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Reaction of Groundnut Advanced Breeding lines to Groundnut Bud Necrosis Disease

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A B S T R A C T

Forty advanced breeding lines were evaluated for reaction to *Groundnut bud necrosis orthotospovirus* (GBNV) in the field and greenhouse in Hyderabad, India during2013 rainy season. Results from natural infection showed eight resistant, 24 moderately resistant and eight moderately susceptible genotypes. There were no genotypes pertaining to highly resistant, susceptible and highly susceptible disease reaction grade. Greenhouse screening with mechanical sap inoculation showed all genotypes highly susceptible at 1:10 infected virus extract dilution, whereas at 1:100, two genotypes were moderately resistant, four moderately susceptible, ten susceptible and 24 highly susceptible. There were no genotypes pertaining to highly resistant and resistant disease reaction grade even at 1:100 infected virus extract dilution.

Introduction

Groundnut is one of the most important food legumes grown in subsistence and commercial farming throughout the tropical, sub-tropical and warm temperate regions of the world (Nwokolo, 1996), with an annual world production of 41.19 Mt from 24.71 Mha (FAOSTAT, 2014). Predominantly rainfed cultivation in marginal landsof many Asian and sub-Saharan African countries, poor seed systems, and the occurrence of many economically important insect pests, fungal diseases, and viral diseases at different stages of crop growth are primary factors responsible for low yields in groundnut (Reddy *et al.*, 1992).

GBNV belongs to family *Tospoviridae* and responsible for causing Groundnut Bud Necrosis Disease (GBND) in groundnut (Reddy, 1991). GBNV is an economically important *Tospovirus* and its distribution is confined to South and Southeast Asian countries namely China, India, Nepal, Pakistan, Sri Lanka and Thailand (Dwivedi *et al.*, 1995). The name *Tospovirus* (*renamed Orthotospovirus*) (Adams *et al.*, 2017, Briese *et al.*, 2016*)* was given after the discovery of *Tomato spotted wilt virus* (TSWV) in Australia in 1915.The disease was first recorded in India at Indian Agricultural Research Institute in 1949 (Reddy *et al.*,

1995). GBND in India until 1990 was reported to be caused by TSWV. Serological comparisons and sequencing of nucleic acids revealed the existence of several distinct Tospoviruses and GBNV was found to be serologically distinct from other Tospoviruses such as TSWV and *Impatiens necrotic spot orthotospovirus* (INSV) (Reddy *et al.,* 1995). This virus is mechanically transmissible, but in nature, it is transmitted by the vector *Thrips palmi* in persistent manner (Vijayalakshmi, 1995).

Symptoms initially appear on young quadrifoliates as mild chlorotic mottle or spots, which develop into necrotic or chlorotic rings and streaks. This is followed by death of terminal bud. Secondary symptoms are stunting, auxiliary shoot proliferation, and malformation of leaflets (Reddy *et al.,* 1995). However, the symptomatology varies depending on the strain, host species and genotype, and is also influenced by environmental factors such as temperature.

Thrips-transmitted Tospoviruses cause significant losses in yield and quality of produce from vegetable, legume and ornamental crops in many parts of the world (Mumford *et al.,* 1996; Pappu, 1997; Pearce, 2005; Persley *et al.,* 2006). GBND became economically important during the late 1960's when incidences up to 100% were recorded in many groundnut growing regions of the country. Incidence of GBND ranging from 5 to 80%, and yield losses of up to 50%, worth more than $89 million in India alone, have been reported (APS, 2013). Substantial decrease in plant stand occurs, during infection at early stages of crop growth leading to considerable yield losses, but infection at later stages may still cause significant losses in the yield and quality of produce (Culbreath *et al.,* 2003).

In India, 80% of groundnut sowing is taken up in *kharif* season (June-September) and sometimes with late onset of monsoon, July-August sowings are usually in practice. Maximum thrips populations were observed from 2nd week of July to end of August resulting in complete crop loss (Vijayalakshmi, 1995). There is no practically feasible control measure currently available for GBNV in groundnut. However, by using certain cultural practices such as adjustment of planting date coinciding with low levels of thrips activity, intercropping with fast growing cereals (Reddy *et al.,* 2000) and close planting (Basu, 1995; Buiel and Parlevliet, 1996; Wongkaew, 1995), the disease incidence can be reduced. Control of this virus disease through crop rotation and removal of alternate weed hosts have met with limited success (Rao *et al.,* 2013). Efforts to control vector with insecticides have been mostly unsuccessful. Indiscriminate use of insecticides is leading to the development of resistance in vector. In this context, genetic resistance remains the most economical method for the resource poor farmers. So far, the released varieties are found to be susceptible to GBND. Identification of GBND resistant sources in newly developed advanced breeding lines which are agronomically superior would help in recommending and release of these genotypes for GBND endemic locations. Keeping in view the economic importance of the disease in most of the groundnut growing areas and lack of available resistance sources to GBND, present work has been taken up.

