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Production of F_1 hybrid seeds from a 6x6 diallel of papaya (*Carica papaya* L.)

[Pengeluaran biji benih F_1 hibrid daripada kacukan dialel 6x6 betik (*Carica papaya* L.)]

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Key words: *Carica papaya* L., papaya, hybrid, compatibility, seed, variety

Abstrak

Enam induk varieti inbred betik iaitu Sunrise Solo, Eksotika, L19, Paris, Subang 6 dan Morib dikacukkan secara dialel untuk menilai keserasian dan keupayaan pengeluaran biji benih F_1. Empat pusingan kacukan dilakukan apabila pokok berumur 9, 12, 15 dan 18 bulan. Keupayaan pengeluaran biji benih sesuatu varieti yang digunakan sebagai induk betina atau jantan diukur dari segi bilangan biji benih yang terbentuk di dalam buah, peratus biji benih yang tidak bernas yang termasuk biji benih yang bercambah dalam buah dan biji benih yang timbul dalam air (embrio yang kurang terbina).

Keputusan menunjukkan bahawa kesemua kombinasi antara enam induk ini serasi dan pengeluaran biji benih bergantung pada umur pokok. Buah daripada pokok yang lebih tua mengeluarkan lebih biji benih dan peratus biji benih pracambah yang lebih tinggi juga.

Keputusan daripada ANOVA menunjukkan bahawa varieti-varieti berbeza dari segi pengeluaran biji benih apabila digunakan sebagai induk betina atau jantan. Eksotika dan L19 banyak mengeluarkan biji benih (melebihi 1 000 biji/buah) manakala Paris mengeluarkan biji benih yang paling sedikit (690 biji). Perbezaan juga timbul apabila varieti digunakan sebagai induk jantan, khususnya dalam mempengaruhi kejadian biji benih pracambah di dalam buah. Induk jantan L19 tidak diingini kerana 27% biji benih yang terbentuk daripada pensenyawaan dengan debunga L19 bercambah di dalam buah.

Pada amnya, Eksotika, Sunrise Solo dan L19 menghasilkan biji benih yang paling banyak oleh sebab kadar kejadian buah yang tinggi dan buahnya mengandungi biji benih yang banyak. Hasil biji benih tiga varieti ini daripada 100 pendebungaan berjulat antara 75 795 dan 94 746 biji. Bagaimanapun, peratus biji benih bernas L19 lebih rendah kerana peratus biji benih pracambah yang tinggi. Dianggarkan bahawa kos pengeluaran biji benih hibrid dengan menggunakan induk yang lemah seperti Paris dan Morib dalam pengeluaran biji benih mungkin 2.5–3 kali ganda kos pengeluaran benih Eksotika yang sedang dijual pada harga RM3 000/kg.

Abstract

Six papaya inbreds namely Sunrise Solo, Eksotika, L19, Paris, Subang 6 and Morib were crossed in a diallel to evaluate their compatibility and production of

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F_1 seeds. Four rounds of crosses were carried out when trees were 9, 12, 15 and 18 months old. The seed production capacity of inbreds used as females or males was assessed by the number of seeds formed in the fruit, the percentage of non-viable seeds which included pre-germinated seeds and 'floaters' which had underdeveloped embryos.

The results showed that all combinations between the six inbreds were compatible and seed production appeared to be related to the age of the trees. Fruit from older trees bore more seeds but there was also a higher percentage of pre-germination in these seeds.

The ANOVA showed that inbreds differed in their production of seeds, both when used as female or male (pollen source). Eksotika and its sib line L19 produced abundant seeds (in excess of 1 000 seeds/fruit) while Paris was the least seedy (690 seeds). Differences also arose when inbred lines were used as pollen donors, particularly in influencing the occurrence of pre-germinated seeds in fruit. L19 was an undesirable male parent because 27% of the seeds developed as a result of fertilization by L19 pollen pre-germinated in the fruit.

Overall, Eksotika, Sunrise Solo and L19 were highest in gross seed yield because of high fruit set and large number of seeds per fruit. The seed yield per 100 pollinations from these three inbreds ranged from 75 795 to 94 746 seeds. However, the percentage of viable seeds of L19 was lower because of high percentage of pre-germinated seeds.

