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# Characterization of multiple disease resistance in melons (*Cucumis melo* L.) against *Meloidogyne incognita*, *Fusarium oxysporum* and tomato leaf curl Palampur virus

Dalvir Singh Dhami[1], Sukhjeet Kaur[2], Abhishek Sharma[2], Sat Pal Sharma[2], Narpinderjeet Kaur Dhillon[1] and Sandeep Jain[1]

[1]Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India and [2]Department of Vegetable Science, Punjab Agricultural University, Ludhiana, Punjab, India

**Research Article**

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**Corresponding author:**
Sukhjeet Kaur;
E-mail: sk-randhawa@pau.edu

## Abstract

Melon is one of the important cucurbitaceous crops being cultivated widely in India and known for its delicious fruits. Crop is threatened by different biotic stresses including nematodes, fungi and viruses. The use of host resistance is the most economical, eco-compatible and long-lasting strategy to combat plant diseases. Keeping in mind this objective, 64 melon genotypes were screened against the prevalent *Meloidogyne incognita*, *Fusarium oxysporum* and tomato leaf curl Palampur virus (ToLCPalV) individually as well as with combined inoculations under artificial conditions. Out of 64 genotypes, three genotypes, MCPS, SM2012-1 and WM11 were found moderately resistant to *M. incognita*, nine genotypes (MM-KP15103, MM327, MM121103, KP4HM15, MM Sel.-103, SM2013-2, SM2012-1, SM2013-9 and WM11) recorded a resistant reaction against Fusarium wilt while four genotypes, WM11, SM2012-1, SM2013-9 and SM2013-2 exhibited a highly resistant reaction against ToLCPalV. A dendrogram constructed based on the resistance response of all the genotypes divided the genotypes into two groups and all resistant genotypes (MM1804, MM120103, SM2012-1, MM121103, SM2013-2, SM2013-9, WM11 and MM Sel.103) clustered in group II. The resistant genotypes when subjected to simultaneous inoculations of all three pathogens showed an increase in disease severity for each pathogen which negatively altered the resistance response of different genotypes. However, the genotypes SM2012-1, SM2013-9, SM2013-2 and WM-11 showing multiple disease resistance exhibited a good level of resistance even after combined inoculations of three pathogens. This study is the first to our knowledge identifying multiple disease resistance against root-knot nematode, Fusarium wilt and tomato leaf curl Palampur virus in muskmelon.

## Introduction

Melon (*Cucumis melo* L.) is a highly cherished cucurbitaceous crop, cultivated for its sweet and flavourful fruit. The crop has a considerable area under cultivation in tropical and subtropical parts of the world. The total production of melons throughout the world is 28.46 million tonnes (FAOSTAT, 2020). India ranked third in production (1.33 million tonnes), growing over an area of 59000 ha (FAOSTAT, 2020). The melon crop is very sensitive and its sustainable production is threatened by different pests and pathogens including, root-knot nematodes (RKNs), *Fusarium* wilt and begomovirus(s). Under Punjab conditions, the muskmelon crop remains in the field from the month of March until June and during this period the average temperature varies from minimum 19.92–26.76 °C to maximum 22.73–43.28 °C. The incidence of RKN and fusarium wilt increases as the temperature starts rising from February onwards. Further, as the population of whiteflies (virus vector) begins to increase due to warmer temperatures, the begomovirus incidence also starts appearing.

RKNs of the *Meloidogyne* genus are reported to cause more than 30% yield losses in highly susceptible vegetable and fruit crops including melons (Sikora and Fernandez, 2005). These nematodes cause intensive galling on the soft roots of melon plants and hamper the uptake of water and nutrients. The galled roots become more prone to other soil-borne pathogens due to the mechanical injuries caused to the roots because of penetration by the nematode larvae and due to alerting host physiology because of nematode feeding (Francl and Wheeler, 1993). Besides RKNs, a soil-inhabiting fungal pathogen, *Fusarium oxysporum* is another major constraint in melon production which may cause yield losses up to 90% in the worst affected area (Chattopadhyay and Sen, 1996). The pathogen colonizes the roots of the plant which is then followed by its extensive growth in the xylem vessels leading to wilting and then eventually death of the plant (Oumouloud *et al.*, 2013). The nematode-infected melon

roots are easily invaded by the *F. oxysporum* forming disease complexes that become difficult to manage and cause serious losses to the farmers (Dhami *et al.*, 2022).

In addition to soil-borne pathogens, different species of begomovirus (ssDNA virus), including melon chlorotic leaf curl virus, squash leaf curl virus, watermelon chlorotic stunt virus, tomato leaf curl New Delhi virus, tomato leaf curl Palampur virus, transmitted by whiteflies *Bemisia tabaci* are prevalent and associated with melons (Brown *et al.*, 2001; Hagen *et al.*, 2008; Sufrin-Ringwald and Lapidot, 2011; Sobh *et al.*, 2012; Venkataravanappa *et al.*, 2021). Dhkal *et al.* (2020a, 2020b) reported tomato leaf curl Palampur virus and tomato leaf curl New Delhi virus infecting melon crops in India. Recently, Dhami *et al.* (2023) identified ToLCPalV infecting melon crop in Indian Punjab. The viruses produce different types of symptoms, including mosaic and yellowing in young leaves coupled with leaf curling, reduced internodal length, vein swelling and roughness of fruit skin and fruit may show longitudinal lesions and cracks (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.

In the field, the melon crop is affected by all three pathogens (*Meloidogyne* spp., *Fusarium* and begomoviruses) during the cropping season, resulting in economic losses. Moreover, the interaction effect of these pathogens has been reported to cause a greater reduction in growth parameters and enhanced the disease severity as compared to the infection caused by them individually (Dhami *et al.*, 2022). In the present scenario of restricted application of chemicals due to harmful effects on the environment and society, the utilization of natural host resistance has been the most effective, sustainable and economical strategy for integrated disease management. Considering the importance of the crop and damage caused by these pathogens, in this study, melon genotypes were screened for resistance against RKN, *M. incognita*, *F. oxysporum* f. sp. *melonis* and tomato leaf curl Palampur virus prevalent under Indian Punjab conditions. A set of melon genotypes comprising popular cultivars and germplasm/breeding lines with promising horticultural traits generated through a conventional muskmelon breeding programme at the Department of Vegetable Science, Punjab Agricultural University (PAU), Ludhiana were used in this study. To our knowledge, this is the first attempt to identify multiple disease resistance against these pathogens in muskmelon.

