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Transmission of Begomovirus and Groundnut Bud Necrosis Virus Infecting Melons in Punjab

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Abstract: Viral diseases were reported to be a major constraint in the cultivation of melons under Punjab conditions. During this study yellows and leaf curl symptoms were recorded on muskmelon crop which was due to the infection of begomovirus whereas, in watermelon crop infection of groundnut bud necrosis virus (GBNV) was recorded that leads to the production of necrosis symptoms. Transmission of begomovirus and GBNV was tested through mechanical transmission, aphid transmission, whitefly transmission and seed transmission. In muskmelon crop where begomovirus was associated with yellows and leaf curl symptoms, 100% transmission was recorded through whiteflies whereas this virus has not shown transmission through other remaining methods. In whitefly based transmission, symptoms were observed 11 days after inoculation (DAI). In watermelon only 1% transmission of GBNV was observed through seeds whereas this virus was not transmitted through other tested methods. Symptoms were observed in the infected plants 34 days after sowing (DAS). Information regarding the mode of transmission of viral diseases could be very useful for their effective management.

Keywords: Watermelon, Muskmelon, Transmission, Begomovirus, Groundnut bud necrosis virus

Melon (*Cucumis melo* L.) is an important vegetable crop that can be used in several ways viz. as fresh vegetable, dessert fruit, cooked, dried or processed for juice and flavoring. Seeds of melons can be roasted and consumed like nuts. Melon seeds are the source of high quality cooking oil and high-protein seed meal (McCreight et al 2011). The world production of melons in 2019 was estimated to be 27.50 million tonnes from 1.03 million ha of land, however, in India production of melons was estimated to be 1.27 million tones which was 4.6% of world's production from 0.06 million ha area (Anonymous 2019). Throughout the world melons are attacked by more than 30 viruses that include both DNA and RNA containing viruses (Zitter et al 1996).

Mosaic, yellows, fruit malformation, puckering of leaves, mosaic and blistering on fruits, yellowing of veins and veinlets, leaf narrowing, mosaic mottling, malformation of stem and leaves, appearance of chlorotic spots on leaves, vein banding, leaf filiformity, leaf resetting, necrosis and enations were some important symptoms produced as a result of viral infection on cucurbits (Holkar et al 2016, Nagendran et al 2017 and Dhkal et al 2020). Worldwide these symptoms were reported to be produced by the infection of viruses belonging to the genera *Begomovirus*, *Potyvirus*, *Cucumovirus*, *Tospovirus*, *Tobamovirus*, *Tymovirus*, *Nepovirus* and *Polerovirus* (Liu et al 2009, Zitter and Murphy 2009, Abdalla et al 2012, Sobh et al 2012, Dreher et al 2012, Mansilla et al 2013, Johnson et al 2013, Holkar et al 2016 and Dhkal et al 2020).Viruses infecting cucurbits are known to be transmitted by different insect vectors, however some of these viruses are also known to be transmitted by seed (Tobias et al 2008, Simmons et al 2011 and Reingold et al 2015). During 2017-18, yellows and leaf curl symptoms on muskmelon crop whereas necrosis symptoms in watermelon were observed on the stem and leaf of infected plants. Earlier in Punjab these symptoms were not commonly observed on the muskmelon and watermelon crop so no information about its transmission is available. Current study was designed to identify the transmission of necrosis (in watermelon) and yellows and leaf curl (in muskmelon) symptoms under Punjab condition.

MATERIAL AND METHODS

Detection of viruses associated with the symptoms: For the detection of virus associated with leaf curl and yellows symptom of muskmelon *Begomovirus* specific primers developed by Rojas et al (1993) viz. PALIc (5'ACNGGNAARACNATGTGGGC3') and PARIv (5'GGNAARATHTGGATGGA3') were used as begomovirus was earlier reported to be associated with muskmelon crop in Punjab (Dhkal et al 2020). The PCR amplification was carried out in a thermal cycler with initial cycle of denaturation at 94ºC for 1 minute, annealing at 52ºC for 1.5 minutes and elongation of 72ºC for 2 minutes, followed by 35 cycles each consisting of denaturation at 94ºC for 50 sec, annealing at 52ºC for 45 sec followed by extension at 72ºC for 1.5 min and final extension for 15 minutes at 72ºC. After the completion of

the reaction, the products were kept at -20ºC prior to gel analysis. Amplified DNA fragments were electrophoresed in 1.0 percent agarose gel. However, in watermelon Groundnut bud necrosis virus (GBNV) specific antisera procured from Agdia Inc (Elkhart, USA) were used as Holkar et al (2016) reported the association of this virus with necrosis symptoms of watermelon. The procedure for DAS/TAS-ELISA given by Clark and Adams (1977) was used as per manufacturer's instruction. End point absorbance readings (OD) were taken by ELISA reader (Tecan, Austria) at 405 nm.

