Research Articles

Indian Journal of Agricultural Sciences **88** (10): 1502–9, October 2018/Article

Screening of elite eggplant (*Solanum melongena*) genotypes for bacterial wilt (*Ralstonia solanacearum*) in field conditions and their genetic association by using SSR markers

P S KHAPTE[1], T H SINGH[2] and D C LAKSHMANA REDDY[3]

ICAR-Central Arid Zone Research Institute, Jodhpur, Rajasthan 342 003

Received: 05 May 2018; Accepted: 26 June 2018

ABSTRACT

Bacterial wilt (*Ralstonia solanacearum*) of eggplant (*Solanum melongena* L.) has been a major production constraint in the tropics and sub tropics of the world. Lack of understanding about host, pathogen and environment perhaps one of the reasons for limited success in controlling this disease. In present investigation, six elite genotypes of eggplant were screened against bacterial wilt in field conditions and later SSR screening was done to identify markers linked for resistant and susceptible lines, and their genetic clustering. At field level, the genotypes like CARI-1, IIHR-7 and IIHR-500A had shown resistant to bacterial wilt and recorded good yield. Further, 245 SSRs markers used for screening had shown good amplification, however only 37 primers were polymorphic, and microsatellite allele sizes were determined at their 74 loci. The average polymorphic information content was 0.315 and it ranged from 0.239 to 0.375. The SSR emh21J12 shown 170 bp band for the resistant genotypes and 160 bp band for the susceptible ones. Another SSR emf01K16 gave the unique banding pattern in resistant genotypes at 250 bp and susceptible at 260 bp. Dendrogram analysis classified these six genotypes into three main clusters. Cluster I consists of IIHR-575, IIHR-108 and IIHR-500A, where IIHR-500S was solitary. The cluster II consists Rampur local and IIHR-7 whereas, cluster III was solitary comprising CARI-1. It was revealed that the co-dominant markers such as SSR proved to be high effective tool in discriminating between resistant and susceptible genotypes, and classifying these genotypes based on genetic diversity. Hence, the field and molecular markers screening reveals that eggplant genotypes IIHR-7, IIHR-500A and CARI-1 are resistant to bacterial wilt and these resistant genotypes can be used for further breeding programme, and the identified SSR markers can be useful tool for marker assisted selection for bacterial wilt in eggplant.

Key words: Bacterial wilt, Eggplant, Microsatellite, Molecular markers, *Solanum melongena*, Yield

Eggplant or brinjal (*Solanum melongena* L.) is an important solanaceous crop of sub-tropical and tropical regions of the world. It is an important vegetable grown in India and other parts of the world (Khapte *et al*. 2012, Frary *et al*. 2007). Eggplant has ayurvedic medicinal properties and is good for diabetic patients. It has been recommended as an excellent remedy for those suffering from liver complaints (Shukla and Naik 1993). It is rich in vitamin A, folic acid, phosphorus and protein (Gopalan *et al*. 2007). India is home for almost all types of brinjal cultivars, found anywhere in the world (Martin and Rhodes 1979). A large number of eggplant cultivars are grown in India, as eggplant has got consumer preference based on fruit colour, size and shape and it varies from region to region. The world acreage of eggplant is about 1.86 million ha with an estimated production of 49.78 million tonnes and productivity of 26.70 tonnes/ha. In India it is cultivated in an area of about 0.71 million ha with an estimated production of 13.55 million tonnes and productivity of 19.10 tonnes/ha (Anon. 2014).