## Materials and methods

### *Germplasm resources and experimental site*

A total of 64 melon genotypes, belonging to different horticultural groups and possessing distinct fruit traits were selected for the present study (online Supplementary Table S1). The experiments were conducted at Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana, during the months of March to June in the year 2019 and were repeated during the same months in the following year 2020. The average maximum and minimum temperature were 31.4 and 16.78 °C during the year 2019 and 30.08 and 17.96 °C respectively during the year 2020. The genotypes were screened individually against RKN, *M. incognita*, *F. oxysproum* f. sp. *melonis* and tomato leaf curl Palampur virus (ToLCPalV) under artificial conditions and the promising genotypes showing resistance to individual pathogens were then screened again by inoculating all three pathogens simultaneously to see their combined effect on host resistance. The melon cultivar, 'Punjab Sunehri' which was susceptible to *M. incognita*, *F. oxysproum* and tomato leaf curl Palampur virus was used as a standard susceptible check.

### *Screening against root-knot nematode (*Meloidogyne incognita*)*

Seeds of all the melon genotypes including susceptible check (Punjab Sunehri) were sown in plastic plug trays filled with steam-sterilized coco-peat, perlite and vermiculite mixture in 3:1:1 ratio. At the 3–4 leaf stage, ten plants of each genotype were transplanted in eight-inch diameter pots (two plants per pot) filled with steam-sterilized soil. After one week of transplanting, when plants were established, inoculations were performed with freshly hatched second-stage juveniles maintained in pure culture at two juveniles per gram of soil making holes with the help of a glass rod near the roots of the plant. Five replications were maintained for each line along with a susceptible check. Plants were watered regularly and properly maintained. After 50 days of inoculations, observations were recorded on the number of knots per root system, number of egg masses per root system and the root-knot index was calculated as per the (0–5) scale as per Taylor and Sasser (1978), where RGI 1 = 0 galls/eggs per root, 1 = 1–2 galls/eggs per root, 2 = 3–10 galls/eggs per root, 3 = 11–30 galls/eggs per root, 4 = 31–100 galls/eggs per root, 5 = more than 100 galls/eggs per root. Each genotype was categorized as resistant or susceptible as per the category index based on RGI, where RGI 0 = immune, 1 = resistant; 2 = moderately resistant; 3 = moderately susceptible; 4 = susceptible; 5 = highly susceptible. The final RKN population was estimated at the end of the experiment in a 250 cc soil sample taken from individual pots and washed using modified Cobb's sieving and decanting method (Whitehead and Hemming, 1965). The reproduction factor (Rf) was calculated as the ratio of the final nematode population to the initial nematode population (Rf = Pf/Pi).

### Screening against Fusarium oxysproum *f. sp.* melonis

The same set of 64 melon genotypes along with a susceptible check (Punjab Sunehri) was sown in plastic plug trays filled with steam-sterilized coco-peat, perlite and vermiculite mixture in 3:1:1 ratio. Twenty seeds were sown for each genotype and 3–4 leaf stage seedlings were inoculated with a pure culture of *F. oxysproum* f. sp. *melonis* taken from the Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The pure culture was multiplied on steam-sterilized sand and chickpea medium contained in 500 ml flasks. For the preparation of spore suspension, chickpea and sand medium was meshed and dissolved in double-distilled water. Spore concentration was determined by using a haemocytometer and adjusted to approximately $1 \times 10^6$ spores/ml by diluting with sterile distilled water. The inoculations were done at $1 \times 10^6$ spores of *F. oxysproum* f. sp. *melonis* per 1 ml of water at the 3–4 leaf stage of the plants. Plants were inoculated artificially by injecting spore suspension in the root zone of the seedlings in the plugged trays (Boyhan *et al.*, 2001). Plants were properly maintained and watered as required. The categorization of all the genotypes as resistant or susceptible was done on the basis of final observations taken 21 days post-inoculation using a 0–5 scale given by Zhang *et al.* (2008) for seedling stage screening, where 0 = all plants alive (highly resistant); 1 = one cotyledon leaf becomes yellow (resistant); 2 = two cotyledon leaves become yellow (moderately resistant); 3 = two cotyledon leaves and one true leaf become yellow (moderately susceptible); 4 = wilting from stem end (susceptible); 5 = dead plant (highly susceptible).

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The melon seedlings with different symptom grades produced by the *F. oxysproum* f. sp. *melonis* have been shown in online Supplementary Fig. S1. The per cent disease index was also computed by using the following formula:

$$\text{Per cent disease index (PDI)} = \frac{\text{The total sum of numerical ratings}}{\text{Total number of plants} \times \text{Maximum disease grade}} \times 100$$

### Screening against tomato leaf curl Palampur virus

A similar set of genotypes was screened against begomovirus and tomato leaf curl Palampur virus (ToLCPalV). The plants of the susceptible melon cultivar, 'Punjab Sunehri' infected with ToLCPalV virus, maintained in an insect-proof cage were used as a source of inoculum. Tomato leaf curl Palampur virus was confirmed by PCR amplification of total DNA extracted from the leaves of virus-infected plants using ToLCPalV-specific primers (Palampur F and Palampur R) (Personal communication, Yogesh Kumar, IHBT, Palampur, Himachal Pradesh, India). The DNA extraction was done with the cetyl tri-methyl ammonium bromide (CTAB) method (Lodhi *et al*., 1994). For inoculations, non-viruliferous whiteflies (*B. tabaci*) (maintained on cotton, *Gossypium hirsutum* L. grown in 10 × 6 cm size plastic pots kept in an insect-proof greenhouse) were collected using an aspirator and were released into the plastic bottle cage made by cutting the bottom end of bottles and replaced by white muslin cloth. A branch of an infected melon plant (grown in a pot) was inserted into the bottle containing non-viruliferous whiteflies and the narrow mouth of the bottle was closed using a cotton plug. These whiteflies were allowed to feed for an acquisition access period of 24 h. After the acquisition period was over, the viruliferous whiteflies were collected and used for the artificial screening of the genotypes. Ten seeds of each genotype were sown directly in pots kept inside the insect-proof cage and seedlings were inoculated twice at weekly intervals at 3–4 leaf stage with ten viruliferous whiteflies per seedling. Observations were taken regularly for symptom appearance up to 6 weeks. Disease severity grade was given to each plant on a 0–5 scale given by Dhkal (2018) (as given below) and the per cent disease index was calculated for each genotype.

