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Resistance of six papaya varieties and their hybrids to malformed top disease

(Kerintangan enam varieti betik dan hibridnya terhadap penyakit daun atas berkerekot)

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Key words: papaya, *Carica papaya*, *Cladosporium oxysporum*, *Thrips parvispinus*, disease resistance, variety, hybrid, GxE effects

Abstrak

Enam varieti inbred betik iaitu Sunrise Solo, Eksotika, Line 19, Morib, Paris, Subang dan 15 hibrid (resiprokal dicampur) diuji di enam lokasi bagi kerintangan terhadap penyakit daun atas berkerekot yang disebabkan oleh kompleks *Cladosporium oxysporum* dan kutu trips.

Hasil daripada ANOVA menunjukkan bahawa genotip, lokasi dan interaksi antaranya ketara. Perbezaan kejadian penyakit antara lokasi disebabkan oleh penanaman betik di lokasi tersebut. Lokasi yang pernah ditanam dengan betik menunjukkan kejadian penyakit yang lebih tinggi (33.8–65.7%) tetapi di lokasi yang belum ditanam dengan betik, kejadian penyakit rendah atau tidak ketara. Bagi kerintangan genotip, tiga kumpulan yang jelas diperoleh.

Kumpulan yang rintang mengandungi varieti Paris, Subang, Morib dan hibridnya manakala kumpulan yang rentan mengandungi varieti Eksotika, Sunrise Solo, L19 dan hibridnya. Kumpulan ketiga mengandungi genotip yang sederhana rintang iaitu hibrid-hibrid yang diwujudkan daripada kacukan antara varieti yang rintang dan varieti yang rentan. Regresi induk-anak (parent-offspring) mendapatkan keterwarisan yang tinggi (h^2 = 94.8%).

Kesan GxE dan kestabilan genotip dinilai secara 'non-parametric ranking'. Didapati bahawa GxE pada umumnya ditunjukkan oleh kumpulan yang mengandungi hibrid-hibrid yang diwujudkan daripada kacukan antara varieti yang rintang dan varieti yang rentan. Kebanyakan genotip yang rentan atau rintang menunjukkan prestasi atau kedudukan (ranking) yang konsisten di semua lokasi. Daripada kajian ini, langkah-langkah serta merta dan jangka panjang dicadangkan untuk membaiki varieti Eksotika dari segi kerintangan terhadap penyakit daun atas berkerekot.

Abstract

Six inbred varieties of papaya, i.e. Sunrise Solo, Eksotika, L19, Morib, Paris and Subang and 15 of their hybrids (reciprocals mixed), were tested over six environments and rated for their resistance to malformed top disease caused by *Cladosporium oxysporum* - thrip complex.

The combined ANOVA showed that environment, genotype and their interaction had significant differences. Environment differences in occurrence of

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the disease were related to the cropping history of papaya in the plots. Environments where papaya had been cultivated previously showed high incidence of the disease ranging from 33.8% to 65.7% while the disease was rather negligible in non-papaya cultivated areas. For genotypes, three distinct groups, viz. resistant, susceptible and moderately tolerant, were found in the expression of malformed top disease.

The resistant group consisted of Morib, Paris, Subang and their hybrids, the susceptible group consisted of Eksotika, Sunrise Solo, Line 19 and their hybrids while the moderately tolerant was made up of hybrids developed from crosses between resistant and susceptible parents. Parent-offspring regression showed a high heritability estimate (h^2 = 94.8%).

GxE effects and stability of genotypes in expression of the disease over environments were evaluated with a non-parametric ranking method. It was found that the major portion of the GxE was exhibited by moderately tolerant hybrids generated by crosses between the resistant and susceptible parents. On the other hand, most of the susceptible or resistant genotypes showed consistent performance in disease ranking through all the environments tested. From this study, some steps, immediate as well as long-term, to overcome the problem of susceptibility of Eksotika to malformed top disease were discussed.

