Evaluation of Screening Procedures to Identify Peanut Resistance to Peanut Bud Necrosis Virus (PBNV)

V. Pensuk[1], N. Daengpluang[1], S. Wongkaew[2], S. Jogloy[1], and A. Patanothai[1]*

ABSTRACT

Six peanut genotypes (ICGV 86388, IC 34, IC 10, JL 24, Khon Kaen 60-1, and Khon Kaen 4) were evaluated for reaction to peanut bud necrosis virus (PBNV) in the field and in the greenhouse in Thailand in 2000 and 2001. The objectives of this study were to (a) investigate if disease score or the area under the disease progress curve (ADPC) would be effective in identifying peanut genotypes resistant to PBNV, (b) determine the appropriate time for assessing PBNV resistance under field conditions by means of disease incidence, and (c) identify peanut genotypes having stable resistance to PBNV by considering their responses to mechanical inoculation and field infection. Results from natural infection by PBNV indicated that differences among the genotypes could be observed by 40 d, but not 30 d after sowing (DAS). Genotypes ICGV 86388, IC 34, and IC 10 had lower field disease incidence than JL 24, Khon Kaen 60-1, and Khon Kaen 4. The proportion of treatment mean square for disease incidence was highest at 50 or 60 DAS depending on the trial, indicating that assessment of disease incidence at these dates could best differentiate the reaction of these genotypes to PBNV. ADPC was an alternative to disease incidence for comparing genotypes affected by PBND in the field, as resistant and susceptible genotypes were readily identified. Mostly, a scoring based on disease severity did not facilitate identifying PBNV resistance in the genotypes used in this study. Greenhouse tests yielded similar results to field disease incidences, indicating the usefulness of greenhouse tests in identifying PBNV resistance. Genotypes ICGV 86388, IC 34, and IC 10 were identified as potential resistant sources for breeding for resistance to PBNV.

Key Words: *Arachis hypogaea* L., disease resistance, disease scores, disease screening, *Tospovirus*.

Peanut bud necrosis disease (PBND) is an economically important virus disease of peanut (*Arachis hypogaea* L.) in Southern and Southeast Asia. PBND is caused by peanut bud necrosis virus (PBNV) (10), a distinct member of the genus *Tospovirus* of the family *Bunyaviridae*, which is transmitted by *Thrips palmi* Karny (13). The virus is not seed-transmitted but the seed from infected plants may fail to germinate or produce a less vigorous plant (2, 17, 19). Plants infected early in development may not produce any pods, while plants infected in later stages produce some pods (16). The disease can cause yield losses of over 50% (1, 10) and losses up to 90-100% have been observed (16). While PBND incidence in major peanut-growing areas of India ranges from 5 to 80% (1, 10), it is a relatively new disease in Thailand, being most prevalent in the northeastern and eastern regions. The incidence of PBND in some locations in Thailand has been as high as 90% and these infected crops had a total yield loss. PBND has become an economically important disease in Thailand because of its severity and wide distribution (20).

Host plant resistance offers the best long-term strategy for PBNV management, and significant efforts have been invested in screening peanut accessions for resistance. Peanut genotypes have been studied intensively for resistance to PBNV at ICRISAT, Patancheru, India (10) and also at several other research centers in India (1, 8, 16). Promising lines are being used in a PBNV resistance breeding program, but complete resistance (immunity) has not been found within *A. hypogaea* (2). Selected lines were registered and released because of their resistance to *T. palmi* and/or PBNV (9, 11). However, none of these lines have been tested under the field conditions in Thailand. Resistance in several peanut genotypes to PBNV appears to be quantitative in reducing disease incidence when it is determined as the percentage of plants showing systemic symptoms (2).

Screening procedures to identify peanut genotypes with resistance to PBNV have relied upon natural field infection or mechanical infection (2, 3, 10). In regard to field infection, only disease incidence has been used as a means to assess the peanut accessions. The potential of other means to evaluate resistance has not been well documented. This study was conducted to (a) determine if disease score or the area under the disease progress curve could provide information on resistance to PBNV, (b) determine the appropriate time for assessing PBNV resistance under field conditions in Thailand by means of disease incidence, and (c) identify peanut genotypes having stable resistance to PBNV based on their responses to mechanical inoculation and natural field infection.

