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Screening of tomato genotypes against bacterial wilt (*Ralstonia solanacearum*) and validation of resistance linked DNA markers

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Abstract

Tomato yield losses are mainly caused due to bacterial wilt (*Ralstonia solanacearum)* throughout the world. The disease management is mainly done through chemicals which lead to health risks. Among non-chemical strategies, development of resistant varieties can be a good alternative. The present study was conducted to screen the fifty seven different tomato genotypes against bacterial wilt using artificial inoculation technique under greenhouse conditions. The plants showing symptoms were examined using ooze test. Morphological and molecular characterization of the bacterial strains isolated from infected plants was carried out and bacterial phylotype I was determined using phylotype-specific multiplex PCR. The bacterial-infected tomato genotypes were categorized into highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, highly susceptible and extremely susceptible lines. Seven tomato genotypes viz.RIL-118, Indam-1004, Arka Samrat, PKM-1, PED, EC-802390, and EC-816105 were found highly resistant to bacterial wilt. These genotypes were also evaluated for plant growth, yield and yield-related traits and fruit quality traits under field conditions. Maximum production (2533 g/plant) was observed for the genotype Arka Rakshak followed by EC816156 (2486 g/plant).while genotype EC815157 (200 g/plant) exhibited lowest production. In addition, fourteen bacterial wilt linked markers were validated for these genotypes where SCAR marker, SCU176-534 was found to be linked with the bacterial wilt resistance significantly. This study will be significant and useful in increasing tomato production and to develop new resistant tomato varieties through marker assisted breeding.

Keywords Bacterial wilt · DNA Marker · Phylotype · Pmx-PCR · *Ralstonia solanacearum* · Tomato

Abbreviations

TZC	Triphenyltetrazolium chloride
SMA	Single marker analysis
SSR	Simple sequence repeats
SCAR	Sequence characterized amplified regions

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Introduction

Tomato *(Solanum lycopersicum* L.) is a major vegetable crop grown throughout the world and is a good source of vitamin A, C and E and contains large quantity of water, calcium and niacin (Olaniyi et al. 2010). It is highly beneficial in reducing the risks of cancer, cardiovascular diseases and osteoporosis (Bhowmik et al. 2012). It has short duration and low input costs because of which it is preferred for cultivation by the farmers. Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the major and widespread diseases throughout the world causing heavy yield losses (Yabuuchi et al. 1995; Aslam et al. 2017a). Apart from tomato, the pathogen also severely infects other solanaceous vegetables (Rao and Sohi 1977; Sinha 1979; Mansfield et al. 2012; Aslam et al.

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2017b). *R. solanacearum* survives in soil saprophytically (Elphinstone et al. 1996) and enters plant roots either through mechanical wounds or natural occurring wounds at root axils during lateral root emergence (Álvarez et al. 2010; Vasse et al. 1995). High-level growth of *R. solanacearum* inside the root leads to wilting symptoms and ultimately causes plant death (Genin 2010). In addition to its lethal effects in tomato, the *R. solanacearum* able to survive in soils for many years and forms latent infections within indigenous weeds (Hayward 1991). Therefore, it is highly difficult to eradicate this bacterium (Hayward 1991; Wenneker et al. 1999).The variability of *R. solanacearum* species has been classified on the basis of different criteria including host range, utilization of different carbon sources and 16S rRNA sequence. Bacterial wilt in tomato is widely distributed in tropical, subtropical and some warm temperate regions of the world (Hayward 1991, 2005; Kelman 1953; Dutta and Rahman 2012). It causes devastating wilt over 450 plant species belonging to 54 families, covering both monocots and dicots (Wicker et al. 2007; Hayward 1991). This disease has been reported to cause 26% loss of fresh fruit production in hybrid tomatoes (Hartman et al. 1991; Dharmatti et al. 2009) and yield losses could reach up to 90.62% (Ramkishum 1987; Dharmatti et al. 2009). Singh et al. (2014) reported the yield losses from 35 to 65% in tomato in low lands during post-paddy cultivation in the dry season (December to May), 60 to 82% in the uplands, and 75 to 90 % under polyhouse cultivation. The disease is generally controlled by various strategies such as crop sanitation, chemicals, disease-free planting material and crop rotation. However, these strategies have one or the other drawbacks and are not successful and the use of chemicals is avoided due to various health risks associated with them. Thus, development of resistant varieties has been employed as an alternative solution to overcome these disadvantages to control bacterial wilt disease (Wang et al. 1998). For development of bacterial wilt resistant varieties, a large number of genotypes have to be screened. Identification of resistant genotype is among the cheapest, simplest and most environmentally safeways to control bacterial wilt disease (Hayward 1991; Mansfield et al. 2012). In addition, the use of molecular markers can increase the efficiency of conventional plant breeding by identifying markers linked to the trait of interest (Collard et al. 2005). Hence, there is a need to identify the DNA marker associated with bacterial wilt disease resistance (Truong et al. 2015; Danesh et al. 1994; Wang et al. 2012; Cao et al. 2009 and Maio et al. 2009). As there is scanty information about the resistant tomato germplasm and DNA marker associated with bacterial wilt disease, therefore, the major objective of the present study was to assess the degree of resistance among the available tomato germplasm against *R. solanacearum* and validate the DNA markers for bacterial wilt resistance in fifty seven genotypes which will prove useful in future for breeding prospective and marker assisted selection.

