JOURNAL OF PURE AND APPLIED MICROBIOLOGY, FEBRUARY 2014. Vol. **8**(1), p. 749-752

Biological Characterization and Detection of Groundnut Bud Necrosis Virus (GBNV) in Different Parts of Tomato

Gurupad B. Balol and M.S. Patil

Department of Plant Pathology , University of Agricultural Sciences, UAS., Dharwad, Karnataka, India.

(Received: 08 May 2012; accepted: 14 June 2012)

Tomato bud blight disease caused by Groundnut bud necrosis virus (GBNV) belongs to genus *Tospovirus* of the *Bunyaviridae*. This is economically important disease affecting tomato crop. Virus causes, necrotic spots on leaves petiole and stems, chlorotic spots on fruits, bud blight and finally wilting of the plants. Virus was mechanically transmitted on to cow pea cv. C152. leaves and produced chlorotic as well necrotic local lesions. Reverse transcription-polymerase chain reaction (RT-PCR) was used for studying the presence of GBNV in different parts, i.e., roots, midrib, fruit pericarp and leaves of tomato bud blight field sample. Amplification of 831bp GBNV-CP by RT-PCR with degenerate primers revealed the positive reaction for GBNV infection in different parts, i.e., roots, midrib, fruit pericarp and leaves of tomato.

Key word: GBNV, GBNV-CP, RT-PCR, Detection, Bud blight.

Groundnut bud necrosis virus (GBNV) causes bud blight disease in tomato. The genus Tospovirus of the family Bunyaviridae is composed of 19 species described so far, and of them 14 have been identified from Asia[1]. GBNV is the most economically important virus affecting a variety of crops such as peanut, potato, tomato, soybean, urdbean, mungbean and cowpea.[2,3,1]

The Tospovirus infection on tomato is called as bud blight, the species of groundnut bud necrosis virus (GBNV) identified as the causal agent of the tomato bud blight[4]. The disease was first observed in tomato variety marglobe in Nilgiri hills during 1964.The disease is characterized by bronze or purple coloured leaves, severe necrosis of buds, petioles, and pale yellow or red concentric rings turning into necrosis on fruits[5]. In karnataka this disease on tomato causes severe yield losses every year[6]. In India host range and serological studies indicated that tomato *Tospovirus* is considered as a strain of GBNV and designated as a GBNV – To. It is believed to be restricted to the Indian sub-continent. However, reports of GBNV from other parts of Asia[7] and spread by polyphaguous insect vector, *Thrips palmi* to other parts of the world [4]. An unusual disease of tomato characterized by leaf mottling and necrotic streaks on veins, shortened internodes, necrosis of terminal buds, and concentric rings on fruits was observed during surveys conducted from 2010 to 2011 in Godagari Upzila, Rajshahi district, Bangladesh[8].

Cowpea cultivar C-152 was used as a virus indicator assay host where the inoculated leaves produced chlorotic lesions[9,10,11,12]. On cowpea cv C-152 GBNV produced concentric local lesions on the inoculated leaves within 4 to 5 days after inoculation.

During 2011-12 tomato plant showing typical symptoms of bud blight, chlorotic spots on fruits, necrotic spots on leaves, necrotic streaks on stems and bronzing of leaves(Figure 1. A-F) were collected and taken for biological characterization and molecular detection of GBNV

* To whom all correspondence should be addressed.
E-mail: gurupadbalol@gmail.com

using reverse transcription-polymerase chain reaction (RT-PCR) is presented below.

MATERIALS AND METHODS

Source of samples

Bud blight infected Tomato sample was collected from Dharwad Farm. Then the leaf samples were subjected to biological characterization and sample from different parts, i.e., roots, midrib, fruit pericarp and leaves were subjected to RT-PCR detection.

Mechanical transmission

Infected leaves of tomato were washed with distilled water, blotter dried and macerated in chilled 0.05 M phosphate buffer containing 0.2% β-mercaptoethanol. The extract obtained from the macerate was inoculated on to cowpea (*Vigna unguiculata* cv. C-152) at the primary leaf growth stage, using celite powder as an abrasive. The mechanical sap inoculation procedure was as described by[13].

