

Intergeneric Hybridisation between Litchi (*Litchi chinensis* Sonn.) and Longan (*Dimocarpus longan* Lour.)

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The breeding barriers between commercial litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan* Lour.) cultivars were investigated by conducting reciprocal pollinations. This work has shown that it is possible to generate intergeneric hybrids using litchi as the female parent. Investigation of comparative *in vivo* pollen tube growth demonstrated that there is discrimination against cross- compared to self-pollen at all sites in the pistil. Pollen tubes were frequently observed in the ovary after cross-pollination in litchi but rarely in longan. Fruit production was reduced after crossing in both longan and litchi. Isozyme analysis using phosphoglucose isomerase revealed that hybrid progeny only developed when litchi was the maternal parent. Morphologically the hybrid plants were similar to the maternal parent but leaves were smaller. Three types of seeds developed in litchi following pollination with longan pollen. These were (1) normal seeds with a developed testa and embryo, (2) seeds with aborted embryos but normal testa development, and (3) seedless fruit where the ovule remained the same size as at anthesis without further development of embryo or testa. The potential germplasm available to improve these crops within the Sapindaceae is discussed.

Key words: Litchi, *Litchi* Longan, *Dimocarpus*, hybridisation, isozyme.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan* Lour.) are subtropical and tropical trees cultivated for the edible aril of their fruits. Both species are members of the Nephelieae in the Sapindaceae along with other commercial fruit tree species including rambutan (*Nephelium lappaceum* L.) and pulasan, or meritam [*Nephelium ramboutan-ake* (Labill.) Leenh.]. The breeding barriers within and between genera of this subfamily have never been investigated. Longan can be readily distinguished from litchi in having a perianth whorl in the flowers and tufts of trichomes as opposed to single trichomes on leaves and stems. The mature fruit of longan have green to brown skin as opposed to the red skin of litchi.

Intergeneric hybrids have been reported to occur naturally in China and are referred to as lungly (Groff, 1921). There have been no detailed investigations to determine if these trees were true hybrids or represent the morphological diversity of these species.

Both longan and litchi have asynchronous sexual phases during the flowering of a single panicle. The first flowers to open possess anthers only (functionally male), followed by flowers with staminodes and fertile pistils (functionally female), and finally flowers with anthers and rudimentary pistils (functionally male). This assists in making controlled pollinations since there is no need to emasculate the functionally female flowers.

The aim of this study was to investigate the pollen–pistil interactions between the litchi and longan and quantify the

efficiency of reciprocal crosses in producing fruit and viable seed.

MATERIALS AND METHODS

Pollen tube growth

Pollinations were performed in research orchards at Alstonville, N.S.W. Agriculture, over the years 1988–91. Intergeneric breeding barriers were investigated in litchi (*Litchi chinensis* Sonn.) cv. Bengal and cv. Kwai May Pink with longan (*Dimocarpus longan* Lour.) cv. Macleans Ridges and cv. Duan Yu by monitoring pollen tube growth in reciprocal crosses. The trees used were clonally propagated and 5–8 years old. Inflorescences about to commence the female phase of flowering were enclosed in paper bags tied closely around the supporting branch with polyethylene marker tape. Twenty flowers, were either self- or cross-pollinated or left unpollinated (controls) and tagged (see Tables 1 and 2 for combinations tested). The bags on the unpollinated controls were removed for comparable times as the pollinated panicles to monitor the amount of undirected pollination occurring. Care was taken to ensure no biotic vectors visited flowers while bags were removed. Any male flowers undergoing anthesis at the time of bagging were removed and panicles were checked daily to ensure that no male flowers underwent anthesis while female flowers were receptive. Pollen was obtained from male flowers with unopened anthers collected on the morning of the day on which pollination was to be performed. Flowers were placed in open Petri-dishes in the laboratory and the anthers

allowed to dehisce. Pollen that adhered to anthers was used in pollinations by brushing against the stigma. Flowers were collected after 5 d and fixed in acetic acid: alcohol 1:3 (v/v), softened, stained with decolorized aniline blue, and viewed by fluorescence microscopy as described in McConchie and Batten (1991). The pollen tube growth was scored at three positions in the pistil, (1) stigma, (2) central style, and (3) in the ovary by counting the number of tubes present.

Fruit and seed production

In the first 2 years all the female flowers in a panicle were used for cross- or self-pollination. Unpollinated panicles were prepared as described above and used as controls. The same pollen source, derived from one to five trees of a single cultivar, was used for an entire panicle. In litchi cv. Bengal, two panicles on three trees were either self or cross pollinated or left unpollinated. In litchi cv. Kwai May Pink the treatments were replicated in the same way on five trees. The three crossing treatments that were performed on longan cv. Duan Yu were applied to six panicles on a single tree. Fruit were allowed to develop to maturity. At harvest, after recording weight of fruit and seed, the seeds were germinated in sand.

In the third year, in an attempt to obtain an increased number of hybrid seeds from litchi cv. Bengal, branches bearing cross pollinated flowers were cinctured by removing 10 mm of bark, approximately 1 m below the panicle at the completion of the female phase flowering. All unopened male floral buds were also removed. In all experiments, both cinctured and uncinctured, every female flower on a panicle was pollinated. Total soluble solids of the mature fruits were determined in sucrose equivalents (°Brix), using a hand-held refractometer.

