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Identification of resistance to *Peanut bud necrosis virus* (PBNV) in wild *Arachis* germplasm

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Summary

Eighty three wild *Arachis* germplasm accessions, belonging to 24 species of five sections and one natural hybrid derivative of a cross between the cultivated and a wild *Arachis* species, were evaluated along with a susceptible groundnut cultivar for resistance to *Peanut bud necrosis virus* (PBNV) in a replicated field trial at ICRISAT, Patancheru, India. Thirty days after sowing, the percentage of infected plants were recorded for all the accessions and subsequently young leaflets from all these accessions were tested for the presence of the virus by enzyme linked immunosorbent assay (ELISA). One accession each of *A. benensis* and *A. cardenasii*, and two accessions of *A. villosa*, in the section *Arachis*, two accessions of *A. appressipila* in the section *Procumbentes*, and one accession of *A. triseminata* under section *Triseminatae* were not infected by PBNV. These seven field-resistant accessions were tested under glasshouse conditions for virus resistance by mechanical sap inoculations. One accession of *A. cardenasii* and two accessions of *A. villosa* did not show systemic infection. Similarly, in another glasshouse test, where 13 *A. cardenasii* accessions of section *Arachis* were evaluated, two accessions did not show systemic infection. In all these resistant accessions, the inoculated leaves showed infection, but the systemic leaves did not show the presence of virus in spite of repeated mechanical sap inoculations. So, the resistance in these accessions appears to be due to a block in systemic movement of the virus. To our knowledge this is the first report on the identification of resistance to PBNV in wild *Arachis* species. Since both *A. cardenasii* and *A. villosa* are the progenitors of cultivated groundnut and can be hybridised with the latter, the resistant accessions are being utilised in conventional breeding programmes to transfer PBNV resistance to widely adapted groundnut cultivars.

Key words: Peanut bud necrosis disease, *Peanut bud necrosis virus*, wild *Arachis* species, field resistance

Introduction

Peanut bud necrosis disease (PBND) is an economically important virus disease of groundnut (*Arachis hypogaea* L.) in south and southeast Asia. It is caused by *Peanut bud necrosis virus* (PBNV) (Reddy *et al.*, 1991). The disease causes crop losses exceeding 89 million US dollars in India alone (Anon., 1992). None of the many cultivated groundnut genotypes that have been tested for resistance to PBNV were found to be resistant (Reddy, 1998).

PBND incidence can be minimised by some cultural practices such as adjustments to planting dates to coincide with low levels of vector activity, maintenance of plant densities in the range of 300 000 to 350 000 ha^{-1}, and intercropping with fast growing cereals such as maize and pearl millet, and frequent application of insecticides, such as dimethioate aimed at controlling the insect vector. The majority of the small farmers are unable to adopt these cultural practices because of various constraints. Therefore, growing resistant cultivars is the best option and, to some extent, farmers now grow cultivars which show field resistance to PBND due to resistance to the thrips vector (*Thrips palmi*). In all such PBND resistant cultivars, resistance is expressed as reduced incidence and, under high disease pressure, they succumb to infection as much as the susceptible genotypes (Dwivedi *et al.*, 1995; Reddy, 1998). Therefore, it is essential to identify a high degree of resistance or immunity to PBNV either in cultivated groundnut or in wild *Arachis* spp. In this paper we present data on the occurrence of resistance to PBNV in some of the wild *Arachis* species that can be intercrossed with groundnut.

Materials and Methods

Screening of wild Arachis *species under field conditions*

During the 1997 rainy season, 83 wild *Arachis* germplasm accessions, belonging to 24 species of five

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sections, and a natural hybrid derivative, *A. batizogaea* (*A. batizocoi* × *A. hypogaea*) along with a known susceptible groundnut cultivar, JL 24 (Table 1) were sown in a replicated trial using a randomised block design on the ICRISAT farm at Patancheru. This experiment was sown in six blocks and each genotype was sown in three replications. Seeds were pre-treated with thiram (dimethyldithiocarbamate) to prevent fungal infection and sown in a single 9 m row in each plot with a row-to-row spacing of 1.5 m and plant-to-spacing of 1 m within the rows. Disease incidence was recorded on the basis of visual symptoms at three-week intervals, starting from one month after germination.