Materials and methods

Germplasm collection, Isolation of *R. solanacearum* and preparation of bacterial inoculums

Bacterial isolates of *R. solanacearum* from infected plants were collected from University of Agricultural Sciences, Bangalore, India. The presence of pathogen was tested by placing longitudinal sections of the collar portion containing vascular tissues from infected plants in a test tube containing clean water. A streaming test was carried out in infected tomato plant to diagnose the presence of *R. solanacearum*. The outer parts of the infected tissue were removed with a sterilized scalpel and the small pieces were placed in distilled water for 10-15 minutes. The inoculation loop was dipped in the ooze and streaked on triphenyltetrazolium chloride (TZC) media containing 10g/L peptone, 1g/L casein hydrolysate, 10g/L dextrose, 18g/L agar, and 5 ml of 1 % TZC (Kelman 1954). The streaked plates were incubated at 31°C for 24-36 hours. The virulent (pink colour at the centre with fluidal in nature) colonies were isolated and then suspended in sterilized distilled water in screw capped vials and stored at room temperature. The bacterium was further mass cultured on TZC Broth (peptone- 1g, dextrose- 1g, casamino acid-0.1g, water-100ml and TZC-0.005%) at 30°C for 24 hours. The inoculum was prepared by suspending the bacterial growth from 523-medium in sterile distilled water. The concentration of the inoculum was adjusted to 1.35 by taking the OD at 600 nm.

Identification of *R. solanacearum* and phylotype determination

Total bacterial genomic DNA was isolated using method of Amnion Biosciences Private Limited. The 16S rDNA was amplified further using primers OLI 1 (seq), Y2 (seq), Y1 (seq), Y2 (seq) (Table 1) (Seal et al. 1993). A phylotype-specific multiplex PCR (Pmx-PCR) was carried out as described by Fegan and Prior (2005) using a set of four phylotype-specific forward primers (Nmult: 21:1F; Nmult: 21:2F; Nmult: 22: InF and Nmult: 23: AF) with a unique and conserved reverse primer (Nmult: 22: RR) (Table 2). The PCR reaction consisted of2μl of 50ng/μl genomic DNA, 1μl of 50mM $MgCl_2$, 0.8μl of 10mM deoxyribonucleotide triphosphate mix, 1U of Taq DNA polymerase, 17.7μl of 1×PCR buffer, forward and reverse primers, each 1.5μl of 10μmol/L. It was performed in a Mastercycler® nexus gradient at initial denaturation of 94°C for 5 min, 94°C for 15 sec, 59°C for 30 sec, and 72°C for 30 sec (35 cycles) and a final extension of 72°C for 10 min. The amplified fragments were visualized on 1.5%agarose gel electrophoresis.

Table 1 List of 16S rRNA primers and sequence information

Sl. No.	Universal primers	Sequence of the primer (5' to 3')	Expected amplicon size
1	OLI1	GGGGGTAGCTTGCTACCTGCC	~300bp
2	Y2	CCCACTGCTGCCTCCCGTAGGAGT	
3	Y1	TGGCTCAGAACGAACGCGGCGGC	292bp
4	Y2	CCCACTGCTGCCTCCCGTAGGAGT	

