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Changes in biochemical and mineral constituents associated with jelly seed in mango (*Mangifera indica*) cv. 'Dashehari'*

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India is the largest producer of choicest mango varieties in the world. However, the trade of north Indian cultivars is limited due to their lower/poor shelf life, alternate bearing habit, physiological disorder, etc. Among these 'Dashehari' is the most valued mango cultivar known for its aroma and taste. It suffers from a wide range of physiological disorders which reduce its quality production resulting into a limiting factor for export. The softening of tissues around stone (jelly seed) is one of the serious problems in 'Dashehari' causing quality deterioration in fruits upon ripening. It is characterized by loosening and disintegration of pulp and jelly formation with tissue turning brown around stone during the senescence. The problem is severe when fruits are harvested at late maturity. Some of the mango varieties like 'Alphonso' and 'Mallika' exhibit flesh disorders like spongy tissue (Katrodia 1988; Singh *et al*. 2006). It has been reported by Selvaraj *et al*. (2000) that spongy tissue affected ripe mango fruits exhibit higher levels of N, Fe, phenol, starch and acidity and lower levels of carotenoids, total soluble solids, sucrose, ascorbic acid, soluble protein, K and Ca. However, the information on changes in biochemical and mineral constituents in 'Dashehari' mango affected with tissue softening is lacking. Therefore, present study was undertaken to investigate changes in biochemical and mineral constituents, particularly changes in protein profile, associated with softening of tissue in mango cv. 'Dashehari'.

The study was carried out in Plant Physiology Laboratory of Central Institute for Subtropical Horticulture, Lucknow, located at latitude 26.55°N and longitude 85.59°E. Dashehari mango fruits harvested at late maturity from experimental farm of the Institute were washed, surface dried and kept for ripening under ambient conditions (35±2°C, 65±5% RH). Ripened fruits were cut open and soft tissues around the stone were collected from jelly seed infected fruits, while healthy tissues were collected from normal fruits. The pulp of unripe fruit was taken for comparison in protein profile study. Homogeneous pulp from fruits in triplicate of same tree of each category were taken for evaluating the biochemical, mineral and protein profiling study.

Protein content in the pulp of ripened and soft tissues was assayed by the method of Lowry *et al*. (1951). SDS-PAGE analysis in different pulp was carried out by the modified method of Hurkman and Tanaka (1986). Protein was extracted from the sample using 2.5 ml extraction buffer containing 0.5 M Tris (*p*H 8), sucrose 0.4 M, 2 mM EDTA. 1% PVP, 1% 2-mercaptoethanol, 1 mM phenyl methyl sulfonyl fluoride (PMSF). SDS-PAGE (12%) and Native-PAGE (10%) were performed according to the protocols described by Laemmli (1970) and the protein samples were resolved in these gels. The α-amylase activity was assayed by the modified procedure of Bernfield (1955). The crude enzyme extract was prepared by homogenizing 5 g of pulp in 0.05 M phosphate buffer (pH 6.9) at 40 °C and the final volume was raised to 50 ml. One ml of crude enzyme extract was added to the reaction mixture containing 0.5 ml of 1.0% starch in phosphate buffer with 0.007 M NaCl and incubated for 30 min at 35°C. The reaction was terminated by the addition of 1.0 ml of 3,5-dinitro-salicylic acid. The final volume was made to 10 ml and absorbance was measured at 540 nm. Enzyme activity was expressed as mg/g maltose/30 minute. The total antioxidant capacity of samples was determined by the ferric reducing ability of plasma (FRAP assay) procedure as described by Benzie and Strain (1999). The procedure involves the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion to a blue coloured complex Fe^{2+}/TPTZ in the presence of sample (bioactive compounds – antioxidants). The freshly prepared FRAP reagent contained 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and 300 mM acetate buffer (*p*H 3.6) in the ratio of 1:1:10, and warmed at 37°C for 10 and 5 minutes, respectively, before and after adding of sample extract. The total phenol content was determined colorimetrically using Folin- Ciocalteu's phenol reagent as per the method described by Singleton *et al*. (1999) having

