Spongy tissue in 'Alphonso' mango. III. Radiotracer evidence for increased mobilization of water from mesocarp to seed

Incidence of spongy tissue (ST) in 'Alphonso' mango (*Mangifera indica* L.) was found to be clearly associated with the onset of precocious germination events in the recalcitrant seed and the disorder could be manipulated by altering seed metabolic activity through plant growth regulators (PGRs)[1]. Absence of ST in fruits with damaged embryonic axis or funiculus connection at the hilum due to mango stone weevil (MSW) infestation provided unequivocal evidence in support of the causative role of seed in this malady[2]. Biochemical changes occurring in the mesocarp and seed during ST formation further supported the pivotal role of seed in initiating ST[3]. It was observed that a significant loss of water from the ST-affected region of the mesocarp was accompanied by a proportionate gain in seed and the extent of water lost determined the degree of sponginess[2,3]. However, the questions remained as to: (i) whether the mobilization of water from mesocarp to seed was occurring randomly or from a pre-destined region, and (ii) how the exogenously applied PGRs (GA_3 and PBZ) were able to regulate amylase activity in the seed without affecting the same in the mesocarp, in spite of diffusing through the same.

Experiments were therefore, conducted on developing Alphonso mango fruits using tritiated water ($_1H^3$) and PGRs in order to address the above points.

Experiments were carried out during 2007–08 on Alphonso mango trees maintained in the Institute orchard. Three sets each of 100 developing fruits were dip-treated at around 60% maturity with GA_3 (1 g l^{-1}), paclobutrazol (1 g l^{-1}) and water, for 30 s followed by tritiated water containing 5 µCi ml^{-1} of $_1H^3$ and APSA 80 (@ 0.2 ml l^{-1}), a non-ionic adjuvant, after ten days. Fruit maturity was computed as a percentage of the ratio of the number of days from fruit set to full maturity (110 d), and the time between pre-harvest tritiated water treatment and ripeness was about three weeks. Fruits were harvested at 85% maturity and ripened under ambient conditions (mean maximum temperature of 28–30°C and RH of 60–70%) before slicing them to record ST incidence. Moisture content of mesocarp and seed tissue samples was determined gravimetrically. Rate of respiration was measured using LICOR 6200 Portable Photosynthesis System (LICOR, Lincoln, NE, USA) equipped with LICOR 6250 CO_2 analyser. Mesocarp and seed tissues (20 g) were placed inside the 250 ml chamber of the system and the difference between initial and final concentration of CO_2 during 30 s was used to compute the rate of respiration as mg CO_2 kg^{-1} h^{-1}. Electrical conductivity (EC) of mesocarp and seed tissues was measured with a conductivity meter (ELICO model CM-180) and expressed as dS m^{-1}. Amylase activity was assayed in seed and mesocarp tissues as previously described[3]. Calcium content of mesocarp and seed tissues was estimated using Atomic Absorption Spectrophotometer (Perkin Elmer Model 5000) and expressed as mg 100 g^{-1} dry wt. For measurement of radioactivity, mesocarp and seed tissues (10 g) were homogenized in an aqueous medium (100 ml) and centrifuged at 10,000 rpm for 15 min. To 1 ml of the clear supernatant, 14 ml of Cocktail 'W' (10 g 2,5-diphenyl oxazole (POP), 0.25 g 1,4-Bis (5-phenyl oxazol) benzene (POPOP) and 100 g naphthalene dissolved in 1 l of 1,4-dioxan) was added and mixed thoroughly prior to detection. Radioactivity was measured using a liquid scintillation counter (Packard 1900 TR) and expressed as the number of disintegrations per second (dps). ST incidence was computed based on data from ten replicates with ten fruits per treatment and five fruits were sampled from each treatment and replicate for the remaining studies. The data were subjected to ANOVA adapting the Fisher's analysis of variance technique[4].