Screening of tomato genotypes for bacterial wilt *R. solanacearum*

The fifty seven tomato genotypes were obtained from different institutions and raised on vermicompost filled trays. Twenty-five days old seedlings of tomato were used for inoculation. Before inoculation, the plants were starved for 24 hours by avoiding watering. The tertiary roots of the seedling sectioned using sterilized scissors and plant root dipped in bacterial suspension (*R. solanacearum*) in concentration of OD_{600} 1.35 for twenty minutes before transplanting. The entire experiment was carried out under glasshouse conditions in a controlled completely randomized design (CRD) for bacterial wilt screening with four replications where each replication consisted of five plants. Periodical observations were made on bacterial wilt symptom expression and graded with disease rating scale of 0–5. The observation was repeated on healthy seedlings after forty five days of inoculation through axil puncturing (7ml of inoculums was injected at axil part using sterile syringe). ANOVA (F test) was carried out to compare the mean disease index in tomato genotypes. The bacterial wilt resistance of each genotype was evaluated by following modified disease rating scale of Winstead and Kelman (1952) and Aslam et al. (2017a, b)(Supplementary Table 1 and Supplementary Table 2).

Phenotypic characterization of tomato genotypes

For Phenotypic characterization, all the genotypes were grown under field conditions in randomized complete block design (RCBD) with three replications in kharif season 2014.Phenotypic data was recorded for various quantitative and qualitative parameters such as plant height (cm), number of branches per plant, fruits per cluster, fruit length (mm), fruit width (mm), single fruit weight (g), total number of fruits, total yield per plant(g), fruit locules, and total soluble solids [TSS (%) was measured using a hand refractometer (Swastik Scientific Co., Mumbai, India) and fruit shelf life (days). It was measured as the number of days from breaker stage to the fully ripen stage i.e. first symptoms of deterioration and excessive softening (Yogendra and Gowda 2013).

Molecular characterization of tomato genotypes

Genomic DNA was extracted from the young leaves of tomato genotypes using a modified CTAB method (Saghai-Maroof et al. 1984). The fourteen DNA markers associated with the bacterial wilt were used in this study and association of DNA marker with bacterial wilt resistance loci was analysed using single marker analysis (SMA) (Table 3).

Table 2 List of primers used for multiplex PCR

Sl. No.	Primer name	Primer sequence (5'-3')	Expected amplicon size	Remarks
1	Nmult:21:1F	CGTTGATGAGGCGC GCAATT	144bp	Phylotype I (Asiaticum)
2	Nmult:21:2F	AAGTTATGGACGGT GGAAGC	372bp	Phylotype II (Americanum)
3	Nmult:22:nF	ATTGCCAAGACGAG AGAAGA	213bp	Phylotype IV (Tropical)
4	Nmult:23:AF	ATTACGAGAGCAAT CGAAAT	91bp	Phylotype II (African)
5	Nmult:22:RR	TCGCTTGACCCTAT AACGAA		Amorce reverse unique
6	759R	GTCGCCGTCAACTC ACTTTCC	280bp	Universal *R. solanacearum* specific primers
7	760F	GTCGCCGTCAGCAA TGCGGAATCG		

Table 3 Bacterial wilt linked SSR markers used in detecting polymorphism between the parents

Sl. No.	Primer name	Marker type	Forward sequence (5'-3')	Reverse sequence (5'-3')	Annealing temperature (°C)
1	SCU176-534	SCAR	TTGAACCAAGAATCTATTCG	GAACTTGAATGCCTACCAAA	45.6
2	SCU176-1190F1R1	SCAR	TGCGGATACTATCGGAAATA	CAACTCATTTCAGTCCGATT	55
3	SCU176-1190F2R2	SCAR	TCACTCGGTGAGTCAATAGAT	TTTGCCGATGTTATCATGT	55
4	SLM12-2	SCAR	ATCTCATTCAACGCACACCA	AACGGTGGAAACTATTGAAA GG	55
5	SLM12-10	SCAR	ACCGCCCTAGCCATAAAGAC	TGCGTCGAAAATAGTTGCAT	55
6	TSCARAAG/CAT	SCAR	AGAAGGTCACGGCGAGA	TGAGTCCTGAGTAACTGG	48.1
7	TSCARAAT/CGA	SCAR	TAGATGGAATCCAATATCAGG	AACCACAGTGAAGGAATATACA	52.6
8	TG564	SSR	TGAGGTGCAAATGGGGTAGTG	GCAATGAAGGCCTACAGATGAC	52
9	TG230	SSR	TTGCAGAAGCAACCCTTGAC	TACTTCTCCCCATTCCATGC	50
10	LEaat002	SSR	GCGAAGAAGATGAGTCTAGA GCATAG	CTCTCTCCCATG AGTTCT CCTCTTC	59.5
11	SSRKAU11	SCAR	TGTTGGTTGGAGAAACTCCC	AGGCATTTAAACCAATAGGT AGC	56.5
12	LEat006	SSR	CAT AATCACAAGCTTCTTTCGCC A	CATATCCGCTCGTTTCGTTA TGTAAT	61.3
13	SSR 20	SSR	GAGGACGACAACAACAACGA	GACATGCCACTTAGATCCACCA	58.9
14	SSR128	SSR	GGTCCAGTTCAATCAACCGA	TGAAGTCGTCTCATGGTTCG	55