Molecular detection

Total RNA isolation

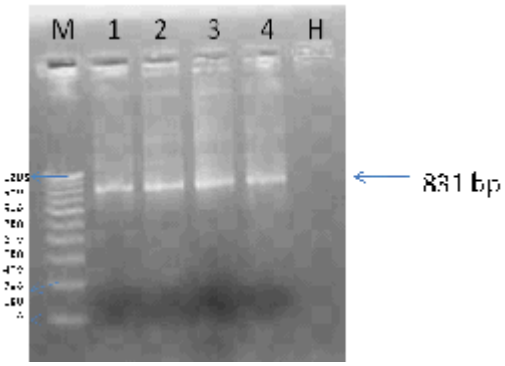
Different parts, i.e., roots, midrib, fruit pericarp and leaves of GBNV-infected tomato plants were taken for RNA isolation. RNA from corresponding healthy sample was also extracted to be used as negative control. Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA

RT-PCR for amplification of *CP* genes

cDNA was synthesized from mRNA through reverse transcriptase in a 20 ¼l reaction mixture containing 5 ng of total RNA isolated from the infected and non infected samples of tomato. M-MuLV RT-Kit from Bangalore Genei was used for RT-PCR. The degenerate primers pair GBNV-CP.F 5' ATGTCTAMCGTYAAGCAVCTHAMCG 3' and GBNV-CP.R5' TTACAMTTCCARM GAAGKRCHAG 3'was used for amplification of *CP* gene of GBNV. Amplification was performed in an automated Thermocycler (JH, BIO , Germany) programmed for one cycle 5 min as initial denaturation at 94ºC and 35 cycles involving 30 s of denaturation at 94ºC, 1 min annealing at 52ºC, 2 min for extension at 72ºC, followed by one cycle of final extension for 10 min at 72ºC. RT-PCR amplified products were analyzed by electrophoresis in 1% agarose gel at 60V for 1 h and staining with ethidium bromide.

Fig. 1. Bud blight disease symptoms on tomato includes wilting of plants and deformation of fruits (A) , necrotic spots on leaves (B), bud blight (C), leaf bronzing and stunting of plants (D), necrotic streaks on stem (E) and Chlorotic spots on fruits and reduction in fruit size (F).

Fig. 2. Mechanical transmission of GBNV from bud blight tomato sample on cow pea



lane M: 100 bp Marker Lane 1= root, lane 2= midrib, lane 3= fruit pericarp, lane 4= leaf from bud blight infected tomato samples , and lane H: Healthy sample (negative control).

Fig. 3. Molecular detection of GBNV in different parts of tomato

Symptomatology

In the field, tomato bud blight infected plants showed bronzing of leaves, chlorotic and necrotic rings on leaves and stems and necrosis on the growing bud. The disease caused death of the plant or drastic reduction in marketable yield of the fruits. The result are in confirmation with earlier findings in tomato crop[14,15,5].

Transmission

The causal virus was sap transmitted from field infected plants to healthy plants of cow pea cv.C-152, generally used as diagnostic hosts for tospoviruses. On inoculated leaves of cowpea, chlorotic as well necrotic lesions appeared within 7 to 8 days (Figure. 2). The GBNV tomato was easily detected by using indicator plants such as cowpea C- 152. The results of virus detection were the similar to the earlier findings of *Tospovirus* detection [16,17].

RT-PCR: Different parts, i.e., roots, midrib, fruit pericarp and leaves of GBNV-infected tomato plants (Figure. 3) gave positive results in RT-PCR. RT-PCR products obtained with the primer pair GBNV-CP.F / GBNV-CP.R in agarose gel electrophoresis revealed presence of amplicons of ~ 831bp corresponding to *CP* genes of GBNV.

RT- PCR based detection of GBNV virus suggested the association of an isolate of GBNV with bud blight disease of tomato. The result are in confirmation with earlier findings of the samples collected from Kerala[4,] Dharwad [6], and from Pune and Rahuri (Maharashtra), Coimbatore (Tamil Nadu) and Kanpur (UP)[5,18,19,17] in tomato and other crops. Detection of virus result is in confirmation with earlier findings[20.] From the present study and previous review it can be concluded that the technique is of great significance in detection of GBNV on different parts, i.e., roots, midrib, fruit pericarp and leaves of-infected tomato plants using RT-PCR.

ACKNOWLEDGEMENTS

Corresponding author is thankful to ICAR, New Delhi, India for financial assistance in the form of ICAR-SRF.

REFERENCES

1. Pappu H.R., Jones, R.A.C. and Jain, R.K., Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. *Virus Research;* 2009: **141**: 219-236.
2. Akram, M., Jain, R.K., Chaudhary, V., Ahlawat, Y.S. and Khurana, S.M.P., , Comparison of groundnut bud necrosis virus isolates based on the movement protein (NSm) gene sequences. *Ann. Appl. Biol.*,2004: **145**: 285-289.
3. Jain R.K., Bag S., Umamaheshwaran K. and Mandal. B., Natural infection by Tospoviruses of cucurbitaceous and fabaceous vegetable crops in India. *Journal of Phytopath.*, 2007: **155**: 22 25.
4. Umamaheswaran, K., Jain, R.K. Bhat, A.I and Ahlawat, Y.S. Biological and molecular characterization of a *Tospovirus* isolate from tomato and its relationship with other

tospoviruses. Indian Phytopath, 2003: **56:** 168-173.

