ORIGINAL ARTICLE

Evaluation of ten peanut genotypes for resistance to *Peanut bud necrosis virus* (PBNV)

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Abstract

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Reactions of peanut genotypes to *Peanut bud necrosis virus* (PBNV) under sap-inoculation and natural field conditions may be different. Ten peanut genotypes (KK 60-3, KKU 72-1, KKU 72-2, Luhua 11, Tainan 9, JL 24, IC 10, IC 34, ICGV 86031 and ICGV 86388) were evaluated for their reactions to PBNV under field conditions in Thailand in 2001. The objectives of this study were (i) to determine breeding potential of the selected peanut genotypes in terms of PBNV disease resistance, and (ii) to explore the usefulness of disease incidence, disease score and area under disease progress curve (AUDPC) as the assessments of resistance for effectively discriminating susceptible and resistant genotypes of peanut. Significant differences were found between susceptible and resistant peanut genotypes as identified by disease incidence, disease score and AUDPC. Disease score and AUDPC tended to be equally effective and slightly better than disease incidence in identifying peanut genotypes resistant to PBNV. None of the resistance parameters could differentiate

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genotypes in the resistant group. IC 10, IC 34, ICGV 86031 and ICGV 86388 were identified as good sources of PBNV resistance. Among susceptible genotypes, KK 60-3 was more resistant than the others and could be used as a parent in peanut breeding programs.

Key words : *Arachis hypogaea* L., disease incidence, disease score, AUDPC

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การประเมินถั่วลิสง 10 พันธุ์ ในด้านความต้านทานต่อโรคยอดไหม้ที่เกิดจากไวรัส

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ปฏิกิริยาของพันธุ์ถั่วลิสงต่อโรคยอดไหม้ในสภาพการปลูกเชื้อด้วยน้ำคั้น และสภาพไร่อาจแตกต่างกัน ได้ประเมินปฏิกิริยาต้านทานต่อโรคยอดไหม้ของถั่วลิสง 10 พันธุ์ (KK 60-3, KKU 72-1, KKU 72-2, Luhua 11, Tainan 9, JL 24, IC 10, IC 34, ICGV 86031 และ ICGV 86388) ในสภาพไร่ในปี พ.ศ. 2544 โดยมีวัตถุประสงค์เพื่อหาพันธุ์ต้านทานต่อโรคยอดไหม้ที่เกิดจากไวรัสเพื่อใช้ในการปรับปรุงพันธุ์ และเพื่อศึกษาลักษณะเปอร์เซ็นต์ต้นที่เป็นโรค ความรุนแรงของโรคยอดไหม้ และพื้นที่ใต้กราฟการเป็นโรค (area under disease progress curve - AUDPC) ว่าสามารถใช้เป็นเกณฑ์ในการคัดเลือกพันธุ์ต้านทานได้หรือไม่ จากผลการทดลองพบว่าลักษณะเปอร์เซ็นต์ต้นที่เป็นโรค ความรุนแรงของโรค และพื้นที่ใต้กราฟการเป็นโรค สามารถบ่งบอกความแตกต่างระหว่างพันธุ์อ่อนแอและพันธุ์ต้านทานได้ดี ลักษณะความรุนแรงของโรคและพื้นที่ใต้กราฟการเป็นโรคมีแนวโน้มว่ามีประสิทธิภาพ ในการบ่งบอกความแตกต่างระหว่างพันธุ์อ่อนแอและพันธุ์ต้านทานได้ใกล้เคียงกัน และดีกว่าเปอร์เซ็นต์ต้นที่เป็นโรคเพียงเล็กน้อย เปอร์เซ็นต์ต้นที่เป็นโรค ความรุนแรงของโรค และพื้นที่ใต้กราฟการเป็นโรคไม่สามารถบ่งบอกความแตกต่างระหว่างพันธุ์ในกลุ่มพันธุ์ต้านทาน พันธุ์ IC 10, IC 34, ICGV 86031 และ ICGV 86388 มีความต้านทานต่อโรคยอดไหม้ในสภาพไร่ เหมาะที่จะใช้เป็นแหล่งความต้านทาน ส่วนพันธุ์ KK 60-3 มีความต้านทานต่อโรคยอดไหม้ดีกว่าพันธุ์อื่น ๆ ในกลุ่มพันธุ์อ่อนแอ