Statistical analysis

The mean and variance of all the qualitative and quantitative traits mentioned above were subjected to statistical analysis by SAS 9.3 (SAS 2002) and mean was compared with the help of ANOVA. The statistical analysis was carried out using STATISTICA software 7.0 (online free version). The single marker analysis was performed by using SPS Software Version 18.0. Chicago: SPSS Inc.

Results

Characterization of isolates of *R. solanacearum*

Molecular characterization of bacterium was performed by *R. solanacearum* specific DNA markers where the genomic DNA was subjected to PCR and the amplification resulted in a ~300 base pair (bp) product for OLI1 and Y2 primers (Fig. 1) and 292bp product for Y1 and Y2 primers (Fig. 2). Further, the Pmx-PCR of bacterial isolate yielded 144bpand 280bp amplicons indicating that the isolate was *R. solanacearum* and belonged to phylotype I. (Fig.3).

Screenings of tomato genotypes for bacterial wilt *R. solanacearum*

During screening in glass house, the wilt symptoms appeared in three to four days after inoculation. The disease was further confirmed by bacterial ooze test, isolation of bacteria from infected plants and based on the colony morphology and peculiar symptom of wilting of leaves. The mean sum of squares for disease index was calculated and significant differences were observed among the genotypes (Supplementary Table 3). On the basis of mean disease index, seven genotypes viz. RIL118, INDAM1004, PKM-1, EC802390, PED, Arka Samrat and EC816105 appeared as highly resistant. EC802395 was the only genotype found to be resistant while twelve genotypes were categorized as moderately resistant to the bacterium. On the other hand, twenty genotypes were rated as moderately susceptible and five viz. RIL130, RIL108, RIL 119, EC816102 and EC316108 showed susceptible reaction. Ten genotypes appeared as highly susceptible and among them Pusa Ruby and IC39457 were found to be extremely susceptible (Table 4).

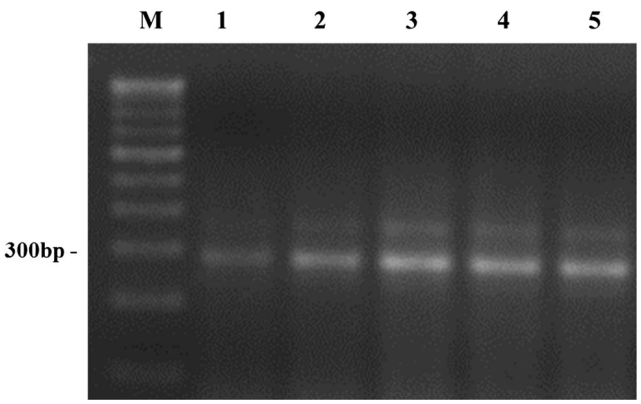

Fig. 1 Electrophoretic analysis of PCR amplified DNA from five isolates of *R. solanacearum* using the primers OLI1 &Y2. The expected amplicon size with primer combination OL1 and Y2 was~300bp

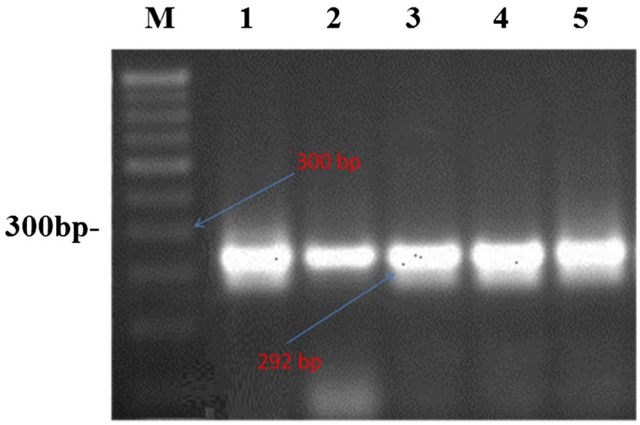

Fig. 2 Electrophoretic analysis of PCR amplified DNA from five isolates of *R. solanacearum* using the primers Y1 and Y2. The expected amplicon size with the primer combination Y1 and Y2 was 292bp