*Short note

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Gallic acid (Aldrich, St. Louis, MO) as standard. Total flavonoids were estimated by the method described by Dae-Ok *et al*. (2003) and total carotenoids in the pulp extracted with acetone was estimated as per method described by Lichtenthaler (1987). Estimation of starch was followed by method of McCready *et al*. (1950). The total and reducing sugars were estimated by the method of Yemm and Willis (1954). Nitrogen content in dry matter was estimated by micro Kjeldahl's method (AOAC 2000). One gram of dry matter was digested with tri-acid mixture (nitric acid : sulphuric acid : perchloric acid 10:1:1, v/v) and the digested materials was used to estimate the minerals. The phosphorous content of the digest was determined colorimetrically by vandomolybdate yellow colour method described by Jackson (1973). Potassium, Ca, Fe, Zn, Cu and Mn were estimated using atomic absorption spectrophotometer (AAS).

The data presented in Table 1 clearly revealed that upon ripening, the protein content in healthy pulp is higher (0.83%), while the affected fruit pulp showed significantly low (0.480%). Lower level of protein in affected tissue in comparison with healthy tissue may be attributed to hydrolysis of storage protein. Similar type of results was also reported in spongy tissue affected Alphonso fruit (Katrodia 1988). The electrophoretic pattern of protein showed distinct differences in banding patterns (Fig 1) in the normal, affected and unripe fruits. Higher intensity of high molecular weight protein was noticed in healthy tissue of fruit. An interesting observation was the increase in a low molecular weight protein (3 kDa to 6.5 kDa) band with higher intensity in senescence pulp (softening affected) which was missing in unripe and healthy ones. Two prominent additional bands of 39 kDa and 41 kDa were observed in soft tissues, whereas these bands were at low intensity in unripe and riped healthy fruits. 10% SDS and native-PAGE (non-denaturing PAGE) confirms the proteolytic degradation occurred in soft tissues during the ripening. These findings suggest that the increase in low molecular weight protein in senescence pulp could be due to selective degradation by protein kinases and substrates

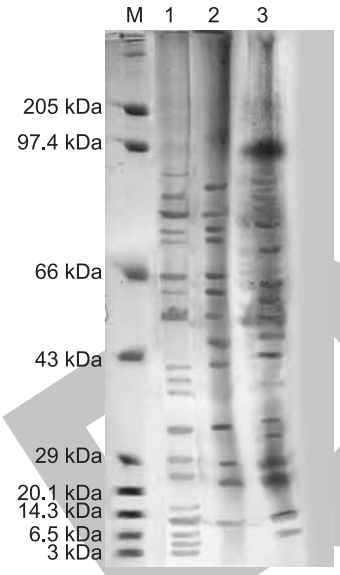

Fig 1 Protein profile of mango pulp on 10% SDS-PAGE M, Marker; 1, soft; 2, unripe; 3, normal

or inhibition of specific enzymes activities by unfavorable micro-environments. Softening related enzymes, pectin methyl esterase [PME], polygalacturonase [PG] were also found to be elevated in affected tissue as reported in our earlier studies (Singh and Pathak 2008). The affected tissue exhibited much higher α-amylase activity with substantial low level of starch and higher level of reducing sugar content as compared to healthy tissue. However, level of difference in total sugar content was non-significant. The low level of starch in affected tissue was attributed to high amylase activity. Owing to higher metabolic activity in affected tissue, total phenols increased substantially. However, total carotenoids decreased significantly in affected tissues as compared to the healthy ones. On the other hand minimum level of total flavonoids was found in both types of tissue. The marginal difference in the total antioxidant capacity in terms of ferric reducing power (FRAP assay) was observed among both types of tissue. The antioxidant capacity of the fruit is attributed to the combined effect of total phenol, flavonoid, carotenoid and other

Table 1 Total antioxidant, biochemical and mineral constituents in normal and soft tissue of ripe mango cv. 'Dashehari'

