Vegetable Science (2021) 48 (2) : 136-141

A molecular clue towards fusarium wilt resistant gene in melon (C*ucumis melo* L.) germplasm

Sayeed AH Patel*, Ajmer Singh Dhatt, Sat Pal Sharma and Abhishek Sharma

Received: October 2021/ Accepted: January 2022

Abstract

Fusarium wilt [*Fusarium oxysporum* f. sp. *melonis* (*Fom*)], is one of the most serious diseases of muskmelon in India. Fifty accessions of muskmelon and its relatives collected from diverse sources were subjected to molecular screening with *Fom*1 and *Fom*2 gene specific markers. Presence of *Fom*1 (R) gene was confirmed using RG-9-2 marker where as susceptible allele was confirmed with another set of primer S-TAG/GCC-470. Out of 50 germplasm lines, 16 lines were found to be positive. Among which, eight were homozygous and eight were heterozygous in nature. The S-TAG/GCC-470 could only able to confirm susceptible allele in exotic lines but was failed to confirm the same in Indian accessions. While for *Fom*2 gene, a combination of R-408 (R allele) and S-342 (S allele) were used. Six genotypes contained homozygous resistant gene while only three lines showed heterozygous nature for *Fom*2 gene. Only two genotypes *viz.*, SM-2012-12 and IC-267359 contained both the resistant genes, whereas Kajri Sel.1, IC-267375, MM-314 and MM-4305 contained *Fom*1 gene. These results conclude the presence of novel resistant gene in Indian melon gene pool and there is an opportunity for developing new gene based markers for these novel genes which will be useful to strengthen the resistance against newly evolving pathogenic races.

Key words: Muskmelon, Fusarium wilt, *Fusarium oxysporum* f.sp. *melonis*, *Fom*1, *Fom*2

Introduction

Muskmelon, a member of genus *Cucumis* and family Cucurbitaceae is grown in temperate, sub-tropical and tropical regions of the world (Kesh and Kaushik 2021). Globally, it is cultivated in 1.05 million ha area with 27.3 million MT production and 26.1 t/ha productivity (FAO 2018). Indian muskmelon exhibits a wide range of morphological, physiological and biochemical diversity as being a broad primary centre (Dhillon et al. 2012). Already reported Indian resistance germplasm are PI 124111 (Thomas 1986, Kenigsbuch and Cohen 1989), PI 124112 (Kenigsbuch and Cohen 1992), MR-1 (Choudhary et al. 2020), PI 164723, etc.

Melon is prone to attack of many diseases that affect the yield and quality of fruits. In muskmelon, among major limiting factors, fusarium wilt caused by *Fusarium oxysporum* f. sp. *melonis* is the most devastating. It is controlled by two major genes (*Fom*1 and *Fom*2). While two *Fom*1 complementary genes *i.e. Fom*3 (Zink 1991) and *fom*4 (Oumouloud et al. 2012) was also reported. But the evolution of pathogen is much faster than the major resistant gene. Thus, genetic diversity of germplasm is important as dynamic resistant gene can withstand new strains of pathogen (Thrall and Burdon 2002). Still a wide variability is present in the melon accessions which are treasure for many resistant gene(s). These Indian germplasms must be dwelled for resistance against newly evolving pathotype which can further be used in breeding programs. Thus in the present study, we screened germplasm with Fom gene (*Fom*1 and *Fom*2) linked marker along with differentials.

Materials and Methods

The plant materials of 50 melon accessions (including 3 *Fom* differentials) were collected from diverse sources (Table 1). The morphological field screening was performed and mentioned by Patel et al. (2016) in detail and the final reaction score was used in this paper. In the seeds of local collections and *Fom* differentials were sown in plug tray of 5cm cell size containing cocopeat: perlite: vermiculite (2:1:1). Genomic DNA was isolated from first two true leaf using Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson 1980). The quality and quantity of extracted DNA was assayed in 0.8% agarose gel and visualized under UV trans-illuminator using Alpha Innotech Multi Imager gel