Materials and Methods

Field screening

During *kharif* 2013, 40 advanced breeding lines along with a resistant check ICGV 86031 and known susceptible check JL 24 were sown in a replicated field trial using a Alpha Lattice Design on the ICRISAT farm at Patancheru, India. Seeds were pre-treated with Thiram (dimethyldithiocarbamate) to

prevent from any seedborne and soilborne fungal infections. Field lay-out consists of three rows of 4 m length with a row to row spacing of 60 cm and plant to plant spacing of 25 cm within the rows for each line. GBNV susceptible check, JL 24 was planted all around the field to create epiphytotic conditions. Recommended package of practices was followed to raise the crop and to promote a natural infection of GBNV. The crop was not sprayed with any insecticide to encourage thrips movement and infestation. The reaction of entries under field conditions was assessed by recording the disease incidence and disease severity at fortnightly intervals, starting from 30 days after sowing (DAS) to 90 DAS. The test genotypes were grouped into six distinct groups using 0-5 scale (Sunkad *et al.,* 2000) based on disease incidence. These include highly resistant (0 to 1.0%); resistant (1.1to 5.0%); moderately resistant (5.1to 10.0%); moderately susceptible (10.1to 25.0%); susceptible (25.1to 50.0%); highly susceptible (50.1 and above). Disease severity (DS) score of 1-5 were also given by randomly tagging five plants per treatment 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 distortion and stunting, and 5 = plants showing severe necrosis and stunting (Pensuk *et al.,* 2002).

Greenhouse screening

GBNV (ICRISAT isolate) maintained on groundnut plants was used for preparation of the inoculum. In *kharif* 2013, the same 40 genotypes were also evaluated for GBNV resistance by mechanical inoculation (using a 10^{-1} and 10^{-2} dilution of infected plant extract) under controlled greenhouse conditions. The plants were raised in plastic pots (5" diameter) @ 3 plants pot^{-1}. Each genotype was grown in three replications, six plants (two pots) per replication. Virus inoculum was freshly prepared from the infected leaves of groundnut ground in a chilled mortar and pestle using phosphate buffer (0.05M, pH 7.0) @ 1:10 (w/v) and 1:100 (w/v). The virus inoculum was rubbed onto all of the opened leaves of 8 to 10 day old test seedlings and rinsed with deionised water. All the pots were maintained at 25^{o}Cand 75% RH in a controlled greenhouse for uniform infection. The observations that were recorded included disease incidence and disease severity as described earlier.

Enzyme Linked Immunosorbent Assay (ELISA)

Direct antigen coating (DAC) ELISA was carried out to detect the presence of GBNV in all the test genotypes that were challenged with the virus in greenhouse experiments, and for the confirmation of natural infection of plants in field experiment studies as suggested previously (Hobbs *et al.,* 1987).

Greenhouse maintained GBNV (ICRISAT isolate) on groundnut served as known positive control and healthy leaves of groundnut as healthy control. All leaf samples were ground using carbonate buffer, 0.01M, pH 9.6 with sodium diethyl dithiocarbamate (DIECA) as antioxidant. Polyclonal antiserum of GBNV with 1:20,000 dilution was used. ALP-labelled anti rabbit IgG was added at a dilution of 1:5000 and absorbance values at 405 nm were measured using 'Bio RAD iMark' ELISA reader after 30 min. of reaction. The readings were considered positive if they were five times more than the healthy samples (-ve control).

Statistical analysis

ANOVA was performed using PROC MIX SAS 9.3 software (SAS Institute Inc., Cary, NC, USA) to determine the difference in disease incidence and severity data collected in field experiment.

Results and Discussion

Screening for field resistance to GBND

Disease incidence

The average GBND incidence in the tested genotypes ranged from 2.57 to 22.71 % compared to 4.04 % in ICGV 86031(resistant check) and 25.45 % in JL 24 (susceptible check) (Table 1). With regard to per cent GBND incidence in the field, four genotypes *viz.,* ICGV 07220 (2.57 %), ICGV 00350 (2.64 %), ICGV 00351(3.36 %), ICGV 00211 (4.02 %) were found to be resistant and significantly superior to the resistant check ICGV 86031 (4.04 %). Out of the 40 genotypes tested, eight genotypes *viz.,* ICGV 00201, 00211, 86699, 03042, 07220, 06146, 00350 and ICGV 00351 were resistant (disease incidence of 2.57 to 4.99 %). Twenty four genotypes *viz.,* ICGV 00187, 00189, 00191, 00202, 00203, 00206, 00213, 00241, 00246, 00247, 03057, 06100, 07222, 05155, 02266, 87846, 00348, 93260, 93261, 89280, 92195, 92035, ICGS 76 and ICR 48 were moderately resistant (5.13 to 9.93 %). Eight genotypes *viz.,* ICGV 99058, 99072, 00162, 86590, 91114, 00308, 93468 and ICGS 44, were moderately susceptible (10.21 to 22.71 %). There were no genotypes pertaining to highly resistant, susceptible and highly susceptible disease reaction grade.