It was estimated that the cost of producing hybrid seeds using low seed-yielding inbreds like Paris and Morib may be 2.5–3 times that of Eksotika, whose hybrid seed is currently sold at RM3 000/kg.

Introduction

Papaya cultivars can be classified into two broad categories, viz. dioecious which consists of male and female trees and gynodioecious which consists of hermaphrodite and female trees. Because of separation of sexual parts in different trees, dioecious papaya cultivars are enforced out-crossers, and their genetic makeup is usually heterozygous. On the other hand, the gynodioecious cultivars are usually homozygous purelines, propagated through seeds that are produced from self-pollination of hermaphrodite flowers. The world renowned Solo papaya and the recently developed Eksotika are homozygous purelines bred through generations of inbreeding. Purelines have advantages to some extent in that there is genetic uniformity and because there is no inbreeding depression, the seeds can be reproduced by simple self-pollination of hermaphrodite flowers.

Recently, however, there has been interest in papaya breeding in exploiting heterosis or hybrid vigour. Chang and Wu (1974), and Subramanyam and Iyer (1984) demonstrated the existence of heterosis when papaya cultivars were crossed. The latter workers reported that there was significant heterosis for all vegetative characters as well as in yield and its components. Heterosis for yield up to 111% over the better parent was reported in certain combinations. More recently, Chan (1990) also reported heterosis in yield and its components from closely related sib crosses. There appears to be good potential for the use of F_1 hybrid varieties for papaya. This area of breeding is still underexploited as evident from the still predominant use of inbred lines and open-pollinated varieties, and the absence of any commercial F_1 hybrids for papaya in the world today.

For successful exploitation of F_1 hybrids, two requirements must be fulfilled.

The first is that there must be a technically feasible seed production scheme with favourable cost benefit ratio. For some crops, there must be a suitable genetic mechanism e.g. cytoplasmic male sterility or distinct separation of androecium and gynoecium e.g. dioecy that would allow for easy, controlled hybridization on a large scale. The economics and prospects for commercialization of F_1 hybrid seeds are very important. Bond (1989) had discussed these aspects for the production of hybrid field bean (*Vicia faba* L.). The second requirement is that the hybrid must be superior to the better parent or other contemporary varieties.

There are no genetic mechanisms for male sterility to aid hybridization in papaya but the production of seeds using hand pollination and emasculation may still be viable because of the abundance of seeds arising from each pollination. This paper examines the production of F_1 seeds through a complete diallel cross involving six inbreds of papaya. The compatibility of the crosses, seed set and quality of seeds obtained from the six inbred parents are discussed. This is the first step towards understanding and building a technically feasible F_1 seed production scheme for papaya.

Materials and methods

Six inbred pure lines, viz. Sunrise Solo, Eksotika, L19, Paris, Subang 6 and Morib which have undergone at least five generations of selfing, were used in the experiment. The inbred lines were planted at MARDI, Serdang in August 1988, using Randomized Complete Block Design with four replicates and 20 trees/plot.

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 round 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 round respectively giving a total of 648 crosses for the entire experiment. This worked out to 108 pollinations per parent.

The crosses were done from early morning till noon daily. Well-developed female flowers with the corolla still closed were chosen from each inbred for crossing. The corolla was carefully opened and a dehisced anther, held with a pair of forceps, was rubbed gently onto the stigma to effect pollination. After the introduction of pollen, the female flower was closed with a wax paper envelope secured with a staple and labelled accordingly.

The pollinated fruit were harvested at index 3–4 (about half yellow) and the seeds were extracted and sarcotesta removed, and a count of total seeds in the fruit, the number of seeds that float in water (an indication of underdeveloped embryos) and the number of pre-germinated seeds (indicated by a break in the testa, sometimes with emergence of the radicle) was 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 taken. The ANOVA was performed using the General Linear Model in SAS.

