Comparative reaction of popular high yielding spanish and virginia bunch groundnut varieties to *Tobacco streak virus*

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Abstract Reaction of five spanish (JL 24, TMV 2, Kadiri 6, Kadiri 9 and Anantha) and a virginia (Kadiri 7 Bold) groundnut cultivars was studied against *Tobacco streak virus* (TSV) by sap inoculation using different age group of plants [7–84 days after sowing (DAS)]. Among different cultivars, incubation period varied from 4 to 28 days and high incubation period recorded in Kadiri 7 Bold. The percent infection decreased with increase in the age of the plants in all the cultivars as it ranged up to 100 % in both pre-flowering (7–21 DAS) and flowering stage (28–63 DAS) plants followed by 50–100 % in maturity stage plants (70–84 DAS) except Kadiri 7 Bold. Similarly, 100 % wilting was observed in pre flowering stage plants against no wilting in maturity stage plants. However, cultivars differed in per cent wilting of flowering stage plants by recording maximum wilting (100 %) in JL24, Kadiri 6 and minimum (25.0 %) in Kadiri 7 Bold. Both localized (necrotic spots, veinal necrosis) and systemic (petiole necrosis, necrotic spots on young leaves, top growing bud and stem necrosis, axillary shoot proliferation, stunting, peg necrosis, pod necrosis, wilting of plant) symptoms induced by TSV were similar among all cultivars without any new symptoms. Prolonged stage of axillary shoot proliferation was observed for the first time in all the

cultivars. In maturity stage plants of Kadiri 7 Bold, Kadiri 9 and Anantha, systemic symptoms restricted to leaf and petiole necrosis only. Virus titer varied significantly with the age of plants and inoculum harvest at days post inoculation and least virus titer recorded by Kadiri 7 Bold at all stages of infection. Among different cultivars, Kadiri 7 Bold was least susceptible/tolerant to TSV by registering higher incubation period with less per cent infection, wilt and titer.

Keywords Groundnut cultivars · *Tobacco streak virus* (TSV) · Resistance and symptoms

Introduction

Groundnut (*Arachis hypogaea*) crop is occupied 6 m ha with production of 5 m t in India [4]. The major groundnut growing states in India are Gujarat, Andhra Pradesh (A.P), Tamil Nadu (T.N), Karnataka and Maharashtra. Productivity of groundnut is very low (857 kg/ha) in India when compared to the productivity of world (2,149 kg/ha). This could be attributed to not only rainfed nature of crop but also biotic stresses like viral diseases. Among different viruses infecting groundnut, *Groundnut bud necrosis virus* (GBNV) and *Tobacco streak virus* (TSV) are causing severe loss in yield. TSV belongs to the genus *Ilarvirus* of family *Bromoviridae* and virions characterized by quasi-isometric or occasionally bacilliform particles measuring about 27–35 nm in diameter [7].

TSV caused an epidemic on groundnut crop during rainy season of 2000 at Anantapuram district of A.P state by affecting 2,25,000 ha and loss estimated to the tune of Rs. 300 crores. The causal organism was identified as TSV and the disease was named as peanut stem necrosis disease

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(PSND) [12]. Anantapuram district is the largest producer of groundnut where it is growing nearly 7 lakh ha every year as rainfed crop during rainy season. This district was identified as hot spot area for PSND as 2–16.5 % recorded during 2004, 5–25 % in 2006 and 2–25 % in 2007 [1–3]. So far, the disease seems to be confined to the states of A.P, T.N, and Karnataka only, but its occurrence in other groundnut growing areas can not be ruled out. The virus has become increasingly important on wide range of vegetables, fiber and oilseed crops from A.P, T.N, Karnataka and Maharashtra [8].

Resistant sources are not available against TSV in the presently grown groundnut cultivars [9, 10]. Groundnut varietal reaction against TSV was also not attempted critically. Moreover, biology of groundnut TSV isolate was not characterized using different cultivars of groundnut since its epidemic on groundnut in India. Hence, present study is focused on reaction of popular groundnut cultivars against TSV including virus titer and distribution by taking different age group of susceptible (JL 24, TMV 2, Kadiri 6) and field tolerant (Anantha, Kadiri 9, Kadiri 7 Bold) groundnut cultivars belonging to spanish and virginia group.

