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Search for Potential Sources of Resistance among Interspecific Derivatives of Peanut against Peanut Bud Necrosis Disease

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A B S T R A C T

Out of 419 peanut interspecific derivatives, screened over four seasons in epiphytotic field (Hot spot location) and glasshouse conditions, seven genotypes *viz.*, CS-43, CS-54, CS-55, CS-73, CS-77, CS-82 and CS-104 were confirmed as potential resistant with least disease incidence, high dry pod yield, desirable yield and pod features besides longer DFSA, DLSA and t_{50}. Further significant positive correlation was found between t_{50} and DFSA, PBND (%) and DLSA. However, negative correlation was recorded between PBND (%) and t_{50}, PBND (%) and DFSA. In glasshouse test, all cross inoculated with PBNV and subsequently produced leaves of resistant genotypes showed presence of virus revealed the block in the systemic virus movement. Perhaps this is first attempt in searching for PBND resistance in interspecific derivatives of peanut and these genotypes can be better utilized in resistant breeding programme for PBND resistant agronomically high yielding varieties.

Introduction

Peanut (*Arachis hypogaea* L.) also known as groundnut, is considered as poor man's *badam* due to its dual qualities *viz.*, high oil (44-56%) and protein (22-30%) content and is grown under varied crop production systems. Due to several biological and environmental constraints the average productivity is often below one tonne per hectare (Mace *et al.*, 2006). Presently growing most of the cultivars have been released on the basis of its superior yield and acceptable seed quality performance. If there is a restriction in use of pesticides, farmers has to agree with lower yields or use resistant varieties (Gopal *et al.*, 2010). In recent years, peanut bud necrosis disease (PBND) is considered as important disease of peanut in south and south-east Asia (Lava kumar *et al.*, 2007a; Reddy *et al.*, 1992). PBND is caused by *Peanut bud necrosis virus* (PBNV) and vectored by *Thrips palmi* Karny in a propagative manner (Vijayalakshmi *et al.*, 1995). The PBND incidence ranged from 5-80

% and reported to cause yield loss up to 50% with worth of more than $ 89 million in India alone (Ghanekhar *et al.,* 1979; Reddy *et al.,* 1991; Patil 1993; Reddy *et al.,* 2000). In India, on the basis of PBND severity, Raichur (Karnataka, India) has been identified as one among the seven "hot-spots" for PBND (Basu 1995). In field condition, genotypes can reacts differentially to PBND incidence due to cumulative effects of resistance to virus and resistance to vector (Gopal *et al.,* 2010). The decrease in the yield loss with delayed infection may be attributed to adult plant resistance (Nagarajan and Muralidharan 1995).

Several studies have identified number genotypes as a resistant or moderately resistant in field test but meager attempts were made on categorisation of genotypes on the basis glasshouse tests, yield and pod features. In contrast, several wild *Arachis* species have been identified as resistant to PBND, due to interspecific compatibility there has been limited success in transferring resistance in to cultivated peanut (Murthy and Jhanvi, 1983). Hence, identification of genotypes with low disease incidence both in hot spot locations and glasshouse condition along with desirable pod features of farmers is need of the hour. Keeping this in view, 419 interspecific derivatives of peanut were screened rigorously under epiphytotic field condition for four cropping seasons and once in glasshouse condition by infective sap inoculation.

Materials and Methods

Field conditions

About 419 interspecific derivatives of peanut were collected from Directorate of Groundnut Research (DGR, Junagadh, Gujarat, India) and were evaluated for PBND resistance by employing infector row technique under epiphytotic condition during *kharif*- 2010 and *rabi*/summer-2010-11 seasons. A susceptible check (cv. KRG-1) was planted with spacing of 45 and 15 cm between rows and plants respectively and initial plant count was recorded in all genotypes at 15 DAS and infected plants were marked with bamboo sticks (0.5 m) to avoid missing of early infected plants as they usually die (Gopal *et al.,* 2010). The PBND reaction in each genotypes was recorded a week before harvest of the crop. Finally, PBND incidence (%) was calculated as follows and genotypes were designated into different categories following 0-5 scale (Gururaj Sunkad *et al.,* 2001).

$$\text{PBND incidence (\%)} = \frac{\text{Number of PBND plants}}{\text{Total number of plants observed}} \times 100$$

Scale	Per cent incidence	Disease reaction
0	0 to 1 %	Highly resistant
1	1.1 to 5 %	Resistant
2	5.1 to 10 %	Moderately resistant
3	10.1 to 25 %	Moderately susceptible
4	25.1 to 50%	Susceptible
5	50.1 % and above	Highly susceptible

The twenty genotypes which behaved as resistant to PBND with desirable yield and pod features during 2010-11 were selected and screened further under epiphytotic field condition during *kharif*- 2011 and *rabi*/summer- 2011-12 seasons.