Introduction

Bunchy and malformed top of papaya was first reported to be due to infestation by thrips (*Thrips parvispinus* Carney) (Vijaysegaran 1986), but later Lim (1989) found that the disorder was caused by an interaction of thrips and a normally saprophytic fungus, *Cladosporium oxysporum*. Thrips feeding on young developing leaves provided the entry sites for infection by Cladosporium fungus.

Infection usually begins on the very young leaves at the apex of the papaya shoot. When the leaves expand, they exhibit the typical symptoms of shot-holes, spotting, streaking and distortion of the lamina. In more severe cases, the top may be completely blighted with only stubs remaining at the end of the petioles. Yield is severely affected in infected plants.

Bunchy and malformed top of papaya was first observed after the Eksotika variety was released and grown on a commercial scale in 1985. The disease does not appear to be serious on other local cultivars. The Eksotika papaya is very popular as an export fruit but its cultivation is made difficult because of the variety's susceptibility to this disorder. Control using suitable fungicides and insecticides appear to be effective (Lim 1989) but added costs are incurred. Resistance breeding offers the long-term solution.

This study reports the relative resistance of six papaya varieties and their hybrids to malformed top disease. This is a prelude to the objective of transferring resistant genes from suitable donor parents to the Eksotika variety.

Materials and methods

Six varieties, i.e. Sunrise Solo (Sun), Eksotika (Eks), Line 19 (L19), Subang (Sub), Morib (Mor) and Paris (Par) and 15 hybrids (reciprocals mixed), were used in the experiment. The six parental varieties have been inbred for at least five generations and are regarded as purelines with homozygous makeup. The 21 genotypes were tested at six MARDI stations located at Serdang, Bukit Tangga, Pontian, Kuala Kangsar, Kluang and Kundang. The soil at Serdang, Bukit Tangga, Kuala Kangsar and Kluang was clay loam while Pontian was on peat and Kundang was on sand-tailings.

The plantings were staggered at the six stations beginning from 17 January 1991 in Serdang, 2 May 1991 in Pontian and Kluang, 31 May 1991 in Kuala Kangsar, 11 September 1991 in Kundang and 26 November 1991 in Bukit Tangga.

At each location, the 21 genotypes were planted in a Randomized Complete Block Design in three replicates and with 10 plants per plot.

The planting distance was 1.8 m between plants and 2.8 m between rows. No fungicide spray was applied for the first 2 months of growth. Irrigation was provided at Serdang, Kuala Kangsar and Bukit Tangga.

Rating of malformed top incidence was carried out after 2 months from transplanting in the field. Each of the 10 plants in a plot was given a rating of 0–10 depending on the severity of the disease and the sum of the rating of each plot is the percentage of disease incidence for the genotype in that plot.

The data were processed with an IBM 4382–11 using SAS. The ANOVA was based on a mixed model with genotype as fixed effect and environment, random. The variance components and the error terms for testing of significance of the various effects are shown in *Table 1*.

The analysis of GxE and stability of genotypes in expression of the disease was done with a non-parametric method proposed by Huhn (1979). The two non-parametric statistics S_i^3 and S_i^6 were computed based on ranks of genotypes in each environment expressed as follows:

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

$$S_i^6 = \sum_j \frac{|r_{ij} - \bar{r}_{i.}|}{\bar{r}_{i.}}$$

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

Results and discussion

The ANOVA showed that environment, genotype and their interaction were highly significant in influencing the occurrence of malformed top disease *(Table 1)*.

The disease score *(Table 2)* was highest in Kundang (65.7%) and relatively high in Kuala Kangsar (35.2%) and Serdang (33.8%). The cropping history of the plots on which the experiments were carried out showed that all these three locations had been cropped with papaya for at least 3 years previously *(Table 2)*. The incidence was, however, rather low or negligible in locations where there had been no previous cultivation with papaya. This shows that the disease inoculum or the vector (thrip) that predisposes the plants to the disease builds up markedly after one or two crops of papaya.

