(2.21–3.38 t ha^{-1}) with inferior sink capacity. The entries are being multiplied for nomination to the national programme for wide testing.

They have diverse genetic base involving 15 to 20 different parents from various countries like India, Thailand, Sri Lanka, Cambodia, Vietnam and Myanmar. They also possess resistant genes inherited from *Oryza nivara* and many useful genes for biotic, abiotic stresses and superior grain quality from different land races. For example, IR 73232 has a parent like Khao Dawk Mali, Benong, Pa Chiam, Bhasamanik, Fortuna, FR 13A, Arikarai, Milek Kunning and Mudgo; IR 73236 has Gam Pai, Tadukan, Vellaikar, Kitchili Samba, Tsai Yuan Chung and Marong Paroc as parents; parents like Arikarai, Niaw Sampatong, Eravapandi, Seraupbesar 15, Tetep, Cina and Latisail are included in IR 67624; IR 70242 has land races like Kong Phlouk, Rathu, Heenati, Marong Paroc, Sinawpagh, Nahng Mon S4, Tsai Yung Chung, Thekkan, Eravapandi, Cina and Latisail in its parentage and land races like Basmati, CRM6, Chow Sung, Seraupbesar 15, Mudgo, Slo 17, Gam Pai 15 and improved Sabarmati were found in the ancestry of IR 70418.

IR 36 is the most adapted, stable and successful variety developed at IRRI for irrigated ecosystem and grown widely in many rice-growing countries by virtue of its wider genetic base, inherited from 18 different parents from 11 countries. Therefore, it is not surprising to note that the promising cultures identified during the present investigation with inherent high yield potential due to increased sink capacity and diverse genetic base, are expected to show greater adaptability and stability of performance under varied heterogeneous, harsh and unpredictable environments of deep-water ecology. Future breeding programmes for any unfavourable ecosystem may follow this direction.

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Spongy tissue in Alphonso mango – significance of *in situ* seed germination events

Alphonso, the most delicious variety of mango (*Mangifera indica* L.) known for its excellent texture, taste and aroma, accounts for nearly 60% of the mango export trade from India. However, the export trade is plagued by incidence of a physiological disorder known as 'spongy tissue' (internal breakdown), characterized by unripe, acidic, pale yellow/white, corky tissue with or without air pockets associated with an unacceptable off-flavour in certain regions of the mesocarp (pulp) adjacent to the endocarp (stone). Fruits affected by this disorder do not show any external symptoms and the malady is detected only after cutting the fruits open, posing a challenge for quality control in export. Spongy tissue occurrence is more prevalent in the coastal Konkan region of Maharashtra, the natural habitat of this variety, than in other inland regions of India[1].

Extensive investigations have been carried out over the past five decades to understand the cause of this disorder, without success[2,3]. The reasons attributed to the incidence of this disorder include factors as diverse as ecological, nutritional, environmental, microbial, physiological and biochemical. However, the problem has remained unsolved since all these studies were confined to measuring the effects rather than the cause of the malady. Past investigations on the physiological and biochemical aspects confined to the fruit mesocarp failed to provide any clue on the nature of the malady. Our preliminary observations revealed that spongy tissue incidence was closely associated with physiological status of the seed during fruit maturation. The seed from spongy-tissue-affected fruits showed faster and higher rate of germination, while a small percentage (2) of fruits even exhibited vivipary. Incidentally, flesh breakdown in mango cv. Tommy Atkins that is similar to spongy tissue in Alphonso mango, was attributed to the disconnection of vascular strands (funiculus) between peduncle and endocarp (stone), even while the fruit was attached to the tree, making it dependent on the mesocarp for its supplies[4]. This led us to believe that the seed could play a key role in spongy tissue formation, thus making a paradigm shift from the earlier thinking.