Yield parameters and pod features

Genotypes which showed field resistance to PBND in four cropping seasons (*kharif*- 2010, 2011 and *rabi*/summer- 2010-11, 2011-12) were examined for their yield and its

parameters *viz.*, dry pod yield, shelling per cent, 100 seed weight, sound matured kernels, oil content and pod parameters.

a) Dry pod yield: Dry pods collected from five randomly selected plants in each genotypes are weighed and average weight was calculated.

b) Shelling per cent: 100 g of dry pods were taken randomly from each genotype and the kernels obtained after shelling of these pods were weighed and shelling per cent was calculated as follows.

$$\text{Shelling (\%)} = \frac{\text{Kernel weight}}{\text{Pod weight}} \times 100$$

c) 100 seed weight: 100 kernels from each genotype were selected randomly and their weight was recorded in grams.

d) Sound matured kernels (SMK): 100 kernels were taken randomly and number of sound matured kernels was counted among them to record per cent SMK

e) Oil content: 30 g kernels of each genotype was taken randomly and subjected for analysis of oil content through Nuclear Magnetic Resonance method (Ramamurthi *et al.*, 1985) at MARS, Raichur, Karnataka, India.

f) Pod features: The pod features of each genotype was studied based on the observation of pod like beak, constriction and reticulation etc.

Glasshouse conditions

Genotypes which documented as PBND resistant along with good yield and pod features during *kharif*- 2010 and *rabi*/summer-2010-11 seasons were selected for screening in glasshouse study.

There are two experiments were conducted to know the resistance of the genotypes to PBND. A PBNV isolate which was maintained on diagnostic host plant (Cowpea cv.C-152) was used as a source inoculum at a dilution of 1/100.

In both experiments ten day old seedlings were mechanically inoculated by using PBNV infective plant sap (1:10 w/v) with inoculation buffer (potassium phosphate-0.05 M) at pH 7.2 (Lava kumar *et al.*, 2007b).

Experiment no.1

In first experiment the relation between t_{50} (Days taken by plants to reach 50% of their final incidence), DFSA (Days taken for first symptom appearance), DLSA (Days taken for last symptom appearance) and PBND (%) incidence in peanut genotypes were studied. Each genotype was sown in 20 pots consisting 3seedlings/pot along with KRG-1 and inoculated mechanically with PBNV infective sap. The number of infected plants in each genotype was recorded on alternate days from 5^{th} day of inoculation until 25 days after inoculation. The t_{50} was determined by calculating the days taken by plants to reach 50% of their final incidence and DFSA, DLSA and PBND incidence (%) were calculated. The data collected in glasshouse test were analysed statistically using IBM SPSS statistics software.

Experiment no.2

The second experiment was conducted to know the resistance mechanism associated with resistant genotypes. Here each genotype was sown in five pots consisting of 3seedlings/pot and a PBNV infective sap was inoculated to all genotypes along with KRG-1. The mean per cent of infection in inoculated and subsequently produced leaves was recorded based on the symptomatic expression

from all five pots of each genotype. All the inoculated and systemically infected leaves were employed to direct antigen-coating enzyme linked immunosorbent assay (DAC-ELISA) know the presence of PBNV.

Results and Discussion

Field conditions

Screening of 419 interspecific derivatives of peanut under epiphytotic field conditions in two rainy and two post-rainy seasons (2010-2012) revealed occurrence of promising sources of resistance to PBNV. The susceptible check (cv. KRG-1) was recorded PBND incidence of 52% in rainy and 49.5% in post-rainy seasons besides 100% of incidence in glasshouse tests and this amount disease pressure was adequate for screening the genotypes. The several peanut genotypes with field resistance to PBND have been identified (Amin, 1985; Reddy *et al.,* 1991; Dwivedi *et al.,* 1993, 1995; Gururaj Sunkad *et al.,* 2001).

