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RESEARCH ARTICLE

Genetic analysis of resistance to tomato leaf curl New Delhi virus in muskmelon (*Cucumis melo* L.)

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Abstract

The genetics and mode of gene action for resistance to tomato leaf curl New Delhi virus (ToLCNDV) in muskmelon (*Cucumis melo* L.) are elucidated in the current work for the first time under conditions of natural epiphytotic and whitefly-mediated challenge inoculation. In order to accomplish this investigation, susceptible parent (DOM 115) and resistant parent (DSM 132) were utilized to produce the F_1, F_2, BC_1P_1 and BC_1P_2 populations. According to generation mean analysis, genetic dominance may be more significant for the manifestation of ToLCNDV resistance in the cross between DOM 115 and DSM 132 in both screening techniques. The F_1 mean was skewed toward the resistant parent and considerably lower than the midparent value. The significance of scaling test proved the existence of epistatic interaction. Estimates of six parametersm, d, h, i, j, and l from generation mean analysis in the cross between DOM 115 and DSM 132 revealed that non-allelic interactions predominated, mostly of additive ×dominance (j) type with negative sign in natural epiphytotic screening and dominance × dominance [l] type with negative signs in challenge inoculation screening methods respectively. Under natural epiphytotic and whitefly mediated challenge inoculation screening procedures, complementary types of epistasis have been found because both components, dominance [h] and dominant × dominance [l], were oriented in the same direction. Above results indicated that the recombination breeding, and hybridization followed by selection at later generations could be used to improve resistance to ToLCNDV.

Keywords: ToLCNDV, Muskmelon, Generation mean analysis, Epistasis

Introduction

Whitefly-transmitted virus diseases are a serious risk for agriculture as they have been continuously emerging (Navas Castillo et al. 2011, 2014). Whitefly transmitted viruses are a significant barrier to the profitability of many horticultural crops and have the potential to have a drastic impact on crop yield. Recently, the spreading of emerging virus in North India such as Tomato leaf curl New Delhi virus (ToLCNDV) in melon has caused great concern among farmers.

In India, tomato (*Solanum lycopersicum* L.) was the first crop to be reported with ToLCNDV (Papidam et al.1995; Srivastava et al. 1995). Later, it was identified on various hosts in nearby nations, especially vegetable species of the *Cucurbitaceae* and *Solanaceae* families (Chang et al.2010; Jyothsna et al. 2013). The whiteflies (*Bemisia tabaci*) are a persistent carrier of the ToLCNDV virus (Jyothsna et al. 2013). Melons (*Cucumis melo*) exhibit curling, severe mosaic of young leaves and vein swelling, short internodes, and rough fruit skin as ToLCNDV related symptoms (Juarez et al. 2014).

Control methods for begomovirus diseases are heavily centred on vector management in many parts of the world. The establishment of whitefly populations is restricted by a number of strategies, such as the use of insecticides and physical barriers. Additionally, cultural practices like virus-free transplants, crop-free periods, weed control, and

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rouging of infected plants are recommended for managing whiteflies (Lecoq and Desbiez 2012; Seal et al. 2006). The management of this disease is not always completely successful with these vector control techniques. Therefore, it is crucial to develop resistant varieties and hybrids to this disease. ToLCNDV disease information and its genetic architecture, however, are crucial for developing the breeding strategy for the enhancement of this trait before producing the desired variety or hybrids.

The inheritance pattern of leaf curl diseases caused by begomovirus, particularly ToLCNDV in melon, was very scanty. According to Islam et al. (2010), a single dominant gene controlled the resistance to the tomato leaf curl New Delhi virus in the advanced breeding lines DSG-6 and DSG-7 of the sponge gourd. Saez et al. (2017) assessed the genetic regulation of ToLCNDV in melon in F_2 and BC_1 populations derived from WM-7 × Pinonet Piel de Sapo (PS), and after phenotyping these populations for ToLCNDV, they found that resistance to ToLCNDV was controlled by a dominant gene. In the melon accession «IC-274014,» Romay et al. (2019) reported that a single recessive gene controls resistance to ToLCNDV. *Cucumis sativus* resistant accession CGN23089 and *Cucurbita moschata* accession PI 419083 both exhibit monogenic recessive genetic control for ToLCNDV resistance (Saez et al 2020, 2021). It has been revealed that chillies have a monogenic recessive gene for resistance to the chili leaf curl virus (Rai et al. 2014; Maurya et al. 2019).

