Spongy tissue in Alphonso mango. II. A key evidence for the causative role of seed

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Spongy tissue in Alphonso mango was traced to the seed, which due to its recalcitrant nature, switches over to germination mode during fruit ripening phase drawing nutrients from the mesocarp. However, the question remained as to whether the changes in seed lead to spongy tissue formation in mesocarp or vice versa. To answer this question, Alphonso mango fruits naturally infested with mango stone weevil (*Sternochetus mangiferae* Fab.) were studied, as the weevil feeds exclusively on the seed within the stone. Absence of spongy tissue in fruits infested by mango stone weevil, where funiculus and embryonic axis were found damaged by feeding activity of the weevil provided unequivocal natural evidence supporting the causative role of seed in this intricate physiological disorder.

Keywords: Alphonso mango, mango stone weevil, seed, spongy tissue.

THE genesis of spongy tissue (ST) in Alphonso mango was a poorly understood phenomenon for several decades until a mechanism involving the seed was proposed, which explained all known facts reported on this physiological disorder[1]. Occurrence of vivipary in a few ST-affected Alphonso mango fruits provided a clue for the possible involvement of seed in ST formation. Physiological studies confirmed a shift of seed to the germination mode in ST-affected fruits as revealed by their faster and higher germination percentage compared to stones from healthy fruits. Biochemical analysis of seeds from healthy and ST-affected fruits also corroborated these observations as revealed by increased hydration, higher activities of hydrolases, DNA and RNA levels in seeds from ST-affected fruits. Subsequently, it was observed that the incidence of ST could be significantly reduced by pre-harvest treatment of fruits with paclobutrazol, a growth-retardant and enhanced by GA_3, a growth promoter as compared to untreated fruits, which supported the hypothesis that the seed played a decisive role in ST formation[1]. All the same, the question remained as to whether the changes in seed led to ST formation in mesocarp or vice versa. In this communication, we present evidence for the direct involvement of seed in ST formation based on critical observations in Alphonso mango fruits infested with mango stone weevil (MSW).

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Sternochetus mangiferae (Fabricius) (MSW) is a common pest of mango in Southern India and all the cultivars are reported[2,3] to be susceptible to infestation varying between 5 and 87%. The female weevil lays eggs randomly on marble/pea-sized fruits and the larva develops within maturing seed in about two months. The larva mines in a zig-zag manner and feeds entirely on the seed within the stone, sometimes reducing the germinability greatly. The degree of MSW infestation varies with location[4].

An examination of seeds from MSW-infested stones showed that the funiculus connection (vascular strands) at hilum (Figure 1 *f*) and/or the embryonic axis (top portion of the seed, Figure 1 *a*) in healthy fruits was found damaged by the weevil. In the present study, about 15% of sampled fruits was found infested with MSW. Out of a total of 424 MSW-infested stones, 83% was from healthy fruits where the weevil damaged either funiculus connection at hilum or embryonic axis, rendering the seed independent of the mesocarp. Of the remaining 17% stones from ST-affected fruits, funiculus connection at hilum (Figure 1 *f*) and embryonic axis were intact even though rest of the seed (bottom portion of the seed, Figure 1 *a*) was found damaged.

In another experiment, 300 fruits each of Alphonso and Totapuri (ST-free) mango varieties were given pre-harvest dip treatments at ten day intervals between 50 and 70% maturity stages with GA_3 (2000 ppm) and GA_3 (1000 ppm) + tungstate (10 mM) solutions to enhance the metabolic activity of seed and the results were compared. Tungstate is an inhibitor of abscisic acid (ABA) biosynthesis[5,6] and is, therefore, expected to alter the ratio of growth promoters to inhibitors in favour of GA_3, thereby increasing seed metabolic activity leading to ST formation. Fruits were harvested at 85% maturity, ripened under ambient conditions (28–30°C and 70–80% RH) and observed for ST incidence. A marked increase in ST incidence with GA_3 treatment (70.5–71.7%) over the corresponding control fruits (45.8%) of Alphonso mango was consistent with the results reported earlier[1]. Surprisingly, there was no induction of ST in Totapuri fruits. A closer examination of stones revealed that the funiculus connection at hilum was intact in Alphonso mango (Figure 1 *c*), while it was cut-off in Totapuri (Figure 1 *b*). Hilum marks the point at which the seed is attached to the ovary tissue via funiculus. Thus, Alphonso fruits in which either the funiculus connection at hilum and/or the embryonic axis was damaged due to random feeding activity of the MSW, were free from ST while those fruits in which the seed had shifted into germination mode with an intact funiculus and embryonic axis were affected by ST.

