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Symptomatology and particle morphology studies of necrosis causing *Peanut bud necrosis virus* (PBNV) and *Tobacco streak virus* (TSV) isolates of blackgram and greengram of Andhra Pradesh

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Abstract
Peanut bud necrosis virus (PBNV = *Groundnut bud necrosis virus* - GBNV) and *Tobacco streak virus* (TSV) are causing similar necrosis symptoms on Blackgram and greengram resulting in confusion of disease diagnosis at field level and it is necessary to differentiate the diseases as the viruses are transmitted by different thrip species with varying patterns where management practices also differ. Hence, the present study has been taken up for symptomatology of the necrosis causing viruses under field and glasshouse conditions and particle morphology. Field collected symptomatic samples tested by DAC-ELISA with respective antisera. Under field conditions, necrosis/leaf curl disease in blackgram and greengram was manifested with similar symptoms such as veinal chlorosis associated leaf curl. These veins later turned to necrotic resulting in downward curling of leaves where necrotic veins were clearly observed on the under surface of the leaf, occasionally, chlorotic spots turning to necrotic, leaf necrosis leading to death of growing bud. Early infection caused death of the plant. Most of the TSV positive plants were completely necrotic leading to death revealing the rapid necrosis than PBNV. Under glasshouse conditions upon mechanical inoculation, PBNV isolates produced chlorotic, necrotic lesions, chlorotic, necrotic spots, veinal necrosis, leaf necrosis and leaf curl in blackgram; and chlorotic, necrotic rings, chlorotic, necrotic lesions; concentric chlorotic and necrotic rings; veinal necrosis, smalling of leaves, stunting, mosaic mottling and apical necrosis were observed with TSV inoculations, whereas greengram genotypes showed chlorotic and necrotic lesions, leaf chlorosis, necrosis, veinal chlorosis, necrosis, chlorotic spots and leaf curl with PBNV infection while TSV showed concentric chlorotic, necrotic rings; veinal, stem and apical necrosis; chlorotic and necrotic lesions. Particle morphology studies revealed quasi spherical virus particles measuring 80-110 nm and 25-30 nm diameter, in the leaf dip preparations of PBNV and TSV isolates of blackgram and greengram by electron microscopy, respectively.

Keywords: Symptamatology, necrosis, particle morphology, electron microscopy, TSV, PBNV, blackgram, greengram

Introduction
Blackgram (Urdbean - *Vigna mungo* L. Hepper) and greengram (Mungbean - *Vigna radiata* L. Wilczek) 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. 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) transmitted by *Thrips palmi* (Karny) in a propagative manner (Sreekanth *et al.*, 2002) [20] was considered to be a major threat, causing 40% yield loss (Nene, 1972) [12]. Recently, *Tobacco streak virus* (TSV) has also been reported to be a cause of leaf curl symptoms on blackgram (Prasada Rao *et al.*, 2003; Ladhalakshmi *et al.*, 2005) [14, 10] and greengram (Prasada Rao *et al.*, 2003) [14] paving confusion in field diagnosis to assess the disease incidence.

Keeping this in view, the present investigation the present study was taken up for the complete understanding for the diagnosis of viruses causing similar necrosis.

Materials and Methods

Symptomatology

Symptoms observed on field infected blackgram and greengram plants tested positive to PBNV and/or TSV antisera by DAC-ELISA and infectivity tests were recorded. Symptomatology studies were carried out under glasshouse conditions on susceptible checks of blackgram (LBG-20) and greengram (K-851). One hundred and twenty plants each were grown in pots (2plants/pot) in glasshouse and inoculated by four virus isolates mechanically. A set of ten plants were inoculated at three growth stages, 7 DAS, 14 DAS and 21 DAS. All the inoculated plants were maintained under glasshouse conditions and observed for the development of symptoms. Un-inoculated blackgram and greengram plants were kept as controls.

Particle morphology

The virus was examined under electron microscope by modified Brande's leaf dip method with slight modifications. Small 2 to 3 mm diameter bits of infected leaf tissue were crushed on a clean glass slide in a drop of phosphate buffer and 10µl of sap homogenate was taken with a micro pipette and was placed on parafilm covered glass slide. The copper grid was placed on the surface of the droplet, ensuring the grid surface becomes wet. After 30 sec, the excess fluid was blotted off by filter paper and the grid was washed from the filmed surface, using a continuous flow of 15-25 drops of double distilled water to remove the sap and then dried again using filter paper. Staining was done by placing the filmed surface of the grid on a drop of 2% uranyl acetate (pH 6.5). Excess stain was drained and dried. After drying, it was examined under electron microscope. Transmission electron microscopy studies were done at IARI, New Delhi and Ruska Lab, Rajendranagar, Hyderabad.