ST incidence was significantly reduced by pre-harvest treatment of fruits with PBZ (2.5%), while it increased with GA_3 application (61.5%) compared to 38.5% incidence in control (Figure 1). The tritium counts in ST-affected fruits were higher by 189% in mesocarp and by 75% in seed compared to those of healthy fruits. ST-affected fruits, exhibiting radicle emergence had higher tritium counts in both mesocarp (529%) and seed (216%) compared to healthy fruits. The tritium counts were higher by 528% in mesocarp and by 387% in seed of fruits induced for ST incidence through pre-harvest GA_3 application compared to healthy fruits (Table 1). Incidentally, tritium counts in the mesocarp tissue of MSW-infested fruits were similar to those of healthy fruits (data not shown).

The moisture content of ST-incident mesocarp tissue was significantly lower compared to healthy tissue irrespective of the treatment, whereas it was significantly higher in the seed of ST-affected and GA_3-treated fruits than healthy and PBZ-treated fruits (Figure 2 *a*). Amylase activity in healthy mesocarp tissue was high compared to that incident with ST,

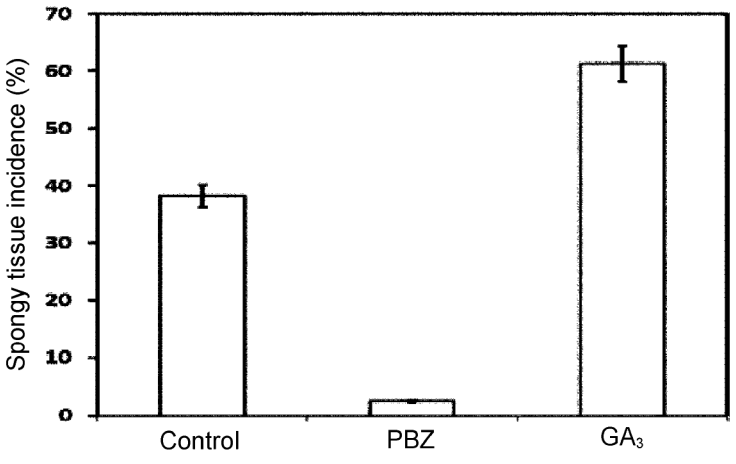

Figure 1. Effect of pre-harvest application of paclobutrazol and GA_3 on the incidence of spongy tissue in 'Alphonso' mango.

whereas it was significantly higher in seed of ST-affected fruits irrespective of treatment (Figure 2 *b*). The PGRs did not affect amylase activity in the mesocarp tissue of fruits in which seeds were damaged by MSW (data not shown). The respiration rate of healthy mesocarp tissue was significantly higher than that incident with ST irrespective of the treatment. On the contrary, seed from ST-affected and GA_3-treated fruits showed significantly higher respiration rate than that of healthy and PBZ-treated fruits (Figure 2 *c*). In ST-affected fruits, the EC of both mesocarp and seed tissues was significantly higher than that of healthy fruits irrespective of the treatment (Figure 2 *d*). In healthy and PBZ-treated fruits the pH of mesocarp tissue was significantly higher than that of ST-affected fruits, whereas the seed tissue of ST-affected and GA_3-treated fruits had significantly higher pH than that of healthy and PBZ-treated fruits (Figure 2 *e*). The calcium content of the healthy mesocarp tissue was significantly higher than that incident with ST, whereas in seed it was significantly higher in ST-affected and GA_3-treated fruits than that of healthy and PBZ-treated fruits (Figure 2 *f*).

The significant decrease in moisture content of mesocarp coupled with its gain in seed of ST-affected fruits indicated increased mobilization of water from mesocarp to seed and was confirmed by the significantly higher number of tritium counts in seed of ST-affected fruits compared to healthy fruits. An upregulation of seed metabolic activity with GA_3 accompanied by a significant increase of tritium counts and downregulation of seed metabolism with PBZ resulting in a decline of tritium counts in seed (Table 1) established the direct relationship between mobilization of water from the mesocarp and metabolic activity of the seed. Incidentally, in fruits infested with MSW the number of tritium counts in the mesocarp tissue was similar to that of healthy fruits (data not shown). The pivotal role of seed in ST formation was further proved by the fact that ST incidence was significantly higher in fruits where the seed had switched over to germination mode, as reflected by its metabolic activities (Figure 2). Disassociation of vasculature between seed and tree besides providing a trigger for the seed to shift into germination mode, also ensures that the programme for embryonic growth that is maintained by vascular flow is overruled by the one for germination[5,6]. Owing to its recalcitrant nature[7–11], the embryo in mango is metabolically demanding and sustains physiological activity throughout ontogeny entailing mobilization of water from the mesocarp to the seed and the consequent progression of germination-associated events. Similar results showing an increase of seed moisture content concurrent with its decline in the mesocarp and its relation to internal breakdown disorder of avocado fruits[12] possessing a recalcitrant seed[13] have been reported.