Disease severity

The average GBND disease severity in these genotypes ranged from 1.99 to 4.32 compared to 2.33 in ICGV 86031 (resistant check) and 4.67 in JL 24 (susceptible check). The genotypes ICGV 00187 (2.00), ICGV 00191 (2.00), ICGV 00201 (1.99), ICGV 00202 (2.00), ICGV 00206 (2.00), ICGV 00211 (2.00), ICGV 00213 (2.00), ICGV 00247 (1.99), ICGV 86699 (2.01), ICGV 07222 (2.01), ICGV 07220 (2.00), ICGV 06146 (1.99) and ICGV 87846 (2.00) showed less disease severity compared to resistant check ICGV 86031 (2.33). Of all the genotypes tested, none of them showed high disease severity compared to susceptible check JL 24 (4.67) indicating the superiority of JL 24 as susceptible check. The disease severity was in the range of 1.99 - 3.02 in resistant genotypes, 1.99 - 4.01 in moderately resistant genotypes and 2.66 - 4.32 in moderately susceptible genotypes.

DAC-ELISA

Leaf samples of few genotypes showing resistant, moderately resistant and moderately susceptible disease reaction were randomly collected, along with resistant (ICGV 86031) and susceptible (JL 24) check and the samples were subjected to ELISA test for further confirmation of field reaction. The resistant genotypes *viz.,* ICGV 03042, 00350 and ICGV 00351 gave negative reaction to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.157 - 0.354 confirming their resistant reaction grade. The moderately resistant genotypes *viz.,* ICGV 00187, 00189, 00213, 00241, 05155, 02266, 93261, 89280, 92195, 92035 and ICR 48 gave 16.66 to 66.66 % infection with GBNV antiserum and the absorbance values at 405 nm was in the range of 0.137 - 2.910 confirming their moderately resistant reaction.

The moderately susceptible genotypes *viz.,* ICGV 99058, 99072, 00162, 86590, 91114, 00308, 93468 and ICGS 44 gave 100 % infection with GBNV antiserum and the absorbance values was in the range of 1.669 - 3.427 confirming their moderately susceptible reaction. The genotypes ICGV 86031 (resistant check) and JL 24 (susceptible check) gave zero and 100 % infection respectively with GBNV antiserum which was in conformity with their disease reaction under field conditions.

Screening for resistance to vector and virus

Disease incidence

The average disease incidence at 1:10 virus concentration ranged from 64.71 to 100 % compared to 72.22 % in ICGV 86031 (resistant check) and 94.44 % in JL 24 (susceptible check) at 21 DAI (Table 2).

The average disease incidence at 1:100 virus concentration ranged from 5.56 to 100 % compared to 26.67 in ICGV 86031 (resistant check) and 77.78 % in JL 24 (susceptible check) (Table 3).

The data revealed that out of the 40 genotypes tested at 1:100 dilution, two genotypes *viz.*, ICGV 00213, 06146were moderately resistant (disease incidence of 5.56 and 7.14 %), four genotypes *viz.*, ICGV 03057,07222, 07220 and ICGS 76 were moderately susceptible (11.11 – 25 %), ten genotypes *viz.*, ICGV 00187, 00191, 00202, 00203, 03042, 06100, 05155, 93260, ICGS 44 and ICR 48 were susceptible (26.67 – 50 %) and twenty four genotype *viz.*, ICGV 99058, 99072, 00162, 00189, 00201, 00206, 00211, 00241, 00246, 00247, 86590, 86699,91114, 00308, 02266, 87846, 93468, 00348, 00350, 00351, 93261, 89280, 92195 and ICGV 92035 were highly susceptible (52.94 – 100 %).

There were no genotypes pertaining to highly resistant and resistant disease reaction grade.

Disease severity

The average GBND disease severity in these genotypes at 1:10 virus concentration ranged from 2 to 5 compared to 4 in ICGV 86031 (resistant check) and 5 in JL 24 (susceptible check). At 1:100 virus concentration disease severity ranged from 2 to 4 compared to 2 in ICGV 86031 (resistant check) and 4 in JL 24 (susceptible check).