Microscopy

To compare the indumentum on the abaxial leaf surfaces, fresh leaf samples were mounted on stubs using double-sided adhesive tape and examined on a Joel 8900F SEM operated at 5 kV. Measurements of trichomes were also made using a graticule and dissecting microscope. Flowers were examined and photographed using a Wild M420 Makroskop (Heerbrugg, Switzerland).

Isozyme analysis

Isozyme analysis was carried out on parental trees, six randomly selected self progeny from longan and litchi and all hybrid progeny using starch gel electrophoresis. About 10⁻⁴ m² of leaf tissue was crushed in 0.3 ml chilled 0.05 M Tris-citrate buffer, pH 7.5 containing 12% soluble polyvinyl pyrrolidone (PVP-40), 1.0% bovine serum albumin and 0.01 M 2-mercaptoethanol. The extracts were adsorbed on to 5 × 7 mm wicks of filter paper (Whatman 3M) through a single layer of Miracloth (Calbiochem). The wicks were arranged in a Petri dish kept chilled over a bed of ice until loaded onto starch gels.

Isozymes were separated using horizontal starch gel electrophoresis on gels containing 12.6% hydrolysed potato

starch (Sigma). Phosphoglucose isomerase (PGI) isozymes were resolved using the pH 8.0, lithium-borate buffer system where the gel buffer was 0.6 M tris-citrate with 0.007 M LiOH and 0.05 M sodium borate and the tray buffer was 0.2 M sodium borate with 0.028 M LiOH. Electrophoresis was carried out at 4 °C, 200 V, 40 mA and the gels were incubated in the staining medium at 37 °C until bands appeared. The gels were stained for PGI in a solution containing 0.13 M NADP, 0.02 M methyl thiazolyl tetrazolium, 0.01 M phenazine methosulphate, 5.0 ml 1 M tris-HCl pH 8.0, 10.0 ml 0.1 M MgCl₂, 10.0 ml 0.018 M fructose-6-phosphate and 5.0 ml glucose-6-phosphate dehydrogenase (10 units ml⁻¹) added just prior to staining and 100 ml of water (Torres, Soost and Diedenhofen, 1978). After staining, the gels were rinsed in distilled water and fixed in 50% ethanol for 1 h before recording the results.

The alleles which specify the migrating enzymes or sub-units, were designated by letters, depending on their mobility as described in Torres *et al.* (1978). The fastest was called 'F' and the slowest, 'S'. When more than one zone representing a locus for a given enzyme existed, the locus encoding the anodal (fastest migrating), form was designated 1 and the next 2 etc.

RESULTS

The flowers and indumentum of litchi and longan were manifestly different (Fig. 1A–D). Litchi flowers had a united calyx covered with uniseriate trichomes and no corolla (Fig. 1A). The anther filaments of litchi lacked ornamentation. The flowers of both cultivars of longan had free calyx and corolla parts and the number of petals on a flower varied from 0 to 6 (Fig. 1B). The pistils of litchi and longan have one to four locules each with an ovule. Usually only one locule develops per fruit containing a single seed. Litchi and longan could be readily distinguished vegetatively by the indumentum along the mid-rib and vascular bundles on the abaxial side of their leaves. Litchi had uniseriate filiform trichomes, 100–400 µm long (Fig. 1C) while longan had multiseriate stellate trichomes usually with more than three branches. Each individual branch of a trichome in longan rarely exceeded 100 µm in length (Fig. 1D). The trichomes in litchi and longan were covered with fine 1–2 µm ridges.

Under the microscope the pistils of longan and litchi after cross-pollination appeared morphologically similar. The path taken by pollen tubes to the ovary in litchi has been previously described by McConchie, Batten and Vithanage (1994) and is essentially the same in longan. Cross-pollen invariably germinated on the stigma surface and most pollen grains produced a small twisted pollen tube that had a collar of callose where they first contacted the stigma papillae (Fig. 2A). Most of these pollen tubes were arrested after penetrating the tissue below the papillae. Pollen tubes that entered the stigma branches and style which were arrested in this zone had irregular callose deposits along the length of the pollen tube (Fig. 2B). Occasionally pollen tubes grew through the pistil and entered the embryo sac. These pollen tubes morphologically resembled pollen tubes grown in self-pollinated pistils of longan and litchi in having

