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Production of supernumerary plants from seed fragments in *Garcinia gummi-gutta*: evolutionary implications of mammalian frugivory

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Seeds of *Garcinia gummi-gutta* follow 'garcinia-type' of germination in which the primary root and shoot emerge from the opposite ends of the seed. The embryo, which fills up the seed, is an elongated hypocotyl with vasculature connecting the two poles. In a series of experiments we observed that removal of primary root at different stages of development does not affect germination or seedling growth; any seed fragment that contains vasculature produces a root and shoot irrespective of its size and position with precise polarity; and, a seed from which a seedling has germinated is capable of producing another seedling. We propose that the regenerative capacity of seed fragments could be a unique strategy for exploiting mammalian frugivory for seed dispersal.

Keywords: Frugivory, *Garcinia gummi-gutta*, germination, root–shoot polarity, seed dispersal, seed fragments.

INTENSE competition in the tropical evergreen forests has led to evolution of varied life-history strategies among different species. In what could probably be another interesting strategy, we report that any fragment of a seed of *Garcinia gummi-gutta*, a tropical evergreen tree, can independently produce root and shoot provided it has some vascular tissue. We discuss the evolution of this feature as a unique way of exploiting mammalian frugivory for seed dispersal.

G. gummi-gutta (L.) Robson (family Guttiferae, also called Clusiaceae) is an evergreen tree of medium size. It grows on the humid slopes along the wet evergreen forests of the Western Ghats, India and Sri Lanka[1] up to an altitude of 1000 m amsl[2]. Trees reach approximately 18 m in height and 70 cm in diameter. Like other *Garcinia* species, *G. gummi-gutta* is dioecious[3], but has a natural sex ratio[4] of 1 : 1. In India, population densities are highest in Uttara Kannada district, Karnataka, which is the northern part of the range of this species[4]. Fruit is the economically important part of the tree. The pulp of the fruit rind, also known as 'kokum', is used in curries as a souring condi-

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ment, in preparing a refreshing drink rich in antioxidants and is known to have antiseptic properties. Dried seeds yield kokum butter, rich in protein and fat. The oil is traditionally used for treating skin diseases. The fruiting season extends from June to August, which coincides with the peak of southwesterly monsoon. Fruits are fleshy and bear five to seven seeds. The seeds are 23.31 ± 2.81 mm in length, 11.90 ± 1.21 mm in breadth, 23.31 ± 2.84 mm in thickness and have a fresh weight of 1.15 ± 0.15 g ($n = 200$). Seeds are reported to show dormancy owing to an impermeable seed coat[5,6]. A study was carried out to observe the effect of seed coat on germination by sowing seeds with and without seed coat separately in a sand bed ($n = 100$ for each case), which was watered twice a day. Seeds with intact seed coat took approximately four months to initiate germination (i.e. emergence of primary root), whereas in the case of seeds without the coat, germination started by three weeks (± 6 days).

Germination in *G. gummi-gutta* begins with the emergence of the primary root (PR) at the distal end of the seed followed by the appearance of a shoot from the proximal end ('proximal' end of the seed refers to the end towards the peduncle, whereas the other end is the 'distal' end). Subsequently, prior to leaf differentiation, an adventitious root (AR) originates from the base of the shoot. This pattern has already been described as 'garcinia-type' of seed germination[7]. The PR along with the seed disintegrates over a six-month period and eventually the adventitious root takes over as the main root system of the plant. An experiment was set up using decoated seeds to ascertain the probable role of PR in seed germination. In the first treatment, the distal end of the seeds was cut-off before allowing them to germinate ($n = 100$). In the second treatment, the PR was severed just after emergence but before the appearance of the shoot at the other end ($n = 100$). One hundred decoated seeds were used as control. Seeds were allowed to germinate in sterile, moist germination paper under room temperature (25 ± 2°C). It was expected that the treatments would significantly reduce the percentage of germination over the control. However, contrary to our expectations, shoot and AR were produced in 96% of seeds in the first treatment and in 95% each in the second treatment and the control. In the first treatment a new PR was formed after 42 days in 22% of the seeds and in 96% by 77 days. In the second treatment, organization of a new PR was first noticed by 50 days and by 80 days in 95% of the seeds. In both cases, shoot initiation occurred first. Thus, removal of PR had no effect on seed germination.