DAC-ELISA

The genotypes showing moderately resistant, moderately susceptible and susceptible reaction at 1:100 dilution of virus concentration were selected for ELISA test. ICGV 86031 (resistant check) and JL 24 (susceptible check) at 1:10 and 1:100 dilution of virus concentration were also tested by ELISA. The moderately resistant genotypes *viz.*, ICGV 00213 and ICGV 06146 gave positive reaction with 6.11 and 28.57 % infection with GBNV antiserum and the absorbance values at 405 nm was in the range of 0.090 – 1.624 confirming their moderately resistant reaction grade. The moderately susceptible genotypes *viz.*, ICGV 03057, 07222, 07220 and ICGS 76 gave positive reaction with 12.5 - 50 % infection with GBNV antiserum and the absorbance values at 405 nm was in the range of 0.100 – 1.841 confirming their moderately susceptible reaction grade. The susceptible genotypes *viz.*, ICGV 00187, 00191, 00202, 00203, 03042, 06100, 05155, 93260, ICGS 44 and ICR 48 gave positive reaction with 73.33 – 93.75 % incidence to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.094 – 1.941 confirming their susceptible reaction grade. The resistant check ICGV 86031 at 1:10 virus concentration and 1:100 virus concentration gave positive reaction with 93.33 and 38.09 % infection to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.407 - 2.559 and 0.088 - 1.820 respectively. The susceptible check JL 24 at 1:10 virus concentration and 1:100 virus concentration gave positive reaction with 100 and 85.71% infection to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.593 - 2.218 and 0.397 –2.129 respectively.

The typical symptoms of GBNV such as chlorotic or necrotic spots on leaves, thrips

injury on leaves, severe chlorosis of top leaves, bushy and stunted growth, severe necrosis and death of bud subsequently death of plants along with vector *T. palmi* was observed during 30 - 60 DAS. Significant differences in disease incidence were observed at different stages of the crop. Although, there were significant differences in disease incidence among genotypes at 30 DAS, some of the resistant lines could not be differentiated from susceptible lines. The mean disease incidence was low at 30 DAS and reached peak levels at 60 DAS when the crop was at flowering. The young plants are more succulent and attract the thrips for feeding. Thereafter, constant or gradual increase in disease incidence was observed at senescence stage. In natural conditions, the decrease in susceptibility of the plant with the age of the crop may be due to increase in resistance of plants to the virus infection. Significant differences in *T. palmi* populations at different stages of green gram crop were reported (Sreekanth *et al.,* 2002).

Low population (15.6) was observed at 15 DAS and thereafter increased progressively up to 45 DAS to reach higher levels (72.1). At 60 DAS, population dwindled to lower levels (17.1) almost similar to the levels at 15 DAS. Since assessment at 45 and 60 DAS for disease incidence clearly differentiated groundnut genotypes for resistance to GBND, the appropriate time for assessment could be considered by the magnitude of genotypic variations in disease incidence.

Significant difference in disease incidence was found between genotypes ICGV 91114 and ICGV 99058, 99072, 00162, 86590, 00308, 93468, ICGS 44. This might be due to difference in genetic makeup and leaf characters such as hairiness, glossy, smooth etc. that resist the vector feeding on them and subsequent block in movement of virus once it enters the plant. The genotypes with thick leaves, glossiness and hairiness showed less disease incidence compared to genotypes having thin, smooth and non-glossy leaves.

In our study, late sowing of the genotypes fairly coincided with the reasonably high vector populations. Yet, our findings indicate that low disease incidence in these genotypes is due to their superiority in curtailing the thrips feeding and subsequently disease incidence. Field resistant varieties reported here are not immune to the disease but have reduced disease incidence under field conditions. Resistance in these genotypes might be due to non-preference by the thrips vector and/or resistance to GBNV infection or multiplication and spread. Similar findings were opinedby (Amin 1985) that resistance in case of groundnut cv Robut 33-1 is due to resistance to the vector, perhaps combined with resistance or tolerance to GBNV.

Resistant genotypes reduced the rate of epidemic development with considerable reduction in the incidence of GBNV (Culbreath *et al.*, 1993; Buiel and Parlevliet, 1996). So, the genotypes showing high resistance or resistance response could be used as seed material after screening of genotypes further in different trials.

All the genotypes were highly susceptible to GBNV at higher virus concentration (1:10 dilution of the infected tissue). Previous reports were also indicated the same (Rao *et al.,* 2006, Dwivedi *et al.,* 1995). The genotypes ICGV 00213, 03057, 07220, 06146, ICGS 76 and ICR 48 showed no disease incidence at 7 DAI for both 1:10 and 1:100 virus concentrations indicating their longer incubation period. At 1:10 virus concentration, due to high disease pressure these genotypes showed highly susceptible disease reaction at 21 DAI. At 1:100 virus concentrations, these genotypes showed moderately resistant and moderately susceptible disease reaction except ICR 48 which showed susceptible disease reaction.

