

Genetic analysis of resistance in tomato cultivars against early blight

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Abstract

Tomato crops are parasitised by a large and ever increasing number of pathogens of which, early blight disease in tomato caused by *Alternaria solani* is an important pathogen in world. In this study, two tomato varieties namely NDT-96 and GP-5 which are tolerant and susceptible to early blight, respectively, were used. To investigate a marker linked to resistance, 25 SSR markers were screened for polymorphisms among the above mentioned two tomato varieties. Of these, SSR 286 revealed a significant polymorphic band of 108bp in NDT-96. Crosses between NDT-96 and GP-5 were carried out to produce F1 hybrid and selfing of F1 plants to produce F2 hybrid. For phenotypic studies, both *in vitro* and *in vivo* studies were carried out by randomly selecting plants and the ratio was 1:1 in 20 F1 hybrids and 3:1 in 30 F2 plants was proved by chi-square test. Spearman's rank correlations between scores for early blight resistance in F1 and F2 plants show that the infection of early blight was similar and the optimal evaluation stage for early blight resistance is 7 days after inoculation. Segregation of SSR 286 was detected in both F1 and F2 generations by performing bulk segregation analysis and by screening of all 120 genotypes of F1 and 145 genotypes of F2 generation plants. A total of 14 QTLs were mapped on chromosomes 7, and 11 including an early blight QTL (linked to SSR 286) having LOD score I which conferred tolerance to leaf lesions in the field.

Keywords: Linkage analysis; Marker assisted selection (MAS); QTL mapping; Simple sequence repeats (SSR); Tomato.

Introduction

The plant diversity in the Solanaceae family is great and several species of economic

importance, e.g., tomatoes (Tambarussi et al. 2009) belong to it. Most commercial cultivars of tomato are susceptible to early blight, a devastating fungal disease of tomato in the world. Early blight (EB) caused by *Alternaria solani* Sorauer is a 3-phased disease that can produce collar rot, leaf blight (early blight) and fruit rot. Alternaric acid is one of the major toxins found in the fungal culture filtrates (Brian et al. 1952) which causes chlorosis and necrosis and therefore it plays a major role in early blight symptoms and defoliation. Symptoms produced by an aqueous solution of crystalline alternaric acid when introduced into the plants are identical with symptoms produced by crude fungus filtrates. (Pound et al. 1951) From our previous studies it has been shown that isolation of alternaric acid by a simple and rapid method gave a good quantity of purified form of alternaric acid (Patel et al. 2011a). Therefore, alternaric acid isolated by this method is used here for both *in vitro* and natural infection assays.

The detached-leaflet method was first used by Douglas (1922) to compare the response of tomato varieties to inoculation with an undetermined species of *Alternaria*. Subsequently, the use of detached leaflets for comparing the virulence of strains of *A. solani* and evaluating the resistance of tomato genotypes has been reported to be either unsuccessful or successful (Wellman 1943 & Locke 1948). In the present study, the detached-leaflet method and natural infection assays would be useful for chi-square (χ^2) test and the data of early blight reaction of natural infection assays would be helpful in estimating Spearman's rank correlations between F1 and F2 tomato progenies. From our previous studies it has been shown that tomato varieties NDT-96 is tolerant and GP-5 is susceptible to early blight (Patel et al. 2011b). As conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization so NDT-96 and GP-5 are used in the present study as parents for plant breeding.

The development of the polymerase chain reaction (PCR) (Saiki et al. 1988) has led to the development of many techniques to detect polymorphism at the DNA level (Powell et al. 1995 & Rafalski et al. 1996). Of these techniques, some require the use of sequence-specific primers, such as SSR or microsatellites (Powell et al. 1996). The microsatellite markers (SSR) are short (mostly 2–4 bp) tandem repeats of DNA sequences and are useful due to their high degree of polymorphism and co-dominant character of heredity. (He et al. 2003) The use of microsatellite polymorphisms to study the genetic diversity and variability was described for a number of plant species especially in tomato (Wang et al. 2006). They are mostly used as markers for genomic mapping, variety identification and marker-assisted selection in tomato. (He et al. 2003) SSR markers have potential application in diversity analysis, fingerprinting, gene mapping and quantitative trait locus (QTL) identification (Rakshit et al. 2010). Marker assisted selection (MAS) is now used to enhance traditional breeding programs and allows the breeder to achieve early selection of a trait in a breeding program. It is particularly useful when the trait is under complex genetic control, or when phenotypic trials are unreliable or expensive. One of the aims of genetic studies is to associate the genotype with the heritable phenotype (Botstein & Risch 2003). The production of genetic linkage maps has enabled quantitative trait loci (QTL) to be mapped. QTL mapping generally involves the use of structured populations and

relatively distant markers that can segregate with the QTL. Thus, QTL provides a wide genetic region within which the gene is located.