Department of Vegetable Science, Punjab Agricultural University, Ludhiana 141004

*Corresponding author; Email: sayeed-coavc@pau.edu

Table 1: List of melon germplasm accessions

S. No.	Designation	Var group	Source Country	S. No.	Designation	Var group	Source Country
1	MS-1	*melo*	India	26	Punjab Sunehri	*melo*	India
2	Hari Patti	*melo*	India	27	IC-267357	*melo*	India
3	SM-2012-12	*momordica*	India	28	IC-320114	*melo*	India
4	NDM-21	*melo*	India	29	IC-267359	*melo*	India
5	IC-267397	*melo*	India	30	KP-9	*momordica*	India
6	MM-601	*melo*	India	31	MM-4021	*melo*	India
7	MM-2013-1	*inodorus*	Afghanistan	32	MM-3864	*melo*	India
8	MM-2013-2	*inodorus*	Afghanistan	33	MM-3917	*melo*	India
9	NDM-18	*melo*	India	34	MM-4216	*melo*	India
10	IC-267375	*melo*	India	35	MM-4276	*melo*	India
11	IC-267379	*melo*	India	36	Kajri Sel. 1	*melo*	India
12	MM-3968	*melo*	India	37	KP_4HM-15	*melo*	India
13	SM-2013-1	*momordica*	India	38	MM-321	*melo*	India
14	Pusa Madhuras	*melo*	India	39	MM-201	*melo*	India
15	MM-202	*melo*	India	40	MM-314	*melo*	India
16	MM-136	*melo*	India	41	MM-312	*melo*	India
17	IC-274034	*momordica*	India	42	Canary Yellow	*melo*	Canada
18	Hara Madhu	*melo*	India	43	IC-255414	*melo*	India
19	IC-267364	*momordica*	India	44	WM-2014-1	*callosus*	India
20	IC-267378	*momordica*	India	45	EinDor	*reticulatus*	Israel
21	MM-4305	*melo*	India	46	MM-137	*melo*	India
22	MM-4279	*melo*	India	47	MM-315	*melo*	India
23	MM-28	*melo*	India	48	MS-5	*melo*	USA
24	Narika Col-1	*melo*	India	49	F-65	*reticulatus*	Israel
25	IC-320165	*momordica*	India	50	Hemed	*cantalupensis*	Israel

documentation system software programme from Alpha Innotech, California, USA. The Experiment was conducted at the Molecular Laboratory, Department of Vegetable Science, Punjab Agricultural University, Ludhiana, India.

In order to establish the utility in Indian germplasm, *Fom* linked marker (Table 2) was used for *In vitro* amplification. The presence of *Fom*-1 resistant gene was confirmed by CAPS marker RG9-2 (Restricted allele of PI 414723) and RG10-1 (Restricted allele of Védrantais) after using restriction enzyme *Dde*I while susceptible allele was confirmed by S-TAG/GCC-470 (STS) marker. Similarly, *Fom2* gene was confirmed with STS (STS411) and set of SCAR (*Fom*2-R_{408} and *Fom*2-S_{342}) markers (Joobeur et al. 2004). Polymerase Chain Reaction (PCR) was performed in an Eppendorf Master Cycler. PCR analysis was carried out using linked markers with component of 1.5µl $MgCl_2$ (25mM), 5µl PCR buffer (5X), 0.5µl dNTP mix (25mM), 2µl forward and reverse primer each (10mM), 0.3µl Taq polymerase (5U/µl), 2µl template DNA (100ng/µl) and 6.7µl doubled distilled water in 20µl reaction. The thermo cycler profile had initial denatured (94ºC) for 4 min followed by 35 cycles of denaturation (94ºC) for 40sec, annealing (50-55ºC) for 1 min, extension (72ºC) for 1 min and final extension (72ºC) for 10 min. The PCR products were separated on 1.5% agarose gel and visualized under UV trans-illuminator using Alpha Innotech Multi Imager gel documentation system software programme from Alpha Innotech, California, USA. The amplification of markers and morphological field screening was scored as + and R for homozygous resistant, - and S for homozygous susceptible, +/- and He for heterozygous and NA for no amplification.

