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SUPEROXIDE DISMUTASE AND PEROXIDASE ACTIVITIES IN RIPENING MANGO (*MAGNIFERA INDICA* L.) FRUITS

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SUMMARY

Loss in fresh weight and rise in respiratory activity along with ethylene peaks have been recorded in ripening mango cultivar, *Baneshan.* Antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD) were noticed to be very high at mature green stage, which significantly declined as the ripening proceeded. These antioxidant enzymes exhibited higher activity in the epicarp over mesocarp at all the stages of ripening. The isoenzyme pattern of SOD was observed to be the same at all the stages of ripening in both epicarp and mesocarp, but intensity of bands differed. Peroxidase also exhibited difference in isoenzyme pattern and intensity of bands. There was only one band at mature green stage, whereas three bands were recorded at partial ripe and full ripe stages, however, the intensity of bands appeared were high in epicarp over mesocarp.

Key words: **Ethylene, mango, peroxidase, respiration, superoxide dismutase**

INTRODUCTION

Fruit ripening is a metabolically active process and not a simple degradative reactions, which results in the transformation of fruit into an edibile product. The metabolic changes during ripening are highly enzyme mediated phenomena and involvs the expression of new genes (Grierson 1985). Antioxidant enzymes like SOD and POD have major role in the scavenging of free radicals, responsible for membrane deterioration in aging tissues (Kellog and Fridovich 1975). Univalent reduction of molecular oxygen to O^-_2 and H_2O_2 during ageing of tissues, senescence of flower petals and fruit ripening is a natural phenomenon to protect the biological system. Antioxidant enzymes like SOD, catalase and POD scavenge the above free radicals (Fridovich 1989). Superoxide dismutase activity has been reported in seeds, seedlings, leaves and fruits of higher plants (Giannopolitis and Ries 1977) but little work has been carried out during various developmental stages of plant organs. Changes in some antioxidant enzymes during plant senescence have been reported earlier (Pastorio and del Rio 1994), but there are not many studies on the role of antioxidant enzymes in ripening process.

An interesting feature of mango fruit ripening is the occurrence of differential softening and a differential distribution of cell wall softening and respiratory enzymes (Lazan *et. al.* 1993). There is however, no report on antioxidant enzymes activities and their distribution in relation to ethylene production and respiratory burst in mango fruits. This study was, therefore, undertaken to study the distribution of antioxidant enzymes during ripening of mango cv. *Baneshan*.

MATERIALS AND METHODS

Mature, unripe fruits of mango (cv. *Baneshan*) were procured from the mango orchard, APAU, Sanga Reddy, Andhra Pradesh and brought to IARI, New Delhi. Fruits of uniform size were selected and kept in lab at 25 ± 1°C. Six fruits were sampled for measurement at mature green (2 DAH), partial ripe (4 DAH) and full ripe (6 DAH) stages.

Loss in fresh weight was recorded daily till full ripe stage. The rate of respiration was measured with the help of an infra-red gas analyzer (ADC, England) in a specially designed chamber. Ethylene produced from intact fruits was estimated by enclosing the fruits in 1 liter chambers for 1 h and then 1 ml of sample was taken from this chamber and ethylene was assayed in a Hewlett Packard gas chromatograph (5890, model). Superoxide dismutase (SOD) activity in the epicarp and mesocarp was estimated at the respective ripening stages following the method of Dhindsa *et al.* (1981) with a little modification. Mango epicarp (0.2 g) of about 2 mm depth tissue was homogenized with 10 ml phosphate buffer (0.1 M, pH 7.5) and centrifuged at 15000 rpm for 15 min at 4°C. The supernatant was preserved in ice. Each 3 ml of the assay mixture constituted 0.1 ml supernatant, 1.5 ml phosphate buffer (0.1 M, pH 7.8), 0.1 ml Na_2CO_3 (1.5 M), 0.1 ml NBT (2.25 mM), 0.2 ml methionine (200 mM), 0.1 ml EDTA (3 mM), 0.1 ml riboflavin (60 µM) and 0.8 ml distilled water. The sample tubes were illuminated with 15 W fluorescent lamp for 10 min. The other set of tubes lacking enzyme were also illuminated and served as control. A non-irradiated complete reaction mixture did not develop colour and served as blank. Absorbance was recorded at 560 mm and 1 unit of enzyme activity was taken as the quantity of enzyme that reduced the absorbance reading to 50% in comparison with those lacking enzymes.

