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# Spatial Distribution and Identification of Begomovirus(es) Infecting Muskmelon in Punjab, India

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***Abstract*:** Muskmelon (*Cucumis melo* L.) has considerable share in overall cucurbit vegetable cultivation in Punjab state. The successful production of the crop is threatened by number of biotic stresses. Among these, whitefly transmitted begomoviruses are very important. In order to know the prevalence of these viruses under Punjab conditions, major muskmelon growing areas in different districts *viz*., Jalandhar, Kapurthala, Ludhiana, Fatehgarh Sahib, Sangrur and S.A.S. Nagar were surveyed. Further, confirmation of the prevalent virus(es) was done by PCR assays using virus specific markers. The perusal of the data revealed that district wise maximum incidence of begomovirus was observed in Sangrur (48.93%) and minimum in Ludhiana district (24.25%). In Kapurthala, S.A.S. Nagar, Jalandhar and Fatehgarh Sahib districts, 32.37, 30.89, 26.41 and 26.00% incidence of the virus was recorded, respectively. The PCR analysis with begomovirus specific primers, PALIc1960 and PARIv722 confirmed the presence of begomovirus(es) in 65% of the collected samples. Further, PCR assay of the selected samples using primers specific to tomato leaf curl New Delhi virus, tomato leaf curl Palampur virus and squash leaf curl virus confirmed the association of these viruses in different muskmelon growing regions of Punjab.

***Keywords*:** Muskmelon, Begomovirus, Tomato leaf curl New Delhi virus, Tomato leaf curl Palampur virus, Squash leaf curl virus

Muskmelon crop is highly ranked in cucurbit cultivation around the world. Worldwide production of muskmelon is 28.61 million tonnes. In India, 75 thousand ha area is under muskmelon cultivation with a production of 1.478 million tonnes per annum making India the third largest muskmelon producing country in the world (FAOSTAT 2021). In Punjab about 139.79 thousand tonnes muskmelon is being produced from an area of 7.01 thousand ha used for growing the crop (Anonymous 2021). In the state, the muskmelon crop is sown after mid-February and harvesting is carried out till June. The average temperature varies from; min. 19.92-26.76°C; to maximum 22.73-43.28°C during this period. As the temperature starts rising in March onwards, the population of begomovirus vector *i.e.,* whitefly also begins to increase. Which then blowout the begomovirus in muskmelon fields and symptoms of begomovirus start to appear. Muskmelon crop is prone to various viral diseases and is reported to be attacked by different group of viruses (Kang and Sandhu 2007). Among these, circular, single standard DNA viruses of genus begomovirus, family Geminiviridae, are the most important vegetable viruses infecting various cucurbits including muskmelons (Moriones and Navas-Castillo 2000). Distribution and importance of begomoviruses have been increased in tropical and subtropical areas of the world in recent decades causing considerable yield loss in many economically important crops (Varma and Malathi 2003). Begomoviruses are transmitted by whiteflies *Bemisia tabaci* (Hemiptera: Aleyrodidae) in persistent, non-circulative manner and infect dicotyledonous crops. Begomoviruses symptomatology include, mosaic, yellowing of new leaves, leaf curling, stunting, increased vein thickness, rough fruit skin and longitudinal fissures on fruits (Mnari-Hattab et al 2015). The virus-infected plants remain stunted and weak as compared to the healthy plants and considerable yield losses are endured. Tomato leaf curl New Delhi virus and tomato leaf curl Palampur virus were found to be prevalent in different melon growing regions of the world (Malik et al 2011, Mnari-Hattab et al 2015). Furthermore, melon chlorotic leaf curl virus, cucurbit leaf crumple virus, squash leaf curl virus and watermelon chlorotic stunt virus, are also established to infect melons (Sobh et al 2012). Begomovirus(es) have wide host range and pose stringent obstacle to successful cultivation of vegetable crops. In order to extrapolate the occurrence, degree of manifestation and identification of begomovirus(es) in muskmelon crop in Punjab state, the present study was carried out.