Table 2: Molecular markers linked to *Fom*1 and *Fom*2 allele in melon

Marker No.	Marker Name	Type	Sequence (Forward)	Sequence (Reverse)
Markers linked to *Fom*1 allele				
M_1	RG9-2	CAPS	TCTGTTGGAAGCGTTTGATG	TTGGCTCCAAATCATTTAGCTT
M_2	RG10-1	CAPS	CCTGTACTCTTGAAATCGAACAA	TTGTGGAAGACTAAAAGAGGTTCA
M_3	S-TAG/GCC-470	STS	GAATTCTAGACTGAGCTTATAAACC	TTAAGCCTAAAAGGAATGGCCCCC
Markers linked to *Fom*2 allele				
M_4	STS411	STS	TTTCTAAAATTTACCATCATTGGAG	AATGGCAAATTCAACCTTCAC
M_5	Fom2-R_{408}	SCAR	GAGAAATTTGCAATGGGTGG	TTACACTATTATTGCTCAACTTGC
M_6	Fom2-S_{342}	SCAR	ATGAAAAGAAAAGATAACGACGA	ATTGCTCTAAGTTGATCATATTCTG

Results and Discussion

Germplasm resistant to pathogenic race(s) is an economic and sustainable way to maintain yield plateau (Mundt 2014, Bailey-Serres et al. 2019). DNA sequence based technology is being widely followed to avoid hindrance while selecting genotypes based on phenotypic characters (Nadeem et al. 2018) and protecting important germplasm (Ismail et al. 2020). In muskmelon, genes *i.e*. *Fom*1 and *Fom*2 provide resistance against *Fusarium oxysporum* f. sp. *melonis* race 2 and 1, respectively. The DNA amplification depicted presence of *Fom-1* gene in 23 accessions (Table 3 and Plate 1) out of which fifteen (IC-267397, MM-3013-2, Pusa Madhuras, Hara Madhu, MM-4305, MM-28, IC-320165, IC-320114, IC-267359, Kajri Sel 1, MM-314, MM-312, MM-315, MM-137 and Hemed) accessions were homozygous and eight (SM-2012-12, NDM-21, IC-267375, MM-3968, MM-202, MM-4021, MM-4276 and WM-2014-1) heterozygous allele. Both the CAPS marker performed similarly in the all the melon germplasm except MM-4021 which was found highly susceptible under field condition while at genomic level, RG9-2 predicted heterozygous and RG10-1 produced homozygous resistant fragment. These results were in accordance with Brotman et al. (2005) as RG10-1 marker could not able to distinguish properly as like CAPS marker NBS1 and 62 thus put MM-4021 germplasm in complex state which need further detailed study with diverse marker type. Under this situation, RG9-2 (lie in exon 2 of NB-LRR region) can be found reliable (Brotman et al. 2012; Oumouloud et al. 2015).

Additionally, susceptible allele of *Fom*-1 gene was confirmed by using another set of primer S-TAG/GCC-470 (SCAR marker) as reported by Tezuka et al. (2009). But S-TAG/GCC-470 did not discriminate any accessions within var *melo*, *momordica* and *inodorus*. Demarcation in var *callosus* and *cantalupensis* accession was not envisaged firmly as only one accession each was included in the study. While Tezuka et al. (2009) reported that S-TAG/GCC-470 predicted three of five *cantalupensis* lines and no genotypes in var. *chinensis, conomon* and *makuwa*. It amplified a single fragment of 347 bp in susceptible exotic accessions. Bakker et al. (2006) observed that R genes in *Arabidopsis* have greater nucleotide variability due to mutation and frequent recombination in the genome. In two diverse haplotypes of perennial rye-grass (*Lolium perenne* L.) there was one SNP in every 10 bp LRR region (Xing et al. 2007). This variability could be due to various genetic mechanisms like frequent recombination, point mutation and unequal crossing-over (gene duplication, deletion) responsible for genetic variation within R genes (Oumouloud et al. 2013).

