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Field performance of selected papaya hybrids for tolerance to dieback disease

(Prestasi ladang bagi hibrid betik terpilih untuk kerintangan terhadap penyakit mati rosot)

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Abstract

Papaya (*Carica papaya*) is one of the most important export fruits in Malaysia, producing 78,000 metric tonnes in 2003. However, the two cultivars commonly grown in Malaysia, namely, Sekaki and Eksotika, are very susceptible to papaya dieback disease, resulting in a reduced production to approximately 31,000 metric tonnes in 2013. Breeding for resistance varieties and hybrids is the best long-term sustainable measure to overcome this problem. MARDI initiated a breeding programme for developing papayas with resistance to dieback disease. Ten papaya F_1 hybrid seeds were developed via crossing within six elite parents, which were found resistant to papaya dieback disease or had high qualities. These hybrids were tested over 'hot-spot' disease locations at MARDI Serdang, Selangor, to evaluate their field tolerance, fruit characteristics and plant performance. Hybrid I (Viorica x P15), Hybrid C (P15 x Dwarica), Hybrid J (Viorica x Dwarica) and Hybrid D (Viorica x Eksotika) appeared to be the most well-balanced hybrids with the best disease tolerance character, high yield and total soluble solids (TSS) and optimum plant height. Hybrid I was found to be the most promising hybrid in terms of high tolerance level (1.6 out of 5.0), high yield (39.9 kg/plant), high TSS (12.1%) and moderate plant height.

Keywords: *Carica papaya*, F_1 hybrid, resistance varieties, papaya dieback disease, papaya varieties

Introduction

Papaya (*Carica papaya* L.) was once called 'the fruit of the angels' by Christopher Columbus due to its sweet delicious musky undertone taste. Like other types of tropical fruits, papaya also contains high vitamins A and C, minerals, flavonoids and antioxidants (Ayoola and Adeyeye 2010). The production of papaya is particularly attractive for fruit crop diversification in Malaysia due to its yield potential, high demand in local market and potential for export. However, the Malaysian papaya industry was threatened by a lethal disease known as papaya dieback disease, which led to a decline in local papaya production. FAOSTAT (2017) reported that papaya fruit production in Malaysia was reduced nearly 60% while the export value declined up to 70% from 2003 to 2011 (Rogayah et al. 2018). Although the production of papaya fruit was increased from 32,000 tonnes in 2013 to 84,000

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metric tonnes in 2017 (FAOSTAT 2017), yet the disease still persisted and attacked the papaya plantation, in Malaysia. Two cultivars commonly grown in Malaysia, Sekaki and Eksotika, are very susceptible to this disease (Suhaina et al. 2017; Rogayah et al. 2018).

Papaya dieback disease (PDD) is currently known as the most threatening disease of papaya in Malaysia caused by *Erwinia mallotivora* bacteria (Noriha et al. 2011). This disease is believed to be spread by wind and the entry into host is usually through injury points. Known also by other names like bacterial crown rot and bacterial canker, this disease has been reported mostly in West Indies, Virgin Islands to Venezuela, Java, Taiwan and the Mariana Islands (Ollitrault et al. 2005). In Malaysia, this disease was first detected in Johor in 2003 (Noriha et al. 2011), which then spread three years later to five other states, leaving a trail of destruction of 800 ha and a loss of USD58 million (Maktar et al. 2008). Early symptoms include water-soaked spots on petioles and stems, followed by yellowing and few black spots on leaves and fruits. Then, wilting of the leaves, petiole and stem occurred with brown lesions on fruits and cankers on stems. In more severe stages, the brown lesions start spreading to the crown, leading to dieback as well as death of trees. These symptoms have also been explained by Nishijima (1994) and Noriha et al. (2011).

