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Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage

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Abstract: The ability of triadimefon (TDM), a triazolic fungicide, to alter the biochemical constituents and thereby minimizing the days required for sprouting in white yam (*Dioscorea rotundata* Poir.) tubers during storage under (30±2) °C in the dark, was studied. TDM at 20 mg/L was given to tubers by dipping the tubers in treatment solution containing 20 mg/L TDM on 10, 25 and 40 d after storage (DAS). Starch, sugars, protein, amino acid contents as well as protease and α-amylase activities were estimated on 15, 30 and 45 DAS from two physiological regions viz., apical and basal regions of the tubers. In normal conditions (control) sprouting occurred on 70 to 80 DAS. The starch content decreased, while protein, amino acid, sugar contents and protease and α-amylase activities were increased due to TDM treatment and led to early sprouting.

Key words: Biochemical constituents, Enzyme activities, *Dioscorea rotundata*, Sprouting, Storage, Triadimefon (TDM)
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INTRODUCTION

Roots and tubers were critical components in the diet during the early evolution of mankind and were the most important food crops of very ancient origin in the tropics and sub tropics, associate with human existence, survival and socio-economic history (Asha and Nair, 2002). Yams (*Dioscorea* spp., Dioscoreaceae) are the third most important tropical "root" crop after cassava and sweet potato (Fu *et al*., 2005). White yam (*Dioscorea rotundata* Poir.) plant produces tubers and bulbils, which are edible and have economic importance (Omonigho and Ikenebomeh, 2000). The tubers of various species of *Dioscorea* constitute one of the stable carbohydrate foods for the people in many tropical countries (Akissoe *et al*., 2003). Many different forms and cultivars of the edible yam species are available in different areas and it is likely that these differ in composition and nutritional values (Bhandari *et al*., 2003). *Dioscorea* spp. also have medicinal properties as their tubers contain diosgenin, a biochemical precursor in the synthetic production of progesterone and other corticosteroids (Albrecht and McCarthy, 2006).

The wide spread cultivation and large-scale production of white yam are hindered by the comparatively long dormancy period of the tubers. To sprout the tubers, for producing seed material, minimum 70 to 80 d after storage (DAS) is required (Muthukumarasamy and Panneerselvam, 2000). In order to develop a competent method for sprouting in tuber crops, application of plant growth regulator (PGR) is a previously established technique (Paul and Ezekiel, 2003). During storage of tubers, especially at higher temperatures, ageing takes place gradually and this is associated with an increase in abscisic acid (ABA) content and a decrease in gibberellic acid (GA) content, as reported in potato by Burton *et al*.(1992).

Increase in GA content of tubers is reported to inhibit little tuber formation and sprouting in potato (Ezekiel *et al*., 2000). These reports show clearly that if GA content of tubers is decreased and ABA content increased, the sprouting process can be initiated in tubers. Some unknown factors other than ABA might also be involved in dormancy-release in tubers (Xu *et al*., 2006).

Triazole compounds have both fungitoxic and plant growth-regulating properties (Fletcher *et al*., 2000). In addition, they can also protect plants against various environmental stresses (Voesenek *et al*., 2003). Triazoles adversely affect the isoprenoid pathway, and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution, and increasing cytokinin levels (Kamountsis and Chronopoulou-Sereli, 1999). Some previous work 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, increased antioxidant potentials and enhancement in alkaloid production (Muthukumarasamy and Panneerselvam, 1997; Muthukumarasamy *et al*., 2000; Panneerselvam *et al*., 1997; 1998; Jaleel *et al*., 2006). Triadimefon (TDM) [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-yl)-2-butanone], a triazole fungicide, having PGR properties, is reported to inhibit GA biosynthesis and increase ABA and cytokinin contents (Fletcher *et al*., 2000). It is necessary to investigate the efficacy of this compound in the induction of early sprouting of white yam tubers during storage in order to minimize the days required for sprouting. Hence, the aim of the present work was to estimate the ability of TDM in early initiation of sprouting process and to analyse the changes in the biochemical constituents due to TDM treatment of white yam tubers during storage under (30±2) °C in the dark.

