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Evaluation blackgram and greengram germplasm for resistance against tobacco streak virus (TSV) and peanut bud necrosis virus (PBNV) isolates of blackgram and greengram of Andhra Pradesh under glasshouse conditions

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Abstract
All the blackgram and greengram genotypes screened under glasshouse conditions for resistance against PBNV and TSV showed hundred per cent infection with a range of symptoms from chlorosis to necrosis showing local and systemic infections. All the blackgram genotypes screened for PBNV and TSV were graded as highly susceptible as cent per cent disease incidence recorded. PBNV sap inoculations on blackgram showed zero per cent mortality in all the genotypes except in LBG-726 (10%) whereas TSV inoculations showed per cent mortality ranging from 0-80. LBG-731 and LBG-727 showed zero per cent mortality while LBG-726 and LBG-755 showed maximum mortality (80%) followed by LBG-20, LBG-22, LBG-611, LBG-738 and LBG-756 with 70%, LBG-623, Sarala and Berham with 60% and LBG-15, -685, -693, -745 and LBG-748 with 50%. Remaining blackgram genotypes showed per cent mortality less than 50. Artificial inoculations on greengram genotypes under glasshouse conditions with PBNV and TSV showed 100% disease incidence and graded as highly susceptible and the per cent mortality recorded was ranged from 0-20 and 0-100, respectively. Majority of the greengram genotypes inoculated with PBNV showed zero per cent mortality, while 10% mortality was observed in LGG-410, -460, -495, -496, -505, TRAM-1C, TRAM-5, TM-96-2, K-851 and Dhowli. Maximum per cent mortality (20%) was recorded in Kopergoan and LGG-490. TSV inoculations showed minimum per cent mortality (0%) in LGG-490 while maximum (100%) was recorded in LGG-499, LGG-503, LGG-505 and WGG-37 followed by Kopergoan and LGG-407 (90%), LGG-496 (88.8%) , K-851 (80%), LGG-480 (60%), LGG-481 (57.1%), LGG-485 (60%), LGG-503 (70%), LGG-469 (55.5%), LGG-495 (50%), LGG-497 (50%), TRAM-IC (50%) mortality. Hundred per cent disease incidence in glasshouse screening can be since there is no chance of escaping from the virus. Whereas under field conditions, there are chances to escape from viruliferous thrips in case of PBNV and escape either from lodging of TSV infected pollen or thrip visit or both.

Keywords: Evaluation, resistance, glasshouse, *Tobacco streak virus* (TSV), *Peanut bud necrosis virus* (PBNV) isolates, blackgram, greengram.

Introduction
Among pulses, blackgram (urdbean - *Vigna mungo* L. Hepper) and greengram (mungbean - *Vigna radiata* L. Wilczek) are major grain legumes and are cultivated in both upland and rice fallows in India. Blackgram and greengram have been subjected to the attack of several biotic stresses such as fungi, bacteria and viruses, affecting the productivity and among them, viral diseases became great menace and are the great yield reducers. Necrosis caused by viruses is posing a serious threat to *Vigna* species in Andhra Pradesh. Over the years, the incidence of the disease gradually increased to about 70-100 per cent. In the recent years, viruses causing necrosis, transmitted by thrips has assumed epidemic proportion and became a serious production constraint in blackgram and greengram especially in upland areas during all the seasons *i.e, kharif, rabi* and summer in Andhra Pradesh. Of several viral diseases attacking greengram and blackgram leafcurl disease caused by *Peanut bud necrosis virus* (PBNV) (= *Groundnut bud necrosis virus* – GBNV) (Amin *et al*., 1985) [1] transmitted by *Thrips palmi*

(Karny) in a propagative manner (Sreekanth *et al.*, 2002a) [11]. Recently, *Tobacco streak virus* (TSV) has also been reported to be a cause of leaf curl symptoms on blackgram (Prasada Rao *et al.*, 2003d; Ladhalakshmi *et al.*, 2005) [6] and greengram (Bhat *et al.*, 2002c; Prasada Rao *et al.*, 2003d) [2] paving confusion in field diagnosis to assess the disease incidence. Although both the viruses cause necrotic symptoms and are transmitted by thrips, the method of transmission and the virus vector relationship vary and hence need different approaches of management practices. It is necessary to identify and differentiate necrosis-causing viruses and their incidence on blackgram and greengram to follow the appropriate management practices. The most economical way to manage these necrosis-causing viruses is to grow resistant varieties. Hence varieties and germplasm available have to be screened against both these viruses for resistance.

