

Microsatellite Marker Associated with Fusarium Wilt Resistance in Tomato

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Abstract: Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is a major constraint of tomato production all over the world. Progress in selection for fungal-resistant lines can be speeded up by the use of molecular markers associated with genes of resistance. Eight microsatellite regions were used to amplify the DNA of tomato lines (3 resistant, 2 susceptible and 3 tolerant). Among loci initially analyzed, SSR-67 was highly polymorphic in the resistant, tolerant and susceptible population. The marker selected is inherited and segregated in Mendelian fashion as demonstrated on a panel of 25F₂ and 20F₁ offspring derived from a cross of two divergent cultivars GP-10 (wilt resistant) and GT-2 (wilt tolerant).

Key words: Genotyping · Microsatellite marker · Resistance · Tomato

INTRODUCTION

Traditional methods of plant breeding have made a significant contribution to crop improvement, but they have been slow in targeting complex traits like grain yield, grain quality and drought resistance. To meet the great increase in food production necessitated by population growth and the higher standards of living expected by most of the developing countries, biotechnology brings new and powerful tools to plant breeders. One method receiving growing attention is the mapping of chromosomal regions affecting qualitative or quantitative traits. Polygenic characters, which were very difficult to analyze using traditional plant breeding methods can now, be tagged using molecular markers (DNA markers). Molecular markers allow geneticists and plant breeders to locate and follow the numerous interacting genes that determine a complex trait. Genetic linkage maps can provide a more direct method for selecting desirable genes *via* their linkage to easily detectable molecular markers [1].

In the last decade, PCR-based markers have become diagnostic tools for marker-assisted selection (MAS) in plant breeding. To be helpful for breeders, a molecular marker should be tightly linked with a trait allele, amplified in a reproducible, easy and cost-effective manner [2]. In tomato, a few microsatellite markers have been developed

[3] and the number of SSR markers available for molecular breeding is still small and only a limited number of SSR markers have been mapped to the tomato genome [4]. It is desirable, therefore, to develop more SSR markers for genetic mapping and marker-assisted selection, since the SSR markers developed to-date are not evenly distributed and do not cover the entire genome.

The present study is aimed to develop molecular markers for identification the resistant tomato cultivars against Fusarium wilt. The marker assay could also be as a method of choice for large scale screening of tomato cultivars.

MATERIALS AND METHODS

Heamsona, Gujarat-1, Gujarat-2, GP-10 (wild), Junagadh ruby, KS-17, NDT-96 and Pusa ruby cultivars of tomato (*Solanum lycopersicum*) were cultivated in the open field (Anand agriculture university, Anand) by planting 50 seeds per variety. They were maintained by drip-irrigation and necessary care.

Genomic DNA was extracted in bulk from young fresh leaves of each cultivar, using the phenol-chloroform method described by Sambrook *et al.* [5]. A total of 8 microsatellite regions from Suliman-Pollatsche [6] and SOL Genomics Network (SGN) of Cornell University, USA,

located on chromosomes 7 and 11 of tomato were chosen for the present study. These microsatellite regions were assayed with the template DNA of eight different tomato cultivars. PCR-amplification was carried out in 12.5 μ l (Microlitre) of reaction mixture, containing 7 μ l distilled water, 1.25 μ l of 10X buffer with 15 mM MgCl₂, 2 μ l of 100 ng template DNA, 1.5 μ l of primer, 1 μ l dNTP mix and 0.25 μ l Taq DNA polymerase. PCR was performed in a thermal cycler (Eppendorf). Amplification was performed as follow: initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 30 sec., annealing for 45 sec, extension at 72°C for 45 sec and finally extension at 72°C for 5 min. PCR products were size-separated on a 2.5% agarose gel in 1 \times TAE buffer at 50 V, stained with ethidium bromide (1 μ g ml⁻¹) and visualized on a GelDoc (α innotech).

An F_{1,2} mapping population was developed from a cross between a wild tomato accession (GP-10) and a Gujarat tomato cultivar (GT-2) at Vegetable section, Anand Agriculture University, the cultivated parent representing a pure line selection from a landrace being the female in the cross. The F_{1,2} populations consisting of 45 individuals were screened for the segregation of the marker to establish a marker for identification of resistant cultivars.

The two cultivars; GP-10 x GT-2 were used to generate the genetic map of tomato. The F₁ and F₂ populations consisted of 45 individuals and segregated for one Fusarium wilt marker. DNA was extracted as described earlier. Aliquots (2.5 g of DNA) of each individual homozygous phenotypically based on bioassay were bulked together. The number of individuals in each bulk varied between 9 (tolerant) and 11 (resistant) plants at F₁ and 6 (tolerant and susceptible) to 13 (resistant) at F₂ level, respectively. The bulks were screened with SSR 67 primer [FP: 5'GCACGAGACCAAGCAGATTA3' RP: 5'GGGCCTTTCCTCCAGTAGAC 3'].

