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Experimental and natural host range studies of *Peanut bud necrosis virus* (PBNV) isolates of blackgram and greengram of Telangana using DAC-ELISA

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Abstract
In the present study, blackgram PBNV isolate was used to study the experimental and natural host range. The studies on experimental host range by mechanical inoculations revealed that 32 plant as well as weed species could be infected out of 36 tested. Groundnut produced apical necrosis systemically while Tomato showed initially water-soaked lesions turning into necrosis. Localized necrotic lesions on *Cajanus cajan* followed by no systemic infection. *Solanum melongena* developed chlorotic rings and leaf necrosis symptoms. Localized concentric chlorotic rings were seen on *N. tabacum* cv. Samsun whereas chlorotic and necrotic lesions and veinal necrosis on *N. glutinosa*. *N. rustica* showed concentric chlorotic rings, necrotic rings, leaf distortion and stunting. Chlorotic lesions, mosaic, leaf chlorosis symptoms were observed on *Vicia faba. Chenopodium quinoa* developed chlorotic lesions while *C. amaranticolor* produced necrotic rings. *Datura stramonium* showed chlorotic lesions initially turning to necrotic. PBNV blackgram isolate could not infect *Abelmoschus esculentum.*, *Helianthus annuus* cv.PAC-36, *Trigonella foenum-graecum*, *Gossypium herbaceum*, *Crossandra infundibuliformis*, *Tagetes patula*, *Amaranthus* spp., *Croton sparsiflorus. Cyamopsis tetragonoloba*, *Luffa acutangula*, *Cucumis sativus*, *Cucurbita moschata*, *Lagenaria siceraria*, *Momordica charantia*, *Corchorus* spp., *Euphorbia geniculate*, *Parthenium hysterophorus*, *Tridax procumbens* and *Euphorbia hirta* were neither produced any visible symptom nor tested positive to PBNV by DAC-ELISA. Under natural host range studies, out of 44 suspected as well as random plant species collected and tested by DAC-ELISA, PBNV was detected in 13 samples, comprising economically important crops and several weeds.

Keywords: Host range, *Peanut bud necrosis virus*, blackgram, greengram

Introduction
Of several viral diseases attacking greengram and blackgram necrosis 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*., 2002) [6] was considered to be a major threat, causing 40% yield loss (Nene, 1972) [2]. PBNV is evolving with time and emerging as new threat to a wide host range of both agricultural and horticultural crops throughout India. In the present study, the PBNV isolates of blackgram and greengram were tested on a different crop and weed species to determine experimental and natural host range as knowledge of the sources of the virus inoculum plays a crucial role in plant virus disease management.

Materials and Methods
Experimental host range under glasshouse conditions
Crop and weed species belonging to different families were tested under glasshouse conditions by mechanical inoculation with the four isolates *viz.*, PBNV-BG and PBNV-GG. Ten plants (2 plants/pot) of each species were sap inoculated. Cucurbits and legumes were inoculated at the primary leaf stage and others at 2-4 leaf stage. The plants were kept under observation for symptom expression. Development of local and systemic symptoms was recorded up to 30 days. Irrespective of symptoms produced, all inoculated and newly emerged leaves were indexed back on local lesion assay host (cowpea cv. C-152) and tested also by DAC-ELISA with respective antiserum.

Natural host range under field conditions
Crop and weed species samples were collected randomly from the fields of Tandur and experimental fields of NBPGR-RS, Hyderabad. The samples collected were tested by DAC-ELISA using both PBNV antiserum.

Results

Experimental host range

The studies on experimental host range of PBNV-BG and PBNV-GG isolates under glasshouse conditions by mechanical inoculation revealed that 32 plant as well as weed species could be infected out of 36 tested (Table 1). Among the plant species inoculated with PBNV-BG and PBNV-GG isolates, 18 showed infection. Symptoms expressed on the experimental host plants ranged from chlorotic, necrotic lesions/rings, chlorotic, necrotic concentric rings, leaf and stem necrosis, chlorotic spots, leaf curl, apical necrosis to death with both the PBNV isolates inoculations.