The use of genotypes with different backgrounds, virus isolates, the timing of inoculation, and screening techniques used to determine resistance are all contributing factors to the contradictory reports regarding the inheritance pattern of ToLCNDV resistance. Therefore, it is crucial to understand how resistance to a particular virus is passed from generation to generation before designing a proper breeding procedure. For the purpose of producing ToLCNDV resistant varieties of muskmelon, knowledge of the nature and magnitude of the gene actions associated in resistance to ToLCNDV can be helpful. There are currently no reports on the genetics of ToLCNDV inheritance in muskmelon available in India. In order to identify the types of gene action for resistance to ToLCNDV in natural epiphytotic and challenge inoculation conditions, a genetic analysis study was conducted.

Materials and methods

Population development

Tomato leaf curl New Delhi virus resistant accession DSM 132 (*Cucumis melo* var. *callosus*) and DOM 115 (*Cucumis melo* var. *conomon*), commonly known as oriental melon is being grown in Southern India for curry making, that is highly susceptible to ToLCNDV were the two plant species used in the experiment. In our previous two years of natural epiphytotic and challenge inoculation screening between July and October of 2019 and 2020, this resistant accession DSM 132 showed a high resistance to ToLCNDV. To generate the F_1s, DOM 115 was crossed with the resistant accession DSM 132 during the Kharif season of 2020 (July-October). During the main growing season (march to June) of 2020, the resulting F_1 progeny were crossed with the parents to produce BC_1 (backcrossed with susceptible parent DOM 115, BC_2 (backcrossed with resistant parent DSM 132), and self-pollinated to produce F_2. These six generations were utilised to examine the genetics of ToLCNDV resistance under both natural epiphytotic and challenge inoculation conditions.

Screening under natural epiphytotic conditions

The ICAR-IARI, New Delhi is a hotspot for the tomato leaf curl New Delhi virus disease of cucurbits due to its special geographic and climatic characteristics (latitude, 20^0 40′ North; $77^0$13′ East longitude; altitude, 228 m above mean sea level; average temperature, 25-32 °C during *kharif*). The major pathogen ToLCNDV, which is spread by the insect vector whitefly, is more common in natural conditions during the Kharif season. As a result, once the insects visit the plants, the infection occurs in the genotypes that are susceptible. A randomised complete block design with three replications was used to set up the experiment. Seeds of all the six populations were sown at a spacing of 120 cm between the rows and 45 cm between the plants. Except for the use of pesticides, which would have otherwise decreased the whitefly population the ToLCNDV vector all of the recommended cultural measures for growing a healthy crop were followed.

In the field, 30 plants of each P_1, P_2 and F_1 (Pusa Sarda × DSM 132) whereas 200 plants of F_2 population and 60 plants each of BC_1 (F_1×Pusa Sarda) and BC_2 (F_1× DSM 132) populations were screened under conditions of high disease pressure and high epidemics of whitefly, the insect vector for disease transmission, during the *kharif* . A standardized 6-point interaction phenotypic scale was used to evaluate plants (Sohrab 2005), where 0 = No symptoms (immune, I); 1 = mild mosaic pattern in young leaves covering > 10% area(resistant, R); 2 = mosaic pattern in young leaves covering > 25% area(moderately resistant, MR); 3 = mosaic pattern in young leaves covering > 50% area, blistering and puckering of leaves(moderately susceptible, MS); 4 = widespread mosaic pattern in young leaves covering > 75% area, distortion of leaves (susceptible, S); 5 = widespread mosaic pattern in young leaves covering > 75% area, distortion of leaves and stunting of the plants. Total four scorings were taken at fifteen days interval after 30 day
s after sowing. Plants were categorized based on their disease reaction (Fig. 1).