| Symptoms | Symptom severity grade | Percent Disease Index (%) | Disease reaction |
|---|---|---|---|
| Symptomless | 0 | 0 | Highly resistant (HR) |
| Pin-head-sized chlorotic spots | 1 | 0.1–5 | Resistant (R) |
| Mild mosaic and blistering | 2 | 5.1–20 | Moderately resistant (MR) |
| Blistering and misshapen leaf | 3 | 20.1–50 | Moderately susceptible (MS) |
| Severe yellows and blistering | 4 | 50.1–75 | Susceptible (S) |
| Severe curling of leaf, yellow vein mosaic and chlorosis small flowers and no healthy or small fruit set | 5 | >75 | Highly susceptible (HS) |

Six weeks after inoculation, the DNA from all the genotypes was extracted using the same method mentioned above and subjected to PCR amplification with the same primer to observe the presence or absence of the TolcPalV.

### Screening of selected melon genotypes for multiple disease resistance

Based on the screening against individual pathogens, 14 melon genotypes showing varying degree of resistance against individual pathogens were selected and further screened for disease reaction to *M. incognita*, *F. oxysporum* and ToLCPalV by inoculating all three pathogens simultaneously. Twenty seeds of each selected genotype were sown in pots (two seeds per pot) containing sterilized autoclaved soil and kept inside an insect-proof cage. Simultaneous inoculations of *M. incognita*, *F. oxysporum* f. sp. *melonis* and ToLCPalV were done at the 4–5 true leaf stage. Inoculation of *M. incognita* and ToLCPalV was done using the same techniques as described earlier in the screening of genotypes against these pathogens individually. However, *F. oxysporum* f. sp. *melonis* was inoculated by mixing sand-chickpea media mass culture of the fungus at the rate of 10 g per kilogram of the soil in the rhizosphere. The observations on RGI and per cent virus disease index were recorded 45 days after inoculation as described earlier, while the *Fusarium* wilt disease severity was recorded as per the (0–4) scale given by Kaur (2005) for full-grown plants, where 0 = no disease (highly resistant); 0.1–1.0 = stunting (resistant); 1.1–2.0 = yellowing (moderately resistant); 2.1–2.0 = necrosis (susceptible); >3.0 = wilting (highly susceptible).

### Statistical analysis

The average of the data recorded on disease parameters during both years was used to calculate the standard errors of means using Microsoft Excel 2010. The mean disease severity grade of the melon genotypes against all three pathogens inoculated individually was used to generate a dendrogram and heat map of the melon germplasm under the present study using R-Studio software.

## Results

### Screening against root-knot nematode (M. incognita)

Screening of melon genotypes against *M. incognita* revealed that out of 64 genotypes, three genotypes *viz*., MCPS (RGI = 2.0; Rf = 1.06), SM2012-1 (RGI = 1.89; Rf = 1.01) and WM11 (RGI = 2.0; Rf = 1.09) were found moderately resistant to *M. incognita* (online Supplementary Table S2 and Table 1). Eight genotypes *viz*., MM120103, MM3964, SM2013-9, MM121103, MM Sel.-103, MM720-2, CTN 1820 and MM1804 were found moderately susceptible with RGI ranging from 2.22 to 2.89 and Rf ranging from 1.13 to 1.34. Among others, eight genotypes (MM608, MM403, Punjab Sunehri, MM617, MM619, MM Var.-2, KP4HM15 and Rio Gold) were found highly susceptible (RGI = 4.11–4.56; Rf = 1.50–1.97) and all other genotypes exhibited susceptible reaction to *M. incognita*. The roots of moderately resistant genotypes were comparatively healthy with a lesser number of galls and egg masses (Fig. 1a).

### Screening against F. oxysporum f. sp. *melonis*

The same set of 64 melon genotypes when screened against *F. oxysporum* f.sp. *melonis* revealed nine genotypes comprising

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**Table 1.** Reaction of melon genotypes against root-knot nematode (*Meloidogyne incognita*)

| Genotypes | No. of genotypes | No. of galls per root system | Category index (0–5 scale) | Reaction |
|---|---|---|---|---|
| – | Nil | 0 | 0 | Immune |
| – | Nil | 1–2 | 0.1–1.0 | Resistant |
| MCPS, SM2012-1, WM11 | 3 | 3–10 | 1.1–2.0 | Moderately resistant |
| MM120103, MM3964, SM2013-9, MM121103, MM Sel.-103, MM720-2, CTN1820, MM1804 | 8 | 11–30 | 2.1–3.0 | Moderately susceptible |
| MM610, MM607, MS-5, Bobby $F_1$ Hybrid, MM902-1, MM904, Hara Madhu, MS-1, MM681, MM10316, MM621, MH-41, Pusa Sharbati, MM672-1, MM675-3, MM201, MM Var.-1, DP Sel.-1, MM675, MM703, SM2013-2, MM677, MM625, MM916-1, MM-KP15103, MM1805, MM327, MM1831, MM1903, MH-51, MM604, Pusa Madhuras, MM721, MM4216, MM331, MM676-1, MM911, CY2012-21, MM4305, MM673-3, Punjab Hybrid, MM1803, Inthanon F1 Hybrid, MM603, MM616 | 45 | 31–100 | 3.1–4.0 | Susceptible |
| MM608, MM403, Punjab Sunehri, MM617, MM619, MM Var.-2, KP4HM15, Rio Gold | 8 | More than 100 | 4.1–5.0 | Highly susceptible |

