3. MATERIALS AND METHODS

3.1. F_1 SEED PRODUCTION

Two experiments were carried out to study the seed production of hybrid papaya. The first used female flowers for the production of seeds while the second used hermaphrodite flowers.

3.1.1. Seed production using female flowers

Six inbred purelines, viz. Sunrise Solo, Eksotika, L19, Paris, Subang 6 and Morib which have undergone at least five generations of selfing, were used in the experiment. Details of the genotypes are described in section 3.2.1. The inbred lines were planted at MARDI, Serdang on 28 August 1988, using Randomized Complete Block Design with four replicates and 20 trees/plot. Agronomic practices followed that described in section 3.2.4.

Four rounds of crossing were carried out, each stretching over a period of 3 months because of staggered flowering. The first round was done in May-June 1989 when the 9-month-old trees started fruiting. The second, third and fourth rounds of crossing were done when the trees were 12, 15 and 18 months old respectively. During each round, each inbred line was crossed with all others including selfing (i.e. 6 x 6 = 36 crosses). The number of times the inbreds were crossed in each round depended on the availability of flowers. In the first round, this was repeated 4 times, in the second, 5 times, in the third, 5 times and in the fourth, 4 times. The total number of crosses worked out to be 36 x 4 in the first round, 36 x 5, 36 x 5 and 36 x 4 for the second, third, and fourth rounds respectively giving a total of 648 crosses for the entire experiment. This worked out to 108 pollinations per parent.

3.1.2. Seed production using hermaphrodite flowers

The same six inbred lines were used in this second experiment. The seeds were sown at two locations viz. Serdang on 17 January 1991 and at Pontian on 2 May 1991. The soil in Serdang is clay loam while in Pontian it is predominantly peat. The seedlings were planted in a Randomized Complete Block Design with four blocks and 15 trees per plot. Agronomic practices followed that described in section 3.2.4. Controlled pollinations were carried out when the trees were six months old.

Four rounds of crossing were carried out. In every round, each of the six inbred lines acting as seed parent had a hermaphrodite and a female flower crossed in a diallel fashion with pollen obtained from the six inbreds. This was replicated three times at the two locations. In all, there were 1728 crosses which came from 2 locations x 6 seed parents x 6 pollen parents x 2 sexes x 3 replicates x 4 rounds.

3.1.3. Crossing procedure

The crosses were done from early morning till noon daily. Well-developed female and hermaphrodite flowers with the corolla still closed were chosen from each inbred for crossing. For hermaphrodite flowers, emasculation was done by forcing open the corolla tube and the 10 anthers were delicately removed with minimal injury using a pair of forceps. To effect pollination, a dehisced anther held with a pair of forceps was rubbed gently onto the stigma. The pair of forceps was dipped in ethyl alcohol after every pollination to prevent pollen contamination in the following cross. After the introduction of pollen, the flower was closed with a wax paper envelope secured with a staple and labelled accordingly.

3.1.4. Data on seed production and quality

The pollinated fruits were harvested at index 3-4 (about half yellow) and the seeds were extracted and sarcotesta removed. Counts of total seeds in the fruit, the number of seeds that float in water (an indication of under-developed embryos) and the number of pre-germinated seeds (indicated by a break in the testa, sometimes with emergence of the radicle) were made. done. The floaters and pre-germinated seeds will affect the quality and viability of seeds in storage. The time of pollination till maturation of the fruit was also recorded. The data processing was performed using the General Linear Model in SAS package.

3.2. PERFORMANCE OF F_1 HYBRIDS IN GxE STUDIES

3.2.1. Genotypes

Six inbred varieties viz. Sunrise Solo, Eksotika, Line 19, Subang, Morib and Paris and 15 of their hybrids obtained from a half diallel cross between the inbreds, were used in the trial. The seeds of the inbred parents were obtained from MARDI's papaya germplasm seed

repository while the hybrid seeds were derived from crosses that were made earlier in the studies on production of F_1 seeds (see section 3.1.). The names of the inbred varieties will be abbreviated as So, Ek, 19, Su, Mo and Pa respectively, for convenience in reference later. For example the inbred parent Sunrise Solo will be referred to as So x So while its hybrid with Paris will be abbreviated as So x Pa.