Phenotypic characterization under field conditions

The mean sum of squares for plant growth, yield, yield attributing traits and fruit quality traits were calculated and significant differences were observed among the genotypes for all the characters (Supplementary Table 4). The mean performance of tomato genotypes used in the present study indicated that no single parental genotype was superior in respect of all the traits studied (Supplementary Table 5). The maximum yield per plant was observed in genotype Arka Rakshak (2533 g) followed by EC816156 (2486 g) and EC802398 (2430 g) while, EC815157 (400 g) exhibited lowest yield followed by EC816103 (445 g) and EC802392 (549 g). RIL-160, RIL-119 and RIL-118(70 Days) recorded maximum fruit shelf life followed by EC802395, EC802404, EC802400 and EC816107 (50 Days) while it was lowest for Pusa Ruby (19 Days) (Supplementary Table 5).

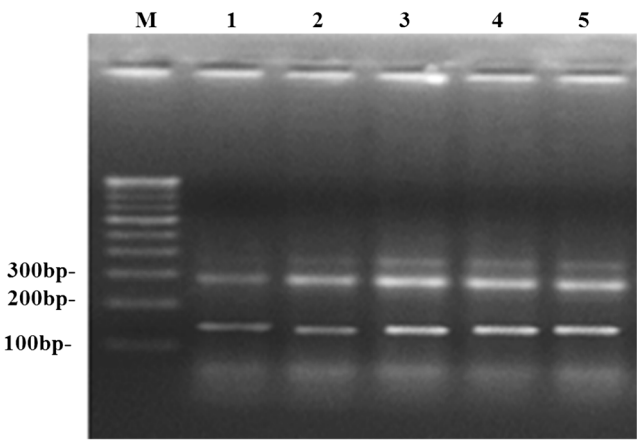

Fig. 3 PMX-PCR using a phylotype specific primer showing PCR product of 280bp amplicon for bacterial isolates and 144bp amplicon for phylotype I

Molecular characterization of tomato germplasm

Bacterial wilt disease linked SSR and SCAR markers were used for molecular characterization and single marker analysis was performed between the marker loci and relevant traits. The results revealed that out of the fourteen markers analysed, one marker, SCU176-534 was found to be associated with bacterial wilt resistance (Supplementary Table 6). The PCR amplicon of 400bp was found to be associated with susceptible parent while 370bp depicted resistant parent (Fig. 4).

Discussion

With pink/ light red colored colonies on TZC media after 24 hours of inoculation confirmed the presence of bacterial isolate, *R. solanacearum* which is similar to the previous reports by Rahman et al. (2010); Ahmed et al. (2013); Popoola et al. (2015); Kumar et al. (2017). Partial sequences of 16S rDNA and 16S rRNA genes are excellent targets for identification of bacteria at the species level as they are species-specific and available in multiple copies in microbial genome (Woese 1987). In the present study, a ~300 base pair (bp) product was obtained for (OLI1& Y2) and 292bp amplicon for (Y1 & Y2) on PCR amplification confirming that all the isolates were *R. solanacearum*. These findings were in accordance with Seal et al. (1993); Chandrashekara et al. (2012a, b) and Kumar et al. (2017). The bacterial isolate was further characterized by a set of four phylotype-specific forward primers (Nmult:21:1F: Nmult:21:2F: Nmult:22:InF: and Nmult:23:AF:) with a unique and conserved reverse primer (Nmult:22:RR:) and set of *R. solanacearum* specific primers (759R and 760F) giving 144bp and 280bp amplicon products (Fegan and Prior 2005; Prior and Fegan 2005; Wang et al. (2013); Popoola et al. (2015) and Kumar et al. (2017). These results revealed that all isolates were *R. solanacearum* and belonged to phylotype I. These results were supported by Fegan and Prior (2005); Prior and Fegan (2005). Based on the disease index (Aslam et al. (2017a, b), the fifty seven genotypes screened were categorized into seven types - highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, highly susceptible and extremely susceptible. The disease confirmation was proved by identification of the colony morphology, peculiar symptom of wilting of leaves maintaining their green color (Fig. 5), bacterial ooze test and isolation of bacteria from infected plants. Ooze test is the quick diagnostic test for detection of bacterial wilt (Manasa et al. 2015). In the present study, out of fifty seven genotypes screened for phenotype, seven genotypes were found to be highly resistant, one genotype was resistant, twelve genotypes were moderately resistant, twenty genotypes were moderately susceptible, five genotypes were susceptible, ten genotypes were highly susceptible and two genotypes were extremely