On the contrary, there was no induction of ST in Totapuri even though the seed was in an advanced stage of germination (Figure 1 *d*). It was also observed that funiculus in Totapuri was always found desiccated and cut-off at hilum (Figure 1 *b*) irrespective of MSW infestation

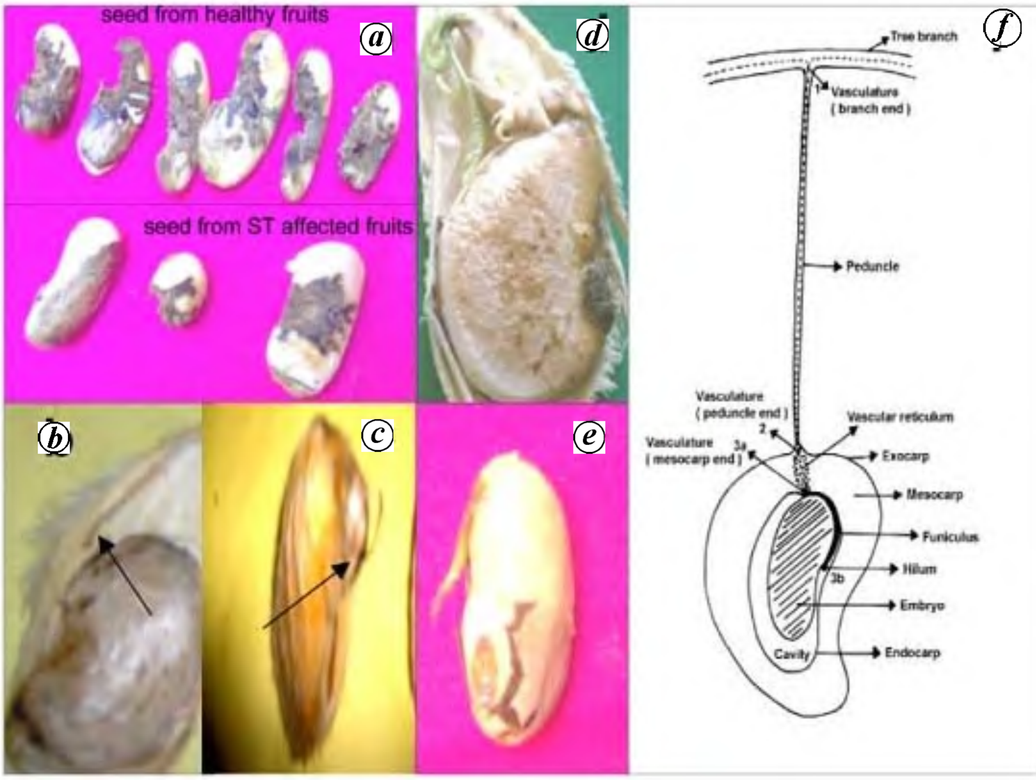

Figure 1. ***a***, MSW-infested healthy and spongy tissue-affected Alphonso mango seed. ***b***, Funiculus detached at hilum in Totapuri mango seed. ***c***, Funiculus intact at hilum in Alphonso mango seed. ***d***, Totapuri seed in an advanced stage of germination inside stone of mature fruit. ***e***, Radicle emergence in seed at fruit maturity in spongy tissue-affected Alphonso mango fruit. ***f***, LS of Alphonso mango fruit showing vasculature and different parts of fruit.

thus preventing the seed from drawing nutrients from the mesocarp. Therefore, it is obvious that the shift of seed into germination mode along with an intact funiculus connection at hilum is essential for initiation of spongy tissue. It is likely that Totapuri seed, by virtue of its higher moisture content (43.29%), does not depend on the mesocarp for initiation of pre-germination events, unlike Alphonso mango in which the seed moisture was lower (32.63%). Incidentally, the moisture content of the Totapuri seed (43.29%) was higher than that of ST-affected Alphonso mango (42.04%), showing its self-sufficiency to initiate germination events without depending on the mesocarp. That the Totapuri seed was not deriving moisture from the mesocarp was also evident from the predominance of plumule growth as against radicle growth in Alphonso mango (Figure 1 *d*).