Successful cultivation of eggplant crop has been hindered due to infestation of many insect pests and diseases. Among these, bacterial wilt disease is the most devastating and a limiting factor caused by *Ralstonia solanacearum* throughout the tropical, sub-tropical and temperate regions of the world (Hayward 1991, Gopalakrishnan *et al*. 2014); it is the second most important plant pathogen bacterial disease (Mansfield *et al*. 2012). Bacterial wilt is an important disease of many plant species especially Solanaceae (Liu *et al*. 2016); it perpetuates in the soil, enters the plant through the roots, progressively invades the stem vascular tissues and blocking of the vessels by bacteria, and finally it leads to partial or complete wilting of plant (Sarkar and Chaudhuri 2016). Understanding the host and pathogen is a pre requisite for devising proper strategies for control of disease. Since,

[1]Scientist (e mail: khaptepratap@gmail.com), Division of Integrated Farming System, ICAR-Central Arid Zone Research Institute, Jodhpur 342 003. [2]Principal Scientist (e mail: thsingh@iihr.res.in), Division of Vegetable Crops, [3]Scientist (e mail: lreddy@iihr.res.in), Division of Biotechnology, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka 560 089.

breeding for resistance remains the best control strategy. In India, bacterial wilt is of major concern and serious in parts of Karnataka, Kerala, Odisha, Maharashtra, Madhya Pradesh, Chhattisgarh, West Bengal and Himachal Pradesh (Rai *et al*. 1975, Rao *et al*. 1976, Sarkar and Chaudhuri 2016). Yield losses in eggplant due to bacterial wilt ranging from 65 to 70% (Das and Chattopadhyay 1953).

Developing commercially acceptable eggplant varieties and hybrids with resistance to bacterial wilt (BW) has been a goal of many breeding programmes (Yang *et al*. 2014, Gopalakrishnan *et al*. 2014, Bainsla *et al*. 2016). In view of this, it is essential to identify the lines possessing resistance to BW and their combining ability with lines having good horticultural attributes. Simple sequence repeats (SSRs) or microsatellite markers are ideal DNA markers for marker assisted selection, genetic mapping and population studies, due to its hypervariability, multiallelic, co-dominant nature, reproducibility, genomic abundance and coverage (Kalia *et al*. 2011). These SSRs polymorphisms at individual loci are amplified and detected by PCR, using locus-specific flanking region primers where the sequence is known (Amin *et al*. 2010). Hence, search for resistant source and incorporating the genes in the commercial cultivars would be the best approach (Bainsla *et al*. 2016).

For molecular breeding, it is necessary to detect polymorphisms among cultivars and breeding lines. However, in solanaceous plants, a low frequency of polymorphism among cultivars and intraspecific lines has been reported (Nunome *et al*. 2003, Stagel *et al*. 2008). In eggplant, SSR markers have been developed recent past and are being mainly used for assessing the genetic diversity and genome similarity in the related species (Nunome *et al*. 2003, 2009, Demir *et al*. 2010, Fukuoka *et al*. 2012, Chourey *et al*. 2017), genetic diversity of eggplant and wild species (Caguiat and Hautea 2014); parent specific SSRs (Liu *et al*. 2016), protection of eggplant germplasm (Prohens *et al*. 2011) and hybrid purity testing (Kumar *et al*. 2014). Studies pertaining to application of SSR markers for identification of diseases like BW in eggplant are very meagre, literature survey reveals that SSRs in eggplant has used either molecular diversity or hybrids purity analysis. Hence, the present study was aimed with an objective to screen the elite eggplant genotypes (advance breeding line) in field condition for BW and to identify SSR markers that can be used for discriminating resistant and susceptible genotypes, and to know their genetic diversity.

MATERIALS AND METHODS

The present study was carried out at ICAR-IIHR, Bengaluru, India. The six elite eggplant genotypes in different colour and shape background were used for screening for growth, yield and bacterial wilt (BW) in field condition during 2014 and further they were also screened for BW using SSR markers during 2015. These genotypes were preliminary screened by our research group (Gopalakrishnan *et al*. 2014). The pedigree and characters of eggplant genotypes used in the study are given in Table 1.