Arachis hypogea infected with PBNV isolates produced chlorotic rings, concentric chlorotic rings, necrotic rings, leaf necrosis on inoculated leaves and chlorotic rings, chlorotic lesions, leaf necrosis, stem necrosis, apical necrosis systemically, leading to death. *Lycopersicon esculentum* showed initially water-soaked lesions turning into necrosis with an appearance of purple-coloured lesions coalesced following death with infection due to both the PBNV isolates. No PBNV isolate could infect the inoculated leaves of *Gossypium* spp. *Abelmoschus esculentum.*, *Helianthus annuus* cv.PAC-36, *Trigonella foenum-graecum*, *Gossypium herbaceum*, *Crossandra infundibuliformis*, *Tagetes patula*, *Amaranthus* spp., *Croton sparsiflorus* showed no infection upon inoculation with the PBNV isolates.

No symptoms were produced on *Parthenium hysterophorus* and *Tridax procumbens* by the PBNV isolates of blackgram and greengram. Localized necrotic lesions, fewer but bigger in size were observed on *Cajanus cajan* with PBNV-BG and PBNV-GG inoculations followed by no systemic infection. *Solanum melongena* developed chlorotic rings, concentric chlorotic rings and leaf necrosis symptoms with PBNV-BG and PBNV-GG infection.

Different species of *Nicotiana* spp. tested in experimental host range showed varied symptoms. PBNV-BG and PBNV-GG produced only localized concentric chlorotic rings, which were bigger in size and fewer in number on *N. tabacum* cv. Samsun. Both the isolates, PBNV-BG & PBNV-GG showed chlorotic and necrotic lesions and veinal necrosis on inoculated leaves of *N. glutinosa*, followed by deformed newly emerging leaves. *N. rustica* showed concentric chlorotic rings, necrotic rings on inoculated leaves and chlorotic rings, concentric chlorotic rings, leaf distortion, stunting with PBNV-BG and PBNV-GG. PBNV-BG and PBNV-GG produced chlorotic lesions, mosaic, leaf chlorosis on *Vicia faba*.

Chenopodium quinoa developed chlorotic lesions with PBNV-BG & PBNV-GG while *C. amaranticolor* produced necrotic rings with PBNV isolates. *Euphorbia geniculata* showed no infection with both the PBNV isolates.

Datura stramonium showed chlorotic lesions initially turning to necrotic which increased in size and coalesced giving papery appearance while newly emerged leaves showed chlorotic lesions, mosaic, deformation with PBNV-BG and PBNV-GG infection.

In the present study, *Cyamopsis tetragonoloba*, *Luffa acutangula*., *Cucumis sativus*, *Cucurbita moschata*, *Lagenaria siceraria*, *Momordica charantia*, *Corchorus* spp. and *Euphorbia hirta* were not found to be infected (neither produced any visible symptom nor tested positive to PBNV in ELISA) upon sap inoculations of PBNV of blackgram and greengram under glasshouse conditions.

Natural host range

Out of 44 suspected as well as random plant species collected and tested by DAC-ELISA, PBNV was detected in 13 samples, comprising economically important crops and several weeds (Table 2). The symptoms were ranging from chlorosis to necrosis of different plant parts. The test plants that showed positive reaction to PBNV are *Arachis hypogaea*, *Lycopersicon esculentum*, *Vigna unguiculata*, *Vigna mungo*, *Vigna radiata*, *Citrullus lanatus*, *Capsicum annuum* and *Solanum melongena.* Among ornamental weed species, *Vinca minor*, *Tagetes patula*, *Phyllanthus madraspatensis*, *C. trilocularis*, *Achyranthes aspera* and *Tephrosia hirta* are infected with both the PBNV isolates under natural conditions.

Arachis hypozaea plants showed leaf and stem necrosis and apical necrosis both in PBNV infected plants. *Lycopersicon esculentum* expressed water-soaked lesions turning to purple, necrosis and apical necrosis was observed with PBNV infection. Chlorotic spots, veinal chlorosis, necrosis and leaf curl symptoms were observed in *V. unguiculata* with PBNV infection under natural conditions. Few of the samples collected from *T. patula* showing leaf and stem necrosis as well as stunting reacted positive to PBNV. *Solanum melongena* showed chlorosis and mosaic mottling with PBNV infection while *Vinca minor* showed necrotic lesions, leaf necrosis and stem necrosis. *Croton sparsiflorus* showed leaf curl and mosaic mottling in *Citrullus lanatus* with PBNV infection.