Materials and methods

Maintenance of virus isolates

Necrotized field samples collected from Anantapuram district of A.P were subjected to direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA) [6]. Polyclonal antisera (1:4,000) developed against the coat protein (CP) of TSV at Unit of Virology; Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi was used. ELISA positive groundnut samples for TSV were used for sap inoculation on cowpea (*Vigna unguiculata* cv. Pusa Komal), a diagnostic host for TSV [11]. First true leaf stage seedlings (5 days old) with uniform growth were selected for inoculation. ELISA positive groundnut field samples macerated using pestle and mortar in 0.01 M phosphate buffer (pH 7.2, 1:1, w/v) containing 0.1 % β -mercaptoethanol as extraction buffer. The cowpea seedlings were dusted with Celite prior to inoculation. The inoculum was applied directly onto the upper surface of the leaves. After inoculations, the seedlings were washed under tap water and kept at National Phytotron Facility (NPF), IARI, New Delhi. Local lesions developed after 3–4 days post inoculation (DPI) were used for further inoculation by taking single lesion and subsequently maintained pure virus culture and designated as GN-AP3-08. Above virus culture produced typical symptoms of TSV upon re-inoculation on healthy groundnut. In addition to above isolate, two more epidemic groundnut TSV isolates collected

during 2000 and 2004 from hot spot area (Anantapuram, A.P) were obtained from the National Bureau of Plant Genetic Resources (NBPGR), Hyderabad. Thus, three groundnut TSV isolates (GN-API-00; GN-AP2-04; GN-AP3-08) were maintained on cowpea at NPF, New Delhi.

Sap inoculation

Six popularly grown cultivars (JL-24, TMV2, Kadiri 6, Anantha, Kadiri 7 Bold, Kadiri 9) belonging to spanish/virginia group were used for the study. For each cultivar, 24 pots were maintained by sowing at same time. Sap inoculation was made at the age of 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84 DAS for each cultivar and maintained respective healthy controls. Groundnut TSV isolate (GN-AP3-08) was used for sap inoculation for all the cultivars. Age of the sap inoculated seedlings was categorized into three stages as pre-flowering (7–21 DAS), flowering (28–63 DAS) and maturity (66–84 DAS) plants. Flowering stage also includes peg and pod initiation stages. The procedure used for sap inoculation was followed as described earlier for cowpea. Carborundum (320 grit) was used instead of Celite due to rough texture of leaf surface. All the seedlings were maintained at 26 ± 2 °C throughout the experiment under glasshouse conditions at Unit of Virology, IARI, New Delhi. Symptoms were recorded up to harvest stage (Table 1).

Monitoring of virus titer

Localized symptomatic leaf samples were collected and bulked separately after harvest at 7, 14, 21 DPI from each interval of sap inoculation (7–84 DAS) and subjected to DAC-ELISA test. Two replications were maintained for each sample in ELISA plate. Absorbance values ($A_{405\text{ nm}}$; 1 h) obtained were analyzed using SAS statistical program (SAS Institute Inc., NC 27513, USA) (Fig 1).

Virus distribution

The groundnut cv. JL 24 was used to study the distribution of virus in different plant parts. Sap inoculation was made at flowering stage plants (30 days old) as early age plants could not yield pods. TSV titer was monitored by DAC-ELISA using localized as well as systemic infection.

Results

Incubation period

Incubation period ranged from 4 to 7 days among pre-flowering stage plants of different cultivars. Minimum (4–5 days) and maximum (7 days) incubation period

Table 1 Effect of sap inoculation of *Tobacco streak virus* (TSV) at different growth stages of groundnut cultivars

Cultivar	Plant stage/age (DAS)	Incubation period (days)	% Infection ^a	% Wilting ^b	Visual observation ^c												
					Local				Systemic								
					1	2	3	4	5	6	7	8	9	10	11	12	
JL 24	Pre-flowering/7-21	4-5	100	100	+	+	+	+	+	+	+	+	+	+	+	+	+
	Flowering/28-63	5-7	100	25.0-100	+	+	+	+	+	+	+	+	+	+	+	+	+
	Maturity/70-84	7-12	63-100	0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
TMV 2	Pre-flowering/7-21	4-5	100	100	+	+	+	+	+	+	+	+	+	+	+	+	+
	Flowering/28-63	6-7	100	0.0-75.0	+	+	+	+	+	+	+	+	+	+	+	+	+
	Maturity/70-84	7-12	63-88	0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
Kadiri 6	Pre-flowering/7-21	5-6	100	100	+	+	+	+	+	+	+	+	+	+	+	+	+
	Flowering/28-63	6-8	100	0.0-100	+	+	+	+	+	+	+	+	+	+	+	+	+
	Maturity/70-84	10-14	86-100	0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
Anantha	Pre-flowering/7-21	6-7	100.0	100.0	+	+	+	+	+	+	+	+	+	+	+	+	+
	Flowering/28-63	7-8	75-100	0.0-63.0	+	+	+	+	+	+	+	+	+	+	+	+	+
	Maturity/70-84	10-14	50-75	0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
Kadiri 9	Pre-flowering/7-21	7	100.0	75.0-100	+	+	+	+	+	+	+	+	+	+	+	+	+
	Flowering/28-63	7-21	63-100	0.0-75.0	+	+	+	+	+	+	+	+	+	+	+	+	+
	Maturity/70-84	21	50-63	0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
Kadiri 7 Bold	Pre-flowering/7-21	7	100	38-100	+	+	+	+	+	+	+	+	+	+	+	+	+
	Flowering/28-63	7-28	63-75	0.0-25.0	+	+	+	+	+	+	+	+	+	+	+	+	+
	Maturity/70-84	28	50.0	0.0	+	+	+	+	+	+	+	+	+	+	+	+	+

