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Immuno-nucleobased diagnosis of *Tospoviruses* infecting tomato and its reactions on different tomato genotypes

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Abstract

Tospoviruses are phytopathogens responsible for significant worldwide crop losses.Tospoviruses are emerging threats of tomato production in globally. The present study was undertaken to identify tospoviruses and their interaction with vector population in Bangladesh which help to develop a durable integrated control package. Among the member of tospoviruses infecting tomato Groundnut bud necrosis virus (GBNV) has been identified based on DAC-ELISA and PCR from Godagari, Rajshahi District with the percent disease incidence 55 to 80%. We tentatively identified Thrips palmi as a vector of GBNV in Bangladesh. Thirteen varieties of tomato has been tested against GBNV and revealed that most the tomato varieties are susceptible against GBNV. The percent disease incidence has been observed 20 to 100 % among the tested BARI released tomato varieties.

Key-Words: Tospoviruses, Tomato, Genotypes

Introduction

Tomato (*Solanum lycopersicon*) is an economically important vegetable crop for resource-poor farmers in Bangladesh cultivated widely in winter crop season (November to February) and small scale protected cultivation in summer season (March to August) . Tomato cultivation faces a lot of fungal, bacterial, viral and nematode disease problems in Bangladesh which leads to low yield as compare to other tomato growing country in the globe. The devastating occurrence of viral diseases of tomato might be the limiting factors for low yield of tomato in Bangladesh. So far, 17 different plant viruses have been recorded in tomato in Bangladesh (Akanda and Rahman, 1993; Akanda 1994; Akanda *et al.* 1999; Akhter *et al.*, 2012). Among the viral pathogens of tomato Tospoviruses (family *Bunyaviridae*, genus *Tospovirus*) has emerge as a new threat of treat of tomato production in Asian sub-continent including Bangladesh (Mandal *et al*.,).

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In Bangladesh, spotted wilt or bud necrosis disease of tomato has emerged as a significant problem, and most of the tomato cultivars are susceptible (Farooq and Akanda, 2007). During 2011 and 2012, an epidemic of *Groundnut bud necrosis virus* seriously affected cultivation of tomato in the Rajshahi district the leading tomato producing region of Bangladesh (Akhter *et al.*, 2012). Member of the Tospoviruses present in tomato in Bangladesh includes *Tomato spotted wilt virus* (TSWV) and *Groundnut bud necrosis virus* (GBNV) but their vector population (Thrips) yet to be identified. It has been reported that worldwide distribution of other member of tospoviruses like *Capsicum chlorosis virus* (CaCV) and *Watermelon bud necrosis virus* (WBNV) lead epidemics of tomato production. So, the project has been designed to find out clear picture of tospoviruses infecting tomato and their interaction with vector population in Bangladesh which help to develop a durable integrated control package.

Material and Methods

The survey was conducted at five locations namely Garipur, Premtoli, Mohisalbari, Harisankorpur and Bidirpur of Godagari Upazilla of Rajshahi District the

leading tomato producing region in Bangladesh. Characteristics symptoms of tospovirus and percent disease incidence have been noted systematically. Number of thrips both adult and larvae were counted from randomly selected infected tomato plant with the help of magnifying glass. Five plots of each location white and yellow sticky traps have been set up to trapped thrips species. Thrips population as well as disease incidence data has been taken every five days interval. Symptomatic samples have been brought out in the Fruit Research Station, Plant Pathology Lab for further use.

Mechanical inoculation of Tospoviruses

The tomato leaf samples representing characteristics symptoms collected from five different locations of Godagari Upazilla were mechanically inoculated on to Tomato, *Nicotiana tabacum*, *N. benthamiana*, following the methods described by Yeh *et al.* (1984). In the mechanical inoculation 0.01 M potassium buffer, pH 7.1 containing 0.05% sodium sulphite and 600-mesh carborandum powder were used. The inoculated plants were grown and maintained in an insect proof net house . Twenty replicated plants of each test plant species were inoculated for each symptom as well as healthy leaf samples. The same number of plants representing each group was used as non-inoculated control. The photographs of symptomatic and asymptomatic plants were taken for further illustration.