The objectives of this investigation were: (1) To screen a marker linked to resistance in NDT-96 (2) To study the segregation of marker linked to resistance in F1 and F2 plants. (3) To study early blight resistance evaluation in F1 and F2 plants by *in vitro* and natural infection assays. (4) To study linkage and QTL mapping of marker linked to resistance for early blight in tomato.

Materials and methods

Fungal culture and its maintenance:

Pathogenic strain *Alternaria solani* was isolated from an infected tomato plant from Anand Agriculture University, Anand. *A. solani* isolate was grown on potato dextrose agar (PDA) plates and these plates were incubated at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until sporulation. After sporulation the organism was identified and maintained at 4°C until further use.

Fungal Culture Filtrate (FCF) production and its analysis:

An 8 mm mycelial mat was cut with a sterile cork borer from 1 month old *A. solani* culture, grown on a PDA plate and transferred into a 250ml Erlenmeyer flask containing 100ml of autoclaved potato-dextrose broth (PDB). Flasks were incubated from 21 days at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in static submerged condition. Thereafter, the broth was filtered using Whatman filter paper No. 1.

Isolation of Alternaric acid:

For development of crystals, inoculation and maintenance of *A. solani* was done by the method as described above in FCF production. Mycelial mat from 18 days old culture grown on PDB was removed and the broth was filtered using Whatman filter paper No. 1. The pH of the filtrate was adjusted to between 3.0-3.5 by 1N HCl and extracted with equal volume of ethanol. The colourless solution obtained was dissolved by adding drop by drop boiling carbon tetrachloride (2ml) using a glass dropper. Residual ethanol was removed by evaporation and crystalline alternaric acid appeared on cooling.

Plant materials:

The following two tomato (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum* Mill.) varieties were included in our studies: (i) NDT-96 and (ii) GP-5. Seeds of NDT-96 variety were obtained from Anand Agriculture University, India and seeds of GP-5 were obtained from Junagadh Agriculture University, India. The surface of tomato seeds was sterilized in 70% ethanol for 2 min, and thoroughly washed with sterile distilled water. (Jung et al. 2005) Thereafter, seeds of both varieties were treated with 1% H_2O_2 (Jaskani et al. 2006) and allowed to germinate in Petri plates at 28°C for 2 days and sown in plastic cups filled with cocopeat initially and after one week the seedlings were transferred to plastic cups filled with soil. The plants were allowed to grow for four weeks and watered daily. The plants were maintained in field. An interspecific cross between NDT-96 and GP-5 was made to produce F1 seeds. Further, F1 plants were selfed to produce F2 plants.

DNA isolation and marker analysis:

For the SSR analysis, DNA was isolated from fresh tissue (young leaves) of NDT-96 and GP-5 according to method Doyle & Doyle (1990). A total of 25 SSR primers were surveyed (Table 1). Primers for SSR 45 to SSR 637 and Tom 144-Tom 196 were obtained from the Sol Genomics Network at www.sgn.cornell.edu. The selection of primer was random. Primers for Tom 8- 9 to Tom 57-58 were obtained from literature (Rajput et al. 2006). PCR were done in 12- μ l volumes containing the following: 1 μ l DNA, 50 ng/ μ l; 0.75 μ l, 10X assay buffer; 1.5 μ l, 2.5 mM dNTPs; 0.5 μ l, 15 mM MgCl₂; 0.25 μ l, 3 Units Taq DNA Polymerase (Banglore Genei); 1 μ l, 10 picomoles/ μ l each of forward and reverse primers and 7 μ l of sterile water. The PCR conditions were as follows: 95°C for 1 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 1 min extension at 72°C for 2 min. A final extension 72°C for 5 min was included. The PCR products were electrophoresed in a 3% agarose gels with ethidium bromide at 50 V for 2-3 h. The gel was then observed on a UV transilluminator. A 100bp DNA marker ladder was used for visual comaparison of the bands. A polymorphic band was observed in NDT- 96 with SSR 286 marker. Thereafter, DNA samples were sent for sequencing to AN Xplorigen Technologies Pvt. Ltd. The sequence obtained after sequencing was submitted to GenBank. The GenBank accession number after acceptance of sequence is GU930288. BLAST analysis was done of the sequence obtained.

Table 1 SSR primers along with their sequence used for the screening of two tomato cultivars.

Sr. No.	Name of Marker	Sequence (5'-3')
	SSR 45	F:TGTATCCTGGTGGACCAATG R: TCCAAGTATCAGGCACACCA
	SSR 46	F:TGTATCCTGGTGGACCAATG R: TCCAAGTATCAGGCACACCA
	SSR 52	F:TGATGGCAGCATCGTAGAAG R: GGTGCGAAGGGATTTACAGA
	SSR 67	F:GCACGAGACCAAGCAGATTA R: GGGCCTTTCCCTCCAGTAGAC
	SSR 76	F:ACGGGTTCGTCTTTGAAACAA R: CCACCGGATTCTTCTTCGTA
	SSR 80	F:GGCAAATGTCAAAGGATTGG R: AGGGTCATGTTCTTGATTGTCA
	SSR108	F:TGTGTTGGATGTTTGGCACT R: GCCATTGAACTTGCAGAGA
	SSR 136	F:GAAACCGCCTCTTTCCTTG R: CAGCAATGATTCCAGCGATA
	SSR 181	F: CAATCGAAACCGACGATACA R: GGTAGATCTGGATCGAGGAGG
	SSR 241	F:TCAACAGCATAGTGGAGGAGG R: TCCTCGGTAATTGATCCACC

