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Molecular Detection of Bulb-Associated Potyviruses in Garlic and their Field Response

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A B S T R A C T

An experiment was designed to detect garlic bulb associated viruses through RT-PCR and to evaluate the response of genotypes against viruses under field condition. The present study was conducted at Bihar Agricultural University, Sabour, Bhagalpur, Bihar during 2019-20 to study the incidence of bulb-associated Potyviruses in garlic through molecular diagnosis. In order to confirm the presence of virus in stored garlic, RT-PCR based assay was used. The suspected leaves of genotypes were tested for the virus. Majority of genotypes were found positive with potyvirus by producing 300 bp band. Stored garlic bulbs were also tested for the virus and out of 14 genotypes, 8 were found positive with potyvirus. Under field condition, maximum genotypes showed the highest incidence at 90 days after sowing e.g. G-282 with 20.7% followed by G-1 with 15.0% disease incidence. Among all the entries, three genotypes, i.e., 499, 516 and 493 showed no any disease symptoms. The present study will be helpful to manage the crop by selecting virus-free garlic. Moreover, timely application of control strategies may be followed based on the field response of disease.

Introduction

Garlic (*Allium sativum* L.) is one of the important vegetables in bulb crops. It belongs to family Amaryllidaceae under Asparagales order. Garlic crop is severely affected by several biotic and abiotic stresses. Among the biotic stresses, diseases play a significant role in reducing crop productivity and quality production. The major diseases of garlic in India are downy mildew (*Peronospora destructor*), purple blotch (*Alternaria porri*), Stemphylium blight (*Stemphylium vesicarium*), rust (*Puccinia porri*), white rot (*Sclerotium cepivorum*), Botrytis rot (*Botrytis porri*), bacterial soft rot (*Erwinia carotovora* pv. *carotovora*), slippery skin (*Burkholderia gladioli* pv. *allicola*), onion yellow dwarf (*Onion yellow dwarf virus*), leek yellow stripe (*Leek yellow stripe virus*), garlic mosaic (*Garlic mosaic virus*) and Iris yellow spot (*Iris yellow spot virus*). In India, six viruses

belonging to four different taxonomic groups namely, Allexivirus e.g. *Garlic virus X* (*Garv-X*) (Baranwal *et al.,* 2011), Potyvirus e.g. *Onion yellow dwarf virus* (*OYDV*) (Ghosh and Ahlawat, 1997) and *Leek yellow stripe virus* (*LYSV*) (Gupta *et al.,* 2013), Carlavirus e.g. *Garlic common latent virus* (*GarCLV*) (Majumder and Baranwal, 2009) and *Shallot latent virus* (*SLV*) (Majumder *et al.,* 2008), and Orthotospovirus e.g. *Iris yellow spot virus* (*IYSV*) (Gawande *et al.,* 2010) have been found to infect the crop. Mixed viral infections produce mosaic pattern, chlorotic streaking, twisting with curling of leaves and stunting of plants which result in the formation of small bulbs and cloves. Yield loss up to 78% has been reported due to mixed infection of viruses (Lot *et al.,* 1998; Conci *et al.,* 2003; Lunello *et al.,* 2007).

Among the viral diseases, onion yellow dwarf disease is commonly infecting onion and garlic crops. The virus belongs to the genus Potyvirus under Potyviridae family. The virus is transmitted by aphids (*Myzus persicae*) in a non-persistent manner. Additionally, the virus is also transmitted by garlic bulbs. The disease has been reported to cause a detrimental effect on the growth of the plants and consequently on bulb production. Moreover, *Leek yellow stripe virus* (*LYSV*) is also an important virus of the Potyvirus genus infecting garlic widely and induces severe symptom (Van Dijk, 1993). It causes mosaic and yellow stripes on leaves.

The typical symptoms of yellow streaks, mosaic pattern and stunted growth were observed in garlic varieties at Bihar Agricultural University (BAU), Sabour, Bhagalpur. Considering the emerging issue, an investigation was planned and focussed on molecular detection of potyviruses in garlic genotypes and their response under field condition.