Maintenance of plants for transmission study: For sap and insect transmission nursery of muskmelon and watermelon was raised in 15 x 10 cm and 100 gauge thick polyethylene bags filled at the base with equal proportions of well-rotted manure and soil. Two seeds per bag of the Punjab Sunehri variety in muskmelon and Barmeri variety in watermelon were sown to a depth of 1.5 cm in the first half of February. Seedlings were maintained as mentioned in the Package of Practices for Growing Vegetables, PAU, Ludhiana (Anonymous 2017). One month old two true leaf stage seedlings were then used for the transmission studies at a vegetable research farm, Department of Vegetable Sciences, in the second half of April 2018. For seed transmission, seeds were sown in 96 well plug trays.

Transmission

Sap transmission: Young leaf of the identified infected plants was crushed in phosphate buffer (pH 7.0) (0.03 M Na_2HPO_4 containing 0.2 per cent Na-diethyldithio-carbamate (DIECA) (1:4), 400 mesh carborundum (75 mg/ml) + activated charcoal (75 mg/ml) and then this sap was used to inoculate the healthy seedlings of muskmelon (Punjab Sunehri) and watermelon (Barmeri) at two true leaf stage. After inoculation plants were observed for the symptom development regularly.

Insect Transmission

Aphid transmission: Non-viruliferous aphids (*Aphis gossypii*) were raised on healthy Chinese cabbage. These non-viruliferous aphids were then starved in the Petri dish. These starved aphids were fed on the virus infected leaf of muskmelon for 10 min acquisition access time (AAT), then transferred to healthy muskmelon (Punjab Sunehri) and watermelon (Barmeri) test plants of two true leaf stage for 2hrs. Inoculated plants were then kept in insect proof cage and observed for the symptom appearance regularly and observed up to 35 days at weekly intervals. After 35 days of inoculation the youngest leaf of the plant was taken for further serological and molecular analysis.

Whitefly transmission: The non-viruliferous whitefly (*Bemisia tabaci*) was reared on virus free cotton plants. These non-viruliferous whitefly was collected in plastic bottle and left on infected twig of muskmelon for 24hrs acquisition access time (AAT). These viruliferous whitefly were transferred to healthy muskmelon (Punjab Sunehri) and watermelon (Barmeri) test plants of two true leaf stage for 24hrs of Inoculation feeding Period (IFP). The inoculated plants were sprayed after 24 hrs of inoculation using insecticide. These inoculated plants were kept in an insect proof cage and observed up to 35 days at weekly intervals for appearance and type of symptoms. After 35 days of inoculation the youngest leaf of the plant was taken for further serological and molecular analysis.

Seed transmission: Seeds were collected from infected fruits of highly susceptible varieties of muskmelon (Punjab Sunehri and Hara Madhu) and watermelon (Barmeri) showing typical virus symptoms. The seeds (100 seeds/ variety) were sown under insect proof cage in sterilized soil and observed for symptom expression. After germination of seeds, seedlings were observed up to 35 days at weekly intervals for the appearance of symptoms. The observations on days for first symptom appearance, number of plants infected and type of symptoms produced were recorded. After 35 days of inoculation the youngest leaf of the plant was taken for further serological and molecular analysis.

RESULTS AND DISCUSSION

Identification of viruses associated with symptoms: *Begomovirus* specific primers *viz*. PALIc(5'ACNGGNAARACNATGTGGGC3') and PARIv (5'GGNAARATHTGGATGGA3') amplified a specific product of ~1280 bp (Fig. 4) in the muskmelon samples showing yellows and leaf curl symptoms that confirms the association of begomovirus with these symptoms. GBNV was found to be associated with necrosis symptoms of watermelon after the ELISA test.