Table 1 Pedigree and characters of eggplant genotypes used in the study

Genotype	Characters
CARI-1	It is a local collection from Andaman and Nicobar Islands, India. The plant are semi spreading type, fruits are light green and oblong in shape.
IIHR-108	A pure line selection from IIHR-193, developed at IIHR, Bengaluru.Spreading plant habit bearing green small fruits borne on clusters.
IIHR-7	An advanced breeding line developed from derivative of cross between BWR-21×IIHR-322. The plants are spreading and fruits are green long.
Rampur Local	It is a local collection from Kanpur area of Uttar Pradesh. The plants are tall and spreading, flowers are white in colour and fruits are dark green in colour.
IIHR-500A	It is a local collection from Gokak sub division of Karnataka. The plants are well branched, fruits are solitary bearing habit and purple with white stripes.
IIHR-575	It is a local collection from Rahuri, Maharashtra. The plants are dwarf and spreading, fruits are oval in shape and having dark purple with white strips.

The experimental field is located at an altitude of 890 m above MSL, 13° 58' N latitude and 78° E longitude. Where the average monthly rainfall was 82.23 mm and mean high and low temperature of 29.6ºC and 19.2 ºC respectively. The eggplant genotypes was raised in pro-trays in sterile coco-peat media. Further, one month old seedling of these genotypes was transplanted to wilt-sick soil which had a pathogen population of 1.0×10^8 cfu (colony forming unit) per g soil. The genotypes were replicated thrice with 30 plants in each replication, in randomized block design spacing at 60 × 50 cm for evaluation during June-December, 2014. Genotype screening for BW was done in infested soil to permits the assessment of field resistance by allowing the infection process to take place under natural environments.

The regular observations were recorded on incubation period and per cent BW incidence. To measure, length of the incubation period, an average of 10% of wilted plants from each genotype was taken (Atabug and Juan 1981) and bacterial infectivity was confirmed by the ooze test, as also by isolating the bacterium on triphenyl tetrazolium chloride medium (Gopalakrishnan *et al*. 2014). The wilt symptom appears at the time of flowering, which is approximately 30 to 40 days after transplanting. The wilted plants per accession were recorded and graded on 0-5 scale, as per Hussain *et al*. (2005) and Gopalakrishnan *et al*. (2014). The rating scale is described in Table 2.

The data pertaining to plant height (cm), primary branches, fruit length (cm), fruit diameter (cm), fruit number, average fruit weight (g), fruit yield (t/ha.) and BW incidence (%) were recorded. Fruit yield is an exponential on the basis of per plant yield. The package of practices for successful cultivation of the crop was followed. Out of 30 plants, five plants were selected arbitrarily from each replication

Table 2 Rating scale for bacterial wilt in eggplant

Rating	Bacterial wilt incidence (%)
0	Highly resistant with no wilt symptom
1	Resistant with 1 -10% wilted plants
2	Moderately resistant with 11 -20% wilted plants
3	Moderately susceptible, with 21-30% wilted plants
4	Susceptible with 31- 40% wilted plants
5	Highly susceptible with > 40% wilted plants

were tagged for the purpose of recording observations on aforementioned characters.

In succeeding season, the seeds material of these elite genotypes CARI-1, IIHR-108, IIHR-7, Rampur Local, IIHR-500A and IIHR-575 were obtain from selfed plant which are maintained at Division of Vegetable Crops, IIHR, Bengaluru. Seeds were sown in pro-trays in coco-peat media and healthy seedling were raised of these genotypes. Genomic DNA was extracted using a modified cetyl trimethylammonium bromide (CTAB) method as described by Doyle and Doyle (1987). The SSR markers were selected from the sequence information available in the published literature (Nunome *et al*. 2009). The details of the 37 polymorphic primers with their sequence is presented in Table 3 were chosen for performing analysis. PCR reactions were performed in a 15 µL volume mixture with 1X buffer (20 mM $MgCl_2$, 1 mM dNTP's, 0.5U *Taq* polymerase. A 1-µM forward and reverse primer and 20-ng template DNA in a Primus advanced 96 thermal cycler was used. A touchdown PCR protocol was applied, consisting of a 94°C/5 min denaturation, 11 cycles of 94°C /30 s, 60°C /30 s decreasing by 0.5°C per cycle, and 72°C /60 s, followed by 30 cycles of 94°C/30 s, 55°C /30 s, and 72°C/60 s. The final extension was carried at out 72°C for 10 min and PCR products were stored at 4°C. PCR products were separated on 3% high-resolution agarose, the gel was stained with ethidium bromide, and the bands were scored. Amplicon sizes were determined by comparison with a 100-1000 bp ladder loaded in a well.