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Peanut bud necrosis disease (PBND) caused by *Peanut bud necrosis virus* (PBNV) is an economically important virus disease of peanut (*Arachis hypogaea* L.) in Southern and Southeast Asia (Dwivedi *et al*., 1995). PBNV is a distinct tospovirus, which causes disease in major economic crops worldwide, and is transmitted by thrips (*Thrips palmi* Karny) in a persistent manner (Reddy *et al*., 1995). In India alone, yield losses by the disease have been estimated to be over 89 million US dollars (Naidu *et al*., 1999). The disease occurs throughout the year but disease incidence varies depending on season, location and year (Buiel *et al*., 1995). In Thailand, Wongkaew (1990) reported the first occurrence of the disease in the peanut growing area at the Nam-Un irrigation project in Sakon Nakhon province, where few infected plants were observed in 5 rai (1 rai = 0.16 ha). In disease surveys made in succeeding years, Wongkaew and Chuapong (1993) reported that the disease increased at an alarming rate and could be found in most peanut growing areas in Thailand especially in the dry season. In addition, many weeds and economic crops have been reported as reservoir hosts of both the virus and its vector (Wongkaew, 1993). This leads to the economic importance of the disease.

The potential peanut crop losses caused by

the disease have been well recognized in many peanut-producing countries in the semi-arid tropics. Pesticide application to control the insect vectors is not effective because of the continual migration of thrips into peanut fields from surrounding areas. Application of systemic pesticides is not effective because the movement of the pesticides is too slow to reach terminal buds and kill viruliferous thrips. Frequent sprays are needed to achieve effective control. Moreover, small scale peanut growers can not afford the extra expense. Early planting, late planting and other cultural practices have been recommended but these practices have inherent problems. Early planting is not practical for irrigated peanut following rice and late planting can adversely affect quality and yield of peanut if rains start early in late April. Biological control of the disease under field conditions has not been well documented, although there has been a report that *Orius tantillus* (Motschulsky) is a natural enemy of *Thrips palmi* Karny (Calilung *et al*., 1997)

Host plant resistance offers an effective component of disease management. Buiel (1996) and Pensuk *et al*. (2002a) reported independently that a polygenic system is involved in the inheritance of resistance to the bud necrosis virus in peanut. Extensive studies have been carried out at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), India, and many peanut lines were released because of their resistance to the thrips vector and PBNV. Some of these lines have been tested in Thailand for potential use as released cultivars or parents in the peanut breeding programs (Pensuk *et al*., 2002a, 2002b; Chuapong,1997; Sarawat *et al*.,1999). For practical breeding programs aiming at developing PBNV resistant cultivars, reproducible, reliable and consistent screening procedures are important. In screening for PBNV resistance in peanut, only disease incidence (percent infected plants) was used by many researchers (Buiel, 1996; Buiel *et al*., 1995). Although Pensuk *et al*. (2002b) reported the use of disease score and area under disease progress curve (AUDPC) as indicators of PBNV resistance, additional information is required before making general conclusions.

The objectives of this study were (i) to determine breeding potential of selected peanut genotypes in terms of PBNV resistance and (ii) to explore the usefulness of disease incidence, disease score and area under disease progress curve (AUDPC) as the assessment of resistance for effectively discriminating susceptible and resistant genotypes of peanut.

Materials and Methods

Evaluation of peanut lines for PBNV resistance

Eight peanut genotypes (KK 60-3, KKU 72-1, KKU 72-2, Luhua 11, Tainan 9, JL 24, IC 10, IC 34, ICGV 86031, ICGV 86388) and susceptible check cultivars (Tainan 9 and JL 24) were evaluated for their reactions to PBNV. The origin and description of these lines/cultivars are presented in Table 1. Field evaluation was performed in the peanut growing area of Kalasin province in Northeast Thailand, during January to May in 2001, where high incidence of PBND was observed during the last three years. A randomized complete block design with 10 replications was used in this experiment. Tested materials were planted on a raised bed in a single-row plot, 7.5 m long and 0.5 m apart with 25 plants. Seeds were sown and then seedlings were thinned to obtain one plant per hill at 20 days after sowing (DAS). Captan (a fungicide) was used in combination with ethephon 0.02% for seed treatment to break putative dormancy and to ensure uniformity of germination, but neither fungicide nor insecticide was used during the crop cycle. Other cultural practices were followed according to recommendation for irrigated peanut in Thailand. Briefly, these included mechanical weed control at 20 days after planting, application of chemical fertilizer (12-24-12 of N-P_2O_5-K_2O) at the rate of 25 kg rai^{-1} at 20 days after planting and gypsum ($CaSO_4$) at the rate of 50 kg rai^{-1} at peak flowering (Department of Agriculture, 1994). Furrow irrigation was supplied as needed. Tainan 9 was planted as a border surrounding the experimental site to ensure secondary spread of the disease.