## MATERIAL AND METHODS

**Survey for distribution and prevalence of begomovirus:** In order to assess the incidence and prevalence of begomovirus in muskmelon, surveys were conducted in

different muskmelon growing districts of Punjab *viz.,* Ludhiana, Sangrur, Kapurthala, Jalandhar, S.A.S. Nagar and Fatehgarh Sahib in the year 2019-20. Diagnosis of the disease was based on typical symptoms i.e., stunting, curling, yellowing of plants in the field. Symptomatic leaf samples were collected and brought to laboratory for analysis and detection of the virus(es). For virus incidence, observations were recorded on young leaves of randomly selected 5-10 plants from four corners C1, C2, C3, C4 and one central patch C5 from each field and per cent incidence was calculated. Virus disease severity grade was measured on a (0-5) scale given by Kumar et al (2006). Where, symptom severity grade 0 = no visual symptoms; 1= 0-5% curling of upper leaves; 2= 6-25% curling of leaves and swelling of veins; 3= 26-50% curling puckering and yellowing of leaves and swelling of veins; 4= 51-75% leaf curling and stunted plant growth and blistering of internodes; 5= More than 75% curling deformed small leaves, stunted plant growth with small flowers and no or small fruit set. Disease incidence (DI) and per cent disease index (PDI) i.e., disease severity was then calculated using the following formulas:

$$\text{Per cent diseases incidence (DI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Per cent diseases index (PDI)} = \frac{\text{Sum of all the numerical ratings}}{\text{Total number of plants} \times \text{Maximum disease grade}} \times 100$$

**Detection and identification of virus(es) infecting muskmelon:** Symptomatic leaf samples were collected in ice box from different districts during the survey and were categorised according to the symptom variability. One set of leaf samples was stored in -80°C for further studies. The all the samples were categorised based on their symptoms. To identify the viruses associated with these samples DNA based detection methods were used. Total nucleic acid from the young symptomatic leaves of muskmelon collected during survey was isolated using the cetyl trimethyl ammonium bromide (CTAB) method (Lodhi et al 1994). Presence of ssDNA viruses was confirmed using begomovirus specific PALIc1960 and PARIv722 primers (Rojas et al 1993). The PCR amplification was done in a thermal cycler with initial-denaturation at 94ºC for 1 minute, 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 1.30 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 PCR products were electrophoresed in 1.0 per cent agarose gel. For further confirmation, the isolated DNA of selected begomovirus positive samples was again subjected to PCR amplification using tomato leaf curl New Delhi virus (ToLCNDV), tomato leaf curl Palampur virus (ToLCPalV) and squash leaf curl virus (SqLCV) specific primers (Table 1).

## RESULTS AND DISCUSSION

**Prevalence and incidence of begomovirus(es) infecting muskmelon:** In Punjab, nursery of muskmelon is sown around mid-January to mid-February and transplanted in the field around 2nd fortnight of February till mid-March. The crop remains in the field from March till the June. The surveys were conducted during the mid-season of the crop to record incidence and prevalence of begomovirus(es). Perusal of data revealed that the highest incidence of begomovirus(es) (48.93%) was observed in Sangrur district, while Ludhiana district recorded the lowest begomovirus incidence (24.25%). Similarly, Sangrur district had the maximum (28.16%) and Ludhiana district had minimum (14.35%) per cent disease index (PDI) (Table 2). Further, it was observed that frequency of occurrence of begomovirus was 100 per cent in all the muskmelon growing districts of Punjab under study. In majority of muskmelon growing areas being surveyed in the current study farmers used to follow potato-muskmelon-rice as crop rotation.