The six inbred parents are gynodioecious (having both hermaphrodite and female trees) and have been self-pollinated for at least five generations. They are regarded as purelines with homozygous genetic makeup. The general description of the varieties are as follows:

3.2.1.1. Sunrise Solo, Eksotika and Line 19

These three are described together because they have very similar genetic background and features. As noted earlier, Eksotika and Line 19 are sib lines derived from the backcross breeding programme with the Sunrise Solo as the recurrent parent. Both of them are Solo-like with the exception that they have better local adaptation, larger fruits and higher yields. All the three varieties are suited for local table fruit and export markets because of their high sugar content, attractive orange-red flesh, good flavour and petite size (*Plate 3.1*). Between the two sib lines Eksotika and Line 19, some minor differences exist. Line 19 is more resistant to fruit freckles than Eksotika (formerly Line 20) (Chan and Toh, 1988), but it has a very high incidence of fruit carpellody (Chan, 1992). Since these three varieties have very close genetic backgrounds, the crosses between them will be considered as sibs rather than hybrids.

3.2.1.2. Morib

Morib was selected from a single tree in 1972 in the west coastal town of Morib, about 60 km from Kuala Lumpur.

The striking features of this variety are its bright yellow colour of the immature fruits and the dwarf stature of the tree. A one-year old tree of Morib is usually about half the height of normal papayas. The medium-sized fruits ripen with an attractive crimson blush (*Plate 3.2*). The flesh is pinkish red, with poor texture and flavour and low sugars. The variety does not have commercial value except perhaps as an ornamental and for breeding to improve fruit cosmetics and low-bearing height (Chan, 1980).

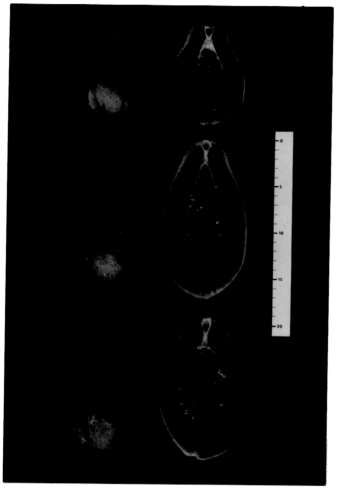

Plate 3.1 Sunrise Solo (top), Line 19 (middle) and Eksotika (bottom) fruits

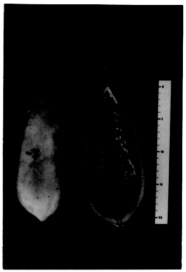

Plate 3.2 Morib fruit

Plate 3.3 Paris fruit

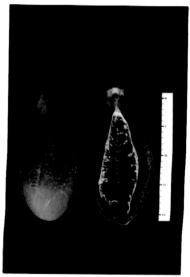

Plate 3.4 Subang fruit

3.2.1.3. Paris

Paris or Semangka Paris introduced from Indonesia in 1978, is a variety suited for papain extraction (Daryono and Muhidin, 1974). It is a very vigorous variety and produces large fruits with thick, red flesh. The fruit shape is an interesting even cylinder with the top and bottom of almost equal diameters (*Plate 3.3*). However, Paris did not appear to be suited for fresh fruit consumption because of its very low sugars and its rather insipid taste (Chan, 1980).

3.2.1.4. Subang

Subang was originally sourced in 1972 from a small papaya-growing township of Subang, about 20 km from Kuala Lumpur. It was the conventional variety for table fruit for many years prior to the advent of Eksotika. It is still popular at hawkers' stalls where it is usually served in long slices packed in plastic sleeves. The fruits are large and elongated with a narrower neck than base (*Plate 3.4*). The flesh is firm, attractive red and has low to medium sugar content. Subang is now recommended for processing but its yield, however, was unstable (Chan, 1985).