Based on the mean incidence over four seasons, 20 genotypes recorded less than 5% of incidence and these were chosen for evaluation of resistance to PBNV under glasshouse conditions (Table 1). Among 20 field resistant genotypes, the eleven genotypes *viz.,* CS-83 (11%), CS-86 (14%), CS-92 (13%), CS-94 (16%), CS-120 (11%), CS-137 (12%), CS-156 (14%), CS-202 (12%), CS-246 (12%), CS-262 (13%) and CS-268 (18%) exhibited moderately susceptible reaction to PBNV by recording more than 10% incidence under glasshouse conditions which were recorded less than 5% incidence in field conditions (Table 1) suggest that the resistance is of quantitative nature and reduced the disease incidence in the crop. There are nine genotypes *viz.,* CS-43, CS-45, CS-51, CS-54, CS-55, CS-73, CS-77, CS-82 and CS-104 which exhibited as resistance reaction in field behaved as moderately resistant in glasshouse tests. The results are in accordance with earlier findings where in genotypes *viz.,* ICGV-0009, ICGV-86699, ICGV-88329, ICGV-91177, ICGV-91234 and ICGV-94252 exhibited partial resistance to PBNV under epiphytotic field and laboratory tests (Gopal *et al.,* 2010). Among nine genotypes, seven genotypes *viz.,* CS-43, CS-54, CS-55, CS-73, CS-77, CS-82 and CS-104 recorded promising yield and yield parameters like pod yield per plant (20.6-25.4g), shelling per cent (71-74%), 100 seed weight (31-35g), SMK (89-72%) and oil content (38-40%) and also desirable pod features of farmers (Table 2).

Glasshouse conditions

1) Correlation among t_{50}, days taken for first symptom appearance (DFSA), last symptom appearance (DLSA) and PBND (%) incidence indicated significant positive correlation between t_{50} and DFSA (0.812), negative correlation between t_{50} and PBND (%) (-0.723). Similarly, a positive correlation between PBND (%) and DLSA (0.604) and negative correlation between PBND (%) and DFSA (-0.602) also supports the fact that cultivar with high disease pressure will have delayed termination of disease and also early initiation of the disease (Table 3) (Buiel and Parlevliet, 1995).

2) The screening of field resistance genotypes under glasshouse condition revealed the maximum mean per cent of infection of 25% in inoculated leaves of CS-268 and minimum of 14% in CS-45 and CS-51. The subsequently produced leaves of CS-43, CS-45 and CS-77 recorded low systemic infection and CS-268 was noticed high infection of 13% whereas susceptible check (cv. KRG-1) was recorded 100% of infection (Table 4).

Table.1 Days to reach 50% incidence (t_{50}), days to first (DFSA) and last (DLSA) appearance of PBND and final PBND incidence in 20 peanut genotypes which showed different levels of PBND resistance in glasshouse and field test

					PBND (%)			
					Field test[d]			Glasshouse test
Sl. No.	Genotypes	t_{50}	DFSA	DLSA	Rainy season[a]	Post-rainy season[b]	Overall mean[c]	
1.	CS-43	13	10	14	3.3	5.1	4.2	9
2.	CS-45	14	11	14	3.5	4.8	4.1	8
3.	CS-51	11	9	12	1.7	1.6	1.6	9
4.	CS-54	13	10	13	3.3	3.5	3.4	8
5.	CS-55	12	9	13	1.7	1.6	1.7	9
6.	CS-73	11	8	12	3.4	2.0	2.7	10
7.	CS-77	14	9	15	4.0	2.9	3.4	10
8.	CS-82	11	8	12	2.0	2.7	2.3	10
9.	CS-83	12	9	14	3.8	4.3	4.1	11
10.	CS-86	11	8	16	2.2	1.6	1.9	14
11.	CS-92	10	7	15	3.3	3.8	3.5	13
12.	CS-94	9	7	15	4.6	3.7	4.1	16
13.	CS-104	12	8	13	2.9	2.4	2.7	10
14.	CS-120	13	10	14	3.8	4.1	3.9	11
15.	CS-137	12	10	14	3.0	4.2	3.6	12
16.	CS-156	11	9	13	2.4	5.6	4.0	14
17.	CS-202	12	8	14	3.7	2.7	3.2	12
18.	CS-246	12	9	14	1.1	1.2	1.2	12
19.	CS-262	11	8	13	1.2	1.8	1.5	13
20.	CS-268	10	8	16	3.1	3.3	3.2	18
21.	KRG-1[e]	8	7	20	52	49.5	50.8	100

a – Mean of two rainy seasons, 2010 and 2011
b – Mean of two post-rainy seasons, 2010-2011 and 2011-2012
c – Mean of 4 (2 rainy and 2 post-rainy) seasons, 2010-2012
d – Infector row method
e – Susceptible genotype

Table.2 Incidence of PBND, dry pod yield, yield parameters and pod features of different peanut genotypes