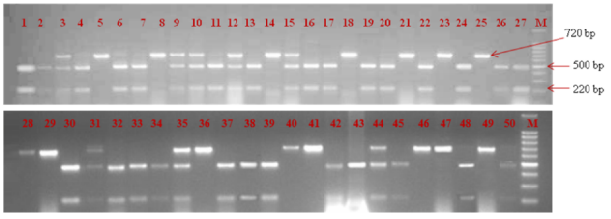

Plate 1: RG9-2 (CAPS marker) linked to *Fom-1* (R) locus in melon germplasm

For *Fom*-2 (R) gene, STS411 (STS marker) and a combination of SCAR markers *viz.*, Fom2-R_{408} (R allele) and Fom2-S_{342} (S-allele) were used as reported by Joobeur et al. (2004) and Oumouloud et al. (2012), respectively. STS411 was found closest to *Fom2* gene at an interval of 75 kb. Joobeur et al. (2004) observed two recombination event present in between STS411 and SSR430 (map-based cloning) at a distance of 5.5 kb. The success of target gene position marker mostly depends on recombination frequency in the gene of targeted trait (Durrett et al. 2002). In our germplasm, STS411 identified seven homozygous resistant and three heterozygous loci. Similarly, Oumouloud et al. (2012) reported that Fom2-R_{408} and Fom2-S_{342}, an allele-specific DNA marker could be used as co-dominant markers in multiplex PCR, but did not worked in our laboratory, therefore, individual PCR was run for each allele. Among 50 genotypes, six lines *i.e*. SM-2012-12 (*momordica*), MM-3013-1 (*inodorus*), IC-267359 (*melo*), KP-9 (*momordica*), MM-3917 (*melo*) and F-65 (*reticulatus*) were found with homozygous dominant allele while only three lines *i.e*. IC-267364, IC-267378 and WM-2014-1 showed heterozygous fragment for *Fom*2 gene (Table 3 and Plate 2 (R allele); Plate 3 (S allele).

The line IC-267378 was found homozygous resistant with STS411 whereas heterozygous with Fom2-R_{408} and Fom2-S_{342} (SCAR) marker vice-versa in IC-320114 line *i.e* heterozygous with STS411 and homozygous

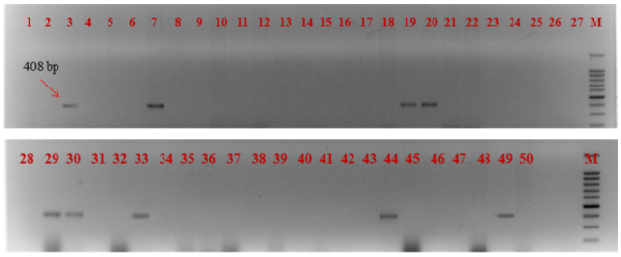

Plate 2: Fom2-R_{408} (SCAR marker) linked to *Fom-2* (R allele) locus in melon germplasm

Note: M= 100bp ladder

susceptible with Fom2-R_{408} and Fom2-S_{342}. This difference could be due to high recombination frequency (Tezuka et al. 2009) at close distance. Thus SCAR markers were found more reliable to identify *Fom-2* gene especially in Indian germplasm. Under wilt sick field condition, 9 lines were found highly resistant and 3 were moderately resistant whereas rests were in range of moderately to highly susceptible (Table 3).