The amplified products were separated on 3% high resolution agarose gel and allele sizes were determined by comparison with a 100-1000 bp ladder (Fermentas) and data were analyzed using UVI-PROPLATINUM 2.0 software (ver.12 Cambridge, UK). The allele frequency analysis module of Cervus 3.0 (Kalinowski *et al*. 2007) was used to generate allele number (K), allele frequencies, expected heterozygosity (He) and polymorphic information content (PIC) (Botstein *et al*. 1980). The genetic cluster analysis was performed using DARwin5 and an unweighted pair group method with arithmetic mean (UPGMA) dendrogram was prepared using an unweighted pair group average, 1-Pearson method.

Data for field parameters were statistically analyzed by analysis of variance using the SPSS software package (SPSS version 22 for Windows, 2013). To separate treatment means within each variable measured, Duncan's multiple range test was performed at P = 0.05.

RESULTS AND DISCUSSION

The growth and yield parameters of the eggplant genotypes under the study is presented (Table 4 and 5). The genotype IIHR-7 outperformed and was statistically significant for plant height, fruit length, fruit number and fruit yield, moreover, it was resistant to bacterial wilt. The genotype IIHR-7 is an advance breeding line from the cross involving one of the resistant parent have contributed to impart resistance. Among the different lines of eggplant screened by Khapte *et al*. (2012) found that eggplant genotype IIHR-574 were superior for growth and yield traits due to the genetic potential. Further, eggplant genotypes screening for BW by Gopalakrishnan *et al*. (2014) reported that the resistant accessions had longer incubation period compared to the susceptible one which take longer time to produce symptoms. Oliveira *et al*. (2014) reported CNPH 785 as resistant eggplant genotype to BW and suggested its resistance mechanism by depriving the bacterium to multiply or spread in the host. Furthermore, genotypes CARI-1 and IIHR-500A were also resistant to bacterial wilt but they reported lesser yield in comparison to IIHR-7. The genotype IIHR-108 was highly susceptible (92.9%), whereas Rampur Local and IIHR-575 was moderately susceptible (26.0%) and susceptible (35.4%) respectively in field conditions for BW which also reflected on their yield performance (Table 5). The results are in confirmation with finding of Chaudhary and Sharma, (2000), Mondal *et al*. (2013) for screening of eggplant genotypes for bacterial wilt in the field under natural conditions. The eggplant genotype IIHR-7 had shown resistance to BW and also performed better for most growth and yield parameters, it seems to be potential genotype yielding 38.4 tonnes/ha.

The symptoms of BW in field conditions on infected genotypes of eggplant in field conditions (Fig 1). The ooze test were performed on symptotic plant in conical flask containing distilled water, which showed the milky whitish ooze from the vascular tissue of infected genotypes (Fig 2). Thus, the infected plants of genotypes IIHR-108, Rampur Local and IIHR-575 was positive in ooze test.

Among 245 SSR used all of them had shown good

Fig 1 Bacterial wilt infestation in field.