5. Raja, P. and Jain R.K., Molecular diagnosis of groundnut bud necrosis virus causing bud blight of tomato. *Indian Phytopath.*,2006: **59**(3): 359-362.
6. Manjunatha, L., Studies on bud blight disease of tomato Caused by groundnut bud necrosis virus. *M.Sc. Thesis*, 2008: University of Agricultural Sciences, Dharwad. 102pp.
7. Thuan, T. T. and Trung, H. M., 1996, Groundnut virus disease in the Asia-Pacific Region. Summary and recommendations of the fourth meeting of the International working group, Ed. by Reddy, D.V.R. and Gowda, C. L. L., pp. 34 (ICRISAT, Patancheru, A.P, India, 1995).
8. Akhter, M. S.; Holkar, S. K.; Akanda, A. M.; Mandal, B.; Jain, R. K., First report of *Groundnut bud necrosis virus* in tomato in Bangladesh. *Plant Dis.*,2012 : **96**(6): 917-918.
9. Ghanekar, A.M., Reddy, D.V.R., Iizuka, N., Amin, P.W. and Gibbons, R.W., Bud necrosis of groundnut (*Arachis hypogaea*) in India caused by Tomato spotted wilt virus. *Ann. Appl. Biol.*,1979: **93**: 173-179.
10. Prasada Rao, R.D.V.J., Chakraborthy, S.K., Rajeswari, R. and Reddy, A.S., Occurrence of *Tomato spotted wilt virus* on cowpea (*Vigna unguiculata* (L.) Walp.), chilli (*Capsicum annuum* L.) and brinjal (*Solanum melongena* L.). *Indian J. Pl. Prot*., 1987: **15**: 117-119.
11. Prasada Rao, R.D.V.J., Rajeswari, R., Veerabhadra Rao, M., Raghunathan, V. and Joshi, N.C., Spotted wilt of pea in India. *Indian Phytopath.*, 1985: **38**: 90-93.
12. Prasada Rao, R.D.V.J., Saratbabu, B., Sreekant, M. and Manojkumar, V., ELISA and infectivity assay based survey for detection of peanut bud necrosis virus in mungbean and urdbean in Andhra Pradesh. *Indian J. Pl. Protect.,* 2003: **31**: 26-28.
13. Bhat, A.I., Jain, R.K., Varma, A. and Lal, S.K., Nucleocapsid protein gene sequence studies suggest that soybean bud blight is caused by a strain of groundnut bud necrosis virus. *Curr. Sci*., 2002: **82**: 1389-1392.
14. Noordam, D., 1973. *Identification of Plant Viruses: Methods & Experiments*. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands, 207 pp.
15. Cuppertino, F.P., Lin, M.T., Munaz, J.O., Perdas na producao do pimentao induziadas pelo viros de vira cabeca de tomateiro. *Fitopatologia Brasileria*, 1984: **9**: 397.
16. Diez, M.J., Nuez, F., Jorda, C. Juarez, M. And Ortega, A., Busqueda de fuentes de resistenciaal virus del bronceado del tomato (Tomato spotted wilt virus) para lame jora del tomate ydel pimiento. *Actas de Horticultura*, 1993: **10**: 1286-1271.
17. Louro, D., Detection and identification of tomato spotted wilt virus and impatiens necrotic spot virus in Portugal. *Acta Hort.*,1996: **431**: 99-105.
18. Akram, M. and Naimuddin, Biological characterization and variability of the nucleocapsid protein gene of *Groundnut bud necrosis virus* isolates infecting pea from India. *Phytopathologia Mediterranea,* 2012: **51 (2):** 266"275.
19. Jain, R.K., Pappu, H.R., Pappu, S.S., Reddy, M.K. and Vani, A., Watermelon bud necrosis tospovirus is a distinct virus species belonging to serogroup IV. *Arch. Virol.* 1998: **143**: 1637-1644.
20. Reddy, A.B., Krishna Reddy, M., Salil Jalali, Patil, M.S. and Usharani, Detection of a *tospovirus infecting tomato (Solanum lycopersicum). Indian J. Virol.*,2008: **19**(1): 32-35.
21. Soweha, H. E., Serological and Sero-molecular Studies on Banana Bunchy Top Disease and in Different Parts of Virus-infected Banana Plant. *J. Agri. Soc. Sci*., 2005: **1**(3) 273-275.