Table.1 Disease incidence of groundnut advanced breeding lines for their natural reaction to GBNV infection under field conditions during *kharif* 2013, at ICRISAT, Patancheru

S. No.	Genotype	Per cent Disease Incidence* at				
		30DAS	45DAS	60DAS	75DAS	90DAS
1	ICGV 99058	4.22 (R)	9.32(MR)	11.49(MS)	11.49(MS)	11.49(MS)
2	ICGV 99072	3.95(R)	5.59(MR)	10.65(MS)	10.65(MS)	10.65(MS)
3	ICGV 00162	4.22(R)	6.93(MR)	9.01(MR)	10.75(MS)	11.44(MS)
4	ICGV 00187	0.86(HR)	4.36(R)	6.99(MR)	6.99(MR)	6.99(MR)
5	ICGV 00189	2.42(R)	2.42(R)	6.36(MR)	7.84(MR)	8.58(MR)
6	ICGV 00191	0.66(HR)	4.30(R)	5.89(MR)	6.72(MR)	6.72(MR)
7	ICGV 00201	1.45(R)	3.57(R)	4.99(R)	4.99(R)	4.99(R)
8	ICGV 00202	1.60(R)	5.22(MR)	5.91(MR)	5.91(MR)	6.61(MR)
9	ICGV 00203	0.84(HR)	3.42(R)	5.13(MR)	5.13(MR)	5.13(MR)
10	ICGV 00206	0.03(HR)	2.60(R)	3.65(R)	5.52(MR)	6.56(MR)
11	ICGV 00211	0.81(HR)	1.58(R)	4.02(R)	4.02(R)	4.02(R)
12	ICGV 00213	1.47(R)	4.38(R)	5.93(MR)	5.93(MR)	5.93(MR)
13	ICGV 00241	1.79(R)	4.21(R)	6.34(MR)	7.35(MR)	7.35(MR)
14	ICGV 00246	4.04(R)	6.17(MR)	7.07(MR)	7.07(MR)	7.07(MR)
15	ICGV 00247	2.40(R)	5.53(MR)	7.07(MR)	7.07(MR)	7.07(MR)
16	ICGV 86590	6.38(MR)	9.58(MR)	9.58(MR)	10.23(MS)	10.23(MS)
17	ICGV 86699	0.63(HR)	2.48(R)	3.10(R)	4.33(R)	4.33(R)
18	ICGV 91114	7.98(MR)	19.09(MS)	22.71(MS)	22.71(MS)	22.71(MS)
19	ICGV 00308	3.82(R)	10.72(MS)	10.72(MS)	10.72(MS)	10.72(MS)
20	ICGV 03042	2.08(R)	4.20(R)	4.20(R)	4.92(R)	4.92(R)
21	ICGV 03057	3.34(R)	5.03(MR)	5.71(MR)	5.71(MR)	5.71(MR)
22	ICGV 06100	2.59(R)	4.14(R)	4.92(R)	5.79(MR)	6.67(MR)
23	ICGV 07222	0.71(HR)	3.07(R)	6.04(MR)	6.04(MR)	6.04(MR)
24	ICGV 07220	0.63(HR)	1.25(R)	1.89(R)	2.57(R)	2.57(R)
25	ICGV 05155	2.09(R)	4.40(R)	5.04(MR)	5.87(MR)	6.71(MR)
26	ICGV 06146	1.40(R)	2.18(R)	3.63(R)	4.31(R)	4.31(R)
27	ICGV 02266	3.80(R)	6.94(MR)	6.94(MR)	7.57(MR)	8.20(MR)
28	ICGV 87846	1.22(R)	4.34(R)	6.21(MR)	6.21(MR)	6.21(MR)
29	ICGV 93468	4.09(R)	11.75(MS)	13.08(MS)	13.08(MS)	13.08(MS)
30	ICGV 00348	2.17(R)	2.92(R)	5.90(MR)	7.45(MR)	7.45(MR)
31	ICGV 00350	2.02(R)	2.64(R)	2.64(R)	2.64(R)	2.64(R)
32	ICGV 00351	2.74(R)	2.74(R)	3.36(R)	3.36(R)	3.36(R)
33	ICGV 93260	1.99(R)	3.40(R)	4.73(R)	5.38(MR)	5.38(MR)
34	ICGV 93261	2.47(R)	6.83(MR)	8.08(MR)	8.70(MR)	8.70(MR)
35	ICGV 89280	3.18(R)	7.03(MR)	7.73(MR)	7.73(MR)	7.73(MR)
36	ICGV 92195	2.92(R)	6.51(MR)	7.93(MR)	8.67(MR)	8.67(MR)
37	ICGV 92035	3.74(R)	7.58(MR)	8.30(MR)	9.12(MR)	9.93(MR)
38	ICGS 44	3.40(R)	8.17(MR)	8.89(MR)	9.57(MR)	10.21(MS)
39	ICGS 76	3.00(R)	4.42(R)	4.42(R)	5.12(MR)	5.12(MR)
40	ICR 48	0.03(HR)	1.20(R)	2.51(R)	5.16(MR)	6.47(MR)
41	ICGV 86031 (Resistant check)	1.34(R)	4.04(R)	4.04(R)	4.04(R)	4.04(R)
42	JL 24 (Susceptible check)	4.88(R)	10.88(MS)	18.78(MS)	20.96(MS)	25.45(S)
	Mean of all genotypes	2.51	5.41	6.94	7.51	7.81