Table 3 List of polymorphic SSR markers used in the present study

Primers	Forward sequence	Reverse sequence
emg21I17	ACAACATTTCTAAGGGCCTTCACG	GTTTGGGCATATTTGGCACTTGTTGAAT
emf11A04	ATACATTCTACCCAACTATCCTTCCA	GTTTGTTCGTACTTCATCGTGTTGGC
emf11A03	ATACATTCTACCCAACTATCCTTCCA	GTTTGTTCGTACTTCATCGTGTTGGC
emd18E02	AGTGCTCTGAACTCCTTTCCTTCA	GTTTCCCTAAAAGGAATATGTGCTCTGG
Eggplant37	AGGCCTGTTTCAATCACCTG	TTCTTCCAGAGCATTGCCCAT
Eggplant 45	CTCATAGACTAATAACCATCGAGAAA	ATTTGCATGGGACCTGATCT
Eggplant 46	AATAAAGTTATGCCACAGGGC	CACCCTTCACCACCAACAAT
Eggplant 55	CGATGAGAGGACCGGTAAGA	CCACAACACAACACAACACAA
Eggplant 66	AAATAGGCACCCAATCGAGA	GATGATTTGCTTCCTTCCCA
Eggplant 100	TGAGGTGGAGAGGTGAAGAAA	CCCAACTTCCACCATGACA
Eggplant 103	ACCGCCGAAACATCACTAAA	GTGACCTTGCGCTGGAAAT
Eggplant 105	TTTGATGAAACCCTGCCTTT	TCCCTAACAATGTATGTCGTGAA
Eggplant 109	GAGGTCCAGATTTATCTCAGGG	TAACAGCTGCTCGTCAGAGG
Eggplant 117	TGAGGTGGAGAGGTGAAGAAA	CCCAACTTCCACCATGACA
emb01D10	AAGAATCGGTCCTCTTTGCATTGT	TGCTTTTCACCTCTCCGCTATCTC
emh02A04	ATTGATTTCTAAGCGCACTCGCAC	GTTTAGGGATTGTTCAATTCTGGGTCTG
emf01K16	ATTTGGACAAGAACAAGGATGGCT	GTTTCACTCACAATTCGAGACACTCGGT
emh11H03	ATAACTACCTCAGCCTGTCCCCCT	GTTTGCACTTATTCCTTTTCCATTAGCTC
emf01E10	ACATATCCAACTGACCTCGGAAGA	GTTTAACCGCTTTGTCCCCAAATACAG
emk04N11	ATCTCCCCCTCAACTTTGAACAAT	GTTTGTGTGATATAGCCCAACAATTCAC
emh11I06	ATTTCAAACCGTTCCTCTGCTCTT	GTTTGCACAATCATCAAGGCTCCTCTTT
emk03O04	ATGATTTGGGCAGCCACTTTTGTA	GTTTGGAACCAACTAAACTTAGGGCA
emf11N23	ATGTTCTTCCCTTTTTCCCCTTTT	GTTTCCAAGAAAGAAGAAAACCCCACA
emb01A03	CGGATTTAGAGGACGTTTGGATTG	GTTTGGTGGAGCTCAGCTGTTAGTTTG
emf21P02	ATGAAGCAGATCTTTCGACTGCAC	GTTTAGGCCAAGGATGTCAAACTGGT
emh21J12	ACAGAACAATTCACCAGCAGTCAA	GTTTAGGAACAGGGAAAATCGTATCGGT
emf11B07a	ACGAGAGTTGCTACAGTTAAGGGG	GTTTGGGGACCAAAGTGTATTTTCAAGG
emf21K08	ATCAATGACACCCAAAACCCATTT	GTTTGAAAACCCAATACAAATCCGA
emb01J19	GACAGGGATAGGGGTACGGATAGG	ATCCATGTGATGCCTCGATTTTCT
emh02E08	AGGCGTTCAGCAGAGAAGAAATTA	GTTTGCTTCCTTAAGTGGCATCTGAAA
emh11B19	ATCAAAACCAACCTCCAGTTCTCG	GTTTCAAATCGCAGAGTTCATCCTTCCT
eme05B09	ATGAAAACTCCACTCTACTCTACTCCAC	GTTTGCTAACGTACGCCTCAATTGCTCT
SSR40	TGCAGGTATGTCTCACACCA	TTGCAAGAACACCTCCCTTT
SSR125	CCTAAAGAAGATAGGAAGAAATGCC	TCTCTCCTACTGAAACAACCAA
eme05B10	ATGAAAACTCCACTCTACTCTACTCCAC	GTTTGCTAACGTACGCCTCAATTGCTCT
emh11B18	ATCAAAACCAACCTCCAGTTCTCG	GTTTCAAATCGCAGAGTTCATCCTTCCT
emb01C12	AAAAAGCTCTGCCCAAACAAGC	GACTTTCCTCACTAATTCACAACCA