No infection by PBNV was detected in randomly collected samples of *Cucumis melo*, *A. esculentum*, *H. annuus*, *Carica papaya*, *C. tetragonoloba*, *C. infundibuliformis*, *Cleome viscosa*, *D. arvensis*, *Acalypha indica*, *Crotan sparsiflorus*, *Amaranthus* spp., *Boerhavia erecta*, *D. stramonium*, *P. hysterophorus*, *T. procumbens*, *Phyllanthus madraspatensis*, *Euphorbia hirta*, *Tribulus terrestris*, *Commelina bengalensis*, *Euphorbia geniculata*, *Phyllanthus neruri*, *Calotropis* sp., *Carthamus tinctorius*, *Eclipta alba*, *Argemone mexicana*, *Lantana camera* and *Trichodesma zylanicum* indicating no symptomless infection.

Table 1: Experimental host range of PBNV-BG and PBNV-GG isolates under glasshouse conditions

Host species	Symptoms			
	PBNV-BG		PBNV-GG	
	Local	Systemic	Local	Systemic
Arachis hypogaea cv. JL-24	CR, CCR, NR, LN	CR, CL, LN, AN, SN, D	CR, CCR, NR, LN	CR, CL, LN, AN, SN, D
Lycopersicon esculentum cv. Marutham	WSL, LN, NL, NR	NL, NR, LN, SN, AN, D	WSL, LN, NL, NR	NL, NR, LN, SN, AN, D
Capsicum annuum cv.G-4	CR, CCR, NR, NCR, LN	CR, CCR, NR, NCR, N, SN, D	CR, CCR, NR, CR, LN	CR, CCR, NR, NCR, N, SN, D
Gossypium herbaceum	-	-	-	-
Abelmoschus esculentum.	-	-	-	-
Cajanus cajan	NL	-	NL	-

Solanum melongena L.	CR, CCR, LN	CR, NR, S	CR, CCR, LN	CR, NR, S
Nicotiana glutinosa	CL, NL	LD	CL, NL	LD
N. rustica	CCR, CR	CR, CCR, C, LD, S	CCR, CR	CR, CCR, C, LD, S
N. tabacum cv. Samsun	CCR	-	CCR	-
Cucumis sativus	CL	-	CL	-
Cucurbita moschata	-	-	-	-
Lagenaria siceraria	-	-	-	-
Momordica charantia	-	-	-	-
Helianthus annuus cv.PAC-36	-	-	-	-
Trigonella foenum-graecum	-	-	-	-
Phaseolous vulgaris cv. Top crop	Cl,NL	-	CL, NL	-
Vigna unguiculata cv. C-152	CR, CCR, NR, NCR	-	CR, CCR, NR, NCR	-
Cyamopsis tetragonoloba	-	-	-	-
Vigna radiata	CL, NL	C, LN, LC, CS, AN	CL, NL	C, LN, LC, CS, AN
Vigna mungo	NL	LN, C, CS, LC	NL	LN, C, CS, LC
Vicia faba	CCR, CL, NL	C	CCR, CL, NL	C
Crossandra infundibuliformis	-	-	-	-
Tagetes patula	-	-	-	-
Amaranthus spp.	-	-	-	-
Datura stramonium	CR, LN, CCR	LN, MM, SL, D	CR, LN, CCR	LN, MM, SL, D
Parthenium hysterophorus	-	-	-	-
Corchorus sps.	-	-	-	-
Glycine max	CL, NL	-	CL, NL	-
Tridax procumbens	-	-	-	-
Croton sparsiflorus	-	-	-	-
Commilina bengalensis	-	-	-	-
Chenopodium quinoa.	CL	CL	CL	CL
C. amaranticolor	NR	NR	NR	NR
Euphorbia hirta	-	-	-	-
E. geniculata	-	-	-	-

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, MM-mosaic mottling, SN-stem necrosis, LD-leaf distortion, AN-apical necrosis, LC-leaf curl, CS-chlorotic spots, NS-necrotic spots, VN- veinal necrosis, VC-veinal chlorosis, S-stunting, WSL- water-soaked lesions.