Per cent disease incidence				
Effect	Num DF	Den DF	F Value	Pr > F
GEN	41	77.5	2.63	0.0001
TIME	4	338	94.74	<.0001
GEN*TIME	164	324	1.24	0.0513

*Mean of three replications

SAS analysis was performed and the values mentioned are angular transformed values

R- Resistant; MR- Moderately Resistant; MS- Moderately Susceptible

Table.2 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution

S. No.	Genotype	*GBND Incidence (%) at		
		7 DAI	14 DAI	21 DAI
1	ICGV 99058	46.15(S)	92.31(HS)	92.31(HS)
2	ICGV 99072	73.33(HS)	93.33(HS)	93.33(HS)
3	ICGV 00162	50.00(S)	94.44(HS)	94.44(HS)
4	ICGV 00187	22.22(MS)	88.89(S)	100.00(S)
5	ICGV 00189	50.00(S)	100.00(HS)	100.00(HS)
6	ICGV 00191	38.89(S)	77.78(HS)	83.33(HS)
7	ICGV 00201	44.44(S)	83.33(HS)	83.33(HS)
8	ICGV 00202	42.86(S)	85.71(HS)	85.71(HS)
9	ICGV 00203	16.67(MS)	77.78(HS)	88.89(HS)
10	ICGV 00206	46.15(S)	84.62(HS)	84.62(HS)
11	ICGV 00211	53.33(HS)	73.33(HS)	80.00(HS)
12	ICGV 00213	0.00(HS)	87.50(HS)	93.75(HS)
13	ICGV 00241	50.00(S)	81.25(HS)	87.50(HS)
14	ICGV 00246	62.50(HS)	81.25(HS)	81.25(HS)
15	ICGV 00247	37.50(S)	100.00(HS)	100.00(HS)
16	ICGV 86590	100.00(HS)	100.00(HS)	100.00(HS)
17	ICGV 86699	64.71(HS)	64.71(HS)	64.71(HS)
18	ICGV 91114	72.22(HS)	100.00(HS)	100.00(HS)
19	ICGV 00308	77.78(HS)	94.44(HS)	94.44(HS)
20	ICGV 03042	38.46(S)	61.54(HS)	76.92(HS)
21	ICGV 03057	0.00(HR)	66.67(HS)	66.67(HS)
22	ICGV 06100	9.09(MR)	72.73(HS)	72.73(HS)
23	ICGV 07222	14.29(MS)	28.57(S)	85.71(HS)
24	ICGV 07220	0.00(HR)	55.56(HS)	66.67(HS)
25	ICGV 05155	6.25(MR)	81.25(HS)	87.50(HS)
26	ICGV 06146	0.00(HR)	75.00(HS)	75.00(HS)
27	ICGV 02266	50.00(S)	50.00(S)	100.00(HS)
28	ICGV 87846	25.00(MS)	81.25(HS)	87.50(HS)
29	ICGV 93468	27.78(S)	94.44(HS)	94.44(HS)
30	ICGV 00348	33.33(S)	94.44(HS)	94.44(HS)
31	ICGV 00350	23.53(MS)	100.00(HS)	100.00(HS)
32	ICGV 00351	20.00(MS)	93.33(HS)	100.00(HS)
33	ICGV 93260	66.67(HS)	66.67(HS)	77.78(HS)
34	ICGV 93261	66.67(HS)	94.44(HS)	94.44(HS)
35	ICGV 89280	11.11(MR)	94.44(HS)	94.44(HS)
36	ICGV 92195	16.67(MS)	94.44(HS)	94.44(HS)
37	ICGV 92035	5.88(MR)	100.00(HS)	100.00(HS)
38	ICGS 44	6.25(MR)	93.75(HS)	93.75(HS)
39	ICGS 76	0.00(HR)	92.31(HS)	92.31(HS)
40	ICR 48	0.00(HR)	100.00(HS)	100.00(HS)
41	ICGV 86031 (Resistant check)	33.33(S)	72.22(HS)	72.22(HS)
42	JL 24 (Susceptible check)	44.44(S)	94.44(HS)	94.44(HS)
	Mean of all genotypes	34.46	83.77	88.79

*Mean of three replications
SAS analysis was performed and the values mentioned are angular transformed values
HR – Highly resistant; R- Resistant; MR- Moderately Resistant; S – Susceptible; MS- Moderately Susceptible; HS - Highly Susceptible
DAI - Days After Inoculation