MATERIALS AND METHODS

Plant materials and TDM

Tubers of *Dioscorea rotundata* cv. Sree Priya were obtained from Central Tuber Crop Research Institute (CTCRI), Kerala, India and planted in Botanical Garden of Annamalai University. The tubers were harvested after the death of aerial parts and stored in plastic trays in the dark at room temperature (30~32 °C) and relative humidity (RH) 80%~85%. TDM as BAYLETON 25 WP was obtained from Bayer (India) Ltd., Mumbai.

TDM treatment

In the preliminary experiments, four concentrations [10, 15, 20 and 25 mg/(L tuber)] of TDM were prepared and used for treatment of tubers to determine the optimum concentration at which maximum sprouting occurred. Among these concentrations, 20 mg/(L tuber) was found to enhance the sprouting process and at lower [10 and 15 mg/(L tuber)] and higher [25 mg/(L tuber)] concentrations, there was no significant effect. Therefore 20 mg/(L tuber) was used for this study. The treatment was given by dipping the tubers in TDM solution for 15 s, at three time intervals i.e. 10, 25 and 40 DAS. For each dipping one litre of the solution was used and the dipping was done in three installments with 30 min gap to facilitate maximum absorption of the chemical. The control tubers were dipped in distilled water.

Analysis of biochemical constituents

The biochemical constituents were analysed at three storage periods viz., 15, 30 and 45 DAS in the two physiological regions i.e., apical (1 cm below the tip) and basal (1 cm above the base) regions of the tubers.

Starch and sugars

Cylinders of the tuber tissue were removed by using cork borer and homogenized in 80% ethanol (v/v). The homogenate was refluxed over a water bath and centrifuged. The residue was extracted twice more with 80% ethanol. The supernatant liquids were combined and used for estimation of soluble sugars by the method of Dubois *et al*.(1956). The remaining pellets were used for the estimation of starch by the method of McCready *et al*.(1950). Both estimations were carried out at room temperature (30~32 °C).

Protein and total free amino acid

The protein content was determined by the method of Bradford (1976). Total free amino acid was

extracted and estimated by following the method of Moore and Stein (1948).

Protease (EC 3.4.2.2)

The protease activity was determined by the method described by Prisco *et al*.(1975), using glycine as standard. The enzyme activity was expressed in terms of enzyme units/mg protein [U/(mg protein)]. One U is defined as μmoles of α-NH_2 released per mg protein per hour.

α-amylase (EC 3.2.1.1)

The tuber tissues were excised and 1 g of fresh mass was ground with a pestle in an ice-cold mortar with 10 ml of distilled water at 4 °C. The homogenate was centrifuged at 4 °C for 30 min at 15000 r/min. The supernatant was used to assay α-amylase activity. The protein content in the supernatant was estimated according to Bradford (1976), using bovine serum albumin as standard. The activity of α-amylase (EC 3.2.1.1) was assayed by the method of Bernfeld (1955), using β-limit dextrin as substrate. The enzyme extract was heated at 70 °C for 5 min with 3 mmol/L $CaCl_2$ to inactivate β-amylase as described by Tarrago and Nicolas (1976).

Statistics

The experiment was carried out in completely randomized design (CRD). Each treatment was analyzed with at least seven replicates and a standard deviation (*SD*) was calculated. The data were expressed in mean±*SD* of 7 replicates.