Today, farmers are very reluctant to invest in papaya plantations due to the incidences of dieback disease. Many approaches have been employed to combat the disease but with limited success. Chemical control has been observed inefficient (Frossard et al. 1985), whereas genetic control was seen more promising as some local populations showed high level of resistance/tolerance, particularly in Guadeloupe, Venezuela, Granada and Trinidad (Webb 1983; de Lapeyre and Lyannaz 1992). Viorica is a papaya variety developed by Malaysian Agricultural Research and Development Institute (MARDI) in 2012 that was highly tolerant to papaya dieback disease (Johari and Noraisah 2018). However, Viorica has a low eating quality that does not reach consumers and market taste. Nevertheless, this variety was suitable to be used as a parent in breeding activities towards developing new superior papaya hybrids (Johari and Noraisah 2018). Breeding for resistance is the best approach for creating novel and superior resistant varieties to solve the disease problem and sustain the papaya industry. The Malaysian Agricultural Research and Development Institute initiated a breeding programme for developing papayas with resistance to dieback disease. The objective of this study was to develop new superior papaya hybrids tolerant to papaya dieback disease with high yield and quality through hybridisation between resistant and excellent quality parents.

Materials and methods

Source of planting material

Ten selected F_1 hybrids were developed via crossing within six elite parents (Viorica, Eksotika, Dwarica, Niensee, P15 and M01), which were found resistant to PDD or had high qualities. Dwarica also known as Puerto Rico 217 (PR217) variety and P15 are varieties from the Solo papaya group. The F_1 hybrids cross combinations are listed in *Table 1*.

Table 1. Cross combinations of selected papaya F_1 hybrids

Genotype	Cross combinations
Hybrid A	Eksotika x P15
Hybrid B	P15 x Niensee
Hybrid C	P15 x Dwarica
Hybrid D	Viorica x Eksotika
Hybrid E	P15 x M01
Hybrid F	Viorica x M01
Hybrid G	Eksotika x Niensee
Hybrid H	Niensee x Viorica
Hybrid I	Viorica x P15
Hybrid J	Viorica x Dwarica

Commercial papaya varieties such as Eksotika, Sekaki and Line 19 were used as check varieties. The 2-month-old F_1 hybrid seedlings were planted in the field according to the Randomised Complete Block Design (RCBD) with three replicates, each containing 10 plants per treatment. The standard agronomic practices as well as pest and disease management recommended by MARDI were followed completely (Chan et al. 1994).

Data collection and statistical analysis

Evaluation of field disease incidence was conducted at the papaya dieback disease hot-spot areas at MARDI Serdang. Disease development and data on fruit numbers of each plant in the field was scored and recorded on 9-month-old plants. Fruit count included the young fruits formed just after anthesis. Ten mature fruits at maturity index 2 from each plant were harvested to record the mean fresh fruit weight and total soluble solids (TSS). The TSS was recorded with a hand refractometer (0 – 25% Brix). The yield was calculated from the product of mean fruit number and the mean fruit weight. The height to first fruit was recorded by measuring the distance of the first fruit produced on a plant from the ground. The height to the first fruit was measured immediately before the first harvest. Statistical analysis was determined by Analysis of Variance (ANOVA) using Statistic Analysis Software 9.4 program (SAS 9.4). Analysis of treatment means was carried out using Duncan's Multiple Range Test (DMRT).

Field disease occurrence assessment

Number of healthy and symptomatic was recorded from each plant sample. Disease incidence (%) was valued based on symptoms recorded from each plant sample. The disease incidence (DI) was calculated by using Cooke's (2006) formula as shown below.

$$\text{DI (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \times 100$$

The incidence score from each plant sample was assessed referring to score description (Lasin et al. 2015; Bakar et al. 2018) from *Table 2*. Disease severity (DS) percentage was computed by applying the equation proposed by Horsfall and Barrat (1945) as shown below.

$$\text{DS (\%)} = \frac{\sum(a \times b)}{(N)(Z)} \times 100$$

$\sum(a \times b)$ = Sum of the symptomatic plant and their corresponding score
N = Total number of sampled plants
Z = Highest score

