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Preliminary screening for *Meloidogyne incognita* resistance in selected *Psidium* species

(Saringan awal kerintangan *Meloidogyne incognita* dalam spesies *Psidium* terpilih)

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Key words: *Meloidogyne* sp., root-knot nematode, guava, screening protocols, rootstock

Abstract

The resistance and susceptibility of 19 guava accessions was measured by gall index (GI) as an indicator for plant damage, and host efficiency (R factor) as an indicator for nematode reproduction. The protocol used in this study was developed by USAID-funded Crop Nematode Research and Control Project (CNRCP) with slight modification. A range of *Psidium* species, namely *P. littoralle* var. *longipes, P. arayan*, naturally grown *P. guajava* and *P. guajava* var. Kampuchea (GU 8) were screened against the root-knot nematode (*Meloidogyne incognita*). Results showed that *P. guajava* var. Kampuchea was the most susceptible, while other accessions showed a range of susceptibility. Based on this screening, *P. littoralle* var. *longipes, P. arayan* and *P. guajava* acc. B-12 had shown potential to be used as rootstock.

Introduction

Guava is one of the fruit types that were given priority for cultivation of fruit in Malaysia. The two major areas planted with guava were Johor and Perak. The area planted with guava increased from 856 ha (1986) to 1,745.5 ha (2002) (Anon. 2005). Production of guava in 2000 was 26,000 t for fresh consumption at value of RM26 million at the price of RM1,000/t and 3,000 t for processed products at the value of RM6 million at the price of RM2,000/t. This was generated from production area of 1,800 ha at the yield of 14.4 t/ha for fresh, and 500 ha at the yield of 6 t/ha (Anon. 2004).

The trend of guava cultivation in terms of area and production was increasing from 1986 until 1993 and then declining until 2002 (*Figure 1*). The most serious problem facing the guava industry in Malaysia is the root-knot nematode (*Meloidogyne incognita*). The attack was widespread across the country and all commonly grown guava varieties were reported to be susceptible (Abdul Karim and Yuen 1994).

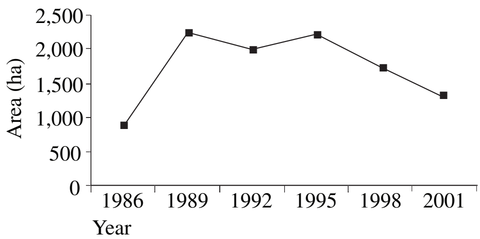

Figure 1. Trend in guava hectareage at Peninsular Malaysia from 1986–2001

Plant resistance is regarded as an extremely feasible method for controlling root-knot nematodes. It is an effective, economical and environmentally safe means of reducing losses from damage caused

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by this nematode. Use of resistant plants enables the grower to control the root-knot nematode without increasing production costs associated with the purchase of expensive chemicals, applicators and in the numerous mechanical operation that go into the production of guava. The importance of controlling root-knot nematodes through genetic means has gained further significance since manufacturing restriction was imposed on fumigants containing 1,2-dibromo-3-chloropropane of their potential carcinogenic effect (Fassuliotis 1979).

Abdul Karim et al. (2002) screened 28 seedlings of guava cultivar free from nematode infection and showed that nematode gave high root galling index and high death rate for the guava cultivars evaluated except in red strawberry guava (*Psidium littorale* var. *longipes*) which showed apparent tolerance. Cuadra and Quincosa (1982) screened five *Psidium* species in Cuba and found that Costa Rican guava (*P. friedrichsthalianum)* was highly resistant to *Meloidogyne* sp.

During the Seventh Malaysia Plan, an IRPA project funded by Ministry of Science and Innovation was implemented to identify and evaluate 10 local guava accessions for resistance/tolerance to the nematode. The seedlings of four accessions showed varying degree of infection when inoculated twice with egg masses (600–900 and 20,000–30,000) of *M. incognita* in the nursery. In fact, Strawberry guava has zero gall indexes, indicating lowest susceptibility infection to the nematode while accessions Paya Rambai and Line no. 6 have the highest index of 8.2, indicating high infection to the nematode. Four accessions namely Air Lanas, Indian guava, Line no. 4 and Strawberry guava were moderately susceptible (Abdul Karim et al. 2002). This trial showed that local accessions have potential resistance/ tolerance to the root-knot nematode.