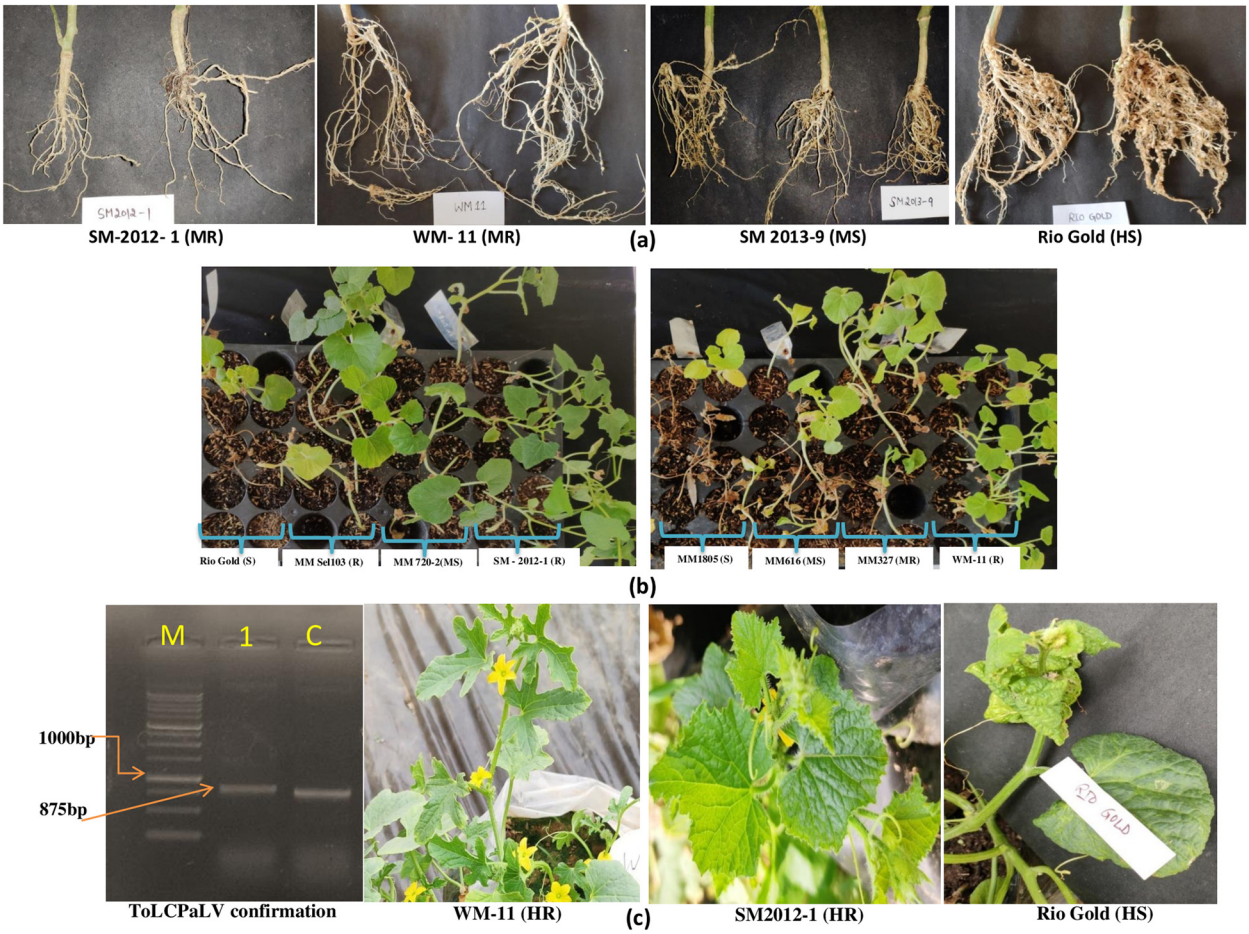


**Figure 1.** Melon genotypes with different levels of resistance to *M. incognita*, *F. oxysporum* f. sp. *melonis* and tomato leaf curl Palampur virus. (a) Roots of moderately resistant genotypes (SM2012-1 and WM-11) showing few root galls while highly susceptible genotype Rio Gold exhibiting numerous galls on the roots due to root-knot nematode infestation; (b) Fusarium wilt resistant genotypes (MM sel.103, SM2012-1 and WM-11) showing healthy green plants while susceptible (MM1805) showing wilting; (c) agarose gel (1%) showing amplicon of 875 bp with ToLCPalV-specific primers (C – positive control, M – marker1000 bp) and resistant genotypes (WM-11 and SM2012-1) showing symptomless healthy plants and highly susceptible genotype (Rio Gold) showing curling and yellowing of leaves.

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five muskmelon genotypes (MM-KP15103, MM327, MM121103, KP4HM15, MM Sel.-103), three snap melon genotypes (SM2013-2, SM2012-1 and SM2013-9) and one wild melon genotype (WM11) with resistant reaction to *F. oxysporum* f. sp. *melonis* with a disease severity grade ranging from 0.5 to 1.0 (online Supplementary Table S2 and Table 2; Fig. 1b). Sixteen genotypes (MM607, MM608, MM327, MM120103, MH-41, MM103616, MM3964, MM675-3, MM201, MM1804, DP Sel.-1, MM703, MH-51, MM911, MM4305 and MM Var.-2) exhibited a moderate level of resistance with a disease severity grade ranging from 1.3 to 1.9. Among others, 13 genotypes *viz*., Bobby $F_1$ Hybrid, MM403, MM616, Hara Madhu, MM621, MM672-1, var.-1, MM675, MM1831, Pusa Madhuras, CY2012-21, Punjab Hybrid and MM720-2 were found moderately susceptible to *F. oxysporum* f.sp. *melonis* with disease severity grade varying from 2.4 to 3.0. Whereas, 22 genotypes were found susceptible and five genotypes were found highly susceptible to *F. oxysporum* f.sp. *melonis*.