Materials and Methods

Blackgram and greengram genotypes were screened against PBNV and TSV isolates under glasshouse conditions by mechanical inoculation. Each ten plants (5plants/pot) were grown and primary leaves were inoculated one week after sowing. The plants were observed for the development of local as well as systemic symptoms. Disease incidence observations were recorded on total plant stand at 15 days after sowing (DAS). Scale based on per cent disease incidence was formulated according to Prakash *et al*. (2000) with modifications to classify the genotypes.

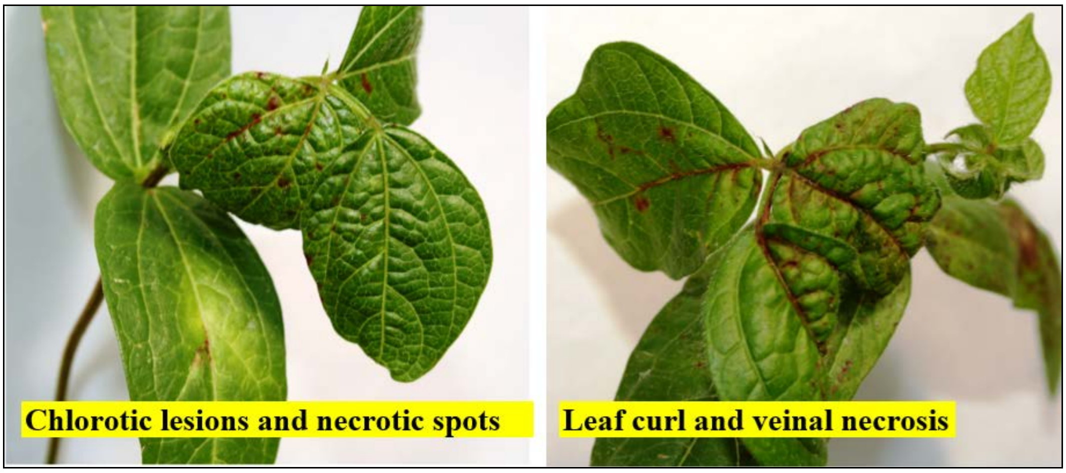
Results

Blackgram and greengram genotypes were screened under glasshouse conditions by mechanical inoculation with PBNV and TSV showed cent per cent disease incidence while per cent mortality was varied with genotype as well as virus.

All the blackgram genotypes screened under glasshouse conditions for resistance against PBNV and TSV showed hundred per cent infection (Table 1) with a range of symptoms from chlorosis to necrosis showing local and systemic infections (Fig 1 and 2). Local symptoms such as chlorotic, necrotic lesions as well as veinal necrosis were observed in all the PBNV inoculated blackgram genotypes with negligible differences. Similarly, chlorotic, necrotic rings, necrotic lesions, concentric chlorotic and necrotic rings as well as veinal necrosis were observed in TSV inoculated blackgram genotypes. PBNV inoculated plants showed systemic symptoms like veinal necrosis, chlorotic, necrotic spots, leaf necrosis and leaf curl while TSV inoculated ones showed smalling of leaves, stunting, apical necrosis and mosaic mottling.

Entire blackgram germplasm screened for PBNV and TSV were graded as highly susceptible as cent per cent disease incidence recorded. PBNV sap inoculations on blackgram showed zero per cent mortality in all the genotypes except in LBG-726 (10%) whereas TSV inoculations showed per cent mortality ranging from 0-80. LBG-731 and LBG-727 showed zero per cent mortality while LBG-726 and LBG-755 showed maximum mortality (80%) followed by LBG-20, LBG-22, LBG-611, LBG-738 and LBG-756 with 70%, LBG-623, Sarala and Berham with 60% and LBG-15, -685, -693, -745 and LBG-748 with 50%. Remaining blackgram genotypes showed per cent mortality less than 50. All the greengram genotypes screened under glasshouse conditions by mechanical inoculations with PBNV and TSV showed hundred per cent disease incidence (Table 2). The greengram genotypes showed local symptoms such as chlorotic and necrotic lesions while TSV showed concentric chlorotic, necrotic rings in addition. Veinal, stem and apical necrosis, chlorotic and necrotic lesion symptoms were recorded with TSV inoculation while leaf necrosis, chlorosis, veinal necrosis, chlorotic spots and leaf curl symptoms were found with PBNV infection (Fig 3 & 4).