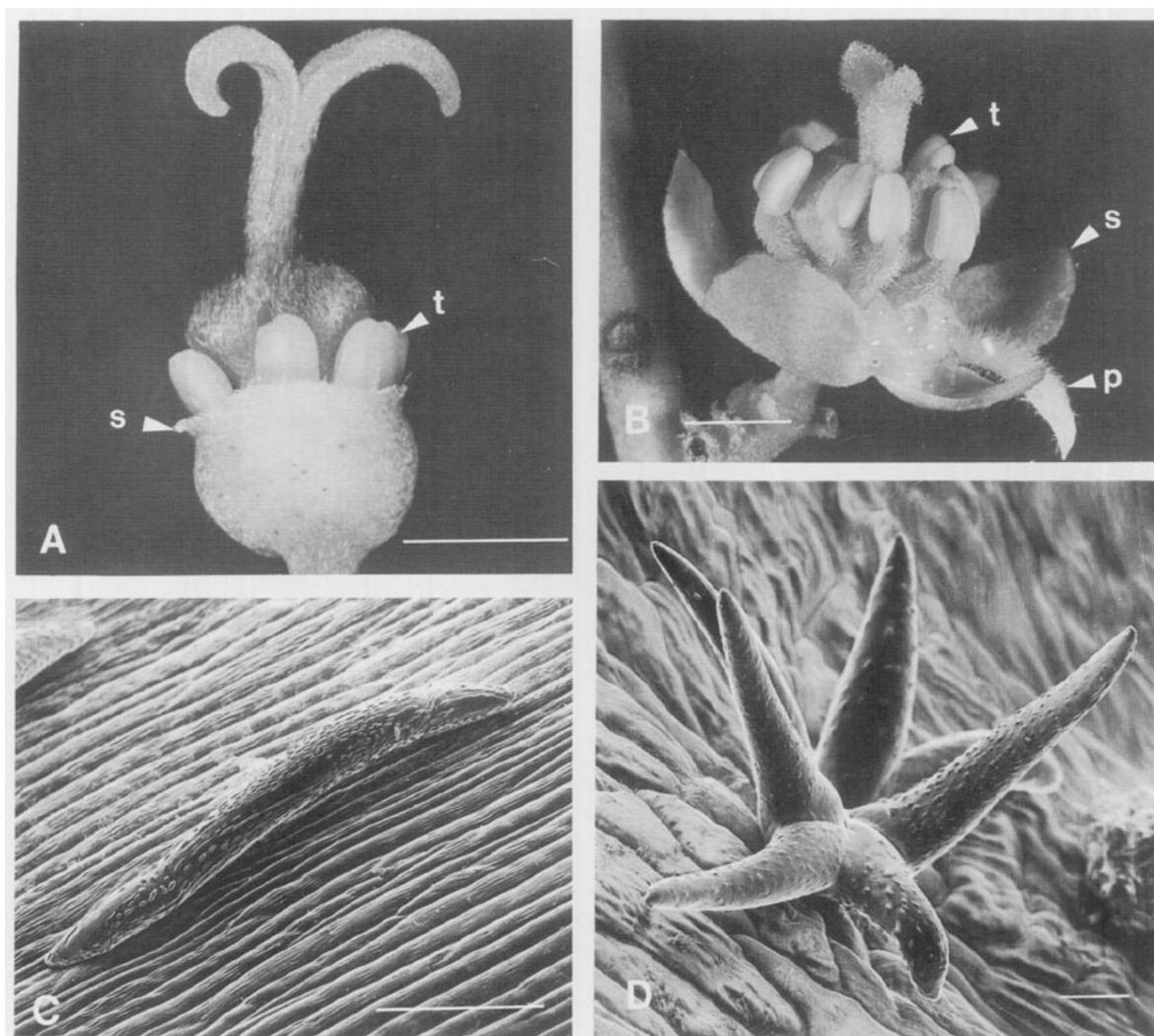


FIG. 1. Flowers and trichomes of litchi and longan. A, Female flower of litchi cv. Bengal with two recurved receptive stigma branches, united style and superior ovary subtended by a whorl of staminodes (t) and reduced united sepals (s) but no petals. Bar = 2 mm. B, Female flower of longan cv. Macleans Ridges with branched stigma, united style and ovary surrounded by a whorl of staminodes (t), petals (p) and free sepals (s). Note the filaments of the staminodes and petals are covered with trichomes. Bar = 2 mm. C, Uniseriate trichome, with fine longitudinally arranged surface ornamentation, on the mid-rib on the abaxial side of a litchi leaf. Bar = 50 μ m. D, Stellate trichomes with fine surface ornamentation on the mid-rib of the abaxial side of a longan side. Bar = 10 μ m.

regularly spaced callose plugs in the upper stigma and style. Callose plugs were absent or distantly spaced in the ovary (Fig. 2C).

After self-pollination in litchi a greater number of pollen grains germinated on the stigma surface than when pollinated with longan pollen (Table 1). Only a small proportion of the pollen tubes successfully penetrated the style and even fewer entered the ovary. However, at all positions in the pistil more pollen tubes were found after self-pollination than cross-pollination. Pollen tube growth was also greater in self-pollinated longan than when crossed with either of the two cultivars of litchi tested (Table 2). However, unlike litchi, a pollen tube was observed only on one occasion to enter the ovary after cross-pollination. Pollen tube growth was not detected on the control unpollinated pistils of longan. A single pollen tube, arrested below the stigma surface, was found on the unpollinated pistils of litchi cv. Kwai May Pink.

On litchi cv. Bengal, marginally more fruit were produced on panicles pollinated with longan pollen than on the unpollinated controls (Table 3). Fruits were not produced on litchi cv. Kwai May Pink when either unpollinated or pollinated with longan pollen. Litchi cv. Bengal had many more female flowers per panicle than litchi cv. Kwai May Pink and retained many more fruits per panicle at harvest. The number of fruits produced by longan after cross-pollination was also greatly reduced compared to self-pollinated panicles and was equivalent to the unpollinated controls (Table 4). Fruit characters were essentially unaffected by pollination treatment (Tables 3 and 4).