Materials and Methods

Six peanut lines (ICGV 86388, IC 34, IC 10, JL 24, Khon Kaen 60-1, and Khon Kaen 4) were evaluated for their reactions to PBNV. ICGV 86388 is a line from ICRISAT with resistance to PBNV and the vector (10, 14). IC 34 and IC 10 are lines that showed low thrips infestation in tests at Khon Kaen (4). IC 34 was derived from the cross [NC Ac 1107 x (NC Ac 2232 x NC Ac 2214)] and IC 10 was derived from the cross Robut 33-1 x NC Ac 2214 (4). NC Ac 2214,

[1]Dept. of Agronomy, Faculty of Agric., Khon Kaen Univ., Khon Kaen 40002, Thailand.
[2]School of Crop Production Technology, Inst. of Agric. Technology, Suranaree Univ. of Technology, Nakorn Ratchasima 30000, Thailand.
*Corresponding author (email: aran@kku.ac.th).

a North Carolina State Univ. germplasm line, is resistant to thrips but has low yield potential and other undesirable traits (11). NC Ac 2232 has been identified as an accession with consistently low symptoms of bud necrosis at ICRISAT (12). ICGV 86388 is a selection from the cross [(Dh 3-20 x USA 20) x NC Ac 2232] (10). JL 24 was used as a susceptible control for PBNV at ICRISAT (2, 10), and Khon Kaen 60-1 and Khon Kaen 4 are adapted lines for Thailand that are susceptible to PBNV (4).

Field evaluation of these lines was conducted in 2000 at a peanut growing area in Kalasin province in Northeast Thailand and repeated at the same location in 2001 and also at another location in the same province, 70 km apart. These locations were selected because of high incidence of PBND in the previous 2 consecutive yr. The same experimental procedures were used for all three tests. The trial was conducted in a randomized complete block design with six replications. A single-row plot, 7.5 m long and spaced 0.5 m apart with 25 plants, was used. The experimental site was surrounded by fields containing naturally infected PBNV hosts to ensure a high viruliferous thrips pressure. Neither fungicide nor insecticide was applied to the crop, and other cultural practices were in accordance with the recommendation for irrigated peanut. Plants were scored visually for PBNV symptoms at 30, 40, 50, and 60 d after sowing (DAS). The disease incidence was determined as the percentage of infected plants. Data were analyzed statistically after being transformed by an arc sine transformation, and the Duncan's Multiple Range Test was used to compare means ($P = 0.01$).

A disease progress curve was constructed for each genotype using disease incidence which was the proportion (0-1.0) of symptomatic plants in the plot. Area under the disease progress curve (ADPC) was calculated for each plot using the formula:

$$ADPC = \sum_{i=1}^{n} [(Y_{i+1} + Y_i / 2](X_{i+1} - X_i) \qquad [Eq. 1]$$

where Y_i = apparent incidence (0-1.0) at the i^{th} observation, X_i = time (days) at the i^{th} observation, and n = total number of observations (15).

Virus scores of 1-5 also were given to individual plants, with 1 = no symptom, 2 = no systemic symptom but with spots on some leaves, 3 = systemic symptoms with top chlorosis but no stunting, 4 = systemic symptoms with strong leaf distortion and stunting, and 5 = plants showing severe necrosis and stunting. Selected photographs representing individual virus scores are shown in Figure 1.