The role of PR in seedling growth and development was investigated by cutting the PR from seedlings at the four-leaf stage in which the AR was already well developed. One hundred seedlings at the four-leaf stage were removed from the polybags and parameters such as shoot length, collar diameter and AR length were measured. For 50 seedlings PR was cut and replanted (treatment), while the remaining 50 seedlings with intact PR were replanted in the polybag (control). At the end of 60 days all the seedlings were carefully removed from the polybag. It was observed that PR had formed in 85% of the treated seedlings. Interestingly, there was no significant difference between treated and control seedlings with respect to shoot length (t test; $P = 0.92$), collar diameter (t test; $P = 0.72$) and AR length (t test; $P = 0.64$). These experiments indicated that neither seed germination nor seedling growth was affected by the removal of PR, and strangely a new PR had become organized irrespective of the stage (seed or seedling) at which it was cut-off.

This type of seed germination and organization of PR could be because the mature embryo is a swollen hypocotyl, completely filling the space within the seed and the cotyledons are either absent or rudimentary, and represented by two small fleshy warts at the apex of the hypo-

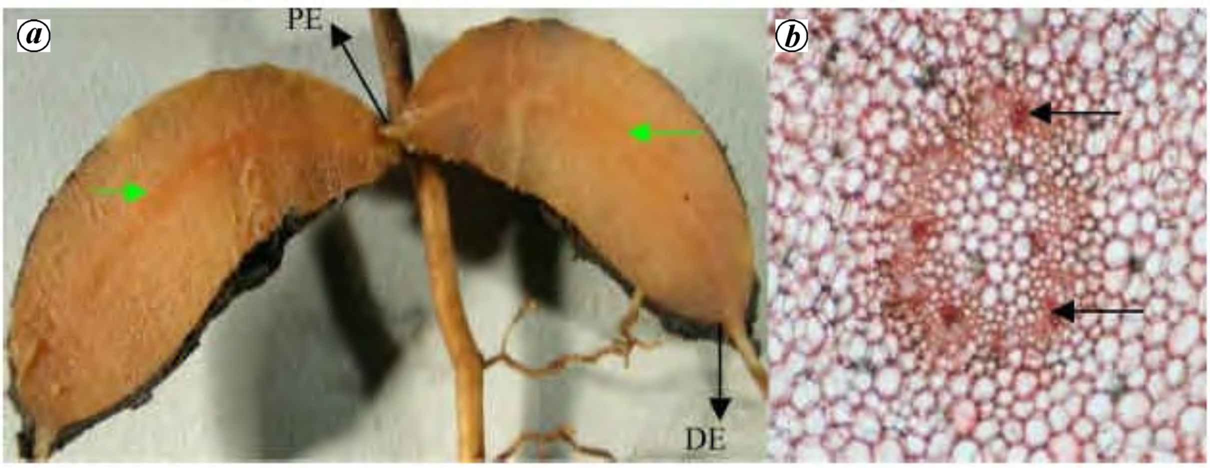

Figure 1. ***a***, Vertical section of the seed attached to a seedling. Two halves have been opened out to show a thin, thread-like vascular strand connecting the proximal and distal ends. A similar structure has also been observed in the ungerminated seed. ***b***, The central part of the seed in cross-section showing a ring of vascular bundles (see arrows) surrounded by parenchymatous cells. PE, Proximal end; DE, Distal end.

cotyl[7]. We have called this embryo encased within the seed coat as the 'seed'. To understand the internal structure of the seed, a vertical section was cut and examined under a hand-lens. It revealed the presence of a central, thin, thread-like structure connecting the two ends of the seed (Figure 1 *a*). Microscopic observation of a transverse section revealed that this thread was a conducting tissue consisting of vascular bundles (Figure 1 *b*) along its entire length. Four or five vascular bundles were observed in the middle of the seed and their number gradually decreased towards the ends. The presence of conducting tissue from the proximal to the distal end of the embryo and organization of the PR in the same place from which it was excised prompted us to cut the seeds transversely into two equal halves (n = 100) and observe the response for PR organization in both halves. Seeds were decoated and the proximal and distal ends were marked before cutting into two. Both fragments of each seed were buried separately in germination trays filled with sand and watered once every two days. Fragments with intact proximal end produced shoot and AR by 47 days after sowing. In the same fraction PR formed from the central portion of the cut end by 80 days in 70% of the seed fragments (Figure 2 *a*). In the fragments with the intact distal end, the PR formed first in 95% of seeds by 70 days and after 95 days bud-like outgrowths organized along the margin of the seed at the cut end (Figure 2 *b*). Subsequently, these buds differentiated into individual shoots, from the base of which AR differentiated (Figure 2 *c*). Longitudinal sections of buds at different stages showed that they were parenchymatous in origin. As the buds developed they established a vascular connection with the central vasculature of the seed. Thus, from two fragments of the same seed two independent seedlings could be obtained (Figure 3). Uncut seeds (control) produced only one seedling.