Screening through challenge inoculation

A total of 140 healthy F_2 seeds of melon cross DOM 115× DSM 132 and F_1, parental genotypes (DOM 115 and DSM

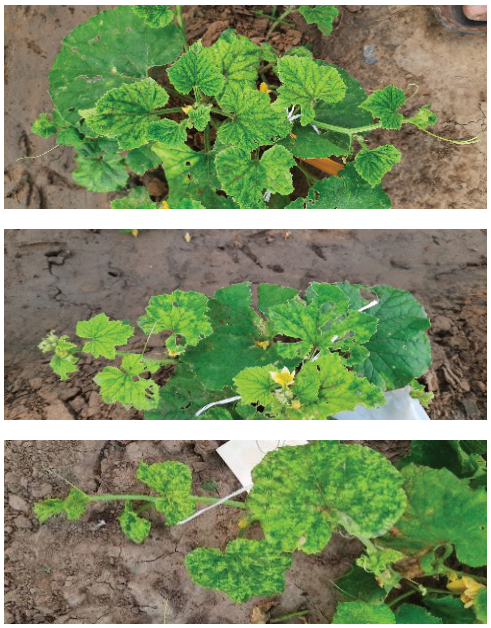

Fig. 1. Various degree of mosaic symptoms observed in susceptible parent DOM 115 under natural epiphytotic screening

132) 30 seeds each were sown in plastic pots of 7.50 × 6.0 cm size. Similarly, 40 BC_1P_1 plants and 40 BC_1P_2 plants were also grown in the same size plastic pots. In controlled environments, whitefly colonies were raised and kept on healthy tobacco plants in insect rearing cages. The optimal whitefly population was achieved by maintaining a 28–35°C temperature, 30–50% relative humidity, and a 14 hours photoperiod. These healthy whiteflies were made viruliferous for screening by allowing them to feed on ToLCNDV-infected muskmelon plants. Whiteflies were considered as virulent after 24 hours of feeding (acquisition access period) on the infected muskmelon plants. They were used for challenge inoculation of 15 days old healthy seedlings (at the two to four true leaf stages) of all the six generations for 24 hours. Each plant was individually inoculated for screening, and 10 viruliferous whiteflies were released for each plant. Plants were evaluated using a standardized 6-point interaction system phenotype scale Sohrab (2005), as explained in the above section (screening under natural epiphytotic conditions). Disease scoring on individual plant was performed from seven days post-inoculation (dpi) and continued at weekly intervals up to 2 months post-inoculation Fig. 2).

Statistical analysis

Chi-square analysis

Under natural epiphytotic and challenge inoculation screening methods individual plants in F_2 and backcross progeny were scored for ToLCNDV diseaseusing Sohrab scale (2005)and classified into resistant (score of 0, 1, 2) and susceptible (score of 3, 4 and 5) categories. In both methods of screening number of plants falling into resistant and susceptible categories were counted subjected to a chi-square test to find out the goodness of fit to various classical mendelian ratios with the assumed phenotypic ratios of F_2 and back cross progenies as suggested by Panse and Sukhatme (1985). The expected values corresponding to the observed values were calculated on the ratio (hypothetical) presumed. The deviations from these were calculated using the chi-square test formula, and subjected to estimate chi-square values for various classical Mendelian ratios as suggested by Panse and Sukhatme (1985).

$\chi^2 = \Sigma(\text{Observed number}-\text{Expected number})^2/\text{Expected number}$

Gene effects for ToLCNDV resistance

As suggested by Hayman (1958), the means and variances were calculated. The A, B, C, and D scaling tests proposed by Mather (1949), Hayman and Mather(1955), were used to determine the presence of epistasis. Just the significance of one or two scaling tests alone indicates that a simple additive-dominance model is inadequate. The Hayman (1958) model was used to calculate the effects of genes. Hayman's notations for the gene effects were [m] for the mean of the F_2 generation, [d] for additive gene effect, [h] for dominance gene effect, [i] for additive × additive gene effect, [j] for additive × dominance gene effect, and [l] for dominance × dominance gene effect. When generation effects were significant, only then was the type of epistasis determined (Kearsey and Pooni 1996). Window Stat version 9.3 was used to do generation mean analysis.