Analysis of GxE

Significant GxE interaction effects indicate that various genotypes showed differential response in disease expression according to the environments tested. Separate ANOVA by locations were done and the results presented in *Table 3*. It can be seen that genotypes indeed behaved differently over the six locations, depending on the disease severity of the environment. At all the locations with the exception of Bukit Tangga, genotypes showed significant difference in malformed top resistance. At Bukit Tangga, however, because of the low disease inoculum level or other factors that did not favour disease occurrence in that environment, all genotypes showed negligible infection, leading to non-significance in genotypic effect at Bukit Tangga.

The nature of the GxE interaction was further examined using two non-parametric statistics proposed by Huhn (1979). The mean values and mean disease ranking for the 21 genotypes and the ranking of the genotypes over five locations are given in *Table 4*. The ranking of the sixth environment, i.e. Bukit Tangga was left out

Table 1. Combined ANOVA for malformed top disease in papaya

Source	df	Variance components	MS
Environment	5	$\sigma^2 + g\sigma^2_{r(e)} + rg\sigma^2_e$	35 962**
Rep (Env)	12	$\sigma^2 + g\sigma^2_{r(e)}$	356
Genotype	20	$\sigma^2 + g\sigma^2_{ge} + re(v)$	5 188**
G x Env	100	$\sigma^2 + g\sigma^2_{ge}$	609**
G x Rep (Env)	240	σ^2	123
Total	377		

Table 2. Malformed top disease incidence at six environments related to their cropping histories

Environment	Disease incidence	Years previously cropped with papaya
Kundang	65.7a*	3
Kuala Kangsar	35.2b	5
Serdang	33.8b	20
Kluang	12.7c	0
Pontian	7.9c	0
Bukit Tangga	0.9d	0

* Mean values with the same letter are not significantly different at $p = 0.01$ according to DMRT

Table 3. ANOVA by environments for malformed top of papaya

Source	df	MS					
		Kundang	Kuala Kangsar	Serdang	Kluang	Pontian	Bukit Tangga
Rep	2	128.5ns	176.2ns	361.5ns	1 306.3**	144.4ns	19.0ns
Gtype	20	1 577.1**	2 965.2**	2 197.6**	1 125.4**	354.9**	17.1ns
Error	40	78.6	282.2	125.2	128.0	106.1	19.0
Total	62						

in this analysis because genotypes did not show significance in that environment *(Table 3)*.

From the mean values of the 21 genotypes *(Table 4)*, it was quite evident that there were three groups of genotypes that showed differences in resistance to malformed top disease. Eksotika, Line 19 and Sunrise Solo were very susceptible to the disease. The hybrids between these genotypes were also very susceptible. That the three genotypes behaved in the same manner may be expected because they, in fact, have very similar genetic backgrounds. Eksotika and Line 19 were varieties developed from a backcross breeding programme which used Sunrise Solo as the recurrent parent (Chan 1987). On the other hand, the local cultivars like Morib and Subang, and the Indonesian variety Paris have very good resistance *(Table 4)*. Hybrids between the resistant cultivars were very resistant too. The third group of genotypes was the moderately tolerant. This comprised hybrids between the susceptible cultivars and the resistant cultivars.