Results

Symptomatology

It is difficult to distinguish symptoms of PBNV and TSV on blackgram as well as greengram under field conditions unless observed in the early stages of infection. The first disease symptoms usually appear within first fortnight after sowing on the leaf as veinal chlorosis either on the secondary or tertiary veins, which later spread to the primary vein. These veins later become necrotic resulting in downward curling of the leaves. The necrotic veins are clearly observed on the under surface of the leaf. Sometimes show chlorotic spots, which later become necrotic finally leading to leaf necrosis and ultimately killing the growing bud. Early infections cause death of the plants. Leaf curl symptoms were prominent in late infected plants. Sometimes plants were severely stunted with reduced internodal length and many axillary shoots. Most of the TSV positive plants were completely necrotic leading to death revealing the rapid necrosis than PBNV. Smalling of leaves was observed on stunted blackgram plants. Mixed infections of the both the viruses were also observed in both the crops where plants were about to die. A range of chlorotic to necrotic symptoms recorded under field conditions in Blackgram (Figure 1) and green gram (Figure 2) are presented here.

On mechanical inoculation of PBNV-BG, PBNV-GG, TSV-BG and TSV-GG at different growth stages *viz.* 7 DAS, 14 DAS and 21 DAS of susceptible cultivars of blackgram (LBG-20) and greengram (K-851) showed a range of chlorotic and necrotic symptoms (Tables 1 & 2, Figure 3 & 4). The range of symptoms observed on PBNV inoculations on blackgram and greengram was chlorosis, veinal chlorosis, veinal necrosis, chlorotic spots, leaf curl and apical necrosis at 7 DAS as well as 14 DAS while apical necrosis was not noticed at 21 DAS. TSV inoculations Blackgram cv. LBG-20 showed a range of symptoms *viz.*, veinal necrosis, leaf necrosis, smalling of leaves, stunting, mosaic, apical necrosis, death at all the three stages while in greengram, symptoms ranged were veinal necrosis, leaf necrosis, stunting, smalling of leaves, apical necrosis, death at 7 DAS, veinal necrosis, chlorosis, stunting, stem necrosis and death at 14 and 21 DAS where smalling of leaves and apical necrosis were absent. Varied mortality of the plants was observed with the virus, crop and the stage of inoculation. TSV-BG inoculations on Blackgram cv. LBG-20 showed 60% mortality at 7 DAS, 44.4% at 14 DAS and 33.3% at 21 DAS while greengram cv. K-851 was observed with 66.6% at 7 DAS, 30% at 14 DAS and 12.5% at 21 DAS. In case of PBNV-BG inoculations, zero per cent mortality as observed in Blackgram cv. LBG-20 while green gram cv. K-851 was recorded with death of 11.1%, 10% and 12.5% plants at 7, 14 and 21 DAS, respectively.

Blcakgram cv. LBG-20 and greengram cv. K-851 plants manifested with a range of chlorotic to necrotic symptoms upon mechanical inoculation of PBNV and TSV isolates under glasshouse conditions were presented in Figure 5, 6, 7 and 8. Differences were observed in number of plants that showed systemic symptoms with the stage of inoculation when inoculated with PBNV-BG. All plants blackgram cv. LBG-20 and greengram cv. K-851 showed with PBNV systemic symptoms at 7 DAS while 88.8% at 14 DAS and 33.3% at 21 DAS in blackgram and 80% at 14 DAS and 37.5% at 21 DAS in greengram was observed while no such differences were observed on TSV-BG inoculations where all the inoculated plants showed local as well as systemic symptoms.