**Identification of begomovirus(es) associated with muskmelon:** Different types of symptoms were observed

**Table 1.** Primers used for detection of tomato leaf curl New Delhi virus, tomato leaf curl Palampur virus and squash leaf curl virus

| Primer | Expected product size | Primer sequence data | Reference |
|---|---|---|---|
| Begomovirus specific primer:<br>PALIc1960<br>PARIv722 | ~1280bp | 5' ACNGGNAARACNATGTGGGC 3'<br>3' GGNAARATHTGGATGGA 5' | Rojas et al (1993) |
| ToLCNDV primer:<br>CRNDv30<br>CRNDc1181 | ~1180bp | 5'GCCCTCAACCAATGAAATTCAC3'<br>5'GAGAGTCTTCAAAACCCAGGTCC3' | Reddy et al (2005) |
| ToLCPalV primer:<br>Palampur F<br>Palampur R | ~875bp | (Personal communication, Yogesh Kumar, IHBT, Palampur, Himachal Pradesh, India) | |
| SqLCV primer:<br>SqLCV F<br>SqLCV R | ~1000bp | (Personal communication, Dr. Abhishek Sharma, PAU, Ludhiana, Punjab, India) | |

during the survey for incidence of begomovirus(es) in muskmelon. These were; leaves showing yellowing only, downward curling of leaves no yellowing, leaves showing yellowing + puckering + curling, yellowing + puckering of leaves, leaves showing yellowing and downward curling, curling + puckering of leaves, yellowing of leaves with reduction in size and stunting of vines (Table 3, Fig. 1). All the leaf samples collected during the survey were segregated based on type of symptoms exhibited and representative samples for each district were selected for detection of begomovirus(es) associated with muskmelon crop in Punjab. In all, 23 symptomatic leaf samples (showing varying symptoms) representing different districts under survey were selected. The selected samples were first subjected to PCR

**Table 2.** Prevalence, incidence, and severity of Begomovirus in major muskmelon growing areas of Punjab

| Village | Global position | Area (ha) | Begomovirus incidence (%) | Begomovirus PDI* (%) |
|---|---|---|---|---|
| Kapurthala district | | | | |
| Sheikhupur | 31.3544°N 75.3609°E | 8.70 | 35.60 (28.00-44.00) ** | 19.36 (14.80-23.60) |
| Biharipur | 31.4040°N 75.1419°E | 10.72 | 28.86 (20.00-36.00) | 15.26 (10.40-20.00) |
| Brindpur | 31.3468°N 75.3565°E | 4.86 | 32.67 (24.00-44.00) | 18.33 (11.60-24.80) |
| | | Mean | 32.37 | 17.65 |
| Jalandhar district | | | | |
| Malsian | 31.1292°N 75.3492°E | 18.62 | 24.73 (16.00-48.00) | 12.36 (6.40-30.80) |
| Chachowal | 31.2249°N 75.6528°E | 6.47 | 39.50 (36.00-44.00) | 23.80 (21.20-26.40) |
| Rupewali | 31.1455°N 75.3033°E | 5.87 | 15.00 (10.00-22.00) | 7.80 (5.20-12.00) |
| | | Mean | 26.41 | 14.65 |
| Ludhiana district | | | | |
| Vegetable Farm, PAU | 30.9041°N 75.8066°E | 0.40 | 35.00 (32.00-40.00) | 21.40 (16.80-24.80) |
| Khwajake | 30.9611°N 75.9224°E | 8.09 | 13.50 (10.00-16.00) | 7.30 (5.60-9.60) |
| | | Mean | 24.25 | 14.35 |
| Fatehgarh Sahib district | | | | |
| Haripur | 31.0629°N 75.5168°E | 8.30 | 26.00 (22.00-32.00) | 14.40 (11.60-18.00) |
| | | Mean | 26.00 | 14.40 |
| Sangrur district | | | | |
| Bhaini Kalan | 30.4681°N 75.9064°E | 2.43 | 50.00 (46.00-54.00) | 29.60 (26.80-31.60) |
| Burj | 30.4780°N 75.8425°E | 3.24 | 44.00 (36.00-52.00) | 26.80 (22.80-30.80) |
| Takhar Kalan | 30.5118°N 75.8229°E | 9.31 | 52.80 (52.00-54.00) | 28.08 (25.60-30.80) |
| | | Mean | 48.93 | 28.16 |
| S.A.S. Nagar district | | | | |
| Tangori | 30.5949°N 76.7059°E | 5.67 | 33.33 (32.00-36.00) | 18.67 (16.80-21.60) |
| Kurara | 30.5993°N 76.7272°E | 2.43 | 28.00 (23.00-32.00) | 13.60 (10.80-16.60) |
| Mote Majra | 30.5856°N 76.6976°E | 4.05 | 31.33 (32.00-34.00) | 13.80 (12.00-15.60) |
| | | Mean | 30.89 | 15.36 |