Fig. 2. Various degree of mosaic symptoms observed susceptible parent DOM 115 under whitefly mediated challenge inoculation.

Table 1. Means and standard errors of six generations of melon cross DOM 115× DSM 132 screened for resistance to tomato leaf curl New Delhi virus under natural epiphytotic and challenge inoculation conditions

Generation	Natural epiphytotic conditions (Disease score)	Challenge inoculation (Disease score)
P_1	4.87 ± 0.06	4.77 ± 0.08
P_2	0.03 ± 0.03	0.17 ± 0.07
F_1	1.47 ± 0.09	1.67 ± 0.09
F_2	2.45 ± 0.11	2.46 ± 0.13
BC_1P_1	3.35 ± 0.14	3.55 ± 0.16
BC_1P_2	1.37 ± 0.09	1.53 ± 0.09

Note: Here, P_1 and P_2 represents susceptible and resistant parents respectively and F_1 is first filial generation, F_2 is second filial generation, BC_1P_1 is backcross with the susceptible parent and BC_1P_2 backcross with the resistant parent.

Table 2. Estimates of chis square values and their probability for classical Mendelian ratio for ToLCNDV resistance in the cross of DOM 115× DSM 132 under natural epiphytotic conditions

Generation	Individual plant reaction							Pooled segregation		Mendelian ratio	χ2 value	p-value
	I	R	MR	MS	S	HS	Total	R	S			
P_1	0	0	0	0	4	26	30	0	30			
P_2	29	01	0	0	0	0	30	30	0			
F_1	0	16	14	0	0	0	30	30	0			
F_2	17	58	27	26	59	13	200	102	98	9:7	2.03	0.15
BC_1P_1	0	05	05	22	20	08	60	10	50	1:3	2.23	0.14
BC_1P_2	09	20	31	0	0	0	60	60	0			

Results and discussion

Mean performance of different generations against ToLCNDV infection

Means and standard errors for parents, F_1, F_2 and backcross generations are presented in Table 1. Under natural epiphytotic and challenge inoculation conditions, the F_1 mean in the cross DOM 115 × DSM 132 was significantly lower than the midpart value. The mean of the F_2 generation was higher than that of the F_1 and BC_2P_2 generations, but lower than BC_1P_1 generations in both the screening methods. In general, under natural epiphytotic and challenge inoculation screening methods, the mean of BC_2P_2 generations in the DOM 115 × DSM 132 cross was lower than the mean of F_1 generations. In both the Field screening and the Challenge inoculation, it was noted that certain F_2 and BC_1P_1, progeny transgressed with extreme mean ToLCNDV disease scoring values compared to either parent. This could be due to contrasting parental means. However, contrasting parents are prerequisites for generation mean analysis (Mather and Jinks 1971). Under both methods of screening, the BC_1P_1 population expressed a lower disease rating than the susceptible parent DOM 115. The parental lines and their progenies different degrees of resistance provided evidence that the disease reaction is a hereditary trait. Genetic dominance may be more significant in both of the screening methods, according to generation mean analysis. According to the mean disease score of F_1 in natural epiphytotic and whitefly mediated screening methods indicated that, cross DOM 115× DSM 132 may be accompanied by dominance effects (Table 1). In natural epiphytotic and whitefly-mediated challenge inoculation conditions, the substantial skewness of the BC_2P_2 means and low variance in BC_2P_2s relative to the BC_1P_1s are indicators of genetic dominance. et al. (2010) recorded a similar kind reaction of the F_1 plants (four resistant × susceptible crosses) towards downy mildew in melon.

Chi-square analysis for goodness of fit to classical Mendelian ratio

Resistant parent, DSM 132 were highly resistant throughout the screening period and did not show any disease symptoms except very mild symptom on single plant under natural epiphytotic screening, whereas very mild symptoms was observed on few plants under challenge inoculation. Under both the method of screening individual plants of the F_1, F_2, BC_1 and BC_2 populations showed differential reactions, starting from no symptoms to severe curling, mosaic, yellowing and stunting of the plants as characteristics of ToLCNDV disease.