### Screening against tomato leaf curl Palampur virus (ToLCPalV)

The PCR amplification of the DNA extracted from the virus-infected melon plants maintained for inoculations showed a DNA product of 875 bp confirming the presence of tomato leaf curl Palampur virus (Fig. 1c). Out of total 64 melon genotypes screened, a wild melon genotype WM11 and three snap melon genotypes, SM2012-1, SM2013-9 and SM2013-2 were found highly resistant to tomato leaf curl Palampur virus and did not show any symptoms (Fig. 1c). Four genotypes (KP4HM15, MM Sel.-103, MH-41 and MM-KP15103) were found resistant with disease severity ranging from 0.1 to 5.0%. Genotypes *viz*., Pusa Madhuras, MH-51, MM120103 and MM121103 recorded moderately resistant reactions with disease severity ranging from 5.1 to 20%. Among others, eight genotypes (MM610, MS-5, MM681, Pusa Sharbati, MM103616, MM1804, DP Sel.-1 and MM1903) were moderately susceptible with disease severity ranging from 20.1 to 50% and 22 genotypes were highly susceptible with disease severity more than 75%. All other genotypes exhibited susceptible reaction with disease severity ranging from 50.1 to 75% (online Supplementary Table S2 and Table 3). However, PCR analysis of the DNA isolated from the symptomless as well as symptomatic genotypes gave PCR amplification of TolcPalV confirming its presence in all the genotypes.

A dendrogram and heat map generated based on the reaction of all the 64 genotypes of melon resulted in the clustering of the genotypes in two broad groups denoted as the group I and group II (Fig. 2). Group I was comprised of a total of 51 genotypes which further segregated into two sub-clusters Ia and Ib. The sub-cluster Ia contained 23 genotypes that were susceptible or highly susceptible to one of the pathogens but were either moderately resistant or moderately susceptible to the other two pathogens. Group Ib comprised 27 genotypes most of which were susceptible or highly susceptible to all three or at least two pathogens under study. Group II included 13 genotypes that were resistant to all three pathogens or were moderately resistant or resistant to at least two pathogens.

### Screening of selected genotypes for multiple disease resistance

Fourteen melon genotypes showing varying degrees of resistance against *M. incognita*, *F. oxysporum* f.sp. *melonis* and *tomato leaf curl Palampur virus* during artificial screening against the individual pathogen, were selected and screened for disease resistance by simultaneously inoculating all three pathogens. As per the data recorded in Table 4, it was found that the melon genotypes SM2012-1 and WM11 showed a moderately resistant reaction (RGI = 2.0) against RKN when inoculated only with *M. incognita* alone, but during simultaneous inoculation of the nematode with *Fusarium* and ToLCPalV, they showed moderately susceptible reaction (RGI = 2.3 and 2.5). Among others, genotypes showing moderately susceptible reaction (MM720-2, MM121103, MM120103) and susceptible reaction (MH-41, SM2013-2, MM721, MM672-1, MM-KP15103) during individual screening against nematode exhibited the same reaction even during the simultaneous inoculation of RKN, *Fusarium* and virus; however, their root gall index was slightly on the higher side during simultaneous inoculation. The three highly susceptible genotypes showed a susceptible reaction with a slight decrease in RGI during the simultaneous inoculation of three pathogens and showed a susceptible reaction.

Irrespective of the inoculation method and associated disease severity scale, the disease reaction of genotypes to fusarium wilt

**Table 2.** Reaction of melon genotypes showing different levels of resistance against *Fusarium oxysporum* f.sp. *melonis*

| Genotypes | No. of genotypes | Symptoms | Disease severity grade (0–5 scale) | Disease reaction |
|---|---|---|---|---|
| – | Nil | No disease, all alive | 0 | Highly resistant |
| MM-KP15103, MM327, MM121103, MM Sel.-103, KP4HM15, SM2012-1, SM2013-2, SM2013-9, WM11 | 9 | One cotyledon leaf becomes yellow | 0.1–1.0 | Resistant |
| MM607, MM608, MM120103, MH-41, MM103616, MM120103, MM3964, MM675-3, MM201, MM1804, DP Sel.-1, MM703, MH-51, MM911, MM4305, MM Var.-2 | 15 | Two cotyledon leaves become yellow | 1.1–2.0 | Moderately resistant |
| Bobby $F_1$ Hybrid, MM403, MM616, Hara Madhu, MM621, MM672-1, MM Var.-1, MM675, MM1831, Pusa Madhuras, CY2012-21, Punjab Hybrid, MM720-2 | 13 | Two cotyledon leaves and one true leaf become yellow | 2.1–3.0 | Moderately susceptible |
| MM610, MS-5, MM902-1, CTN1820, MM904, MS-1, MM681, MCPS, Punjab Sunehri, MM916-1, MM1805, MM1903, MM604, MM619, MM721, MM4216, MM1803, Inthanon F1 Hybrid, MM603, MM331, MM676-1, Rio Gold | 22 | Wilting from stem end | 3.1–4.0 | Susceptible |
| Pusa Sharbati, MM677, MM617, MM625, MM673-3 | 5 | Dead | 4.1–5.0 | Highly susceptible |

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**Table 3.** Reaction of melon genotypes showing different levels of resistance against *tomato leaf curl Palampur virus*

| Genotypes | No. of genotypes | Symptoms | PDI (%) | Disease reaction |
|---|---|---|---|---|
| SM2013-2, WM11, SM2013-9, SM2012-1 | 4 | Symptomless | 0 | Highly resistant |
| MH-41, MM-KP15103, KP4HM15, MM Sel.-103, | 4 | Pin head size chlorotic spots on top 2–3 young leaves | 0.1–5 | Resistant |
| MM120103, MH-51, Pusa Madhuras, MM121103 | 4 | Mild mosaic and blistering | 5.1–20 | Moderately resistant |
| MM610, MS-5, MM681, Pusa Sharbati, MM103616, MM1804, DP Sel.-1, MM1903 | 8 | Blistering and misshapen leaf | 20.1–50 | Moderately susceptible |
| CTN1820, MM904, MM403, MM616, MS-1, MM3964, MM672-1, MM675-3, MCPS, MM201, MM 677, MM617, MM916-1, MM1805, MM604, MM4216, MM676-1, MM911, MM Var.-2, MM673-3, MM720-2, MM1803 | 22 | Sever yellow and blistering | 50.1–75 | Susceptible |
| MM607, Bobby $F_1$ Hybrid, MM608, MM902-1, HM, MM621, Punjab Sunehri, MM Var.-1, MM675, MM703, MM625, MM327, MM1831, MM619, MM721, MM331, CY2012-21, MM4305, Rio Gold, Punjab Hybrid, Inthanon F1 Hybrid, MM603 | 22 | Severe curling of leaf, yellow vein mosaic and chlorosis | >75 | Highly susceptible |