TSV-GG inoculations on blackgram cv. LBG-20 showed 60% mortality at 7 DAS, 40% at 14 DAS and 44.4% at 21 DAS, while greengram cv. K-851 was observed with 70% at 7 DAS, 44.4% at 14 DAS and 12.5% at 21 DAS. In case of PBNV-GG inoculations, zero percent mortality as observed in blackgram cv. LBG-20 while greengram cv. K-851 was recorded with death of 10% plants at 7 DAS and 11.1% at 14DAS and no mortality at 21 DAS. Differences were observed in number of plants showed systemic symptoms with the stage of inoculation when inoculated with PBNV-GG. All the plants of blackgram cv. LBG-20 and greengram cv. K-851 were expressed with PBNV systemic symptoms at 7DAS while 90% at 14 DAS and 33.3% at 21 DAS in blackgram and 77.7% at 14DAS and 55.5% at 21 DAS in greengram was observed while no such differences were observed on TSV-GG inoculations where all the inoculated plants showed local as well as systemic symptoms.

Particle morphology

Transmission electron microscopy of PBNV and TSV isolates of blackgram and greengram by leaf dip preparation showed quasi-spherical virus particles measuring 80-110 nm and 25-30 nm diameters, respectively (Figure 9 & 10).

Discussion

Symptomatology

Both PBNV and TSV ultimately cause necrosis and hence it is difficult to distinguish symptoms of PBNV and TSV on blackgram and greengram under field conditions. The disease symptoms usually appear within first fortnight after sowing on the leaf as veinal chlorosis. The chlorosis becomes necrotic

resulting in downward curling of the leaves where necrotic veins are clearly observed on the under surface of the leaf. In some leaves chlorotic spots appear on part of the leaf or on entire leaf, which later become necrotic leading to leaf necrosis and ultimately killing the growing bud. Death of the plants was observed in case of early infections. Prominent leaf curl symptoms were present in late infected plants. These results were in agreement with the findings of previous authors (Nene, 1972; Bhat *et al*., 2001a; Prasada Rao *et al*., 2003a; Thein *et al*., 2003) [12, 2, 14, 21]. Nene (1972) [12] described that the leaf curl symptoms caused by PBNV in blackgram appear throughout the season, starting within 20 DAS till the plants are able to throw last new leaves. The earliest symptoms are appearance of chlorosis around some lateral view and its branches near the margin of a leaf and leaves show curling of margins downwards although rolling up of a few affected young leaves. The veins show reddish brown discoloration on the under surface which also extends to the petiole. Majority of the plants that show symptoms within five weeks after sowing die due to top necrosis within a week or two. A few plants which escape the death remain stunted and fail to flower. Bhat *et al*. (2001a) [2] reported symptoms of PBNV on greengram and blackgram included necrosis of all plant, bud and pod. Prasada Rao *et al*. (2003a) [4] observed that the first leaf curl symptoms caused by PBNV on mungbean and urdbean usually appeared within 15 days after sowing on the leaf as veinal chlorosis either on the secondary or tertiary veins, which later spread to the primary vein. These veins later become necrotic resulting in downward curling of the leaves. The necrotic veins were clearly observed on the under surface of the leaf. Sometimes, young leaves show chlorotic spots, which later become necrotic finally leading to leaf necrosis and ultimately killing the growing bud. Early infections caused death of plants and leaf curl symptoms were more prominent in late infected plants. Thein *et al*. (2003) [21] observed the similar disease symptoms on mungbean due to PBNV. Sometimes plants were severely stunted with reduced inter nodal length and many axillary shoots. These symptoms are in accordance with the findings of Bhat *et al*. (2001a) [2].

Under field conditions it was observed that TSV infected blackgram or greengram have succumbed due to rapid necrosis compared to PBNV. Smalling of leaves with mosaic mottling was often observed on stunted blackgram plants when compared to greengram. TSV infected urdbean plants exhibited brown necrotic areas in the leaves, petiole, necrosis of stem and drying of the plants from the tip and finally death of the entire plant (Ladhalakshmi *et al.,* 2006) [7]. TSV infection on mungbean under field conditions was characterized by brown necrotic areas in the leaves, petiole, and necrosis of stem and drying of the plants from the tip (Vinod *et al.,* 2007) [23]. The symptoms produced by TSV infection on blackgram and greengram in present investigation were in agreement with TSV symptoms reported by other workers. Contrary to the necrotic symptoms produced by TSV on groundnut, blackgram and greengram, on beans and soybean, it produced mosaic, malformation of leaves, axillary bud proliferation, shortening of internodes, necrotic streaks on the growing buds and dark brown coloured spots on the pods (Costa and Carvalho, 1961; Truol *et al*., 1987; Laguna *et al*., 1988) [5, 22, 8]; mild mottling and mosaic symptoms on potato (Salazar *et al*., 1982) [19]; leaf distortion and mosaic mottling in okra (Krishna Reddy *et al*., 2003b) [3].