Mature green fruits were harvested from trees grown in experimental orchards of the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore and ripened at an ambient temperature of 25 ± 2°C and RH of 70 ± 5% for 1–10 days. Ripe fruits were cut open and classified as healthy or spongy-tissue-affected, based on visual scoring. A large number of fruits (> 1000) was sampled each year and analysed. Thirty stones each from healthy and spongy-tissue-affected fruits were collected and stored at –20°C for biochemical studies and 300 stones of each type were used for germination studies in the field. Observations on various physiological and biochemical parameters were recorded for five successive years (1998–2003).

Acetone powder of the kernel tissue was prepared for assay of amylase[5] and lipase[6] enzymes. Kernel tissue from healthy and spongy-tissue-affected fruits was also analysed for DNA[7], RNA[8], soluble proteins[9], starch[10], total soluble sugars[10], free amino acids[11], total phenols[8], fat[12] and seed moisture[12]. Calcium content in the seed was estimated using Atomic Absorption Spectrophotometer, Perkin Elmer Model 5000. Seed germination was studied with 30 stones each of healthy and spongy-tissue-affected fruits per replication.

In order to elucidate the causative role of seed in spongy tissue formation, pre- and post-harvest treatment of fruits with plant growth regulators was carried out during the fruiting season of 2004. For pre-harvest studies, fruits were treated with GA_3 (seed germination promoter) @ 200 ppm and paclobutrazol (anti-gibberellin) @ 2000 ppm at 55% maturity and the same fruits were treated again at 65 and 75% maturity levels to ensure effective penetration. Fruits on the tree were dipped in the respective growth regulator solution along with a non-ionic adjuvant (APSA-80) @ 0.03% for 30 s, and an absorbent cotton dipped in the respective solution was placed around the peduncle of the fruit to ensure better penetration into seed. Water with adjuvant acted as control. About 400 fruits per treatment were harvested at maturity from three trees of each treatment to record the incidence of spongy tissue formation. For post-harvest studies, fruits harvested at two maturity levels (85 and 95%) were treated with the same growth regulators at the same concentration for 30 min and the incidence of spongy tissue was recorded after the fruits ripened. For all the parameters studied, ten replications were used and the data were statistically analysed to compute the test of significance.

Seed respiration was measured using LICOR 6200 Portable Photosynthesis System equipped with 6250 CO_2 analyser. Respiration rates were computed based on differences in the initial and final concentrations of CO_2 for a period of 30 s and expressed as g CO_2 liberated/kg/h. The heat equivalent of CO_2 was worked out using the conversion factor of 2.55 cal/mg CO_2 and expressed as kcal/kg/h.

Results showed (Table 1) that moisture content was significantly higher in seeds from spongy-tissue-affected fruits (STS) than seeds from healthy fruits (HS), while there was a corresponding decrease in the spongy tissue. Hydration of seed is the first step in activation/synthesis of amylase and lipase enzymes which were found to be significantly higher (45.2 and 26% respectively) in STS over HS. Such an activation of hydrolytic enzymes is the key to initiation of germination-associated events. Analysis of other seed components revealed that STS had significantly lower starch content (33.5%) and higher levels of soluble sugars (27.7%) than HS, consistent with higher amylase activity, thus making it available to the growth and development of the embryo. As duplication of DNA is initiated only after the embryo imbibes water[13], a significant rise in the content of both DNA (76.7%) and RNA (65.5%) in STS indicated the rapid progress of germination events. A substantial increase in the content of soluble protein (43.1%) in STS indicated *de novo* synthesis of various enzymes associated with germination events. Higher levels of free amino acids (52.1%) in STS in comparison with HS may be attributed to hydrolysis of storage protein or to the amination of organic acids. Free amino acids give rise to TCA cycle intermediates, which are essential for the release of instant energy during imbibition[14]. Owing to high metabolic activity in STS, phenols also increased substantially (51.6%). Mobilization of

Table 1. Biochemical composition and germination behaviour of seeds from healthy and spongy-tissue-affected Alphonso mango fruits

