Journal of Experimental Sciences Vol. 1, Issue 3, Pages 10-13 [2010]
www.jexpsciences.com

Regular Article

Alteration of Antioxidative Metabolism Induced by Triazoles in Sweet Potato

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Abstract

The triazole induced changes of antioxidants such as ascorbic acid, α-tocopherol, riboflavin, anthocyanin, and xanthophylls and the activities of antioxidant enzymes like ascorbate peroxidase, superoxide dismutase and catalase in *Ipomoea batatas* L. during initiation and maturation of storage roots were studied in field experiments. Each plant was treated with one liter of aqueous solution containing 20 mg L^{-1} triadimefon and 15 mg L^{-1} hexaconazole on 40, 55 and 70 days after planting (DAP). The treatments were given by soil drenching. The plants were harvested on 45, 60, 75, 90 and 105 DAP and used for analyzing antioxidant contents and antioxidant enzyme activities. It was found that these triazole compounds increase the contents of ascorbic acid, α-tocopherol, riboflavin, anthocyanin, and xanthophylls and activities of ascorbate peroxidase, superoxide dismutase, and catalase activities at 105 DAP. Triadimefon and hexaconazole treatments increased the antioxidation status in all parts of plants when compared to the control.

Key words: Triazole, Sweet potato, Antioxidation, Anthocyanin, Xanthophyll

Abbreviation: DAP, days after planting; CON, control; TDM, triadimefon; HEX, hexaconazole; FW, fresh weight; DW, dry weight; AA, ascorbic acid; APX, ascorbate peroxidase; SOD, superoxide dismutase; CAT, catalase.

Introduction

Sweet potato (*Ipomoea batatas*) is one of the most important food crops in the world [1]. Sweet potato storage roots are used for food, beverages, alcohol fermentation and as natural colorant [2, 3]. Triazole compounds used for their fungi toxicity also have plant growth regulating properties, and thus modulate the balance of important plant hormones including GA, ABA and cytokinins [4, 5]. Triazoles inhibit gibberellin and ergosterol biosynthesis in plants [6] and induce a variety of morphological and biochemical responses in plants, including inhibited shoot elongation, stimulated root growth, and increased cytokinins and ABA, and altered ergosterol biosynthesis [5]. Triadimefon and hexaconazole are triazole compounds with fungicidal and plant growth regulating properties [7, 8]. The application of these triazole compounds can alter the metabolic equilibrium, result in stress-like symptoms in plants [9], but simultaneously, they can protect plants from apparently unrelated abiotic stresses like NaCl stress [10]. The non-enzymatic antioxidants including ascorbic acid were important components of plant antioxidative systems [11, 12]. α-tocopherol was major lipid soluble antioxidant in membranes which can break the chain of lipid peroxidation and acts as cell membrane stabilizer [13]. The antioxidative effects of riboflavin during lipid peroxidation are oxidized by the hydrogen peroxide which acts as an electron acceptor and the hydrogen peroxide, itself then decomposes. The extent of the phenomenon may be proportional to the amount of the antioxidant [14]. Anthocyanin pigments are found in the orange, red and blue colours of fruits, vegetables [15] xanthophylls protect leaves from photo inhibition [16] and certain flavanoids, flavones, flavan-3-oils and hydroxycinnamate have a proven antioxidant capacity [17, 18]. Dioxygenase mediated cleavage of xanthophylls precursor for ABA has been postulated to regulate the formation of xanthophylls in an inducible manner [19].

The non-enzymatic antioxidants include lipid soluble membrane associated antioxidants (α -tocopherol and β–carotene) and water soluble reductants (glutathione and ascorbate). Antioxidative enzymes include superoxide dismutasse (SOD), ascorbate peroxidase (APX) and catalase (CAT).

The present study is to understand the effect of hexaconazole and triadimefon on non-enzymatic antioxidants like ascorbic acid (AA), α-tocopherol, riboflavin, anthocynanin, and xanthophylls, and antioxidative enzymes like APX, SOD and CAT activities in the leaves and storage roots of sweet potato (*Ipomoea batatas* L.).