Therefore, the following study attempted to screen and identify systematically for sources of nematode resistance in other guava accessions. The sources can be used as materials for rootstock multiplication and for breeding programmes with specific objectives. The protocol used in this study was developed by the USAID-funded Crop Nematode Research and Control Project (CNRCP) (Sasser et al. 1984) with slight modification. Preliminary results of the screening on 17 naturally grown guava accessions and related species for their resistance to the root-knot nematode are described and discussed.

Materials and methods

Preparation of inoculum

The species of the nematode was identified by examination of perineal pattern and host differential test. Eggs of *M. incognita* were inoculated on 2-week-old eggplant seedlings grown in autoclaved soil in glasshouse. The glasshouse temperature was at 20–30 °C. The seedlings were harvested 60 days after inoculation. The roots were washed, cut into pieces about 2 cm long and immersed in a 1,000 ml container with 200 ml of 10% sodium hypochlorite (NaOCl) solution. The container was shaken vigorously for 3 min and then the mixture of NaOCl solution and eggs was poured through a 200-mesh sieve under a slow stream of cool tap water for 2 min. The eggs were then suspended in 100 ml of distilled water. To estimate the total number of eggs in the suspension, 5 ml of aliquot of the suspension was taken and the eggs were counted using counting dish. The 100 ml suspension was diluted to get the appropriate number of eggs for inoculation.

Growth and inoculation of guava plants

Seeds of 19 selected guava accessions from naturally grown *P. guajava* were collected from states of Wilayah Persekutuan (3), Johor (1), Pahang (2), Negeri Sembilan (3), Kelantan (1) and Selangor (6). One accession of *P. littorale* var. *longipes* and one accession of *P. arayan* were collected from Selangor and one accession of marcotted plant of commercial clone Kampuchea (GU 8) as a control was bought from Department of Agriculture Serdang. These accessions were collected to be used as test plants to evaluate the protocol whether it can be used to differentiate the response and identified the accessions that have potential resistance to root-knot nematode.

Guava seeds of the selected species were germinated in germinating tray filled with autoclaved soil. After the seedling produced six well develop leaves, the seedlings were transplanted into polybag (17 cm x 7 cm) filled with autoclaved soil. Seedlings to be screened were prepared in four replicates. Once the plants have six well develop leaves, they were inoculated or dispensed in each polybag with 5,000 eggs of a known species of *M. incognita* by using pipette. Plants were grown in greenhouse condition for 60 days. After 60 days, the inoculated plants were harvested and the galling index assessed (Taylor and Sasser 1978).

Damage rating and determination of host resistance

The inoculated plants were harvested by cutting off the tops of guava plants and the roots were washed gently under running tap water. The tops of plants were cut off and the roots were soaked in a solution of 15 mg phloxine in one litre of water for 15–20 min to stain the egg masses. The eggs of nematode turned pink and could easily be seen after phloxine staining. Plant damage and the presence of nematode were assessed by counting the number of gall formed in the root and assigned the rating of galls index or egg masses according to Taylor and Sasser's rating scale (*Table 1*). The egg masses index was used to quantify nematode damage on the plants.

To determine plant host efficiency (susceptibility), nematode reproduction as number of eggs produced was estimated. The roots were cut into pieces about 2 cm long and immersed in a 1,000-ml container

Table 1. Taylor and Sasser's rating scale for the presence of root-knot nematode galls or egg masses on roots

Number of galls or egg masses	Gall index (GI)/ egg mass index (EI)
0	0
1–2	1
3–10	2
11–30	3
31–100	4
100 +	5

Source: Taylor and Sasser (1978)

with 200 ml of 10% NaOCl solution. The container was shaken vigorously for 3 min and then the mixture of NaOCl solution and eggs was poured through a 200-mesh sieve under a slow stream of cool tap water for 2 min.

The extracted eggs were stained with acid fuchsin-acetic acid solution (3.5 g acid fuchsin, 250 ml acetic acid and 750 ml distilled water). The suspension was boiled briefly.

The total numbers of eggs extracted from three egg masses were counted and an average of egg masses in one egg was calculated. Then the total numbers of eggs in the roots of the plant were calculated by timing the average egg masses in one egg with total number of eggs in the root.