Vinod *et al*. (2007) [23] reported mixed infection of PBNV and TSV in mungbean which resulted in reduction in internodal length and axillary shoots with severe stunting, vein necrosis along with chlorotic spots on young leaves and necrotic streaks, reduced lamina area, vein necrosis, yellowing on the leaf margin and chlorotic spots on matured trifoliate leaf. Mixed infections of PBNV and TSV also were recorded in both, blackgram and greengram but unable to study the synergism and sequential appearance of symptoms.

An attempt was made to correlate the symptomatology of PBNV and TSV isolates on blackgram cv. LBG-20 and greengram cv. K-851 at different growth stages *viz*., 7, 14 and 21 DAS upon artificial inoculation under glasshouse conditions with the symptoms produced under field conditions. Both the PBNV inoculations were manifested with symptoms *viz*., chlorosis, veinal chlorosis, veinal necrosis, chlorotic spots, leaf curl, and apical necrosis at 7 DAS as well as 14 DAS, while apical necrosis was not noticed at 21 DAS in both the crops. Both the TSV inoculations on blackgram cv. LBG-20 showed veinal necrosis, leaf necrosis, smalling of leaves with mosaic mottling, stunting, mosaic, apical necrosis leading to death at all the three stages while greengram cv. K-851 showed veinal necrosis, leaf necrosis, stunting and apical necrosis leading to death at 7 DAS, veinal necrosis, chlorosis, stunting, stem necrosis and death at 14 and 21 DAS, where apical necrosis was absent. Early infections caused death of the plants and leaf curl symptoms were more prominent in late infected plants under field conditions (Prasada Rao *et al.,* 2003a) [4]. Similar symptoms were recorded with PBNV infection in blackgram (Nene, 1972) [12]. It can be due to early infections of both PBNV and TSV in blackgram and greengram resulting in death or non-flowering leading to no yield whereas late infections of PBNV in blackgram and greengram did not have significant effect on grain production. In contrast to the present finding, Nene (1972) [12] reported that a few plants, which escaped death remained stunted and failed to flower and blackgram plants infected by PBNV late in the season produced less number of pods containing smaller and lighter grains; some pods did not contain grains at all. The grains produced by diseased plants were unfit both for seed as well as for consumption purposes. However, in the present study, TSV infections produced neither flowers nor grains. In the previous reports, TSV infection on blackgram (Ladhalakshmi *et al.,* 2006) [7] and mungbean (Vinod *et al.,* 2007) [23] was characterized by brown necrotic areas in the leaves, petiole, necrosis of stem and drying of the plants from the tip and finally death of the entire plant under field conditions.

Particle morphology

Electron microscopy of PBNV infected leaves of blackgram and greengram by leaf dip preparation showed quasi-spherical virus particles measuring 80-110 nm diameter. In the previous studies, ultra-thin sections of the PBNV infected leaves of tomato revealed clusters of spherical virus particles of 70-90 nm in diameter in cytoplasm, enclosed in vesicles (Prasada Rao *et al.,* 1980) [13]. Purified preparation on carbon colloidal coated copper grids negatively stained with 2 per cent uranyl acetate, revealed spherical particles of 75 to 85 nm diameter. There were a few or no membranous contaminants present and the GBNV particles had a well-defined, unstained envelope surrounded by an outer layer of projections. No internal structure could be differentiated even in particles penetrated by stain (Mohammed, 1981) [11]. Reddy *et al.* (1991a) [17] examined thin sections of PBNV infected leaf tissue with electron microscope which revealed that virus particles were associated with endoplasmic reticulum. Several particles were seen clustered in the cisternae of endoplasmic reticulum. They reported that crude plant extract and purified preparation should be fixed in 1.5 per cent glutaraldehyde and negative staining with 1 per cent uranyl acetate was preferable to the use of phosphotungstate or

ammonium molybdate. Kulkarni (1996) [6] studied the virus particles of two isolates, GBNV-D and GBNV-R by electron microscope and stated the particle size to be 80 nm in diameter and the particles are roughly spherical.