	SSR 276	F:CTCCGGCAAGAGTGAACATT R: CGACGGAGTACTTCGCATTT
	SSR 285	F:AGTGGCTCTCACCTACTGCG R: CAATTCTCAGGCATGAAACG
	SSR 286	F: AGCTATGGAGTTTCAGGACCA R: ATTCAGGTAGCATGGAACGC
	SSR 304	F:TCCTCCGGTTGTTACTCCAC R: TTAGCACTTCCACCGATTCC
	SSR 565	F: GAGGATGATGAGAACTCGCC R: TCAGAGGCTTCTGGGTCAGT
	SSR 637	F: AATGTAACAACGTGTCATGATTC R: AAGTCACAACTAAGTTAGGG
	Tom 8-9 ATT7	F:GCATTGATTGAACTTCATTCTCGTCC R: ATTTTTGFCCACCAACTAACCG
	Tom 31A-32A TA11	F: AATGTAATGGTGATGCTCTTCC R: CTCGGTTTTAATTTTTGTGTCT
	Tom 41- 42 TCC6	F: GAAATCTGTTGAAGCCCTCTC R: GACTGTGATAGTAAGAATGAG
	Tom 43- 44 TCC6	F: GCAGGAGATAATAACAGAATAAT R: GGTAGAAGCCCGAATATCATT
	Tom 47- 48 AT10	F: CAAGTTGATTGCATTACCTATTG R: TACAACAACATTTCTTCTCCTT
	Tom 49- 50 AT10	F: AAGAACTTTTTGAATGTTGC R: ATTACAATTTAGAGAGTCAAGG
	Tom 57- 58 CT8	F: TCTAAGTGGATGACCATTAT R: GCAGTGATAGCAAATGAAAAC
	Tom 144	F: CTGTTTACTTCAAGAAGGCTG R: ACTTTAACTTTATTATTGCGACG
	Tom 196	F: CCTCCAAATCCCAAACTCT R: TGTTTCATCCACTATCACGA

Early blight resistance evaluation (Phenotyping in F1 and F2 plants):

For phenotyping in F1 and F2 plants, 20 F1 plants and 30 F2 plants were selected. Detached leaf bioassay and *in vivo* bioassay were followed for screening of tolerant and susceptible varieties.

Detached leaf bioassay:

Tomato leaves were washed in tap water. The leaves were surface sterilized with a cotton swab dipped in 4% Sodium hypochlorite solution for 10-15 seconds. They were then washed thoroughly in sterile water and placed in sterile Petri plates containing Whatman No. 1 paper. Thereafter these leaves were treated by alternaric acid crystals and FCF as follows: a) 100µL of crystals along with water was applied gently on leaf surface using a micropipette and 100µL of water was applied on control leaves. b) 100µL of FCF was applied gently on leaf surface using a micropipette and

100 μ L sterile PDB was applied on control leaves. These plates were incubated at 25°C \pm 1°C for 1 to 2 days and the moisture in dishes was maintained throughout the experiment. The visible symptoms were recorded after 24 hours. The experiment was carried out in triplicates.

***In vivo* bioassay:**

Tomato plants were grown and maintained in the way described above for about 4 weeks and was used in *in vivo* bioassay. Tomato plants were treated by toxin and FCF as follows: a) 0.5 ml of crystal solution along with water was injected in the petiole region of the plant. Sterile water was injected in the control plants. b) 0.5 ml of FCF was taken in an injection syringe and this was injected in the petiole region of the plant. Sterile PDB was injected in the control plants. The surface of the leaf was slightly pricked with sterilized syringe in order to enhance the rate of infection. The plants were monitored regularly for the development of visible symptoms. The experiment was carried out in triplicates.

Disease assessment:

Disease severity was rated as previously described (Gaube et al. 2004) by visual assessment based on a 0–9 scale. A disease index (DI), obtained from the average of each leaf value and was used to assess plant resistance. Differences between cultivars were tested using Tukey's Multiple Comparison Test method. The software used was GraphPad Prism Version 3. The data of early blight reaction of detached-leaflet and natural infection assays were tested for significant deviation from the expected Mendelian ratios using chi-square (χ^2) test. In order to evaluate the optimal stage for early blight resistance in F1 and F2 tomato plants, the data of early blight reaction of natural infection assays was tested for Spearman's rank correlations between scores for *Alternaria* leaf blight resistance of F1 tomato plants (2, 3, 5 and 7 days after inoculation) and scores of their self fertilized F2 progeny (3 and 7 days after inoculation).