Materials and Methods

Plant materials and cultivation methods

The cuttings of sweet potato (*Ipomoea batatas* L.) cv CO2 were collected from Tamil Nadu Agricultural University (TNAU) at Tamil Nadu, India. The triazole fungicides hexaconazole and triadimefon were obtained from Rallies and Bayer (India) Ltd., Mumbai respectively. During the study, average temperature was 32/26° C (maximum/minimum) and the relative humidity (RH) varied between 60% and 75%. The experiments were carried out in the field of Botanical Garden, Department of Botany, Annamalai University, at Tamil Nadu, India.

The plants were cultivated during the months of November-February (2003-2004). The sprouts of storage roots were used as seed materials. Thirty cm cuttings of uniform thickness with 3 nodes were used for planting. One and half by 1.5 m plot was prepared for each plant and 105 plots were designed for this study. Vines were trained to grow within the plot. Ground water was used for irrigation to maintain the optimum moisture level in the soil. Completely Randomized Block Design (CRBD) was used for this experiment.

Triazole treatments

Each plant was treated with one liter of aqueous solution containing active principle concentration of 20 mg L^{-1} triadimefon and 15 mg L^{-1} hexaconazole by soil drenching. The treatments were given on 40, 55, and 70 days after planting (DAP). The plants were harvested randomly on 45, 60, 75, 90 and 105 DAP. Leaves and storage roots were used for analyzing the non-enzymatic antioxidants and antioxidative enzymes.

Antioxidants

The ascorbic acid contents were estimated by the method of Omaye et al. [20], α-tocopherol by Backer et al. [21], and riboflavin by Sawhney [22].

Pigments

The anthocyanin content was estimated by the method of Kim et al. [23] and the xanthophylls by Neogy et al. [24].

Antioxidative enzymes

APX (EC: 1.11.1.11) activity was estimated by the method of Asada and Takasaki [25] and APX expressed in units (U= change in 0.1 absorbance min^{-1} mg^{-1} protein). Superoxide dismutase (SOD) EC: 1.15.1.1 by the method of Beauchamp and Fridovich [26] and SOD activity is expressed in units. One unit (U) is defined as change in 0.1 absorbance h^{-1} mg^{-1} protein under the assay condition. CAT (EC: 1.11.1.6) was estimated using the method of Chandlee and Scandalios [27] and expressed in units. (U=1mM of H_2O_2 reduction

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ISSN: 2218-1768

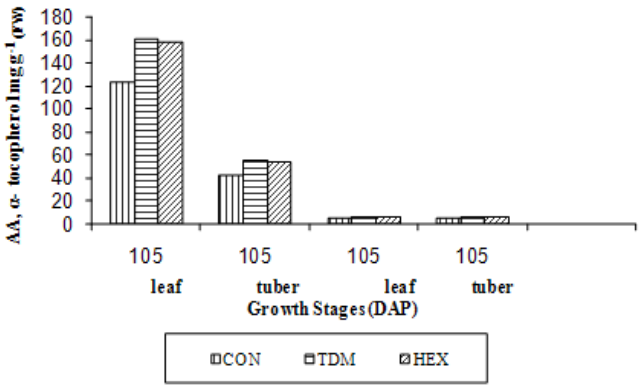
min^{-1} mg^{-1} protein). The enzyme protein was determined by the method of Bradford [28].

Results and Discussion

Effect of triadimefon and hexaconazole on ascorbic acid

The ascorbic acid contents increased in the leaves and storage roots of triazole treated plants (Fig. 1). Triadimefon and hexaconazole caused a profound influence upon the regulatory mechanisms of the plant as a whole including the increase of antioxidants [5, 29, 30]. An increase in ascorbic acid content was reported in uniconazole treated tomato seedlings and paclobutrazol treated *Dioscorea rotundata* poir [30, 31]. Ascorbic acid is an important antioxidant which functions as the terminal antioxidant because the redox potential of ascorbate/monodehydroascorbate pair is lower than that of most of the bioradicals [32].