DAS days after sowing, + presence, - absence

^a % Infection: no. of symptomatic plants/no. of plants inoculated^b % Wilting: no. of dead plants/total plants^c Symptom description: localized (1-3) and systemic symptoms (4-12) recorded on different groundnut cultivars: necrotic spots on leaf (1); veinal necrosis (2); leaf yellowing and wilting (3); petiole necrosis (4); necrotic lesions on young leaves (5); top growing bud and stem necrosis (6); stunted growth (7); axillary shoot proliferation with small leaves (8); flower and peg formation (9); peg necrosis (10); pod necrosis (11); pod size reduction (12)

Fig. 1 Graphic view of effect of sap inoculation of TSV on different age group of (7–84 DAS) groundnut cultivars (a JL 24; b TMV 2; c Kadiri 6; d Anantha; e Kadiri 9; f Kadiri 7 Bold) on virus titer as determined by DAC-ELISA at 7, 14 and 21 days post inoculation (DPI)

recorded in JL24, TMV2 and Kadiri 7 Bold respectively. Similarly, in all the cultivars tested, incubation period varied from 5 to 28 days and 7 to 28 days in flowering and maturity stage plants respectively. Further, incubation period is directly proportional to the age of the plants in all the cultivars (Table 1).

Per cent infection

Early infection did not allow to bear flower and pods as 100 % infection observed in pre flowering stage plants in all the cultivars. Flowering stage plants also showed 100 % infection among all the cultivars except Anantha, Kadiri 9 and Kadiri 7 Bold. However, maturity stage seedlings of different cultivars showed 50–100 % infection only and least per cent infection recorded in Kadiri 7 Bold (Table 1).

Wilting percent

Wilting was not observed in maturity stage plants belonging to different cultivars against 100 % wilt in pre-flowering stage plants in all the cultivars. However, flowering stage plants of different cultivars showed considerable differences in percent wilting as maximum of 25–100 % wilting in JL24 and minimum of 0.0–25 % wilting in Kadiri 7 Bold were recorded (Table 1).

Symptoms

TSV induced both localized and systemic symptoms in all the cultivars. The localized symptoms recorded among different cultivars were necrotic spots on leaf and veinal necrosis, leaf yellowing and wilting. The systemic symptoms observed were petiole necrosis, necrotic spots on young leaves, top growing bud and stem necrosis, axillary shoot proliferation with reduced size of leaflets having general chlorosis and stunted growth, peg necrosis, pod necrosis with reduced in size and wilting of whole plants. Localized infection was observed by all cultivars at all age group of plants. There was no flower and pod formation observed in pre flowering stage plants in all cultivars due to severe infection at an early age. Similarly, systemic infection was observed at all age group of plants of different cultivars. However, there were no systemic symptoms observed in the maturity age group of plants in the cultivars Kadiri 7 Bold, Kadiri 9 and Anantha as symptoms restricted to petiole necrosis only (Table 1).

