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Incidence of Self- and Cross-pollination in Two Hevea brasiliensis *Clones*

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Two isozyme loci, Est-1 *and* Ugp-1, *were used to estimate the incidence of self- and cross-pollination in open-pollinated seeds of two* Hevea brasiliensis *clones (RRIM 513 and RRIM 600) planted in monoclonal blocks of RRIM 623 and PB 217 respectively. The proportion of outcrossed seeds were 59.3% for RRIM 513 and 62.1% for RRIM 600.*

The rubber tree, *Hevea brasiliensis,* has often been described as an outbreeding species. Although a few *Hevea* clones are highly self-fertile, hand-pollination studies showed that most clones had higher rates of fruit-set when crossed than when selfed[1]. Hand-pollination is not ideally suited for the assessment of outcrossing since the pollination event itself is disregarded and fruit-set success hinges only on the events occurring from the time pollen is deposited on the stigma. On the other hand, the effectiveness of open pollination has a major bearing on the eventual success of natural fruit-set. To date, very limited information is available on the outcrossing rates of this entomophilous crop from an open-pollination study. The only estimate we have on the proportion of selfs and crosses in the young seedling stage comes from the data on yellow recessive mutants of PB 5/51. Simmonds[2] estimated the outcrossing rate of this clone to be from 72% to 84%.

More precise knowledge of crossing/selfing in rubber could be derived from the use of genetic markers. In tree species, isozymes have been predominantly used to estimate breeding system parameters, for example Brotschol and Namkoong[3] have found that outcrossing rates in *Liriodendron tulipifera* (Magnoliaceae) averaged 0.55 in two coastal populations. Levels of heterozygosity or homozygosity of isozyme loci have sometimes been correlated with economically important traits such as the growth rate[4]. Such correlations are considered to be invaluable in forest tree breeding programmes where long generation times are common[5]. While knowledge of *Hevea* pollination behaviour would be useful to the breeder seeking to improve latex yield, the increasing acceptance of the rubber tree as a timber species has added greater significance to the acquisition of this information. The vigour of *Hevea* seedling trees (as compared with clonal buddings) makes them more suitable for incorporation in afforestation programmes. It is not improbable then that more *Hevea* seed gardens could in future be set up to meet the demand for quality rubber seeds. In the present study, isozymes were used to estimate the outcrossing rates in two *Hevea* trees planted in monoclonal plantings.

MATERIALS AND METHODS

Plant Materials

The study was based on the outcrossing rates of two trees planted in two commercial

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rubber estates. The trees from which seeds were collected were rogue trees growing in otherwise monoclonal blocks. The rogue tree growing in a planting of RRIM 623 was identified by clonal inspectors as RRIM 513. The other rogue tree was RRIM 600 planted in a field of PB 217. A check was also made by clonal inspectors to ensure that the experimental area (within at least 30 m radius from the identified rogue) was free from other rogue trees.

Mature fruit capsules were harvested from the trees just prior to dehiscence. Fruits of two seedings seasons (February and August 1993) were bulked for RRIM 513 while fruits from RRIM 600 were harvested during the main seeding season (August 1993). Synchronised flowering had been observed in the rogues and in the surrounding trees during the preceding flowering seasons in both locations.

Seeds were germinated in sowing beds in the nursery and later transferred into polybags. Newly expanded mature leaves of 3 month-old seedlings were excised for isozyme analyses.

Enzyme Extraction and Electrophoresis

About 0.5 g of leaf tissue (central vein discarded) were ground thoroughly in 1.5 ml of 0.07*M* Tris-HCl, pH 7.4, containing 10% w/v polyvinylpyrrolidone (PVP-40) in an ice-cold mortar. The homogenate was poured into microcentrifuge tubes and centrifuged for 30 min at 58 g in a pre-cooled Sigma 201 M microcentrifuge. The clear supernatant was then transferred into a new tube and stored at –70°C until use. The extracts were absorbed onto 3.5 mm × 10 mm wicks of Whatman 1M filter paper and loaded onto 12% starch gels. Isozymes were separated using horizontal starch gel electrophoresis using 2 different buffer systems. For esterase isozymes *(Est-1)*, the gel buffer consisted of 0.05% histidine adjusted to pH 6 with 1*M* Tris while the electrode buffer consisted of 0.15*M* Tris and 0.05*M* citric acid (pH 6.6) according to Lebrun and Chevallier[6]. When the gels were to be stained for uridine diphosphogluconate pyrrophosphatase *(Ugp-1)*, the electrode buffer employed was 0.04*M* citric acid adjusted to pH 6.1 with N-(3-amino-propyl) morpholine while the gel buffer was similar but diluted to one twentieth concentration according to Wickneswari and Norwati[7].