In the third year of experiments cincturing control panicles did not enable unpollinated flowers to set fruit and all flowers on these branches abscised within 2 weeks of anthesis. As a result of cincturing the four treated litchi panicles pollinated with longan pollen produced a total of 42 fruit. Only three of these fruit contained normal seeds

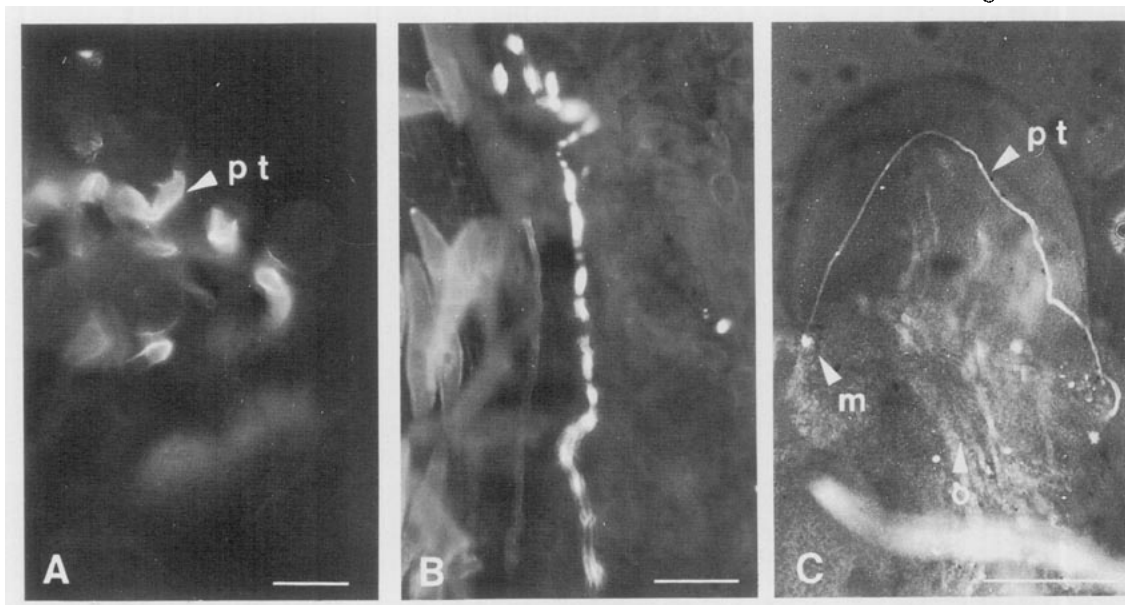


FIG. 2. Fluorescence micrograph of longan pollen tubes at different positions in the pistils of litchi cv. Kwai May Pink 5 d after pollination. Stain: Decolourized aniline blue. A, Pollen on the stigma surface with pollen tubes arrested after penetrating below the papillae. Note the collar of callose where the pollen tubes (pt) contact the papillae and the non-fluorescent tip when arrested. Bar = 50 μ m. B, Pollen tube with irregular callose deposits in the style. Bar = 50 μ m. C, Pollen tube (pt) growing across the obturator (o) to the micropyle (m). Bar = 0.5 mm.

TABLE 1. Mean number of pollen tubes in the pistil of litchi 5 d after selfing or cross-pollination with longan pollen after 5 d \pm s.e. (average of 20 flowers containing two ovaries per flower). Control plants received no pollination

Cross	Female flower	Litchi cv. 'Bengal'	Litchi cv. 'Bengal'	Litchi cv. 'Bengal'	Litchi cv. 'Kwai May Pink'	Litchi cv. 'Kwai May Pink'	Litchi cv. 'Kwai May Pink'
	Pollen source	Litchi cv. 'Bengal'	Longan cv. 'Macleans Ridges'	Control	Litchi cv. 'Kwai May Pink'	Longan cv. 'Macleans Ridges'	Control
Position in pistil	Stigma branches	130.6 \pm 10.0	88.1 \pm 7.75	0	80.2 \pm 16.14	67.7 \pm 21.5	0.05
	Style	10.4 \pm 1.9	4.85 \pm 1.00	0	9.6 \pm 1.4	2.8 \pm 0.5	0
	Ovary	0.3 \pm 0.07	0.13 \pm 0.05	0	0.6 \pm 0.09	0.2 \pm 0.07	0

TABLE 2. Mean number of pollen tubes in the pistil of longan 5 d after selfing or cross-pollination with litchi pollen after 5 d \pm s.e. (average of 20 flowers containing two ovaries per flower). Control plants received no pollination