These six inbreds appear to represent a diverse genetic background ranging from the Solo types with small fruits of high eating qualities to the large-fruited Paris that appeared only suited for papain extraction. There is large phenotypic variation in plant stature, fruit skin colour, vegetative vigour and yield among the varieties. The crosses between the Solo, Eksotika and Line 19 will also give an opportunity to evaluate the performance of sibs against the inbreds on one end and the hybrids on the other.

3.2.2. Environments

Six environments representing diverse agro-ecological variation, were used for testing the performance of the 21 genotypes. All were MARDI research stations located at Serdang, Bukit Tangga, Kuala Kangsar, Kundang, Kluang and Pontian. The locations of these stations and the rainfall patterns during the period of experimentation are shown in *Figure 3.1*. The stations are mainly on the west coast of Peninsular Malaysia because the east coast is not recommended for papaya cultivation because of strong winds and floods during the year-end monsoon.

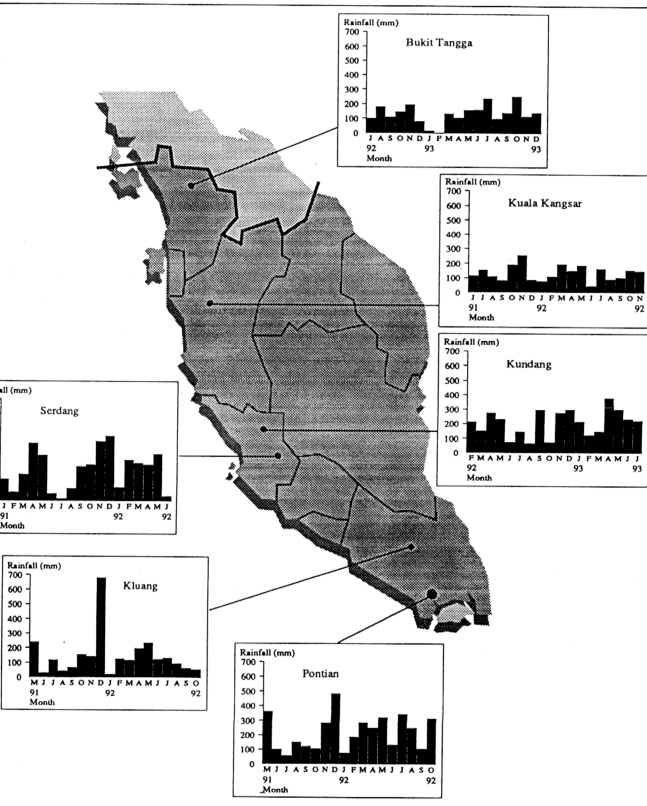

Figure 3.1 Locations and rainfall patterns of the six environments

Nieuwolt *et al.* (1982) established three basic agro-ecological zones, i.e. Zone 1 (3-4 months annual drought), Zone 2 (1 - 2 months annual drought) and Zone 3 (no distinct drought). The six locations covered a full representation of these three agro-ecological zones (*Table 3.1*).

Two of the environments were situated on soils which were considered marginal for crop cultivation.. Pontian is on peat soil, a partly decomposed, wood-debris organic medium with low pH and generally high water table. Kundang is on tin-tailings, a predominantly sandy soil left over after tin-mining operations. The tailings are poor in nutrients and low in cation exchange capacity. The other four are on normal mineral soils.

With regard to the cropping history of the experimental sites, three of them were previously planted with papaya while pasture, pineapple and secondary forest occupied the other three areas prior to the trial (*Table 3.1*).

The plantings at the various environments were carried out from 17 January 1991 and completed on 26 November 1991. However, due to waterlogging at Kundang and severe drought at Bukit Tangga which resulted in heavy casualties, the trials at these two locations were repeated on 15 February 1992 and 22 July 1992 respectively.