Peroxidase activity was determined by the guaiacol reaction (Simon *et al.* 1074). The unit of peroxidase activity was determined, and modified from Kokkinakis and Brooks (1979). Units/g fresh weight = 1000 x C x A/ min. In this equation C represents the concentration (g/ml) of tissue per final reaction mixture, and A is the change in absorbency.

Superoxide dismutase isoenzymes pattern was obtained by staining the non-dinaturating gels (Burk and Oliver 1992) with minor modifications. Fruits were washed with distilled water. The epicarp tissues at 2 mm depth and mesocarp tissues at 10 mm depth from epicarp were immediately homogenized (1 g fruit tissue + 2 ml extraction buffer) at 0 – 4°C in extraction buffer containing 96 mM Tris-HCl, 13% (v/v), glycerol, 0.6% (w/v), PVPP, 5 mM DTT and 1 mM PMSF in 1: 10 (w/v) ratio. The extracts were loaded on a non-denaturing gradient (7.5-15%) PAGE at 50 µg protein/lane. Pre-running was performed for 20 min at 70 V to remove the ammonium per sulfate. Electrophoresis run was carried out at 50 to 60 V in a mini cold lab at 4°C for 6 h. Gels were stained by incubating in dark in 50 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA, 0.2% TEMED, 3 mM riboflavin and 0.25 mM NBT for 30 min at room temperature with constant shaking. After incubation, gels were rinsed and placed in distilled water and exposed to light under 400 WHP sodium lamp, 60 cm above gel (300 E m^{-2} s^{-1}) for five to ten min. at 25°C. SOD activity was visibly compared by the intensity and size of the achromatic bands against a blue background.

Peroxidase isoenzymes pattern was estimated by staining gels as per method of Misra and Fridovich (1977). After the electrophoresis peroxidase zones were stained by soaking gels in 2 mM dianisidine in 10 mM potassium phosphate buffer (pH 7.2) for 1 h followed by a 15 min incubation in 0.1 mM H_2O_2.

The brick red coloured peroxidase activity became visible within 1 min, but the bands continued to intensify for 15 min. The peroxidase activity was estimated by comparing the intensity and the size of the peroxidase zones.

RESULTS AND DISCUSSION

Continuous loss in fruit weight was observed from the day of harvest to full ripe stage. However, per day loss in fresh weight increased up to 8th day and thereafter, decreased. Respiration rate increased after the harvest with a sudden rise in respiratory burst reaching a peak by

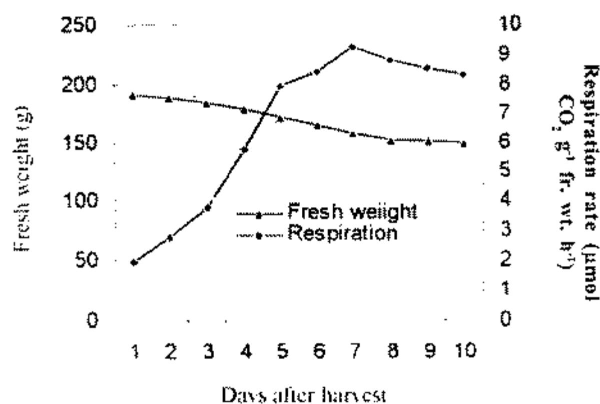

Fig. 1. Fresh weight and respiration rate during ripening in mango cv. *Baneshan*

7th day and then showed a declining trend (Fig. 1). These results indicated that there was typical climacteric pattern of respiration during ripening of mango cultivar *Banishan*. The respiration rate was opposite in trend to loss in fresh weight suggesting the consumption of biomass in the respiratory process which is the major pathway for carbon skeleton for synthesis various metabolites. Similarly ethylene production also increased from the days of harvest and showed two typical peaks at 3rd and 7th day after harvest (Fig. 2). The rise in respiration and enhanced ethylene production are two most important events during early stages of fruit ripening (Burg 1968).