Materials and Methods

Molecular detection of potyviruses in garlic

Detection of virus from garlic leaves using RT-PCR

Suspected leaves from garlic genotypes were collected (432, 352, 498, 305, 516, 417 and 141) from vegetable field of BAU, Sabour. Total RNA of all the samples was extracted using RNeasy RNA Isolation Kit (Qiagen, Germany). One-step reverse transcription PCR (RT-PCR) was performed using Titanium One-Step RT-PCR Kit (Clontech, USA). Additionally, total RNA was also processed for cDNA by using GoScript Reverse Transcription System (Promega, USA) for further investigations. A 25 µl reaction was prepared by adding 2.5 µl 10X one-step buffer, 0.5 µl 50X dNTP mix, 0.25 µl recombinant RNase, 12.5 µl thermostabilizing reagent, 5 µl GC melt, 0.5 µl Oligo (dT) primer, 0.5 µl 50X Titanium (TaqRT) enzyme, 2.25 µl RNase-Free water and 0.5 µl forward and reverse primer each along with RNA templates. A set of Potyvirus specific primer oligo 1n TGGTHTGGTGYA THGGARAAYGG and oligo 2n TGGTHT GGTGYATHGGARAAYGG was used in RT-PCR (Marie-Jeanne *et al.,* 2000). The PCR was performed in Surecycler, Agilent, USA programmed with 50˚C for 60 minutes for cDNA synthesis, 94˚C for 5 minutes for initial denaturation, 94˚C for 30 seconds for second denaturation, 49˚C for 30 seconds for annealing and 68˚C for 1 minute for extension. After 35 cycles, the final extension was performed at 68˚C for 2 minutes. The amplified products were analyzed by 1% agarose gel electrophoresis using gel documentation system, UV Tech Cambridge. Among the positive samples, four were sequenced.

Detection of virus from garlic bulbs using RT-PCR

Upon maturity of crop, bulbs were collected from earlier tagged plants (14 genotypes). The harvested bulbs were stored as per the standard procedure. From each lot, five bulb cloves were selected for RNA isolation. Total RNA of all bulb cloves was isolated using SV total RNA Isolation System (Promega, USA). One-step RT-PCR assay was performed as earlier used kit and specific primers.

Field response of different garlic genotypes against viruses

Response of different garlic genotype against viruses was assessed in natural field condition. A field experiment was designed in randomized block design (RBD) in 3 replications and conducted during winter (Rabi) season of 2019-20 at the Vegetable Research Farm, BAU, Sabour. Each plot was maintained in 4.5 m x 1.5 m size. Spacing of row to row and plant to plant was 15 cm and 10 cm respectively. Under the study, 20 garlic genotypes were screened against viruses. Based on the characteristic symptoms, plants were tagged and the disease incidence was recorded at periodic intervals.

Results and Discussion

Molecular detection of viruses using RT-PCR in different genotypes

Garlic plants from all the twenty genotypes showing abnormal mosaic and yellow streaks symptoms and twisting in the leaves (Fig. 2) were collected from the Vegetable Research Farm, BAU, Sabour. Each sample was tested against potyvirus. In each genotype, the collected samples were found positive. The number of positive samples out of total tested plants was detected more in 5 genotypes namely G-1, G-189, G-282, G-323 and G-50 as compared to other genotypes. In G-1, out of 12 tested plants, 7 were found positive. Similarly, 10 out of 14 in G-189, 6 out of 9 in G-282, 7 out of 12 in G-323 and 8 out of 12 in G-50 were found positive (Table 1). The gel electrophoretic profile of RT-PCR product (~300 bp) of different genotypes was depicted (Fig. 1).