Table 4 Performance of eggplant genotypes for growth and fruit parameters

Genotype	Plant height (cm)	Primary branches	Fruit length (cm)	Fruit diameter (cm)
CARI-1	102.8^{b}	7.1	13.4^{b}	9.1^{a}
IIHR-108	86.8^{d}	7.0	14.8^{ab}	2.9^{c}
IIHR-7	113.6^{a}	8.6	17.7^{a}	3.1b^{c}
Rampur Local	94.5^{c}	7.6	15.3^{ab}	2.7^{c}
IIHR-500A	83.5^{e}	9.3	16.0^{ab}	8.6^{a}
IIHR-575	78.6^{f}	8.0	8.6^{c}	4.1^{b}
Significance	***	NS	**	**

[a]Mean values of three replicates within columns are separated using Duncan's multiple range test P = 0.05. NS-Non significant; Significance **, *** at P< 0.01 or 0.001, respectively.

Table 5 Performance of eggplant genotypes for yield and bacterial wilt incidence

Genotype	Fruit number (plant^{-1})	Average fruit weight (g)	Fruit yield (tonnes/ ha)	Bacterial wilt incidence (%)
CARI-1	18.0^{d}	285.0^{a}	31.6^{ab}	0.0^{d}
IIHR-108	34.6^{b}	34.1^{d}	8.3^{c}	92.9^{a}
IIHR-7	38.0^{a}	32.9^{d}	38.4^{a}	0.0^{d}
Rampur Local	31.3^{bc}	36.0^{d}	28.4^{b}	26.0^{c}
IIHR-500A	20.6^{d}	146.6^{b}	32.3^{ab}	0.0^{d}
IIHR-575	30.0^{c}	71.6^{c}	26.4^{b}	35.4^{b}
Significance	**	***	**	***

[a]Mean values of three replicates within columns are separated using Duncan's multiple range test P = 0.05. Significance **, *** at P< 0.01 or 0.001, respectively.

amplification however only 37 primers were polymorphic and microsatellite allele sizes were determined at these 74 loci. The number of alleles were 2 per locus and expected heterozygosity varied between 0.303 and 0.545 with an average of 0.437 whereas, observed heterozygosity varied between 0.000 and 0.833 with an average of 0.049. The average polymorphic information content (PIC) was 0.315 and it ranged from 0.239 to 0.375 (Table 6). This indicates that the genotypes used in the present study were homozygous due to their low observed heterozygosity values. In earlier finding on the 20 eggplant genotype diversity using SSR markers, the average PIC value was noted as 0.24 (Mangal *et al*. 2016). SSR allele size values across laboratories may vary by one to four base pairs due to different analytical and rounding methods (This *et al*. 2004). These polymorphic primers can further be used for screening of mapping population of the resistant and susceptible crosses for bacterial wilt. The present finding also corroborate with the results of Lakshmana Reddy *et al*. (2012) where they used 39 SSR markers and recorded PIC ranged from 0.343 to 0.794 in five eggplant genotypes using SSR markers.

Fig 2 Bacterial ooze from infected plant.