* Per cent disease index; ** Values in parenthesis are range of the parameters

analysis with begomovirus specific PALIc1960 and PARIv722 primers (Rojas et al 1993) to confirm the presence of begomovirus(es). The results showed that about 65 per cent samples were positive for presence of begomovirus(es) showing desired amplicon of ~1200bp with PALIc1960 and PARIv722 primers (Table 3, Fig. 2). This confirmed the association of begomoviruses with muskmelon crop in Punjab. The same set of samples when subjected to PCR analysis again using tomato leaf curl New Delhi virus (ToLCNDV), tomato leaf curl Palampur virus (ToLCPalV) and squash leaf curl virus (SqLCV) specific primers, all the three begomoviruses (ToLCNDV, ToLCPalV and SqLCV) were found to be infecting muskmelon crop under Punjab conditions. The PCR analysis also revealed mixed infection of more than one virus in some samples (Table 3). Tomato leaf curl New Delhi virus (ToLCNDV) was found to be more prevalent in Jalandhar, Kapurthala, Sangrur and Fatehgarh Sahib district. One sample from Ludhiana district also showed presence of ToLCNDV. However, no amplification of ToLCNDV was observed from all the four samples subjected to PCR analysis from S.A.S. Nagar (Table 3, Fig. 3). ToLCPalV was found more prevalent in S.A.S. Nagar as four samples out of total five samples were found positive (Fig. 4). Prevalence of ToLCPalV was also there in muskmelon

**Table 3.** Symptom variability and summary of marker-based detection of Begomovirus(es) infecting muskmelon in different districts of Punjab

| Leaf symptoms | Begomovirus | ToLCNDV | ToLCPalV | SqLCV |
|---|---|---|---|---|
| Jalandhar district | | | | |
| Curling | - | + | - | - |
| Yellowing, puckering, curling | - | + | - | + |
| Yellowing, stunting, mosaic like symptoms | + | + | + | + |
| Kapurthala district | | | | |
| Yellowing | + | - | + | - |
| Yellowing, puckering | + | + | - | - |
| Yellowing, curling | + | + | + | - |
| Sangrur district | | | | |
| Curling, puckering | + | + | - | - |
| Yellowing, mosaic like symptoms | + | - | + | + |
| Yellowing, stunting, small leaves | + | + | + | + |
| Fatehgarh Sahib district | | | | |
| Yellowing, puckering | - | + | - | + |
| Curling | + | + | - | - |
| Yellowing, curling | + | + | - | + |
| Curling | + | + | - | - |
| S.A.S Nagar district | | | | |
| Yellowing | + | - | + | + |
| Yellowing | + | - | + | + |
| Yellowing | - | - | - | - |
| Yellowing | - | - | + | - |
| Yellowing | + | - | + | - |
| Ludhiana district | | | | |
| Yellowing | + | - | + | + |
| Yellowing | - | - | - | - |
| Yellowing | - | - | - | - |
| Yellowing, small leaves | - | - | - | + |
| Curling | + | + | - | - |

ToLCNDV=Tomato leaf curl New Delhi Virus; ToLCPalV=Tomato leaf curl Palampur virus; SqLCV= Squash leaf curl virus; (+) = presence of virus; (-) = absence of virus

growing areas of Jalandhar, Kapurthala, Sangrur and Ludhiana. However, none of the sample from Fatehgarh Sahib found positive for ToLCPalV. Squash leaf curl virus was also found associated with muskmelon crop in all the districts under study; expect Fatehgarh Sahib, where none of the four samples showed presence of SqLCV (Table 3; Fig. 5).