A total of 200 F_2 plants generated from the cross of DOM 115×DSM 132 were screened for ToLCNDV disease response under natural epiphytotic method of screening; 102 plants were found to be resistant and 98 plants to be susceptible. The backcross with the susceptible parent was segregated into 10 resistant plants and 50 susceptible plants. Backcross plants with a resistant parent were found to be resistant. When F_2 population data were subjected to chi-square analysis, complementary gene interaction between two independent genes was found to be fulfilled and

Table 3. Estimates of chi-square values and their probability for classical Mendelian ratio for ToLCNDV resistance in the cross of DOM 115×DSM 132 under challenge inoculation

Generation	Individual plant reaction							Pooled segregation		Mendelian ratio	χ2 value	p-value
	I	R	MR	MS	S	HS	Total	R	S			
P_1	0	0	0	0	07	23	30	0	30			
P_2	25	05	0	0	0	0	30	30	0			
F_1	0	10	20	0	0	0	30	0	30			
F_2	16	34	22	19	36	13	140	72	68	9:7	1.42	0.23
BC_1P_1	0	03	03	06	25	03	40	06	34	1:3	2.13	0.14
BC_1P_2	0	20	19	01	0	0	40	39	01			

χ2 at 0.05 with 1 df=3.84.

Table 4. Estimates of gene effects based on scaling test for tomato leaf curl New Delhi virus under natural epiphytotic and whitefly mediated challenge inoculation conditions in the cross DOM 115 × DSM 132

Screening method	Scale			
	A	B	C	D
Natural epiphytotic screening	0.37 ± 0.30	1.23** ± 0.21	1.98** ± 0.48	0.19 ± 0.27
Whitefly mediated challenge inoculation Screening	0.67 ± 0.34	1.22** ± 0.21	1.56** ± 0.57	-0.16 ± 0.32

*, ** Significant at p < 0.05 and p < 0.01, respectively

Table 5. Estimates of gene effects, type of epistasis for resistance to tomato leaf curl New Delhi virus in DOM 115 × DSM 132 melon cross screened in natural epiphytotic and challenge inoculation conditions

Parameter	Natural epiphytotic	Challenge inoculation
m	2.45** ± 0.10	2.46** ± 0.13
d	1.98** ± 0.17	2.03** ± 0.18
h	-1.37* ± 0.55	-0.48 ± 0.66
i	-0.38 ± 0.54	0.32 ± 0.65
J	-0.43* ± 0.17	-0.28 ± 0.19
l	-1.21 ± 0.83	-2.21* ± 0.93
Epistasis	Complementary	Complementary

*, ** Significant at p < 0.05 and p < 0.01, respectively

revealed a 9:7 (resistant: susceptible) segregation pattern with a chi-square value of 2.03 and a percent probability of 15.00. Chi-square analysis of BC_1 population data revealed a 1:3 (resistant: susceptible) segregation pattern with a chi-square value of 2.23 and a percent probability of 14.00 per cent. However, all of the BC_2 plants were segregated in a 1:0 ratio (Table 2).

A total of 140 F_2 plants from the cross of DOM 115 × DSM 132 were assessed for ToLCNDV disease response under the challenge inoculation; 72 plants were found to be resistant and 68 plants to be susceptible.The backcross with the susceptible parent was segregated into 06 resistant plants and 34 susceptible plants. Backcrosses with resistant parents resulted in the presence of resistance in all plants. When F_2 population data were subjected to chi-square analysis, the segregation pattern was revealed to be 9:7 (resistant: susceptible), with a chi-square value of 1.42 and a % probability of 23.00. With a chi-square value of 2.13 and a probability of 14%, examination of BC_1 population data revealed a segregation pattern of 1:3 (resistant: susceptible). All of the BC_2 population's plants, however, were segregated in a 1:0 ratio (Table 3). The F_2 and BC_1P_1 populations derived from DOM 115× DSM 132 best fit with 9:7 ratio and 1:3 ratio, respectively, in both the screening methods. This suggests the involvement of two independent genes with complementary gene interactions for resistance to ToLCNDV in the genetic background of *Cucumis melo* var. *callosus* genotype, DSM 132 (Tables 2 and 3).