The presence of potyvirus was tested for 14 garlic entries. The virus was detected in 8 out of 14 garlic entries such as Local Garlic Collection-1, G-41, G-282, IC-375416, AC-50, AC-283, RUAG and ACC-40. The maximum viral infection (>10%) was found in G-41 and the moderate infection (>5-10%) was observed in Local Garlic Collection-1, IC-375416, AC-283 and ACC-40. The least infection (1-5%) was shown by G-282, AC-50 and RUAG and among 14 entries, there were 6 entries e.g. BRG-13, BRG-14, AC-200, Yamuna Safed, Godavari and Bhima Purple which did not amplify and showed negative result with Potyvirus specific primer (Table 2).

Field response of different garlic genotypes

Field response of each genotype against virus was assessed at periodic intervals. It was observed that G-1, G-189, G-282, G-50, 432, 141, BRG-13 and BRG-14 showed disease symptoms appeared after 30 days of sowing. Among them, genotype G-50 showed maximum disease incidence (6.7%) on 30th day followed by genotype G-282 (5.3%). After 45 days of sowing, genotypes G-323, IC-395680 and BRG-10 first showed disease symptoms. Among them, maximum disease incidence was observed for BRG-10 (2.6%) followed by genotype IC-395680 (1.3%). The genotypes BRG-8, 498, 305, 417, 352 and BRG-7 showed the delayed disease symptom, i.e., 60 days after sowing. The maximum disease incidence was found in BRG-8 with 2.7% incidence followed by 352 (2.6%), 305

(2.4%), 417 (2.2%) and 498 (2.0%). Maximum genotypes showed the highest incidence at 90 days after sowing e.g. G-282 with 20.7% followed by G-1 with 15.0% disease incidence. Among all the entries, three genotypes, i.e., 499, 516 and 493 showed no disease symptoms (Table 3).

Table.1 Molecular detection of virus using RT-PCR in different genotypes

S.No.	Name of genotypes	Potyvirus specific primer (No. positive/Total tested plants)
1.	G-1	07/12
2.	G-189	10/14
3.	G-282	06/09
4.	BRG-8	03/13
5.	G-323	07/12
6.	G-50	08/12
7.	499	03/10
8.	IC-395680	05/14
9.	498	04/11
10.	432	06/14
11.	141	03/09
12.	516	04/08
13.	305	04/09
14.	417	03/11
15.	BRG-10	05/12
16.	352	04/12
17.	493	04/13
18.	BRG-7	05/11
19.	BRG -13	02/12
20.	BRG -14	03/14

Table.2 Testing of viruses in stored garlic bulbs of different entries harvested from infected crops

S.No.	Name of entries	Infection of Potyvirus
1.	Local Garlic Collection-1	++
2.	BRG-13	-
3.	BRG-14	-
4.	G-41	+++
5.	G-282	+
6.	IC-375416	++
7.	AC-50	+
8.	AC-200	-
9.	AC-283	++
10.	RUAG	+
11.	Yamuna Safed	-
12.	Godavari	-
13.	ACC-40	++
14.	Bhima Purple	-

(-) No amplification, (+) 1-5% infection, (++) >5-10% infection, (+++) >10% infection