Fig. 1: Triazole induced changes in ascorbic acid, α- tocopherol content in the leaf and tuber of sweet potato. Values are mean ± S.D. of three samples



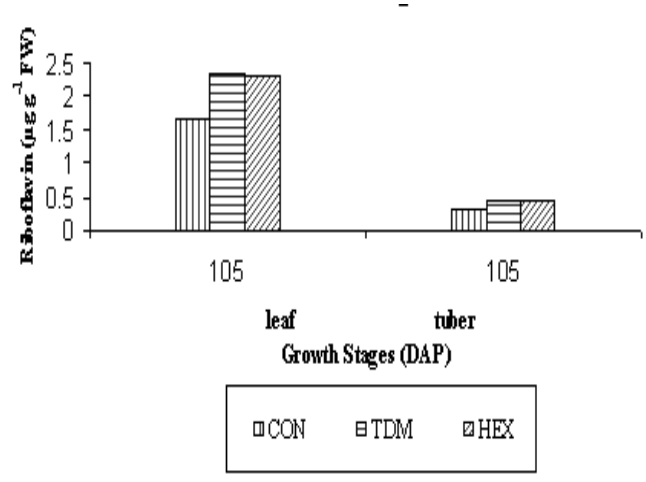
Effect of triadimefon and hexaconazole on α- tocopherol

α- tocopherol contents in the HEX and TDM treated plants were higher than those of controls in all growth stages (Fig. 1). The TDM treated plants showed an increased α- tocopherol content in leaves, stems as well as in roots, thus showed very good antioxidant potentials in different parts of the plant [30, 31, 33].

Effect of triadimefon and hexaconazole on riboflavin

Riboflavin content increased with age in the leaves and storage roots of sweet potato (Fig. 2). For triadimefon and hexaconazole treated sweet potato plants, storage roots showed an increased riboflavin content as compared to the control. Increase of riboflavin content in TDM and HEX treated plants can decrease the membrane degradation due to oxidation of lipid component of the membrane by the reactive oxygen species. It is involved in the prevention of lipid peroxidation, is oxidized and acts as an electron acceptor [34] in TDM treated *C. roseus* [33].

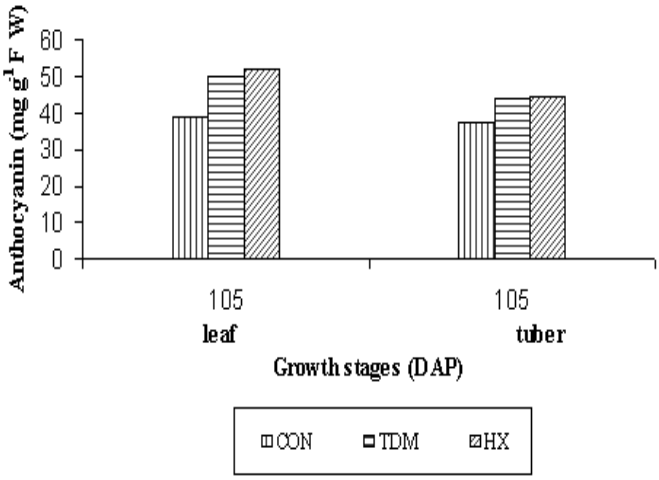
Fig. 2: Triazole induced changes in riboflavin content in the leaf and tuber of sweet potato. Values are mean±S.D. of three samples



Effect of triadimefon and hexaconazole on anthocyanin

Triazole treatments increased the anthocyanin content to a higher level in the leaves when compared to storage roots (Fig. 3). Anthocyanin pigments are widespread in the plants, like fruits, flowers, leaves, roots and storage organs [15]. Concentration of anthocyanin was higher in sweet potato storage roots [35]. Triadimefon increased the anthocyanin content in radish cotyledons and its effect can be comparable to that produced by cytokinin [36]. Treatment with ABA stimulated the accumulation of anthocyanin and phenolics as well as ethylene production [37]. Triazoles induced an transients increase in ABA in rice plants [38]. The transient increase in ABA induced by TDM and HEX might have increased the anthocyanin content in the storage roots of triazole treated sweet potato.

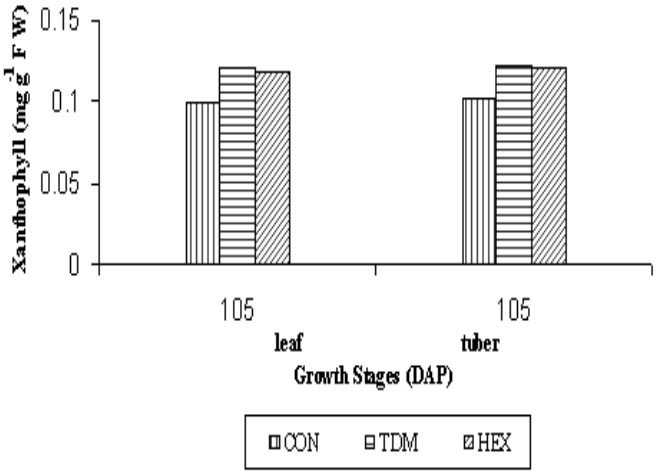
Fig. 3: Triazole induced changes in the anthocyanin content in the leaf and tuber of sweet potato. Values are mean±S.D. of three samples