Disease scores, 1-5 for PBND symptoms

Table 1. Origin and description of eight peanut genotypes and standard check cultivars.

Genotypes	Origin	Description
Susceptible group		
KK60-3	Introduced as germplasm (a released cultivar in Thailand)	Virginia group, semi-spreading plant type, resistant to rust, leaf spot, leaf miner, thrips and jassids
KKU72-1	Introduced as segregating germplasm from cross of NC 17090 x B1 from NCSU (a released cultivar)	Virginia group and semi-spreading plant type
KKU72-2	Introduced as segregating germplasm from cross of NC 17090 x B1 from NCSU (a released cultivar)	Virginia group and semi-spreading plant type
Luhua 11	Introduced as germplasm from China	Spanish group, erect plant type and semi-early maturity
Resistant group		
IC 10	Introduced as germplasm resistant to thrips from cross of Robut 33-1 x NC Ac 2214	Virginia group, semi-spreading plant type, small seed and purple seed coat
IC 34	Introduced as germplasm resistant to thrips from cross of NC Ac 1107 x (NC Ac 2232 x NC Ac 2214)	Virginia group, semi-spreading plant type, small seed and purple seed coat
IGCV 86031, ICGV 86388	Introduced as germplasm resistant to PBND and thrips from ICRISAT	Spanish group, erect plant type and late maturity
Susceptible check cultivar		
Tainan 9	A released cultivar in Thailand	Spanish group, erect plant type and semi-early maturity
JL 24	Introduced as standard susceptible cultivar for PBND incidence from ICRISAT	Spanish group, erect plant type and semi-early maturity

with 1 = no disease symptom, 2 = spots on some leaves but no systemic symptom, 3 = systemic symptom but no stunting, 4 = systemic symptom with stunting and 5 = severe necrosis as described by Pensuk *et al*. (2002b), were recorded on ten plants in a single plot at 30, 40, 50, 60 and 70 DAS. The typical characteristics of PBNV disease symptoms are necrosis at the growing buds, short internodes, petiole bending at upper leaves, stunting and proliferation of axillary branches. The symptoms usually occur on early infected plants (less than one month old), giving a stunted appearance on the plants. The infected plants at early growth stages often die. To specify different evaluation dates, bamboo sticks with different colors were placed near main stems of diseased plant as markers. Disease incidence was determined as the percentage of infected plants in the plot. At 80 days after planting we made a visit to the experimental site and found that the disease was still increasing so final disease incidence was determined at 90 DAS using all plants in the plot but disease score was not recorded because of spreading growth habit of peanut.

Area under disease progress curve (AUDPC) was also calculated for each genotype using disease incidence (arcsine-transformed data), which was the proportion (0-1.0) of symptomatic plants in the plot using the formula:

$$AUDPC = \sum_{i=1}^{n} \left[Y_{i+1} + Y_i \right] / \left[T_{i+1} + T_i \right] \quad \text{[Eq. 1]}$$

where: Y_{i+1} = apparent incidence (0-1.0) at the i^{th} observation, T_i = time (days) at the ith observation, n = total number of observations (Shaner and Finney, 1977).

Data were tested for variance heterogeneity and normal distribution according to Snedecor and Cochran (1980). Because variances of some entries were larger than the others, disease score data were log-transformed (log(x+1)), and disease incidence data were arcsine-transformed (asin(sqrt(x*0.01)) *57.3) before analysis of variance was performed and comparisons among means were done using Duncanís multiple range test ($p<0.05$). The transformation of data was accomplished by Microsoft Excel program.