Table 3: Validation of *Fom*1 and *Fom*2 allele specific molecular marker for 50 melon accessions

S. No.	Genotypes	Field score	*Fom*1 allele			*Fom*2 allele		
			M_1	M_2	M_3	M_4	M_5	M_6
1	MS-1	HS	-	-	-	-	-	+
2	Haripatti	MS	-	-	-	-	-	+
3	SM-2012-12	HR	+/-	+/-	-	+	+	-
4	NDM-21	HR	-	-	-	-	-	+
5	IC-267397	HS	+	+	-	-	-	+
6	MM-601	MS	-	-	-	-	-	+
7	MM-3013-1	HS	-	-	+	+	+	-
8	MM-3013-2	HS	+	+	-	-	-	+
9	NDM-18	HS	+/-	+/-	-	-	-	+
10	IC-267375	HR	+/-	+/-	-	-	-	+
11	IC-267379	HS	-	-	-	-	-	+
12	MM-3968	MS	+/-	+/-	-	-	-	+
13	SM-2013-1	MR	-	-	-	-	-	+
14	Pusa Madhuras	HS	+	+	-	-	-	+
15	MM-202	HR	+/-	+/-	-	-	-	+
16	MM-136	MS	-	-	-	-	-	+
17	IC-274034	MS	-	-	-	-	-	+
18	Hara Madhu	MS	+	+	-	-	-	+
19	IC-267364	MS	-	-	-	+/-	+	+
20	IC-267378	HS	-	-	-	+	+	+
21	MM-4305	MR	+	+	-	-	-	+
22	MM-4279	HS	-	-	-	-	-	+
23	MM-28	MS	+	+	-	-	-	+
24	Narika Col 1	HS	-	-	-	-	-	+
25	IC-320165	MS	+	+	-	-	-	+
26	Punjab Sunehri	HS	-	-	-	-	-	+
27	IC-267357	HS	-	-	-	-	-	+
28	IC-320114	HS	+	+	-	+/-	-	+
29	IC-267359	HS	+	+	-	+	+	-
30	KP-9	HS	-	-	-	+	+	-
31	MM-4021	HS	+/-	+	-	-	-	+
32	MM-3864	MS	-	-	-	-	-	+
33	MM-3917	MS	-	-	-	+	+	-
34	MM-4216	MR	-	-	-	-	-	+
35	MM-4276	MS	+/-	+/-	-	-	-	+
36	Kajri Sel. 1	HR	+	+	-	-	-	+
37	KP_4HM-15	HR	-	-	-	-	-	+
38	MM-321	HR	-	-	-	-	-	+
39	MM-201	MS	-	-	-	-	-	+
40	MM-314	HR	+	+	-	-	-	+
41	MM-312	HS	+	+	-	-	-	+
42	Canary Yellow	HS	-	-	+	-	NA	NA
43	IC-255414	HS	-	-	-	-	-	+
44	WM-2014-1	HR	+/-	+/-	-	+/-	+	+
45	Ein Dor	MS	-	-	+	-	-	+
46	MM-137	HS	+	+	-	-	-	+
47	MM-315	HS	+	+	-	-	-	+
48	MS-5	HS	-	-	-	-	-	+
49	F-65	HS	-	-	+	+	+	-
50	Hemed	HS	+	+	-	-	-	+

Note: + & R: Homozygous resistant, - & S: Homozygous susceptible, +/- & He: Heterozygous, NA: No amplification

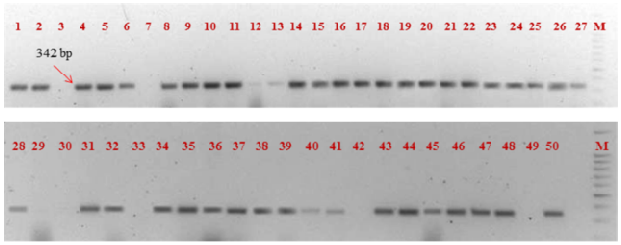

Plate 3: Fom2-S_{342} (CAPS marker) linked to *Fom-2* (S allele) locus in melon germplasm

Conclusion

From the above study, it was concluded that the differential cultivars i.e. Hemed (Fom1 gene), F-65 (Fom2 gene) and EinDor were found to be susceptible under sick plot which indicates the presence of another race other than Race 0, 1 and 2. Whilst there is a clue of novel resistance gene present in those lines which showed resistance against under wilt sick plot.