Table 4 Screening of tomato (*Solanum lycopersicum* L.) genotypes against bacterial wilt (*Ralstonia solanacearum*)

Sl. No.	Tomato Genotypes	Source	Mean Disease Index	Reaction
1	RIL-160	UAS, Bangalore	0.67	Highly susceptible
2	RIL169	UAS, Bangalore	0.69	Highly susceptible
3	RIL127	UAS, Bangalore	0.66	Highly susceptible
4	RIL130	UAS, Bangalore	0.51	Susceptible
5	RIL108	UAS, Bangalore	0.5	Susceptible
6	RIL119	UAS, Bangalore	0.54	Susceptible
7	RIL118	UAS, Bangalore	0.07	Highly resistant
8	L121	IIHR, Bangalore	0.47	Moderately susceptible
9	ANAGA	Kerala Agricultural University	0.33	Moderately resistant
10	PUSA RUBY	IARI, New Delhi	0.94	Extremely susceptible
11	SANKRANTI	UAS, Bangalore	0.42	Moderately susceptible
12	VAIBHAV	UAS, Bangalore	0.49	Moderately susceptible
13	ARKA MEGHALI	IIHR, Bangalore	0.4	Moderately resistant
14	IC39457	IIHR, Bangalore	1	Extremely susceptible
15	ARKA ABHA	IIHR, Bangalore	0.66	Highly susceptible
16	INDAM1004	Indo American Hybrid Seeds India Pvt. Ltd. Bangalore	0.09	Highly resistant
17	EC816101	IIHR, Bangalore	0.41	Moderately susceptible
18	NS2535	Namdhari Seed Pvt. Ltd. Bangalore	0.41	Moderately susceptible
19	EC816097	IIHR, Bangalore	0.43	Moderately susceptible
20	ARKA VIKAS	IIHR, Bangalore	0.43	Moderately susceptible
21	CRA66	TNAU, Coimbatore	0.31	Moderately resistant
22	PKM-1	Ashoka Seed Pvt. Ltd. Bangalore	0.08	Highly resistant
23	ARKA ALOK	Ashoka Seed Pvt. Ltd. Bangalore	0.68	Highly susceptible
24	HAWAII-7998	IIHR, Bangalore	0.31	Moderately resistant
25	KASI VISHESH	IIHR, Bangalore	0.47	Moderately susceptible
26	KASI AMRUT	IIHR, Bangalore	0.47	Moderately susceptible
27	EC802395	IIVR, Varanasi	0.3	Resistant
28	EC802393	IIVR, Varanasi	0.4	Moderately resistant
29	EC802403	AVRDC, Hyderabad	0.71	Highly susceptible
30	EC802401	AVRDC, Hyderabad	0.68	Highly susceptible
31	EC802404	AVRDC, Hyderabad	0.65	Highly susceptible
32	EC802400	AVRDC, Hyderabad	0.4	Moderately resistant
33	EC802396	AVRDC, Hyderabad	0.68	Highly susceptible
34	EC802398	AVRDC, Hyderabad	0.4	Moderately resistant
35	EC802391	AVRDC, Hyderabad	0.4	Moderately resistant
36	EC802390	AVRDC, Hyderabad	0.06	Highly resistant
37	S22	AVRDC, Hyderabad	0.31	Moderately resistant
38	PED	AVRDC, Hyderabad	0.12	Highly resistant
39	EC802397	AVRDC, Hyderabad	0.39	Moderately resistant
40	EC802392	AVRDC, Hyderabad	0.41	Moderately susceptible
41	EC802399	AVRDC, Hyderabad	0.43	Moderately susceptible
42	EC816103	AVRDC, Hyderabad	0.5	Moderately susceptible
43	ARKA SAMRAT	AVRDC, Hyderabad	0.08	Highly resistant
44	ARKA RAKSHAK	AVRDC, Taiwan	0.31	Moderately resistant
45	EC816102	AVRDC, Taiwan	0.52	Susceptible
46	EC816099	AVRDC, Taiwan	0.41	Moderately susceptible
47	EC816156	AVRDC, Taiwan	0.44	Moderately susceptible
48	EC816098	AVRDC, Taiwan	0.78	Highly susceptible
49	EC816107	AVRDC, Taiwan	0.43	Moderately susceptible