Genotyping in F1 and F2 plants:

An F1:2 mapping population was developed from a cross between a NDT-96 and GP-5. Two methods were followed. a) All the 120 genotypes of F1 and 145 genotypes of F2 generation were scored visually using the two methods described above and screened with SSR 286 primers for the presence and absence of the SSR band b) Bulk DNA samples from all the varieties were prepared according to Michelmore et al. (1991). Aliquots (50ng of DNA) of each homozygous individual based on bioassay were bulked together. The bulks were screened with SSR 286 primer. The number of individuals in each bulk varied between 10 (tolerant) and 10 (susceptible) plants at F1 and 13 (tolerant) to 6 (susceptible) at F2 level, respectively.

Linkage analysis:

Out of 25 SSR markers 15 were used for linkage analysis and QTL mapping because the position of Tom 144-Tom 196 and Tom 8- 9 to Tom 57-58 on chromosome were

not known. The genetic map was constructed with MapMaker version 3.0b (Lander et al. 1987 & Lincoln et al. 1992). Linkage groups were created with a LOD score of 3.0 and a recombination fraction of 0.5 by the “group” command. The Following Tomato Specific Interactive Maps were scanned (Whole Map scanning) to retrieve the statistical data of our interest against requested marker data sets. Tomato-EXPEN 1992: *S. lycopersicum* cv. VF36 x *S. pennellii* LA716 type F2, 1992; Tomato-EXPIMP 2008: *S. lycopersicum* TA492 x *S. pimpinellifolium* LA1589; Tomato-EXHIR 1997: *S. lycopersicum* TA209 x *S. habrochaites* LA1777 type BC1, 1997; Tomato-EXPEN 2000: *S. lycopersicum* LA925 x *S. pennellii* LA716 type F2. 2000; Tomato-LXCHM 2007: *S. lycopersicum* LE777 x *S. chmielewskii* CH6047 type F2 and Tomato-EXPIMP 2001: *S. lycopersicum* TA209 x *S. pimpinellifolium* LA1589 type RI, 2001. The linkage groups which were assigned to chromosomes based on the microsatellite consensus map of Tomato-EXPEN 2000: *S. lycopersicum* LA925 x *S. pennellii* LA716 type F2.2000 (<http://www.sgn.cornell.edu>). Briefly, the “group” command was used to assign marker loci into linkage groups (LOD \geq 4.0). Subsequently, 3-point and multipoint linkage analyses and the “order” and “compare” commands were used to find the best order of the loci within each group. The “ripple” command was used to verify the order (LOD \geq 3.0). Markers Successfully Screened after Interactive Map Search (For Final Map Construction) Screened Markers List on Chromosome 07 were: SSR 286, SSR 181, SSR 241, SSR 285, SSR 52, SSR 304, SSR 276, SSR 565, SSR 45 and SSR10 and on Chromosome11 were: SSR 76, SSR 136, SSR 46, SSR 80 and SSR 67. Once the linear arrangement of marker loci along each chromosome was determined, the recombination frequencies between markers were estimated. The Kosambi mapping function (Kosambi 1944) was used to convert recombination frequencies to map distances in centiMorgan (cM). The genetic map was drawn by the software Mapdraw (Liu & Anderson 2003).

QTL mapping:

QTLs were detected by the segregation data of all markers in 145 F2 plants using the software package MAPMAKER/QTL version 1.1b (Lincoln et al. 1993) with a 2.0 LOD threshold. Mapping of QTLs and estimation of their effects were performed using the interval mapping method (Lander et al. 1987). Gene action was determined on the basis of the average level of dominance by using the criteria described by Stuber et al. (1987). For map enrichment adjacent markers were selected (Table 2).

Table 2 Selected Adjacent Marker List (For Map Enrichment)