Acknowledgement

The authors are thankful to University Grants Commission (UGC), New Delhi for providing fellowship (Grant No. F1-17.1/2013-14/MANF-2013-14-MUS-MAH-23366) and facilities created under DST-PURSE and FIST programme for conduct of research study.

सारांश

फ्यूजेरियम विल्ट (*फ्यूजेरियम ऑक्सीस्पोरम* एफ. एस.पी. *मेलोनिस* (फोम)), भारत में खरबूजे की सबसे गंभीर बीमारियों में से एक है। विभिन्न स्रोतों से एकत्र किये गये खरबूजा और उसके सम्बन्धित प्रजातियों के 50 प्रविष्टियों को फोम1 और फोम$_2$ जीन विशिष्ट मार्कर्रों के साथ आण्विक जाँच किया गया। आर.जी.–9–2 मार्कर का उपयोग करके फोम$_1$ की उपस्थिति से प्रतिरोधी जीन की उपस्थिति की पुष्टि की गयी। जहाँ अति संवेदनशील एलील की पुष्टि एस.–टी.ए.जी–जी.सी.सी.–470 के दूसरे समूच्य के साथ की गयी जननद्रव्य की 50 लाइनों में से 16 लाइनें सकारात्मक पाई गयी जिनमें से 8 समयुग्मजी और 8 विषमयुग्मजी प्रकृति वाली थी। एस.–टी.ए.जी–जी.सी.सी.–470 केवल आयातित लाइनों में अतिसंवेदनशील एलील की पुष्टि करने में सक्षम था, लेकिन भारतीय प्रविष्टियों में इसकी पुष्टि करने में विफल रहा, जबकि फोम$_2$ जीन के लिए आर.–408 (प्रतिरोधी एलील) और एस.–342 (संवेदनशील एलील) के संयोजन का उपयोग किया गया। कुल 6 प्रभेदों में समयुग्मजी प्रतिरोधी जीन उपस्थित थी जबकि केवल 3 प्रभेदों में फोम$_2$ जीन के लिए विषमयुग्मजी प्रकृति पायी गयी। केवल 2 प्रभेदों–एस.एम.–2012–12 एवं आईसी267359 में दोनों प्रतिरोधी जीन शामिल थे जबकि कजरी सिलेक्शन–1, आईसी267375, एम.एम.–314 और एम.एम.–4305 में फोम$_1$ जीन था। यह परिणाम भारतीय जीन समूह में प्रतिरोधी जीन की उपस्थिति को समाप्त करते हैं और उन जीनों के लिए नये जीन आधारित मार्कर विकसित करने का अवसर प्रदान करते हैं जो नयी विकसित रोगजनक जाति के विपरित प्रतिरोध को मजबूत करने के लिए उपयोगी होंगे।

References

Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GED and Schroeder JI (2019) Genetic strategies for improving crop yields. Nature 575: 109-118.

Bakker EG, Toomajian C, Kreitman M and Bergelson J (2006) A genome-wide survey of R gene polymorphisms in Arabidopsis. Plant Cell 18: 1803-1818.

Brotman Y, Kovalski I, Dogimont C, Pitrat M, Portnoy V, Katzir N and Perl-Treves R (2005) Molecular markers linked to papaya ring spot virus resistance and Fusarium race 2 resistance in melon. Theor Appl Genet 110: 337-345.

Brotman Y, Normantovich M, Goldenberg Z, Zvirin Z, Kovalski I, Stovbun N, Doniger T, Bolger AM, Troadec C, Bendahmane A and Cohen R (2012) Dual resistance of melon to Fusarium oxysporum races 0 and 2 and to Papaya ring-spot virus is controlled by a pair of head-to-head-oriented NB-LRR genes of unusual architecture. Mol Plant 6: 235-238.

Choudhary H, Yadav RK and Maurya SK (2020) Principles and Techniques for Rapid Improvement of Muskmelon for Yield, Fruit Quality and Resistance to Biotic Stresses. In: Accelerated Plant Breeding, Volume 2. (Eds. Gosal S and Wani, S.), Springer, Cham. https://doi.org/10.1007/978-3-030-47298-6_14.