was elevated under simultaneous inoculation of all three pathogens. Genotypes, KP4HM15, SM2013-9, SM2013-2, MM-KP15103 and MM121103 were observed to become moderately resistant to fusarium wilt under simultaneous inoculation, whereas these genotypes showed resistant reaction when inoculated with *F. oxysporum* f.sp. *melonis* alone. The other two resistant genotypes WM11 and SM2012-1 showed resistant reactions even during simultaneous inoculations; however, the disease severity grade was increased from 0.5 to 0.8 in WM11 and from 0.6 to 0.8 in genotype SM2012-1. The moderately resistant genotype, MM120103 showed a moderately susceptible reaction after combined inoculation of all three pathogens. Susceptible genotypes (Rio Gold, MM721 and MM619) and two moderately susceptible genotypes (MM672-1 and MM720-2) became more vulnerable and produced highly susceptible reactions to fusarium wilt, under the effect of simultaneous inoculation of RKN, *Fusarium* and ToLCPalV.

For ToLCPalV, disease severity was found to be slightly higher when the virus was inoculated with RKN and *Fusarium* simultaneously. Two highly resistant genotypes (SM2013-9 and SM2012-1) and three resistant genotypes (KP4HM15, MH-41 and MM-KP15103) showed a moderately resistant reaction. However, two highly resistant genotypes (WM-11 and SM2013-2) showed resistant reactions even under the influence of all three pathogens. MM121103 maintained the moderately resistant disease reaction but MM120103 became moderately susceptible from moderately resistant. Highly susceptible genotypes (Rio Gold, MM721 and MM619) and susceptible genotypes (MM720-2 and MM672-1) exhibited severe symptoms and gave highly susceptible disease reactions.

## Discussion

The present study demonstrated the identification of muskmelon genotypes with multiple disease resistance against *M. incognita*, *F. oxysporum* f.sp. *melonis* and ToLCPalV. It was observed that during the screening for each pathogen individually, out of a total 64 genotypes, three genotypes comprising a snap melon genotype (SM2012-1), a wild melon genotype (WM-11) and melon genotype, MCPS were found with moderate levels of resistance against *M. incognita*. Most of the other genotypes showed moderately susceptible to highly susceptible reactions against the nematode. For fusarium wilt, one melon genotype (KP4HM-15), three snap melon genotypes (SM2013-2, SM2012-1 and SM2013-9) and one wild melon genotype (WM11) showed resistant reaction while others exhibited a moderate resistance to the susceptible reaction against the fungus. Three snap melon genotypes (SM2012-1, SM2013-9 and SM2013-2) and one wild melon genotype (WM11) also showed a high level of resistance against ToLCPalV; while genotypes, KP4HM15, MM Sel.-103, MH-41 and MM-KP15103 showed resistant reactions against the ToLCPalV.

Almost all the cultivated cucurbits are susceptible to RKNs and it is very infrequent to get complete resistance against RKNs in cultivated cucurbit species, especially *C. melo* and *C. sativus* (Siguenza *et al.*, 2005; Mukhtar *et al.*, 2013). However, resistance to *Meloidogyne* spp. has been reported in different accessions of the wild species of the genus *Cucumis viz*., *C. africanus*, *C. anguria*, *C. metuliferus*, *C. myriocarpus*, *C. moschata*, *C. postulatus*, *C. subsericeus* and *C. zeyheri* (Fassuliotis, 1970; Punja *et al.*, 2001; Walters *et al.*, 2006; Pofu and Mashela, 2011; Guan *et al.*, 2014; Liu *et al.*, 2015; Expósito *et al.*, 2017). Apart from these, Indian snap melon landraces, i.e. *C. melo* var. *momordica* commonly known as '*Phut*' are reported to possess resistance or tolerance against different biotic (fungal and viral diseases, nematodes and insect pests) and abiotic stresses (drought, soil salinity and high temperature) (Dhillon *et al.*, 2014). A high level of resistance against RKN has been reported in snap melon accession IC 274023 by Dhillon *et al.* (2007). Roy *et al.* (2012) reported that among the 43 wild melon accessions screened against RKN, two accessions (WM 8 and WM 16) showed segregation for nematode resistance. Indian snap melons are also reported to possess varying levels of resistance to distinct groups of viruses. Sáez *et al.* (2017) reported resistance to begomovirus, tomato leaf curl New Delhi virus in *C. melo* var. *momordica* and wild melon *C. melo* var *agrestis* genotypes. Dhkal in 2018 also reported resistance against tomato leaf curl Palampur virus in melon genotypes, WM11, KP4HM15, MM Selection 103, SM2012-1, MH27, MH51 and WM1607. Romay *et al.* (2019) screened 31 melon accessions against two begomoviruses: melon chlorotic mosaic virus (MeCMV) and tomato leaf curl New Delhi virus (ToLCNDV), and reported five accessions (IC-274014, WM7, WM9, PI-124112 and PI-282448) resistant against these viruses. Resistance against ToLCNDV was observed to be controlled by