Artificial inoculations on greengram genotypes under glasshouse conditions with PBNV and TSV showed 100% disease incidence and graded as highly susceptible and the per cent mortality recorded was ranged from 0-20 and 0-100, respectively. Majority of the greengram genotypes inoculated with PBNV showed zero per cent mortality, while 10% mortality was observed in LGG-410, -460, -495, -496, -505, TRAM-1C, TRAM-5, TM-96-2, K-851 and Dhowli. Maximum per cent mortality (20%) was recorded in Kopergoan and LGG-490. TSV inoculations showed minimum per cent mortality (0%) in LGG-490 while maximum (100%) was recorded in LGG-499, LGG-503, LGG-505 and WGG-37 followed by Kopergoan and LGG-407 (90%), LGG-496 (88.8%) , K-851 (80%), LGG-480 (60%), LGG-481 (57.1%), LGG-485 (60%), LGG-503 (70%), LGG-469 (55.5%), LGG-495 (50%), LGG-497 (50%), TRAM-IC (50%) mortality.



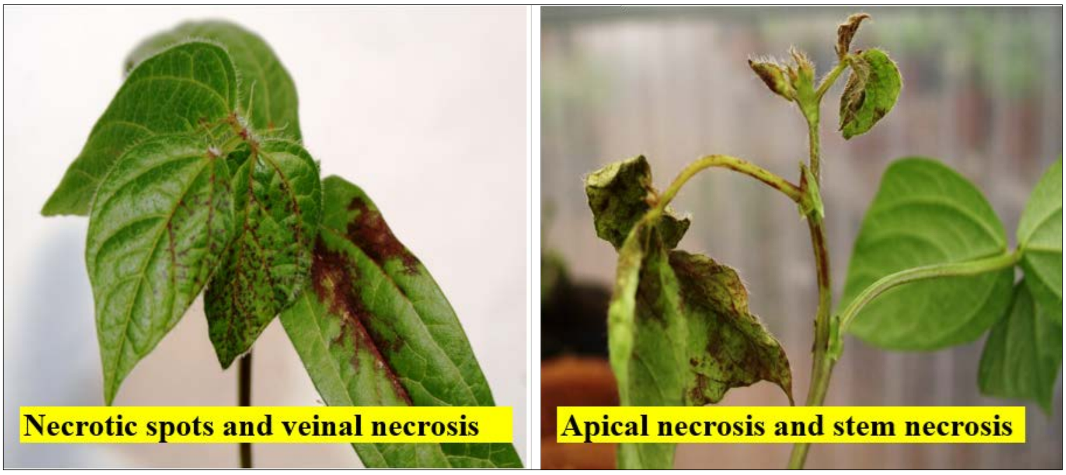


Fig 1: Symptoms induced by PBNV in blackgram upon mechanical inoculation under glasshouse conditions.

