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Molecular screening of tomato (*Solanum lycopersicum* L.) genotypes for resistance alleles against important biotic stresses

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Abstract: Molecular markers are vastly used as tool for screening of disease resistant/tolerant plant genotypes in early stage of growth in an environment-independent manner. In tomato (*Solanum lycopersicum* L.), the major biotic stresses like tomato yellow leaf curl virus, *Fusarium* wilt, tomato spotted wilt virus and root knot nematode cause severe crop loss. Recently, DNA based molecular markers for the screening of resistance alleles for the above-mentioned diseases have been reported in tomato. In the present study, a total of eighteen tomato genotypes have been screened for the presence of possible resistance alleles, using sequence characterized amplified region (SCAR) molecular markers. Resistance allele-specific bands for *Fusarium* wilt disease, tomato spotted wilt disease and partial resistance allele-specifc band for root knot disease have been identified in some of the genotypes used in the present study. However, none of the genotypes was found to contain *Ty3* resistance allele-specific band for resistance to tomato yellow leaf curl disease. Thus, possible resistance sources have been identified for three out of the four biotic stresses, mentioned earlier. Thus, the present study has screened the 18 tomato genotypes at molecular level for presence of resistance alleles for biotic stress, which might be further evaluated and explored in future tomato breeding programmes, targeting biotic stress resistance in tomato. At the same time, the study documents the applicability of molecular markers for rapid disease screening in tomato in an environment independent manner.

Keywords: *Fusarium* wilt, Root knot disease, Sequence characterized amplified region (SCAR), Tomato

INTRODUCTION

Breeding for biotic stress resistance has remained as one of the most important area of research for the crop breeders. However, screening the genotypes for tolerance/resistance to a particular disease is often problematic. Proper development of disease generally depends on three major factors: a. a susceptible host, b. a virulent pathogen and c. presence of congenial environmental conditions for disease development. As presence of congenial environment for the pathogen is necessary for the occurrence and severity of a particular disease, screening the genotypes for biotic stress resistance becomes extremely difficult in absence of the aforementioned environment. As it is well known that different plant pathogens require different environmental conditions to reach optimum severity in disease development (Peries 1971), screening the genotypes for multiple disease resistance becomes more difficult. For this reason, use of molecular markers to identify possible disease resistance alleles has become crucial. Being free from the environmental effects, molecular markers provide a unique opportunity to accurately screen a large number of genotypes in a reproducible and rapid manner. Till date, a huge number of molecular markers have been developed in different vegetable crops (available at www. solgenomics.net) and particularly in tomato (Foolad 2007).

Production of Tomato (*Solanum lycopersicum* L.), one of the most important vegetable crops of the world, is often jeopardized due to the heavy infestation of different diseases. Among them, the tomato yellow leaf curl virus (TYLCV) is a devastating one. This viral disease is transmitted through several species of white fly (Fauquet *et al.*, 2008). Another example is the wilt disease caused by the tomato spotted wilt virus (TSWV). This disease is transmitted through thrips and causes severe stunting and necrosis of stem and leaves (German *et al.*, 1992). Apart from these viral diseases, root knot disease caused by nematodes and wilt disease caused by *Fusarium oxysporum* f. sp. *Lycopersici* are serious threat to tomato cultivation. Through exploring the large collection of tomato germplasm and wild relatives, several resistance gene sources for different diseases have been identified (Van Ooijen *et al.*, 2007), and suitable molecular markers for screening

the presence of resistance alleles of the aforementioned diseases have been reported (El Mehrach *et al.*, 2005; Arens *et al.*, 2010; Shi *et al.*, 2011). Hence, exploring these molecular markers for screening the presence of disease resistance alleles becomes an important aspect in tomato breeding programme.