Transmission electron microscopy of TSV infected leaves of blackgram and greengram by leaf dip preparation showed quasi-spherical virus particles measuring 25-30 nm diameters. In the earlier reports under electron microscope, TSV virions were observed as spherical or isometric particles ranging from 25 to 32 nm in diameter (Salazar *et al*., 1982, Cook *et al*., 1999; Ramiah *et al*., 2001; Reddy *et al*., 2002) [19, 4, 16, 18]. Mc Daniel *et al*. (1992) [9] observed quasi-spherical particles of 27 nm and 32.8 nm in diameter, respectively. Numerous quasi spherical virus-like articles appeared to be distorted and had diameters ranging from 25 to 35 nm, and their general condition was poor in a range of negative stains (Reddy *et al*., 2002) [18]. Electron microscopy studies of TSV infected groundnut by Prasada Rao *et al.* (2003) [14] revealed spherical virus particles in the range of 25-35 nm in diameter through purified preparations. The purified preparations of TSV from infected soybean leaves from Brazil, negatively stained with uranyl acetate revealed spherical particles measuring 28 nm diameter under electron microscope (Almeida *et al.,* 2005) [1]. Electron microscopic observation of sap from TSV infected bhendi leaves revealed isometric particles (Merin Babu *et al*., 2005) [10]. The purified preparations of the TSV from infected blackgram leaves from Tamilnadu through electron microscope showed isometric particles (Ladhalakshmi *et al.,* 2006) [7]. Thus, the shape and size of particles of PBNV and TSV isolates of blackgram and greengram were in corroboration with the previous reports.

Table 1: Symptomatology of PBNV-BG and TSV-BG isolates of blackgram on susceptible checks, cv. LBG-20 and greengram cv.K-851.

Crop	PBNV-BG						TSV-BG					
	I/T	D/T	Local	No.*	Systemic	No.*	I/T	D/T	Local	No.*	Systemic	No.*
Blackgram cv. LBG-20												
7DAS	10/10	0/10	CL, NL, VN, LN	10/10	C, VN, VC, CS, LC, AN	10/10	10/10	6/10	CR, CCR, NR, VN	10/10	VN, LN, SL, S, M, AN, SN, D	10/10
14DAS	9/9	0/9	CL, NL, VN, LN	9/9	C, VN, VC, CS, LC, AN	8/9	9/9	4/9	CR, CCR, NR, VN	9/9	VN, LN, SL, S, M, AN, SN, D	9/9
21 DAS	9/9	0/9	CL, NL, VN, LN	9/9	C, VN, VC, LC	3/9	9/9	3/9	CR, CCR, NR, VN	9/9	VN, LN, SL, S, M, AN, SN, D	9/9
Greengram cv. K-851												
7DAS	9/9	1/9	CL, NL, VN, LN	9/9	C, VN, VC, CS, LC, AN, D	9/9	9/9	6/9	CR, CCR, NR, NCR, VN	9/9	VN, LN, S, SL, AN, D	9/9
14DAS	10/10	1/10	CL, NL, VN, LN	10/10	VN, VC, C, CS, LC, AN, D	8/10	10/10	3/10	CR, CCR, NR, NCR, VN	10/10	VN, C, S, SN, D	10/10
21 DAS	8/8	1/8	CL, NL, VN, LN	8/8	VN, VC, C, LC, CS, D	3/8	8/8	1/8	CR, CCR, NR, NCR, VN	8/8	VN, C, S, SN, D	8/8

* No. of plants showed local/systemic symptoms, I/T=Infected/Total plants D/T=Dead/Total plants

C-chlorosis, CR-chlrotic rings, D-death, NR-necrotic rings, NCR-necrotic concentric rings, CCR-chlorotic concentric rings, NL-necrotic lesions, CL-chlorotic lesions, SL-smalling of leaves, M-mosaic, S-stunting, SN-stem necrosis, AN-apical necrosis, LC-leaf curl, CS-chlorotic spots, NS-necrotic spots, VC-veinal chlorosis, VN-veinal necrosis

Table 2: Symptomatology of PBNV-GG and TSV-GG isolates of greengram on susceptible checks, cv. LBG-20 and greengram cv.K-851.