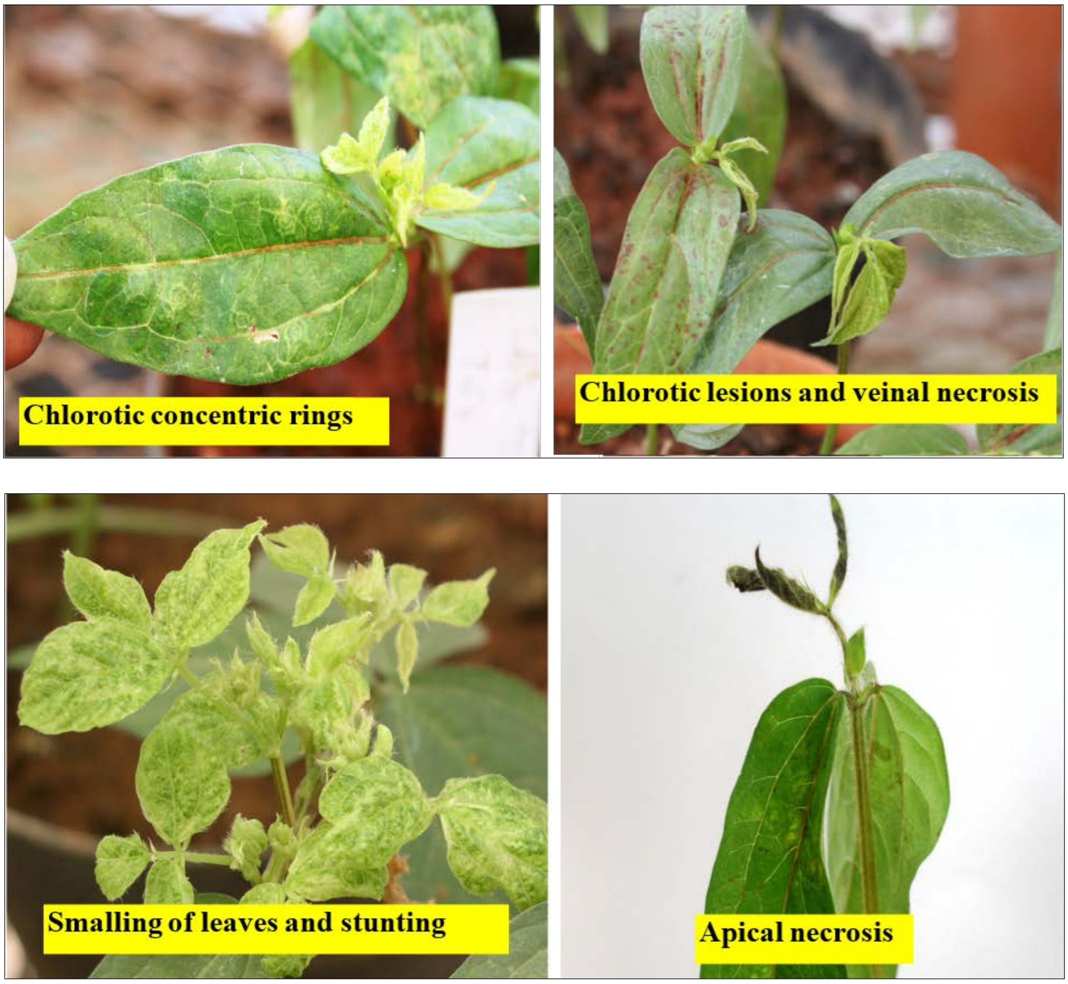


Fig 2: Symptoms induced by TSV in blackgram upon mechanical inoculation under glasshouse conditions