Table.3 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:100dilution

S. No.	Genotype	*GBND Incidence (%) at		
		7 DAI	14 DAI	21 DAI
1	ICGV 99058	50.00 (S)	58.33(HS)	58.33(HS)
2	ICGV 99072	78.57(HS)	85.71(HS)	85.71(HS)
3	ICGV 00162	60.00(HS)	60.00(HS)	73.33(HS)
4	ICGV 00187	22.22(MS)	27.78(S)	44.44(S)
5	ICGV 00189	52.94(HS)	52.94(HS)	52.94(HS)
6	ICGV 00191	38.89(S)	38.89(S)	38.89(S)
7	ICGV 00201	47.06(S)	47.06(S)	52.94(HS)
8	ICGV 00202	33.33(S)	33.33(S)	33.33(S)
9	ICGV 00203	18.75(MS)	25.00(MS)	37.50(S)
10	ICGV 00206	35.29(S)	58.82(HS)	58.82(HS)
11	ICGV 00211	47.06(S)	52.94(HS)	52.94(HS)
12	ICGV 00213	0.00(HR)	5.56(MR)	5.56(MR)
13	ICGV 00241	57.14(HS)	64.29(HS)	64.29(HS)
14	ICGV 00246	55.56(HS)	72.22(HS)	77.78(HS)
15	ICGV 00247	40.00(S)	46.67(S)	53.33(HS)
16	ICGV 86590	100.00(HS)	100.00(HS)	100.00(HS)
17	ICGV 86699	70.59(HS)	88.24(HS)	88.24(HS)
18	ICGV 91114	72.22(HS)	72.22(HS)	72.22(HS)
19	ICGV 00308	82.35(HS)	82.35(HS)	82.35(HS)
20	ICGV 03042	50.00(S)	50.00(S)	50.00(S)
21	ICGV 03057	0.00(HR)	0.00(HR)	11.11(MR)
22	ICGV 06100	9.09(MR)	27.27(S)	36.36(S)
23	ICGV 07222	8.33(MR)	25.00(MS)	25.00(MS)
24	ICGV 07220	0.00(HR)	11.11(MS)	22.22(MS)
25	ICGV 05155	6.25(MR)	25.00(MS)	37.50(S)
26	ICGV 06146	0.00(HR)	0.00(HR)	7.14(MR)
27	ICGV 02266	50.00(S)	50.00(S)	100.00(HS)
28	ICGV 87846	25.00(MS)	56.25(HS)	56.25(HS)
29	ICGV 93468	27.78(S)	55.56(HS)	66.67(HS)
30	ICGV 00348	33.33(S)	55.56(HS)	55.56(HS)
31	ICGV 00350	22.22(MS)	61.11(HS)	77.78(HS)
32	ICGV 00351	16.67(MS)	61.11(HS)	61.11(HS)
33	ICGV 93260	44.44(S)	44.44(S)	50.00(S)
34	ICGV 93261	66.67(HS)	72.22(HS)	72.22(HS)
35	ICGV 89280	11.11(MS)	55.56(HS)	72.22(HS)
36	ICGV 92195	16.67(MS)	61.11(HS)	61.11(HS)
37	ICGV 92035	5.56(MR)	72.22(HS)	72.22(HS)
38	ICGS 44	6.67(MR)	46.67(S)	46.67(S)
39	ICGS 76	0.00(HR)	25.00(MS)	25.00(MS)
40	ICR 48	0.00(HR)	25.00(MS)	33.33(S)
41	ICGV 86031 (Resistant check)	6.67(MR)	13.33(MS)	26.67(S)
42	JL 24 (Susceptible check)	66.67(HS)	77.78(HS)	77.78(HS)
	Mean of all genotypes	34.17	48.66	54.21

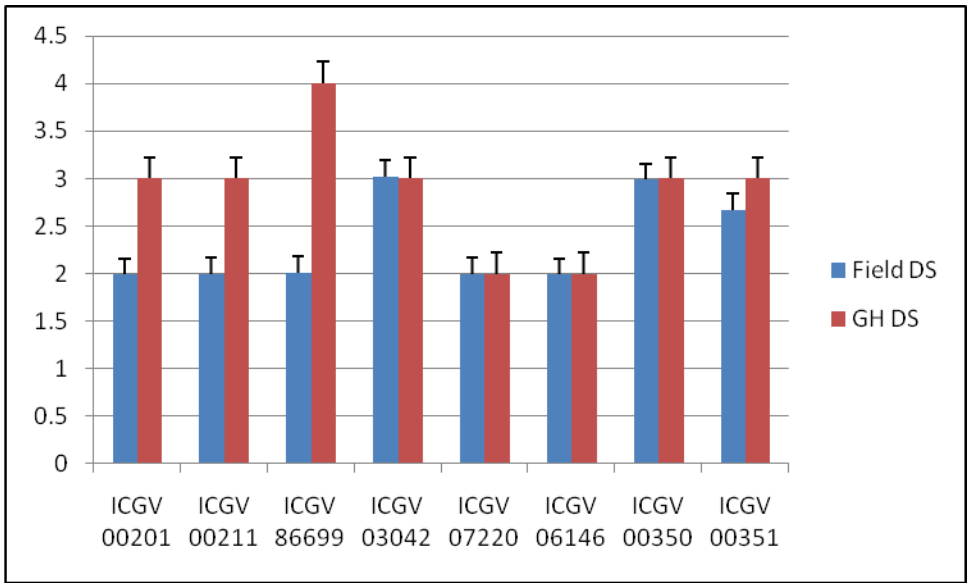
*Mean of three replications

SAS analysis was performed and the values mentioned are angular transformed values

