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Short Communication

Extraction of the NBS-LRR Class Resistance Gene (I2 Gene) from the Heamsona Tomato Cultivar

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Three races of *Fusarium oxysporum* f. sp. *lycopersici* race 1, 2 and 3 are identified depending on the avirulence protein or effector protein secreted by fungal pathogen during the host colonization in tomato. These effector proteins are recognized by the host innate immune system based on R gene expressions that are I1, I2 and I3 in tomato for each races. Amongst the three, I2 protein has been cloned and characterized for the incompatibility against race 2 type of the pathogens. In India race 1 type of *F. oxysporum* f. sp. *lycopersici* observed commonly which require presence of I1 gene in tomato plant for the incompatibility reactions but in the present study, I2 gene was partially isolated from the tomato cultivar Heamsona and observed to be resistance against race 1 type of pathogen.

Key words: Fusarium wilt, race, R-gene, resistance, tomato.

INTRODUCTION

Resistance to pathogens is suspected to involve a specific interaction between a resistant plant and a pathogen. These molecules are encoded by resistance (R) genes in plant and each resistant protein typically initiates a defense response in the presence of one pathogen-derived elicitor protein that is known as avirulence (Avr) determinant. The genetic relationship between R and Avr pro-teins was elegantly stated in the gene for gene hypothe-sis (Flor, 1971) and this type of plant defense is now described as the plant innate immunity.

Number of polymorphic resistance genes has been identified in the tomato species and each confers resistance against a subset of *Fusarium oxysporum* f.sp. *lycopersici* (Fol) strains. It includes I (for Immunity), I1, I2 and I3 (Huang and Lindhout, 1997). Races of Fol are numbered as per the R gene that is effective against them: the I gene and the (unlinked) I1 gene are effective against race 1 type of *F. oxysporum* f. sp. *lycopersici* that is if tomato plant encompasses it then it will show resistance against race 1 pathotype; similarly race 2 overcomes I and I1, that is it will infect the tomato plant encompassing I and I1 gene and will kill it, but is stopped by I2, while race 3 overcomes I, I1 and I2 but is blocked by I-3 (Rep et al., 2005). So it states that to achieve the resistance against the Fol pathotype or race, the plant must encompass the respective R gene against it.

The I2 locus conferring resistance to race 2 of the soil-borne fungus *F. oxysporum* f sp *lycopersici* is the only gene which is characterized in tomato. The selective am-plified restriction fragment polymorphism (AFLP) posi-tional cloning strategy was used to identify I2 in the tomato genome (Simons et al., 1998). As the gene responsible for the race 2 resistance has been

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Abbreviations: R, Resistance; **Avr,** avirulence; **AFLP,** amplified restriction fragment polymorphism; **RFLP,** restriction fragment length polymorphism; **NCBI,** National Center for Biotechnology Information.

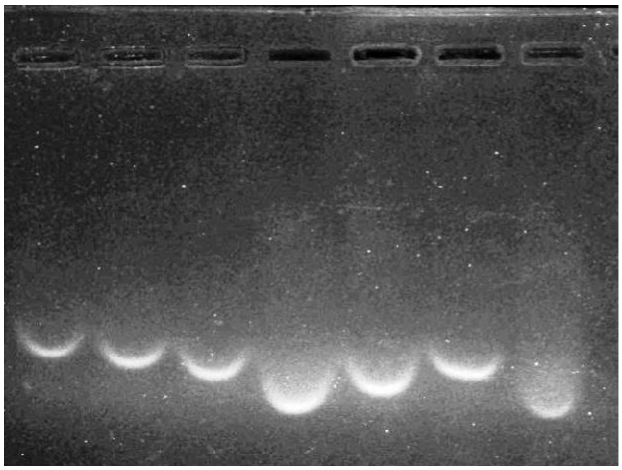

Figure 1. Screening of resistant and suceptible cultivars through I1 primers showed amplicon in all the cultivars.

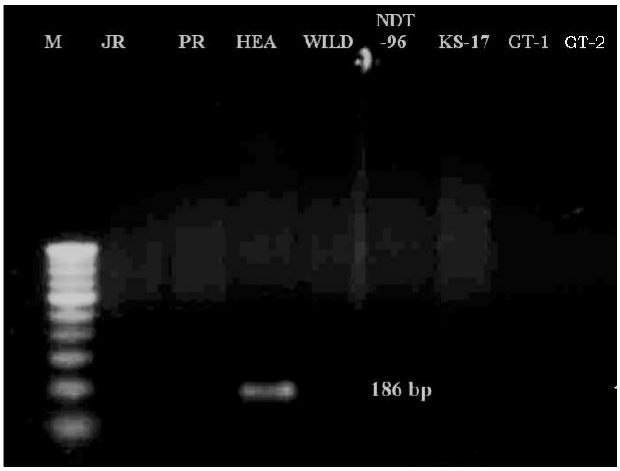

Figure 2. Screening of resistant and suceptible cultivars through I2 LRR primer showed amplicon of size 186 bp in Heamsona cultivar. (M=100bp ladder).

characterized, it will be easy to achieve the resistance in other cultivars by genetic engineering but what is the case with the other races for which the gene has not been charecterized. In this study, we focused on isolation of resistance gene from Indian tomato cultivar to reveal that I2 gene can be employed to achieve resistance against race 1 pathogen.