Seed component	Seed from healthy fruit	Seed from spongy-tissue-affected fruit	SEm (±)	CD ($P = 0.01$)
Seed moisture (%)	32.63	42.04	1.92	5.49
Pulp moisture (%)	81.31	72.59	0.64	1.82
Calcium (ppm)	346.80	453.70	16.04	45.88
DNA (µg/g FW)	231.49	409.07	6.56	18.76
RNA (mg/g FW)	2.35	3.89	0.10	0.29
Soluble protein (mg/g FW)	63.91	91.44	2.15	6.16
Free amino acids (mg/g FW)	0.94	1.43	0.09	0.27
Amylase (mg glucose liberated/g/h)	1.55	2.25	0.10	0.28
Starch (mg/g FW)	634.06	421.40	31.37	89.75
Total soluble sugars (mg/g FW)	75.84	96.86	2.30	6.59
Lipase (mg fatty acid liberated/g/h)	67.19	84.69	2.80	8.02
Fat (%)	8.98	6.25	0.17	0.49
Total phenols (mg/g FW)	44.04	66.77	1.63	4.66
Mean no. of days to germination	26.90	17.80	0.38	1.08
Germination (%)	60.50	78.37	1.85	5.29
Rate of respiration (mg CO_2/kg/h)	395.03	805.09	106.03	297.63
Heat of respiration (kcal/kg/h)	1.01	2.05	0.27	0.76
Ä*T* (°C)*	0.64	1.74	0.15	0.42

*Mean difference of temperature between seed and chamber.

lipids and phenolics associated with antioxidant activity has been reported in germinating seeds of the tropical tree, *Pangium edule*[15]. Thus, all the parameters studied clearly indicated a shift of STS to the germination mode at pre-harvest stage itself. The faster and higher rate of stone germination (29.5%) was a result of the already progressing germination-associated events in mature–ripe fruits.

A significant increase in spongy tissue incidence was observed in pre-harvest treatment of fruits with GA_3 (70.2%), while there was a considerable reduction in incidence with paclobutrazol (16.4%) treatment compared to 51.5% incidence in the control. Besides an increase in the incidence of spongy tissue, GA_3 treatment also resulted in higher intensity of spongy tissue, wherein >70% of the fruit mesocarp was affected. Post-harvest treatment of fruits with plant growth regulators did not have any influence on spongy tissue formation (Table 2).

Both healthy and affected fruits were noticed not only on the same tree or branch but also on the same panicle, as also reported by Desai[16]. Panicle-wise analysis revealed that the spongy tissue incidence in fruits was 5.7 in one-fruit, 29.5 in two-fruit, 18.7 in three-fruit, 16.3 in four-fruit and 20.8% in five-fruit panicles, indicating variable and localized competition for resources (water, nutrients and photo-assimilates), affecting fruit maturity. Incidence of spongy tissue in excess of 90% was observed in fruits that reached physiological maturity and ripened on the tree[17]. In this study, over 70% fruits with spongy tissue were observed at late mature–ripe harvest stage irrespective of the year, age of tree, orchard spacing, fruit position and size. Therefore, in the Konkan belt, it is a commercial practice to harvest Alphonso fruits at around 80% maturity status to avoid spongy tissue.

The degree of recalcitrance exhibited by seeds is reported to vary[18]. Most recalcitrant seeds like mango show no developmental arrest and proceed directly to germination phase due to the absence of maturational drying and the consequent metabolic quiescence[19,20]. Vascular disconnection from the tree presumably puts the seed under stress for resources, including water, prompting its shift into germination mode. This could probably explain the variable nature of incidence of internal breakdown occurring in only a few mango varieties like Alphonso and Tommy Atkins.