Table 4 (continued)

Sl. No.	Tomato Genotypes	Source	Mean Disease Index	Reaction
50	EC815157	AVRDC, Taiwan	0.43	Moderately susceptible
51	EC816100	AVRDC, Taiwan	0.47	Moderately susceptible
52	EC316108	AVRDC, Taiwan	0.52	Susceptible
53	EC816104	AVRDC, Taiwan	0.38	Moderately resistant
54	EC816106	AVRDC, Taiwan	0.43	Moderately susceptible
55	EC816105	AVRDC, Taiwan	0.11	Highly resistant
56	EC802402	AVRDC, Taiwan	0.43	Moderately susceptible
57	EC802394	AVRDC, Taiwan	0.41	Moderately susceptible

susceptible. Our score of resistant genotypes for bacterial wilt were significantly higher in comparison to a recent study of Aslam et al. (2017a). They have screened thirty tomato cultivars for their resistance to bacterial wilt and observed none of the cultivars were immune or highly resistant to *R. solanacearum*. They found two cultivars namely Early King and Lerica as resistant (R), and four cultivars viz. Red Hero, Giant Cluster, Red Ruby and Red Stone as moderately resistant (MR). Eleven cultivars each appeared as moderately susceptible and susceptible, while two cultivars (Bonny Best and Roma VF) were assessed as highly susceptible (HS) to the bacterium. Sangrit et al. (2011) reported thirty tomato varieties tested against bacterial wilt incidence under artificial inoculum and found that none of the resistant cultivars were detected under the rainy season. In another study, Aslam et al. (2017b)were assessed twenty eight chili cultivars for their relative resistance to a highly virulent strain of *R. solanacearum* biovar 3 (RsBd6). They reported two cultivars namely Skyline-II and Hifly as highly resistant, Sanam as the only resistant cultivar while twelve cultivars were categorized as moderately resistant to the bacterium. On the other hand, seven cultivars were rated as moderately susceptible and three as susceptible. They also reported two cultivars, Talhari and Maxi, as highly susceptible and California Wonder as extremely susceptible. Nath et al. (2015) adopted root inoculation technique which is similar to the present study except age of seedlings and bacterial inoculum level. In the present study, the disease index of Pusa Ruby and Arka Vikash was similar to the previous report by Nath et al. (2015).Such studies were also reported by Singh et al. (2015) and Tiwari et al. (2012). In the present study, double the concentration of bacterial inoculum (OD_{600}= 1.35) was used in comparison to previous report by Kishun and Chand 1990; Chellemi et al. 1994; Nguyen and Ranamukhaarachchi 2010; Tiwari et al. 2012 where they have used lower bacterial inoculum (OD_{600}=0.775). Double the bacterial inoculum concentration from the present study was found to be highly effective. In addition, the present study also adopted both inoculation methods i.e. root dipping and axil puncturing method which puts more selective pressure in comparison to other previous studies. It is revealed from the present study that both inoculation methods and inoculation concentrations were helpful to identify the resistant lines against bacterial wilt.

Wang and Lin (2005) reported that the stability of bacterial wilt resistance in tomato is highly affected by pathogen density, pathogen strains, temperature, soil moisture and presence of root knot nematode. Winstead and Kelman (1952) reported that the plant age, inoculum concentration, inoculation method and temperature also affected bacterial wilt incidence. In the present study, the inoculation time of twenty minutes was