The performance of the parental lines and their progeny in the current experiment with regard to ToLCNDV resistance has provided a brief insight into the genetic nature of the resistant lines. Both screening methods' chi-square analyses revealed that the resistance in the DOM 115×DSM 132 cross is dominant since almost all of the F_1 generation was dominant and the F_2 generation was segregated in a 9(R):7(S) pattern, suggesting that the resistance may be controlled by two separate genes that interact in a complementary manner. However, it was also segregating in 1(R):3(S) in BC1P1, which may be explained by the presence of two independent dominant genes that determine resistance in DSM 132. Islam et al. (2010) observed that the chi-square value for ToLCNDV resistance in the cross of DSG-7× NSG-1-11 was found to fit to a 9(R):7(S) ratio. The sponge gourd's highly cross-pollinated nature, which maintains a heterozygous balance even after generations of selfing, is what causes this form of segregation into resistant and susceptible.

Estimation of gene effects for ToLCNDV resistance

The DOM 115× DSM 132 cross was subjected for scaling test to check for epistasis using natural epiphytotic and whitefly-mediated challenge inoculation screening methods. The results are shown in (Table 4). B and C were significant in

natural epiphytotic screening and challenge inoculation screening methods in the cross DOM 115×DSM 132. It was evident from the scaling test results for the DOM 115 × DSM 132 cross that the resistance to ToLCNDV disease did not follow a simple Mendelian inheritance pattern, suggesting that epistatic interactions such as additive × additive (i) additive×dominance (j) and dominance × dominance (l) may be present and play a significant role in the expression of the trait (Rai et al. 2020).

For the cross between DOM 115 and DSM 132, the estimations of various genetic components, including mean (m), additive (d), dominance (h), additive × additive (i), additive × dominance (j) and dominance × dominance (l), are provided in (Table 5). Results of non-allelic interaction for both the screening method were interpreted using the methodology of (Hayman and Mather 1955). Significant values of the gene effects (d), (h), and (j) under natural epiphytotic conditions indicated the presence of additive, dominant, and additive×dominance kinds of gene interactions, respectively. Significant values of the gene effects (d) and (l) in the whitefly-mediated challenge inoculation screening method showed the presence of an additive and dominant ×dominance types of gene interactions. In the cross, DOM 115 × DSM 132 the values of [h] and [l] attained the same signs which suggested a complementary type of epistasis in both methods of screening (Table 5). The aforementioned findings imply that non-additive components are predominant for both methods of screening and forthe breeding of tomato leaf curls New Delhi virus disease-resistant varieties. These components must be considered carefully.

The dominance (h) and the additive × dominance (j) component was significant in the negative direction and contributing to resistance in the natural epiphytotic screening, where as in case whitefly-mediated challenge inoculation conditions the dominance×dominance (h) component was significant in the negative direction and contributing to ToLCNDV resistance. Presence of complementary epistasis in both the screening methods indicated that the parents selected for crossing were diverse, allowing the breeding programme to attain a considerable level of heterosis and genetic gain (Rai et al. 2020).

In both the screening methods, the magnitude of the dominant effect was more than the additive effect, along with a higher magnitude of dominance × dominance but mostly with a negative sign compared to the other two interactions. This indicated that the most effective breeding method for improving this population for ToLCNDV resistance was heterosis breeding and recombination breeding, followed by a selection of transgressive segregants. The presence of a complementary type of epistasis for the ToLCNDV resistance trait in the cross of DOM 115× DSM 132 under natural epiphytotic and whitefly mediated challenge inoculation screening methods was promising, as this type of epistasis would produce new recombinants with late disease appearance and least disease infection, resulting in higher yield. As a result, the hybrid breeding strategy might be used to increase the ToLCNDV resistance in this cross. Senjam et al. (2018) reported the complementary gene action for percent disease incidence (PDI) of *yellow vein mosaic virus* disease in Okra. Inheritance of *yellow vein mosaic virus* resistance in an interspecific okra hybrid revealed mendelian segregation in accordance with the condition controlled by two complementary dominant genes. The resistant plant may be heterozygous for either or both of the dominant and resistant genes when the varieties succumb to a viral disease (Dhankar et al. 2005). A complementary type of epistasis for the two crosses in tomato was observed by Rai et al. (2020) as both the components viz., dominance [h] and dominance × dominance [l] were in similar direction, where they observed the preponderance of non-allelic interactions.

