*Jl. Rubb. Res. Inst. Sri Lanka* (1986) 65, 1-13

# FLORAL BIOLOGY OF RUBBER AND RECOMMENDATIONS FOR INCREASES IN SEED YIELD FOR PLANT BREEDING

*by*

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ABSTRACT

*Research was conducted into the floral biology of rubber by fluorescence microscopy and analysis of the breeding records. The results of the research were developed into the following major recommendations for the improvement of the crossing methodology.*

1. *Change the time of controlled hand pollination from the morning to the afternoon.*
2. *Instruct the pollinators on the importance of handling the flowers gently.*
3. *Pollinate no more than four flowers per inflorescence.*
4. *Establish a breeding orchard where the trees are not tapped and the plant breeder has full control over the management of the trees.*
5. *Use clones with high female fertility as female parents.*
6. *Use trees over 10 years of age as female parents.*

## INTRODUCTION

The breeding of improved clones of rubber was commenced at the Rubber Research Institute of Sri Lanka (RRISL) in the 1930s. In order to obtain crosses between high yielding parents a method of controlled hand pollination was developed. This method was improved by the plant breeder Mr W. E. Manis in 1959 and is still used today for production of breeding progeny.

Rubber trees are monoecious with a male to female flower ratio of between 10:1 and 19:1 under Sri Lankan conditions (Attanayake *et al.*, 1987). The flowers are 5–10 mm in length with the female flowers larger than the males and situated

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terminally on the inflorescences (Heusser, 1919). Flowers of both sexes have five yellow perianth segments which are fused for two-thirds of their length. The female flower has a large pistil normally with three fused carpels each containing a single ovule and surmounted by a stigma lobe. Pistils with four or five carpels are also produced occasionally and have a corresponding number of ovules and stigma lobes. The male flower has a central staminal column with 10 anthers in two whorls of five. The flowers are scented and are insect pollinated (Ferwerda, 1969). They do not have nectaries but extrafloral nectaries occur on the petioles. The ripe fruit contains three seeds, and pods with less than the full complement of seeds are rarely carried to maturity.

A major problem encountered in the breeding of rubber is the low fruit set resulting from controlled hand pollinations. This low yield of seed of known parentage has limited progress in both rubber improvement and the understanding of rubber genetics. Nevertheless considerable improvements in latex yield have been achieved as a result of plant breeding and the Sri Lankan clones are amongst the highest yielding in the world. The problem is not confined to Sri Lanka but is reported also in Malaysia where the mean percentage fruit set was 3% for the main flowering season and 8% for the second season during the period 1969 to 1980 (Harihar & Yeang, 1984). In Sri Lanka there is only one flowering season which extends from the end of February to the beginning of April.

Previous work involving a comparison of the standard method of controlled hand pollination in the morning prior to anthesis, and hand pollination in the afternoon coinciding with anthesis showed that afternoon pollination may result in higher fruit set (Attanayake & Dharmaratna, 1984). There were also differences in pollen germination with greater viability in the afternoon than in the morning. Moreover, natural fruit set following open pollination in the afternoon (0.6%) was higher in clone RRIC 102 than the fruit set resulting from controlled hand pollination in the morning (0.1%) (Attanayake *et al.*, 1987). Insect visitors to the flowers included *Stomorina discolor* and *Drosophila sp.* The viability of the pollen may also influence hand pollination efficiency. *In vitro* pollen germination under Sri Lankan conditions results in less than 30% germination (Samaranayake *et al.*, 1979; Alagoda & Attanayake, 1984) in contrast to over 80% germination reported from Malaysia using the same pollen germination medium (Majumder, 1964). Recent work in Malaysia has suggested that the conventional hand pollination technique results in the deposition of insufficient pollen grains on the stigma (Harihar & Yeang, 1984).

Rubber trees show varying levels of self-incompatibility from complete sterility to apparently full fertility (Morris, 1929; Ferweda, 1969). The genetic base of cultivated rubber is very narrow and the major clones are often closely

related. It is possible that both self-sterility and inbreeding effects may be affecting seed yields. The aim of this work was to investigate the possible reasons for poor fruit set and to formulate improvements to the hand pollination technique.

## MATERIALS AND METHODS

In order to assess the magnitude of the problem the mean percentage initial fruit set at approximately one month following pollination and the mean percentage of mature fruit were calculated from the RRISL Annual Reviews and the breeding records.