Disease score (mean) was also assessed using the incidence score data recorded from each plant sample and measured by the following formula (Horsfall and Barrat 1945):

$$\text{Disease score} = \frac{\sum(a \times b)}{N}$$

Table 2. Bacterial Dieback score description

Score	Description of symptoms
0	No symptoms (free from disease)
1	Water-soaked spots on petioles and stems
2	Yellowing on leaves. Few black spots on leaves and fruits
3	Wilting of the leaf followed by petiole and stem (flag leaf symptoms). Brown lesion on fruits and canker on stems
4	Brown lesion spreads to crown and leads to dieback. Only a few leaves/leaf stalks on papaya trees
5	Stems and all plant parts become rotten causing death of papaya trees (plant death)

$\sum$(a x b) = Sum of the symptomatic plant and their corresponding score
N = Total number of sampled plants

The translation of disease score (Cueva et al. 2017) is listed in *Table 3* below.

Table 3. Bacterial dieback disease score translation

Disease score	Disease score translation
0	Resistant
0.1 – 1.0	Highly tolerant
1.1 – 2.0	Tolerant
2.1 – 3.0	Susceptible
3.1 – 5.0	Highly susceptible

Rank-sum method analysis

For simultaneous selection of disease tolerance and good fruit attributes of a balanced genotype, a rank-sum method was used. In this method, all the genotypes were ranked for four characters, disease score, yield, TSS and height to first fruit. The best value of each character was set as the lowest number while the worst value was set as the highest number. The ranking of the four characters were summed to give the rank-sum which will be used to select the best hybrids.

Results and discussion

Analysis of variance

Table 4 shows the results of the analysis of variance (ANOVA) of the 13 genotypes tested in PDD hot-spot areas at MARDI Serdang. The mean squares for disease incidence, disease severity, fruit weight, fruit number, yield, TSS and height to first fruit are presented in *Table 4*. In the case of disease occurrence, significant differences between genotypes were detected on disease severity but not on disease incidence data. The ANOVA proposed that there was variation on disease severity within these genotypes but not on disease incidence value.

In the ANOVA for yield, fruit quality and plant performance, genotypic differences were highly significant in all characters. The ANOVA also showed that there was no significant differences within the replicates for each character analysed which suggests that the samples used for each replicate were uniform.

Table 4. Analyses of variance for disease occurrence, yield, fruit quality and plant performance

Source	df	MS values						
		Disease incidence	Disease severity	Fruit weight	Fruit number	Yield	TSS	Height to first fruit
Replicate	2	319.37^{ns}	284.33^{ns}	4174^{ns}	46.59^{ns}	43.23^{ns}	0.0823^{ns}	10.40^{ns}
Genotype	12	296.44^{ns}	195.33*	405380**	440.23**	416.39**	0.8067**	153.27**
Error	24	194.28	91.35	3831	32.20	29.72	0.1438	4.72
Total	38							

*Significantly different at $p \leq 0.05$
**Significantly different at $p < 0.01$
^{ns}Not significant

Papaya dieback disease occurrence in 'hot-spot' areas

Table 5 highlights that the disease occurrence of papaya dieback was maximum on Eksotika variety with incidence and severity levels of 100% and 62.8%,

respectively. Meanwhile, the lowest disease incidence and disease severity was observed on Hybrid C and Hybrid I with 66.7% and 32.4% respectively. Disease severity was also relatively lower compared to disease incidence. All hybrids showed better percentages consistently on disease severity compared to check varieties except for Hybrid G. Similar results have been reported by Ollitrault et al. (2005) where the 'Solo x Gua1' and 'Solo x Gua2' hybrids presented lower percentages of disease severity compared to their parents (Solo variety).

Table 6 shows that the disease score of hybrids B, C, D, E, F, I and J were in the values ranging from 1.6 to 2.0. The results suggested that these hybrids were tolerant to the dieback disease occurrence, while Sekaki and Eksotika were susceptible and highly susceptible respectively. However, Lasin et al. (2015) reported that the highest disease severity among cultivars was recorded on Sekaki rather than Eksotika during the survey conducted on disease severity of papaya dieback disease in Peninsular Malaysia. Chan (2008) stated that the levels of tolerance in dieback disease were variable and inherited in an additive manner subjected to strain virulence.