Transmission of *Begomovirus* associated with yellows disease of muskmelons: Among these four methods, *Begomovirus* was successfully transmitted through whitefly. In whitefly-based transmission, symptoms were observed 11 days after inoculation (DAI), whereas cent per cent plants showed symptoms after twenty one days of inoculation (Table 1). Mechanically inoculated plants were found to be healthy even after one month of inoculation (Fig. 1). Viruliferous aphids that were fed on infected plants (Fig. 2) also did not produce any symptoms on healthy inoculated plants even after 30 days. Similarly, no symptoms were observed on the plants grown from the seeds collected from infected fruits even after four weeks of sowing (Table 1). In whitefly inoculated plants first symptoms initiated as chlorotic spots, later turns to mild puckering and yellow vein mosaic symptoms 14 DAI on young leaves that later on convert into

severe yellow vein mosaic symptom at 21 DAI and the whole leaf turned yellow/ bleached at 28 DAI. In some plants older leaves get curled downward at 14 DAI and these curled leaves turn completely yellow after 28 days of inoculation (Fig. 3). Presence of this *Begomovirus* in whitefly inoculated plants were further confirmed by *Begomovirus* specific primers PALIc and PARIv (Rojas et al 1993) that amplify *Begomovirus* specific band of ~1280bp in whitefly inoculated plants DNA (Fig. 4).

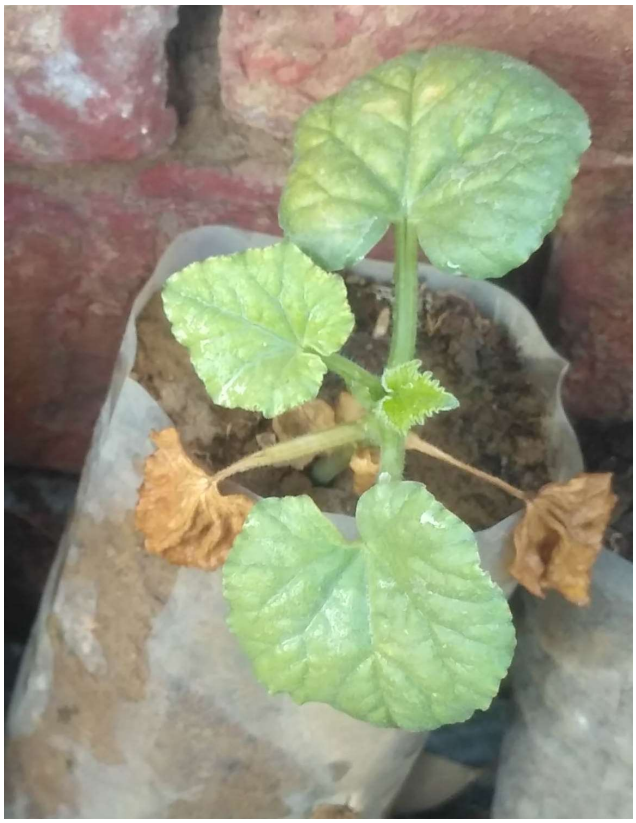

Fig. 1. Mechanically inoculated plant after 14 days of inoculation

Many workers reported transmission of different *Begomoviruses* through whitefly in various vegetable crops (Hidayat and Rahmayani 2007 and Ghanim et al 2007). Brown and Nelson (1985) reported a new whitefly transmitted *Begomovirus viz.* watermelon curly mottle virus infecting watermelon and lettuce. They tried to transmit this virus to various indicator plants through mechanical and whitefly inoculation and observed that mechanical transmission of virus from infected lettuce plant to indicator hosts did not occur, whereas isolates from watermelon produces symptoms on indicator plants *viz.* red kidney bean, big max pumpkin and zuchini squash plants. They also reported that virus isolates from both lettuce and watermelon were efficiently transmitted through whitefly to various indicator host plants. Similarly other workers also reported the transmission of *Begomovirus* by whitefly in different crop