In the non-parametric analysis of GxE for stability, the statistics S_i^3 and S_i^6 are presented in *Table 4*. Low values for a genotype would indicate that the genotype

Table 4. Ranking and stability of genotypes for malformed top disease incidence over five environments

Genotype*	Mean	Mean rank	Rank at environments**					S_i^3	S_i^6
			Kun	KK	Ser	Klu	Pon		
Eks x Eks	64.4	1	1	1	1	1	1	0.00	0.00
Eks x L19	52.8	2	2	3	4	2	2	2.50	1.50
Eks x Sun	50.0	3	3	4	2	3	4	1.00	1.00
Sun x Sun	47.8	4	6	2	6	4	3	3.25	1.75
L19 x L19	40.6	5	4	6	5	6	5	0.60	0.60
L19 x Sun	37.8	6	5	9	3	5	9	4.83	1.83
Eks x Par	30.0	7	8	5	8	11	14	10.14	2.14
Sub x Sun	25.6	8	13	10	9	9	8	3.87	1.13
Sun x Par	25.0	9	9	11	7	12	15	5.89	1.44
Mor x Sun	25.0	10	15	7	10	10	10	3.40	0.80
Eks x Sub	23.3	11	7	12	11	13	7	3.36	1.00
Eks x Mor	22.8	12	10	15	12	7	6	6.16	1.33
L19 x Par	18.3	13	11	21	13	8	16	7.84	1.38
Mor x L19	18.3	14	12	13	14	14	11	1.00	0.42
L19 x Sub	14.4	15	14	17	15	15	12	0.93	0.40
Mor x Mor	13.9	16	19	8	16	16	13	5.12	0.87
Sub x Sub	10.5	17	16	14	18	17	17	0.65	0.29
Sub x Par	7.8	18	17	18	17	18	18	0.11	0.11
Par x Par	6.7	19	18	19	19	19	19	0.05	0.05
Mor x Sub	6.7	20	20	16	20	20	20	0.80	0.20
Mor x Par	5.6	21	21	20	21	21	21	0.05	0.05

*Eks = Eksotika
L19 = Line 19
Sun = Sunrise Solo
Par = Paris
Mor = Morib
Sub = Subang

**Kun = Kundang
KK = Kuala Kangsar
Ser = Serdang
Klu = Kluang
Pon = Pontian
Bukit Tangga was excluded because there were no differences between genotypes

varied little in the order of ranking for disease incidence throughout the environments. Regarding the susceptible group which consisted of six genotypes, i.e. Sunrise Solo, Eksotika, Line 19 and their three hybrids, they consistently showed high susceptibility, frequently occupying the top six ranks at all the environments. There was some slight re-ordering of ranks depending on environments, but for Eksotika, the 0 value for S_i^3 and S_i^6 indicated that it was consistently the most susceptible variety in all environments.

For the resistant group of genotypes consisting of Paris, Subang, Morib and their three hybrids, they more or less retained the six most resistant ranks throughout the five environments. Their resistance was consistent throughout the test environments as evidenced by the low values of their stability estimates. A notable exception was perhaps Morib which showed slight susceptibility at Kuala Kangsar and Pontian, and this caused its stability parameters to be high compared with the other genotypes in the resistant group.

The area where GxE was mostly detected perhaps came from the moderately resistant group comprising genotypes formed from hybridization between the resistant and susceptible parents. The stability statistics ranged from $S_i^3 = 0.93$ and $S_i^6 = 0.40$ for L19 x Sub hybrid to $S_i^3 = 10.14$ and $S_i^6 = 2.14$ for Eks x Par hybrid *(Table 4)*. Many other genotypes in this group showed high S_i^3 and S_i^6 values,

indicating considerable re-ordering of rankings and large deviations from their expected (mean) ranking ($\bar{r}_{i.}$).

Parent-offspring regression

The heritability estimate (h^2) for malformed top resistance was estimated by the parent-offspring regression. It showed a relationship of $y = 0.948x - 4.873$ *(Figure 1)*. The high heritability (94.8%) indicated that malformed top resistance was controlled by few additive genes and that offspring performance can be accurately predicted from the performance of its two parents. However, a fairly large negative value of the intercept (–4.873) suggested that offsprings (hybrids) were usually lower in disease expression than the predicted mean of the two inbred parents. This was probably due to existence of hybrid vigour which may have subdued the expression of the disease in hybrids.