A Reichert Microstar IV microscope with vertical fluorescence illuminator was purchased by FAO and UNDP for the work. The microscope was set up for pollen tube work and found to be satisfactory for routine observation. Experiments were conducted on pistils of *Hevea nitida* and *H. brasiliensis* to set up a suitable filter system for the fluorescence microscope and to develop the following technique for studying pollen tube growth in rubber pistils, following the methods of Martin (1959).

Fixation : Whole pistils were fixed in Carnoy's fixative (6 parts alcohol, 3 parts choloroform, one part acetic acid) for 24 hours.

Hydration : Whole pistils were hydrated via 70% alcohol, 30% alcohol and two changes of distilled water, each for 10 minutes.

Softening : Whole pistils were softened in 0.8 N sodium hydroxide for 20 minutes at 60 °C.

Staining : Whole pistils were stained for 18 hours in 0.1% aniline blue in 0.1 N tripotassium phosphate.

Mounting : Each pistil was mounted on one microscope slide. The stigma was removed by cutting just below the point of attachment to the ovary. The number of stigma lobes (corresponding to the number of carpels and ovules) was recorded. The stigma was placed face up on the microscope slide. The ovules were dissected from the softened ovary and placed on the slide. The ovary wall was removed from the slide. The stigma and ovules were lightly squashed in 80% glycerol under separate coverslips. The stigma generally required more squashing than the ovules.

Observation: The slides were observed using Reichert filter combination 1713 with 490 nm exciter filter, 510 nm dichroic mirror and 520 nm barrier filter. This technique permitted the clear obseravation of all pollen grains on the stigma, of

pollen tubes penetrating the stigma (Fig. 1) and of pollen tubes penetrating the ovules (Fig. 2). The ovules were dissected from the ovary as the outer surface of the rubber pistil is pubescent and this obscured the observation of the ovules within.

Pollinations were conducted on eight year old trees at the Eladuwa State Plantation and the Belmont Estate, Padukka. Both plantations are in commercial production and the breeding trees had been tapped up until the time of flowering. Tapping was discontinued during the flowering and fruiting period. The trees for pollination were watered three times a week with 4 gallons to the base of each tree. They were also sprayed with Benlate to control the *Oidium* fungus which attacks the flowers. The frequency of spraying varied between three times a week and once in every ten days depending on the weather conditions. Scaffolding was erected to allow access to the canopy of selected trees to be used as female parents. The parental clones were all high-yielding latex producers. One experienced pollinator conducted all the crosses.

Hand pollinations were conducted using the conventional method and pistils were harvested at one hour, three hours, one day and at two days following pollination. Flowers were also labelled but not pollinated to allow open pollination to occur. These were harvested at one day and at two days following labelling.

Pollinations were conducted using the conventional (morning) method and in addition flowers were bagged in the morning and pollinated in the afternoon of the same day at the time of normal anthesis. The flowers were sealed with cotton wool plugs as usual. Flowers were fixed at three hours after pollination.

Pistils were fixed, processed, and observed by fluorescence microscopy. Pollen grains, pollen tubes and ovules were counted in each pistil. For statistical analysis some of the pollen tube data was transformed to square roots. Results were compared by calculation of standard errors, by t-test analysis or by analysis of variance. Photographs were taken using an Olympus Vanox microscope.

It was observed that variable numbers of pollinations were conducted per inflorescence ranging from one to twelve. The relationship between the number of pollinations per inflorescence and the number of initial fruits set and mature fruits harvested was calculated from the experimental breeding records for 1985 and 1986. The diurnal temperature range in the canopy of the rubber trees was small although relative humidity (RH) varied between morning and afternoon (Table 1). Mean maximum and minimum temperatures and RH recorded at Dartonfield are also presented.