It was of interest to ascertain whether smaller seed fragments would also produce root and shoot. Each decoated seed was cut transversely into two unequal fragments from the proximal end with the smaller fraction being 1, 2, 3, 4 or 5 mm in length (n = 50). Similar fragments were also taken from the distal end of the seed and kept in sterile moist germination papers at room temperature. All fragments greater than 1 mm in length produced root and shoot. Additionally, the proximal end invariably produced shoot and AR while the distal end gave rise to PR irrespective of the size of the fraction (Figure 4). This led us to further verify root–shoot polarity in fragments in which

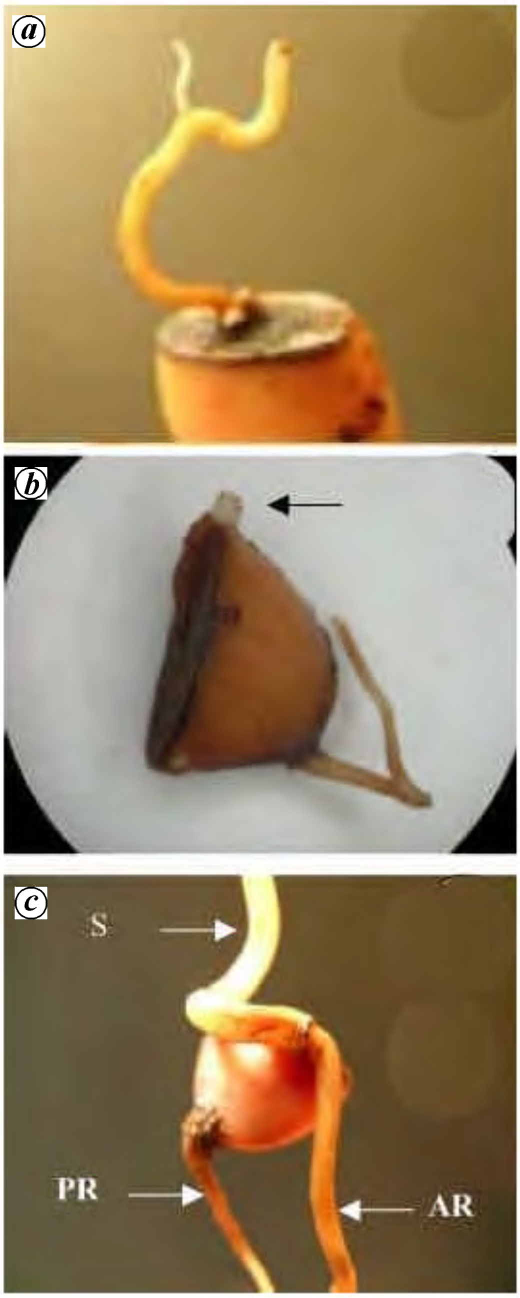

Figure 2. *a*, Primary root (PR) organizing from distal cut end of the seed. *b*, Bud-like outgrowths from the cut margin of the seed cut transversely into half. *c*, Bud-like outgrowth differentiated into shoot and adventitious root (AR). S, Shoot.

Figure 3. Independent seedlings developed from each of two fragments of the same seed.

the original proximal and distal ends were removed. To investigate this, seeds were cut from both the ends at a distance of 1, 2, 3, 4 and 5 mm ($n = 100$) respectively. The middle fragments thus obtained were marked for their corresponding proximal and distal ends and then observed for root shoot emergence by placing them in moist germination paper (25 ± 2°C). The proximal end of the middle fraction invariably produced shoot and AR whereas the distal end differentiated a PR, thus maintaining polarity (Figure 4 *d*).

Driven by curiosity, 100 decoated seeds were vertically cut into two equal halves and kept in sterile moist germination paper at room temperature (25 ± 2°C). Interestingly, each half invariably produced PR from the distal end, and shoot and AR from the proximal end.

These results raised another fundamental question – is a seed which has already germinated into a seedling capable of forming another seedling? Decoated seeds attached to three-month-old seedlings were detached ($n = 50$) and their ends were trimmed before placing them in moist germination paper. To our surprise, 80% of them produced PR, shoot and AR.