Interestingly, among the 37 polymorphic primers, two SSR primers emh21J12 and emf01K16 showed polymorphism and unique band with respect to resistant and susceptible genotypes in the present study (Fig 3). In case of primer emh21J12 the resistant genotypes (CARI-1, IIHR-7 and IIHR-500A) showed unique band at 170bp and susceptible genotypes (IIHR-108, Rampur Local and IIHR-575) at 160bp. Further, another primer emf01K16 also discriminated between resistant at 250bp and susceptible genotypes at 260bp. In tomato, SCAR marker SCU176-534 was developed for early selection of bacterial wilt resistant lines in tomato breeding program (Truong *et al*. 2015) and in eggplant SCAR marker linked to a bacterial wilt resistant identification by Cao *et al*. (2009). Similarly, the SSR markers emh21J12 and emf01K16 has been found linked

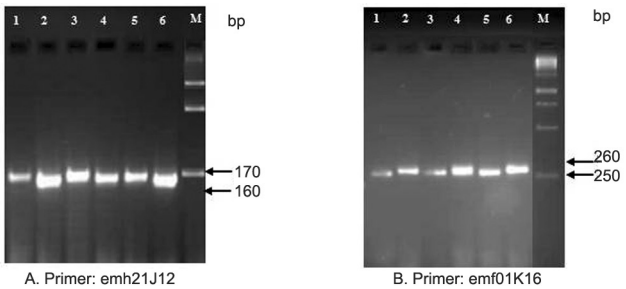

Fig 3 SSR primers with unique banding pattern. Lane 1-6: CARI-1, IIHR-108, IIHR-7, Rampur Local, IIHR-500A and IIHR-575; Lane M, Molecular weight. marker.

Table 6 Prominent features of the microsatellites loci analysis

Locus	Number of alleles	H_E	H_O	PIC
emg21I17	2	0.303	0.000	0.239
emf11A04	2	0.485	0.000	0.346
emf11A03	2	0.530	0.167	0.368
emd18E02	2	0.530	0.833	0.368
Eggplant37	2	0.409	0.500	0.305
Eggplant 45	2	0.485	0.000	0.346
Eggplant 46	2	0.485	0.000	0.346
Eggplant 55	2	0.303	0.000	0.239
Eggplant 66	2	0.303	0.000	0.239
Eggplant 100	2	0.303	0.000	0.239
Eggplant 103	2	0.485	0.000	0.346
Eggplant 105	2	0.303	0.000	0.239
Eggplant 109	2	0.303	0.000	0.239
Eggplant 117	2	0.303	0.000	0.239
emb01D10	2	0.303	0.000	0.239
emh02A04	2	0.303	0.000	0.239
emf01K16	2	0.545	0.000	0.375
emh11H03	2	0.485	0.000	0.346
emf01E10	2	0.485	0.000	0.346
emk04N11	2	0.545	0.000	0.375
emh11I06	2	0.545	0.000	0.375
emk03O04	2	0.545	0.000	0.375
emf11N23	2	0.303	0.333	0.239
emb01A03	2	0.303	0.000	0.239
emf21P02	2	0.303	0.000	0.239
emh21J12	2	0.545	0.000	0.375
emf11B07a	2	0.545	0.000	0.375
emf21K08	2	0.545	0.000	0.375
emb01J19	2	0.303	0.000	0.239
emh02E08	2	0.545	0.000	0.375
emh11B19	2	0.545	0.000	0.375
eme05B09	2	0.545	0.000	0.375
SSR40	2	0.303	0.000	0.239
SSR125	2	0.485	0.000	0.346
eme05B10	2	0.545	0.000	0.375
emh11B18	2	0.545	0.000	0.375
emb01C12	2	0.545	0.000	0.375
Mean	2	0.437	0.049	0.315

H_E: Expected heterozygosity, H_O: Observed heterozygosity, PIC: Polymorphic information content.