Table.3 Disease incidence and symptoms of garlic genotypes against viruses

S.No.	Genotypes	Disease incidence (%) in garlic at periodic intervals					Symptom
		30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	
1.	G-1	3.2	6.3	8.5	12.2	15.0	Twisting and mosaic
2.	G-189	1.5	3.3	5.0	6.7	7.9	Twisting
3.	G-282	5.3	12.6	17.4	18.0	20.7	Twisting and streaking
4.	BRG-8	0.0	0.0	2.7	3.1	3.6	Mosaic
5.	G-323	0.0	1.2	1.9	2.2	3.8	Twisting
6.	G-50	6.7	9.0	11.3	12.5	13.3	Twisting and streaking
7.	499	0.0	0.0	0.0	0.0	0.0	-
8.	IC-395680	0.0	1.3	2.2	2.7	3.5	Mosaic
9.	498	0.0	0.0	2.0	2.8	3.2	Mosaic
10.	432	2.2	3.7	5.0	5.9	6.5	Mosaic
11.	141	3.5	5.9	6.4	6.7	7.5	Twisting and mosaic
12.	516	0.0	0.0	0.0	0.0	0.0	-
13.	305	0.0	0.0	2.4	3.5	4.4	Mosaic
14.	417	0.0	0.0	2.2	3.0	4.2	Mosaic
15.	BRG-10	0.0	2.6	4.7	6.2	7.4	Yellow streaking
16.	352	0.0	0.0	2.6	3.5	4.0	Mosaic
17.	493	0.0	0.0	0.0	0.0	0.0	-
18.	BRG-7	0.0	0.0	1.4	2.7	3.7	Mosaic
19.	BRG-13	1.0	1.5	2.3	2.5	3.3	Twisting and streaking
20.	BRG-14	1.3	2.2	2.6	2.8	3.4	Twisting and streaking

(DAS - Days after sowing)

Fig.1 Gel electrophoretic profile of RT-PCR product of different genotypes (1) G-1 (2) G-189 (3) G-282 (4) BRG-8 (5) G-323 (6) G-50 (7) 499 (8) IC-395680 (9) 498 (10) 432 (11) 141 (12) 516 (13) 305 (14) 417 (15) BRG-10 (16) 352 (17) 493 (18) BRG-7 (19) BRG-13 (20) BRG-14

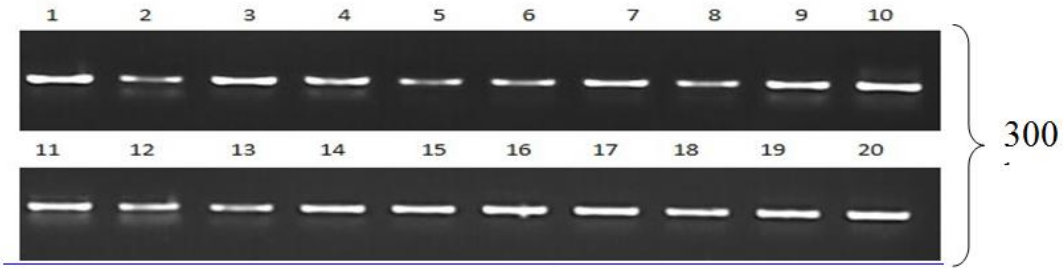

Fig.2 (a) Yellow streaks on leaves (b) Mosaic pattern on leaves (c) Twisting of leaves Testing of viruses in stored garlic bulbs from infected crops

In order to detect the *Leek yellow stripe virus* (Potyvirus), PCR assay was performed. Various sets of primers were used for PCR to amplify full-length genome of *LYSV* (Gupta *et al.,* 2017). Development of multiplex RT-PCR for simultaneous detection of garlic viruses was done by Nam *et al.*, (2015) for the detection of *Garlic virus D* from garlic plants which showed the yellow stripes, dwarfing and mosaic symptoms. The PCR product was

sequenced and it showed the maximum identity (91%) with CP gene of *Garlic virus D* (Khan *et al.,* 2016). Garlic is commonly grown by bulb which may have the possibility of virus inoculum. Viruses associated with garlic belong to genus Potyvirus which may persist in bulbs and provide the inoculum for subsequent crops. The viruses associated with garlic bulbs under stored conditions were confirmed by RT-PCR (Mann and Minges, 1958). Field response of different genotypes was assessed, most of the entries showed the symptoms with varying degree of aggressiveness. The present study elaborated the RT-PCR based detection in garlic for the association of bulb-associated virus which will encourage for the selection of healthy seed materials. Since, the virus was detected in garlic bulbs, therefore, it will be an important footstep for selection of virus-free garlic stock in garlic cultivation. Based on the field response, few entries or genotypes may be suggested for the resistant breeding programmes.

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