Symptoms with yellowing, curling, leathery leaves, reduced leaf size and stunted growth clearly indicated the

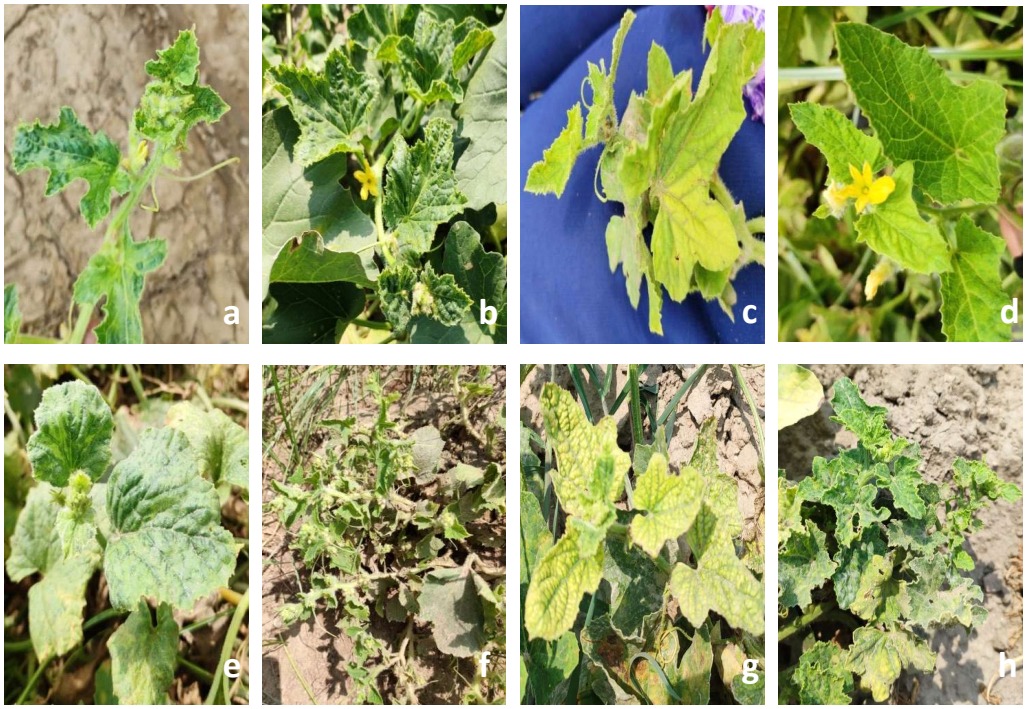


**Fig. 1.** Begomovirus symptom variability observed during the survey. (a & b): upward curling and puckering of leaves; (c & d): yellowing of leaves; (e): downward curling & puckering of leaves with reduction in size; (f): upward curling and stunting of vine; (g & h): yellowing, puckering and vein clearing

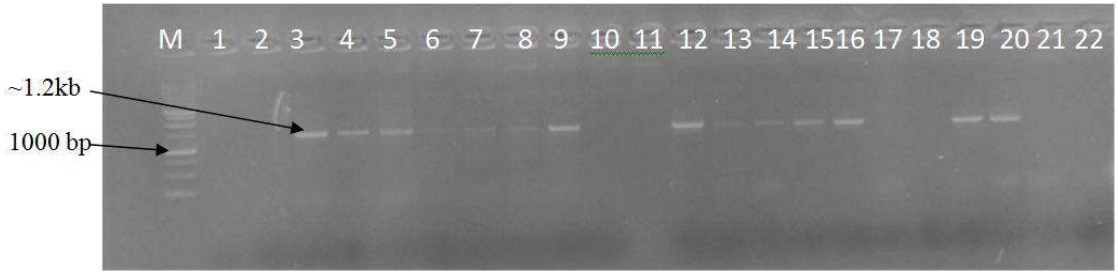


**Fig. 2.** Agarose gel (1%) showing amplicon of ~1.2kb with PALIc1960 and PARIv722 primer specific to begomovirus in different samples collected during the survey. (M-marker - 1000bp; 1-22 samples)