RESULTS AND DISCUSSION

The two physiological regions (apical and basal) were demarcated in yam tubers as described previously (Isamah *et al*., 2000). Sprouting occurs mainly in the apical region. The starch content varied in the two physiological regions of white yam tubers during storage (Fig.1a). The highest content was seen in early storage periods (15 DAS). It declined slightly at 30 DAS and decreased further towards the onsetting of sprouting (45 DAS in TDM treated and 70 to 80 DAS in control tubers). This result is in accordance with reports of Faboya and Asagbra (1990). TDM at 20 mg/L concentration reduced the starch content in both apical and basal regions of the tubers on 15, 30 and 45 DAS. A significant decrease was noted in the basal region of the tubers on 45 DAS under TDM treatment. The decrease in starch content can be correlated to the necessity of starch breakdown to initiate sprouting. Sprouting was initiated due to TDM treatment on 45 DAS. Reduced starch content is the prerequisite to initiate the sprouting process (Ravindran and Wanasundera, 1992). Reduced starch content is reported in potato during sprouting (Davies and Ross, 1984). The breakdown of starch is essential to enhance sprouting in order to utilize and translocate the food reserves from tuber (Edelman *et al*., 1968).

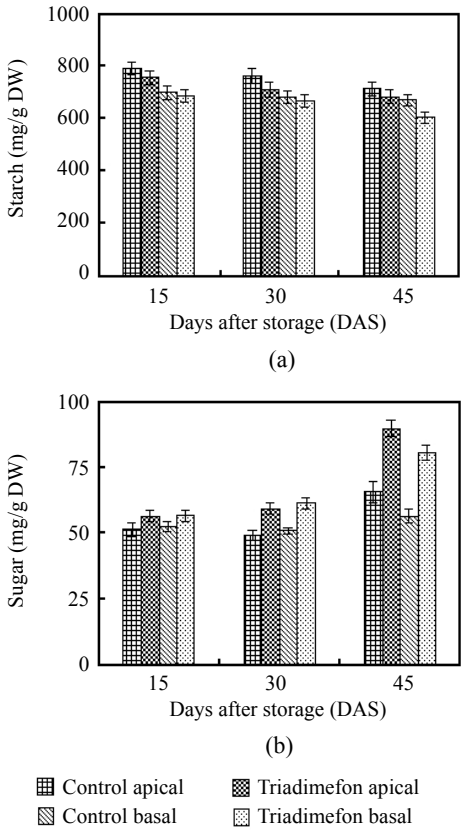

Fig.1 Triadimefon induced changes in the starch (a) and sugar (b) (mg/g DW) contents in apical and basal regions of *D. rotundata* tubers during storage
Values are mean±*SD* of 7 replicates

A variation in the sugar content was seen in apical and basal regions of the tubers on all storage periods except 15 DAS. The sugar content increased due to TDM treatment, which facilitate sprouting (Fig.1b). An increase of sugar content during sprouting was observed in potato by Isherwood and

Burton (1975). A decrease in starch and an increase in sugar content were reported in *Crocus sativus* corms during sprouting (Chrungoo and Farooq, 1985). Sprouting behaviour of *Lilium* bulblets cultivated under low temperature conditions showed rapid starch hydrolysis accompanied by accumulation of sugars (Miller and Langhans, 1990). The accumulation of sugars could influence the sprouting and bud growth in tubers (Xu *et al*., 2006).

The protein content was highly increased due to TDM treatment in both basal and apical regions of white yam tubers on 15, 30 and 45 DAS (Fig.2a). At the time of sprouting, the protein content increased to a maximum extent at the apical region but it declined at the basal region. Protein synthesis was proved to be one of the essential processes associated with sprouting (Jayakumar *et al*., 1993). The amino acid content was more or less the same in apical and basal regions of the tubers (Fig.2b). A rapid increase was seen on 45 DAS in TDM treated tubers, which is helpful to initiate sprouting. Increased amino acid content was reported in white yam tubers during sprouting by Splittstoesser and Rhodes (1973).

The protease activity is directly related to sprouting. Increased protease activity was found in TDM treated white yam tubers during storage (Fig.3a). The increase in protease activity and amino acid content with a concomitant reduction in the protein content of the basal region was observed during sprouting. Izundu and Fasidi (1991) reported similar observation of *Dioscorea dumetorum*. Stored protein in the tuber is hydrolyzed in the form of amino acid by the enzyme protease during the time of sprouting (Muthukumarasamy and Panneerselvam, 1996).