In the present study, we have screened 18 tomato genotypes (including 2 hybrids, 8 lines and 8 exotic collections) through co-dominant/dominant sequence characterized amplified region (SCAR) markers for identifying the resistance alleles corresponding to TYLCV, tomato spotted wilt virus (TSWV), root knot disease and fungal wilt caused by *Fusarium oxysporum* f. sp. *Lycopersici* race 0 and race I. Through analysis, these 18 tomato genotypes have been characterized in documenting the presence of resistance alleles for TSWV, root knot disease, and *Fusarium* wilt disease. Thus, the present study will help to select tomato genotypes for target oriented breeding programme and serve as the platform for adopting marker assisted selection (MAS) in segregating tomato population.

MATERIALS AND METHODS

Plant materials: Total eighteen genotypes of tomato, including two hybrids, eight lines and eight exotic collections were used in the present study. Name and source of these genotypes are mentioned in Table 1.

Isolation of genomic DNA and polymerase chain reaction: Genomic DNA from the young leaves of tomato plants was isolated using the standard CTAB (cetyl-triethyl-ammonium-bromide)-method (Doyle and Doyle 1990). Following isolation, the quality and quantity of the DNA was checked through 0.8% (w/v) agarose gel electrophoresis using TAE (Tris-acetate-EDTA) buffer system. Concentration of the genomic DNA, isolated from all the genotypes was brought to uniformity (50 ng/µl) by dilution. For, polymerase chain reaction (PCR), 100 ng of genomic DNA was taken as template. PCR was performed in 12 µl reaction volume, containing genomic DNA (100 ng), 1.2 µl of 10X PCR buffer (Bangalore Genei), 2.5 mM $MgCl_2$ (Bangalore Genei), 0.1 mM of dNTP mix (Bangalore Genei), 0.4 µM each of corresponding forward and reverse primers and 0.4 units of *Taq* DNA polymerase (Bangalore Genei). PCR amplification was performed using the thermal profile consisting of an initial denaturation at 94 ˚C for 4 min followed by 35 cycles of 40 s at 94 ˚C, 30 s at annealing temperature, 60 s at 72 ˚C, and ended with final extension at 72 ˚C for 10 min followed by hold at 4 ˚C. Details of the specific primers along with the specific annealing temperatures used in this study are documented in Table 2. In each PCR, an identical tube lacking any genomic DNA was used as –ve control (water control) in order to validate the genomic origin of the amplicon(s). Amplified PCR products were subjected to electrophoresis in 1.5% (w/v) agarose gel using TAE buffer system and imaged through gel documentation system. The 100 bp ladder (Merck Biosciences) was used as standard DNA molecular weight marker.

RESULTS AND DISCUSSION

Screening for the *Ty3* loci: In previous studies (Agrama and Scott 2006; Ji *et al.*, 2007), four loci leading to resistance to begomovirus have been documented on chromosome 6 in tomato. Among these loci, three loci including the *Ty-3* have been reported to be introgressed from *Solanum chilense* LA2799 and LA1932 (Ji *et al.*, 2007). Keeping the difference of the introgressed region in mind, two different *Ty-3* alleles have been annotated (*Ty-3* for the LA2799 derived allele and *Ty-3a* for the LA1932 derived allele). At the same time, a co-dominant sequence characterized amplified region (SCAR) marker has been reported (Ji *et al.*, 2007), which can distinguish between the *Solanum lycopersicum ty-3* allele and the *S. chilense Ty-3* allele. Recently, a new type of introgression (*Ty-3b*) has been documented and a co-dominant SCAR marker (P625-F2/ P625-R5) has been developed that can differentiate between *ty-3*, *Ty-3a* and *Ty-3b* alleles (http://www.plantpath.wisc. edu/GeminivirusResistant

Table 1. Names and sources of the tomato genotypes used in the present study.