Sl. No.	Genotypes	PBND (%)	Pod yield per plant (g)	Shelling (%)	100 seed weight	Sound matured kernels (%)	Oil content (%)	Pod feature
1.	CS-43	4.2	21.9	73	33	92	38	D
2.	CS-45	4.1	20.2	67	28	75	32	ND
3.	CS-51	1.6	21.5	72	32	88	39	ND
4.	CS-54	3.4	20.6	74	33	89	39	D
5.	CS-55	1.7	22.1	73	32	90	38	D
6.	CS-73	2.7	21.4	72	32	92	41	D
7.	CS-77	3.4	25.4	73	31	91	40	D
8.	CS-82	2.3	24.9	71	32	89	38	D
9.	CS-83	4.1	22.5	74	34	94	39	D
10.	CS-86	1.9	26.7	75	33	88	37	D
11.	CS-92	3.5	23.9	72	34	91	41	D
12.	CS-94	4.1	24.4	70	29	87	37	D
13.	CS-104	2.7	22.6	74	35	89	40	D
14.	CS-120	3.9	23.3	73	33	87	42	D
15.	CS-137	3.6	26.6	73	32	89	37	D
16.	CS-156	4.0	25.6	72	35	91	42	D
17.	CS-202	3.2	26.3	72	34	88	40	D
18.	CS-246	1.2	22.1	75	32	91	38	D
19.	CS-262	1.5	22.4	74	33	87	39	D
20.	CS-268	3.2	21.2	67	30	87	38	D
21.	KRG-1[c]	50.8	17.0	65	28	70	35	ND

D – Desirable
ND – Non desirable
C – Susceptible check

Table.3 Correlation coefficient of four epidemiological parameters t_{50}, PBND%, DLSA and DFSA

	t_{50}	PBND %	DLSA	DFSA
t_{50}	1			
PBND%,	-0.723[a]	1		
DLSA	-0.108	0.604[a]	1	
DFSA	0.812[a]	-0.602[a]	-0.164	1

a – indicates significance at 0.01% level

Table.4 Screening of peanut genotypes for resistance to PBNV under glasshouse condition

Sl. No.	Genotypes	Inoculated leaves		Subsequently produced leaves	
		Per cent infection[a]	ELISA test	Per cent infection[b]	ELISA test
1.	CS-43	15.0	+	6.0	+
2.	CS-45	14.0	+	6.0	+
3.	CS-51	14.0	+	7.0	+
4.	CS-54	18.0	+	8.0	+
5.	CS-55	16.0	+	9.0	+
6.	CS-73	19.0	+	8.0	+
7.	CS-77	16.0	+	6.0	+
8.	CS-82	18.0	+	7.0	+
9.	CS-83	18.0	+	8.0	+
10.	CS-86	22.0	+	9.0	+
11.	CS-92	21.0	+	9.0	+
12.	CS-94	23.0	+	10.0	+
13.	CS-104	17.0	+	7.0	+
14.	CS-120	18.0	+	8.0	+
15.	CS-137	19.0	+	8.0	+
16.	CS-156	20.0	+	9.0	+
17.	CS-202	21.0	+	8.0	+
18.	CS-246	20.0	+	9.0	+
19.	CS-262	22.0	+	11.0	+
20.	CS-268	25.0	+	13.0	+
21.	KRG-1[c]	100.0	+	40.0	+

a – Mean per cent of infection from inoculated leaves
b – Mean per cent of infection from subsequently produced leaves
C – Susceptible check
+: Virus present

Field conditions

Host plant resistance is considered to be the most practical and effective method for the management of viral diseases in crops. Among 419 interspecific derivatives screened, nine genotypes recorded as resistant along with promising yield and pod features and

these genotypes offer promising sources for resistance to use in breeding programmes to develop PBND resistant agronomically high yielding varieties. Complete resistance to PBNV has not been found among cultivated groundnut (Reddy *et al.,* 1991). The results indicated that there was considerable variability in genotypes for resistance to PBNV. In natural condition, field resistant genotypes took a longer time for expression of symptoms and virus multiplication The genotypes CS-21, CS-77, CS-83, CS-85, CS-86, CS-124, CS-180 and CS-222 have been registered has potential germplasm under the plant germplasm registration system of Indian Council of Agricultural Research. These genotypes can be exploited in development of PBND resistant varieties.

Glasshouse conditions

1) Correlation among t_{50}, DFSA, DLSA) and PBND (%) incidence indicated that the earlier the initiation of the symptom early to reach the maximum disease pressure. The cultivar with ability to delay the initiation of symptom will have less final disease pressure (Gopal *et al.,* 2010). So, these epidemiological may very useful in finding out the resistant sources against PBND where, the disease resistance seem to be quantitative nature and there is no complete resistance available against the virus and most of the available sources are of field resistant only.

2) The inoculated and subsequently produced leaves contain PBNV detected by ELISA. So, it appears that resistance mechanism may involve a block in the systemic virus movement or resistance of genotypes to thrips vector. However systemically infected leaves showed less systemic spread than susceptible varieties (Buiel 1995). Many genotypes *viz.,* ICG5044, ICG1656 and ICG799 showed resistance to the vector (Reddy *et al.,* 1983) but they were susceptible to PBNV when tested in glasshouse condition.

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