HR – Highly resistant; R- Resistant; MR- Moderately Resistant; S – Susceptible; MS- Moderately Susceptible; HS - Highly Susceptible

DAI - Days After Inoculation

Fig.1 Disease severity of certain groundnut advanced breeding lines for their reaction to Groundnut bud necrosis disease (GBND) under greenhouse and field conditions



The above results indicate longer incubation period of virus inside the host plant which may be due to unsuitable environment in the host plant or may be due to block in movement of virus inside the plant due to host defense response. Young tissue and young plants are more susceptible while mature tissue and plants are highly resistant to GBNV (Buiel and Parlevliet, 1996). Disease incidence decreased and incubation period increased with the age of plants and leaves. This type of resistance (mature plant and tissue) occurs irrespective of the susceptibility level of the genotype to GBNV. However, this type of resistance develops earlier in the resistant than in the susceptible genotype.

In the present study, none of the groundnut genotypes screened under artificial inoculated conditions using sap of the virus were highly resistant or resistant to the GBND. This could be attributed to the high inoculum pressure of the virus. However, the reaction of these genotypes may change, if the screening is attempted with lower virus concentration of 1:100 or 1:1000 (Rao *et al.,* 2003; Kalyani *et al.,* 2005).

The resistant and susceptible genotypes could not be clearly differentiated by using disease severity scoring alone. This was even comparable with earlier results of some of the researchers (Pensuk *et al.,* 2002; Buiel and Parlevliet, 1996) who reported the disadvantage of using disease severity scoring due to the highly variable symptoms caused by GBNV that are not primarily genotype specific. Disease incidence is more advantageous than disease score because it is easy to evaluate (Kesmala *et al.,* 2006). Moreover, field evaluation of lines is complicated initially by the non-uniformity of disease distribution in the field resulting from random distribution of vectors.

At 1:10 virus concentration, the highly susceptible group of genotypes has 2-5 disease severity rating. While, at 1:100 virus concentrations, the moderately resistant and moderately susceptible group of genotypes had 2 severity rating, the susceptible and highly susceptible reaction group has 2-4 as their severity rating. This clearly shows the drawback in using disease severity as a parameter to measure the disease.

The percent infection to GBNV antiserum and the absorbance values at 405nm clearly differentiated the resistant and susceptible check at 1:10 and 1:100 virus concentrations. The ICGV 86031 (resistant check) showed 93.33 percent susceptibility when inoculated with 1:10 dilution of virus concentration and positive reaction with ELISA. This might be due to the high amount of virus applied. In support of our results, a previous study also reported that genotypes ICGV 86031 and ICGV 86388 succumbed to GBND under high disease pressure and recorded substantial yield losses (Reddy *et al.*, 2000).

The genotype ICGV 06146 showed resistant reaction in field and moderately resistant reaction in greenhouse screening. ICGV 00213 showed moderately resistant reaction in both field and greenhouse screening. The genotypes *viz.*, ICGV 07222, 03057 and ICGS 76 showed moderately resistant reaction in field and moderately susceptible reaction in greenhouse. ICGV 00187, 00191, 00202, 00203, 06100, 93260, 05155 and ICR 48 gave moderately resistant reaction in field and susceptible reaction in greenhouse. ICGV 03042 showed resistant reaction in field and susceptible reaction in greenhouse. ICGV 07220 showed resistant reaction in field and moderately susceptible reaction in greenhouse. ICGS 76 showed moderately resistant reaction in field and moderately susceptible reaction in greenhouse (Figure 1).

The genotypic differences may be due to inherent response for resistance and susceptibility to GBNV. The genotypes mentioned above that showed variable degree of resistance under field and greenhouse conditions had Spanish bunch growth habit except ICGS 76 and ICR 48 which had Virginia bunch growth habit.

The genotypes *viz.*, ICGV 00187, 00191, 00202, 00203, 00213, 06146 and ICGV 93260 were also reported as resistant for foliar diseases whereas, the genotypes *viz.*, ICGV 03057, 07222, 07220, 05155 and ICR 48 were drought resistant.

The resistant check (ICGV 86031) used in the study showed resistant reaction in field and susceptible reaction in greenhouse. And the susceptible check (JL 24) showed susceptible reaction in field and highly susceptible reaction in greenhouse. This implies that most probably ICGV 86031 is resistant to vector *T. palmi* and susceptible to GBNV whereas, JL 24 is susceptible to both vector and virus. In our study, the resistance showed by test genotypes could be associated with non-preference of the vector or slower multiplication of virus in the host plant. In any case both the characters are of good value for a resistant genotype. Further screening of these advanced breeding lines in multi-location trails will help in direct release of these genotypes as promising varieties in hot spot locations of the country where GBND is prevalent.

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