In 2000, the same six peanut genotypes also were evaluated for PBNV reactions under greenhouse conditions. The plants were grown in plastic pots, 11.43 cm high x 8.89 cm diam., and filled with a soil mixture (soil, sand, and compost at 2:1:1 v/v/v). Each pot contained three plants. Each genotype was grown in five replications, six plants (two pots) per replication. The PBNV isolate used was collected originally from naturally infected plants in the field at the Kalasin province, Thailand, where the field trial was conducted. The virus clone was isolated from a single lesion that appeared as primary symptom on infected peanut and propagated in Tainan 9 peanut kept in a screened house. Identity of the virus was confirmed by ELISA using PBNV antiserum as a reference (21). The inoculum was prepared by grinding

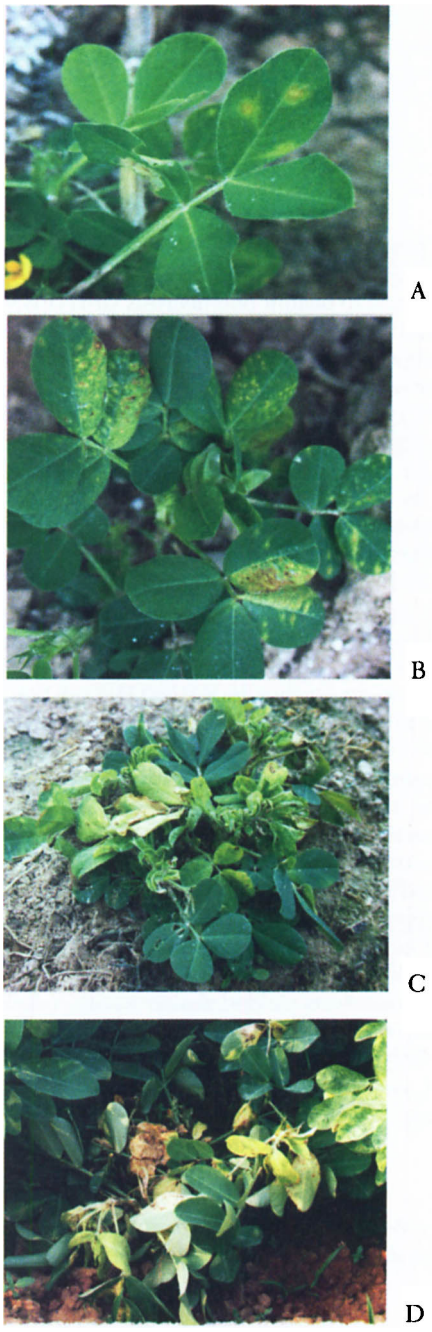

Fig. 1. Symptoms for bud necrosis caused by PBNV in peanut used for disease scores. A = disease score 2, no systemic symptom, spots on some leaves; B = disease score 3, systemic symptoms, top chlorosis but no stunting; C = disease score 4, systemic symptoms, strong leaf distortion, stunt; and D = disease score 5, plants showing severe necrosis and stunting. [Note: Photograph for disease score 1 (no symptom) was not shown].

systemically infected peanut leaves in 0.05 M phosphate buffer, pH 7.0, containing 0.2% 2-mercaptoethanol (1:10, w/v), and 1% celite. This extract was kept chilled during the inoculation. The inoculation was conducted by dripping 200 µL of the inoculum onto the unfolding leaves of each plant and rubbed thoroughly with fingers. Prior to inoculation, the plants were maintained under dark conditions for 3 hr. The

test plants were inoculated twice mechanically (5 and 8 d after planting) to ensure infection, and scored visually for PBNV symptoms at 14 and 28 d after inoculation. Only plants showing systemic symptoms were considered as infected plants. The disease incidence was determined as the percentage of infected plants. Data were analyzed statistically after being transformed by an arc sine transformation, and comparisons among means were made using the Duncan's Multiple Range Test (P = 0.01).