Genotype	Source/ Collected from	Genotype	Source/ Collected from
Badshah	Nunhems[1]	Pusa Ruby	NBPGR[5]
Shahanshah	Nunhems	EC 520075	NBPGR
CLN B	BCKV[2]	EC 520046	NBPGR
Sun Cherry	IIVR[3]	EC 528372	NBPGR
Azad T-5	IIVR	EC 671592	NBPGR
B-9-2	IIVR	EC 529080	NBPGR
CLN-1621 L	IIVR	EC 625644	NBPGR
IIHR 2620	IIHR[4]	EC 620421	NBPGR
ArkaVikas	IIHR	EC 521080	NBPGR

[1]Nunhems = Nunhems India Pvt. Ltd., Bengaluru;[2]BCKV = Bidhan Chandra KrishiViswavidyala, Nadia, West Bengal, India;[3]IIVR = Indian Institute of Vegetable Research, Varanasi, India;[4]IIHR = Indian Institute of Horticulture Research, Bengaluru, India; [5]NBPGR = National Bureau of Plant Genetic Resources, New Delhi, India

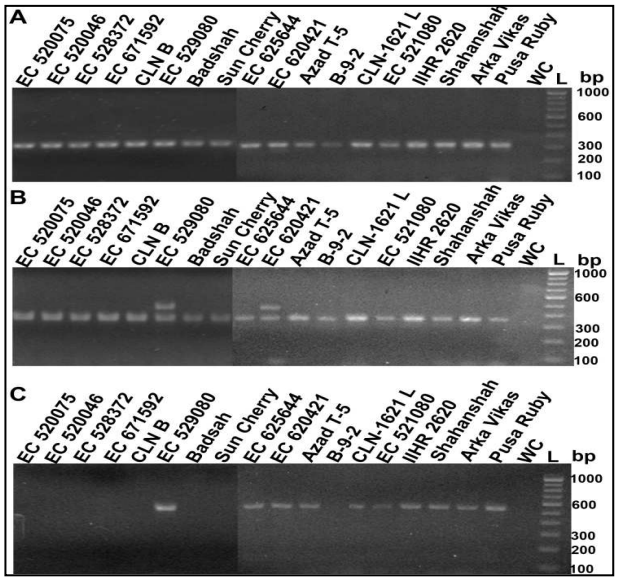

Fig. 1. *Sequence characterized amplified region (SCAR) marker based screening of tomato genotypes. A. Screening for the presence of Ty3 allele conferring resistance to tomato yellow leaf curl disease. B. Screening for the presence of Mi1-2 allele conferring resistance to tomato root knot disease. C. Screening for the presence of Sw-5b allele conferring resistance to tomato spotted wilt disease. WC = water control. L = 100 bp DNA ladder.*

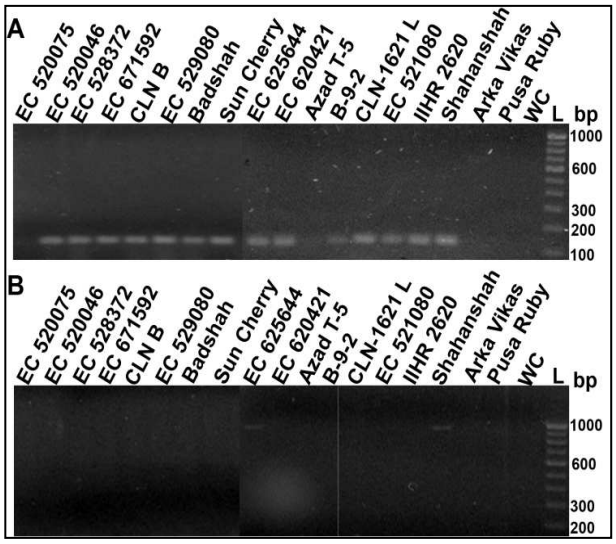

Fig. 2. *Sequence characterized amplified region (SCAR) marker based screening of tomato genotypes for Fusarium wilt disease resistance alleles. A. Screening for the presence of the I allele conferring resistance to tomato wilt disease caused by Fusarium oxysporum f. sp. lycopersici race 0. B. Screening for the presence of the I2 allele conferring resistance to tomato wilt disease caused by Fusarium oxysporum f. sp. Lycopersici race I. WC = water control. L = 100 bp DNA ladder.*