It was consistently observed that seeds from healthy and spongy-tissue-affected fruits were distinctly different in their physiological status and biochemical composition (Table 1). The moisture content of STS was found to be significantly higher than HS, while that in the spongy tissue per se was lower by the same proportion. Viviparous and recalcitrant embryos maintain higher tissue moisture content throughout their ontogeny[21]. The higher levels of free sugars and amino acids in STS create a higher osmotic potential in the seed (embryo), leading to a further drain of water from mesocarp into the seed along an osmotic gradient (Figure 1). Such mobilization of water along an osmotic gradient promotes germination-associated events culminating in radicle emergence, as is observed in about 2% of fruits exhibiting vivipary as also the faster and higher rate of germination observed in STS. The higher levels of germination-related hydrolytic enzymes, DNA, RNA, phenolic acids and early germination indicated an obvious shift of STS to germination mode. Farrant *et al.*[22] and Berjak *et al.*[23] reported that many pre-germination events are triggered in recalcitrant seeds during development on the mother plant itself. Vargas Ramos and Durigan[24] reported radicle emergence in cv. Tommy Atkins and other mango varieties during cold storage and hinted at its possible relevance to internal breakdown.

Table 2. Spongy tissue incidence (%) in pre- and post-harvest treated fruits of Alphonso mango

Stage of treatment	Control	GA_3 (200 ppm)	Paclobutrazol (2000 ppm)
Pre-harvest			
(55–75% mature)	51.5	70.2	16.4
Post-harvest			
85% mature	52.5	49.5	45.2
95% mature	54.0	53.0	48.5

Significantly higher levels of Ca^{2+} and moisture were observed in STS than HS, while a reduction in Ca^{2+} content in the spongy tissue per se in mesocarp was reported by many workers[1,25,26]. Therefore, it may be inferred that the lower levels of Ca^{2+} observed in spongy tissue per se could be due to its drain from the mesocarp along the endocarp into the seed than to its lack of supply. Since calcium has a profound effect on firmness, ethylene production, respiration, storage-life, ripening and decay in many fruits and vegetables[27], it appears likely that mobilization of Ca^{2+} away from the mesocarp into the seed results in the loss of pulp texture and firmness as observed in spongy tissue (Figure 1).

The higher respiration rate of STS (Table 1) coupled with the climacteric respiration in the mesocarp results in the build-up of CO_2 near the endocarp, creating an anaerobic environment. Accumulation of organic acids in STS[25] leads to reduced mesocarp pH and activation of cell-wall degrading enzymes like cellulase, polygalacturonase, etc. (Figure 1). It is also reported that CO_2 accumulation leads to the development of off-flavours, internal pitting[28] and inhibition of ripening[29].

The incidence of spongy tissue was found to vary directly in proportion with seed respiration rate and amylase activity. An increase in seed amylase activity due to GA_3 (1.416) as against a decrease due to paclobutrazol (0.505) treatment indicated its mobilization to the target site (seed). The fact that spongy tissue incidence could be increased by GA_3 (70.2%) and decreased by paclobutrazol (16.4%) as against 51.5% incidence in control showed an apparent role of seed and its amylase activity in inducing spongy tissue formation. Since the post-harvest treatments had no effect on the incidence of spongy tissue (Table 2) in 85 and 95% maturity-grade fruits, it could be inferred that the stage of maturity of the seed is the most critical factor determining spongy tissue formation. This further proves the primary role of seed in inducing spongy tissue incidence and underlines the need to treat fruits before the critical stage (between 50 and 75% maturity) is reached.