Dhillon NPS, Monforte AJ, Pitrat M, Sudhakar P, Singh PK, Reitsma KR, Garcia-Mas J, Sharma A and McCreight JD (2012) Melon landraces of India: contributions and importance. Plant Breed Rev 35: 85-150.

Durrett RT, Chen K and Tanksley SD (2002) A simple formula useful for positional cloning. Genet 160: 353-355.

FAO (2018) Vegetables and Melons Outlook. http://faostat.fao.org Accessed: 5 March 2021.

Ismail A, Mosa KA, Ali MA and Helmy M (2020) Biochemical and Molecular Markers: Unraveling Their Potential Role in Screening Germplasm for Thermotolerance. In: *Heat Stress Tolerance in Plants: Physiological, Molecular and Genetic Perspectives.* (eds. Wani SH and Kumar V). pp. 47-76.

Joobeur T, King JJ, Nolin SJ, Thomas CE and Dean RA (2004) The fusarium wilt resistance locus Fom-2 of melon contains a single resistance gene with complex features. Plant J 39: 283-297.

Kenigsbuch D and Cohen Y (1989) Inheritance of resistance to downy mildew in a gynocious muskmelon. Plant Dis 73: 994-996.

Kenigsbuch D and Cohen Y (1992) Inheritance of resistance to downy mildew in Cucumis melo PI 124112 and commonality of resistance genes with PI 124111F. Plant Dis 76: 615-617.

Kesh H and Kaushik P (2021) Advances in Melon Breeding (*Cucumis melo* L.): An Update. *Scientia Hort* 282: 110045.

Mundt CC (2014) Durable resistance: a key to sustainable management of pathogens and pests. Infect Genet Evol 27: 446-455.

Murray MG and Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321-4326.

Nadeem MA, Nawaz MA, Shahid MQ, Doðan Y, Comertpay G, Yýldýz M, Hatipoðlu R, Ahmad F, Alsaleh A, Labhane N and Özkan H (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equi 32: 261-285.

Oumouloud A, El-Otmani M and Álvarez JM (2015) Molecular characterization of Fom-1 gene and development of functional markers for molecular breeding of resistance to *Fusarium* race 2 in melon. Euphytica 205: 491-501.

Oumouloud A, El-Otmani M, Chikh-Rouhou H, Garce´s-Claver A, Gonza´lez-Torres R, Perl-Treves R and Álvarez JM (2013) Breeding melon for resistance to Fusarium wilt: recent developments. Euphytica 192: 155-169.

Oumouloud A, Mokhtari M, Chikh-Rouhou H, Arnedo-Andrés MS, González-Torres R and Álvarez JM (2012) Characterization of the Fusarium wilt resistance Fom-2 gene in melon. Mol Breed 30: 325-334.

Patel SAH, Vashisht VK and Kaur J (2016) Identification of sources of resistance against fusarium wilt in Indian melon (*Cucumis melo* L.). Ecol Environ Conserv 22: 99-104.

Tezuka T, Waki K, Yashiro K, Kuzuya M, Ishikawa T, Takatsu Y and Miyagi M (2009) Construction of a linkage map and identification of DNA markers linked to *Fom*-1, a gene conferring resistance to *Fusarium oxysporum* f. sp. *melonis* race 2 in melon. Euphytica 168: 177-188.

Thomas CE (1986) Downey and powdery mildew resistance musk melon breeding line MR-1. Hort Sci 21: 32.

Thrall PH and Burdon JJ (2002) Evolution of gene for gene systems in metapopulations: the effect of spatial scale of host and pathogen dispersal. Plant Pathol 51: 169-184.

Xing Y, Frei U, Schejbel B, Asp T and Lübberstedt T (2007) Nucleotide diversity and linkage disequilibrium in 11 expressed resistance candidate genes in *Lolium perenne*. BMC Plant Biol 7: 43.

Zink FW (1991) Origin of Fusarium wilt resistance in Texas AES muskmelon cultivars. Plant Dis 75: 24-26.