Results and discussion

The ANOVA for seed count per fruit, percentage of pre-germinated seeds and percentage of floating seeds is shown in *Table 1*. The most striking result is that effect of ‘round’ of pollination affected all the three characters while inbred parents used as females was significant for seed

Table 1. Analysis of variance for seed yield, pre-germinated seeds and floating seeds

Source	df	Mean square		
		No. seeds per fruit (x 10^5)	% pre-germinated seeds	% floating seeds (x 10^{-3})
Round	3	10.27**	0.3105**	8.1801**
Female	5	5.80**	0.2882**	0.6882ns
Male	5	0.58*	0.2018**	0.2189ns
F*M	25	0.25ns	0.0261ns	0.4421ns
Error	96	0.22	0.0252	0.3708
Total	134			

ns = not significant
* = significant at p >0.05
** = significant at p >0.01

number and percentage of germination. Surprisingly, the effect of inbred parents used as 'males' (or pollen source) was also significant for these two characters. This implies xenic effects in seed development of papaya. The interaction between female x male was not significant, suggesting that the combination of the best female with the best male would provide the best hybrid for the trait in question.

The total degree of freedom in the ANOVA was 134 which was 9 short of the expected 143 derived from 6 males x 6 females x 4 rounds = 144 'plots'. This was because some of the plots did not set fruit at all in spite of pollination of 4–5 flowers in each 'plot'. For unbalanced data sets, the ANOVA was performed using the General Linear Model.

Examining the 'female' effect first, the means of the inbred parents used as female in the seed production are shown in *Table 2*. Eksotika when used as the female parent for F_1 production appeared to be the most prolific seed producer, with an average of 1 137 seeds/fruit. Its sib, L19 which comes from the same breeding population, was also very seedy. Subang, Morib and Solo were rather similar (about 860–870 seeds) but Paris was the least seedy (690 seeds). In the percentage of pre-germinated seeds, L19 and Paris seemed to be the highest. Pre-germinated seeds show a break in the testa and sometimes the radicle has emerged. Such seeds when dried and stored, will not be viable. About 24% of the seeds of L19 and Paris germinated while still in the fruit. Because of this high rate of pre-germination, these two varieties produced a lower percentage of good or 'sound' seeds than the others. Subang and Eksotika, on the other hand, did not have this constraint in seed production.

Table 2. Hybrid seed production as influenced by female and male inbred parents

Inbred parent	Seed yield (No./fruit)	Pre-germinated seed (%)
Female		
Eksotika	1 137.0a	0.64d
L19	1 053.0a	24.74a
Subang	876.2b	0.83d
Morib	870.3b	14.05bc
Solo	861.7b	5.81cd
Paris	690.7c	23.46ab
Male		
Solo	975.8a	4.66c
Eksotika	961.3a	9.32bc
Paris	910.5ab	16.04b
L19	904.0ab	27.18a
Morib	897.8ab	11.50bc
Subang	845.3b	1.60c

Values within column with the same letter are not significantly different from one another at p = 0.01 according to DMRT.

The 'male' effects were significant for seed yield (number of seeds per fruit) and percentage of pre-germinated seeds, suggesting the occurrence of xenic effect of pollen source on seed production for these

two characters. Examining the mean of seed number arising from different pollen sources, it can be seen that Solo and Eksotika pollen increased seed development compared with Subang *(Table 2)*. The effects of pollen source were more evident in the case of their influence on percentage of pre-germinated seeds. Apparently, L19 pollen increased the development of seeds that have a tendency to germinate prematurely in fruit. Paris also showed the same tendency though at a lower percentage. Incidentally, these two varieties also developed high percentage of pre-germinated seeds when they were used as female parents in crosses.

From the results of this experiment, the quantity and quality of F_1 seeds clearly depended on the inbred parent used. Eksotika and L19 were more seedy and have the advantage as female parents for hybridization but the latter may have high proportion of poor quality seeds due to pre-germination. These results are useful in the choice of male or female parent for the crosses. As an example, assuming that there is no reciprocal difference in performance between Eksotika and Paris hybrids, the breeder would certainly use Eksotika as the female parent and Paris as the pollen donor. Not only would seed yield be almost doubled, but the amount of good seeds would also be high because of the negligible percentage of pre-germinated seeds in Eksotika.