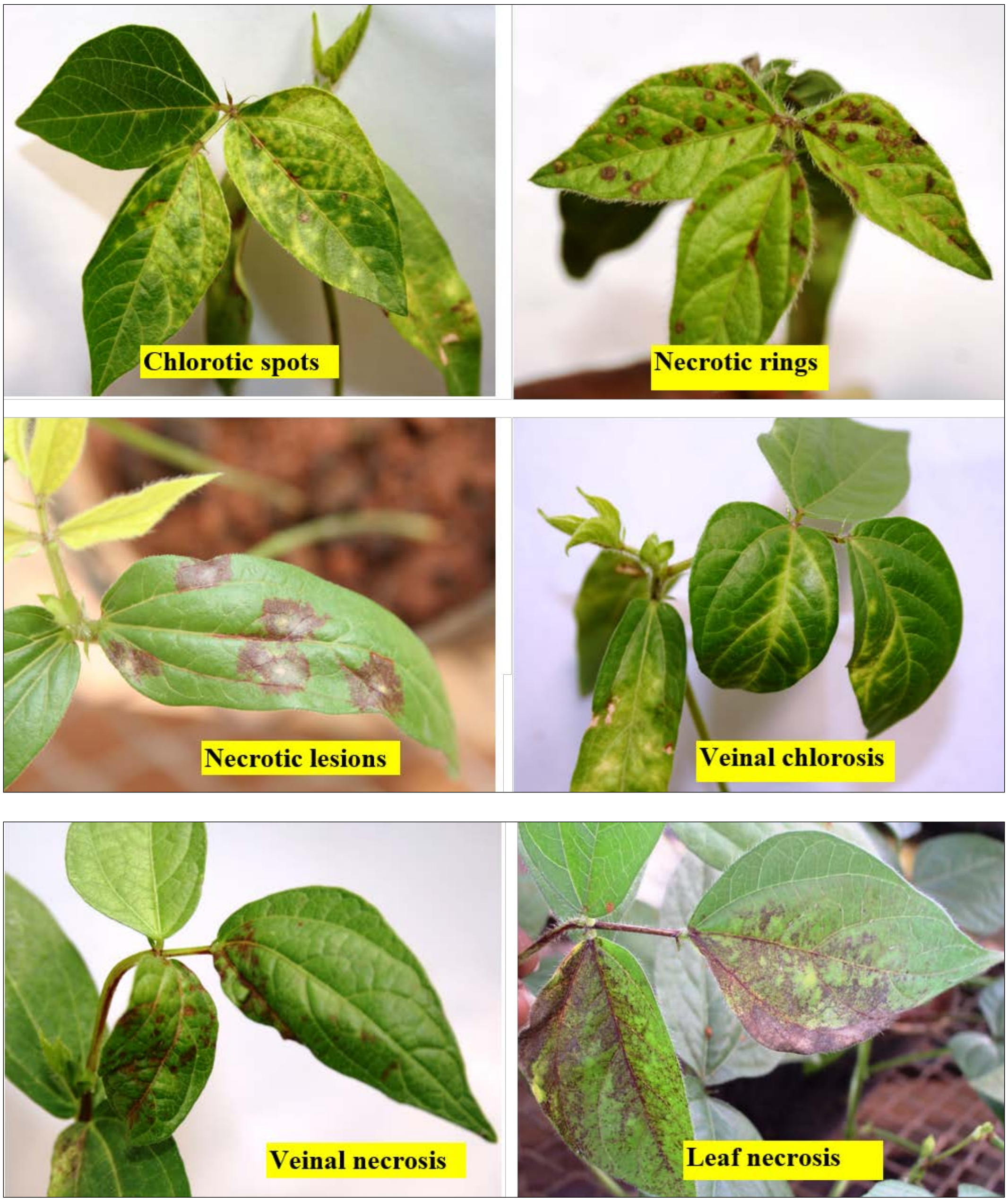


Fig 3: Symptoms induced by PBNV in greengram upon mechanical inoculation under glasshouse conditions.

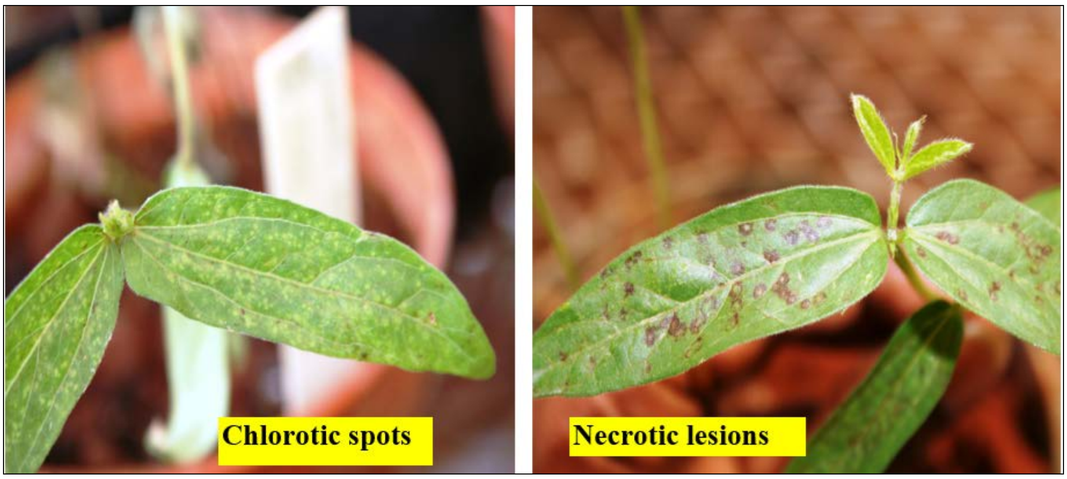


Fig 4: Symptoms induced by TSV in green gram upon mechanical inoculation under glasshouse conditions.

Table 1: Artificial screening of blackgram genotypes by mechanical inoculation for resistance to PBNV and TSV under glasshouse conditions.