Cross	Female flower	Longan cv. 'Macleans Ridges'	Longan cv. 'Macleans Ridges'	Longan cv. 'Macleans Ridges'	Longan cv. 'Duan Yu'
	Pollen source	Litchi cv. 'Bengal'	Longan cv. 'Macleans Ridges'	Control	Litchi cv. 'Bengal'
Position in pistil	Stigma branches	20.1±6.3	33.2±4.9	0	49.5±10.2
	Style	0.85±0.28	3.85±0.71	0	0.85±0.4
	Ovary	0	0.63±0.08	0	0.03±0.05
Cross	Female flower	Longan cv. 'Duan Yu'	Longan cv. 'Duan Yu'	Longan cv. 'Duan Yu'	
	Pollen source	Litchi cv. 'Kwai May Pink'	Longan cv. 'Duan Yu'	Control	
Position in pistil	Stigma branches	36.4±5.7	112.6±18.8	0	
	Style	0.5±0.2	8.75±1.45	0	
	Ovary	0	0.8±0.12	0	

TABLE 3. Results of self- and cross-pollination on fruit production and quality in litchi cultivars. Fruit and seed weights \pm s.e. Control plants received no pollination

Female parent	Litchi cv. 'Bengal'	Litchi cv. 'Bengal'	Litchi cv. 'Bengal'	Litchi cv. 'Kwai May Pink'	Litchi cv. 'Kwai May Pink'	Litchi cv. 'Kwai May Pink'
Pollen source	Litchi cv. 'Bengal'	Longan cv. 'Macleans Ridges'	Control	Litchi cv. 'Bengal'	Longan cv. 'Macleans Ridges'	Control
Flowers pollinated	1186	1766	1026	1152	987	842
No. of panicles used	6	6	6	10	10	10
Fruit harvested (% flowers)	61 (5.1)	6 (0.34)	3 (0.29)	10 (0.87)	0 (0)	0 (0)
Mean fruit weight (g)	19.85 \pm 0.25	21.34 \pm 1.23	19.15 \pm 1.3	19.94 \pm 0.97	—	—
Mean seed weight (g)	2.93 \pm 0.13	2.96 \pm 0.53	3.49 \pm 0.31	2.23 \pm 0.16	—	—

TABLE 4. Results of self- and cross-pollination on fruit production and quality in longan. Fruit and seed weights \pm s.e. Control plants received no pollination

Female parent	Longan cv. 'Duan Yu'	Longan cv. 'Duan Yu'	Longan cv. 'Duan Yu'
Pollen source	Litchi cv. 'Bengal'	Longan cv. 'Duan Yu'	Control
Flowers pollinated	634	667	667
No. of panicles used	3	5	3
Fruits harvested (% flowers)	6 (0.95)	114 (17.1)	6 (0.9)
Mean fruit weight (g)	9.25 \pm 1.00	10.42 \pm 0.19	9.77 \pm 0.40
Mean seed weight (g)	1.59 \pm 0.17	1.88 \pm 0.03	1.79 \pm 0.08

TABLE 5. Comparison of vegetative morphology and zymograms of progeny derived from self- and cross-pollinated litchi cv. Bengal and longan cv. Macleans Ridges

Female parent	litchi	litchi	longan
Pollen parent	litchi	longan	longan
Mean leaf dimensions (mm)			
Length (range)	105.4 (170–72)	31.9 (36.5–27)	57 (77–45)
Width (range)	33.5 (48.5–18)	8.7 (9.5–7.9)	23.5 (32–13)
Hair type	Single hairs	Single hairs	Clumped hairs
Isozyme composition	PGI-1 SS PGI-2 Absent PGI-3 FS or SS	PGI-1 FS PGI-2 FF PGI-3 SS Heterodimer 2F3S	PGI-1 FS PGI-2 FF PGI-3 Absent

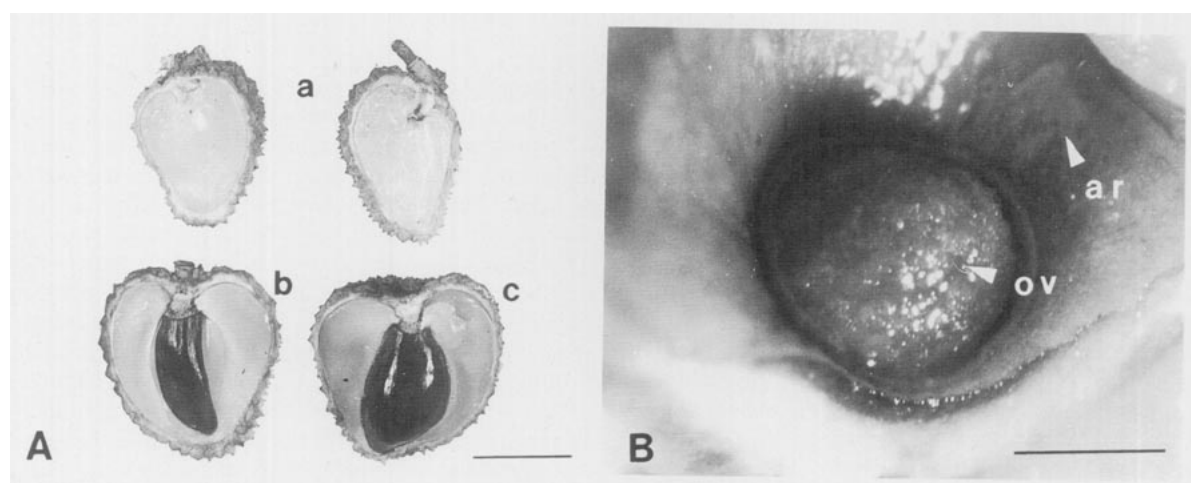


FIG. 3. Fruit of litchi cv. Bengal from cinctured panicles pollinated with longan cv. Macleans Ridges pollen. A, Longitudinal medial slices fruit with: a, no seed or embryo development (two fruit); b, seed development but aborted embryo; c, normal seed and embryo development. Bar = 2 cm. B, Dissected seedless fruit showing the undeveloped ovule (ov) surrounded by aril (ar). Bar = 0.5 mm.