Tomatoes/Markers/MAS-Protocols/P6-25-locus.pdf). Very recently, this SCAR marker has been used for marker assisted introgression of *Ty3* gene in tomato (Prasanna *et al.*, 2014). During the present study, the same primer pair (annotated as TY3SF and TY3SR in this study) was explored to identify the allelic variants at *Ty-3* locus in eighteen tomato genotypes. Following PCR amplification, the monomorphic band of ~320 bp was amplified in case of all the genotypes (Fig. 1A), indicating the absence of both *Ty-3a* and *Ty-3b* resistance alleles in these genotypes.

Screening for the *Mi1-2* allele: The *Mi* locus, introgressed from *Solanum peruvianum* has been documented to be located on tomato chromosome 6 containing open reading frames (ORFs) of two genes (*Mi1-1* and *Mi1-2*) along with a pseudogene (Milligan *et*

Table 2. Details of the primers used in the present study.

Locus	Primer sequence (5´-3´)	Annealing temperature (°C)#	Reference
Ty3	TY3SF: GGTAGTGGAAATGATGCTGCTC TY3SR: GCTCTGCCTATTGTCCCATATATAACC	53	http://www.plantpath.wisc.edu/GeminivirusResistant-Tomatoes/Markers/MAS-Protocols/P6-25-locus.pdf
Mi1-2	RKNSF: GGTATGAGCATGCTTAATCAGAGCTCTC RKNSR: CCTACAAGAAATTATTGTGCGTGTGAATG	55	El Mehrach*et al*. 2005
Sw-5b	TSW5F: CGGAACCTGTAACTTGACTG TSW5R: GAGCTCTCATCCATTTTCCG	55	Shi *et al*. 2011
I	TF0WSF:CGAATCTGTATATTACATCCGTCG T TF0WSR:GGTGAATACCGATCATAGTCGAG	55	Arens *et al*. 2010
I2	TF1WSF:ATTTGAAAGCGTGGTATTGC TF1WSR:CTTAAACTCACCATTAAATC	48	Arens *et al*. 2010

# indicates annealing temperatures used in the present study

al., 1998). Among these two genes, *Mi1-2* only has been reported to be the resistance source against the root knot nematodes *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. For *Mi1-2*, different PCR-based markers have been developed previously (Goggin *et al.,* 2004; Devran and Elekçioglu 2004; Bendezu 2004; El Mehrach *et al.*, 2005). In a recent study (El Mehrach *et al.,* 2005), the primer pair PMiF3 -R3 has been reported to be the most suitable one for routine applications in case of tomato genotypes (Arens *et al.,* 2010). In the present study, the same primer pair (annotated as RKNSF and RKNSR) was used to find resistance sources among the selected genotypes. The *mi1-2* susceptibility allele-specific ~350 bp band was recorded in all these genotypes with two genotypes (EC 529080 and EC 620421) having an additional band of ~510 bp (Fig. 1B). As the expected *Mi1-2* allele-specific single band should be of ~550 bp in size, this ~510 bp band was predicted to be the *Mi-J* allele, which is a variant of the *Mi1* allele. This result was found to be in accordance with the previous study (Arens *et al.,* 2010), where the same *Mi-J* allele has been reported to be amplified in a few tomato genotypes through the same primers. Though the *Mi-J* allele has been documented to confer partial resistance to *M. incognita*, it has been reported to be linked to the *Ty-1* locus responsible for resistance to TYLCV Arens *et al.,* 2010). Thus, the amplification of the *Mi-J* allele might be interesting to find possible TYLCV resistance in these two genotypes.