S. No.	Cultivar/ variety	PBNV						TSV					
		Inf/TP[a]	PDI	D/TP[b]	%M[c]	Local	Systemic	Inf/TP[a]	PDI	D/TP[b]	%M[c]	Local	Systemic
1	LBG-15	9/10	90.0	0/10	0	NL	VN	10/10	100.0	5/10	50	CR,NR	SL,S, MM
2	-17	9/9	100.0	0/9	0	NL	VN	10/10	100.0	2/10	20	CL,NL	VN, SL, S
3	-20	10/10	100.0	0/10	0	NL, VN	VN	10/10	100.0	7/10	70	CL,NL,VN	N,SL,S,M,SN
4	-22	10/10	100.0	0/10	0	NL, VN	VN, LC	9/9	100.0	7/9	77.7	CCR, NR	AN,SL,S,SN
5	-170	9/10	90.0	0/10	0	NL	VN, LC	10/10	100.0	2/10	20	CL, NL	N,SL,S,M
6	-611	10/10	100.0	0/10	0	NL, VN	VN, LC	10/10	100.0	7/10	70	CCR. NR	N,M,AN,SN
7	-623	10/10	100	0/10	0	VN	VN, LC	10/10	100.0	6/10	60	CCR, NR	AN,VN
8	-648	9/9	100	0/9	0	CL,NL,VN	N, VN,LC	9/9	100.0	2/9	22.2	CL, NL, VN	VN,SL,S,M,AN
9	-685	9/10	90.0	0/10	0	NL	VN, N	10/10	100.0	5/10	50	CL, NL, VN	VN,SL,S,M
10	-693	9/10	90.0	0/10	0	NL	VN, LC	10/10	100.0	5/10	50	CCR,VN	AN,VN,SN
11	-709	8/8	100.0	0/8	0	NL, VN	VN,LC,CS	8/8	100.0	1/8	12.5	CL, NL	AN, VN
12	-726	9/10	90.0	1/10	10	NL	VN, LC	10/10	100.0	8/10	80	CCR, VN	AN, VN, SN
13	-727	10/10	100.0	0/10	0	NL	N,VN,LC	10/10	100.0	0/9	0	CL,NL	SL, S, M
14	-728	9/9	100.0	0/9	0	CL, NL	VN	10/10	100.0	1/10	10	CL, NL, VN	SL, S, M
15	-729	10/10	100.0	0/10	0	NL, VN	N,LC,CL	10/10	100.0	3/10	30	CL, NL, VN	AN,SL,S,M
16	-730	8/10	80.0	0/10	0	NL	NL	10/10	100.0	1/10	10	CL, NL	AN,SL,S,M
17	-731	10/10	100.0	0/10	0	CL, NL	VN, N	10/10	100.0	0/7	0	CL, NL	SL,S,M
18	-733	7/7	100.0	0/7	0	NL, VN	VN, N	10/10	100.0	1/10	10	NL, VN	AN,SL,S,M
19	-734	9/10	90.0	0/10	0	CL, NL	VN, N	10/10	100.0	1/10	10	CL, NL	AN,SN,
20	-735	8/10	80.0	0/10	0	CL, NL	VN, CS	10/10	100.0	1/10	10	CL,NL	AN, SL, S
21	-738	8/9	88.8	0/9	0	CL, NL	VN	10/10	100.0	7/10	70	CL, NL	VN,AN,SN
22	-741	10/10	100.0	0/10	0	NL, VN	VN, LC	10/10	100.0	1/10	10	NL, VN	SL,S,M
23	-744	10/10	100.0	0/10	0	NL,CL	N,CS	9/9	100.0	4/9	44.4	CL,NL,VN	AN,SN
24	-745	10/10	100.0	0/10	0	CL, NL	N	10/10	100.0	5/10	50	CL, NL	AN,SL,S,M
25	-746	8/9	88.8	0/9	0	CL, NL	VN, LC	10/10	100.0	2/10	20	CL, NL	AN,SL,S,M
26	-748	10/10	100.0	0/10	0	CL, NL	N	10/10	100.0	5/10	50	CL, NL	AN,AL,S,M
27	-750	9/10	90.0	0/10	0	NL, VN	VN,LC	8/8	100.0	1/8	12.5	NL, VN	AN,SL,S,M
28	-751	10/10	100.0	0/10	0	CCR, NL	N	10/10	100.0	3/10	30	CCR, NL	M, N, AN
29	-752	8/8	100.0	0/8	0	CL, NL	VN,LC,CS	10/10	100.0	2/10	20	CL,NL	VN, AN
30	-753	10/10	100.0	0/10	0	CL, NL	VN,LC	9/9	100.0	3/9	33.3	CL, NL	VN, AN
31	-754	8/8	100.0	0/8	0	NL, CL	N,LC	10/10	100.0	3/10	30	CL,NL,VN	AN,SL,S,M
32	-755	8/10	80.0	0/10	0	NL	VN, LC	10/10	100.0	8/10	80	CCR, NCR	AN,SN
33	-756	9/10	90.0	0/10	0	NL	VN	10/10	100.0	7/10	70	CL, NL	AN, SN
34	TAU-1	10/10	100.0	0/10	0	VN	VN,N,LC	9/9	100.0	3/9	33.3	CCR, NL	AN, SL, S
35	Pant-U- 31	9/9	100.0	0/9	0	NL	VN,N,LC	10/10	100.0	2/10	20	CL, NL, VN	AN, SL, S
36	99-U-19	8/10	80.0	0/10	0	VN, NL	VN,N,LC	10/10	100.0	4/10	40	CL, NL, VN	AN,SL,S,M
37	99-U-21	10/10	100.0	0/10	0	NL	VN,N,LC	10/10	100.0	2/10	20	CL,NL,VN	AN,SL,S,M
38	Berham	9/9	100.0	0/9	0	NL	VN,N,LC	9/9	100.0	6/9	66.6	CL, NL	AN, SN
39	T-9	9/10	90.0	0/10	0	NL, VN	VN,N,LC	10/10	100.0	4/10	40	CCR,CL,NL	AN,SL,S,M
40	Sarala	10/10	100.0	0/10	0	NL, VN	VN,LC,CS,NS	10/10	100.0	6/10	60	CCR,NCR,VN	N, AN, VN

a- Infected / Total plants, b- Dead / Total plants, c- Per cent mortality
CR-chlorotic rings, NR-necrotic rings, NCR-necrotic concentric rings, CCR-chlorotic concentric rings, NL-necrotic lesions, CL-chlorotic lesions, SL-smalling of leaves, M-mosaic, SN-stem necrosis, AN-apical necrosis, LC-leaf curl, CS-chlorotic spots, NS-necrotic spots, VC-veinal chlorosis

Table 2: Artificial screening of greengram genotypes by mechanical inoculation for resistance to PBNV and TSV under glass- house conditions.