Results

Evaluation for PBNV resistance

Disease incidence At 30 day after sowing (DAS), differences among peanut genotypes were not statistically significant (Table 2). Although significant differences among genotypes were found at 40 and 50 DAS, these observation dates were questionable because of high CV values. IC 10 (resistant) and Luhua 11 (susceptible) were significantly different at these dates and, at 50 DAS, the two groups of peanut genotypes could be distinctly differentiated by disease incidence. IC 10, IC 34, ICGV 86031 and ICGV 86388 were resistant, while KK 60-3, KKU 72-1, KKU 72-2 and Luhua 11 were susceptible. At 60 and 70 DAS when disease incidence (ranging from 0 to 24% for IC 10 and Luhua 11, respectively) was higher than that of the previous observation dates, significant difference was also found between the two groups of peanut genotypes. These dates were considered useful because of low CV values and high F-ratios compared to the previous observation dates. Because disease incidence continued to increase after 70 DAS, final incidence was determined at 90 DAS. Difference among resistant genotypes was not found, but significant differences among susceptible genotypes were recorded. Luhua 11 was considered highly susceptible, whereas KK 60-3, KKU 72-1 and KKU 72-2 were considered susceptible. For AUDPC, the results showed that differences among genotypes were similar to the pattern at 90 DAS. The genotypes in the resistant group could not be separated by AUDPC, whereas the genotypes in the susceptible group could be divided into 2 classes. Luhua 11 was considered highly susceptible, whereas KK 60-3, KKU 72-1 and KKU 72-2 were considered susceptible.

Disease score Significant difference among genotypes could be observed as early as 30 DAS (Table 3). However, the differences between resistant genotypes and susceptible genotypes were not clear. Luhua 11 was highly susceptible. At 40 and 50 DAS, differences among genotypes were clearer than those at 30 DAS, but neither group of genotypes could be separated by disease score because some susceptible genotypes were classified as resistant by disease score. As expected, Luhua 11 is the most susceptible, whereas IC 10 is the most resistant. At 60 DAS, significant difference between the two groups was clearly indicated. However, disease score could not differentiate genotypes in the resistant group, whereas, in the susceptible group, Luhua 11 appeared to be highly susceptible but not significantly different from KK 60-3, KKU 72-1 and KKU 72-2. At 70 DAS, significant differences between the two groups were similar in pattern to the assessment at 60 DAS and difference among resistant genotypes could not be differentiated by disease score. However, disease score could separate genotypes in the susceptible genotypes in group into two classes. Luhua 11 was highly susceptible, whereas KK 60-3 was susceptible and KKU 72-1 and KKU 72-2 were intermediate between the two classes.

Discussion

The results of the previous studies and this study indicate that IC 10, IC 34 and ICGV 86388 are consistently resistant to PBNV under field and greenhouse conditions. Agronomic traits of these materials were also evaluated in a different experiment that favored agronomic performance of these lines (data not reported). The results

Table 2. Mean comparison for original and arcsine-transformed PBND incidence (%) and AUDPC of eight peanut genotypes and two susceptible check cultivars from six assessments in Kalasin in 2001.