Immuno-nucleobased detection of Tospoviruses

Direct Antigen Coating ELISA- DAC ELISA

Presence of the virus in lyophilized samples was confirmed by direct antigen coated enzyme-linked immuno-sorbent assay (DACELISA) using polyclonal antibodies (PAb) against GBNV, TSWV, CaCV and WBNV as described by Clark and Bar-Joseph. Leaf tissue (500 mg) was ground in 1 ml extraction buffer and 200 µl samples were used in duplicates for ELISA. The PAb to PRSV was used at 1:2000 dilutions. The reaction was recorded 1 h after adding substrate by an ELISA reader.

PCR-Based Detection of Tospoviruses

Total RNA was isolated from positive symptomatic samples (100 mg) using SV Total RNA Isolation System (Promega) according to the manufacturer's instructions. The resulting RNA preparation was used as a template in RT-PCR. The first strand cDNA was synthesized with with 15.0 µl RNA and 10 pM reverse primer
(5'CTTCTTWGARTGTHCACCATARTCATCC 3') using 2.0 units of IMPROM II reverse transcriptase (Promega, Madison, USA) and 23.0 µl sterile distilled water to make up the volume to 50.0 µl. The 3'-terminal region of N gene of Tospovirus genome was amplified by polymerase chain reaction (PCR) using 20.0 µl cDNA template, 2.5 units of GoTaq Flexi DNA polymerase (Promega, Madison, USA) with 10 pM each of specific primers of Tospoviruses (forward and reverse).

Detection of Thrips species as a vector of Tospovirus

Thrips is a tiny insect and difficult to detect. The eggs are laid with in the plant tissue which is not visible under naked eyes. Identification of thrips species using conventional and molecular method are going on under the project. Transmission test of tospoviruses using thrips vector is also going on.

Screening of tomato varieties against Tospoviruses

As many as thirteen popular BARI released tomato variety namely BARI tomato – 2, BARI tomato – 3, BARI hybrid tomato – 4, BARI hybrid tomato – 5, BARI hybrid tomato – 6, BARI tomato – 7, BARI hybrid tomato – 7, BARI hybrid tomato – 8, BARI tomato – 8, BARI tomato – 9, BARI tomato – 11, BARI tomato – 14 and BARI tomato – 1 has been grown in the Research farm of FRS. To study the vector (Thrips population) and tospoviruses white and yellow sticky trap has been set up in the experimental field and counted the thrips population every morning after the first flowing and correlated the disease incidence and thrips population trapped in the sticky trap.

Results and Discussion

Tospoviruses has recently been introduced as a devastating plant viruses infecting tomato transmitted by thrips. Survey of the Godagari upazilla revealed the characteristics symptoms of tospoviruses i.e. shortening the internode, wilting and necrosis of shoot and growing part of all cultivated tomato cultivars. The percent disease incidence was recorded as 55 to 80%.

Direct Antigen Coating ELISA- DAC ELISA

Twenty five symptomatic samples (five from each location) has been subjected to DAC-ELISA. Three antisera namely GBNV, CaCV and TSWV has been tested in the DAC-ELISA. The ELISA results have been presented in the table 2. After one hour of adding substrate buffer GBNV treated well of the microtitre plate has been developed yellow color which indicated the presence of GBNV in the tomato samples.

PCR-Based detection of Tospoviruses

ELISA positive samples were subjected to PCR amplification using GBNV, CaCV and TSWV specific primers. The presence of <500 bp DNA band by using GBNV primer confirmed the occurring of GBNV at Godagari upazilla. Other two member of tospovirus namely CaCV and TSWV has not been amplified by PCR reaction (Fig.1)

Mechanical inoculation of Tospoviruses

Systemic mottling, wilting and necrosis of shoot and growing leaves has been developed following sap inoculation by ELISA positive sample on to *N tabacum, N. benthamiana* and tomato.

Screening of BARI released tomato varieties against Tospoviruses

In the field condition highest percent disease incidence was observed in BARI tomato-7 and 21 thrips has been traped on to the white sticky trap among the thirteen varieties tested. The lowest disease incidence was observed in BARI tomato -11 and 17 thrips were trapped (Table 4).