The PGRs while significantly affecting amylase activity in the seed could not do so in the mesocarp in spite of diffusing through the same, presumably due to the unfavourable pH of the tissue (Figure 2 *e*). Noticeably, the PGRs also did not affect amylase activity in the mesocarp of MSW-infested fruits in which the embryonic axis and/or the funiculus connection at the hilum had been damaged (data not shown), further validating the causative role of seed in ST-formation.

In healthy and PBZ-treated fruits the EC and pH of mesocarp tissue reflected the maintenance of cell membrane, integrity, whereas in ST-affected mesocarp damage to the cell membrane integrity was expressed as high EC and acidic pH of the tissue (Figure 2 *d* and *e*). The loss of membrane integrity and the resulting leakage of cell constituents leads to the

Table 1. Radioactive counts (dps g^{-1} dry wt) in the mesocarp and seed tissues in 'Alphonso' mango fruits treated with PGRs

Fruit status	Mesocarp	Seed
Healthy	506.32	38.78
Spongy	1466.63	67.98
Spongy (germinating seed)	3185.48	122.57
Spongy (GA_3-induced)	3179.93	188.82
Healthy (PBZ-treated)	494.63	34.42
CD @ 5%	17.91	

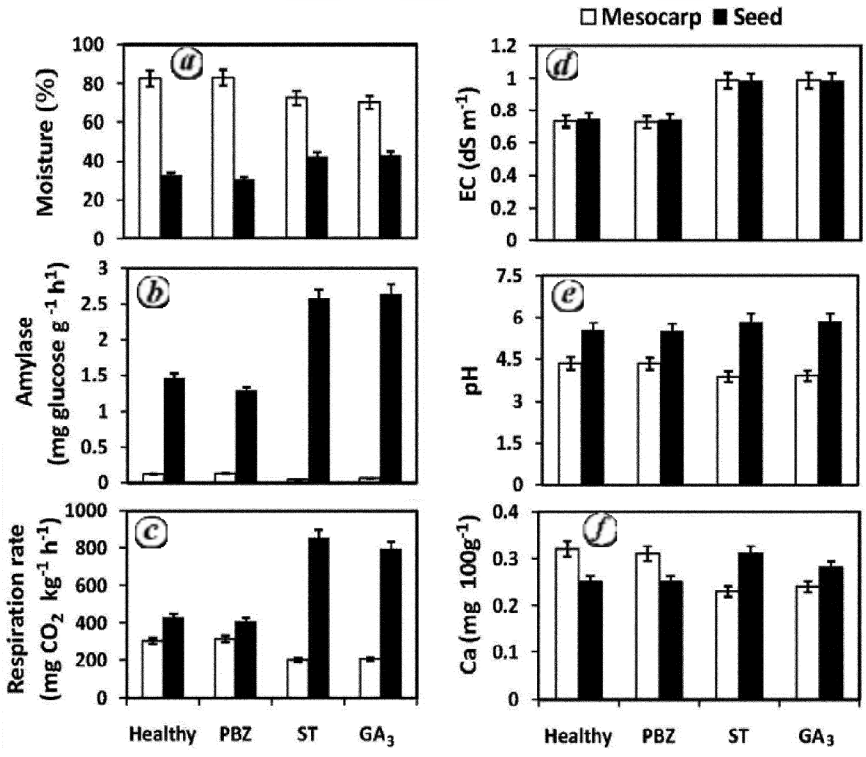

Figure 2. Effect of pre-harvest application of plant growth regulators on (*a*) moisture content, (*b*) amylase activity, (*c*) respiration rate, (*d*) electrical conductivity, (*e*) pH and (*f*) Ca content of mesocarp and seed tissues in healthy and ST-affected fruits of 'Alphonso' mango.