Virus titer

Virus titer significantly varied with the age of plants and inoculum harvest period. In JL24, virus titer was maximum ($A_{405\text{ nm}}$: 2.05–3.00) in 7–42 days old plants at 7 DPI,

followed by 14 DPI ($A_{405\text{ nm}}$: 0.81–2.00) and minimum at 21 DPI ($A_{405\text{ nm}}$: 0.21–1.33). On the other hand, the virus titer in 70–84 days old plants was maximum at 21 DPI ($A_{405\text{ nm}}$: 2.30–2.55) and minimum at 7 DPI ($A_{405\text{ nm}}$: 0.08–0.83), while in 49–63 days old plants, the virus titer was maximum at 14 DPI ($A_{405\text{ nm}}$: 2.12–2.32). More or less similar pattern of changes in virus titer was observed according to the age of plants and inoculum harvest period in all the groundnut cultivars tested. Among different cultivars, least virus titer was recorded by Kadiri 7 Bold ($A_{405\text{ nm}}$: 1.75) followed by Kadiri-9 ($A_{405\text{ nm}}$: 2.42), Anantha ($A_{405\text{ nm}}$: 2.42), JL 24 ($A_{405\text{ nm}}$: 3.0), TMV 2 ($A_{405\text{ nm}}$: 3.0) and Kadiri 6 ($A_{405\text{ nm}}$: 3.0) (Fig. 1).

Virus distribution

In the cultivar JL 24, maximum virus titer recorded in young leaves ($A_{405\text{ nm}}$: 2.20–3.00) and sap inoculated leaves ($A_{405\text{ nm}}$: 2.0–3.00) followed by peg ($A_{405\text{ nm}}$: 1.8–3.00), pod shell ($A_{405\text{ nm}}$: 0.38–3.00), petiole ($A_{405\text{ nm}}$: 1.23–1.75), seed coat ($A_{405\text{ nm}}$: 0.20–1.72), dried seed ($A_{405\text{ nm}}$: 0.20–1.20), flower ($A_{405\text{ nm}}$: 0.48–1.16), root ($A_{405\text{ nm}}$: 0.56–0.74) and endosperm including embryo ($A_{405\text{ nm}}$: 0.05–0.08).

Discussion

Resistance sources are lacking against viral diseases in many crops. In this context, in the present study, reaction of popularly grown groundnut cultivars against TSV was tested by sap inoculation under glasshouse conditions using different age group of plants. Initially, this study was initiated using three groundnut TSV isolates (GN-AP1-00; GN-AP2-04; GN-AP3-08) separately. However, entire sap inoculation study was carried out using a groundnut TSV isolate (GN-AP3-08) as symptoms produced by all the three above groundnut TSV isolates were similar (data not showed). Some of the susceptible (JL 24, TMV 2, Kadiri 6) and field tolerant groundnut cultivars (Anantha, Kadiri 9, Kadiri 7 Bold) were selected for this study based on their disease reaction under field conditions against TSV from hot spot area at Agricultural Research Station, Kadiri, Andhra Pradesh since 2002–2007 [1–3].

Increase in incubation period was observed as the age of plants increased in all the cultivars tested. Thus, incubation period varied from 4 to 28 days depending upon the cultivars tested at all stages of infection. Maximum incubation period (7–28 days) recorded in Kadiri 7 Bold and minimum in JL24 and TMV2 (4–12 days). Similarly, per cent infection decreased as the increase in age of the plants in all the cultivars and least per cent infection was recorded in Kadiri 7 Bold. In contrary, no wilting was observed in

maturity stage plants belonging to different cultivars (Table 1). This could be due to adult plant resistance and observed in many viral infections in agricultural crops. However, for the first time, we are reporting adult plant resistance against TSV in groundnut as it was reported earlier for GBNV in groundnut by Buiel [5]. Some of the typical symptoms of TSV namely necrotic spots, top bud necrosis, axillary shoot proliferation, pod necrosis recorded in the present study were in accordance with the field symptoms described earlier by Reddy et al. [12]. There was no symptom variation observed among groundnut cultivars used in this study. But, cultivars differed in expression of symptoms based on the stage of infection. Among different symptoms, prolonged stage of axillary shoot proliferation for TSV is identified for the first time in the present study as it was described earlier for GBNV in groundnut (Table 1).

Above observations were further supported by monitoring the virus titer at weekly intervals (7, 14 and 21 DPI) from sap inoculated plants of different age groups (7–84 DAS). In pre-flowering stage plants, the virus titer was gradually decreased from 7 to 21 DPI. This could be due to maximum virus holding capacity immediately after sap inoculation (7 DPI) in young plants and subsequently decrease in titer due to quick wilting of sap inoculated leaves. In contrary, virus titer was gradually increased from 7 to 21 DPI in both flowering and maturity stage plants (Fig. 1). This could be due to either more incubation period or adult plant resistance in matured plants. Similar observations were reported by Kalyani et al. [9] as they screened 56 groundnut germplasm accessions from 20 wild *Arachis* spp. in four sections (*Arachis*, *Erectoides*, *Procumbence* and *Rhizomatosae*) and no systemic infection was found in the following accessions 8139, 8195, 8200, 8203, 8205, 11550, CG 8144, ICG 13210 even though 0–100 % infection was there on sap inoculated leaves. TSV was detected in almost all the plant parts except endosperm and embryo by sap inoculation in groundnut cv. JL 24 as Indian TSV isolate was non seed transmissible in legume crops [11, 13].