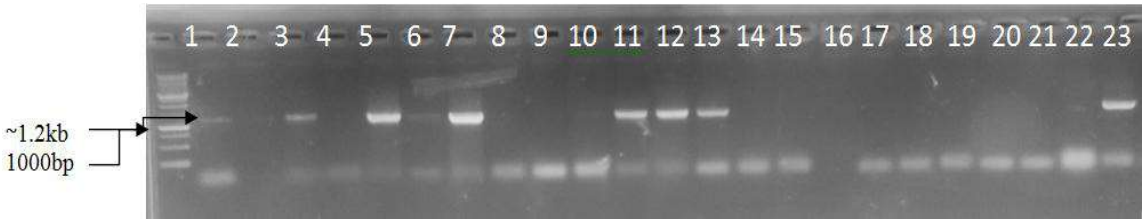


**Fig. 3.** Agarose gel (1%) showing amplicon of ~1.2kb with CRNDv30 and CRNDc1181primer specific toTomato leaf curl New Delhi virus. (M-marker -1000bp; 1-23 samples)


**Fig. 4.** Agarose gel (1%) showing amplicon of ~875bp with primer specific to Tomato leaf curl Palampur virus. (M-marker -1000bp; 1-23 samples)

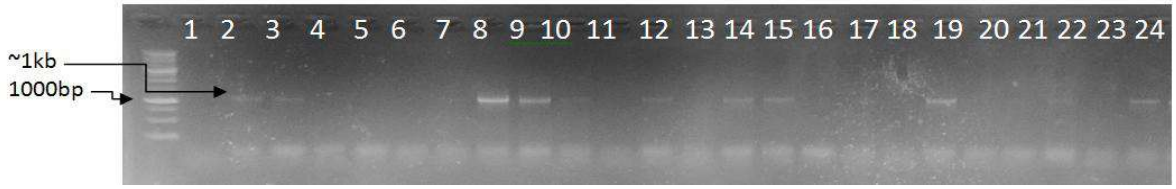


**Fig. 5.** Agarose gel (1%) showing amplicon of 1kb with primer specific to Squash leaf curl virus. (M-marker -1000bp; 1-24 samples)

infection of begomovirus (Sinha et al 2011). The ToLCPalV has been found associated with muskmelon crop and producing a serious yellow leaf curl disease in Pakistan (Malik et al 2011). Yadani-Khameneh et al (2013) reported tomato ToLCNDV infecting muskmelon in Iran for the first time. The infection of ToLCNDV on cucurbit crops like melon, cucumber and zucchini was also described by Mnari-Hattab et al (2015). In Punjab, Sharma et al (2015) first time documented the mixed infection of zucchini yellow mosaic virus (ZYMV) and a (ToLCNDV) in bitter gourd crop. Thereafter, Dhkal et al (2020a, b) confirmed the association of ToLCPalV and ToLCNDV with muskmelon crop causing leaf yellowing and curling in India. Later on, Venkataravanappa et al (2021) also reported ToLCPalV infecting muskmelon and cucumber in Uttar Pradesh.

## CONCLUSIONS

The present study reveals that whitefly transmitted begomoviruses were prevalent in all muskmelon growing areas of Punjab and caused disease incidence ranging from 13.50 to 52.80 per cent in year 2019-20. Distinctive symptoms due to the infection were yellowing, curling, yellowing plus curling, puckering of leaves, mosaic like symptoms, and stunted growth of muskmelon plants. Tomato leaf curl New Delhi virus, tomato leaf curl Palampur virus, and squash leaf curl virus were identified to be associated with muskmelon crop singly or in mixed infection. The information generated during the present study can be helpful in planning management strategies and identifying resistant sources to combat the begomoviruses infecting muskmelon in Punjab. Mixed infection may lead to mutations, recombination, and re-assortment in viral genome which will further result in evolutionary development of new virus strains which may be more contagious and cause severe damage to the crop. In future, whole genome characterization could be done to elucidate begomovirus evolution in Punjab.

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