The scatter diagram of the parent-offspring regression *(Figure 1)* also showed the three distinct groups of genotypes in expression of the disease. The resistant group consisted of three resistant inbreds Paris, Subang and Morib and three of their hybrids while the susceptible group consisted of the three susceptible inbreds i.e. Eksotika, Sunrise Solo, Line 19 and their three hybrids. The moderately tolerant group was made up of nine hybrids from crosses between the three resistant and three susceptible inbreds. The expression of the disease by the susceptible (Sunrise Solo) and the moderately tolerant hybrid (L19 x Par) is shown in *Plate 1*. These distinct groupings of genotypes underlined the fact that malformed top resistance is highly heritable and that improvements to susceptible cultivars like Eksotika can be made easily.

Breeding for malformed top resistance

Breeding for malformed top resistance is urgently required for the Eksotika cultivar because the fruit is popular for export with an annual export value of about RM20 million. Its high susceptibility to malformed top disease had caused great concern and high added costs in its management.

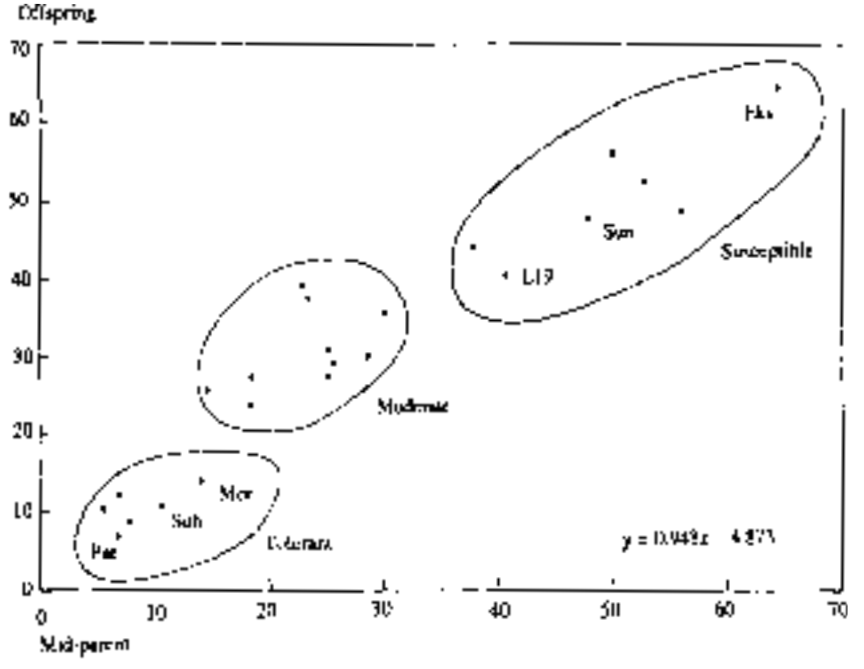

Figure 1. Parent-offspring regression for Cladosporium resistance

Plate 1. Comparison of susceptible Sunrise Solo with a tolerant hybrid

For immediate solutions, it is recommended that Eksotika be planted in areas which have no previous history of papaya cultivation because the present study indicated that disease occurrence will be low to negligible in such areas. In areas where papaya has been grown before, growing Eksotika will incur higher expenditure in disease management. In such areas, some of the tolerant hybrids may be more suited. A very promising one is L19 x Sub hybrid which showed very good tolerance and more importantly, its tolerance was consistent over all environments as indicated by its low S_i^3 (0.93) and S_i^6 (0.40) values.

As a long-term measure, tolerant hybrids which have good fruit qualities may be selfed to generate a segregating F_2 population. Promising progenies from this population which have the qualities of the Eksotika variety and resistance to malformed top may be selected and further inbred for several generations to obtain pure lines. These may be recommended for cultivation or they may be crossed with each other to provide F_1 hybrids with better vigour. Such varieties are expected to be similar to Eksotika in most respects but will have resistance to malformed top disease.

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