Based on the biological parameters tested including virus titer, the virginia cultivar Kadiri 7 Bold is least susceptible to TSV among all the cultivars tested by recording less per cent infection and wilting with high incubation period. Besides, it supported less virus titer at all stages of infection. Similarly, among spanish cultivars tested, Kadiri 9 is least susceptible to TSV. So far, resistance to TSV has not yet been identified in the presently cultivated species [9, 10]. However, in this study, we are reporting least susceptible/tolerant cultivars viz., Kadiri 7 Bold and Kadiri 9 among virginia and spanish groups respectively. Further, present investigation revealed that presently grown popular cultivars in India (Kadiri 6, JL24, and TMV 2) are highly

susceptible to TSV as they showed systemic symptoms even at maturity stage plants by supporting high virus titer. Further research is needed to exploit resistance by screening germplasm both under field and glasshouse conditions. Moreover, it is difficult to start breeding programme without resistance sources as it is lacking in the presently cultivated genotypes of groundnut.

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References

1. Anonymous, Annual progress report 2004, National Research Center on Groundnut, Junagadh; 2004. p. 23.
2. Anonymous, Annual Report 2006, National Research Center on Groundnut, Junagadh; 2006. p. 2.
3. Anonymous, Annual Report 2007, National Research Center on Groundnut, Junagadh; 2007. p. 2.
4. Anonymous, FAO Stat Data 2004. <http://faostat.fao.org/faostat> (2007).
5. Buiel AAM. Quantitative resistance to peanut bud necrosis tospovirus in groundnut. PhD thesis. Wageningen Agricultural University: The Netherlands; 1996.
6. Clark MF, Joseph Bar. Enzyme-linked immunosorbent assay in plant virology. In: Marmorash K, Korowski H, editors. *Methods in virology*, vol. VII. New York: Academic Press; 1984. p. 51–85.
7. Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA. *Virus taxonomy*. VIIIth Report of ICTV, Academic Press: New York; 2005.
8. Jain RK, Vemana K. *Tobacco streak virus* an emerging virus in vegetable crops. In: Rao GP, Kumar PL, Holuguin-Pena RJ, editors. *Characterization, diagnosis and management of plant viruses*. Vegetable and pulse crops, vol. 13. Texas: Studium Press LLC; 2008. p. 203–12.
9. Kalyani G, Reddy AS, Lava kumar P. Sources of resistance to *Tobacco streak virus* in wild *Arachis* (Fabaceae: Papilionoidae). *Plant Dis*. 2007;91:1585–90.
10. Prasada Rao RDVJ, Reddy DVR, Nigam SN, Reddy AS, Waliyar F, Yellamanda Reddy T, Subramanyam K, John Sudheer M, Naik KSS, Bandyopadhyay A, Desai S, Ghewande MP, Basu MS and Somasekhar. 2003. Peanut stem necrosis-a new disease of groundnut in India. *Information Bulletin no. 67*. ICRISAT, Patancheru, Andhra Pradesh, p. 12.
11. Prasada Rao RDVJ, Jyothirmai Madhavi K, Reddy AS, Varaprasad KS, Nigam SN, Sharma KK, Lava Kumar P, Waliyar F. Non transmission of *Tobacco streak virus* isolate occurring in India through the seeds of some crop and weed hosts. *Indian J Plant Prot*. 2009;37:92–6.
12. Reddy AS, Prasada Rao RDVJ, Thirumala Devi K, Reddy SV, Maya MA, Roberts I, Satyanarayana T, Subramaniam K, Reddy DVR. Occurrence of *Tobacco streak virus* on peanut (*Arachis hypogaea* L.) in India. *Plant Dis*. 2002;86:173–8.
13. Vemana K, Jain RK. New experimental hosts of *Tobacco streak virus* and absence of true seed transmission in leguminous hosts. *Indian J Virol*. 2010;21:117–27. doi:[10.1007/s1337-010-0081-0](https://doi.org/10.1007/s1337-010-0081-0).