ELISA

The plants were scored for visual symptoms, 30, 60, and 95 days after germination. Plants that showed typical PBND symptoms were noted and tested along with the plants that did not show any overt symptoms. From symptomatic plants, only young leaflets that showed early symptoms were chosen, and from apparently healthy plants, young leaflets were chosen from at least three branches, pooled and tested. The

Table 1. *Screening of wild* Arachis *germplasm accessions for resistance to peanut bud necrosis disease during the 1997 rainy season at ICRISAT - Patancheru farm*

ICG No.	Section and Species	(n)[a]	Percent infection Field tests[b]	Percent infection ELISA tests	ICG No.	Section and Species	(n)[a]	Percent infection Field tests[b]	Percent infection ELISA tests
	Arachis								
8209	*A. batizocoi*	22	9.1	22.7	8164	*A. kempff-mercadoi*	10	10.0	10.0
8210	*A. batizocoi*	26	11.5	15.4	8959	*A. kempff-mercadoi*	16	18.8	18.8
8211	*A. batizocoi*	13	38.5	53.8	8954	*A. kuhlmannii*	24	12.5	20.8
8958	*A .batizocoi*	21	23.8	33.3	8960	*A. magna*	17	47.1	52.9
13160	*A. batizocoi*	13	23.1	23.1	8197	*A . monticola*	20	75.0	75.0
8901	*A. batizogaea*	26	73.1	73.1	8198	*A. monticola*	22	72.7	77.3
11551	*A. benensis*	24	0.0	0.0	11549	*A. monticola*	22	90.9	90.9
11558	*A. cardenasii*	17	11.8	23.5	13177	*A. monticola*	20	60.0	60.0
11561	*A. cardenasii*	21	9.5	12.0	13178	*A. monticola*	24	95.8	95.8
11563	*A. cardenasii*	12	16.7	16.7	8125	*A. stenosperma*	16	6.3	25.0
11564	*A. cardenasii*	24	0.0	0.0	8137	*A. stenosperma*	29	10.3	17.2
11566	*A. cardenasii*	16	37.5	37.5	8906	*A. stenosperma*	11	18.2	18.2
12165	*A. cardenasii*	16	6.3	25.0	13171	*A. stenosperma*	22	40.9	58.8
13166	*A. cardenasii*	18	16.7	33.3	13172	*A. stenosperma*	28	14.3	21.4
8918	*A. correntina*	17	23.5	29.4	13173	*A. stenosperma*	23	34.8	34.8
8123	*A. duranensis*	12	58.3	66.7	8193	*A. valida*	13	61.5	61.5
8138	*A. duranensis*	14	7.1	21.4	11548	*A. valida*	26	15.4	34.6
8139	*A. duranensis*	21	38.1	33.3	8144	*A. villosa*	8	0.0	0.0
8195	*A. duranensis*	22	59.1	63.6	13168	*A. villosa*	27	0.0	0.0
8196	*A .duranensis*	18	38.9	50.0		*Erectoides*			
8199	*A. duranensis*	30	56.7	60.0	8192	*A. oteroi*	25	20.0	28.0
8200	*A. duranensis*	24	45.8	54.2	8141	*A. paraguariensis*	28	28.6	28.6
8201	*A. duranensis*	19	68.4	68.4	8963	*A. paraguariensis*	15	13.3	33.3
8202	*A. duranensis*	11	45.5	45.5	8970	*A. paraguariensis*	13	76.9	76.9
8205	*A. duranensis*	20	55.0	65.0	8973	*A. paraguariensis*	13	6.9	17.2
8207	*A. duranensis*	18	61.1	66.7	8215	*A. stenophylla*	29	18.8	25.0
8956	*A. duranensis*	18	16.7	33.3		*Heteranthae*	16		
8957	*A. duranensis*	12	58.3	58.3	13167	*A. sylvestris*	13	46.2	46.2
11550	*A. duranensis*	24	8.3	20.8		*Procumbentes*			
11552	*A. duranensis*	22	22.7	31.8	8127	*A. appressipila*	27	7.4	7.4
11553	*A. duranensis*	14	21.4	28.6	8128	*A. appressipila*	27	25.9	25.9
11554	*A. duranensis*	20	55.0	60.0	8129	*A. appressipila*	25	4.0	8.0
11555	*A. duranensis*	15	26.7	26.7	8945	*A. appressipila*	12	0.0	0.0
11556	*A. duranensis*	25	20.0	24.0	8946	*A. appressipila*	22	0.0	0.0
12162	*A. duranensis*	13	46.2	53.8	11560	*A. chiquitana*	18	11.1	6.7
13161	*A. duranensis*	23	28.0	33.3	8191	*A. kretschmeri*	14	64.3	71.4
13174	*A. duranensis*	17	76.5	76.5	11557	*A. matiensis*	29	3.4	13.8
13175	*A. duranensis*	24	45.8	60.9	13163	*A. matiensis*	13	7.7	16.7
13176	*A. duranensis*	30	53.3	53.3	8186	*A. rigonii*	28	39.3	72.2
13182	*A. duranensis*	15	53.3	60.0	8904	*A. rigonii*	23	21.7	26.1
13183	*A. duranensis*	20	50.0	65.0		*Triseminatae*			
13184	*A. duranensis*	18	44.4	55.6	8131	*A. triseminata*	15	0.0	0.0
13185	*A. duranensis*	30	60.0	60.0		*Arachis* (Control)			
13186	*A. duranensis*	16	81.3	81.3		*A. hypogaea*	27	92.3	92.3
8190	*A. hoehnei*	17	41.2	58.3		(cv. JL 24)			
8206	*A. ipaensis*	12	33.3	33.3					
Mean								33.5	36.7
SE (df = 168)								±23.82	±16.41