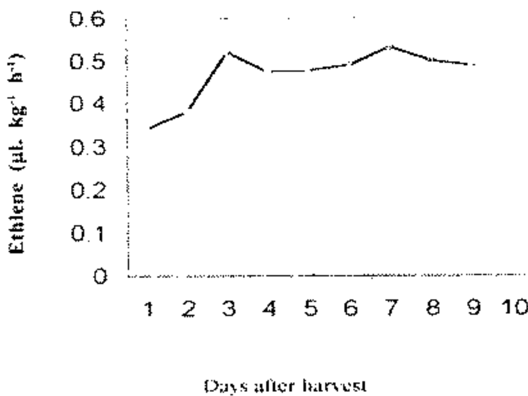

Fig. 2. Ethylene production during ripening in mango cv. *Baneshan*

Superoxide dismutase activity was assayed at mature green (2 DAH), partial ripe (4 DAH) and full ripe (8 DAH) stages both in epicarp and mesocarp of the fruit (Fig. 3). SOD showed maximum activity at mature green stage and gradually declined till full ripe stage. Peroxidase enzyme also showed maximum activity at mature green stage (Fig. 4) and declined through partial ripe and full ripe stages. At all the stages of ripening SOD and POD exhibited higher activity in epicarp over mesocarp. These results indicated that SOD and POD have differential activity at different tissue depth. High SOD activity at mature green and low with the advancement in ripening indicated that SOD is involved in protecting the membrane integrity by scavenging the free radicals, whereas the decline in enzyme activity in partial ripe and full ripe fruits leads to loss in membrane integrity leading to ripening and enhanced perishability. High SOD enzyme activity in the pericarp tissue retarded the membrane permeability (Zhang *et al.* 1997). Similarly its high activity in epicarp over mesocarp maintain epicarp firm and fresh over mesocarp. Because of this differential enzyme activity, fruit ripening was not uniform which was initiated from mesocarp and progressed towards epicarp.

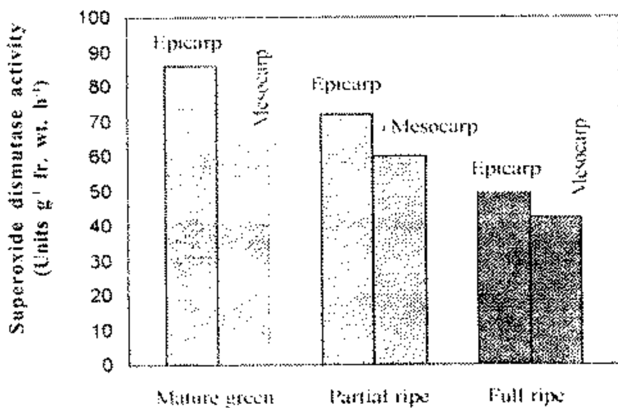

Fig. 3. Superoxidedismutase activity at different stages of ripening in mango cv. *Baneshan*

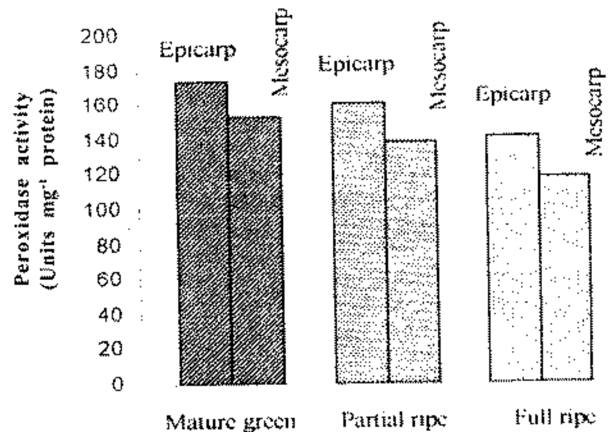

Fig. 4. Peroxidase activity at different stages of ripening in mango cv. *Baneshan*

Isozyme pattern indicated that the SOD activity in the extract is composed of distinct bands. Four SOD bands were obtained at all the stages of ripening in both epicarp and mesocarp tissues in a single electrophoretic run (Fig. 5).