creation of an acidic environment which progressively damages the surrounding tissue due to osmolysis and an increase in the extent of ST. As a consequence, the volume of extracellular water in the ST-affected mesocarp can be expected to be apparently higher than that of healthy mesocarp. The tritiated water that infused through the lenticels during pre-harvest treatment of fruits gets mobilized naturally along the water potential gradient, i.e. from healthy mesocarp tissue with high water potential towards the ST-affected mesocarp tissue with low water potential, and presumably got mixed with the extracellular water resulting in higher number of tritium counts. Owing to the onset of germination-associated events, the seed in ST-affected fruit draws more water from the breakdown-mesocarp tissue, which was reflected in the higher number of tritium counts compared to that of healthy fruits (Table 1). The higher number of tritium counts observed in seed of fruits exhibiting *in situ* radicle emergence clearly indicated that the germinating seed is a stronger physiological sink enabling increased drawl of water from the ST-affected region of the mesocarp to meet its metabolic demands. Owing to its recalcitrant nature and consequent shift into germination mode, the embryo establishes contact randomly at the interface of the endocarp (stone) and mesocarp to draw water for its metabolic activities necessitating the loss of membrane integrity of mesocarp tissue in that region. The higher EC and acidic pH (Figure 2 *d* and *e*) of ST-affected mesocarp tissue was a reflection of the loss of cell membrane integrity resulting in the death of cells and leakage of cellular contents. It is for this reason that ST was initially observed as a tiny necrotic spot on the surface of the endocarp. Thus, higher number of tritium counts in the ST-affected mesocarp tissue in spite of its lower moisture content clearly established that mobilization of water into seed had already begun from this specific region. The seed being relatively dormant and the trigger for germination absent in healthy fruits[1], mobilization of water from mesocarp to seed was slower as revealed by the lower tritium counts (Table 1). Thus, the onset of germination-associated events and the resulting ST incidence were obviously due to an increased mobilization of water from mesocarp to seed and a metabolic reversal as reflected by higher amylase and respiration rate of the seed in ST-affected fruits. A certain amount of gene activation/deactivation was reported to be involved in such a fundamental metabolic reversal[6].

Cell metabolism being tightly coupled to the rate of respiration, the energy thus derived drives all the reactions within a cell. A higher rate of seed respiration and the continuous supply of soluble sugars produced by the action of amylase on starch reserves of the seed during onset of germination ensure production of metabolic energy and biosynthetic precursors to sustain embryo growth and development[14]. A sudden rise in the consumption of O_2 by the fast-respiring seed in germination mode coupled with increased diffusion of CO_2 into the mesocarp create a hypoxic environment around the endocarp, resulting in the decline of amylase activity and incomplete breakdown of starch in the mesocarp of ST-affected fruits. A similar reduction in the activity of amylase under anaerobic conditions has been reported[15]. On the contrary, the increase in amylase activity of seed in GA_3-treated fruits is expected, since GA_3 promotes amylase biosynthesis. Active gibberellins (GAs) are known to promote embryo germination and antagonize ABA activity. Likewise, increased sensitivity to GAs may accompany or precede germination[16]. Thus, an analysis of changes in the activity of amylase revealed that an increased mobilization of water from mesocarp to seed clearly triggered the hydrolytic activities of seed in ST-affected fruits, akin to changes occurring in seed during the onset of germination[8]. A significant increase in Ca^{2+} content of seed coupled with a proportionate reduction in mesocarp was also noticed in ST-affected and GA_3-treated fruits. As discussed earlier, the loss of membrane integrity of ST-affected mesocarp tissue led to the leakage of cell contents, including Ca^{2+} which apparently got mobilized into the seed along with water mimicking Ca^{2+} deficiency in the ST-affected mesocarp. Thus, it is clear that ST of 'Alphonso' mango and other similar physiological disorders believed to be arising due to an apparent localized Ca^{2+} deficiency are, in fact, a manifestation of redistribution of Ca^{2+}, rather than poor uptake[17].