Crop	PBNV-GG						TSV-GG					
	I/T	D/T	Local	No.*	Systemic	No.*	I/T	D/T	Local	No.*	Systemic	No.*
Blackgram cv. LBG-20												
7DAS	9/9	0/9	CL, NL, VN, LN	9/9	C, VN, VC, CS, LC, AN	9/9	10/10	6/10	CR, CCR, NR, VN	10/10	VN, LN, SL, S, M, AN, SN, D	10/10
14DAS	10/10	0/10	CL, NL, VN, LN	10/10	C, VN, VC, CS, LC, AN	9/10	10/10	4/10	CR, CCR, NR, VN	10/10	VN, LN, SL, S, M, AN, SN, D	10/10
21 DAS	9/9	0/9	CL, NL, VN, LN	9/9	C, VN, VC, LC	3/9	9/9	4/9	CR, CCR, NR, VN	9/9	VN, LN, SL, S, M, AN, SN, D	9/9
Greengram cv. K-851												
7DAS	10/10	1/10	CL, NL, VN, LN	10/10	C, VN, VC, CS, LC, AN	10/10	10/10	7/10	CR, CCR, NR, NCR, VN	10/10	VN, LN, S, SL, AN, D	10/10
14DAS	9/9	1/9	CL, NL, VN, LN	9/9	VN, VC, C, CS, LC, AN	7/9	9/9	4/9	CR, CCR, NR, NCR, VN	9/9	VN, C, S, SN, D	9/9
21 DAS	9/9	0/9	CL, NL, VN, LN	9/9	VN, VC, C, LC, CS	5/9	8/8	1/8	CR, CCR, NR, NCR, VN	8/8	VN, C, S, SN, D	8/8

* No. of plants showed local/systemic symptoms, I/T=Infected/Total plants, D/T=Dead/Total plants

C-chlorosis, CR-chlrotic rings, D-death, NR-necrotic rings, NCR-necrotic concentric rings, CCR-chlorotic concentric rings, NL-necrotic lesions, CL-chlorotic lesions, SL-smalling of leaves, M-mosaic, S-stunting, SN-stem necrosis, AN-apical necrosis, LC-leaf curl, CS-chlorotic spots, NS-necrotic spots, VC-veinal chlorosis, VN-veinal necrosis.

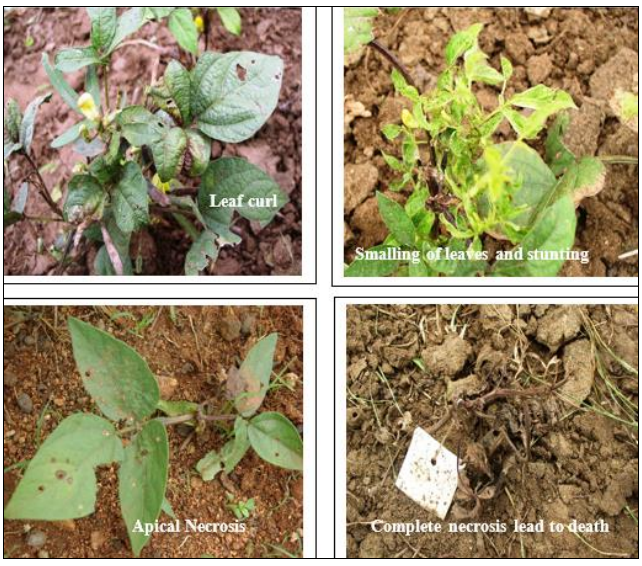


Fig 1: Symptoms produced by blackgram due to necrosis/leaf curl disease under field conditions

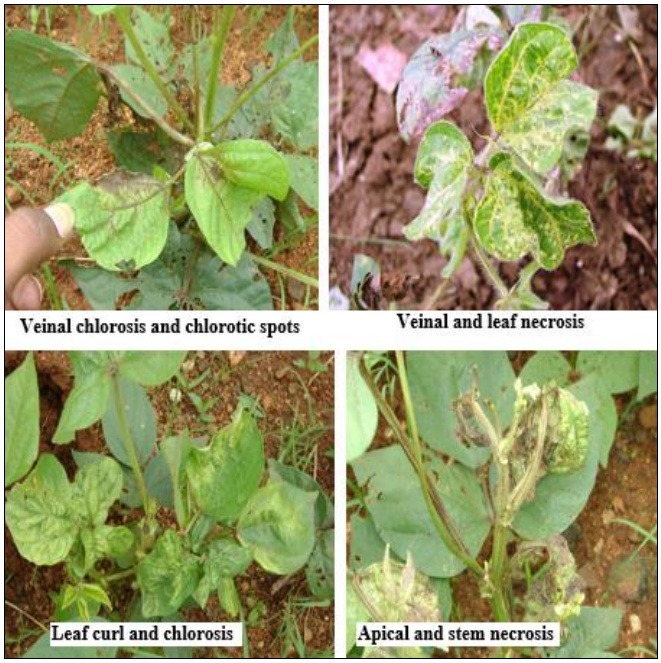


Fig 2: Symptoms produced by greengram due to necrosis/leaf curl disease under field conditions.