Results and Discussion

The levels of disease incidence in the three field trials in 2 yr were not much different. Infected plants at 60 DAS of the three susceptible lines (JL 24, Khon Kaen 60-1, and Khon Kaen 4) averaged 40.1, 35.0, and 42.7% for the trials in 2000, 2001 location 1 and 2001 location 2, respectively. At 30 DAS, although there were significant differences among lines in disease incidence, some of the resistant lines could not be discernible statistically from susceptible lines (Table 1). Similar results also were observed by Chuapong (4) using some of these lines. However, by 40 DAS, two groups of peanut genotypes could be differentiated easily by field disease incidence, with ICGV 86388, IC 34, and IC 10 showing resistance and JL 24, Khon Kaen 60-1, and Khon Kaen 4 showing susceptibility. These results were quite consistent in all three trials (Table 1). The incidences of PBND on IC 34 and IC 10 at 40 DAS in 2000 were slightly lower than those observed at 30 DAS. In this trial, the PBND incidence of IC 10 at 60 DAS also was lower than those observed at the previous assessments. Similarly, reduced disease incidences from an earlier date were observed for JL 24 and Khon Kaen 4 at 60 DAS in the trial at location 1 in 2001, and for ICGV 86388, IC 34, and IC 10 at 40 DAS in the trial at location 2 in 2001. These results could have been due to the hypersensitivity reaction at

Table 1. Peanut bud necrosis incidences of six peanut genotypes observed under field conditions at Kalasin, Thailand and by mechanical inoculation in the greenhouse.

Year	Genotypes	Infected plants[a]						
		30 DAS	40 DAS	50 DAS	60 DAS	GH (14 DAI)[b]	GH (28 DAI)	ADPC[c]
		----------	----------	----------	----------	% ----------	----------	
2000	ICGV 86388	1.39 c	2.75 b	4.78 b	4.75 b	0.00 b	0.00 c	1.06 d
	IC 34	5.69 bc	4.38 b	6.59 b	6.75 b	0.00 b	0.00 c	1.72 d
	IC 10	4.72 bc	4.08 b	5.42 b	2.06 b	0.00 b	0.00 c	1.29 d
	JL 24	11.51ab	23.91 a	33.34 a	38.04 a	70.00 a	80.00 a	8.20 b
	Khon Kaen 60-1	9.71ab	17.58 a	24.51 a	36.46 a	55.53 a	60.00 b	6.52 c
	Khon Kaen 4	20.54 a	32.02 a	42.20 a	45.77 a	69.33 a	79.33 ab	10.74 a
	F ratio[d]	6:1	16:1	16:1	24:1	-	-	-
	C.V. (%)	51.78	39.37	36.63	34.02	37.45	31.84	42.18
2001 (Loc.1)	ICGV 86388	2.00 ab	9.33 ab	4.67 b	3.33 b	-	-	1.67 b
	IC 34	0.00 b	0.69 b	2.03 b	1.36 b	-	-	0.34 b
	IC 10	0.00 b	0.00 b	0.00 b	0.00 b	-	-	0.00 b
	JL 24	14.67 ab	20.67 a	35.33 a	32.67 a	-	-	7.97 a
	Khon Kaen 60-1	13.33 ab	18.67 a	34.00 a	34.67 a	-	-	7.67 a
	Khon Kaen 4	14.81 a	24.19 a	41.67 a	37.56 a	-	-	9.20 a
	F ratio[d]	5:1	8:1	27:1	30:1	-	-	-
	C.V. (%)	99.93	64.06	39.18	39.04	-	-	60.83
2001 (Loc.2)	ICGV 86388	14.00 ab	4.00 b	0.00 b	1.33 b	-	-	1.17 b
	IC 34	4.00 bc	1.33 b	0.00 b	0.00 b	-	-	0.33 b
	IC 10	1.33 c	0.00 b	0.00 b	0.00 b	-	-	0.67 b
	JL 24	18.00 a	18.67 a	21.33 a	38.67 a	-	-	6.83 a
	Khon Kaen 60-1	22.00 a	27.33 a	32.86 a	49.19 a	-	-	9.58 a
	Khon Kaen 4	18.00 a	34.28 a	37.75 a	40.33 a	-	-	10.10 a
	F ratio[d]	9:1	18:1	44:1	28:1	-	-	-
	C.V. (%)	45.82	49.85	39.69	46.47	-	-	60.88

[a]The treatment means are illustrated based on original scale but data analysis is based on transformed data by arc sine. Means in the same columns followed by a common letter are not significantly different at the 1% level.