S. No.	Cultivar/ variety	PBNV						TSV					
		Inf/TP[a]	PDI	D/TP[b]	%M[c]	Local	Systemic	Inf/TP[a]	PDI	D/TP[b]	%M[c]	Local	Systemic
1	LGG-407	10/10	100.0	0/10	0	CL, NL	VN, N, LC	10/10	100.0	9/10	90	CCR,NCR,	AN
2	-410	9/9	100.0	1/9	11.1	CL, NL	VB, N, CL	10/10	100.0	4/10	40	CCR, NCR	VN, AN
3	-458	10/10	100.0	0/10	0	CL, NL	VN, N, CS	9/9	100.0	4/9	44.4	CCR, NCR	AN
4	-460	9/9	100.0	1/9	11.1	CL	NS,VN, CL	10/10	100.0	2/10	20	CR, NR	AN, VN
5	-469	10/10	100.0	0/10	0	CL, NL	VN,NS, LC	9/9	100.0	5/9	55.5	NL	AN, SN
6	-470	9/10	90.0	0/10	0	CL, NL	VN, CS	8/8	100.0	2/8	25	NL, CL	VN, SN, AN
7	-473	9/10	90.0	0/10	0	CL, NL	NS, VN	10/10	100.0	4/10	40	CL, NL	NL,CL,S,AN
8	-474	10/10	100.0	0/10	0	CL, NL	VN, CS	10/10	100.0	2/10	20	NL	VN, CL, AN
9	-476	10/10	100.0	0/10	0	CL, NL	CL,VN, LC	10/10	100.0	2/10	20	NL	AN, SN
10	-479	8/10	80.0	0/10	0	CL	VC, CS	9/9	100.0	3/9	33.3	CCR	AN
11	-480	10/10	100.0	0/10	0	CL, NL	VN, LC	10/10	100.0	6/10	60	NL	AN, SN
12	-481	9/10	90.0	0/10	0	CL, NL	VN, C	7/7	100.0	4/7	57.1	CL, NL	AN, SN
13	-485	9/9	100.0	0/9	0	CL, NL	CL NL, VC	10/10	100.0	6/10	60	CL, NL	VN, CL, AN
14	-487	10/10	100.0	0/10	0	CL, NL	CL, VN	9/9	100.0	4/9	44.4	CL, NL	VN, SN
15	-489	9/10	90.0	0/10	0	CL, NL	VN, N, LC	9/9	100.0	3/9	33.3	CL, NL	SN, AN
16	-490	8/8	100.0	2/8	25	CL, NL	VC, CL	10/10	100.0	0/10	0	CL, NL	VN, AN
17	-491	10/10	100.0	0/10	0	CL, NL	VB, N	9/9	100.0	2/9	22.2	NL	AN
18	-495	9/9	100.0	1/9	11.1	CL	VC, CS	10/10	100.0	5/10	50	CL, NL	VN, AN
19	-496	10/10	100.0	1/10	10	CL, NL	C, VN, VC	9/9	100.0	8/9	88.8	NL	AN
20	497	8/10	80.0	0/10	0	CL, NL	VN, N, LC	10/10	100.0	5/10	50	CL, NL	VN, AN
21	-499	10/10	100.0	0/10	0	CL, NL	VN, LC	9/9	100.0	9/9	100	NL	VN, AN
22	-500	9/9	100.0	0/9	0	CL, NL	VN, LC	10/10	100.0	7/10	70	NL, CR	VN, AN
23	-503	9/10	90.0	0/10	0	CL, NL	VN, N	8/8	100.0	8/8	100	NL	AN
24	-505	10/10	100.0	1/10	10	CL, NL	CS, N	10/10	100.0	10/10	100	NL	AN
25	WGG-37	8/8	100.0	0/8	0	CL, NL	VN, N	10/10	100.0	10/10	100	NL	AN
26	OBGG-35	9/10	90.0	0/10	0	CL, NL	VN, N	9/9	100.0	1/9	11.1	CCR,CL,NL	AN
27	TRAM-1C	9/9	100.0	1/9	11.1	CL, NL	VC, VN, N	10/10	100.0	5/10	50	CL, NL	VN, AN
28	TRAM-5	9/9	100.0	1/9	11.1	CL, NL	VN, NS	10/10	100.0	5/10	50	CL, NL	VN, AN
29	ML-267	10/10	100.0	0/10	0	CL, NL	VN, LC	9/9	100.0	4/9	44.4	NL	AN
30	OUM-115	9/10	90.0	0/10	0	CL, NL	VN, LC	9/9	100.0	3/9	33.3	NL	AN, SN
31	TM-96-2	9/10	90.0	1/10	10	CL, NL	VN, N	10/10	100.0	4/10	40	NL	AN, SN
32	K-851	10/10	100.0	1/10	10	CL, NL	VN, LC	10/10	100.0	8/10	80	NL	AN, SN
33	Kopergaon	10/10	100.0	2/10	20	CL, NL	VN, LC	9/9	100.0	9/9	90	CCR, NCR	AN
34	Dhowli	9/9	100.0	1/9	11.1	CL, NL	VN, N, LC	10/10	100.0	4/10	40	CL, NL	VN, AN