The present study provides conclusive evidence for the increased mobilization of water from mesocarp to seed in ST-affected fruits. Accumulation of higher number of tritium counts in the ST-affected mesocarp revealed that mobilization of water into the seed takes place exclusively from a pre-destined region of the mesocarp, and not randomly. Data on tritium counts from pre-harvest GA_3-treated fruits clearly established that enhancement of seed metabolic activity was coupled with an increased mobilization of water from mesocarp to seed, resulting in ST formation. Pre-harvest treatment of fruits with PGRs unequivocally demonstrated that mobilization of water from mesocarp to seed precedes initiation of ST in 'Alphonso' mango. The present study, besides confirming our earlier observations[1–3], also suggests that the reported deficiency of calcium in the ST-affected mesocarp of 'Alphonso' mango fruits[18,19] is evidently the effect of redistribution of Ca^{2+} and not due to its reduced uptake. The causative role of seed in ST formation of 'Alphonso' mango is further elucidated by the following observations: (i) The tritium counts in the mesocarp tissue of fruits in which the seed was damaged by MSW infestation were similar to that of healthy fruits. (ii) PGRs could not directly affect amylase activity in the mesocarp tissue in spite of diffusing through it, but could regulate the same significantly in seed. (iii) Amylase activity in the mesocarp was not affected by the PGRs in fruits where the seed was damaged by MSW infestation. (iv) Manipulation of seed metabolic activity by PGRs significantly influenced the ST incidence levels in the mesocarp.

The findings of the present study have not only helped in resolving the enigma of ST in 'Alphonso' mango, but have also provided insights to the understanding and management of similar disorders of many other tropical fruits endowed with recalcitrant seeds. The key to manage such internal fruit disorders clearly lies in down-regulation of the metabolism of recalcitrant seed, a common feature of tropical fruit crops.

1. Ravindra, V. and Shivashankar, S., *Curr. Sci.*, 2004, **87**, 1045–1049.
2. Ravindra, V. and Shivashankar, S., *Curr. Sci.*, 2006, **91**, 1712–1714.
3. Shivashankar, S., Ravindra, V. and Louis, L., *J. Hortic. Sci. Biotechnol.*, 2007, **82**, 35–40.
4. Panse, V. G. and Sukhatme, P. V., *Statistical Methods for Agricultural Workers*, ICAR, New Delhi, 1978, p. 108.

5. Wainwright, H. and Burbage, M. B., *J. Hortic. Sci.*, 1989, **64**, 125–135.
6. Dure, L. S. III., *Annu. Rev. Plant Physiol.*, 1975, **26**, 259–278.
7. Berjak, P., Dini, M. and Pammenter, N. W., *Seed Sci. Technol.*, 1984, **12**, 365–384.
8. Berjak, P., Farrant, J. M., Mycock, D. J. and Pammenter, N. W., *Seed Sci. Technol.*, 1990, **18**, 297–310.
9. Farrant, J. M., Pammenter, N. W. and Berjak, P., *Seed Sci. Technol.*, 1988, **16**, 155–166.
10. Pammenter, N. W. and Berjak, P., *Seed Sci. Res.*, 2000, **10**, 301–306.
11. Farnsworth, E., *Annu. Rev. Ecol. Syst.*, 2000, **31**, 107–138.
12. Kalala, M. B., Modi, A. T. and Cowan, A. K., *South African Avocado Grower's Association Year Book*, 2005, **28**, 33–39.
13. Egli, D. B. and Tekrony, D. M., *Seed Sci. Res.*, 1997, **7**, 3–11.
14. Perata, P., Matsukura, C., Vernieri, P. and Yamaguchi, J., *Plant Cell*, 1997, **9**, 2197–2208.
15. Guglielminetti, L., Yamaguchi, J., Perata, P. and Alpi, A., *Plant Physiol.*, 1995, **109**, 1069–1076.
16. Leon-Kloosterzeil, K. M., vande Bunt, G. A., Zeevart, J. A. D. and Koornneef, M., *Plant Physiol.*, 1996, **119**, 233–240.
17. Bangerth, F., *Annu. Rev. Phytopathol.*, 1979, **17**, 97–122.
18. Rane, D. A., Katrodia, J. S. and Kulkarni, D. N., *J. Maharashtra Agric. Univ.*, 1976, **1**, 89–94.
19. Selvaraj, Y., Edward Raja, M. and Rawal, R. D., *Indian J. Hortic.*, 2000, **57**, 183–188.