Total antioxidant and biochemical constituent	Fruit pulp		CD (P = 0.05)	Mineral constituent	Fruit pulp		CD (P = 0.05)
	Normal	Soft			Normal	Soft	
FRAP (mg/g AEAC FW)	14.83	10.53	1.46	Ca (ppm)	382.20	298.90	12.50
Phenol (mg/g FW)	1.82	2.52	0.52	K (ppm)	110.0	80.0	5.85
Flavonoid (mg/g FW)	0.19	0.19		N (%)	0.167	0.187	0.011
Carotenoid (µg/g FW)	39.50	21.50	3.95	P (%)	0.013	0.018	NS
Reducing sugar (%)	7.13	2.68	1.56	Fe (ppm)	27.52	37.22	3.52
Total sugar (%)	20.46	19.46	0.96	Zn (ppm)	9.15	12.29	2.10
α-amylase (mg malt/g FW 20/min)	28.75	60.86	8.95	Cu (ppm)	7.26	7.79	NS
Starch (%)	0.563	0.195	0.250				
Protein (%)	0.830	0.480	0.120				

contributing compounds (Olsson *et al*. 2004). The more phenol accumulated in affected parts as evident in the present finding (Table 1) might became pro-oxidants which ultimately reduced its antioxidant property. The low carotenoid might also be another reason for reducing the antioxidant potential of affected fruits. Greater contribution of phenols to antioxidant activity than that of vitamins and carotenoids was reported by Maria *et al*. (2002).

Data pertaining to the mineral composition of normal and affected tissue (Table 1) clearly revealed that soft tissue had low Ca (298.90 ppm), K (80.00 ppm) and higher N (0.187%), Fe (37.22 ppm) and Zn (12.29 ppm) as compared to normal tissue. Variation in P and Cu was non-significant among the two types of tissue. On the other hand, the level of Mn was found at non-detectable level (data not shown). Shantha (1981) reported that low Ca and K and higher P in internal affected tissues of 'Alphonso' mango and Whangchai *et al*. (2001) reported that low Ca in the fruit could cause of jelly seed in mango. Whereas, Wainwright and Burbage (1989) earlier reported that high Ca levels in tree retard several post harvest disorders. The existence of differences might be a result of redistribution of minerals which takes place as internal breakdown in the tissue develops. In an experiment with Tommy Atkins mangoes reported by Cracknell *et al*. (2004), the degree of internal fruit breakdown (IFB) and total-N content of fruits were remarkably greater in the high N treatments and the incidence of IFB showed a high positive correlation with the fruit N content and negative correlation with Ca content. Our present investigation confirming earlier reports, revealed that presence of high calcium, potassium and low nitrogen levels in normal fruits might retarded the development of the jelly seed disorder when compared with the disordered fruits.

SUMMARY

The present investigation reports the protein profile and changes in biochemical parameters and mineral constituents associated with jelly seed (softening of pulp) in mango cv. Dashehari. The protein content in ripened healthy fruits was found to be 0.83%, while in affected fruit pulp it was noted 0.48%. SDS-PAGE was done to investigate variation in polypeptide banding pattern in the fruit pulp. Unripe fruits exhibited protein band between 20.1 and 66 kDa, while normal ripe fruits showed higher protein band intensity with major distinct bands of 43 kDa and 97.4 kDa. In comparison, the softening affected fruit showed lower molecular weight polypeptide bands of 3 to 6.5 kDa with high intensity. Similar results were obtained in 10% native-PAGE. However, several protein bands observed in healthy fruit (normal and unripe fruit pulp) were found missing in soft tissue. Significantly lower value of total antioxidant capacity and total carotenoid level was also recorded in affected fruit as compared to normal ones but total phenolics increased with no change in flavonoid. Enhanced activity of hydrolytic enzyme α-amylase was also found in affected fruits in comparison to healthy fruits. The soft tissue contained higher level of reducing sugar and low starch content as compared to the normal tissue. Normal fruits had presence of high calcium, potassium and low nitrogen levels which might have retarded the jelly seed disorder.

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