The heat generated during seed respiration was significantly higher in STS fruits, which also maintained a higher temperature difference (Ä*T*, °C) than HS (Table 1). The excess heat thus generated

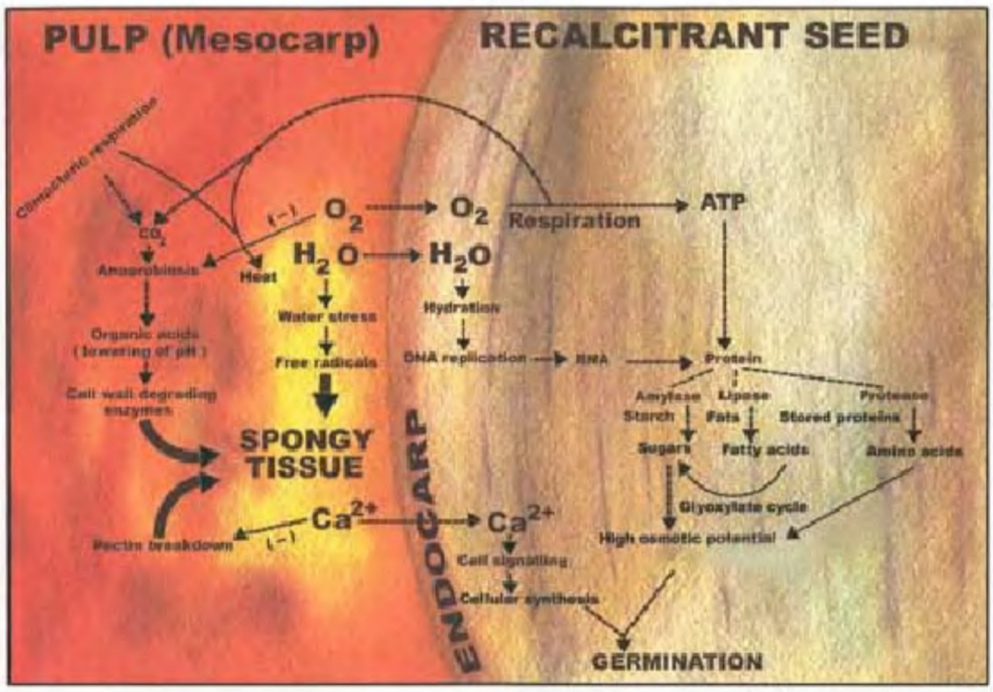

Figure 1. Schematic diagram showing the events leading to spongy tissue formation in Alphonso mango.

would presumably damage the tissues closer to the stone leading to vaporization of water and spongy tissue formation. Apparently, the source of excess heat near the endocarp arises out of increased respiratory activity of the seed rather than accumulation of soil convective heat as postulated by Katrodia[17].

Based on the above observations, we believe that an *in situ* seed germination signal triggers the following sequence of events leading to the formation of spongy tissue in Alphonso mango:

(i) Disconnection of vascular strands (funiculus) between the peduncle and the endocarp during fruit/seed maturation phase.

(ii) Shift of physiologically mature recalcitrant seed to the germination mode.

(iii) Diffusion of water and solutes from mesocarp into seed and consequent initiation of germination-associated events.

(iv) Development of anaerobiosis due to build-up of CO_2 in the mesocarp tissues near the endocarp, arising out of climacteric respiration and that diffusing from the fast respiring seed in germination mode.

(v) Metabolic disturbances in the mesocarp such as increased accumulation of organic acids, reduction of pH, activation of cell-wall degrading enzymes, free radical formation, dissociation of Ca^{2+} from the pectin complex and accumulation of heat of respiration in the vicinity of endocarp leading to.

(iv) Formation of spongy tissue along the endocarp.

All the facts known so far, on the phenomenon of spongy tissue (internal breakdown) in Alphonso mango can be explained by the above sequence of events triggered by the shift of seed into germination mode. The future strategy should therefore aim at manipulating seed dormancy to induce metabolic quiescence and prevent spongy tissue formation. The implications of this study would go a long way in understanding similar fruit disorders in other varieties of mango.