MATERIALS AND METHODS

Plant material

Heamsona, Gujarat tomato -1 (GT-1), Gujarat tomato-2 (GT-2), Pusa ruby (PR), Junagadh ruby (JR), Wild, NDT-96 and KS-17 cultivars of tomato were cultivated in open field at Main vegetable research station, Anand Agricultural University, Anand, Gujarat, India.

Oligonucleotide design

Two sets of oligonucleotide with varying amplicon size were designed using Primer 3.0 software. First set of primer were con-structed for screening of tomato population for presence of I2 gene, within the LRR region of the gene for amplification of twenty two amino acids in two repeats as it is only observed in the active copy
of the I2 gene. Forward primer I2 FP 'TCTCACCTCACTTCGCTTCA' and reverse primer I2 RP 'TAGGGCAATCCTGGATGAAC'. TG 194 a restriction fragment length polymorphism (RFLP) probe sequence was used for the screening of I1 gene isolation amongst the tomato population
(Sarfatti et al., 1991). Forward primer I1 FP 'TAGGGAGACAGCTTGCATGCCT' and reverse primer I1 RP 'CAAGTTGAAGGATATGAGTATTAT'.

DNA isolation and PCR screening of I1 and I2 gene

Genomic DNA was extracted in bulk from young fresh leaves of tomato cultivars, using the phenol - chloroform method described by Oza et al. (2008) and quantified on a spectrophotometer. Amplification was carried out for the screening of I1 and I2 gene in 12.5µl of reaction mixture, containing 7 µl distilled water, 1.25 µl of 10x assay buffer with 15 mM $MgCl_2$, 2 µl of 100 ng template DNA, 1.5 µl of primer, 1 µl dNTP mix and 0.25 µl Taq DNA polymerase. Polymerase chain reaction (PCR) was performed in a thermal cycler. The PCR profile starts with initial denaturation at 93°C for 5 s, followed by 30 cycles of denaturation at 93°C for 30 s., annealing as per the primers for 2 min, extension at 68°C for 4 min and finally extension at 72°C for 5 min. The products were size-separated on a 0.8% agarose gel in 1×TAE (Tris acetic acid EDTA) buffer at 50 V, stained with ethidium bromide (1 $\mu g \cdot ml^{-1}$) and visualized on a GelDoc.

RESULTS AND DISCUSSION

PCR based screening of I1 and I2 gene

In the present study, the marker TG-194 linked to the resistance gene I1 on chromosome 7 was used to determine the presence or absence of the I1 gene in the eight varieties selected because the gene I1 is not characterized. DNA fingerprint of eight different varieties developed with TG-194 probe (Figure 1) could not show the polymorphism, indicating that it is not linked to resistance and cannot be used further for the identification of resistant varieties in breeding programs. Figure 2 shows the fingerprint pattern of eight varieties with primer pairs designed specifically from the LRR region of the I2 gene. An amplicon of 186 bp could be obtained only in Heamsona tomato cultivar which was reported by Parmar et al. (2011) as a resistant entity while others showed no amplicons.

Sequencing and identification of gene

Sequencing of the isolated gene (I2) was done with specific

primer at Xcelaris, Laboratory, Ahmedabad. Subsequently the identification of sequence was done using bioinformatics tool. Searches of the National Center for Biotechnology Information (NCBI) genbank database using BLAST algorithm showed 100% similarity with the I2 active gene copy. The sequence has been deposited in NCBI database (Parmar et al., 2009).

Conclusion

The present investigation has shown application of I2 gene for the development of resistance against race 1 pathotype, which results into resistance only if I1 gene is available in the tomato cultivars. Positive response of I2 gene against race 1 pathotype suggest that both I1 and I2 gene are having some similarity in terms of their expression against avirulence genes, it may be the case that both have identical recognition site for the avirulence gene but it is to be disclosed which can be possible only after its characterization. Present study has showed strategies that can be employed for the eradication of the wilt disease especially when the R gene responsible for the resistance against such pathotype is not characterized.

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