The α-amylase activity was increased due to TDM treatment. The extent of increase was high in basal region, and was higher than that in the apical region during sprouting (Fig.3b). The higher α-amylase activity coincides with the higher sugar content in the tubers during sprouting (Ikediobi and Oti, 1983). Increased α-amylase activity was reported by Panneerselvam (1987) in sprouting rhizomes of *Curcuma* and tubers of *Dioscorea*.

The dormancy period of *Dioscorea* tubers decreased due to TDM treatment. Enhanced breakdown

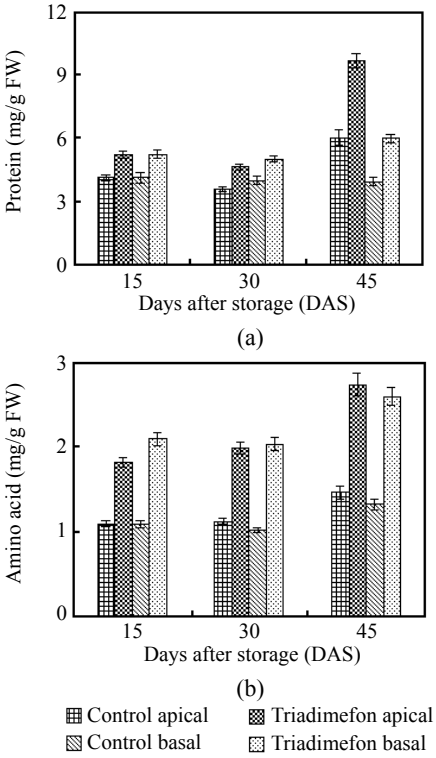

Fig.2 Triadimefon induced changes in the protein (a) and amino acid (b) (mg/g FW) contents in apical and basal regions of *D. rotundata* tubers during storage
Values are mean±*SD* of 7 replicates

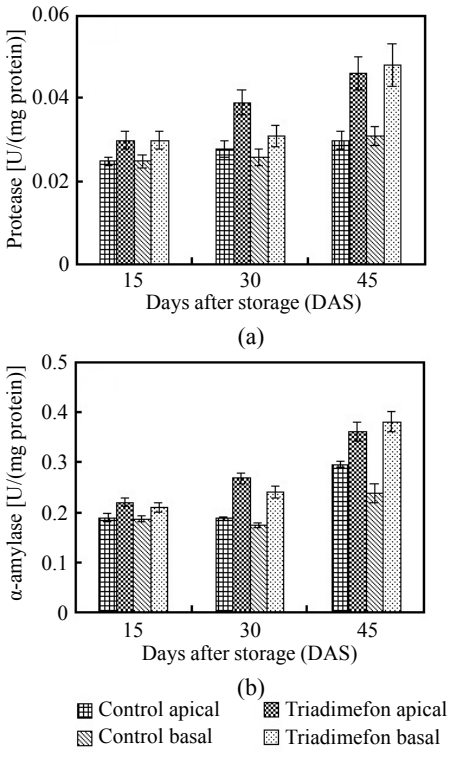

Fig.3 Triadimefon induced changes in the protease (a) and α-amylase (b) (U/mg protein) activities in apical and basal regions of *D. rotundata* tubers during storage
Values are mean±*SD* of 7 replicates

of starch and production of sugars and early initiation of sprouting are the evidence for this. Related observations were made in TDM treated potato, where the little tuber production was increased due to TDM treatment (Paul and Ezekiel, 2003). This result can also be correlated with the findings of Li and Zhang (1999) who reported increased adventitious bud development in triazole treated maize. From the results of this investigation, it can be concluded that the application of TDM can be used for inducting and enhancing of sprouting in white yam tubers by reducing the dormancy period. Further research is required to see whether this effect of TDM can be used to further reduce the time period required for sprouting in yam tubers and to make this technique more acceptable and viable.

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