Table 2: Natural host range of PBNV and TSV in crop and weed samples determined by DAC-ELISA.

Crop/weed species	**PBNV**	
	ELISA	**Symptoms**
Arachis hypogaea	12/57	LN, SN, AN
Lycopersicon esculentum	5/14	WL, PL, N, AN
Vigna unguiculata	1/2	VN, C, LC
Cucumis melo	0/5	Nil
Abelmoschus esculentum	0/5	Nil
Helianthus annuus	0/3	Nil
Gossypium herbaceum	0/3	Nil
Carica papaya	0/5	Nil
Cyamopsis tetragonoloba	0/5	Nil
Crossandra infundibularis	0/5	Nil
Tagetes patula	7/35	LN, SL, S
Ageratum conyzoides	0/32	Nil
Citrullus lanatus	2/3	LC, M
Cleome viscose	0/54	Nil
Digera arvensis	0/45	Nil
Acalypha indica	0/15	Nil
Croton sparsiflorus	0/19	Nil
Amaranthus spp.	0/44	Nil
Boerhavia erecta	0/26	Nil
Datura stramonium	0/29	Nil
Parthenium hysterophorus	0/454	Nil
Chorchorus trilocularis	13/49	NL
Achiranthus aspera	12/27	Nil
Tephrosia hirta	6/20	Nil
Vinca minor	4/17	NL, LN, SN
Tridax procumbens	0/123	Nil
Acanthospermum hispidum	0/11	Nil
Phyllanthus madraspatensis	0/30	Nil
Euphorbia hirta	0/25	Nil
Tribulus terrestris	0/29	Nil
Euphorbia geniculata	0/38	Nil

Phyllanthus neruri	0/35	Nil
Calotropis sp.	0/22	Nil
Carthamus tinctorius	0/10	Nil
Commilina bengalensis	0/35	Nil
Vigna mungo	78/82	VN, C, LC, AN
Vigna radiata	71/92	VN, C, LC, AN
Solanum melongena	28/39	C, LC, M
Eclipta alba	0/14	Nil
Argemone mexicana	0/12	Nil
Lantana camera	0/15	Nil
Capsicum annuum	7/11	NL, SN, AN
Trichodesma zylanicum	0/12	Nil
Cassia auriculata	0/9	Nil

C-chlorosis, CL-chlorotic lesions, NL-necrotic lesions, LN- leaf necrosis, SL-smalling of leaves, SN-stem necrosis, AN-apical necrosis, LC-leaf curl, NS-necrotic spots, VC-veinal chlorosis, VN- veinal necrosis, M-mosaic, WL-water-soaked lesions, PL- purple lesions, N-necrosis.