Genotypic data were evaluated for Mendelian segregation with the phenotypic data using chi-square test by QGene 4.0 software [7]. Genetic linkage maps were constructed in MAP MANAGER QTX Version b16 with the Kosambi map function [8]. Quantitative trait loci analyses were conducted using interval mapping with Genome-wide threshold values for declaring the presence of QTL were determined by 1000 permutations.

RESULTS AND DISCUSSION

The markers hunt started with an application of *in vitro* method that classified the tomato cultivars to

three classes; susceptible, tolerant and resistant. It will be a contrary to do bioassay in present investigation but it is necessary initially because no marker is available as such which can classify the tomato cultivars. Once it has been established it can be employed efficiently without application of bioassay. Leaves of different tomato cultivars five to ten per entity were collected, sized (10 mm) and challenged to ten days old 15X fungal culture filtrate (*Fusarium oxysporum* f. sp. *lycopersici* race 1) on 1% basal agar medium. Disease severity was evaluated after two days which showed that cultivar "LA 3042" remained asymptomatic representing the class of resistance, a resistant check in comparison to "Pusa ruby" with 100% symptoms representing a susceptible class, a susceptible check respectively. While evaluation we found many cultivars with symptom in range of 30 -70% so probably it is the cultivars that tolerate the disease and it was assigned a separate group. Quantitative evaluation of disease symptoms were looked for the correct measurement and grouping of the cultivars, chlorophyll level evaluation was found to be efficient in this case with significant variation over all others methods. As author pointed out initially, still this assay should be checked for the confirmation so biochemical markers like PR proteins that found to be important in their role with plant defense systems were taken into account. Many proteins like peroxidase, PAL, β -1, 3 glucanase, polyphenol oxidase, chitinase and total soluble phenols were checked and it validated the above finding implying the correct grouping of tomato cultivars in to three classes.

The reproducibility of findings was checked at field level to see the reaction of cultivars when challenged with fungus under open environmental condition where it actually faces the problem. The response by each cultivar infers that the cultivars were correctly evaluated at laboratory conditioned. Both these methods were found to be efficient for the grouping of different cultivars but it has limitation that it is tedious, time consuming and uneconomical. These methods are valuable when a small fraction of samples to be evaluated but it is not the case when comes to breeder where lots of cultivars to be processed. Present investigation is an initiation to help the breeder for evaluation of resistant cultivars. As per the phenotypic evaluation by *in vitro* and *in vivo* assay following groups were ascertain to the tomato cultivars. Susceptible: Junagadh ruby, Pusa ruby, Tolerant: Gujarat-1, Gujarat-2, KS-17 and Resistant: GP-10, Heamsona, NDT-96. Based upon the results obtained from the *in vitro* and *in vivo* assay, the selected microsatellite and RFLP regions were analyze to find a molecular marker associated with resistance to wilt. PCR fingerprint of eight tomato

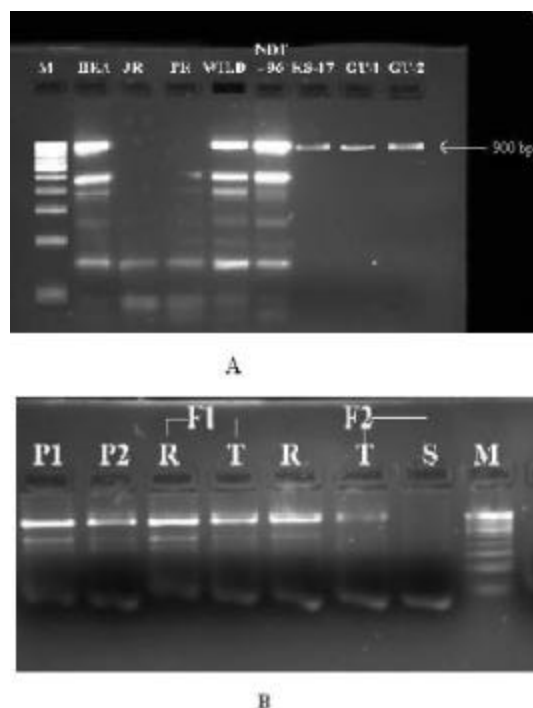


Fig. 1: A) Highly polymorphic profile obtained with use of SSR-67 primer. Resistant cultivars like Wild (GP-10), NDT-96 and Heamsona showed alleles of size 900 + 900 in comparison to tolerant cultivars KS-17, GT-1 and GT-2 and Null allele with susceptible cultivars JR and PR
 B) Bulk segregation analysis of resistant, tolerant and susceptible bulk with parents GP-10 (P1) and GT-2 (P2) using primer SSR-67