Table 5. Means of papaya dieback disease occurrence on F1 hybrids and check varieties

Genotype	Disease incidence (%)	Disease severity (%)
Hybrid A	100.0^{a}	44.8^{abc}
Hybrid B	90.5^{ab}	40.0^{bc}
Hybrid C	66.7^{ab}	39.0^{bc}
Hybrid D	95.2^{a}	38.1^{bc}
Hybrid E	100.0^{a}	40.0^{bc}
Hybrid F	100.0^{a}	40.0^{bc}
Hybrid G	95.2^{a}	52.4^{ab}
Hybrid H	100.0^{a}	45.7^{abc}
Hybrid I	81.0^{ab}	32.4c
Hybrid J	95.2^{a}	40.0^{bc}
Eksotika	100.0^{a}	62.8a
Sekaki	100.0^{a}	50.5^{ab}
Line 19	100.0^{a}	51.4^{ab}

Column means with the same letters are not significantly different at $p \leq 0.05$ according to DMRT

Table 6. Disease score and translation on F_1 hybrids and check varieties

Genotype	Disease score (mean)	Disease score translation
Hybrid A	2.2	Susceptible
Hybrid B	2.0	Tolerant
Hybrid C	2.0	Tolerant
Hybrid D	1.9	Tolerant
Hybrid E	2.0	Tolerant
Hybrid F	2.0	Tolerant
Hybrid G	2.6	Susceptible
Hybrid H	2.3	Susceptible
Hybrid I	1.6	Tolerant
Hybrid J	2.0	Tolerant
Eksotika	3.1	Highly susceptible
Sekaki	2.5	Susceptible
Line 19	2.6	Susceptible

Yield, fruit quality and plant performance

The tolerance or resistance to disease in fruit crops would be rather meaningless without considering yield and quality. *Table 7* illustrates the yield, its components (fruit number and fruit weight) and total soluble solids (% TSS) that are usually indicative of eating quality. Hybrid J showed the highest yield of 45.3 kg/plant due to its precocity in bearing a high number of medium sized fruits. Most hybrids, except for Hybrid A, B and G seemed to bear fruits quite well under PDD-infected conditions with yields ranging from 21.9 to 45.3 kg/plant. These yields were much better compared to the performance of commercial varieties (Sekaki: 22.1 kg/plant and Eksotika: 12.1 kg/plant). These yields were also quite comparable to Eksotika's yield (30 – 40 kg/plant) under non-infected environments as reported by Rogayah et al. (2013).

There were lots of variations in terms of fruit weight displayed in *Table 7*. Hybrid C possessed the lowest fruit weight (372 g) while the highest was shown by Hybrid D (1,411 g). Export markets in the USA, Europe and China preferred the small fruits of the Solo and Eksotika types while the domestic market in Malaysia preferred the medium fruits of the Sekaki variety (Chan 2008). The varieties of the

Solo group are planted in many regions of the world, producing fruits with desirable characteristics for export such as red flesh, small size and weight between 300 and 650 g (da Silva et al. 2017). In Malaysia, small size papayas like Eksotika and Solo own the highest price compared to the large size papaya like Sekaki. Hybrids developed by Viorica or M01 parents resulted in higher fruit weight compared to those formed without using those parents. Among the most tolerant hybrids, only Hybrid I, Hybrid A and Hybrid B showed an insignificant result on fruit weight compared to Sekaki and Eksotika varieties. This suggested that the hybrids can match the weight of commercial papaya varieties.

TSS content of Hybrid I and Hybrid A was significantly higher than Eksotika and Sekaki varieties, which was in contrast with Hybrid J. Eksotika and Sekaki fruits that were not infected with PDD usually had a TSS in the range of 13 to 14% and 11 to 12% respectively (Chan et al. 1991). However, infected fruits were expected to drop in fruit quality, particularly in TSS (Yeh et al. 1988).