[b]GH = greenhouse screening, DAI = days after inoculation.

[c]ADPC = Area under the disease progress curve in a field test. Proportion-days, calculated using incidence proportion (0-1.0) at four evaluations in each genotype.

[d]F ratio is computed by dividing treatment (genotype) mean square by its corresponding error mean square.

the earlier stages of infection. We observed that plants with this type of reaction appeared healthy at the subsequent assessment because of defoliation of spotted leaves. At the early stage of infection, the abscission of the spotted leaves from plants without systemic symptoms occurred with no negative effect on plant growth and development. Thus, those plants became asymptomatic at the next assessment. Plants with systemic symptoms either continued to show moderate symptoms or developed more severe symptoms, but never become healthy. It was observed also that the systemic symptoms routinely appeared at 40 DAS. Thus, data collection at 30 DAS should be considered questionable, and a large number of observations are needed to accurately evaluate resistance in peanut genotypes.

Since assessments at 40, 50, and 60 DAS similarly differentiated peanut genotypes for resistance to PBNV, the appropriate time for assessment could be considered by the magnitude of genotypic variations in disease reactions. This could be determined by the ratio between the treatment mean squares and error mean squares. Such a ratio, in the term of the F ratio, was found to be highest at 60 DAS for disease incidences in 2000 and 2001 location 1, and at 50 DAS in 2001 location 2 (Table 1), indicating that variation among genotypes were largest at these dates. Correspondingly, coefficient of variation (C.V.) was lowest also at the date with the highest F ratio (Table 1). However, the C.V. values for disease incidence at 50 and 60 DAS were not significantly different. Although field disease incidence of PBNV at 60 DAS has been used previously as a suitable time to evaluate PBNV resistance of peanut lines (4), the above results suggest that assessment at 50 or 60 DAS would be suitable equally.

In this study, ICGV 86388, IC 34, and IC 10 were all resistant when evaluated in the greenhouse (Table 1). On the contrary, in the study of Buiel and Parlevliet (3) in which plants were also mechanically inoculated, 52.4% of ICGV 86388 and 96.0% of JL 24 plants became infected. These differences may have been due to the different concentration of inoculum and/or differences due to the virus isolate.

Peanut lines may exhibit different responses when the inoculum concentration varied (4, 10). It was observed that on peanut lines IC 34 and IC 10, necrotic local lesions formed on the inoculated leaves without systemic symptoms developing. These delimited lesions, typical of a hypersensitive response, appeared in 7 to 10 d after inoculation. The inoculated leaves subsequently abscised with no observable negative effect on plant growth and development. The results obtained confirmed the resistance of IC 34 and IC 10 to PBNV infection, which had been reported to be due to thrips resistance (4). Unfortunately, these two lines have undesirable agronomic traits that include small seed size and a violet testa.

While greenhouse screening was effective in differentiating genotypes (Table 1), greenhouse evaluation is not a suitable substitute for field evaluation because reactions in the field may be different from those in the greenhouse (18). Greenhouse screening could be useful for a preliminary screening of large numbers of peanut genotypes and to determine if genotypes were susceptible or resistant to PBNV or just preferred differentially by the thrips vector.

ADPC values based on incidence for PBND were not significantly different among the three resistant genotypes, but significant differences were found between the resistant and the susceptible group (Table 1). In the trial in 2000, the three susceptible genotypes showed significant differences in ADPC values, with Khon Kaen 4 having the highest value followed by JL 24 and Khon Kaen 60-1. These results were not in agreement with other assessment methods (i.e., scoring of severity) that showed similar reactions for these susceptible genotypes (Tables 1 and 2). It seemed that ADPC was a more discernable measurement of PBND. However, in both trials in 2001, differences in ADPC values among these three susceptible lines were not significant statistically, presumably due to high variations as indicated by high C.V. values. It appeared that the ability of ADPC to better differentiate peanut genotypes for PBNV resistance was inconsistent. Nevertheless, ADPC was equally effective

Table 2. Disease scores of six peanut genotypes to bud necrosis caused by PBNV under field conditions at Kalasin, Thailand.