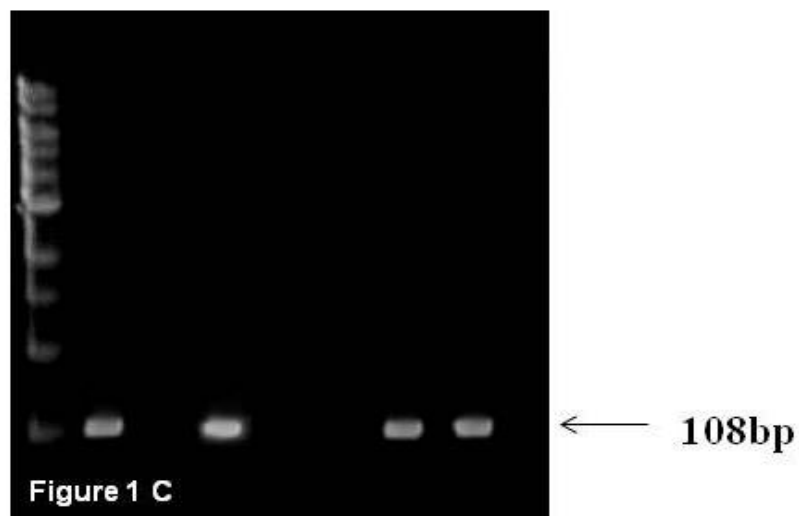
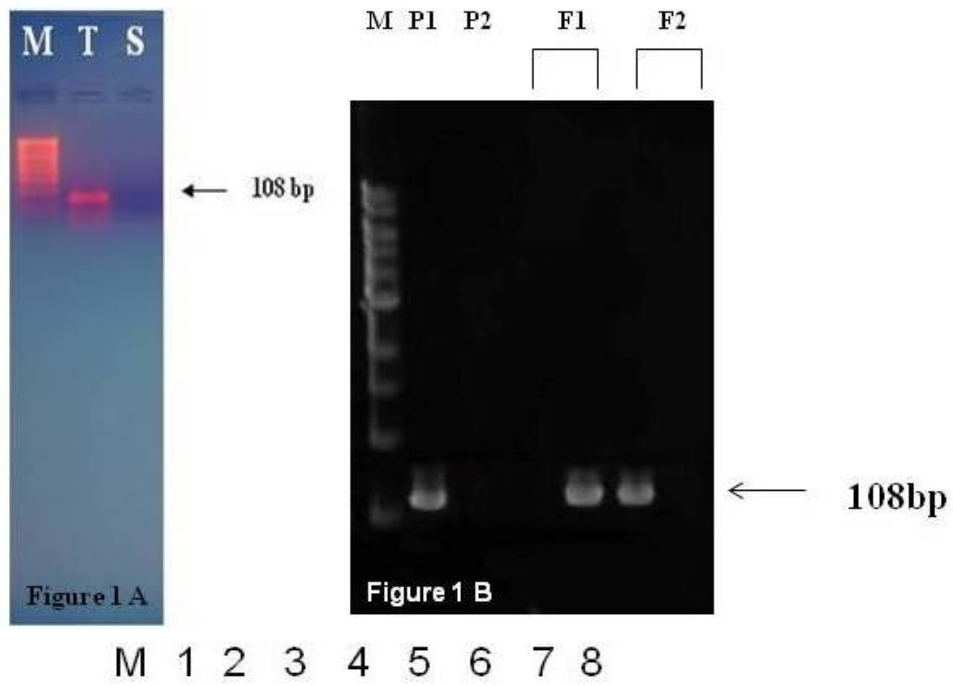
S.No.	Marker	Map	Chromosome	Position (cM)
1	TGS0501	Tomato-EXPEN 2000	7	0.674
2	TES0178	Tomato-EXPEN 2000	7	1.5
3	TES1816	Tomato-EXPEN 2000	7	1.925
4	TGS2602	Tomato-EXPEN 2000	7	2.081
5	TES0584	Tomato-EXPEN 2000	7	2.918
6	TES1008	Tomato-EXPEN 2000	7	3.017
7	TG61	Tomato-EXPEN 2000	7	11.875

8	TES0407	Tomato-EXPEN 2000	7	15.691
9	T1328	Tomato-EXPEN 2000	7	17.336
10	TES0525	Tomato-EXPEN 2000	7	18.197
11	TES1146	Tomato-EXPEN 2000	7	30.328
12	TGS0787	Tomato-EXPEN 2000	7	30.725
13	TGS3163	Tomato-EXPEN 2000	7	43.896
14	TES0680	Tomato-EXPEN 2000	7	44.357
15	T1624	Tomato-EXPEN 2000	7	58.678
16	TG216	Tomato-EXPEN 2000	7	63.184
17	P47	Tomato-EXPEN 2000	11	0
18	TES1975	Tomato-EXPEN 2000	11	9.214
19	T1968	Tomato-EXPEN 2000	11	12.914
20	T1657	Tomato-EXPEN 2000	11	18.667
21	TES1229	Tomato-EXPEN 2000	11	22.173
22	TES1021	Tomato-EXPEN 2000	11	23.048
23	TGS2073	Tomato-EXPEN 2000	11	24.297
24	TES0808	Tomato-EXPEN 2000	11	37.419
25	TES0333	Tomato-EXPEN 2000	11	38.313
26	TGS2887	Tomato-EXPEN 2000	11	39.621
27	T408	Tomato-EXPEN 2000	11	40.21

Results

Molecular marker analysis:

A 3% PCR agarose gel showed the presence of a PCR product of 108 bp present in NDT-96. This particular PCR product was absent in GP-5 (Figure 1 A). BLAST analysis of the marker sequence showed a significant match with –A. *Lycopersicon esculentum* - Homeodomain protein mRNA and B. *Solanum lycopersicum* - cDNA, HTC in leaf (Figure 2).