Figure 1 illustrates that all hybrids produced the first fruit at a height of less than 80 cm from the ground, which was lower than the height of Eksotika and Line 19 varieties except for Hybrid F. The height to the first fruit recorded by Hybrid C (61.7 of a dwarf papaya tree. Paternal parents of Hybrid C which was Dwarica (also known as PR217) was particularly outstanding in dwarf stature and earliness in flowering as reported by Lim and Siti Hawa (2005). Johari et al. (2016) also mentioned that PR217 was chosen as parents in their study due to its small sized fruit, dwarf plant and longer storage life compared to Eksotika. The first bearing height is an important character in papaya as it facilitates easy harvest and extends the economic life of plantation (Storey 1953). The standard economical period of papaya tree can be prolonged if it can produce fruits early while the tree is still short. The fruiting height was governed by multiple factors and the product of any cross could be expected to be intermediate to the parental lines (Muthulakshmi et al. 2007).

Selection for the best genotype

The question remained as to which hybrid to select as there was a set of genotypes tolerant to PDD, a different set with good fruit attributes (high yield and good quality) and perfect plant height. The rank-sum

Table 7. Means of yield and fruit quality of F1 hybrids and check varieties

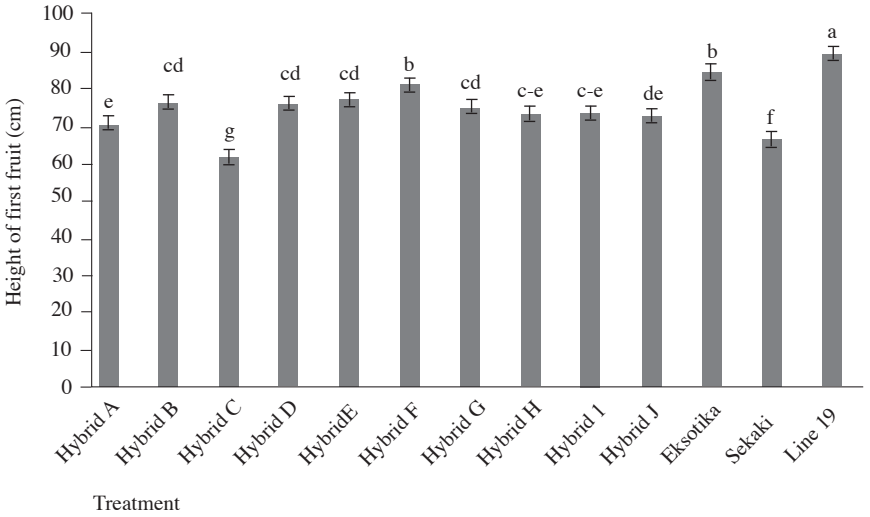
Genotype	Fruit weight (g)	Fruit number	Yield (kg/plant)	TSS (%)
Hybrid A	522.3^{f}	16.2^{f}	8.4^{f}	12.5^{a}
Hybrid B	439.4^{fg}	24.9^{c-f}	10.9^{ef}	11.6^{ab}
Hybrid C	372.8^{g}	58.5^{a}	21.9^{cde}	11.1^{b}
Hybrid D	1,411.1^{a}	20.7^{ef}	29.1^{bc}	11.1^{b}
Hybrid E	1,292.8^{b}	30.5^{cde}	39.4^{b}	11.4^{ab}
Hybrid F	1,284.5^{b}	21.7^{def}	28.0^{c}	11.5^{ab}
Hybrid G	658.3^{e}	27.0^{c-f}	17.8^{de}	11.5^{ab}
Hybrid H	1,271.2^{b}	31.6^{cd}	40.1^{b}	11.1^{b}
Hybrid I	1,182.7^{bc}	33.7^{c}	39.9^{b}	12.1^{a}
Hybrid J	951.9^{d}	47.5^{b}	45.3^{a}	10.1^{c}
Eksotika	511.1^{f}	23.7^{c-f}	12.1^{def}	10.9^{b}
Sekaki	1,150.6^{c}	19.1^{f}	22.1^{cde}	10.8^{b}
Line 19	610.2^{e}	19.7^{ef}	11.9^{def}	11.6^{ab}