Nematode reproduction and host efficiency was assessed by calculating Oostenbrink (1966) reproduction factor: Rf = Pf/Pi, where Pi was the initial inoculums level (5,000 eggs) and Pf was the final number of egg in the root after 60 days. Canto-Saenz's host suitability designation was used as the basis of the scale proposed by Oostenbrink (1966). Host suitability was designated by utilizing a rating system based on the gall index (GI) as an indicator of plant damage and the reproduction factor (Rf) as an indicator of nematode reproduction efficiency on host plat (*Tables 2* and *3*).

Fassuliotis (1979) categorised plant interaction with nematode into four categories. Those were immune, resistant,

Table 2. Host suitability designations proposed by Canto-Saenz

Nematodes reproduction on host (Rf)	Damage to plant (GI)	
	Statistically significant	Insignificant
Efficient	Susceptible	Tolerant
Inefficient	Moderate resistant	Resistant

Source: Canto-Saenz (1983)

Table 3. Quantitative scheme for assignment of Canto-Saenz's host suitability (resistance)

Plant damage (gall index)	Host efficiency (R factor)	Degree of resistance (DR) designation
≤2	≤1	Resistant
≤2	>1	Tolerant
>2	≤1	Moderate resistant
>2	>1	Susceptible

Source: Canto-Saenz (1983)

tolerant and susceptible. An immune plant is one that is able to prevent infection with no disease expression. A resistant plant is a plant that cannot prevent entrance by the parasite but is able to prevent, restrict or retard its development. Tolerance refers to the ability of a plant to endure invasion by the nematode without appreciable symptom expression or damage. A susceptible plant is one that cannot overcome or withstand the injurious effects of the nematode and permits it to develop to its fullest capacity.

The trial was established as a single tree plot using Complete Randomised Design (CRD) with four replicates. Data on total root weight (g), total egg masses in the root, total eggs in the root, and numbers of initial egg inoculated, final eggs produced 60 days after inoculation and gall index were recorded. Data was analysed using SAS 9.1 for Window software (SAS Inst. 1990).

Results and discussion

The modification in this protocol as compared to CNRCP protocol is that it does not use 500-sieve to collect larvae and use an egg mass in the treatment. Analysis of variance shows that all parameters studied were highly significantly different among the accessions (*Table 4*). *Table 5* shows that using the proposed modified protocol, the mean and range of the parameters studied were highly significantly different and contributed to high coefficient of variation for total root weight (37.65), total egg masses in the root (47.72), total egg in the root (45.93), final egg produced (81.91) and host efficiency (82.04). CV value was low only for gall index parameter. Analysis of means for all the parameters studied showed that there was highly significantly different between all the accessions as shown in *Table 6*. Based on all the results, only three accessions out of 19 accessions have the potential resistance to root-knot nematode as shown in *Table 7*.

Performance of accessions

The procedure developed was successful to quantify the ability of individual accession to respond to the nematode attack. The results can be used as guideline to select the most potential accession that is resistant to nematode character. The higher CV for final egg production indicated that very wide variation on the plant performance against nematode that was reproduced inside the root of the plant. The range of 38 to 145,350 eggs produced in different accessions showed that the variation in response to nematode is very wide. The host efficiency of the accession with high CV indicated that the nematode reproduction capacity is affected by the accessions as

Table 4. Means square of analysis of variance for six parameters of 19 Psidium accessions tested for nematode response

Source	df	Total root weight (g)	Total egg masses in the root	Total eggs/ egg mass in the root	Final egg produce (Pf)	Host efficiency (Rf)	Gall Index (GI)
Accessions	18	136.97**	17,184.63**	8,125.62**	1,402,121,558.39**	56.14**	1.60**
Error	57	4.13	3,019.58	2,388.78	233,469,598.28	9.36	0.32
Total	75						

**Significantly different at $p < 0.01$

Table 5. Summary of the parameters studied

Parameter	Mean	Range	C.V (%)
Total root weight	5.39	1.2–33.4	37.65
Total egg masses in the root	115.15	2–385	47.72
Total eggs/egg mass in the root	106.42	5.3–303.7	45.93
Final egg produce (Pf)	18,654.70	38–14,5350	81.91
Host efficiency (Rf)	3.73	0–29.1	82.04
Gall Index (GI)	4.45	2–5	12.64