The significance of crossing 'rounds' for all the variables is of interest. The four rounds were carried out at 3-monthly intervals when the trees were 9, 12, 15 and 18 months old. The variation in seed production characteristics may not, of course, be due to the age of trees alone, but other factors such as environmental effects including weather. However, looking at the means of the four rounds, there appeared to be an interesting trend. Such trends are unlikely to have resulted from environmental effects which tend to be random.

Seed development seemed to increase from round 1 to round 3, plateauing off at round 4 *(Table 3)*. The same trend seems to be true for occurrence of pre-germinated seeds where older trees seemed to have higher incidence. However, the trend in occurrence of floating seeds (poorly developed embryos) was in the opposite direction where seed development became better as trees got older.

When the time taken for fruit development was computed at the various tree ages, it was found that the fruit took longer to ripen when trees were older. There seemed to be a strong correlation between seed development, percentage of pre-germination and time of fruit development which seemed to be logical. When fruit remained on trees longer, the seeds may have passed their stage of physiologic maturity and inhibitors in the fruit or sarcostesta may have lost their effectiveness allowing seeds to germinate. On the other hand, the percentage of floating seeds may have decreased with age because a longer maturation period allows better development of the embryos.

With regard to hybrid seed yield from the six inbred lines, three factors need to be considered:

- the percentage of successful pollinations and fruit set,
- the number of seeds developed in the fruit, and
- the percentage of seeds that are sound and viable i.e. excluding floaters and pre-germinated seeds.

From the results shown in *Table 4*, the best fruit set came from Solo and its related lines, Eksotika and L19, where over 80% of the flowers successfully set fruit. Coupled with the high seed yield per fruit, these three lines topped in gross seed yield. On per 100 pollination basis, the seed yield ranged from 75 795 to 94 746 seeds. Discounting for poor seeds (floaters and pre-germination), their seed yields were still very high, but L19 showed considerable reduction due to

Table 3. Hybrid seed production and time for fruit maturity as influenced by age of trees

Age (months)	Seed yield (no./fruit)	Pre-germ. seeds (%)	Floating seeds (%)	Good seeds (%)	Harvest (days)
9	686.8c	4.27b	4.86a	90.85a	151.3c
12	853.8b	2.70b	2.39ab	94.86a	156.3b
15	1 083.6a	18.07a	1.35b	80.58b	159.1b
18	1 015.2a	21.63a	1.64b	76.72b	163.4a

Table 4. Seed yield and estimated seed costs of hybrids developed from 6 inbred parents

		Seed yield		Good seeds			
Inbred	Fruit set (%)	No./fruit	Per 100 pollinations	%	No.	Compare with Eksotika (%)	Cost (RM/kg)
Solo	87.96	861.7	75 795	91.22	69 140	75.82	3 957
Eksotika	83.33	1 137.0	94 746	96.25	91 193	100.00	3 000*
L19	83.33	1 053.0	87 746	72.37	63 502	69.63	4 308
Paris	63.88	690.7	44 121	74.30	32 782	35.95	8 345
Subang	52.78	876.2	46 246	97.65	45 159	49.52	6 058
Morib	42.59	870.3	37 066	83.77	31 050	34.05	8 811

*MARDI's price of Eksotika II hybrid

high percentage of pre-germinated seeds. The poorest hybrid seed yielder was Morib which had very poor fruit set (42.6%) as well as low seed yield per fruit. Paris had better fruit set, but low seed yield per fruit as well as high proportion of pre-germinated seeds pushed its seed yield down to the same level as Morib. Subang, despite quite poor fruit set, made up this weakness with high percentage of sound seeds.

Eksotika appears to be the best parent to use for hybrid seed production in so far as seed yield is concerned. In comparison, the others were far inferior, particularly Morib and Paris which produced only 34–35% that of Eksotika. The capacity of an inbred to produce hybrid seeds must be given due consideration because it definitely affects pricing of hybrid seeds. Eksotika hybrid seeds are now sold at RM3 000/kg at MARDI. Using this as the yard-stick and assuming that all costs remain the same for the other inbreds, the cost of hybrid seeds using Paris and Morib will be in excess of RM8 000/kg. Whether or not this price will justify the increase in yield or returns to investment using these two as inbred lines for hybrid production remains to be seen. The cost benefit ratio will be computed when the performance of the various hybrids in field trials, currently in progress, becomes known.

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