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Crinum woodrowii Baker (Amaryllidaceae), hitherto assumed to be extinct, rediscovered after a century from Mahabaleshwar, India

The genus *Crinum* L. is represented in India by 12 species, 3 varieties and 1 form[1] of which 3 species and 1 form, viz. *Crinum brachynema* Herb., *C. eleonorae* Blatt. & McC. f. *eleonorae*, *C. eleonorae* f. *purpurea* Blatt. & McC. and *C. woodrowii* Baker are endemic to Mahabaleshwar and adjoining areas[2–7] in Maharashtra. The first one was recently recollected from the Kates Point, Mahabaleshwar after a lapse of 94 years[8] and the remaining three were assumed to be possibly extinct[6,7]. During floristic exploration of the above-mentioned area between 2001 and 2004, we collected and identified *C. woodrowii* Baker after a lapse of hundred years. G. M. Woodrow first collected this species from Mahabaleshwar. Several bulbs of this were sent to Kew (England) supposing them to be *C. brachynema* Herb., but when they flowered at Kew the plant proved to be a new species and was described by Baker[9] as *C. woodrowii*. It has been so far represented only by a single sheet in Calcutta Herbarium (CAL) collected by Woodrow[7] in 1899; after that report it was not collected again from the type locality or elsewhere[7,10–12].

In the present finding, a total of about 150 individuals were seen growing on hill slopes of Kates Point, Mahabaleshwar. Hence we strongly recommend and assign the status of this species as 'critically endangered'. Considering its narrow range of distribution, it is recommended for inclusion in *Red Data Book of Indian Plants*.

A detailed description, ecological observations, photograph (Figure 1 *a–e*) and distribution map (Figure 2) of the species are provided for its easy identification.

Crinum woodrowii Baker in *Bot. Mag.* 124: t. 7597. 1898; T. Cooke, Fl. Pres. Bombay 2: 750. 1907 [3: 257. 1967 (Repr.)].

Type: Holotype: Bot. Mag. 124: t. 7597. 1898.

Tall herbs; bulbs 8.6–16.2 cm in dia., globose–spheroidal, outer tunics brown, membranous. Leaves contemporary with the flowers, sometimes appear after flowering, many (8–17), 45.5–80 cm × 4.5–14 cm, ensiform, flat, bright green, slightly glaucous beneath, glabrous, apex acute, white waxy, scabrous along margin; leaf sheaths forming a pseudostem. Scapes one, rarely two, arising from bulb outside the tuft of leaves, stout, compressed, 53.5–82.5 cm × 1–3 cm, green at base and apex, purple in middle, faintly channelled. Flowers 10–20 in umbel, fragrant; pedicels 1–3 cm long, green with purple tinge. Spathe valves (involucral bracts) two, opposite, 8.7–10 cm × 2.7–3.9 cm, deltoid, obtuse or acute at apex, margin inflexed, often green, purple tinged, nervate, coriaceous. Bracteoles many, 3–8 cm long, filiform, pale yellow or green. Perianth hypocrateriform (salver-shaped); tube 4–8 cm long, terete, curved, green with purple tinge in flowers, purple in buds; segments spreading equally, white, lanceolate, acute at apex, longer than perianth tube, 8.6–10 cm × 1–1.8 cm, purple tinged on dorsal median line, shining. Stamens 6; filaments 6–7.2 cm long, filiform, white in lower half and at tip, red in upper half, shorter than perianth lobes; anther lobes versatile, linear, crescent, 1.2–1.5 cm long, yellow, grey when wet. Ovary oblong, 8–10 mm × 3–4 mm, three-celled, with numerous ovules in axile placentation; ovules sessile; style terete, filiform overtopping the stamens, 15–15.6 cm long, white in lower half, red in upper half; stigma lobed. Fruits irregular in shape, 3–7 cm across, trilocular, finally bursting, peduncle *c.* 3 cm long. Seeds *c.* 3, large, rounded, testa thick, albumen copious.

Fls. & Frts.: May–July.

Distribution: India, Maharashtra State, Satara District, Mahabaleshwar, Kates Point.

Ecology: Growing at an elevation of *c.* 1275 m (latitude 17°56′. 270″N and lon-