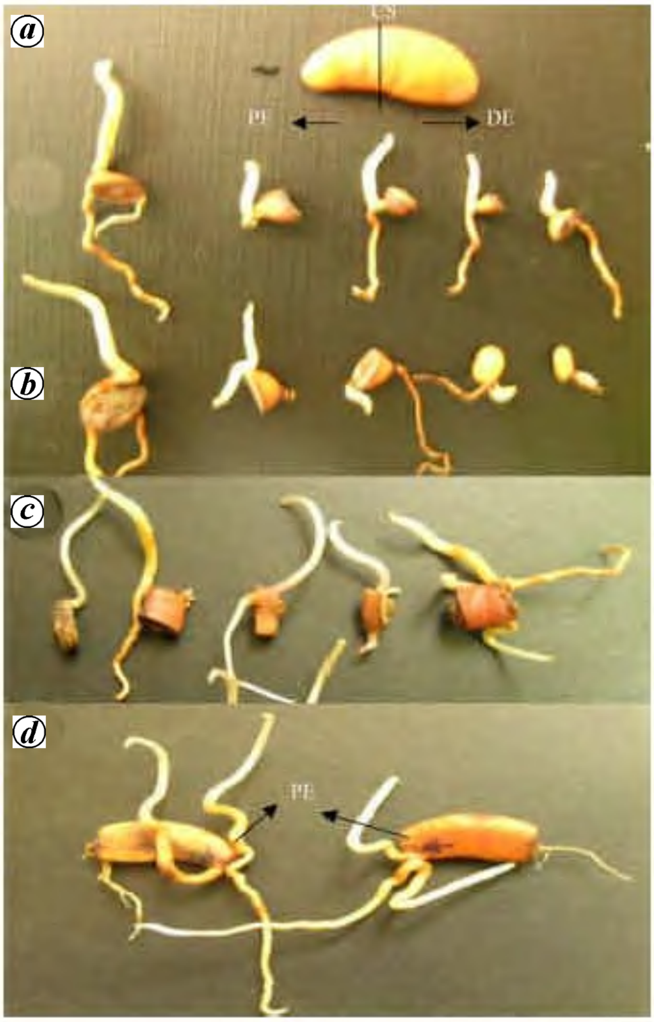

Figure 4. Germinated seed fragments of different sizes (2 to 5 mm) cut along the cross-section: ***a***, Germinated fragments with intact proximal end; ***b***, Germinated fragments with intact distal end; ***c***, Germinated seed fragments from middle portion of the seed with both proximal end and distal end removed; ***d***, Germination of entire seed with the ends cut-off showing root–shoot polarity. CS, Cross-section.

The study shows that fragments of seeds of *G. gummi-gutta* possess an unusual capacity for regeneration. Considering the natural history of *G. gummi-gutta*, it appears that this phenomenon may have evolved to exploit mammalian frugivory for seed dispersal. The mature fruit is medium-sized, yellow, pulpy and acidic in taste that attracts frugivores such as primates, civets and arboreal squirrels, which are the chief seed dispersal agents[4]. Primates (*Presbytus entellus* and *Macaca radiata*) have been found to discard seeds of *G. gummi-gutta* after feeding on the pulp, whereas civets (*Paradoxorus hermaphroditus* and *P. jerdonii*) defecate them[4]. During frugivory, especially primates, there is a considerable probability that at least a few of the 6 or 7 seeds dispersed in the fruit pulp are damaged, as the seed coat is leathery and the kernel is not hard enough to prevent the sharp teeth of frugivores from piercing the seeds. Incidentally, seeds defecated by civets were not found to be scarified[4] and perhaps did not differ from seeds taken from fruits with respect to germination[8,9]. As shown earlier, seeds with intact coat took approximately four months to germinate while seeds devoid of coat germinated in about two to three weeks which suggests that seeds damaged during frugivory have a better chance to germinate during the current monsoon (fruits mature during peak monsoon months of July–August) than those that have not been damaged. Monsoons in southern India last only until late September or early October, and if seeds remain dormant for four months it would result in germination of undamaged seeds only after the monsoon. This would expose the developing seedlings to a tough dry spell of 7 or 8 months. Moreover, a majority of seeds of species in the evergreen forests germinate during the monsoon, which would expose those that germinate after the season to severe competition. Additionally, ants devour seed kernels of *G. gummi-gutta*, which reduces the chances of survival with time[4]. Hence, it appears that germination after monsoon (for the undamaged seeds) may significantly risk seedling establishment and survival. Damaged seeds are also more likely to be the ones that have been successfully dispersed, thus increasing the success rate of those that are dispersed compared to those that are not. We have also shown that seed fragments not only produce root and shoot, but also that they have enough reserve material to support development of complete seedlings. Therefore germination of seed fragments may have evolved as a unique way of exploiting mammalian frugivory for seed dispersal. We believe that *G. gummi-gutta* could be used as a model system for fresh lines of investigation in several branches of science, especially morphogenesis, physiology, seed biology and evolutionary ecology.

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