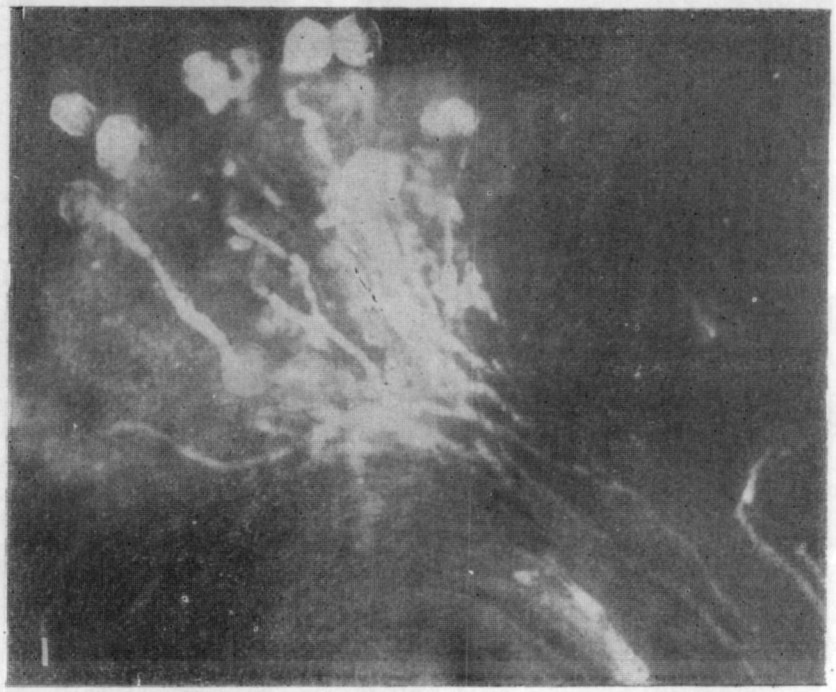

Fig. 1 Fluorescence micrograph of stigma of *Hevea brasiliensis* clone RRIC 102 pollinated with pollen from clone GTI showing pollen grains germinating on the stigma and pollen tubes growing toward the ovary.

Squash preparation stained with aniline blue x 125.

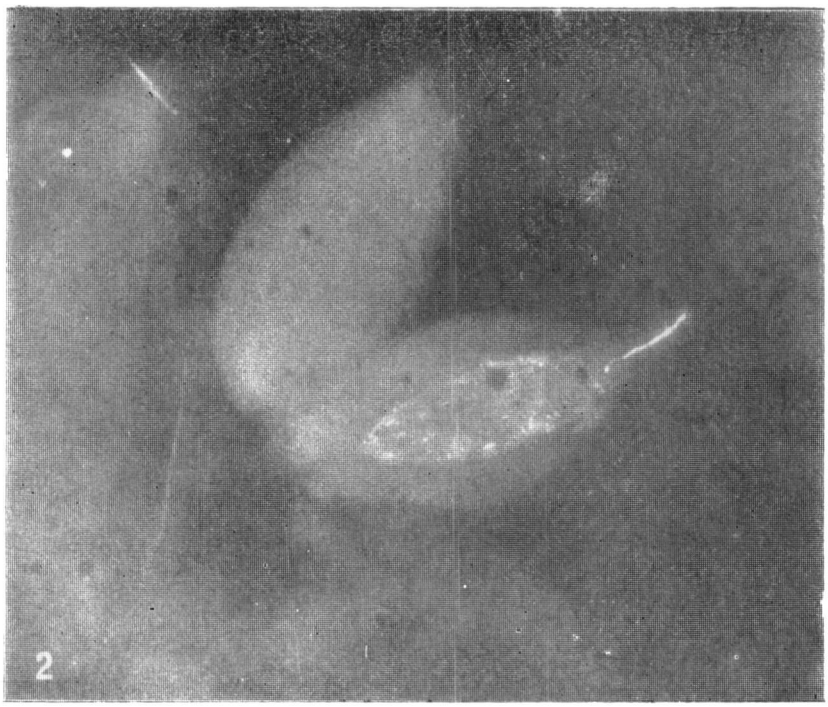


Fig. 2 Fluorescence micrograph of ovule of *Hevea brasiliensis* clone RRIC 102 pollinated with pollen from clone RRIC 101 showing ovule penetrated by a pollen tube. Squash preparation stained with aniline blue x 125.

Table 1. *Temperature and relative humidity recordings during the pollination experiments*

| Recording | Time (h) | Temperature (°C) | Relative humidity (%) |
|---|---|---|---|
| Diurnal readings in tree canopy : | | | |
| Belmont 23.3.87 | 10.00 | 32 | 55 |
| | 12.00 | 34 | 41 |
| | 15.00 | 33.5 | 53 |
| | 18.00 | 30.0 | 71 |
| Eladuwa 24.3.87 | 08.00 | 27 | 70 |
| | 12.00 | 32 | 58 |
| | 15.00 | 31 | 57 |
| 26.3.87 | 08.00 | 28.5 | 81 |
| | 12.00 | 32 | 60 |
| | 15.00 | 30.5 | 69 |

| | Max | Min | 08.30 h | 15.30 h |
|---|---|---|---|---|
| Mean data for pollination period 21.2 - 25.3.87 Dartonfield | 34.76 (33.0 - 36.8) | 20.12 (19.5 - 23.5) | 82.27 (67 - 90) | 58.18 (50 - 65) |