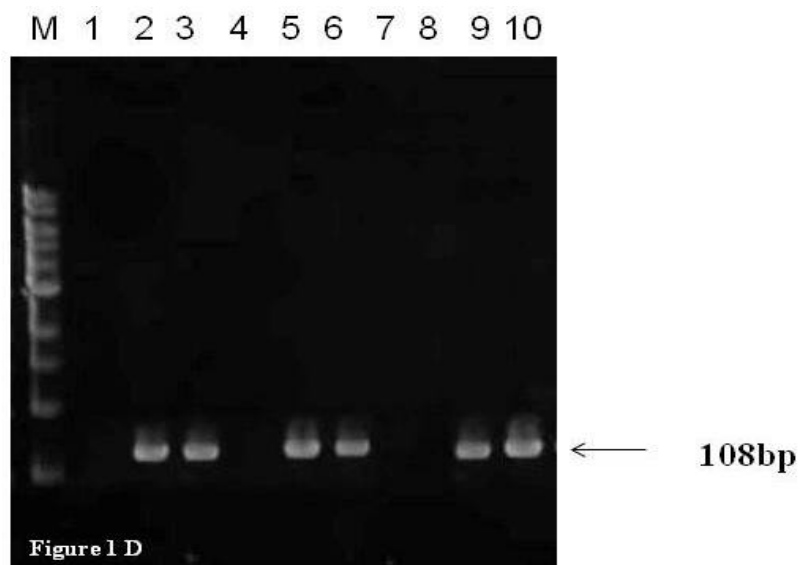


Figure 1 A: PCR gel showing the presence of marker linked to resistance in tolerant variety. Here M= 100 bp DNA ladder, T= Tolerant variety (NDT-96) and S= Susceptible variety (GP-5). B: Showing the bulk segregation analysis of F1 and F2 plants along with parents. Here M = 100bp marker, P1= NDT-96 and P2= GP-5. Here P1 and P2 are controls for F1 and F2 generation tomato plants. C: Showing segregation of 108 bp marker in F1 generation tomato plants. D: Showing segregation of 108 bp marker in F2 generation tomato plants.

BLAST STATISTICS REPORT											
Accession Numbers (gi, gb)	Description	% identity	alignment length	mismatches	gap opens	q _i start	q _i end	s _i start	s _i end	evaluate	bit score
gi 47109629 gb 87014214.1	<i>Lycopersicon esculentum</i> clone 133392f, mRNA sequence	100	59	0	0	23	81	84	142	5.00E-21	110
gi 2529702 gb AF000142.1 LEAF000142	<i>Lycopersicon esculentum</i> class II knotted-like homeodomain protein (LeT12) mRNA, complete cds.	100	59	0	0	23	81	150	208	5.00E-21	110
gi 225916795 db AK329753.1	<i>Solanum lycopersicum</i> cDNA, clone: LEFL10638E08, HTC in leaf	98.28	58	1	0	24	81	171	228	8.00E-19	102

Figure 2: BLAST analysis showed a significant match with – A. *Lycopersicon esculentum* - Homeodomain protein mRNA and B. *Solanum lycopersicum* - cDNA, HTC in leaf.

Early blight resistance evaluation (Phenotyping in F1 and F2 plants):

The SSR marker was verified to fit Mendel segregation ratios by the Chi-square test. Chi square test (χ^2) of the mean DI score of each 20 field-grown F1 plants (20 days after inoculation) and their field-grown self-fertilized 30 F2 progeny 15 and 20 days after inoculation with FCF and alternaric acid of *A. solani* was carried out and it was observed that the F1 generation shows 1:1 ratio and F2 generation shows 1:2:1 ratio (Table 3 A). Spearman's rank correlations between scores for *Alternaria* leaf blight resistance of F1 tomato plants (2, 3, 5 and 7days after inoculation) and scores of their self fertilized F2 progeny (3 and 7 days after inoculation) show that the infection of early blight is similar in both F1 and F2 generations. Spearman's rank correlations between F1 and F2 disease scores showed significant non zero correlation ($P= 0.001$) (Table 3 B).

Table 3 A: Chi square test (χ^2) of 20 field-grown F1 plants (7 days after inoculation) and their field-grown self-fertilized 30 F2 progeny 7 days after inoculation with FCF and alternaric acid. All Chi square test values were highly significant ($P=0.001$ for the experiment).

Table3A	F1 generation		
	Observed N	Expected N	Chi square (χ^2)
1	8	10.0	0.4
2	12	10.0	0.4
Total	20		0.8
	F2 generation		
	Observed N	Expected N	Chi square (χ^2)
1	5	6.0	0.166
2	25	18.0	2.722
Total	30		2.888

Table 3 B: Spearman's rank correlations between scores for *Alternaria* leaf blight resistance of F1 tomato plants (2, 3, 5 and 7days after inoculation) and scores of their self fertilized F2 progeny (3 and 7 days after inoculation). All correlation coefficients were highly significant ($P= 0.001$ for the experiment).