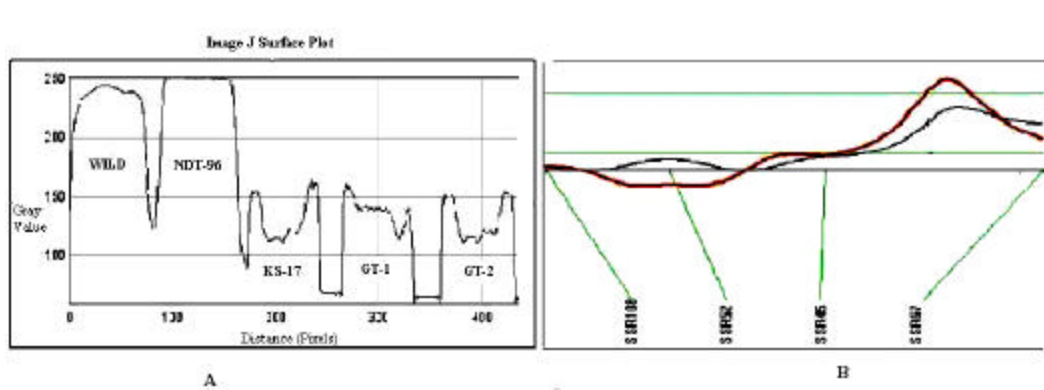


Fig. 2: A) Brand intensity of five cultivars through image J analysis showed resistant cultivars NDT-96 and Wild (GP-10) with double the intensity in comparison to tolerant KS-17, GT-1 and GT-2
 B) Maximum likelihood plot indicating regions of quantitative trait loci associated with disease resistance in 25 inbred lines from wild X GT-2

cultivars were developed using eight different pairs of microsatellite present on chromosome 7, 11 and a RFLP marker TG 194 reported to be linked to I gene [9]. Of the eight microsatellites studied, only SSR 67 was found to be a putative marker for the resistant cultivars of tomato with varying allelic size among three different classes viz. susceptible, tolerant and resistant. SSR 67 could discriminate the resistant cultivars with alleles of size 900+900 bp (reported) in comparison to tolerant cultivars with a single 900 bp allele and susceptible with no amplicon (Fig. 1A). To judge the two alleles of same size and a single allele of similar size among the different cultivars quantitative measurement Image J software (National Institute of Health, USA) was used to quantify the allelic size among the different cultivars. Data obtained clearly exhibit twice the intensity for alleles of resistant cultivars in comparison to the single allele of tolerant cultivars. (Fig. 2A). On the contrary the RFLP marker TG-194 could not show any discrimination between resistant and susceptible tomato cultivars.

There are mainly three races of the fungus *Fusarium oxysporum* f. sp. *lycopersici* is observed all over world. In India race 1 fungus is normally found. Subsequently, in the present investigation I gene was targeted. This study is also significant in light of the recent findings of infection of tomato cultivars containing genes I2 and I3 by race 1 fungus [10] and if the resistance against race 1 is established, it implies that the cultivar is resistant to all the races.

Bulked segregation analysis is employed to identify markers linked to phenotypic characters in plant species for which no NILs exist. In the present study, two bulks were made, each of 9 and 11 of F1 individuals homozygous for alternate alleles as identified through *in vitro* bioassay. The pair of tolerant and resistant bulks with an expected phenotypic ratio of (1:1) was screened for segregation using SSR-67 primer set as a marker. Amplification with SSR-67 generated polymorphism of 900+900 bp alleles with resistant bulk and a single 900bp allele with tolerant bulk. The polymorphism was confirmed by repeated amplification and compared with the two parents (Fig. 1B). F2 progeny were also assessed to validate the co-segregation of the identified marker. F2 level analysis revealed three bulks of 6 susceptible, 13 tolerant and 6 resistant with a phenotypic ratio of 1:2:1. SSR-67 analysis of F2 progeny revealed 900+900 amplicon only in resistant bulk, no amplicon in susceptible bulk and 900 amplicon in tolerant bulk. The allelic polymorphism was confirmed by quantitative measurement using Image J software analysis.

Data obtained through phenotypic evaluation and SSR analysis were subjected to linkage analysis by Mapmanager QTX to establish the correlation between genotypic and phenotypic markers. The phenotypic performance of resistant trait in detached-leaflet assay was designated Ph-RE. The scoring led to expected phenotypic ratio (tolerant: resistant) 1:1 in the F1 population and 1:2:1 in F2 population. There were thirteen cultivars that had no symptoms, six cultivars with symptoms and six cultivars with tolerance in the F2 population, which satisfactorily fit the expected ratio based on chi-square test ($\chi^2=104.27$) significant at $\alpha = 0.01$ and regression analysis ($r^2= 0.9586$). After establishing the Mendelian segregation pattern of inheritance, genotypic marker SSR67 was evaluated for the correlation with the phenotypic marker Ph-RE on the basis of linkage analysis. When the marker and phenotypic data from the F₂ populations were analyzed by interval mapping with Map Manager QTX, QTL peak was found closer to SSR-67 (0.42 cM) than SSR-45 (0.54 cM) with 97.3 cM distance between each others (Fig. 2B).

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