Screening for the *Sw-5b* allele: Among the several resistance alleles for tomato spotted wilt virus (Finlay 1953; Saidi and Warade, 2008), the *Sw5* allele has been reported to be the most important one due to its durability against various tospoviruses and different TSWV isolates collected from different locations like Brazil, Texas, Hawaii, etc. (Stevens *et al.,* 1995). The *Sw5* locus has been mapped near the telomeric region of chromosome 9 (Stevens *et al.,* 1995). Later, five different *Sw5* alleles (*Sw5-a, Sw5-b, Sw5-c, Sw5-d* and *Sw5-e*) and seven homologs of *Sw-5* have been reported to be distributed in chromosome 9 and chromosome 12. Among these alleles, the *Sw5-b* allele has been reported to be the functional one (Spassova et al., 2001). In a recent study (Shi *et al.,* 2011), a specific PCR-based marker for the identification of the *Sw5-b* allele using the Sw5-f2/Sw5-r2 primer pair has been reported. The same primer pair (annotated as TSW5F and TSW5R) has been explored in the present study, where ten genotypes (EC 529080, EC 625644, EC 629421, Azad T-5, CLN-1621 L, EC 521080, IIHR 2620, Shahanshah, ArkaVikas and Pusa Ruby) were found to produce the desired *Sw5-b* resistance allele-derived amplicon of ~540 bp (Fig. 1C).

Screening for the *I* and *I2* alleles: The *I* locus, conferring resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 0 has been reported to be introgressed from *S. pimpinellifolium* and mapped on the short arm of chromosome 11 (Ori *et al.,* 1997; Sela-Buurlage *et al.,* 2001). On the other hand, the *I2* locus conferring resistance to race 1 of *F. oxysporum f.* sp.*lycopersici* has been reported to be introduced from *S. pimpinellifolium* and mapped on the long arm of chromosome 11 (Ori *et al.,* 1997). Recently, PCR-based dominant markers have been established for identifying the *I* and *I2* resistance alleles (Arens *et al.,* 2010). These primer pairs (At2-F3/At2-R3 for *I* and Z1063F/R for *I2*) were explored (annotated as TF0WSF/TF0WSR for *I* and TF1WSF/TF1WSR for *I2*) in the present study to identify these resistance alleles, where all the genotypes, except EC 520075, Azad T 5, ArkaVikash and Pusa Ruby were found to contain the *I* resistance allele-specific ~130 bp amplicon (Fig. 2A). On the other hand, the *I2* allele-specific ~940 bp amplicon was obtained only in case of the genotypes, EC 625644 and Shahanshah (Fig. 2B).

Availability of molecular markers has greatly enhanced the efficiency and accuracy in selection during targeted breeding programmes in different crops. However, prior to application of the molecular markers for marker assisted selection (MAS), it is important to validate them and to characterize the parental breeding lines. This background selection then becomes the platform for further MAS in the segregating materials. In this study, we document the utility of some linked SCAR markers to characterize 18 tomato genotypes for the presence/absence of some important disease resistance alleles. Hence this work will help to adopt MAS in future breeding programmes of tomato, targeted towards the development of disease resistant tomato lines.

Conclusion

Screening for disease resistance is problematic as it is highly dependent on the presence of congenial environment. Furthermore, screening for multiple disease resistance in a simultaneous manner is more difficult. Hence, molecular marker-based screening for the presence of possible disease resistance alleles in different genotypes has become very important. In this article, we have used molecular markers to detect the presence of possible resistance alleles in different tomato genotypes. Through the linked molecular markers, we have identified 2 lines carrying the allele for conferring partial resistance to root knot disease, 10 lines carrying the allele for conferring resistance to spotted wilt disease, 14 lines carrying the allele for conferring resistance to *Fusarium* wilt caused by race 0, and, 2 lines carrying the allele for conferring resistance to *Fusarium* wilt caused by race I. Naturally, the novelty of this study is the identification of the possible donor parents for developing disease resistance in tomato. Observations recored in this study will serve as the platform for developing precise breeding programmes

towards development of diseases resistant tomato genotypes or development of tomato genotypes with pyramided genes for multiple disease resitance through marker assisted approach.

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