Effect of triadimefon and hexaconazole on xanthophylls

Triazole treatments increased the xanthophylls content in the sweet potato leaves and storage roots (Fig. 4). Triadimefon treatment increased the xanthophylls content in the leaves of barley [39]. Xanthophyll protects leaves from photoinhibition [16]. Certain flavanoids, flavones, flavan-3-oils and hydroxycinnamate have a proven antioxidant capacity [17]. Hence the increased anthocyanins might increase the antioxidative potentials in sweet potato when treated with triazoles [33]

Fig. 4: Triazole induced changes in the xanthophyll content in the leaf and tuber of sweet potato. Values are mean±S.D. of three samples

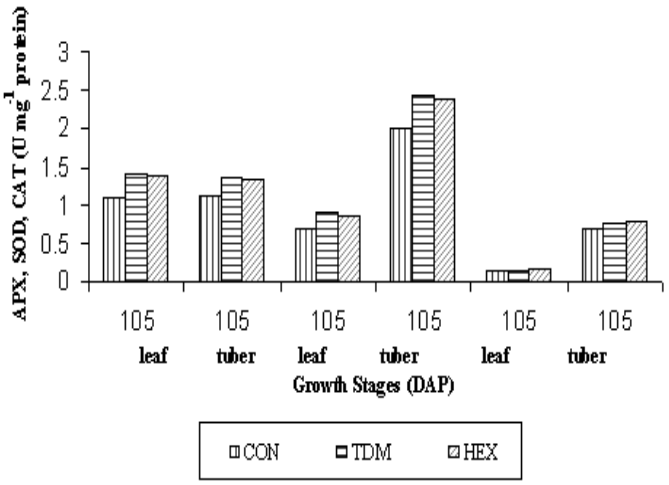


Effect of triadimefon and hexaconazole on ascorbate peroxidase

The activity of APX was higher in leaves as compared to storage roots of sweet potato (Fig. 5). Increased APX activity by TDM and HEX treatments would increase the demand for ascorbate

regeneration. Similar findings were reported in PBZ treated wheat [40, 41]. The increased ascorbic acids in the triazole-treated plants were well correlated with the increased APX contents [33]. Ascorbate peroxidase is the main antioxidant enzyme in the chloroplast which contains superoxide dismutase in *C. roseus* [33].

Fig. 5: Triazole induced changes in the APX, SOD, CAT activity in the leaf and tuber of sweet potato. Values are mean±S.D. of three samples



Effect of triadimefon and hexaconazole on superoxide dismutase

The antioxidative enzyme SOD activity increased with HEX and TDM treatment in the leaves and tubers (Fig. 5). According to Pastori [41] many stress situations caused an increase of the foliar SOD activity [40] and TDM treated radish, *C. roseus* [33,42] uniconazole treated wheat [43] and *Cassia* seedling [44] showed an increased SOD activity.

Effect of triadimefon and hexaconazole on catalase

The CAT activity was also increased in the leaves and storage roots of sweet potato (Fig. 5). The increase of CAT activity observed in TDM treatment is of great importance in plant protective mechanism. The H_2O_2 scavenging system represented by CAT is more important in importing tolerance to oxidative stress as observed in sweet potato and wheat varieties [45, 46, 47, 48]. The result showed that the TDM and HEX treatments enhanced the ROS scavenging capacity by the increased activity of the antioxidative enzymes like APX, SOD and CAT in the sweet potato [30]

Conclusion

In conclusion, our results indicated that the triadimefon and hexaconazole application at low concentration could be a potential tool to increase the antioxidative defense mechanisms in sweet potato.

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Please Cite This Article As: T. Sivakumar, G. M. A. Lakshmanan, P. V. Murali and R. Panneerselvam. 2010. Alteration of Antioxidative Metabolism Induced by Triazoles in Sweet Potato. J. Exp. Sci. 1(3): 10-13.