Genotypes	Disease incidence assessment[1/]												AUDPC[2/]	
	30 DAS		40 DAS		50 DAS		60 DAS		70 DAS		90 DAS			
	Original	Arcsine	Original	Arcsine	Original	Arcsine	Original	Arcsine	Original	Arcsine	Original	Arcsine	Original	Arcsine
Susceptible group														
KK60-3	0.0	0.0	2.0cd	3.6bc	9.0bc	13.3ab	13.0b	18.5bc	16.0bc	20.8a	17.86ab	25.00b	3.24bc	4.60bc
KKU72-1	0.0	0.0	5.0bcd	8.2abc	11.0ab	17.2ab	19.0ab	24.3ab	18.0a	22.4a	18.66a	25.59b	4.40ab	6.09ab
KKU72-2	3.0	4.5	12.0a	16.8a	12.0ab	17.8ab	14.0b	20.7ab	15.0ab	21.5a	19.13a	25.94b	4.70ab	6.83ab
Luhua 11	3.0	4.5	11.0ab	14.8a	17.0a	20.2a	24.0a	27.5a	22.0a	26.1a	28.68a	32.38a	6.45a	7.77a
Resistant group														
IC 10	0.0	0.0	0.0d	0.0c	0.0d	0.0e	0.0d	0.0d	0.0c	0.0c	1.15c	6.16d	0.00d	0.00d
IC 34	1.0	1.5	3.0cd	5.2bc	1.0cd	1.2de	3.0d	5.5cd	3.0c	5.5bc	5.61c	13.70cd	1.00d	1.66d
ICGV 86031	0.0	0.0	0.0d	0.0c	2.0cd	3.7cde	3.0d	5.5cd	2.0c	3.7bc	4.75c	12.59cd	0.60d	1.11d
ICGV 86388	0.0	0.0	0.0d	0.0c	0.0d	0.0e	1.0d	1.8d	1.0c	1.8bc	2.29c	8.70d	0.15d	0.28d
Susceptible check cultivar														
Tainan 9	0.0	0.0	8.0abc	11.5ab	9.0abc	12.3abc	11.0bc	14.8bc	8.0bc	10.3b	16.93b	24.30b	3.20bc	4.47bc
JL 24	0.0	0.0	2.0cd	2.7bc	6.0bcd	10.0bcd	5.0cd	8.2cd	5.0c	8.0bc	10.38bc	18.80c	1.55cd	2.50cd
F-ratio	1.3 ns	1.3 ns	4.3**	4.8**	5.8**	6.1**	9.2**	9.6**	8.4**	9.0**	15.5**	16.9**	10.9**	10.6**
CV (%)	416.5	399.8	156.2	142.4	114.3	102.9	91.1	78.4	97.3	85.1	74.3	55.8	83.1	77.4

Means in the same column followed by a common letter are not significantly different ($p<0.05$).
ns, Not significant, and significant at 0.01 probability level, respectively**
[1/] Calculated from all plants in the plot
[2/] Calculated from 30 DAS to 70 DAS

Table 3. Mean comparison for original and log-transformed PBND scores (1-5) of eight peanut genotypes and two susceptible check cuiltivars of five assessments in Kalasin in 2001.

Genotypes	Disease score assessment									
	30 DAS		40 DAS		50 DAS		60 DAS		70 DAS	
	Original	Log	Original	Log	Original	Log	Original	Log	Original	Log
Susceptible group										
KK60-3	1.00b	0.30d	1.03bc	0.31c	1.21bcde	0.34bcd	1.50b	0.39ab	1.54bc	0.40b
KKU72-1	1.00b	0.30d	1.10bc	0.32bc	1.28abcd	0.35abc	1.55ab	0.40ab	1.64ab	0.42ab
KKU72-2	1.07ab	0.31b	1.31a	0.36a	1.45ab	0.38ab	1.51b	0.39ab	1.56bc	0.41ab
Luhua 11	1.10a	0.32a	1.35a	0.37a	1.53a	0.39a	1.83a	0.45a	1.93a	0.46a
Resistant group										
IC 10	1.00b	0.30d	1.00c	0.30c	1.00e	0.30d	1.00d	0.30d	1.00d	0.30d
IC 34	1.02b	0.30c	1.08bc	0.32bc	1.04de	0.31d	1.12cd	0.32d	1.12d	0.32cd
ICGV 86031	1.00b	0.30d	1.00c	0.30c	1.05de	0.31d	1.14cd	0.33cd	1.12d	0.32cd
ICGV 86388	1.00b	0.30d	1.00c	0.30c	1.01e	0.30d	1.04d	0.31d	1.06d	0.31cd
Susceptible check cultivar										
Tainan 9	1.01b	0.30cd	1.21ab	0.34ab	1.32abc	0.36abc	1.42bc	0.38bc	1.35bcd	0.36bc
JL 24	1.00b	0.30d	1.02c	0.30c	1.17cde	0.33cd	1.19bcd	0.34cd	1.20cd	0.35cd
F-Ratio	2.2*	2.2*	4.4**	4.6**	5.9**	5.4**	6.6**	6.8**	7.3**	7.2**
CV (%)	7.1	5.0	18.2	11.5	20.2	13.7	25.3	16.1	26.4	17.3

Means in the same column followed by a common letter are not significantly different ($p<0.05$)
***,** Significant at 0.05 and 0.01 probability levels, respectively**

indicate that Luhua 11 was susceptible but was the best genotype for several agronomic traits (data not reported).