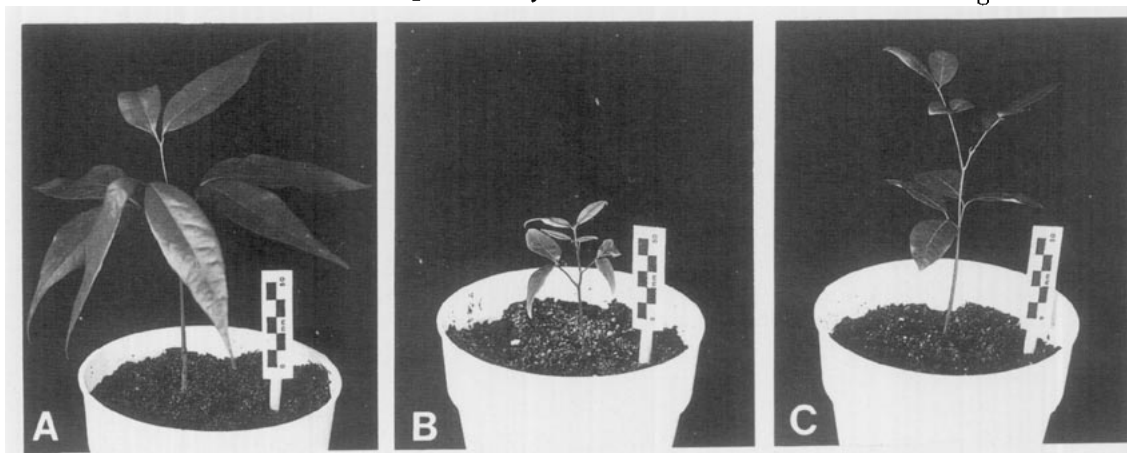


FIG. 4. Seedlings 2 months after sowing, longan cv. Macleans Ridges (A), intergeneric hybrid between longan cv. Macleans Ridges and litchi cv. Bengal (B) and litchi cv. Bengal (C). Bar = 50 mm.

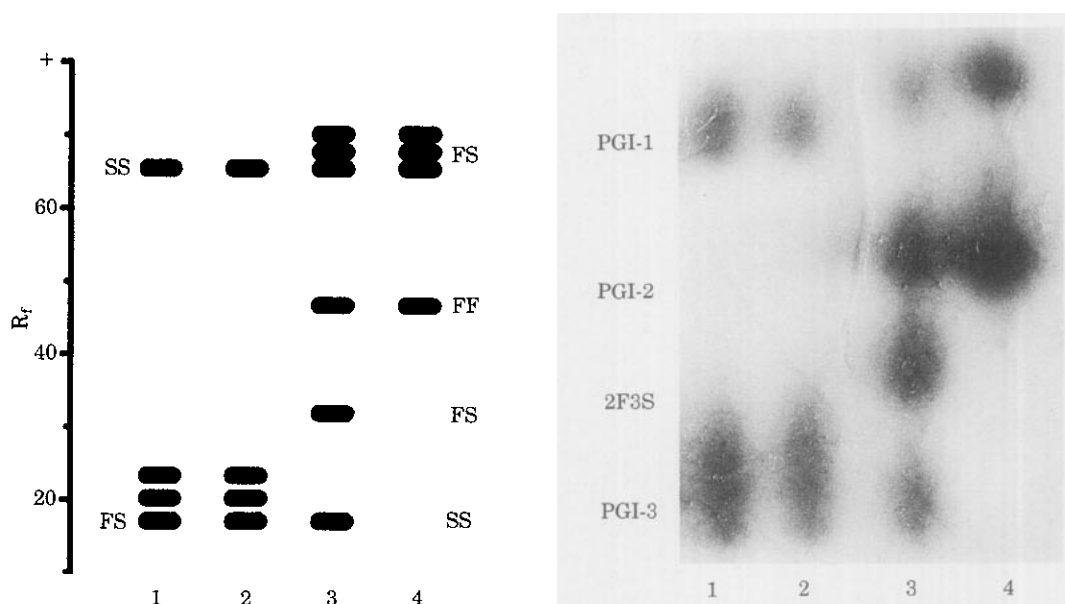


FIG. 5. Zymogram of PGI isozymes. Origin is at the bottom and anode (+) at the top. Lanes 1 and 2 litchi cv. Bengal with PGI-1S/1S, PGI-3F/3S; lane 3, litchi cv. Bengal and longan cv. Macleans Ridges hybrid with PGI-1F/1S, PGI-2F/2F, intergenic heterodimer 2F/3S and PGI-3S/3S; lane 4, longan cv. Macleans Ridges with PGI-1F/1S and PGI-2F/2F. Note PGI-3 is absent in longan cv. Macleans Ridges.