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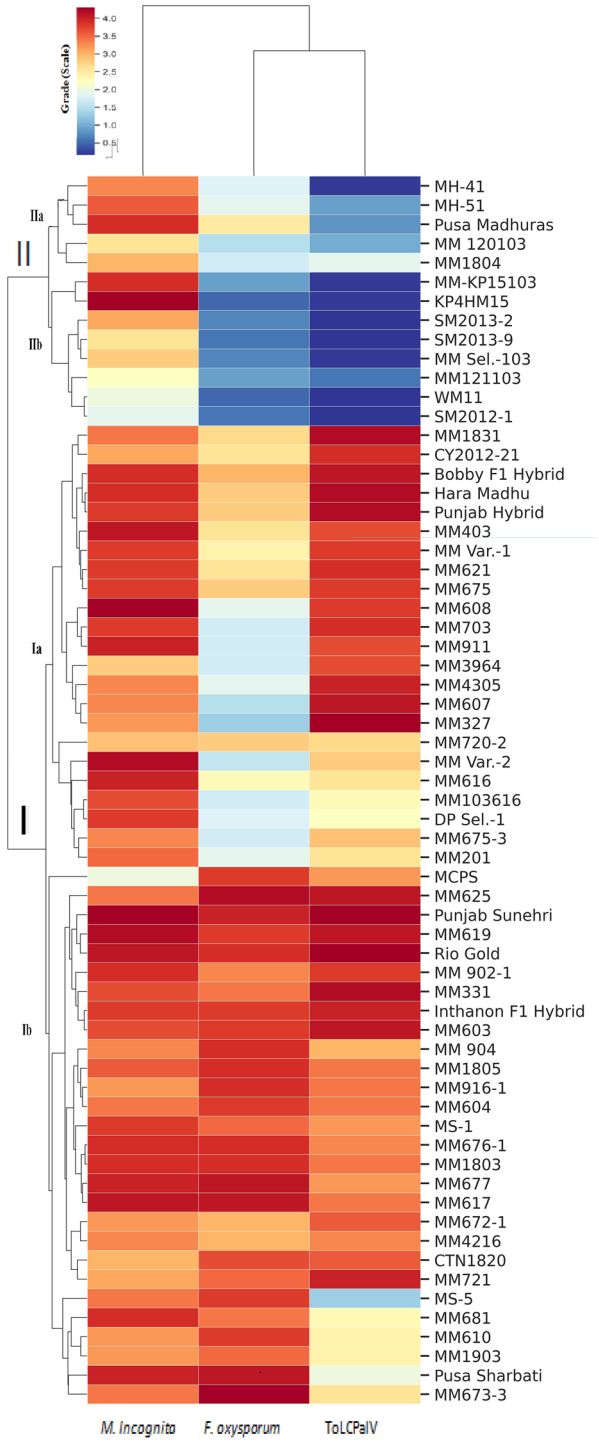


**Figure 2.** Dendrogram and heat map of muskmelon genotypes based on the disease severity grade of the germplasm against all three pathogens under study.

genes *bgm-1*, *Bgm-2* and the dominant gene *tomato leaf curl New Delhi virus resistance gene*. While resistance to MeCMV was controlled by the same *bgm-1* and *Bgm-2* genes coupled with recessive Melon chlorotic mosaic virus resistance gene. In addition to begomoviruses, Indian snap melon landraces are also reported to possess resistance against cucumovirus, cucumber mosaic virus and potyvirus zucchini yellow mosaic virus (Dhillon *et al.*, 2007). Some dominant genes (*Zym-1*, *Zym-2*, *Zym-3*) showing resistance to Zucchini yellow mosaic virus were also detected in snap melon accession (PI 414723) from India (Pitrat and Lecoq, 1984; Danin-Poleg *et al.*, 1997). McCreight and Wintermantel (2008) also reported resistance to cucurbit yellow stunting disorder virus (crinivirus) in Indian snapmelon landraces, PI 313970, Ames 20203, PI 614185 and PI 614213. These findings illuminate the molecular underpinnings of resistance in snap melons and wild melons against different pathogens.

Further, when the selected set of 14 melon genotypes, showing resistance to the individual pathogens was subjected to simultaneous inoculations by all three pathogens (*M. incognita*, *F. oxysporum* f.sp. *melonis* and ToLCPalV) the disease severity score showed variation over the rating score obtained during screening against the individual pathogen. For RKN, the moderately resistant, moderately susceptible as well as susceptible genotypes showed slightly higher root galling index during the simultaneous inoculation, however highly susceptible ones recorded slightly lower RGI. This might be due to damage to roots caused by *Fusarium* infection in highly susceptible genotypes. Fusarium wilt disease severity grade was also enhanced during simultaneous inoculations of all three pathogens in all the genotypes. The moderately resistant genotypes showed moderate susceptibility and moderately susceptible genotypes showed susceptible reactions during combined inoculations. Among the resistant genotypes, WM-11 and SM2012-1 exhibited only a mild increase in disease severity and both the genotypes remained in the resistant category even after the simultaneous inoculations. While genotype KP4HM15 which showed a resistant reaction to fusarium during individual screening showed a moderately resistant reaction during simultaneous inoculations. This variable response may be due to the susceptibility of the genotypes to *M. incognita* as both WM-11 and SM2012-1 are resistant to *M. incognita* but KP4HM15 is highly susceptible to the nematode. The RKNs are known to predispose the host plant roots to other soil-borne fungal and bacterial pathogens either via mechanical injuries to the root tissues by providing entry points to the passive invaders or by changing the host physiology (Khan, 2008). The giant cells or nurse cells formed due to RKN infection remain metabolically more active and contain higher amounts of photosynthates, DNA, RNA and cell organelles (Caillaud *et al.*, 2008). These nutritionally rich giant cells support the multiplication and colonization of the fungal pathogens. The nematode infection also induces the production of some biochemical compound which may neutralize the fungicidal phytoalexins. Besides this, root exudates from nematode-infected plants have been reported to suppress the actinomycetes in rhizosphere (Khan and Sharma, 2020). Hua *et al.* (2019) investigated the reliability of *F. oxysporum* f. sp. *niveum*-resistant genotypes of watermelon against *M. incognita*. The result revealed that *M. incognita* enhanced the susceptibility of all watermelon genotypes to Fusarium wilt. Regmi *et al.* (2022) also reported that wilt disease severity due to *F. oxysporum* f. sp. *lycopersici* was more pronounced in the presence of RKN. The genotypes WM11 and SM2012-1 behaved resistant to fusarium wilt during simultaneous inoculations; this may be because they possess a moderate level of resistance against RKN. Further, increased virus disease severity may be explained by the additional manifestation of RKN (stunting and yellowing) and *Fusarium* (yellowing, wilting and necrosis).