Resistance to PBNV is quantitatively inherited and expressed as reduced disease incidence (percentage of infected plants) in peanut crop, and 3 or more genetic factors were reportedly involved in the inheritance of resistance (Buiel, 1996). Such quantitative trait could not be simply transferred by backcrossing and selection. Therefore, resistant parents with good agronomic traits are preferred. Unfortunately, most resistant lines are inferior in agronomic performance and possess undesirable traits including late maturity, small seed size, spreading growth habit and purple seed coat. Undesirable traits associated with resistant lines can hinder the progress in peanut breeding programs aimed at developing promising peanut cultivars resistant to PBNV.

Some genotypes showed higher variation than the others in the test for heterogeneity. Thus, the data were transformed. The arcsine-transformation could not eliminate the heterogeneity of variances, while the log-transformed data were slighly better than the original ones.

Differences in percent infected plants among peanut genotypes were found at early assessment times, but they did not provide useful information because the CV values were extremely high, and the corresponding F-ratios were low. The appropriate assessment times determined by CV values and F-ratios would be at 60 DAS or later because CV values were lower and F-ratios were higher. Also, peanut genotypes were distinctly differentiated at these dates. Luhua 11 was highly susceptible and IC 10 was the most resistant line, but not significantly different from IC 34, ICGV 86031 and ICGV 86388. In the susceptible group, KK 60-3 was somewhat more resistant than Luhua 11. Assessment at early growth stage is required for an effective selection program, but this may be difficult to achieve because more time is required for infected plants to reach full expression of PBNV symptoms when differences among genotypes can be easily identified.

Screening of peanut for PBNV resistance

relies mainly on field and greenhouse evaluation. The parameter used is only disease incidence. We are interested in disease score and AUDPC if they are as effective as disease incidence in discriminating peanut genotypes for resistance to PBNV. We found that all PBNV resistance parameters were equally effective. Pensuk *et al*. (2002b) reported that disease score was less useful than disease incidence and AUDPC. The contrasting results may be caused by the methods in determining diseased plants. In Pensuk *et al*. (2002b) study, the plants showing local lesions on at least one leaflet were regarded as infected plants, whereas in our study the plants with local lesions that confirmed by systemic symptoms in succeeding evaluations were considered diseased plants. The local lesions could be confounded by the coincidence of peanut yellow spot virus (PYSV) in the fields, but the virus did not produce systemic symptoms in peanut plants. If only systemic symptoms are considered, PBNV symptoms can be easily identified with high accuracy. The previous works showed that samples with visual symptoms from field experiments had positive ELISA readings, whereas asymptomatic samples were free from PBNV (Pensuk *et al*., 2002b; Chuapong, 1997). For tomato spotted wilt virus (TSWV- a related species of PBNV) that causes spotted wilt in peanut, Pappu *et al*. (1999) reported that symptomatic plants were indeed infected with TSWV, whereas healthy looking plants were free from TSWV. Similarly, Culbreath *et al*. (1992) found that positive ELISA results were obtained over 95% of symptomatic samples assayed. Culbreath *et al*. (1993) reported that disease incidence for spotted wilt caused by TSWV in peanut was 2-fold higher when detected by ELISA than by visual symptoms and 98% of infected plants identified by visual symptoms were confirmed by ELISA. The results showed high association between the two methods.

PBNV symptoms are not genotype-specific. Therefore, screening of individual plants with low PBND score will not be effective. Besides, the inheritance of resistance to PBNV is quantitative. Buiel (1996) reported that screening of superior genotypes should be conducted in high or moderate disease pressure. In case of low disease incidence, multi-location testing should be employed. Disease incidence is more advantageous than disease score because it is easy to evaluate. AUDPC can be used when disease incidence is low, but it needs more than one assessment time. In a parallel study, Kesmala (2003) reported that there had been high correlation among PBND incidence, PBND score and AUDPC, indicating that inheritances of the three PBNV resistance parameters are closely related. Our results are limited to the data of one location. However, the results from previous studies indicate that IC 10, IC 34 and ICGV 86388 are consistently resistant to PBNV under field and greenhouse conditions and should be used as parents to transmit PBNV resistance to well-adapted peanut cultivars.

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