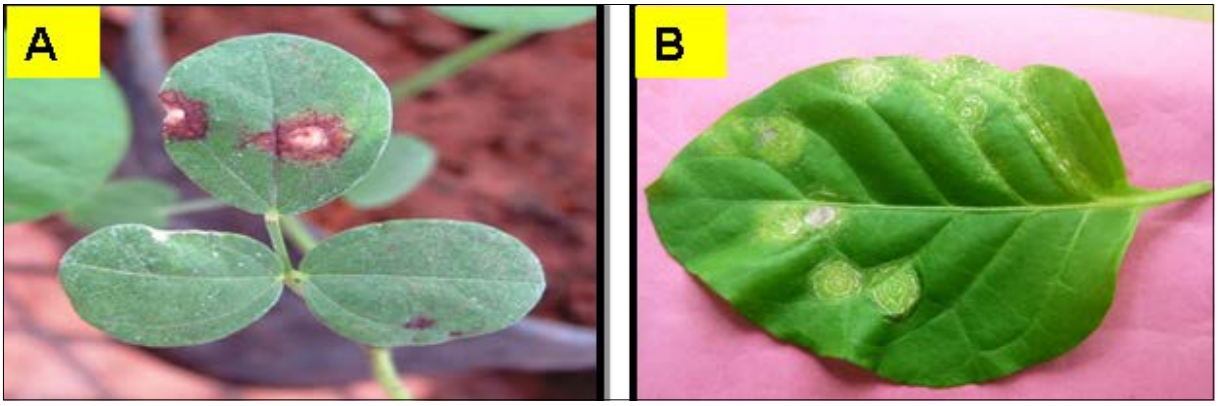
Discussion

Experimental host range

Upon mechanical inoculations, PBNV isolates of blackgram and greengram infected 18 out of 36 plant species tested. Both the PBNV isolates showed similar symptoms on the test plants. PBNV-BG and PBNV-GG produced local and systemic symptoms on *Arachis hypogaea*, *Lycopersicon esculentum*, *Vigna radiata*, *Vigna mungo*, *Nicotiana glutinosa*, N. *tabacum* cv. Samsun and *Datura stramonium*. In contrast, Prasada Rao *et al.* (1980) [4] reported that *A. hypogaea* var. TMV-2, *D. stramonium*, *L. esculentum* var. Pusa Ruby, *V. radiata* var. T-44, *V. mungo*, var. UPU-1 and *N. glutinosa* developed only systemic symptoms upon mechanical inoculation of TSWV (considered to be PBNV) isolated from tomato. *Glycine max* var. Bragg developed only localized infection which is in accordance with the finding of Thein *et al.* (2003) [7] where, mungbean PBNV isolate developed only local symptoms on soybean, whereas, only systemic symptoms were recorded by Prasada Rao *et al.* (1980) [4]. In the present study, local and systemic symptoms were produced by PBNV-BG and PBNV-GG in *N. rustica*, *Vicia faba*, *Chenopodium amarantocolor* and *C. quinoa*, in contrast to the findings of Prasada Rao *et al.* (1980) [4] while appearance of only local symptoms on *V. unguiculata* cv. C-152 (Prasada Rao *et al.*, 1980; Umamaheswaran *et al.*, 2003) [4, 8], and *Cajanus cajan* (Umamaheswaran *et al.*, 2003) [8] was in agreement with the earlier findings. *Solanum melongena* recorded with in the line with the findings of Umamaheswaran *et al.* (2003) [8]. In contrast, Prasada Rao *et al.* (1987) [3] reported only systemic symptoms. *Capsicum annuum* showed both local and systemic symptoms in contrast with the findings of Prasada Rao *et al.* (1987) [3] and Umamaheswaran *et al.*, (2003) [8], where only systemic symptoms were reported. Prasada Rao *et al.* (1987) [3] recorded systemic infection on *C. annuum* var. NP-46 with top necrosis, chlorotic spots and mosaic mottling, whereas chlorotic concentric lesions, necrotic concentric lesions, stem necrosis and top necrosis symptoms were observed in systemic infection along with local infection showing chlorotic and necrotic lesions. *Phaselous vulgaris* cv. Top crop developed only local lesions in contrast to the findings of Thein *et al.* (2003) [7] where both local and systemic symptoms observed. *G. herbaceum* and *H. annuus* showed no infection in the present study in contrast to the findings of Thein *et al.* (2003) [7] where localized infection recorded.

Natural host range

Out of 44 suspected as well as random plant species collected and tested by DAC-ELISA, PBNV was detected in 13 samples, comprising economically important crops and several weeds. PBNV detected in *A. hypogaea* (Reddy *et al.*, 1978) [5], *L. esculentum* (Prasada Rao *et al.*, 1980) [4], *V. unguiculata* (Prasada Rao *et al.*, 1987) [3], *V. mungo* (Nene *et al.*, 1972; Amin *et al.*, 1978) [2, 1], *V. radiata* (Nene *et al.*, 1972; Amin *et al.*, 1978) [2, 1], *S. melongena* (Prasada Rao *et al.*, 1987) [3] and *C. annuum* (Prasada Rao *et al.*, 1987) [3], *confirming* the findings of previous reports made by several workers. In addition, PBNV found in *T. patula*, *C. lanatus*, *Corchorus* sps., *A. aspera*, *Tephrosia hirta* and *Vinca minor*. PBNV was not detected in *H. annuus* L. in contrast to the finding of venkata subbaiah *et al.* (2000) [9].