Fig. 2. Aphids feeding on infected plants of the Muskmelon

Table 1. Transmission of major viruses infecting melons in Punjab

Inoculation method	First symptom appearance	% disease Incidence				Types of symptoms
		14 DAI/S	21 DAI/S	28 DAI/S	35 DAI/S	
Muskmelon cv. Pb. Sunheri ; Virus: *Begomovirus*						
Mechanical transmission	-	-	-	-	-	
Aphid transmission	-	-	-	-	-	
Whitefly transmission	11 DAI	60	100	100	100	Puckering, downward curling, severe yellows, intervenial chlorosis
Seed transmission	-	-	-	-	-	
Watermelon cv. Barmeri; Virus: *Groundnut bud necrosis virus*						
Mechanical transmission	-	-	-	-	-	
Aphid transmission	-	-	-	-	-	
Whitefly transmission	-	-	-	-	-	
Seed transmission	34 DAS	-	-	-	1	Necrotic lesion

(-) : No symptoms observed

plants (Mehta et al 1994, Rubinstein and Czosnek 1997 and Abudy et al 2010). Unlike the finding of this study, Lopez et al (2015) mechanically inoculated tomato leaf curl New Delhi virus to the different cucurbit crops.

Transmission of GBNV associated with necrosis in watermelon samples: For transmission studies in watermelon the GBNV seropositive plants showing necrosis on young leaves and stems were selected as inoculums for

Fig. 3. Symptoms produced by the whitefly transmission of *Begomovirus* associated with yellows disease of Muskmelon. 1) mild puckering and yellow vein mosaic symptoms on young leaf after 14 days of inoculation B) Yellow vein mosaic symptoms on young leaf after 21 days of inoculation C) Complete yellows symptom on leaf after 28 days of inoculation D) Leaf curl symptom after 14 days of inoculation E) Leaf curl symptom after 28 days of inoculation

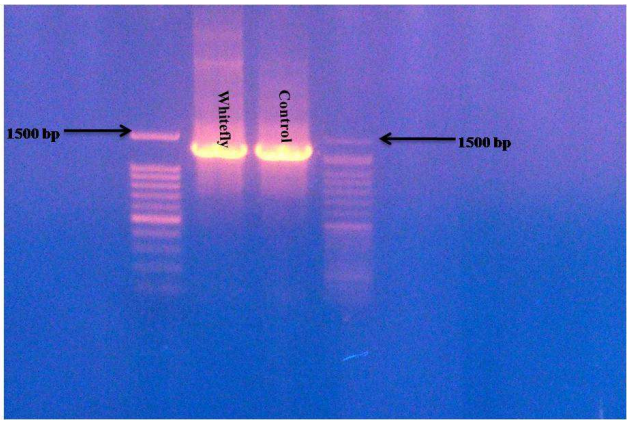

Fig. 4. PCR amplicons obtained from whitefly inoculated and positive sample of muskmelon with *Begomovirus* specific PALIc and PARIv primer pair. Single typical ~1280bp band in whitefly inoculated and positive sample identified the presence of *Begomovirus* in whitefly inoculated plant. Lane 1 in the gel contain 100bp DNA ladder (SimBio) and Lane 4 in the gel contain 50bp DNA ladder (NextGen Life Sciences)

Fig. 5. Necrotic lesion appeared on the seedling grown from infected seeds after 34 days of sowing

transmission studies. Among tested methods virus associated with necrosis showed very low per cent of transmission through seed. The seeds collected from infected fruits showed transmission up to one per cent only. No transmission could be established through mechanical, aphid, and whitefly (Table 1). Necrotic lesion type of symptoms (Fig. 5) appeared on seedlings after 34 days of sowing, whereas no symptoms appeared on the mechanically, whitefly and aphid inoculated plants even 30 days after inoculation. Serologically virus associated with necrosis symptoms was confirmed as GBNV.

In this study, we tried to transmit the virus causing necrotic symptom through mechanical, whitefly and aphid inoculation and were unsuccessful in transmitting the symptoms from diseased to the healthy plants. Low per cent of seed transmission could be due to the difference in the virus species that produced these necrotic symptoms on infected plant.

CONCLUSION

In muskmelon *Begomovirus* was found to be transmitted through whitefly and in watermelon GBNV has shown very low level of transmission through seed. So, in future this information can be used for evaluation of different insecticides for the effective management of these insect vectors that ultimately helps in the management of these viruses. Further studies can be designed to determine the total time taken by insect vectors for the transmission of viruses from diseased plant to healthy plants that will help in developing better spray schedule of insecticides for the effective management of insect vectors and viruses. Use of healthy seeds can also lead to effective management of GBNV in watermelon crop.

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