[a] No. of plants assayed for both visual symptoms and ELISA tests.
[b] Assessed by visual symptoms.

direct antigen-coating enzyme linked immunosorbent assay (DAC-ELISA) procedure similar to that reported by Hobbs, Reddy, Rajeswari & Reddy (1987) was employed. Extracts from leaflets prepared in carbonate buffer, diluted to 1/50, 1/100 and 1/1000 were added to ELISA plates. Polyclonal PBNV antiserum was used at a dilution of 1:10 000. Rabbit Fc-specfic immunoglobulins prepared in goats and conjugated to penicillinase enzyme were added at a dilution of 1:2000. Substrate was penicillin prepared in bromothymol blue (Sudarshana & Reddy, 1989). Absorbance values at 620 nm were determined with a Titertek Multiskan ELISA reader after 30 min of substrate reaction time. Readings were considered to be positive if the absorbance values given by samples were five-fold greater than those given by virus-free control samples.

Screening of field-resistant wild Arachis *species and* A. cardenasii *accessions under glasshouse conditions*

Seven accessions that were not infected under field conditions (considered to be field-resistant) were planted along with a susceptible cultivar JL 24 in a glasshouse. The seeds were treated with thiram and each accession was sown in four 20 cm plastic pots. Each pot containing five seedlings was considered as one replication. A PBNV isolate which was maintained in *A. hypogaea* (cv. JL 24) in a glasshouse was used as a source of inoculum at a dilution of 1/100 (Reddy *et al.*, 1991). All inoculated leaflets and non-inoculated leaflets produced subsequently were tested by DAC-ELISA as described above. Tests were done at fortnightly intervals until the plants were three months old.

Another set of 13 accessions of *A. cardenasii* resistant to foliar fungal diseases were screened for resistance to PBNV. Each accession was represented by 20 plants raised in four 20 cm plastic pots (serving as four replications). Mechanical inoculations and tests for the presence of PBNV were conducted as described above.

Results

Field experiment

Of the 83 accessions and one natural hybrid tested, one accession of each of *A. benensis* (ICG11551) and *A. cardenasii* (ICG 11564), two accessions each of *A. villosa* (ICG 13168 and ICG8144) in the section *Arachis, A. appressipila* (ICG 8945 and ICG 8946) in the section *Procumbentes,* and one accession of *A. triseminata* (ICG 8131) in the section *Triseminatae*, were not infected with PBND under field conditions despite the fact that the plants were exposed to infection throughout the growing season (Table 1).

Glasshouse tests

The seven accessions that showed field resistance were evaluated under glasshouse conditions for virus resistance by sap inoculations. All plants of three genotypes, one accession of A. *cardenasii* (ICG 11564) and two accessions of *A. villosa* (ICGs 13168 and 8144) were free from systemic infection even after repeated sap inoculations and are presumed to be resistant to PBNV. Although the mechanically inoculated leaves of these resistant accessions showed virus replication, the systemic leaves did not contain PBNV detectable by ELISA. So, it appears that the resistance mechanism may involve a block in the systemic virus movement. Moreover, as these accessions were not tested by grafting, we prefer to describe the reaction as resistant and not immune (Cooper & Jones, 1983). Interestingly, less than 10% of plants of *Triseminatae* (ICG 8131) could be infected when nearly 92% plants of the susceptible cv. JL 24 were infected (Table 2). Three accessions, one in the section *Arachis* (*A. benensis*, ICG 11551), and two in the section *Procumbentes* (*A. appressipila*, ICG 8945 and ICG 8946) that were not infected under field conditions, were found to be susceptible to virus infection under glasshouse conditions. It is likely that these accessions have some resistance to the thrips vector.

Of the 13 accessions of *A. cardenasii* tested under glasshouse conditions, ICG 13164 and ICG 13165 did not show systemic virus infection, even after repeated sap inoculations. Therefore, they are presumed to be resistant to PBNV infection. Another accession of *A. cardenasii* (ICG 15175) showed only 11% infection (Table 3) when JL 24 showed 100% infection.