weighing 1.9–3.6 g. The majority of the fruit contained seeds with aborted embryos weighing 0.41–1.03 g and 12 fruit were seedless, with seeds weighing less than 0.01 g (Fig. 3A). In seedless fruit, aril and skin development appeared morphologically normal but all components of the ovule failed to develop after pollination (Fig. 3B). Seedless fruit were small and had a lower sugar content equivalent to 10.2–13.6% sucrose as against 17.5–20.2% in normal fruit. In seedless fruit the aril completely filled the cavity between the ovule and skin.

Morphologically and putative hybrids from litchi had smaller leaves than seedlings derived from selfed flowers (Fig. 4A–C). The indumentum on all six hybrid plants resembled the litchi and did not have the clumped hairs of longan. The seedlings derived from the hybridised longan flowers were indistinguishable from that of selfed flowers.

Starch gel electrophoresis of PGI was used to provide

biochemical confirmation of the hybrid nature of the plants produced from cross-pollinations in this study. PGI is known to be dimeric enzyme in other plants (Torres *et al.*, 1978) and the crops under investigation showed similar patterns. Two well-resolved zones of enzyme activity were detected in the zymograms of each parental plant (Fig. 5). The faster migrating zone designated as *Pgi-1* showed a single-banded phenotype in litchi (SS) and a triple-banded phenotype in longan (FS). The hybrids also showed the triple-banded (FS) phenotype. *Pgi-2* showed a single band in longan but was absent in litchi. The zymograms of the progeny resulting from self-pollinations were identical to their respective parents. On the other hand, litchi zymograms revealed a triple-banded phenotype in the zone, designated *Pgi-3*. In one hybrid, PGI activity was seen in all three zones, in contrast to either of the parents (Fig. 5). In this hybrid, there was also an additional band in an intermediate

region between *Pgi-2* and *Pgi-3* and only a single band at the *Pgi-3* zone. In the other hybrid there was no activity at *Pgi-2* and a triple-banded phenotype at *Pgi-2* and *Pgi-3*.

DISCUSSION

An incompatibility response that discriminated against intergeneric pollen tube growth in reciprocal crosses between litchi and longan was observed in this study. This was evident by fewer pollen tubes being present at all sites in the pistil after cross-pollination compared to selfing. Longan pollen tubes frequently penetrated the ovule in litchi, but, litchi pollen tubes rarely entered the ovary of longan. Fruit development was also reduced in both litchi and longan after cross-pollination. In litchi this decrease could not be completely accounted for by differences in pollen tube growth. McConchie and Batten (1991) showed that the number of fruit produced per panicle in litchi cv. Bengal rarely exceeds 25 irrespective of the number of flowers pollinated. This can usually be achieved by pollinating as few as 100 flowers. In litchi cv. Bengal the mean number of pollen tubes entering each ovule was 0.13 after crossing with longan compared to 0.3 when selfed, a reduction of approximately 60%. Although almost 50% more flowers were cross-pollinated than selfed these flowers yielded less than 10% of the final number of fruit produced by selfing. This suggests that there was a post-zygotic incompatibility mechanism acting between longan and litchi. This would also appear to be the situation in litchi cv. Kwai May Pink in which no fruits developed despite longan pollen tubes entering the ovule.

The hybrid origin of the progeny produced by crossing longan cv. Macleans Ridges with litchi cv. Bengal was confirmed by the PGI isozyme analysis. The hybrid seedlings could be separated from all other progeny at F allele by the presence of the triple banding at *Pgi-1* which is similar to that of longan cv. Macleans Ridges. There was also an additional marker in the intermediate region between *Pgi-2* and *Pgi-3*. It seems likely that this is an intergeneric heterodimer formed by the dimerization of the sub-units corresponding to *Pgi-2* of longan cv. Macleans Ridges and *Pgi-3* of litchi cv. Bengal. The absence of any activity at the *Pgi-3* zone of longan and the re-appearance of an SS phenotype in one of the hybrids may indicate the presence of null alleles in longan at this locus. Further isozyme analyses are needed to confirm this conclusion.

Studies by Batten and McConchie (unpubl. res.) have shown that fruit retention per panicle can be increased in litchi by cincturing the panicle-bearing branches after the female phase. Fruit produced after self-pollination using this technique did not significantly differ in size or seediness compared to self-pollinated control fruit produced on uncinctured branches. Cinctured self-pollinated panicles of litchi cv. Bengal rarely produced any fruit with an aborted embryo. Pollination with longan pollen followed by shoot cincturing produced fruit with seeds where the embryos were predominantly aborted, and a form of seedless fruits that resembled the 'hollow fruit' reported by Huang and Qiu (1987). Previous work by Huang and Xu (1983) reported the occurrence of fruit in litchi where the embryo

aborts during development producing not only a greater proportion of aril but also an enlarged empty testa. In the seedless fruit that developed after pollination with longan pollen the testa had similar dimensions to the integuments of the ovule at anthesis. A similar sized seed is illustrated in the 'hollow' fruit described by Huang and Qiu (1987). This suggests that seed development in the 'seedless' and 'hollow' fruit ceased at comparable stages in development. In normal fruit development testa and endosperm growth occurs prior to aril formation (Huang and Qiu, 1987). However, the complete aril development in the seedless fruit negates the proposition that aril growth is dependent on the life-span of the liquid endosperm (Huang and Qiu, 1987). The cytological events that give rise to these seedless fruit are unclear but it would appear that pollination is required since fruit development did not occur on unpollinated cinctured branches.