ACKNOWLEDGEMENTS. We thank the Director, Indian Institute of Horticultural Research, Bangalore, for providing facilities to undertake the study. We also thank CSIR, New Delhi for providing research grants. M. Lakshmaiah, S. S. A. Qazi and N. K. Kacker provided technical assistance in the field and laboratory. The kind help of Dr N. Sivaprasad, Senior General Manager and Dr D. Padmanabhan, Senior Manager, Radiopharmaceuticals Division, BRIT, Mumbai in standardizing ^{3}H-assay in mango pulp and seed tissues is gratefully acknowledged.

Received 5 February 2010; accepted 27 July 2010

V. RAVINDRA*
S. SHIVASHANKAR
T. MURALEEDHARA REDDY
S. C. KOTUR

Indian Institute of Horticultural Research,
Hessaraghatta Lake Post,
Bangalore 560 089, India
**For correspondence.*
e-mail: vattem_r58@yahoo.com

An unusual composition of the plant species towards zone of ablation (Tipra glacier), Garhwal Himalaya

The snowline in the Himalayas is rising ceaselessly due to regional and global climate change. Upward rising of snowline leads to the formation of moraines. Due to extreme environmental conditions, the existing vegetation of the moraine is scanty and marked with different ecological adaptations. Ablation zone of the glaciers is covered by a thick pile of supra glacial moraine and characterized by several serrac ice sections, melting into pools of supra glacial lakes, ice caves and dead ice mounds because of subsidence and the fast-degenerating nature of the glaciers[1,2]. Dynamic and perennial ice bodies of the Himalaya are directly concerned with climate and climatic changes[3]. Temperature changes observed in the high Himalayas reflect mass and volume of the glacial ice[4,5]. Tipra glacier (30°36′–30°47′N and 079°33′–079°44′E) along with its tributary glaciers, i.e. Ratavan, Saptshring and four other cirque glaciers supply ice melts to the Pushpawati river and makes the valley rich in its unique floral wealth, the renowned 'Valley of Flowers'. Tipra glacier is bounded by a well-preserved series of lateral and recessional moraines (Figure 1 *d* and *h*), indicating past extension of the glaciers[6,7]. High alpine ecosystems are generally sensitive, and even small environmental changes can cause obvious changes in vegetational development[8]. This can largely be attributed to the differences in topography, physiognomic conditions, altitudinal ranges, and different climatic or biotic features[8]. It is evident that when global and regional climatic conditions are changed, plants shift their habitat to optimum adaptive elevation from conventional altitudes[9], thus producing new communities (Table 1).

The study was carried out during April–September 2009. The two broad geomorphic units, viz. glacio-fluvial (3700–3800 m) and glacial zones (3800–4000 m) were identified to study the impact of glacial retreat. The objectives were to examine the extension of plants upslope due to reduced mass and volume of the glacier, together with vegetational composition, succession and shift towards the line of equilibrium (ELA). A total of 30 quadrates (glacio-fluvial = 15 and glacial zones = 15) were randomly laid and studied. The plant species have been identified by consulting the Herbarium of Botanical Survey of India, Dehradun, Herbarium of Forest Research Institute, Dehradun and the Garhwal University Herbarium, Srinagar-Garhwal.

The expansion of plants in the glacial moraines is controlled by the disappearance of glacial mass, volume, area and length[10]. From 1962 to 2002 recession rate[7] of Tipra glacier was 13 m/yr, which increased to 21.3 m/yr during 2002–2008. More recently, the glacier retreat was found to be 45, 83 and 535 m during the years 2006–2008, 2002–2006 and 1962–2002 respectively[6,7]. The areas where glacial retreat (about 663 m) took place during the last 46 years are now occupied by upward shift of vegetation. Plant density in glacial environment is strictly determined by climatic and edaphic factors[1]. The glacio-fluvial zone showed greater number of plant species in a unit area than the glacial zone (Figure 1 *e–h*). It is clear that recessional moraines of the glacio-fluvial zone (Figure 1 *d*) provide a nursery and open-up new habitats and niches[2]. *Trigonella emodi* (5445 individuals/m^2) and *Anaphalis triplinervis* (5355 individuals/m^2)