Discussion

Cultural practices to minimise the effects of vectors transmitting PBNV that are currently available to small-scale farmers are seldom used. Hence, growing resistant cultivars is considered to be the most practical and efficient method to reduce yield losses due to PBND. With the goal of identifying resistant germplasm, over 10 000 *A. hypogaea* lines have been screened under field conditions at ICRISAT from 1979 to 1988. Although many genotypes (e.g. ICG 5044, ICG 1656, and ICG 799) showed resistance to the vector (Reddy, Amin, McDonald & Ghanekar, 1983), they were susceptible to PBNV, when tested in under glasshouse conditions. Genotypes that showed low incidence of PBNV (ICGV 86031 and ICGV 86388) have been reported (Dwivedi *et al.*, 1995). However, under high disease pressure they were infected by PBNV, leading to substantial losses to the crop. Therefore, sources of resistance or immunity to PBNV that can be transferred to *A. hypogaea* need to be identified. In this study, all plants of seven wild species accessions in all replications were free from

Table 2. *Tests on selected wild* Arachis *germplasm for resistance to* Peanut bud necrosis virus *under glasshouse conditions*

ICG No.[a]	Section and Species	Inoculated leaflets		Subsequently produced leaflets	
		Percent Infection[b]	ELISA Tests[c]	Percent Infection[d]	ELISA Tests[c]
	Arachis				
11551	*A. benensis*	93.8	+	56.3	+
11564	*A. cardenasii*	92.3	+	0.0	-
13168	*A. villosa*	92.3	+	0.0	-
8144	*A. villosa*	83.3	+	0.0	-
	Procumbentes				
8945	*A. appressipila*	90.0	+	65.0	+
8946	*A. appressipila*	100.0	+	72.7	+
	Triseminatae				
8131	*A. triseminata*	90.0	+	10.0	+
	Arachis (Control)				
	A. hypogaea (cv. JL 24)	100.0	+	92.3	+
Healthy leaves		0.0	-	0.0	-
	Mean	69.10		29.30	
	SE (df =24)	±6.72		±12.22	

[a] No. of plants assayed ranged from 15-19 for each accession.
[b] Mean percent infection from all inoculated leaves.
[c] In the case of positive reaction ELISA absorbance values were at least five fold higher than the values from healthy controls.
[d] Mean percent of systemic infection.

PBND under field conditions, although the disease pressure was high and the plants were exposed to infection throughout the growing season. Presumably, the resistance in these accessions could be due to non-preference by the thrips vector and/or resistance to PBNV infection or multiplication and spread. Under glasshouse conditions, in all the three accessions of *A. cardenasii* and two accessions of *A. villosa*, although the virus was detected in the inoculated leaves, it did not cause systemic infection. Therefore, the resistance is apparently due to a block in the systemic virus movement. Both A. *cardenasii* and *A. villosa* that have the 'A-genome' in common with the cultivated groundnut, are considered as the progenitors of A.

Table 3. *Tests on selected* Arachis cardenasii *accessions for resistance to* Peanut bud necrosis virus *under glasshouse conditions*

ICG No.[a]	Section and Species	Inoculated leaflets		Subsequently produced leaflets	
		Percent Infection[b]	ELISA Tests[c]	Percent Infection[d]	ELISA Tests[c]
	Arachis				
8216	*A. cardenasii*	80.0	+	100.0	+
11558	*A. cardenasii*	85.7	+	28.6	+
11559	*A. cardenasii*	100.0	+	33.3	+
11561	*A. cardenasii*	100.0	+	60.0	+
11562	*A. cardenasii*	85.7	+	28.6	+
11563	*A. cardenasii*	80.0	+	40.0	+
11566	*A. cardenasii*	88.9	+	22.2	+
12165	*A. cardenasii*	100.0	+	22.2	+
13164	*A. cardenasii*	88.9	+	0.0	-
13165	*A. cardenasii*	90.0	+	0.0	-
13166	*A. cardenasii*	87.5	+	25.0	+
13237	*A. cardenasii*	100.0	+	50.0	+
15175	*A. cardenasii*	88.9	+	11.1	+
	Control				
	A. hypogaea (cv. JL 24)	100.0	+	100.0	+
	Mean	72.04		32.60	
	SE (df=42)	±7.58		±22.17	

[a] No. of plants assayed ranged from 18-20 for each accession.
[b] Mean percent infection from all inoculated leaves.
[c] In the case of positive reaction ELISA absorbance values were at least five fold higher than the values from healthy controls.
[d] Mean percent of systemic infection.

hypogaea, and are cross compatible with the latter. Also, *A. cardenasii* has been successfully used in conventional breeding programs in the past for transferring rust and late leaf spot resistance into agronomically superior groundnut varieties (Moss *et al.*, 1997). Thus, potential exists for transfer of PBNV resistance by conventional breeding methods. To our knowledge, this is the first report of identification of resistance to PBNV in wild *Arachis* germplasm. Efforts are being made currently to transfer the PBNV resistance to widely adapted groundnut cultivars.

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