During fruit development, the seed remains in direct connection with the tree through the funiculus, ensuring food supplies for its growth and development. Normally, mango seed gets physiologically detached from the tree towards maturity due to breakage of vasculature at peduncle end of the fruit (Figure 1 *f*). Seed and fruit growth are naturally phased out in mango to prevent intra-fruit competition. However, the disconnection may also take place at any stage of fruit growth due to inherent competition among various sinks, including other fruits operating concurrently. During fruiting season, inter-fruit competition is the predominant stress factor that leads to disconnection of vasculature between seed and peduncle of weaker sinks (fruits) to prevent any reverse flow of nutrients[7]. Flesh breakdown in mango cv. Tommy Atkins was attributed to disconnection of vascular strands between the peduncle and the seed even while fruits were still attached to the tree, making them dependent on mesocarp for maintenance/survival[8]. Once the seed is cut-off from direct supplies of the tree, it draws nourishment from the mesocarp for its growth, provided the funiculus link between the seed and mesocarp is intact as seen in Alphonso mango (Figure 1 *c*). In Totapuri, the funiculus was found cut-off at hilum (Figure 1 *b*), thus preventing the drain of resources from the mesocarp into the seed. It is, therefore, apparent that funiculus disconnection at hilum explains both the non-response of Totapuri to applied GA_3 and the absence of ST.

To sum up, both the non-response of Totapuri to applied GA_3 and the absence of ST in Alphonso mango fruits where the funiculus connection at hilum and the embryonic axis were damaged by the random feeding activity of MSW, clearly provided unequivocal natural evidence for the pivotal role of seed in ST formation. A significant reduction of ST in paclobutrazol-treated fruits reported previously also revealed the involvement of seed in ST formation[1]. Therefore, it is obvious that ST in Alphonso mango is an effect of the seed shifting into germination mode and not vice versa. It is possible that a similar mechanism may be operating in mango varieties prone to ST formation, while the funiculus disconnection at hilum might be crucial in mango varieties free from spongy ST.

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Onset of sexual maturity in captive-reared endangered Indian seahorse, *Hippocampus kuda*

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The endangered Indian seahorse, *Hippocampus kuda* successfully reared in captive conditions for more than two years, repeatedly spawned and produced F_2 generations. In F_1 males, brood pouch started developing from 55 to 60 days of birth. The courtship behaviour was noted 30 days subsequently and males started receiving eggs in their pouch when their body length attained 101.0 ± 2.0 mm. Females started transferring eggs into the male brood pouch when their body length reached 110.0 ± 2.0 mm. The sexual maturity of male was earlier than that of female. Compared to the age (in days), the size was inferred as a reliable factor to predict the onset of maturity in this species.

Keywords: *Hippocampus*, Indian seahorse, sexual maturity.

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THEORETICAL and experimental studies show that certain life-history characteristics make species more vulnerable to overexploitation[1,2]. Seahorse is an endangered fish and warrants replenishing the stock at least in selected marine habitats. In order to understand the impact of targeted fishing, by catch in trawlers and trade, knowledge of their biology along with population monitoring and fisheries management is required[3]. To a limited extent, attempts were made in this direction in understanding the behavioural and breeding patterns in *Hippocampus whitei*[4]. *H. zosterae* was reported to mature at three months[5]. *H. barbouri*, *H. fuscus*[6], *H. hippocampus* and *H. ingens*[3] reached maturity at four to five months, while many other species such as *H. kuda*[7], *H. spinosissimus*[7], *H. trimaculatus*[8], *H. capensis*[9], *H. erectus*[10] and *H. abdominalis*[11] were reported to initiate breeding behaviour after six to twelve months of birth. There is little attempt towards continuous monitoring for their first maturity and life history under captive condition, except for *H. zosterae*[5]. Considering the importance of conservation and the need to harness the brooder resources in India, breeding and lifecycle observations

Figure 1. Adult *Hippocampus kuda* [female (left) and male (right)].