Table 3.1. Description of environments and planting dates

Environment	State	Soil type	Crop history	Agro-eco zone	Planting date*
Bukit Tangga	Kedah	mineral	forest	Zone 1	26 Nov 91 (22 Jul 92)
K. Kangsar	Perak	mineral	papaya	Zone 2	31 May 91
Serdang	Selangor	mineral	papaya	Zone 3	17 Jan 91
Kundang	Selangor	tin-tailings	papaya	Zone 3	11 Sept 91 (15 Feb 92)
Kluang	Johor	mineral	pasture	Zone 3	2 May 91
Pontian	Johor	peat	pineapple	Zone 3	2 May 91

* repeat planting dates in parentheses

3.2.3. Experimental design and layout

A randomised complete block design replicated three times and with 15 trees per plot was used for the trial at each location. The planting distance was 1.8 m between plants and 2.8 m between rows. The experimental area at each location was 0.47 ha.

3.2.4. Agronomic practices

The seeds were sown in 15 cm x 23 cm perforated polythene bags filled with 1:1:2 mixture of sand, organic matter [chicken dung or palm oil mill effluent (POME)] and top soil. The seedlings were ready for field planting after eight weeks in the nursery, or when they have formed 8 - 12 leaves.

The experimental areas at all the environments were limed with 2 t of ground magnesium limestone [equivalent to the recommended rate of 4 t/ha by Chan *et al.* (1991)]. However, at Pontian, the dosage was doubled because of the very low pH of the peat. The fertiliser rates followed the recommendation of Chan *et al.* (1991) which was 200 g N: 290 g P_2O_5 : 270 K_2O per plant for the first year and 250 g N: 250 g P_2O_5 : 290 K_2O per plant in the second year. This was achieved by adding 200 g triple super phosphate (TSP) in the planting hole and a commercial compound fertiliser of 15:15:15 formulation at a rate of 200 g per tree spread over the first three months. This was followed by 350 g per tree of a 12:12:17 formulation at every two-month interval until termination of the crop. In addition, palm oil mill effluent (POME) was supplemented at a rate of 3 kg/planting hole at every location with the exception of Pontian. At this location, a supplement of 15 g Fetrilon Combi (micronutrients) per tree was given every six months.

All the experimental areas except those at Pontian and Kluang, were irrigated with a drip system. Two drippers, each dispensing at a rate of 2 L/h were used for each tree. The system was switched on when a period of three continuous dry days occurred.

In pest and disease management, benomyl (0.05 % a.i.) was sprayed after the second month from planting to control *Cladosporium oxysporum* which causes malformed top disease (MTD). The fungicide was not used earlier because the trees were evaluated for tolerance to MTD. Control sprays of benomyl against MTD and methamidophos (0.1 % a.i.) for control of thrips were carried out based on severity of visual symptoms and incidence.

3.2.5. Data collection

Five of the 15 plants per plot were randomly tagged for data collection on the following characters:

Vegetative: (at six months after sowing)

1. *Trunk diameter*: Measured with a pair of Vernier calipers at a height of 15 cm from the ground.

2. *Plant height:* Measured with a calibrated 2 m pole as the distance of the shoot terminus from the ground level.

3. *Petiole length and lamina width*: Measured from the leaf that subtended the most recently anthesised flower.

Fruit:

1. *Fruit weight and TSS %*: Ten random fruits from each sample tree were analysed for fruit weight and TSS %. The latter was taken with a hand refractometer measuring Brix 0 - 25^0.

2. *Fruit number and carpellody %*: For estimating fruit number, immature fruits including newly set fruitlets were counted from each sample tree at the 7th month and the 13th month to obtain the fruit number that were expected to be harvested at the end of 12 months (harvest 1) and 18 months (harvest 2) respectively. This estimate was made possible because the youngest fruitlet takes usually five to six months to mature. Fruit counts at the immature stage were necessary and more accurate in yield evaluation because they removed errors due to pilferage which unfortunately was rather rampant in fruit trials. Carpellody % was estimated during fruit counts. The characteristic scars of carpellody can be easily seen even during the fruitlet stage.

Yield components and yield:

1. *Earliness and height of fruiting*

Earliness was measured by the number of days from sowing to the appearance of the first fertile anthesised flower. The height of the first fruit is the distance of the peduncle attachment on the trunk from the ground level.