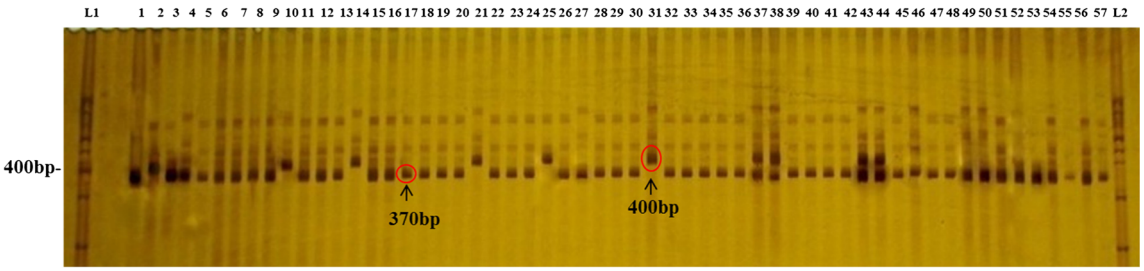

Fig. 4 Poly Acrylamide Gel Electrophoresis (PAGE) image of SCAR marker SCU176-534. 1. RIL-160, 2. RIL-169, 3. RIL-127, 4.RIL-130, 5. RIL-108, 6.RIL-119, 7.RIL-118, 8. L121, 9.Anaga, 10. Pusa Ruby, 11. Sankranti, 12. Vaibhav, 13. Arka Meghali, 14. IC395457,15. Arka Abha, 16. Indam-1004, 17. Ec-816101,18. NS-2535, 19. Ec-816097,20.Arka Vikash, 21.CRA-66, 22.PKM-1, 23. Arka Alok, 24.Hawaii-7998, 25. Kashi-Vishesh, 26. Kashi-Amruth, 27. Ec-802395, 28. Ec-802393, 29.Ec-802403, 30.Ec-802401, 31.Ec-802404,32.Ec-802400, 33.Ec-802396, 34.Ec-802398, 35. Ec-802391, 36. Ec-802390, 37. S-22, 38. PED, 39.Ec-802397, 40. Ec-802392, 41.Ec-802399, 42. Ec-816103, 43. Arka Samrat, 44. Arka Rakshak, 45. Ec-816102, 46. Ec-816099, 47. Ec-816156, 48.Ec-816098, 49.Ec-816107, 50.Ec-815157, 51. Ec-816100, 52.Ec-316108, 53. Ec-816104, 54.Ec-816106, 55.Ec-816105, 56. Ec-802402,57. Ec-802394

Fig. 5 Overview of tomato genotypes showing the bacterial wilt symptoms under greenhouse conditions

used on twenty day old tomato seedlings which were found very effective. The sources of resistance to bacterial wilt disease either monogenic or polygenic and the genetics of resistance to this disease is very complex (Acosta et al. 1964). Tikoo et al. (1990) recorded that simple genetic control may underlie the bacterial wilt resistance in some resistance stocks originating from the tropical areas whereas Grimault et al. (1995) reported that a single dominant gene is involved in the inheritance of resistance to bacterial wilt in tomato. Oliveira et al. (1999) showed the importance of additive gene effects on the resistance against bacterial wilt, while, Monma et al. (1997) reported that the bacterial wilt resistance is partially recessive. Analysis of variance revealed that the variance among genotypes was highly significant indicating the presence of substantial genetic diversity for all the characters. Here, none of the genotype was superior for all the characters. The highest yield performance was recorded for Arka Rakshak whereas it was lowest for EC815157. This result was in agreement with previous findings (Yogendra and Gowda 2013; Kumar and Gowda 2014, 2016; Kumar et al. 2015). The maximum fruit shelf life was recorded in RIL-160, RIL-119 and RIL-118 due to the fact that they were derived from *alc* parent while it was lowest in Pusa Ruby. Yogendra and Gowda (2013) observed the fruit shelf-life *alc* line was 44 days which is significantly higher than that of the other ripening gene mutants *rin* (38days) and *nor* (38.5 days). Similarly, Kumar et al. (2015) identified some of the RILs having fruit shelf-life of more than 60 days. These lines were derived from *alc* parent which is responsible for delayed ripening.

From the present study, out of fourteen DNA Markers studied in tomato germplasm, only one marker, SCU176-534 was associated with the bacterial wilt resistance at significant level $P < 0.0001$. This result was in agreement with the results obtained by Truong et al. (2015) in F_9 lines of *S. lycopersicum* Hawaii 7996 (resistant parent) and *S. pimpinellifolium* WVa 700 (susceptible parent).

Conclusion

The resistant tomato germplasm identified from the present study could be used directly in tomato breeding programs against bacterial wilt. The resistant varieties will be useful as they could either delay the initial infection against bacterial wilt of tomato or slow down the rate of wilting if there is initial infection. The moderately resistant line, EC802398 with high shelf life and good yield performance can be put forward for multi-location trials. The SCAR marker, SCU176-534 found to be associated with bacterial wilt resistance could be used for marker assisted selection in breeding programs.

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