Column means with the same letters are not significantly different at p <0.01 according to DMRT

method was used to simultaneously consider both selection criteria (Chan and Ong 2003). The rankings of genotypes for disease score, yield, TSS and height to first fruit are presented in *Table 8*. The rankings of four characters were combined to give the rank-sum for selecting the promising hybrids. The low rank-sum value represents the superior genotype. The genotype with the lowest rank-sum was Hybrid I followed by Hybrid C, J and D. These hybrids appeared to be the most well-balanced selection. They have the best disease tolerance character, good yield and TSS as well as good plant height.

Conclusion

Considering the economic traits of papaya, Hybrid I (Viorica x P15), Hybrid C (P15 x Dwarica), Hybrid J (Viorica x Dwarica) and Hybrid D (Viorica x Eksotika) showed the best characteristics among the hybrids in terms of tolerance level to papaya dieback



Means with the same letters are not significantly different at p <0.01 according to DMRT

Figure 1 Means of height to first fruit in F_1 hybrids and check varieties

Table 8. Rank-sum for simultaneous selection of PDD tolerance, fruit attributes and plant performance

Genotype	Disease score (1)	Yield (2)	TSS (3)	Height to first fruit (4)	Rank sum (1+2+3+4)	Final rank
Hybrid A	8	13	1	3	25	7
Hybrid B	3	12	3	9	27	9
Hybrid C	3	8	8	1	20	2
Hybrid D	2	5	8	8	23	4
Hybrid E	3	4	7	10	24	5
Hybrid F	3	6	5	11	25	7
Hybrid G	11	9	5	7	32	11
Hybrid H	9	2	8	5	24	5
Hybrid I	1	3	2	6	12	1
Hybrid J	3	1	13	4	21	3
Eksotika	13	10	11	12	46	13
Sekaki	10	7	12	2	31	10
Line 19	11	11	3	13	38	12

disease incidence, yield, fruit quality and plant performance. However, detailed data on shelf life and multilocation trials of selected hybrids need to be collected for better selection of superior papaya hybrids.

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Abstrak

Betik (*Carica papaya*) merupakan salah satu daripada buah-buahan eksport terpenting di Malaysia yang telah menghasilkan 78,000 tan metrik buah pada tahun 2003. Walau bagaimanapun, dua kultivar yang sering kali ditanam dalam negara iaitu Sekaki dan Eksotika adalah sangat rentan pada penyakit mati rosot betik yang telah menyebabkan penurunan hasil kepada lebih kurang 31,000 tan metrik pada tahun 2013. Pembaikbakaan bagi varieti dan hibrid rintang adalah langkah jangka panjang yang terbaik bagi mengatasi masalah ini. MARDI telah memulakan program pembaikbakaan bagi membangunkan betik yang rintang pada penyakit mati rosot. Sebanyak sepuluh biji benih hibrid F_1 betik telah dibangunkan melalui kacukan silang di antara enam induk elit yang dikenal pasti rintang pada penyakit mati rosot betik atau berkualiti tinggi. Kesemua hibrid ini diuji pada plot kajian '*hot-spot*' berpenyakit di MARDI Serdang, Selangor, bagi menilai kadar toleran di lapangan, ciri buah dan prestasi tumbuhan hibrid ini. Hibrid I (Viorica x P15), Hibrid C (P15 x Dwarica), Hibrid J (Viorica x Dwarica) dan Hibrid D (Viorica x Eksotika) muncul sebagai hibrid yang paling seimbang dengan ciri toleran penyakit yang terbaik, hasil dan jumlah pepejal larut (JPL) yang tinggi dan ketinggian pokok yang optimum. Hibrid I adalah hibrid yang paling berpotensi dari segi tahap toleran yang tinggi (1.6 daripada 5.0), hasil yang tinggi (39.9 kg/pokok), JPL yang tinggi (12.1%) dan ketinggian pokok yang sederhana.