Table 3B	F1 (2days)	F1 (3 days)	F1 (5 days)	F1 (7 days)
F2 (3days)	$r_s = 0.43$	$r_s = 0.65$	$r_s = 0.62$	$r_s = 0.66$
F2 (7days)	$r_s = 0.46$	$r_s = 0.61$	$r_s = 0.64$	$r_s = 0.69$

Genotyping in F1 and F2 plants:

All the 120 genotypes of F1 and 145 genotypes of F2 generation were scored visually and screened with SSR 286 primer for the presence and absence of the SSR PCR product (Figure 1 C and 1 D). Bulk segregation analysis was performed with two bulks, a resistant and tolerant bulks produced at F1 level and susceptible, tolerant and resistant bulks at F2 level on the basis of phenotypic analysis of both F1 and F2 plants through *in vivo* bioassay. Figure 1 B show the amplification pattern of two bulks along with parents obtained through the use of SSR-286 as a marker. In bulk segregation analysis, P1 and P2 are controls for F1 and F2 generation tomato plants.

Linkage analysis:

Genetic linkage map of Chromosome 07 and 11 (*Solanum lycopersicum*) with selected SSR markers data sets was constructed and showed that SSR 286 is present on chromosome 7 at a position of 12 cM (Figure 3).

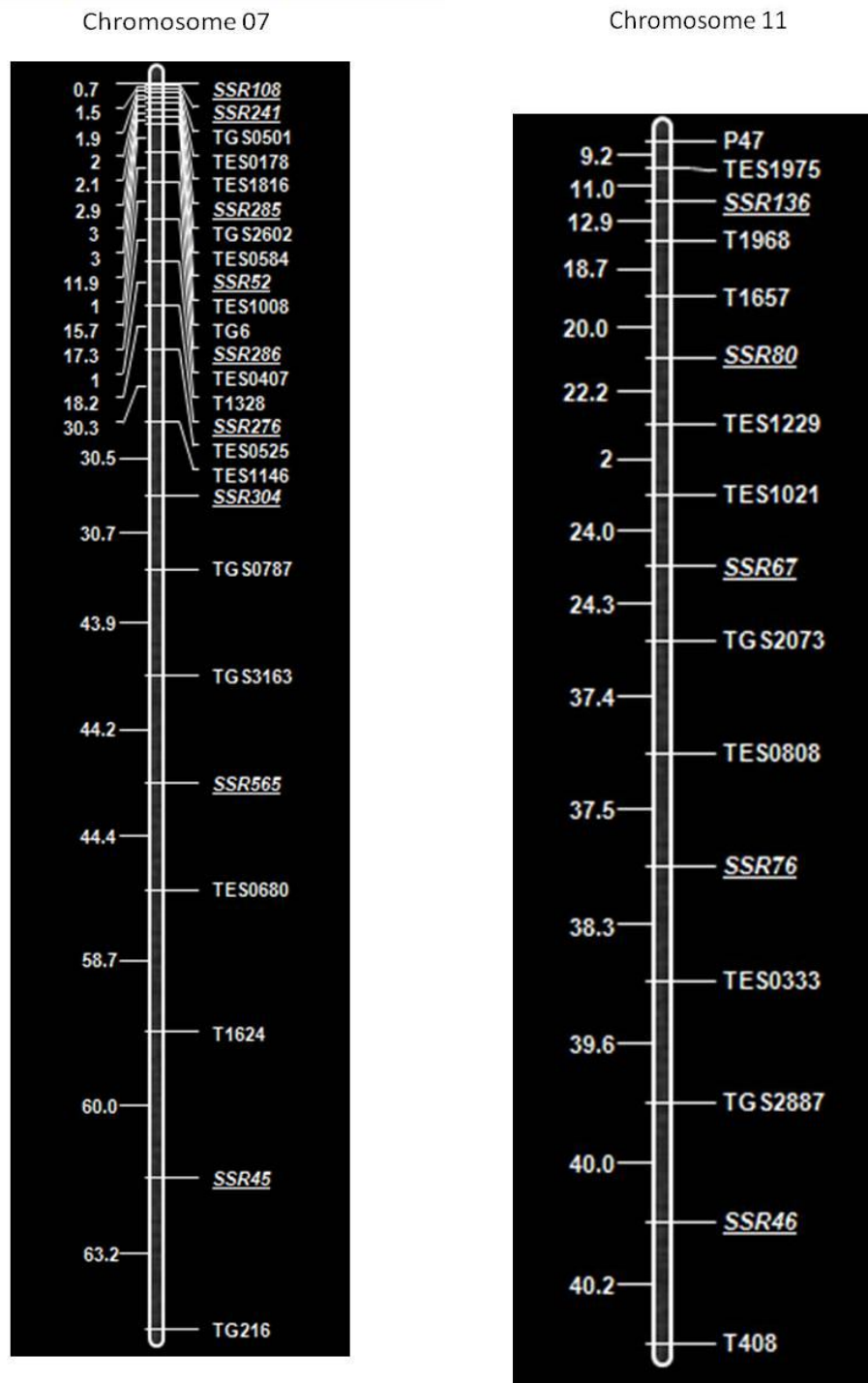


Figure 3: Genetic linkage map of Chromosome 07 and 11 (*Solanum lycopersicum*) with selected SSR markers data sets. Targeted markers are shown as italics underlined on the map. The distances between markers (cM) are listed to the left of each figure part.