a- Infected / Total plants, b- Dead / Total plants, c- Per cent mortality
CR-chlorotic rings, NR-necrotic rings, NCR-necrotic concentric rings, CCR-chlorotic concentric rings, NL-necrotic lesions, CL-chlorotic lesions, SL-smalling of leaves, M-mosaic, SN-stem necrosis, AN-apical necrosis, LC-leaf curl, CS-chlorotic spots, NS-necrotic spots, Vc-veinal chlorosis

Discussion

Glasshouse screening was planned to confirm field resistant germplasm by mechanical sap inoculations. However, all the greengram or blackgram germplasm screened under field conditions showed susceptible reactions. As planned originally, selected germplasm lines of blackgram and greengram were screened by mechanical sap inoculation. The artificial TSWV inoculation method most closely resembles natural infection transmission by thrips (Rosello *et al*., 1999) [10]. Among the three inoculation methods *viz.*, grafting, rubbing inoculation and high-pressure spray, evaluated for their effectiveness in screening groundnut genotypes for resistance to PBNV. Rubbing inoculation method was effective under glasshouse conditions, yielding consistently high percentage of infected plants and inoculation by rubbing twice tended to give a slightly higher percentage of infected plants than a single inoculation (Pensuk *et al*., 2002) [7].

In the present study, the genotypes of blackgram and greengram screened under glasshouse conditions by mechanical inoculation with PBNV and TSV showed hundred per cent disease incidence while varied per cent mortality was recorded with genotype as well as virus. PBNV sap inoculations on blackgram showed zero per cent mortality in all the genotypes except in LBG-726 (10%) whereas TSV inoculations showed per cent mortality ranging from 0-80. LBG-731 and LBG-727 showed zero per cent mortality while LBG-726 and LBG-755 showed maximum mortality (80%). In greengram, the per cent mortality ranged from 0-20 with PBNV and 0-100 with TSV inoculations. Majority of the genotypes inoculated with PBNV showed zero per cent mortality. Whereas 10% mortality was observed in LGG-410, -460, -495, -496, -505, TRAM-1C, TRAM-5, TM-96-2, K-851 and Dhowli. Maximum per cent mortality (20%) was recorded in Kopergoan and LGG-490. TSV inoculations showed per cent mortality ranging from 0-100 in LGG-490 and LGG-505 and WGG-37. These results are complementing with results of field screening under natural conditions showing no resistance among the blackgram and greengram genotypes screened. Similar works have been done by different workers in different hosts. Among 32 urdbean and 30 mungbean genotypes tested, none were found to be resistant upon mechanical inoculations under laboratory conditions except LGG-458 with less than 10% infection (Prasada Rao, 2003) [8], whereas in the present study, LGG-458 was found to be highly susceptible upon mechanical inoculation. Buiel (1993) [3] reported that ICGV 86363, ICGV 86430 and ICGV 86029 were partially resistant to the PBNV and ICGV 86598, 21928 (50) and ICGV 86030 were partially resistant to vector, *T. palmi* when screened both in field and laboratory conditions. Groundnut cultivars and advanced breeding lines (150) evaluated for TSV were found to be susceptible. Three breeding lines *viz.*, ICGV # 92267, 99029 and 01276 showed delayed symptom expression but they succumbed under high disease pressure (Prasadarao *et al*., 2003c; Kalyani *et al*., 2005) [5]. ICGV 86031 and ICGV 86388 showed resistance to PBNV when mechanically sap inoculated with low virus concentration (10^{-2}) (Dwivedi *et al*., 1995) [4]. Hundred per cent disease incidence in glasshouse screening can be due to the fact that there is no chance of escaping from the virus. Whereas under field conditions, there are chances to escape from viruliferous thrips in case of PBNV and escape either from lodging of TSV infected pollen or thrip visit or both.

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