Indian J. Plant Physiol., Vol. 7, No. 1, (N.S.) pp. 44-47 (Jan.-March, 2002)

ACTIVITIES OF ANTIOXIDANT ENZYMES DURING ETHANOL REGULATED RIPENING OF TOMATO FRUITS

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Received on 28 Sept., 2001, Revised on 23 March, 2002

SUMMARY

Mature green fruits of tomato of a *Desi* (local) variety were exposed to ethanol vapour in a closed jar for 24 h. The doses used were 1, 2, 4, 8 and 10 ml of ethanol per kg of fruit. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities were determined in treated and untreated fruits till 10th day after the harvest. By this time untreated (control) fruits were red ripe and treated fruits at different stages of ripening depending upon the dose of ethanol. Assay of enzyme activities showed rapid and early rise in superoxide dismutase activity in untreated fruits by 4th day of the harvest coinciding with half ripe stage of the fruits. A decline in SOD activity was observed later. Lower doses of ethanol i.e. 1 and 2 ml marginally delayed the peak SOD activity but with doses of 4 ml and above, no change in enzyme activity from initial value was noticed till 10th day. Both catalase and peroxidase activities steadily increased as ripening is progressed i.e. till 10th day in untreated and in 1 and 2 ml treated fruits. At higher concentrations of ethanol either no change or slight lower values were noted in the activity of these two enzymes suggesting no change in the condition of fruit from that of mature green stage.

Key words : **Antioxidants, catalase, ethanol, peroxidase, ripening, superoxide dismutase, tomato**

INTRODUCTION

The increase in free space and loss in ability to retain solutes have been demonstrated during fruit ripening. Free radicals and other reactive derivatives of oxygen are inevitable byproducts of biolgical redox reactions. These oxygen species (singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radical) inactivate enzymes and damage important cellular components. Free radical induced lipid peroxidation is considered to be an important mechanism of membrane disintegration during fruit ripening (Ferrie *et al.* 1994). A loss of microsomal membrane integrity was observed during maturation and ripening of muskmelon (Lester and Stein 1993). Ripening tomato fruits displayed increase in ion leakage (Palma *et al.* 1995). According to Huber (1987) ripening is a functionally modified protected form of senescence and mechanism for membrane deterioration during ripening is probably similar to those characterized in senescence (Ferrie *et al.* 1994).

Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) scavenge superoxide radical O_2^- and H_2O_2 respectively and control the level of lipid peroxidation. The inability to quench the free radicals or the reactive oxygen species by scavenging enzymes either due to over production of free radicals or degradation of enzymes during later stages determines the pace of ripening activity. SOD activity changes during the course of fruit ripening and shows an increase after climacteric phase (Rabinowitch and Sklan 1980). Possible involvement of enzyme in the synthesis of ethylene from methionine has

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been suggested (Beauchamp and Fridovich 1970). Rogiers *et al.* (1998) reported that changes in the activities of SOD, CAT and POX are characteristic of the stage of fruit developement and must be induced with the expression of gene to indicate commencement of senescence or ripening. Their magnitude, however, is decided by the availability of substrate i.e. O_2^- and H_2O_2.

Early ripening of harvested fruits causes major post harvest losses in tomato and this problem is yet to be resolved. There is a need of a technology for extending the shelf life of tomato fruits. Ethanol has been reported to inhibit tomato ripening (Kelly and Saltveit 1988). Ethanol production has been reported from many plant tissues under anaerobic (Kimmerer and Kozlowaski 1982) or aerobic (Chang *et al.* 1983) conditions during metabolism. Ethanol application could therefore, be a safe and useful approach for extending shelf life of tomato fruits. The present study was, therefore, undertaken to analyse the effect of dose dependent application of ethanol during ripening of tomato fruits on activities of antioxidant enzymes such as SOD, POX and CAT

MATERIALS AND METHODS

The fruits of a *Desi* (local) variety of tomato grown in the Institute's field, were used for the study. The variety has a high water content, thin peel (skin) and low keeping quality. Mature green fruits of uniform shape and size were selected. About 30-35 fruits weighing ≈2.5 kg were grouped and placed in 5 litre airtight jars. Fruits were exposed to ethanol vapour for 24 h by keeping 0, 1, 2, 4, 8 and 10 ml ethanol in small beakers with filter paper strips dipped into the liquid for gradual adsorption and evaporation inside the jars. The jars were closed immediately after adding ethanol and were left at room temperature (25°C) for 24 h. During the period ethanol was fully vapourized and presumably sufficiently absorbed by the fruits. After the exposure, fruits were taken out and were kept in open trays in the laboratory for various observations and enzyme assay. Samples for the enzyme assay viz. superoxide dismutase, peroxidase and catalase were collected from the pulp portion of treated and untreated fruits at 0, 2, 4, 8 and 10th day after treatment coinciding with breaker, turning, pink and red ripe stages of the untreated fruits. Data were subjected to analysis of variance with the least significant difference calculated at P=0.05. Superoxide dismutase, peroxidase and catalase activities were assayed as per Beauchamp and Fridovich (1971), Zieslin and Zaken (1992) and Teranishi *et al.* (1974) respectively.