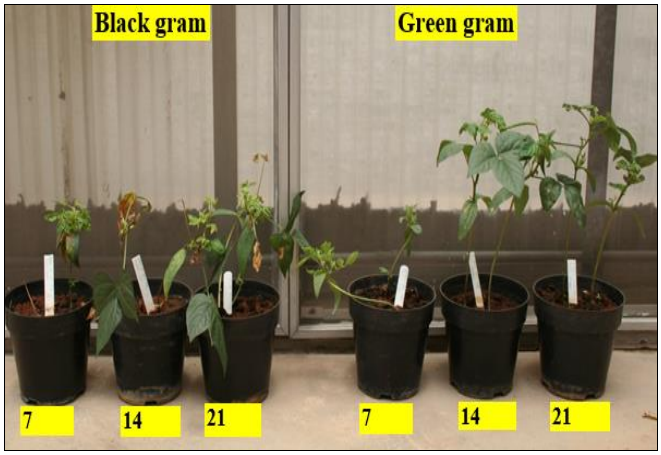


Fig 3: Symptoms induced by TSV on blackgram and greengram upon inoculations at 7DAS, 14DAS and 21 DAS (DAS-Days after sowing)

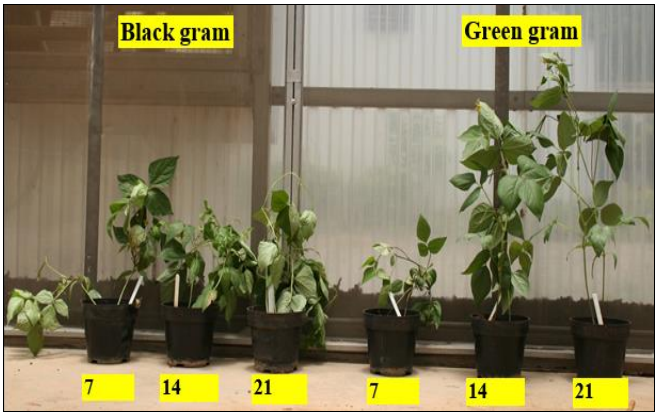


Fig 4: Symptoms induced by PBNV on blackgram and greengram upon inoculations at 7DAS, 14DAS and 21 DAS (DAS-Days after sowing)

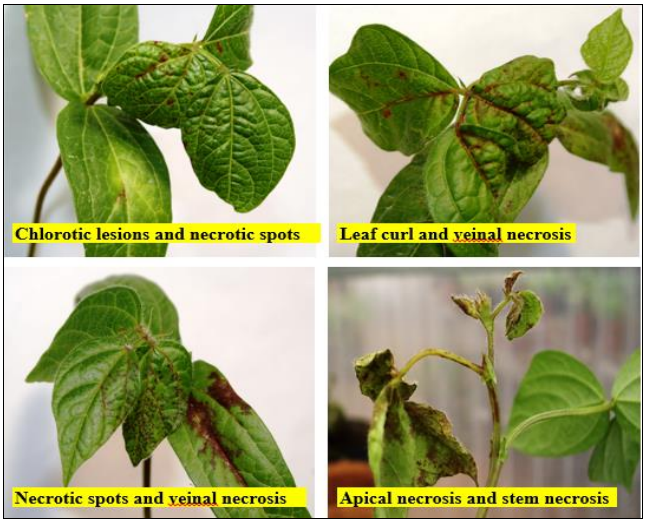
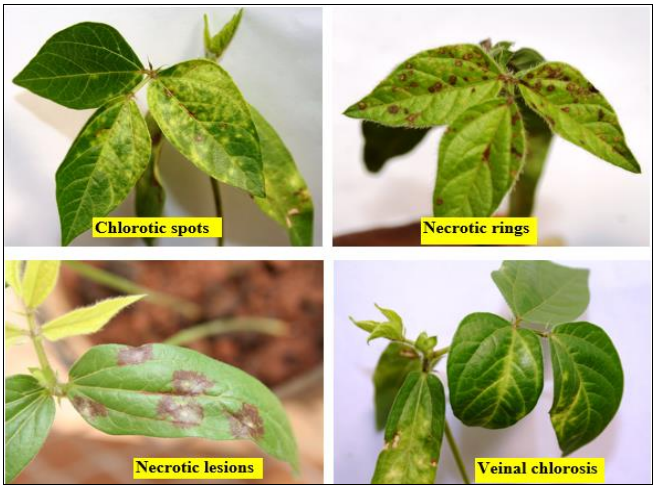