## RESULTS

### Survey of pollination success 1933-1986

The results are shown in Table 2 with data from the initiation of the breeding programme to the present. Success rates over the years are variable, and the percentage mature fruit is often much lower than the percentage initial set due to losses resulting from infection by *Phytophthora meadii*. The highest success rate was achieved during the 1950s with a mean of 6.8% initial set and over 4% mature fruit over the decade. There appears to have been a decline in success rate during the 1970s and 1980s, and the pollination programme was also hampered by rain at flowering during 1983 to 1986. Over the years the female clones used for breeding have changed as higher yielding clones were produced and incorporated in to the plant breeding programme. The origin and pedigree of the clones are shown in Tables 3 and 4 respectively (Fernando, 1966).

Table 2. *Fruit set data from controlled hand pollination of* Hevea brasiliensis *at RRISL from* 1933 – 1986

| Years | Number of years for which data is available | Major female* clones used for breeding | Mean percentage initial fruit set | Mean percentage mature fruit |
|---|---|---|---|---|
| 1933 – 1939 | 2 | Not listed | 4.79 | 0.89 |
| 1940 – 1949 | 4 | PB 86<br>TKD 113<br>PB 5/139 | 3.76 | 2.57 |
| 1950 – 1959 | 7 | LCB 870<br>RRIC 52<br>RRIC 51 | 6.81 | 4.08 |
| 1960 – 1969 | 7 | RRIC 52<br>IAN 45 - 710<br>RRIC 7 | 3.76 | 1.87 |
| 1970 – 1979 | 2 | RRIC 100<br>RRIC 101<br>RRIC 103 | 1.89 | 1.11 |
| 1980 – 1986 | 6 | RRIC 100<br>RRIC 101<br>RRIC 102 | 1.51 | 0.57 |

* See Table 3 for origin of clones

Table 3. *Origin of clones of* Hevea brasiliensis

| Code | Name | Country of origin |
|---|---|---|
| BR | Bogorredjo | Indonesia |
| FA | Ford Motor Company (later clones were labelled IAN following purchase by the Brazilian Government) | Brazil (parental clones from the Far East and Brazil) |
| IAN | Institute Agronomico do Norte | Brazil |
| GT | Gondang Tapen | Indonesia |
| LCB | 's Lands Caoutchouc Bedrijven | Indonesia |
| PB<br>PBIG | Prang Besar isolated garden | Malaysia |
| RRIC | Rubber Research Institute of Ceylon | Sri Lanka |
| TKD | Tijikadoe seed garden | Indonesia |

Table 4. *Pedigree of* Hevea brasiliensis *clones used in pollination experiments.*

| Clone | Pedigree | |
|---|---|---|
| | | .....female parent |
| | | .....male parent |
| GT 1 | Selected seedling | |
| RRIC 100 | .....RRIC 52 - clone of TKD 103 - selected seedling | |
| | .....PB 86 - selected seedling | |
| RRIC 101 | .....Ch 26 | .....BR 2 - selected seedling |
| | | .....BR 2 - selected seedling |
| | .....RRIC 7 - seedling from PBIG | |
| RRIC 102 | .....RRIC 52 - clone of TKD 103 - selected seedling | |
| | .....RRIC 7 - seedling from PBIG | |
| RRIC 110 | .....LCB 1320 - selected seedling | |
| | .....RRIC 7 - seedling from PBIG | |
| RRIC 121 | .....PB 28/59 - selected seedling | |
| | .....IAN 45 - 873 | .....PB 86 - selected seedling |
| | | .....FA 1717 - selected seedling |

## Timing of pollen tube growth

The pollen had started to germinate on the stigma by 3 hours after controlled hand pollination in the morning (Table 5). Pollen tubes had penetrated the ovules by 24 hours after pollination and there was no further increase in ovule penetration. Very few open pollinated flowers had pollen grains on the stigma, but there was a slight increase in pollen tube number 48 hours after labelling.