RESULTS AND DISCUSSION

The SOD activity in untreated fruits increased 2- fold from initial unripe stage when fruits attained turing stage. With further advancement of ripening a drastic reduction was noted by 7th day (pink stage) and at full ripening it became even lower than the values observed at initial stages (Table 1). With the treatment of ethanol a delay in the SOD peak activity was noted and was delayed further as the dose of ethanol increased. For instance, in case of 1 ml of ethanol treatment peak SOD activity was shifted to 7th day after the harvest and with 2 ml ethanol it was observed on 10th day. In fruits exposed to higher doses of ethanol viz. 4 ml, 8 ml and 10 ml, the enzyme activity remained almost unchanged and was at the same level as noted in the unripe fruit. The visual symptoms and the conditions of the fruits treated with higher doses were very similar to that of unripe fruit. It is logical to assume that there was no increase in the production of O_2^- which causes speeding up of the ripening of fruits as also opined by Rogiers *et al.* (1998). In the absence of further rise in the substrate i.e. O_2^- no change in the activity of SOD enyzme was noted.

Peroxidase activity continued to rise in fruits both untreated and exposed to low levels of ethanol (Table 2). In untreated fruits almost 3-fold increase in the enzyme activity was observed at 10th day after the harvest and fruits were ripe by that time. In fruits exposed to lower concentration of ethanol 1 and 2 ml, peroxidase activity was very low and increased slowly upto 10th day. At higher doses of exposure, almost no change in the enzyme activity was observed from 0 day till 10th day.

Pattern of catalase activity is somewhat different than the other two enzymes. In untreated tomato enzyme activity steadily increased with advancement of ripening till fruits become full ripe and about 38% increase in catalase activity was observed over unripe fruits (Table 2). Ethanol treatment suppressed the catalase activity. With lower doses i.e., 1 and 2 ml this inhibition was effective for 4 to 7 days respectively but later on it subsided. Compared to corresponding control ('0' day) a

Table 1. Effect of ethanol treatment for 24 h on SOD activity (units min^{-1} mg^{-1} protein) of tomato fruit harvested at mature green stage.

Ethanol treatments (ml)	SOD activity (units min^{-1} mg^{-1} protein)					Mean
	Days after treatment					
	0	2	4	7	10	
Control	5.070	7.816	10.223	5.360	4.393	6.572
1	5.317	6.367	7.587	9.151	7.491	7.183
2	5.151	5.542	5.883	6.167	8.421	6.233
4	5.126	6.142	5.160	5.712	6.000	5.428
8	5.383	5.375	5.358	5.877	5.925	5.584
10	5.121	5.178	5.233	5.849	5.920	5.460
Mean	5.195	5.903	5.674	6.353	6.358	6.077

	Treatment (T)	Days (D)	TxD
SE(m)	0.0670	0.0612	0.1498
CD (P=0.05)	0.1935	0.1766	0.4326

Table 2. Effect of ethanol treatment for 24 h on peroxidase and catalase activity of tomato fruit harvested at mature green stage.

Ethanol treatments (ml)	Peroxidase activity (mmol H_2O_2 min^{-1} mg^{-1} protein)					Catalase activity (mmol H_2O_2 min^{-1} mg^{-1} protein)				
	Days after treatment									
	0	2	4	7	10	0	2	4	7	10
Control	2.379	4.084	4.261	6.261	7.733	29.083	30.167	32.167	34.583	40.250
1	2.513	3.184	4.099	4.207	5.383	30.750	29.250	29.333	31.583	33.833
2	2.481	2.531	2.986	3.905	4.148	30.500	24.883	27.250	29.417	30.167
4	2.542	2.517	2.555	2.885	3.017	30.000	19.833	20.000	20.667	21.500
8	3.497	3.074	2.861	2.723	2.889	30.333	17.500	19.083	19.500	20.333
10	3.650	3.706	3.735	3.106	2.996	29.975	14.667	15.883	16.667	19.500
Mean	2.844	3.244	3.416	3.848	4.361	30.107	22.708	23.944	25.403	27.597

	Treatment (T)	Days(D)	TxD	Treatment(T)	Days(D)	TxD
SE(m)	0.0147	0.0134	0.0329	0.2076	0.1895	1.3406
CD(P=0.05)	0.0424	0.0388	0.0949	0.5996	0.5473	0.4642

little higher activity was noticed after 10th day in ripe fruits. Though CAT activity increased by about 3.0 mmol H_2O_2 min^{-1} mg^{-1} protein after 10th day from their corresponding control ('0 day) yet it almost maintained similar level after same duration with 2 ml treatment. Fruits exposed to relatively higher doses of ethanol i.e. 4, 8 and 10 ml, showed substantial inhibition of catalase activity and was particularly marked during early days after treatment. The inhibitory effect of ethanol slightly diminished at 7th and 10th day after harvest.

Increase in the activity of peroxidase and catalase during ripening may be in response to increased supply of oxidizing substrate (O_2^-, H_2O_2) that leads to membrane

disintegration (Rhodes 1980). Ethanol reduces this damage most probably either by lowering down the initial production of O_2^- and subsequently H_2O_2, the substrate for catalase and peroxidase enzymes or by removing the H_2O_2 through Halliwell-Asada pathway. As the availability of the substrate becomes limited, *de novo* synthesis of these oxidative stress enzymes depressed. Halliwell-Asada pathway involves enzymes such as ascorbate peroxidase (APOX), dehydro-ascorbate reductase (DAR), glutathione reductase (GR) and the reductant NADPH for removing H_2O_2. This pathway not only exists in chloroplast but also found to be operative in mitochondria and cytoplasm (Edverds *et al.* 1992). It appears that with higher doses of ehanol particularly at later phase of ripening where lower POX and CAT activities have been noticed in this study, the Halliwell-Asada pathway may be operative and activities of GR and APOX may be higher. In carnation free radical scavenger such as sodium benzoate (Halevy and Mayak 1979) and 3, 4, 5-trichlorophenol reduces the lipid peroxidation and resultant ion leakage causing delay in flower sensescence. It was found that delay in senescence was also associated with a blockage of ethylene synthesis. It appears that ethanol in appropriate concentration might be doing the same in the present study.

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