2. *Yield*:

The yields of harvest 1 and harvest 2 were computed from the product of the mean fruit weight (from 10 sample fruits) and the number of fruits counted in harvest 1 and harvest 2 of the same sample tree.

Malformed top disease (MTD) resistance:

Ratings of malformed top incidence were carried out after two months from transplanting in the field. Ten random sample trees out of the fifteen in a plot were given disease ratings from 0 (no disease symptoms) to 10 (most severe) and the sum of ratings in each plot was the percentage of incidence of MTD for the plot.

3.2.6. Statistical analyses

Statistical computations were made with statistical packages in SAS using an IBM mainframe computer (model 4382-11).

3.2.6.1. Analysis of Variance

Analysis of variance was carried out using PROC ANOVA in SAS. The ANOVA was based on a random effects model and was carried out for analysis of the 14 characters. The random effects model is presented as:

$$y_{ij} = \mu + g_i + \epsilon_j + f_{ij} + e_{ij}$$

where y_{ij} = observed value of the ith genotype at the jth environment

μ = overall mean
g_i = effect of the ith genotype (i = 1, k)
ϵ_j = effect of the jth environment (j = 1, n)
f_{ij} = interaction term between the ith genotype and the jth environment
e_{ij} = error term

The analysis of variance was first carried out by environments and later a combined ANOVA was done over the six environments. The expected mean squares and appropriate tests of significance for the effects in both analyses are given in *Table 3.2*.

Table 3.2. Expected mean squares and tests of significance in ANOVA

ANOVA by environments

Source	df	MS	Expected mean squares	F test
Replicate	r-1	M1	$\sigma^2 + g\sigma^2_r$	M1/M3
Genotype	g-1	M2	$\sigma^2 + r\sigma^2_g$	M2/M3
Error	(r-1)(-1)	M3	σ^2	

ANOVA (combined)

Source	df	MS	Expected mean squares	F test
Environment	e-1	M1	$\sigma^2 + r\sigma^2_{ge} + g\sigma^2_{r(e)} + rg\sigma^2_e$	M1+M5/M2+M4
Rep(environ)	e(r-1)	M2	$\sigma^2 + g\sigma^2_{r(e)}$	M2/M5
Genotype	g-1	M3	$\sigma^2 + r\sigma^2_{ge} + re\sigma^2_g$	M3/M4
GxE	(g-1)(e-1)	M4	$\sigma^2 + r\sigma^2_{ge}$	M4/M5
Error	e(g-1)(r-1)	M5	σ^2	

σ^2 = error variance
σ^2_g = genotype variance
σ^2_e = environment variance
σ^2_{ge} = genotype x environment variance
$\sigma^2_{r(e)}$ = replicate within environment variance

3.2.6.2. Analysis of GxE and stability

All characters that showed significance in GxE interaction were subjected to further analysis of GxE and stability using the following methods:

(i) Francis and Kannenberg's (1978) mean and CV distribution:

Genotypic means for all genotypes were obtained from PROC MEAN in SAS package while the CV's were computed in the PROC ANOVA (by genotype). The two values were

plotted in a scatter diagram to evaluate the performance of genotypes on basis of overall mean and variance (stability).

Four quadrants were demarcated by the lines representing the genotypic mean and the CV mean. The quadrants were labelled in the following manner:

Quadrant I: area with above average mean and below average CV
Quadrant II: area with above average mean and above average CV
Quadrant III: area with below average mean and below average CV
Quadrant IV: area with below average mean and above average CV

It was obvious that the genotypes that fall in Quadrant I were most desired and those in Quadrant IV, the least. The definition of 'above average mean' did not necessary imply larger values, but rather it meant 'more preferred'. Thus for characters like earliness (days to flower) and height of fruit, smaller mean values were of higher preference. Therefore, the positions of the quadrants for these two characters will be shifted as compared with the normal circumstances where high mean values were desired.