The almost complete absence of seedless fruit in litchi cv. Bengal in the three treatments: (1) self-pollination no cincture; (2) cross-pollination with longan and no cincture; and (3) self-pollination and cinctured compared to the numerous seedless fruit produced after cross-pollination with longan and cincturing suggests that in this cultivar, seedless fruit are unable to compete with seeded fruit for resources during development. The comparatively low number of fruit with fertile seeds on cinctured or uncinctured panicles of litchi cv. Bengal pollinated by longan supports the proposition that embryo abortion is not caused by competition for resources but by an intrinsic incongruence in the cytological events that follow the entrance of the pollen tube into the embryo-sac.

The relatively high number of fruit produced on panicles which were apparently unpollinated was not a true reflection of the authors ability to regulate pollen supply during cross-pollination, as flowers that were not pollinated remained receptive for several days after anthesis. Stigmas of either longan and litchi pollinated on the first day of anthesis were brown and non-receptive the following day. Prolonged stigma receptivity of unpollinated flowers allowed the female period of anthesis to overlap with the second period of male flowering. Because of the possibility that some contamination might have occurred during controlled pollinations, seedlings were screened for isozyme composition which revealed that only two of the six putative hybrids from litchi had complimentary zymograms. This confirms that despite the pre- and post-zygotic breeding barriers it is possible to produce viable hybrid progeny between litchi and longan. The seedlings that showed complimentary zymograms also appeared morphologically intermediate, while all others resembled seedlings from selfed progeny. None of the seed from the longan fruit produced morphologically intermediate seedlings, suggesting that the progeny arose by accidental self-pollination. This is supported by the pollen tube growth data where only one in eight longan ovules were penetrated by a litchi pollen tube.

It has been proposed by Hogenboom (1975, 1984) that incongruity controls a species' breeding limits. In this theory co-ordination between pollen and pistil is progressively lost as the relationship between species decreases through evolutionary divergence. This has led to the suggestion that

study of pollen–pistil interactions can be used to assess evolutionary relationships between taxonomic groups (Ellis, Sedgley and Gardner, 1991). In crosses between litchi and longan all stages of the reproductive process were inhibited compared to self-pollination supporting the theory of Hogenboom (1975, 1984) that incongruity operates at several levels. Since it has been shown that different temperatures have marked effects on pollen tube growth in litchi (McConchie et al., 1994) and that there are daily fluctuations in microclimatic conditions within the orchards used, we have made no attempt to rank cultivars. It is, however, questionable to suggest that, for example, pollen tube arrest on the stigma surface alone could be used as a measure of taxonomic distance when other stages of fruit set may influence reproductive isolation.

Differences in discrimination between reciprocal crosses also call into question the simplistic use of pollen–stigma interactions to gauge relationships. The success of longan pollen on litchi suggests a close relationship but poor success of litchi on longan suggests greater divergence.

The genus *Litchi* is thought to contain a single species divided into three subspecies (Leenhouts, 1978). *Litchi chinensis* ssp. *chinensis* Sonn. is described as having at least 30 different commercial cultivars but an objective means to determine the relationship of these to each other needs to be developed (Galán Saúco and Menini, 1989). *Dimocarpus* contains six species with *Dimocarpus longan* divided into two subspecies and five varieties (Leenhouts, 1971, 1978) and again there are numerous cultivars. The differences between commercial litchi and longan cultivars cannot be used to separate the genera since petals in *D. fumatus* ssp. *fumatus* (Bl.) Leenh. are absent or severely reduced and trichomes are absent, or if present, not tufted in *D. gardneri* (Thw.) Leenh. The present study has investigated the breeding barriers between the commercial cultivars only. There may also be potential to hybridise other related genera. In the most recent revision of the Nephelieae the seven genera that it contains were divided into two groups depending upon whether they contain a sarcotesta or aril (Leenhouts, 1978). *Litchi*, *Dimocarpus* and *Cubilia* all have an aril along with *Otonophelium* which also has stipules. *Pometia* is also grouped with these genera but has a fleshy mesocarp and stipules. A more extensive investigation is needed to determine the potential germplasm available for breeding these corps.

Several authors have speculated that there is potential for

using other members of the Sapindaceae to improve commercial cultivars. Both litchi and longan have characters of economic value that are not found in the other. For example, litchi fruit are generally larger and more colourful than longan while longan is resistant under Australian conditions to erinose mite (*Aceria litchii* Keifer) which is a debilitating pest of litchi. The creation of intergeneric hybrids will enable the study of segregation of such characters to improve both longan and litchi.

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