Year	Genotypes	Disease score (1-5)[a] 30 DAS	40 DAS	50 DAS	60 DAS
2000	ICGV 86388	1.33 c	1.67 b	2.78 ab	2.83 ab
	IC 34	2.00 abc	2.00 ab	2.78 ab	3.00 ab
	IC 10	1.67 bc	1.72 b	1.86 b	2.00 b
	JL 24	2.36 ab	3.21 a	3.50 a	4.24 a
	Khon Kaen 60-1	2.67 a	3.38 a	3.68 a	3.99 a
	Khon Kaen 4	2.79 a	3.28 a	3.68 a	4.29 a
	F ratio[b]	6:1	6:1	5:1	3:1
	C.V. (%)	26.97	33.11	31.20	37.17
2001 (Loc. 1)	ICGV 86388	1.33 b	1.92 abc	2.00 ab	2.00 ab
	IC 34	1.00 b	1.33 bc	1.50 bc	2.00 ab
	IC 10	1.00 b	1.00 c	1.00 c	1.00 b
	JL 24	1.60 b	2.14 abc	2.18 ab	2.96 a
	Khon Kaen 60-1	1.52 b	2.31 ab	2.33 ab	2.97 a
	Khon Kaen 4	2.42 a	2.86 a	2.57 a	3.15 a
	F ratio[b]	8:1	6:1	7:1	6:1
	C.V. (%)	31.04	35.55	27.15	35.16
2001 (Loc. 2)	ICGV 86388	2.00	1.50 b	1.00 b	2.17 ab
	IC 34	1.50	1.17 b	1.00 b	1.00 b
	IC 10	2.83	1.00 b	1.00 b	1.00 b
	JL 24	2.18	2.22 a	2.94 a	3.38 a
	Khon Kaen 60-1	2.05	2.16 a	2.74 a	3.26 a
	Khon Kaen 4	2.10	2.15 a	2.87 a	3.52 a
	F ratio[b]	NS	16:1	27:1	8:1
	C.V. (%)	79.76	19.94	24.96	41.45

[a]1 = no symptom, 5 = sever necrosis and stunting. Means in the same columns followed by a common letter are not significantly different at the 1% level.

[b]F ratio is computed by dividing treatment (genotype) mean square by its corresponding error mean square.

as disease incidence in differentiating the three resistant genotypes from the three susceptible genotypes. Thus, final incidence and ADPC can be used to differentiate virus resistance in peanut, as previously reported for tomato spotted wilt virus (TSWV) (5, 6, 7).

The results of this investigation indicate the low utility of disease severity scores in determining PBNV resistance. Clear differentiation between the resistant and susceptible genotypes was not obtained from disease scores for all the three trials (Table 2), while these two groups of genotypes were distinctly discerned by disease incidence and ADPC that were concurrently measured in the same plot (Table 1). The disadvantage for using disease score as a measurement of PBNV resistance is due to the highly variable symptoms caused by PBNV that are not genotype specific (2). Additionally, field evaluation of lines is complicated initially by the nonuniformity of disease distribution in the field resulting from random distribution of vectors.

In our study, field disease incidence at 50 or 60 DAS was found to be the most appropriate method to identify resistance to PBNV in peanut genotypes. The use of ADPC was an alternative to disease incidence for comparing genotypes affected by PBND epidemics in the field. Mechanical sap inoculation under greenhouse conditions gave similar results as field disease incidences and, thus, also was useful in identifying PBNV resistance. Field disease scores, however, did not give a good indication of resistance in this study. Based on the results from field disease incidence, ADPC, and greenhouse screening, peanut genotypes ICGV 86388, IC 34, and IC 10 showed potential to be used as parents to introgress PBNV resistance into cultivated peanut in a breeding program.

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