Table 6. Means comparison of six different parameters on 19 *Psidium* accessions

Accession	Total root weight (g)	Total egg masses in the root	Total eggs/ egg mass in the root	Final egg produce after inoculation (Pf)	Host efficiency (Rf)	Gall Index (GI)
W-1	4.05de	205.75ab	179.60a	35,020bc	7.00bc	5.00a
W-2	3.10e	152.25bcd	152.93abcd	22,319bcd	4.48bcd	5.00a
W-3	2.95e	180.50bc	88.58bcdef	16,015bcd	3.20bcd	5.00a
J-1	3.60de	123.50bcdef	167.43ab	22,050bcd	4.38bcd	5.00a
C-1	1.60e	70.25defgh	104.80abcdef	6,549d	1.33d	4.25abc
C-2	4.3de	137.00bcde	116.33abcde	16,339bcd	3.28bcd	4.75ab
B-1	2.48e	57.00efgh	96.58bcdef	626d	1.28d	3.75cd
B-3	3.23e	120.75bcdef	161.43abc	18,255bcd	3.65bcd	4.75ab
B-5	3.05e	95.25cdefgh	164.65abc	164,67bcd	3.30bcd	4.50abc
B-8	6.55cd	180.50bc	117.25abcde	21,485bcd	4.30bcd	5.00a
B-12	4.75de	105.25cdef	43.43ef	4,570d	0.88d	4.50abc
B-13	4.45de	102.00cdefgh	93.00bcdef	10,338cd	2.08cd	4.75ab
N-2	2.80e	81.00defgh	56.90ef	5,818d	1.15d	4.25abc
N-3	2.95e	100.00cdefgh	120.58abcde	12,117bcd	2.43bcd	4.50abc
N-4	3.05e	120.00bcdef	84.25cdef	18,621bcd	3.73bcd	4.00bcd
P. longipes	7.90c	13.00h	50.23ef	710d	0.15d	2.75e
P. arayan	2.65e	27.25gh	25.90f	874d	0.15d	3.25de
J. Gerap	11.45b	279.25a	123.90abcde	36,780b	7.34b	4.50abc
GU 8	27.63a	37.35fgh	74.23def	83,845a	16.78a	5.00a

Mean values with the same letter in the same column are not significantly different ($p < 0.05$)

Table 7. Preliminary result of screening of *Psidium* accessions for root-knot nematode

Accession	Initial egg inoculum (Pi)	Final egg produced after inoculation (Pf)	Host efficiency (Rf)	Gall Index (GI)	Degree of resistance designation
W-1	5,000	35,020	7.00	5.00	Susceptible
W-2	5,000	22,319	4.48	5.00	Susceptible
W-3	5,000	16,015	3.20	5.00	Susceptible
J-1	5,000	22,050	4.38	5.00	Susceptible
C-1	5,000	6,549	1.33	4.25	Susceptible
C-2	5,000	16,339	3.28	4.75	Susceptible
B-1	5,000	6,269	1.28	3.75	Susceptible
B-3	5,000	18,255	3.65	4.75	Susceptible
B-5	5,000	16,467	3.30	4.50	Susceptible
B-8	5,000	21,485	4.30	5.00	Susceptible
B-12	5,000	4,570	0.88	4.50	Moderate resistant
B-13	5,000	10,338	2.08	4.75	Susceptible
N-2	5,000	5,818	1.15	4.25	Susceptible
N-3	5,000	12,117	2.43	4.50	Susceptible
N-4	5,000	18,621	3.73	4.00	Susceptible
P. longipes	5,000	710	0.15	2.75	Moderate resistant
P. arayan	5,000	874	0.15	3.25	Moderate resistant
J. Gerap	5,000	36,780	7.34	4.50	Susceptible
GU 8	5,000	83,845	16.78	5.00	Susceptible

shown in *Table 5*. Gall index with low CV indicated that the small variation i.e. from 2 to 5 only (*Table 5*).