RESULTS AND DISCUSSION

Genotypes of Clones

Preliminary investigations revealed *Est-1* and *Ugp-1* to be two of the most polymorphic isozyme loci among five *Hevea* clones. Band resolution of *Est-1* and *Ugp-1* were excellent for characterising the genotypes of the open-pollinated progenies (*Figures 1a* and *1b*). *Est-1* was suitable for detecting outcrossed progenies in RRIM 513 planted in a monoclonal block of RRIM 623 whereas *Ugp-1* was a suitable marker for detecting outcrossed progenies in RRIM 600 planted in a monoclonal block of PB 217. The isozyme genotype classification of open-pollinated progenies in RRIM 513 and RRIM 600 are summarised in *Table 1*.

Inter-clonal Crosses

The rogue trees of the clones RRIM 513 and RRIM 600 were opportunistically used for the assessment of outcrossing rates in open-pollinated seeds of *Hevea*. The female flowers in each rogue tree could be self-pollinated by pollen from its own male flowers or cross-pollinated by pollen from male flowers of the surrounding trees. Two isozyme markers were used to determine the proportion of selfed and crossed progenies from the two rogue trees.

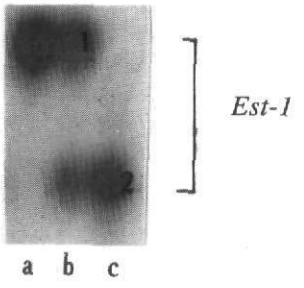

Figure 1A. Zymogram of Est-1 *locus showing the different isozyme patterns in progenies of the rogue RRIM 513. Self-pollination is indicated in* a *(Genotype 11), while cross-pollination is indicated in* b *(Genotype 12). (Genotype 22 of the male parent, RRIM 623, is depicted in* c*).*

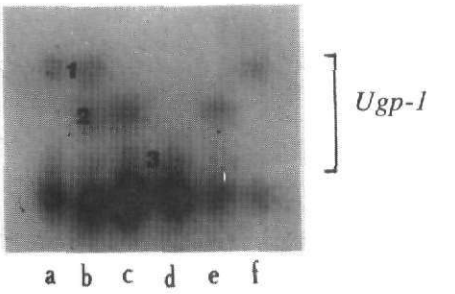

Figure 1B. Zymogram of Ugp-1 *locus showing the different isozyme patterns in progenies of the rogue RRIM 600. Self-pollination is indicated in* b *(Genotype 12),* e *(22) and* f *(11), while cross-pollination is indicated in* a *(Genotype 13) and* c *(23). (Genotype 33 of the male parent, PB 217, is depicted in* d*).*

Estimates of the proportion of selfs and crosses in both RRIM 513 and RRIM 600 *(Table 2)* showed moderate outcrossing in these clones, with 59.3% and 62.1% incidence of cross-pollination respectively. The outcrossing rates obtained in our study were slightly lower than that reported by Simmonds[2] who estimated 72%–84% outcrossing for PB 5/51 at young seedling stage of yellow recessive mutant following open-pollination. However after taking into consideration the possibility of severe zygotic elimination of selfs, he calculated the lowest rate of outcrossing at pollination could have been as low as 26%.

Hevea, in general, appears to exhibit inbreeding depression to a certain extent. The rather conservative figures obtained in our study also indicate in part the cross-compatibility of clone RRIM 513 with

TABLE 1. ISOZYME GENOTYPE CLASSIFICATION

Genotype Classification at *Est-1*			
Rogue RRIM 513	Monoclonal RRIM 623	Selfs in RRIM 513	Crosses in RRIM 513
11	22	11	12
Genotype Classification at *Ugp-1*			
Rogue RRIM 600	Monoclonal PB 217	Selfs in RRIM 600	Crosses in RRIM 600
12	33	11, 22, 12	13, 23

TABLE 2. ESTIMATION OF OUTCROSSING IN RRIM 513 AND RRIM 600

Clone	Seeds harvested	Seeds germinated	Plants derived from self-pollination	Plants derived from cross-pollination	Estimated outcrossing (%)
RRIM 513	73	59	24	35	59.3
RRIM 600	65	58	22	36	62.1

RRIM 623 and clone RRIM 600 with PB 217. In a study of hand-pollinated selfs and crosses carried out between 1937 and 1970 for various parental combinations, 3.1% fruit-set success was recorded for selfs and 4.5% for crosses[1]. The extent of self-compatibility in different clones and even the extent of cross-compatibility with pollen from the surrounding clones would influence viable selfs and crosses.

Various other factors can also influence the rates of outcrossing estimated in the present study. Temporal variation in interspecific crosses has been observed in *Acacia auriculiformis* and *A. mangium* with values ranging from 0.7% to 21.7% between different seed production periods[8]. Furthermore, as an entomophilous species, the availability and activity of pollinators could have an important bearing on *Hevea* fruit-set. Pollinator behaviour and stand density were also found to be related to outcrossing rates in *A. auriculiformis*[9].

Isozyme markers can aid in the evaluation of seed garden designs for adequate pollen dispersal and optimal fruit set. This study successfully used isozyme markers to detect self- and cross-pollination in Hevea clones. Since the present study was limited to the results from only two trees with two isozyme markers, a comprehensive study utilising a multilocus approach to give a more reliable estimate of intraspecific crossing rate in a commercial *Hevea* seed garden is being undertaken.

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