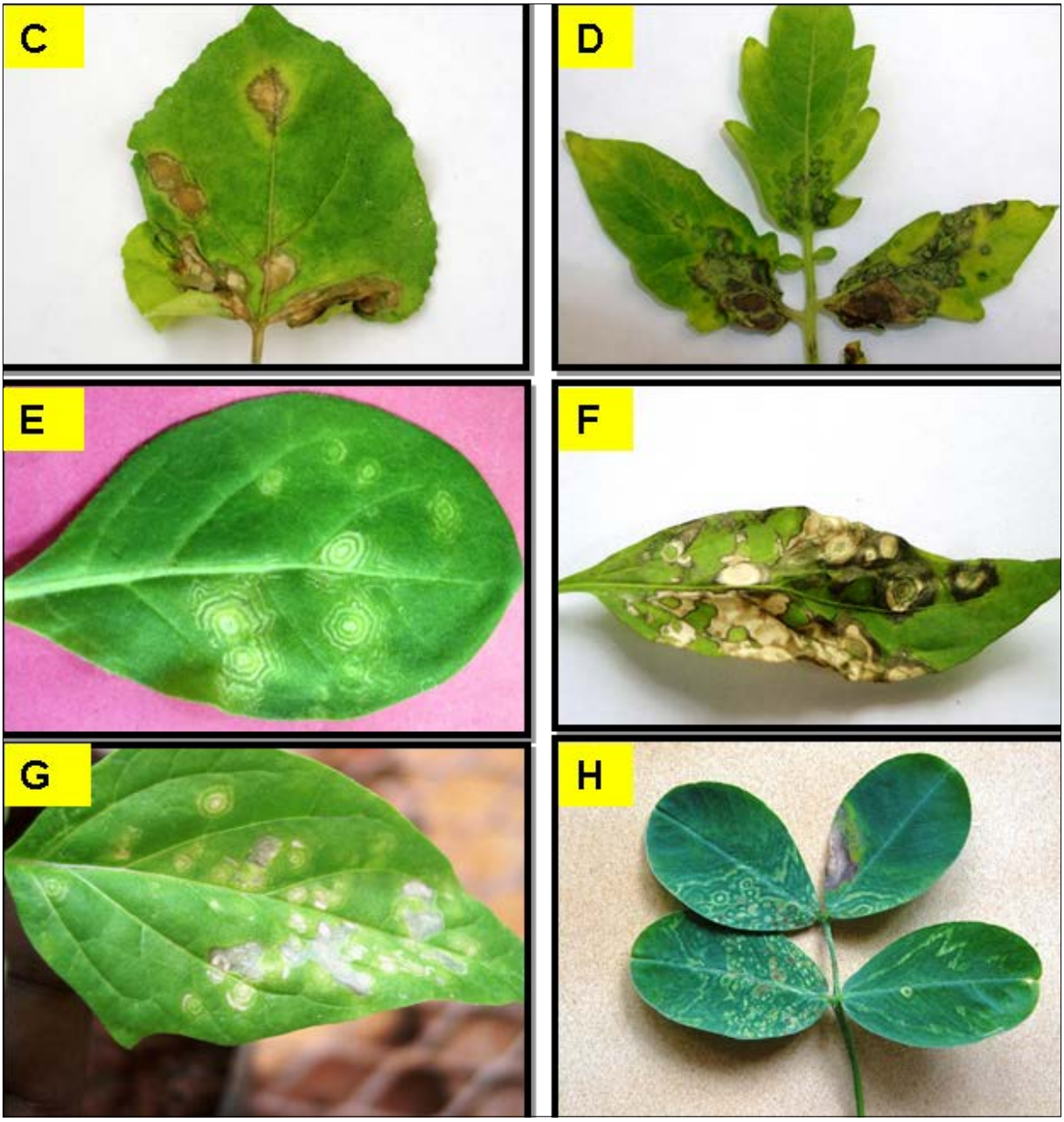


Fig 1: Symptoms produced by PBNV BG and GG isolates upon mechanical inoculations under glasshouse conditions. A. *Vicia faba*, B. *Nicotiana tabacum* cv. Samsun, C. *Nicotiana rustica*, D. *Lycopercon esculentum cv. Marutham*, E. *Nicotiana glutinosa*, F. *Capsicum annum cv. G4*, G. *Datura stramonium*, H. *Arachis hypogea*

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