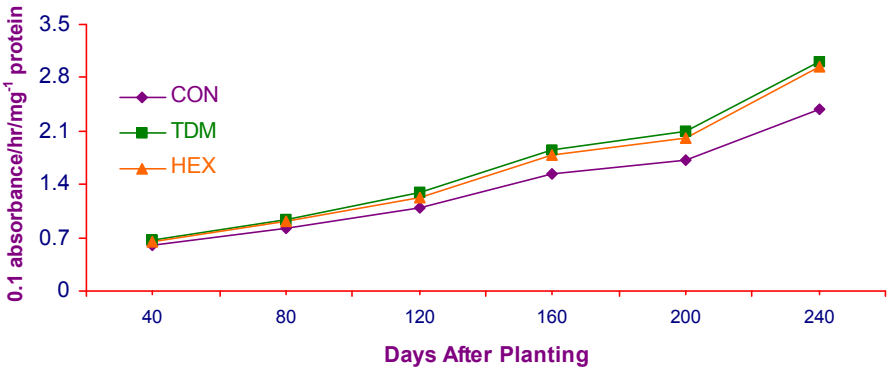

Fig. 1: Triazoles induced changes in the superoxide dismutase activity in the leaves of Tapioca

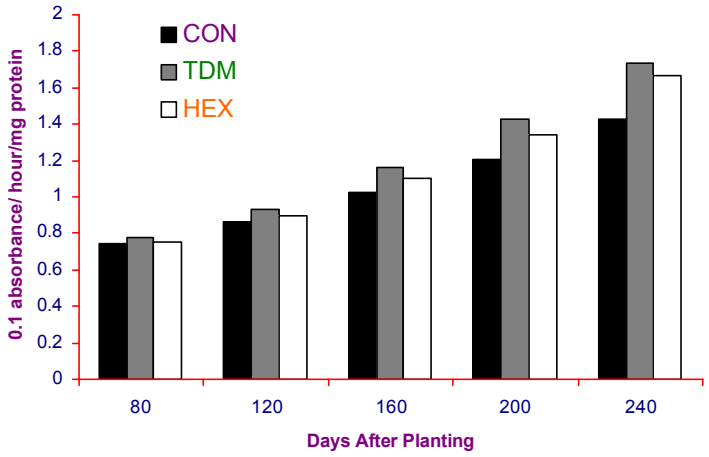

Fig. 2: Triazoles induced changes in the super oxide dismutase activity in the tuber of Tapioca

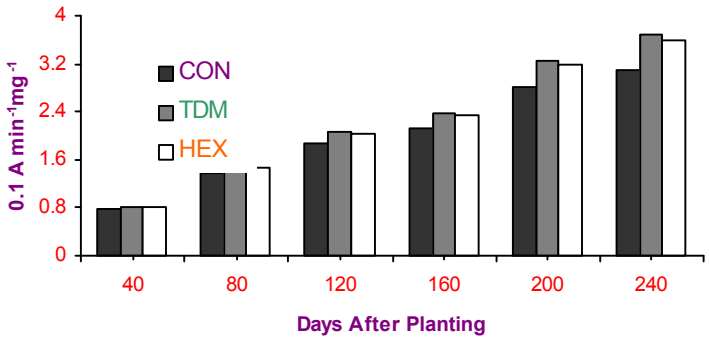

Fig. 3: Triazoles induced changes in the ascorbate peroxidase activity in the leaf of Tapioca

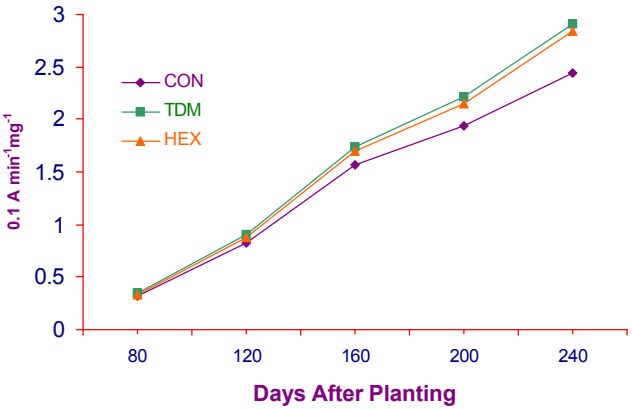

Fig. 4: Triazoles induced changes in the ascorbate peroxidase activity in the tuber of Tapioca

of superoxide anion radical (O_2^-) with great efficiency resulting in the production of H_2O_2 and O_2 [24,25]. Propiconazole treatment protected seedlings from damage and the stress protection is mediated by an increased activity of antioxidant enzymes [26]. Similar observations were observed that triazole treatment increased the activity of superoxide dismutase, glutathione reductase and ascorbate peroxidase in the leaves and roots of plants [27-30].

Ascorbate Peroxidase

Leaf (Fig. 3): The APX activity increased with the age in the leaves of control and treated plants. In both the treatments the APX activity increased to a larger extent when compared to control and it was 119.32 and 116.15 percent over control respectively on 240 DAP.

Tuber (Fig. 4): In the tubers of tapioca triazole treatments increased the APX activity to a larger extent when compared to control. The APX activity increased in triadimefon and hexaconazole treatments to 118.89 and 116.15 percent over control respectively on 240 DAP.

The ascorbate peroxidase activity increased in the triazole treated tapioca plants when compared to control. Similar observation was made in triazole treated plants [30-33]. The enzyme of glutathione ascorbate cycle has been implicated in mitigating the effect of reactive oxygen species [28-29]. Antioxidant enzymes such as ascorbate peroxidase, superoxide dismutase and antioxidant metabolites like ascorbate, glutathione, carotenoids are involved in scavenging reactive oxygen species [28]. The triazole compounds enhanced the free radical scavenging capacity in treated plants including the levels of carotenoids, ascorbate superoxide dismutase and ascorbate peroxidase [8-13].

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