POD isozyme pattern at different stages of fruit ripening showed different number of bands (Fig. 6). At mature green stage, there was only one distinct band both in epicarp and mesocarp, whereas at partial ripe and full ripe stages three bands were recorded. However,

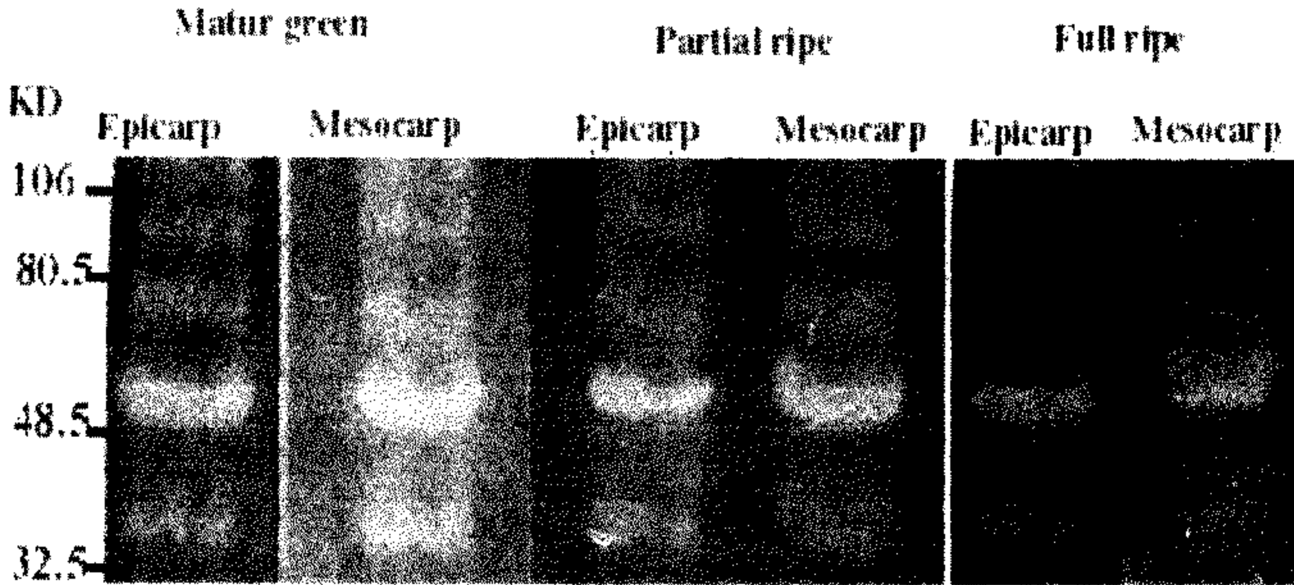

Fig. 5. Non-denaturing polyacrylamide gels stained for Superoxide dismutase activity

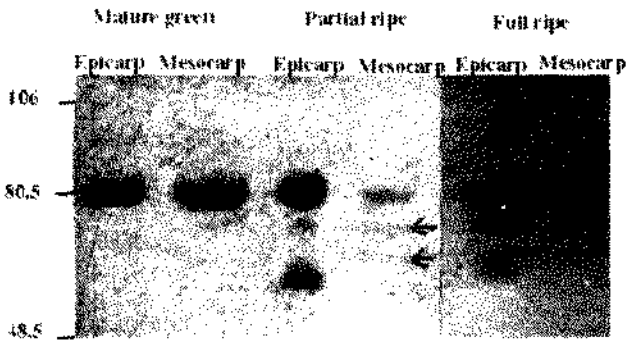

Fig. 6. Non-denaturing polyacrylamide gels stained for peroxidase activity

quantitatively banding pattern in epicarp tissue was more intense over mesocarp at both partial ripe and full ripe stages. The data on enzyme activities and isozyme pattern suggested that these antioxidant enzymes are differentially expressing at different depth inside the mango fruit. Similarly differential softening and a differential distribution of cell wall softening enzymes have been reported by Lazan *et al.* (1990). High POD activity is thought to scavenge the hydrogen peroxide evolved during the process of ripening. It has been noted that hydrogen peroxide is consumed by peroxidases, which are present in many plants (Saunders *et al.* 1964).

Similarly the variation in SOD activity has been reported during maturation and ripening of tomato (Rabinowitch and Sklan 1980) and cucumber and pepper (Rabinowitch and Sklan 1981). These enzymes are thought to scavenge toxic free radicals and thus reduces their inhibitory effect, however, with the progress of ripening, the activities of these enzymes decreased which may be due to disintegration of enzyme proteins.

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