Exploiting host plant resistance through resistance breeding is a cost-effective, environmentally friendly, and long-term method to viral disease management. Virus resistance breeding is still confronting new hurdles as new viruses and strains develop, leading to the breakdown of resistance in previously released resistant varieties (Sanwal et al. 2016). This clearly shows that virus-resistant breeding is a continuing activity, and understanding the pattern of gene inheritance in different genotypes is critical for an effective breeding strategy. The majority of our virus resistance breeding programs attempt to use vertical resistance genes. However, there is always the possibility that resistance will be overcome by viral genome mutations. Our future resistance breeding programs should also try to use horizontal resistance governed by polygenes. Multiple resistance genes can be pyramided, either to the same or different diseases, and the genotype of the recurrent parent can be recovered through background selection. This would extend the life of the resistant cultivars. The majority of resistance genes are introduced into cultivated plants from wild relatives. Conventional breeding will likely pass some wild traits to the recipient parent and the target gene (s). Wild traits persist in the following generations due to their close genetic link. Conventional antiviral breeding is important for crop improvement, but it typically requires large crop growing populations over multiple generations, which is a time-consuming and tedious approach and molecular marker assisted breeding can accelerate the process of selection.

To study the genetics of ToLCNDV resistance under natural epiphytotic and challenge inoculation conditions, populations were generated by crossing a resistant parent DSM 132 with a susceptible parent DOM 115. However, in our study, we obtained high level of resistance in DSM 132 accession with higher magnitude of dominance ×

dominance model of inheritance involving at least two major complementary dominant genes. According to a review of the literature, there are various reports regarding the kind of resistance and the number of genes controlling the ToLCNDV viral infection, showing quantitative inheritance (QTLs). In such cases, we should map the ToLCNDV resistance QTL using modern genomic approaches such as genotypic by sequencing, association mapping, and QTL sequencing. Through genotyping by sequencing Seaz et al. (2017). identified the Three QTLs (one major on Chromosome 11 and two minor on chromosome 2 and 12) controlling the resistance to ToLCNDV in *Cucumis melo* using segregating populations derived from resistant sources (wild agrestis group) and a susceptible (belonging to the subsp. melo). In marker-assisted breeding for ToLCNDV resistance in melon, the SNP marker D16, which is closely linked to the resistance gene on chromosome 11, can be utilized. ToLCNDV resistant segments (five QTLs) in *Cucumis melo* accessions NCIMB 42,585 (QTL-5), NCIMB 42,506 (QTL-11), NCIMB 42,705 (QTL-1) and NCIMB 42,625 (QTL11 & QTL12) were recently patented. Resistance QTL-5 on chromosome 5 is flanked by markers KASP06 and KASP01 and has been successfully introgressed into commercially susceptible cultivars (Nunhems B.V. Patent: US20190225983A1). Large marker collections, high-throughput genotyping methods, and high-resolution mapping populations will enable breakthroughs in gene mapping and identification of the underlying genes for ToLCNDV viral resistance, providing markers useful for effective marker-based selection procedures and pyramiding of resistance genes for imparting a durable resistance to ToLCNDV.

Authors contribution

Conceptualization of research (HC, PK, BM,GPM,AUS); Designing of the experiments (PK,HC,RKY); Contribution of experimental materials (BM, HC); Execution of field/ lab experiments and data collection (PK, HC,BM,GPM); Analysis of data and interpretation (PK, DCM); Preparation of manuscript (PK, HC,RKY).

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