(ii) Hühn's (1979) Non parametric ranking indices:

Two indices i.e. S_i^3 and S_i^6 were computed based on the variation in rank orders of the genotypes over environments. Their computations were as follows:

$$S_i^3 = \Sigma_j (r_{ij} - \bar{r}_{i.})^2 / \bar{r}_{i.}$$

$$S_i^6 = \Sigma_j | r_{ij} - \bar{r}_{i.} |^2 / \bar{r}_{i.}$$

r_{ij} = rank of ith genotype in jth environment
$\bar{r}_{i.}$ = mean of ranks over all environments for the ith genotype

Both indices were useful because S_i^3 was inclined towards selection for stability, while S_i^6 showed more biasness towards selection of desirable means (Leon, 1986; Kang and Pham, 1991). Large indices in genotypes would indicate wide fluctuations in rank orders of the genotype across environments implying therefore, lower stability.

(iii) Rank sum and rank product indices:

Two sets of ranks were required for computation of these indices proposed by Kang (1988) for rank sum and Schuster and Zschoche (1981) for rank product. The first set of ranks

was used to indicate the mean performance of the genotypes. In this case, the mean values of all the 21 genotypes for a particular character were ranked accordingly, the most desired given a rank of 1 and the worst, a rank of 21.

The second set of ranks was to indicate the variance of the genotypes (and their relative stability). Shukla's (1972) stability variance (σ_i^2) was used for this purpose and was computed from the following:

$$\sigma_i^2 = \frac{1}{(g-1)(e-1)(e-2)} \; e(e-1)\,\Sigma_j(\bar{Y}_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + \bar{Y}_{..})^2 - \Sigma_i\Sigma_j(\bar{Y}_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + \bar{Y}_{..})^2$$

where σ_i^2 = stability variance of the ith genotype
g = number of genotypes
e = number of environments
$\bar{Y}_{ij}$ = mean of ith genotype in the jth environment
$\bar{Y}_{i.}$ = mean of ith genotype across all environments
$\bar{Y}_{.j}$ = mean of jth environment across all genotypes
$\bar{Y}_{..}$ = overall mean

The within environment variance (σ_o^2) was used to test the significance of the stability variance. Large values of σ_i^2 will indicate more instability of the genotype.

The stability variances of the genotypes were again ranked, with the lowest (most stable) being accorded a rank of 1 and the most instable, a rank of 21.

The rank sum proposed by Kang (1988) was the sum of the rank of the mean with the rank of the stability variance for each genotype. The rank product proposed by Schuster and Zschoche (1981) was obtained by multiplying the two ranks together. They have used ecovalence (W_i^2) as the statistic for stability but in this analysis, the stability variance (σ_i^2) was used instead. According to Kang *et al.* (1987), both the statistics can be used as they ranked genotypes identically.

3.2.6.3. Simultaneous selection of mean and stability:

The three methods were considered together in awarding scores to determine the overall best performers. For the mean and CV distribution, the best quadrant (1) was awarded 0 points while quadrants 2, 3 and 4 were given 5, 10 and 15 points respectively. These scores were added to the ranks obtained for S_i^3 and S_i^6 and the rank sum and rank products. The

genotypes with the lowest scores were adjudged the best overall performer in terms of superior means and good stability.

3.2.6.4. Heterosis

Heterosis was measured by the percentage of the hybrid performance which was better or worse compared with its mid or better parent i.e.

$$H_{mp} = (F_1 - MP) / MP \times 100$$

$$H_{bp} = (F_1 - BP) / BP \times 100$$

where H_{mp} = heterosis estimate (%) over mid-parent
Hb_p = heterosis estimate (%) over better parent
F_1 = hybrid mean
MP = mid-parent mean
BP = better parent mean

Heterosis estimates over environments were averaged from the heterosis means of the 18 plots (6 environments x 3 replicates) while heterosis estimates at each environment were averaged from the three plot means (replicates).

A 't' test was carried out to test the significance of the heterosis estimates. In the case of mean heterosis over environments, the estimate was tested against the standard error (S.E.) derived from the 18 plot means over environments (df = 17). In the case of mean heterosis at each environment, they were tested against the S.E. estimated from the three plot means (df = 2).