Conclusion

Tospoviruses has recently been introduced as a devastating plant viruses infecting tomato transmitted by thrips. Survey of the Godagari upazilla revealed the characteristics symptoms of tospoviruses i.e. shortening the internode, wilting and necrosis of shoot and growing part of all cultivated tomato cultivars. The percent disease incidence was recorded as 55 to 80%. Direct Antigen Coating ELISA- DAC ELISA: Twenty five symptomatic samples (five from each location) have been subjected to DAC-ELISA. Three antisera namely GBNV, CaCV and TSWV have been tested in the DAC-ELISA. PCR-Based detection of Tospoviruses: ELISA positive samples were subjected to PCR amplification using GBNV, CaCV and TSWV specific primers. The presence of <500 bp DNA band by using GBNV primer confirmed the occurring of GBNV at Godagari upazilla. Other two member of tospovirus namely CaCV and TSWV has not been amplified by PCR reaction Mechanical inoculation of Tospoviruses Systemic mottling, wilting and necrosis of shoot and growing leaves has been developed following sap inoculation by ELISA positive sample on to *N. tabacum, N. benthamiana* and tomato

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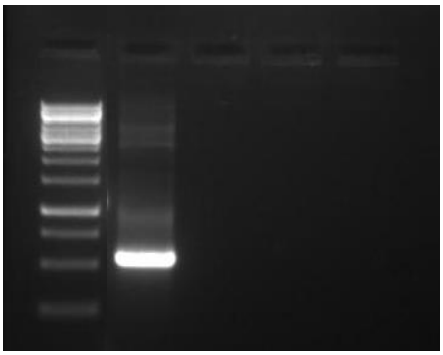

Lane 1= DNA Ladder
Lane 2= GBNV
Lane 3= CaCV
Lane 4= TSWV
Lane 5= Healthy tomato leaf

Fig. 1: PCR based detection of Tospoviruses infecting tomato

Table 1: Percent disease incidence of Tospoviruses at Godagari upazila

Location	Variety	Percent Disease Incidence
Garipur	Salamat	70
	Lyco	65
	Bangabir	55
	Aviruchi	65
	Sabal	60
Kakonhat	Salamat	75
	Lyco	65
	Bangabir	50
	Aviruchi	60
	Sabal	75
Mohisalbari	Salamat	70
	Lyco	65
	Bangabir	65
	Aviruchi	75
	Sabal	75
Harisankorpur	Salamat	60
	Lyco	65
	Bangabir	65
	Aviruchi	60
	Sabal	80
Bidirpur	Salamat	75
	Lyco	70
	Bangabir	60
	Aviruchi	55
	Sabal	65

Table 2: ELISA based detection of Tospoviruses infecting tomato

Isolates[a]	Antisera used[b]		
	CaCV	GBNV	TSWV
KS	-	++	-
KL	-	++	-
KB	-	++	-
KA	-	++	-
KSA	-	++	-
MS	-	++	-
ML	-	++	-
MB	-	++	-
MA	-	++	-
MSA	-	++	-
BS	-	++	-
BL	-	++	-
BB	-	++	-
BA	-	++	-
BSA	-	++	-
HS	-	++	-
HL	-	++	-
HB	-	++	-
HA	-	++	-
HSA	-	++	-
BSA	-	++	-

BBAN	-	++	-
BAV	-	++	-
BSAB	-	++	-

A= Isolates

B= CaCV *Capsicum chlorosis virus; GBNV = Groundnut bud necrosis virus; TSWV= Tomato spotted wilt virus*

-Negative in serology test ++ positive in serology test

Table 3: Inoculation Test

Name of the plant	Symptoms Appeared	Percent inoculation positive
N. tabacum	Systemic mottling, wilting of shoot and top leaves following sap inoculation	10/6
N.benthaminia	Do	10/8
Tomato	Necrosis of shoot and top leaves	10/10

Table 4: Screening of tomato varieties against Tospovirus diseases

Name of the variety	Thrips/trap	Disease incidence
BARI Tomato-2 (Ratan)	17	33.33 (35.21) cde
BARI Tomato-3	21	43.33 (41.14) c
BARI hybrid Tomato-4	28	33.33 (35.00) cde
BARI hybrid Tomato-5	22	41.33 (40.18) c
BARI hybrid Tomato-6	34	30.00 (33.20) de
BARI Tomato-7	21	100 (89.96)a
BARI hybrid Tomato-7	16	26.66 (30.98) ef
BARI hybrid Tomato-8	19	43.33 (41.14)c
BARI Tomato-8 (Shila)	11	25.00 (29.91) ef
BARI Tomato-9 (Lalima)	30	70.00 (56.98)b
BARI Tomato-11 (Jhumka)	17	20.00 (26.55)f
BARI Tomato-14	19	40.00 (39.22)cd
BARI Tomato-15	17	43.33 (41.14)c

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