QTL mapping:

Nine significant QTLs were identified on chromosome 7 and five significant QTLs were identified on chromosome 11 of tomato (and validated) for early blight resistance. The QTL for SSR 286 with LOD score I (Table 4).

Table 4 Confidence / Positional Assignment of Screened Markers. Here the Interactive map against respective markers was Tomato-EXPEN 2000. Based on the map an early blight QTL (linked to SSR286) having LOD score I was calculated.

Sr. No.	Name of Marker	Position on chromosome (Tomato) Identified	Position (cM)	Confidence
1	SSR 108	7	0	I
2	SSR 241	7	0	F(LOD3)
3	SSR 285	7	2	CF(LOD3)
4	SSR 52	7	3	F(LOD3)
5	SSR 286	7	12	I
6	SSR 276	7	18	I(LOD2)
7	SSR 304	7	30.5	I
8	SSR 565	7	44.2	I(LOD2)
9	SSR 45	7	60.5	F(LOD3)
10	SSR 76	11	40	F(LOD3)
11	SSR 136	11	24	I
12	SSR 46	11	37.5	I
13	SSR 80	11	30	I
14	SSR 67	11	11	F(LOD3)

Discussion

Tomato is an important horticultural crop worldwide, being the second most important fruit after potato in the world. Its production is hindered by fungal diseases (Panthee & Feng 2010). The causal agent of early blight disease, *A. solani* is an important pathogen in tomato representing a risk to crop production. Currently, this disease is primarily controlled through cultural practices and heavy use of fungicides, however, the use of genetic resistance is being sought by tomato breeders and the industry. Resistant cultivars are the most convenient way to control early blight disease. (Cali et al. 2011). To date, simple and/or complex resistance to this disease are being identified and characterized, and considerable progress is being made toward development of resistant tomatoes.

Locke (1948) used detached leaflet assays for evaluation of early resistance as a means to circumvent the influence of growth habit, which may affect the reaction of plants in the field or glasshouse. In the present study, the result of detached leaf bioassays showed similar symptoms as displayed by the *A. solani* infected leaves in

field. The symptoms appeared very fast and were distinct. In our studies, in the laboratory as well as in field conditions, FCF and fungal toxin has been used in screening for resistance. Thus, in the present study, both detached-leaf assay and *in vivo* assay has been helpful in screening and evaluating F1 and F2 generation tomato plants as the infection parameters were similar in both conditions. The phenotypic ratio was 1:1 in F1 hybrid and 1:2:1 in F2 plants was proved by chi-square test. Spearman's rank correlations of natural infection assays applied to results obtained with both F1 plants and their progeny (F2 plants), indicated that the optimal evaluation stage for *Alternaria* leaf blight resistance in tomato is similar and 7 days after inoculation.

SSRs provide highly informative markers because they are co-dominant (unlike RAPDs and AFLPs) and generally highly polymorphic (Daugrois et al. 1991; Devi et al. 2002 & Dickerson et al. 1992). Furthermore, the ease and speed of genetic analysis based on SSRs enhance the ability to make a greater number of SSRs available to the scientific community, at least for most of the species of social and economical value, such as tomato. As codominant and locus specific markers, SSRs have been widely used as tools in genotype identification and population genetic studies in tomato (Chaerani et al. 2007). In the present study, the presence of 108bp band in tolerant variety has been useful as a marker for marker-assisted selection in tomato. Segregation of this molecular marker linked to resistance was observed to segregate in both F1 and F2 generation by performing bulk segregation analysis and by screening of all 120 genotypes of F1 and 145 genotypes of F2 generation plants. In the present study, we found one SSR marker (SSR 286) to be associated to early blight in tomato populations. This SSR marker was further useful for MAS for early blight resistance in tomato breeding programs and segregated in F1 and F2 populations. With the aid of a genetic linkage map, Foolad and co-workers (Foolad et al. 2002 & Zhang et al. 2003) have identified and estimated the magnitude of QTL effects in a *Solanum habrochaites* (syn. *L. hirsutum*) resistance source using backcross populations. Thus, it is necessary to exploit the moderate, but useful degree of resistance within *L. esculentum* species, until true resistance is transferred from wild species without linkage drag. In the present study using interval mapping and selective genotyping approaches, a genetic linkage map was constructed and nine significant QTLs were identified on chromosome 7 and five significant QTLs were identified on chromosome 11 of tomato (and validated) for early blight resistance. The QTL for SSR 286 with LOD score I was potentially useful in marker assisted breeding programs.

Thus, SSR-286 is linked to the resistance to *A. solani*. This information can be further useful for investigation that the gene linked to the SSR marker could be an R gene present in a region of the genome that's enriched in resistance genes. The present study is important for selecting plants with quantitative resistance in the early phase of a breeding program.

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