The screening for resistance against individual pathogens has been done earlier but the present work is the first attempt to identify melon genotypes possessing multiple disease resistance against more than one pathogen. The snap melon genotypes, SM2012-1, SM2013-9 and SM2013-2 were found resistant to both Fusarium wilt as well as ToLCPalV. The genotype SM2012-1 also showed moderate resistance to RKN, *M. incognita*.

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**Table 4.** Screening of promising muskmelon genotypes for multiple disease resistance against root-knot nematode, fusarium wilt and *tomato leaf curl Palampur virus*

| | | Disease reaction when inoculated individually | | | | | | | Disease reaction when inoculated simultaneously | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RKN | | *Fusarium* | | ToLCPalV | | | RKN | | *Fusarium* | | ToLCPalV | | |
| S. No. | Genotypes | RGI (0–5) scale | Reaction | DSG (0–5) scale | Reaction | DSG (0–5) scale | PDI (%) | Reaction | RGI (0–5) scale | Reaction | DSG (0–4) | Reaction | DSG (0–5) scale | PDI (%) | Reaction |
| 1 | KP4HM15 | 4.3 ± 0.33 | HS | 0.5 ± 0.33 | R | 0.2 ± 0.19 | 4.45 | R | 3.5 ± 0.40 | S | 2.0 ± 0.50 | MR | 0.6 ± 0.20 | 11.11 | MR |
| 2 | MH-41 | 3.3 ± 0.33 | S | 1.8 ± 0.19 | MR | 0.2 ± 0.19 | 4.44 | R | 3.5 ± 0.50 | S | 1.3 ± 0.29 | MR | 0.4 ± 0.51 | 8.89 | MR |
| 3 | SM2013-9 | 2.6 ± 0.19 | MS | 0.6 ± 0.19 | R | 0.0 ± 0.00 | 0.00 | HR | 2.8 ± 0.40 | MS | 1.2 ± 0.29 | MR | 0.3 ± 0.33 | 6.67 | MR |
| 4 | Rio Gold | 4.1 ± 0.19 | HS | 3.9 ± 0.19 | S | 4.4 ± 0.33 | 88.89 | HS | 3.6 ± 0.51 | S | 3.2 ± 0.29 | HS | 4.3 ± 0.29 | 86.67 | HS |
| 5 | SM2013-2 | 3.1 ± 0.33 | S | 0.7 ± 0.19 | R | 0.0 ± 0.00 | 0.00 | HR | 3.3 ± 0.58 | S | 1.5 ± 0.87 | MR | 0.2 ± 0.18 | 4.45 | R |
| 6 | MM721 | 3.1 ± 0.38 | S | 3.5 ± 0.38 | S | 4.0 ± 0.38 | 80.00 | HS | 3.3 ± 0.58 | S | 3.3 ± 0.29 | HS | 4.1 ± 0.19 | 82.22 | HS |
| 7 | MM720-2 | 2.9 ± 0.19 | MS | 2.8 ± 0.33 | MS | 2.7 ± 0.19 | 53.33 | S | 3.0 ± 0.00 | MS | 3.2 ± 0.29 | HS | 4.0 ± 0.33 | 80.00 | HS |
| 8 | WM-11 | 2.0 ± 0.19 | MR | 0.5 ± 0.00 | R | 0.0 ± 0.00 | 0.00 | HR | 2.5 ± 0.40 | MS | 0.8 ± 0.76 | R | 0.2 ± 0.19 | 4.44 | R |
| 9 | MM672-1 | 3.2 ± 0.19 | S | 3.0 ± 0.19 | MS | 3.6 ± 0.19 | 57.78 | S | 3.5 ± 0.40 | S | 3.3 ± 0.25 | HS | 3.9 ± 0.19 | 77.78 | HS |
| 10 | SM2012-1 | 1.9 ± 0.34 | MR | 0.6 ± 0.19 | R | 0.0 ± 0.00 | 0.00 | HR | 2.3 ± 0.58 | MS | 1.0 ± 0.00 | R | 0.3 ± 0.00 | 6.67 | MR |
| 11 | MM-KP15103 | 3.9 ± 0.19 | S | 0.9 ± 0.19 | R | 0.2 ± 0.19 | 4.45 | R | 4.0 ± 0.00 | S | 1.3 ± 0.29 | MR | 0.4 ± 0.19 | 8.89 | MR |
| 12 | MM121103 | 2.2 ± 0.33 | MS | 0.9 ± 0.33 | R | 0.6 ± 0.19 | 20.0 | MR | 2.7 ± 0.58 | MS | 2.0 ± 0.25 | MR | 1.0 ± 0.33 | 20.00 | MR |
| 13 | MM120103 | 2.6 ± 0.33 | MS | 1.5 ± 0.33 | MR | 1.0 ± 0.19 | 11.1 | MR | 2.8 ± 0.60 | MS | 2.2 ± 0.50 | MS | 1.2 ± 0.19 | 24.44 | MS |
| 14 | MM619 | 4.2 ± 0.35 | HS | 3.8 ± 0.00 | S | 4.1 ± 0.19 | 82.22 | HS | 3.6 ± 0.51 | S | 3.2 ± 0.29 | HS | 4.3 ± 0.00 | 86.67 | HS |

RKN, root-knot nematode; DSG, disease severity grade; ToLCPalV, *tomato leaf curl Palampur virus*; RGI, root gall index; PDI, per cent disease index; HR, highly resistant; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; HS, highly susceptible.

Also, wild melon genotype WM11 exhibited resistance against all three pathogens. Besides, genotypes MH-41, MM120103, MMKP15103, KP4HM15 and MM Sel. 103 were found resistant to both *F. oxysporum* f.sp. *melonis* and ToLCPalV. Further, simultaneous inoculations of promising genotypes with all three pathogens showed that the attack of one pathogen alters the host's response to another pathogen. However, genotypes that were resistant to more than one pathogen showed a good level of resistance even during simultaneous inoculations of the pathogens. The resistant genotypes identified in this study may be utilized as donor parents for strengthening the resistance breeding programme of the melon crop.

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