Fig 5: Symptoms induced by PBNV in blackgram upon mechanical inoculation under glasshouse conditions

Fig 6: Symptoms induced by TSV in blackgram upon mechanical inoculation under glasshouse conditions



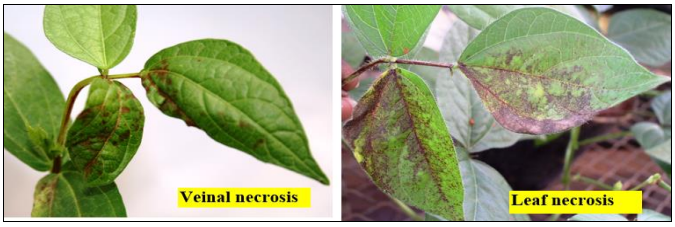


Fig 7: Symptoms induced by PBNV in greengram upon mechanical inoculation under glasshouse conditions

Fig 8: Symptoms induced by TSV in green gram upon mechanical inoculation under glasshouse conditions

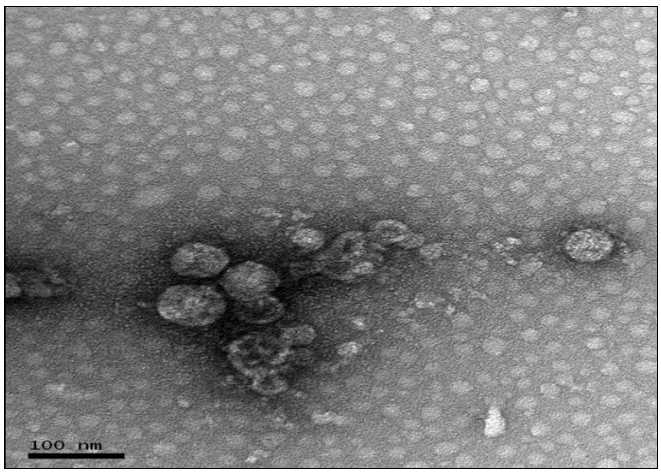


Fig 9: Electron micrograph of *Peanut bud necrosis virus* (PBNV)

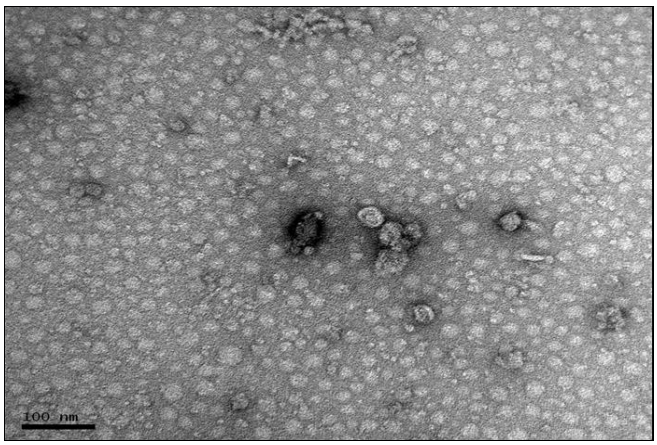


Fig 10: Electron micrograph of *Tobacco streak virus* (TSV)

Conclusion

Viral diseases, particularly those caused by *Peanut bud necrosis virus* (PBNV) and *Tobacco streak virus* (TSV), pose significant threats to blackgram and greengram production, especially in Andhra Pradesh. Both viruses cause necrotic symptoms, with TSV often resulting in more rapid plant mortality. Through mechanical inoculation studies, the research highlighted a range of chlorotic and necrotic symptoms across different growth stages, showing higher mortality in TSV infections. The particle morphology studies confirmed the presence of quasi-spherical virus particles, in line with previous findings. Overall, this research provides a detailed understanding of the symptomatology and viral impact, aiding in better diagnosis and management of these diseases in Vigna species.

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