Table 5. *Timing of pollen tube growth in* Hevea brasiliensis *clones RRIC* 100 *and RRIC* 121.

| Time after pollination (hours) | Mean number of pollen grains on stigma | Mean number of pollen tubes in stigma | Mean number of pollen tubes per ovule | Percentage of pistils with all ovules penetrated by a pollen tube |
|---|---|---|---|---|
| Controlled hand pollination | | | | |
| 1 | 18.14 ± 7.14 | 0.00 | 0.00 | 0.00 |
| 3 | 14.52 ± 2.45 | 0.24 ± 0.10 | 0.00 | 0.00 |
| 24 | 31.60 ± 3.50 | 12.67 ± 1.26 | 0.68 ± 0.05 | 40.00 ± 7.3 |
| 48 | 20.16 ± 2.97 | 7·24 ± 0.91 | 0.61 ± 0.06 | 33.33 ± 7.0 |
| Open pollination | | | | |
| 24 | 0.53 ± 0.43 | 0.48 ± 0.43 | 0.03 ± 0.02 | 0.00 |
| 48 | 0.90 ± 0.47 | 0.58 ± 0.31 | 0.11 ± 0.05 | 7.50 ± 4.2 |

## Time of pollination

Controlled hand pollination in the afternoon, at the normal time of anthesis, resulted in a greater number of germinated pollen grains in stigmas than morning pollination (Table 6). There was little variation in temperature between morning and afternoon but relative humidity was more variable. Nevertheless it is considered unlikely that climatic conditions were responsible for the difference in pollen germination and tube growth. Maturity of the flower is the most likely explanation.

Table 6. *Effect of time of pollination on pollen tube growth in pistils of* Hevea brasiliensis *clone RRIC* 121.

| Time of pollination | Mean number of pollen grains on stigma | Mean number of pollen tubes in stigma |
|---|---|---|
| Morning | 14.52 | 0.24 |
| Afternoon | 13.68 | 3.08 |
| [a] Significance | NS | *** |

[a] NS : Not significant
*** : Probability = 0.01

Relationship between number of pollinations and number of fruits set per inflorescence

A survey of the 1985 and 1986 pollination data relating the number of pollinations performed per inflorescence to the number of intial fruits set showed that the highest percentage set resulted from between one and four pollinations per inflorescence (Table 7). There was no effect of pollination number per inflorescence on the percentage of mature fruit, but it is possible that the results were influenced by *Phytophthora* infection of the pods.

There did not appear to be any effect of the Benlate spray on the efficiency of pollinations or on pollen tube growth.

## DISCUSSION

The standard controlled hand pollination method was found to be reasonably efficient with ample numbers of pollen grains deposited on the stigma and up to 40% potential fruit set. The pollen tubes had reached the ovary by one day after pollination thus confirming the fruit set observarions of Majumder (1964) in Malaysia.

The research into the floral biology and breeding system of rubber has resulted in a number of recommendations for the improvement of the crossing methodology (Sedgley & Attanayake, 1988).

At present the controlled hand pollinations are conducted between 08.30 and 12.00 hours, Pollination between 12.00 and 16.00 hours, coinciding with the natural time of anthesis, gives higher pollen tube growth. The inflorescence should

Table 7. *Percentage fruit set and mature fruit per inflorescence calculated from* 1985 *and* 1986 *data for* Hevea brasiliensis *clones PB* 86, *RRIC* 101 *and RRIC* 104

| Number of pollinations per inflorescence | Number of inflorescences pollinated | Number of inflorescences with X fruit X = 1 | X = 2 | X = 3 | Total number of flowers | Total number of fruits | Percentage fruit |
|---|---|---|---|---|---|---|---|
| Initial set | | | | | | | |
| 1 – 4 | 481 | 51 | 9 | 2 | 1291 | 75 | 5.81 ± 0.65 |
| 5 – 8 | 171 | 16 | 7 | 2 | 967 | 36 | 3.72 ± 0.61 |
| 9 – 12 | 13 | 5 | 0 | 0 | 126 | 5 | 3.97 ± 1.74 |
| Mature fruit | | | | | | | |
| 1 – 4 | 481 | 18 | 1 | 0 | 1291 | 20 | 1.55 ± 0.34 |
| 5 – 8 | 171 | 7 | 3 | 1 | 967 | 16 | 1.65 ± 0.41 |
| 9 – 12 | 13 | 2 | 0 | 0 | 126 | 2 | 1.59 ± 1.11 |

be bagged just prior to anthesis to prevent contamination of both male and female flowers. The bags should be removed for pollination and the flowers then sealed using a cotton wool plug as in the current method.