Analysis of means showed that all parameters were significantly different among the 19 accessions (*Table 6*). Root weight was highest in GU 8 (27.63 g). Total egg masses in the root were high in Jambu Gerap (279.25) and lowest in *Psidium littoralle* var. *longipes* (13.00). The accession W-1 had the highest total eggs in the root. Final egg production after the inoculation of 5,000 eggs was highest in GU 8 and the lowest in C-1, B-1, B-12, *P. littoralle* var. *longipes*, *P. arayan* and N-2. The highest host efficiency occurred in GU 8 while the lowest in C-1, B-1, B-12, N-2, *P. littoralle* var. *longipes*, *P. arayan*. The highest galling index was found in W-1, W-2, W-3, J-1, and GU 8 (5.0) while the lowest gall was in *P. littoralle* var. *longipes* (2.75).

Results showed that commercial clone GU 8, that was used as control in the test, was the most vulnerable to nematode attack because it had the highest Rf value (16.78). This was expected as GU 8 was planted using vegetative propagation (marcott) and was known to be susceptible to the root knot nematode. However, observation of the Rf value showed that plants propagated by seeds were less vulnerable to the nematode attack as compared to the plant propagated by vegetative means. These results supported the proposal to use resistant rootstock for the propagation of guava. Only three accessions namely *P. guajava* acc. B-12, *P. littoralle* var. *longipes* and *P. arayan* showed potential resistance to nematode. According to Canto-Saenz (1983), the three accessions were designated as moderate resistant where their Rf values are 0.88, 0.15 and 0.15 respectively (*Table 3*). The moderate resistant means that the root attacked by the nematode will be damaged, but the nematode will not survive or multiply inside the root of the plants.

Preliminary evaluation of the growth character showed that *P. littoralle* var. *longipes* and *P. arayan* were not suitable for rootstocks. *Psidium littoralle* var. *longipes* had slow growth and spreading growth habit. The species did not produce upright single stem for efficient propagation. *Psidium arayan* has the potential resistance to nematode and observation showed that the plant growth habit is slow and their graft compatibility is not yet tested and still questionable because of inter-species compatibility. Only *P. guajava* acc. B-12 has the potential to control the root-knot nematode and graft-compatible with commercial clone of guava because they are from the same species.

Conclusion

There is a wide variation in terms of plant response to the root-knot nematode attack. The protocol was able to differentiate the response of guava accessions and this can be used to evaluate the response of individual accession to root-knot nematode attack. Preliminary results of this study showed that the protocol developed was successful in screening the resistance of *Psidium* species to the root-knot nematode. All *P. guajava* accessions, except accession B-12, were susceptible to the nematode, with commercial clone (GU 8) was the most susceptible. *Psidium littoralle* var *longipes* and *P. arayan* showed potential resistant characters to the root-knot nematode but their potential as rootstock for guava cultivation is still questionable. *Psidium guajava* acc. B-12 had the potential resistant to the nematode and as an interim recommendation it could be used as a rootstock for guava commercial clones. Gall index (GI) measured the suitability of the guava accessions as host of the root-knot nematode, thus may indicate resistance and susceptibility. Reproduction factor on the other hand measured the ability of the nematode to produce on the screened accessions, which indicate tolerance. When combined together, the Canto-Saenz's suitability designation was able to determine if the accessions screened are susceptible or otherwise.

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Abstrak
Kerintangan dan kerentanan 19 aksesi jambu batu telah diukur dengan indeks puru (GI) sebagai penunjuk kerosakan pokok dan kecekapan perumah (R factor) sebagai penunjuk pembiakan nematod. Protokol yang digunakan dalam kajian ini telah dibangunkan oleh USAID melalui pembiayaan Crop Nematode Research and Control Project (CNRCP) dengan sedikit pengubahsuaian. Beberapa spesies *Psidium* iaitu *P. littorale* var. *longipes*, *P. arayan* dan tumbuhan liar *P. guajava,* dan *P. guajava* var. Kampuchea (GU 8) telah disaring terhadap serangan nematod puru akar (*Meloidogyne incognita*). Keputusan menunjukkan bahawa *P. guajava* var. Kampuchea adalah aksesi yang paling rentan manakala aksesi-aksesi lain menunjukkan satu julat kerentanan. Berdasarkan saringan ini, *P. littorale* var. *longipes*, *P. arayan* dan *P. guajava* acc. B-12 telah menunjukkan keupayaan untuk diguna pakai sebagai pokok penanti.

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