Damage to the stigmas was observed following controlled hand pollination with differences between operators in the amount of damage inflicted (Sedgley & Attanayake, 1988). Damaged stigmas supported reduced pollen tube growth. The pollinators should be instructed to place the staminal column and the cotton wool plug with a light touch and not to press down on the stigma during the pollination process.

Inflorescences maturing more than two fruits are rarely observed. Analysis of fruit set data indicated that pollination of between one and four flowers per inflorescence was the optimum. At present up to 12 flowers are pollinated per inflorescence. This is a waste of labour as so many flowers could not mature fruit even if all were successfully fertilised.

At present the breeding trees are located on commercial plantations and are tapped until the time of flowering. Research with other tropical tree crops suggests that this will adversely affect the fruiting ability of the trees. Depletion of the reserves will result at a time when the tree needs to build up starch to support the fruit crop.

Fruit production is a large drain on the reserves of a tree. Work with a number of tree crops has shown that starch is stored in the trunk and branches. Starch reserves decrease during the period of fruit growth and development and increase between fruit harvest and fruit set following the next flowering season (Schdefield *et al.*, 1985). Some fruit crops, such as avocado, are irregular bearers and produce a heavy crop only in alternate years. High fruit crops follow years of high starch accumulation and low fruit yields follow years of low starch accumulation. Thus the period between fruit harvest and fruit set is imporatant for the replenishment of the starch reserves of the tree to support the subsequent fruit crop. The trees used for controlled hand pollination in Sri Lanka are all tapped up until the time of flowering. Tapping is a drain on the resources of the tree and it is likely that tapped trees are unable to store sufficient starch reserves to support a heavy fruit crop. Thus the management of the trees used for pollination may be an important factor in fruit set success.

Breeding and selection of rubber in Sri Lanka has resulted in large increases in latex production and RRIC 121, for example, can yield up to 4000 kg per hectare under experimental conditions. It is possible that selection for latex yield has been achieved at the expenses of other characters, including fruit production. There is

some evidence for this as high yielding clones grow vigorously during the early years but growth increment is reduced following the commencement of tapping. Lower yielding clones then catch up with the high yielders in terms of tree size and girth. This suggest that latex yield has been achieved at the expenses of growth rate (Jayasekera, personal communication, 1987). Recent breeding at the RRISL has largely involved trees of the high-yielding RRIC 100 series and this may be one of the reasons for the reduced fruit set success observed during the 1970s and 1980s.

Differences between clones in female fertility have been observed by fruit set and by fluorescence microscopy. In order to achieve maximum seed yields only highly fertile clones such as RRIC 101 should be used as female parent. Clones of low female fertility should be used as male parents.

The breeding trees are currently pollinated at the relatively young age of eight years. Trees of 10 years and older would be expected to give higher seed yields. They would also produce more flowers and thus facilitate the pollinators' task of selecting suitable flowers for pollination. This would improve the efficiency of the operation and also allow the pollination of larger number of flowers per desired cross. This is important for the understanding for the genetics of the crop.

The RRIC 100 series clones currently used for plant breeding are all related and future generations will be inbred to some extent. This may be a factor responsible for low seed yields and is also likely to result in reduced increase in latex yield from future breeding. New germplasm is needed to widen the genetic base. This approach has already been commenced at the RRISL by the importation of wild material from Brazil. This material should be evaluated and superior individuals incorporated into the breeding programme as soon as possible. High yielding clones from overseas breeding programmes should also be used as parents.

## RECOMMENDATONS

1. Controlled hand pollination method.
   - 1.1 Change the time of pollination from the morning to the afternoon.
   - 1.2 Instruct the pollinators on the importance of handling the flowers gently.
   - 1.3 Instruct the pollinators to pollinate no more than four flowers per inflorescence.

2. Management of plant breeding programme.
   - 2.1 Establish a breeding orchard where the plant breeder has full control over the management of the trees.
   - 2.2 Select the clones to be used as female parents on the basis of their fertility.
   - 2.3 Pollinate older trees.
   - 2.4 Widen the genetic base of the breeding material.

ACKNOWLEDGEMENTS

Facilities for the work were provided by FAO, UNDP and RRISL. Thanks to the members of the Genetics and Plant Breeding Department for assistance throughout the project. Thanks also to Mr L. W. Amaratunga of the Plant Pathology Department for help with the photomicroscopy and to Mr W. N. Wickremasinghe of the Biometry Department for the statistical analysis.

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