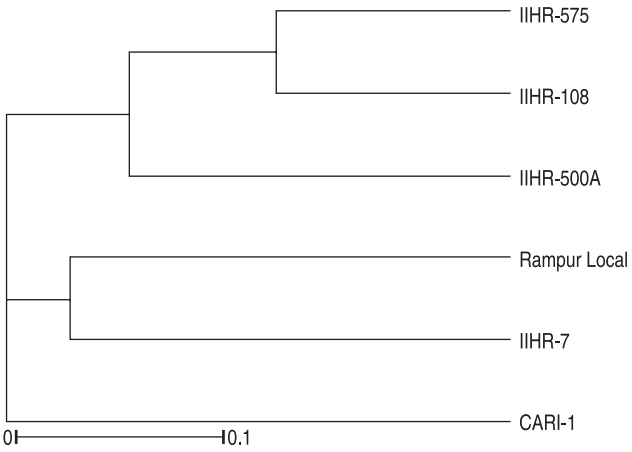

Fig 4 Dendrogram demonstrating the genetic relationships among eggplant genotypes using 37 SSR markers.

to bacterial wilt resistant genotypes in present study and could be used for early selection of bacterial wilt resistant line in eggplant.

The dendrogram classified six eggplant genotypes into three main clusters (Fig 4). Cluster I consists of IIHR-575, IIHR-108 and IIHR-500A this is perhaps due to genetic similarity between genotypes despite of their diverse origin, where IIHR-500A was solitary. The cluster II consists Rampur local and IIHR-7 both the genotypes bear long green colour fruits which concord them in single cluster.The cluster III was solitary comprising Island originated genotype CARI-1 which bears the fruit of 285 g, it is genetically dissimilar to rest of genotypes which are originated from main land. In this study, co-dominant marker SSRs proved to be highly effective tools in classifying between these six genotypes. The clustering of genotypes concord to the collection site and fruit shape, and colour traits; the result are in confirmation with the findings of Verma *et al*. (2012) in eggplant genotypes using six SSR markers. Genetic diversity analysis using SSRs markers were also reported by Nunome *et al*. (2003); Stagel *et al*. (2008) and Demir *et al*. (2010) in eggplant. The diverse genotypes on the basis of fruit colour and shape can be further incorporated in breeding programme for developing hybrids resistant to BW in eggplant.

In conclusion, the genotypes CARI-1, IIHR-7 and IIHR-500A were identified as resistant to bacterial wilt (BW) in field conditions having pathogen count of 1.0×10^8 cfu/g soil and were also confirmed resistant to BW by SSR markers (emh21J12 and emf01K16) with unique banding pattern. The present study uses the potential of co-dominant SSR markers in discriminating bacterial wilt resistant and susceptible genotypes. The polymorphic primers can further be used for mapping of bacterial wilt resistant gene in eggplant. The clustering pattern generated clearly segregated the genotypes of eggplant from main land and Andaman and Nicobar Island. The elite eggplant genotypes which had recorded high and bacterial wilt resistant could be used for further bacterial wilt resistant breeding programme.

ACKNOWLEDGEMENT

The support of Director, ICAR-CAZRI, Jodhpur and ICAR-IIHR, Bengaluru for professional attachment training and conducting research at ICAR-IIHR, Bengaluru is duly acknowledged.

REFERENCES

Amin A, Razvi, S M, Dar Z A, Zafar G, Bhat M A, Mir M R, Wani N and Bhat K A. 2010. Molecular markers and their application in improvement of vegetable crops. *International Journal of Current Research* **4**: 38–48.

Anonymous. 2014. National Horticulture Board, Indian Horticulture Database, 2013, Gurgaon, p 251.

Atabug R R and Juan M O S. 1981. Screening of tomato accessions for bacterial wilt resistance. *Philippines Phytopathology* **17**: 63–6.

Bainsla N K, Singh S, Singh P K, Kumar K, Singh A K and Gautam R. 2016. Genetic behaviour of bacterial wilt resistance in eggplant (*Solanum melongena* L.) in tropics of Andaman and Nicobar Islands of India. *American Journal of Plant Science* **7**: 333–8.

Botstein D, White R L, Skolnick M and Davis R W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics* **32**: 314–31.

Caguiat X G I and Hautea D M 2014. Genetic diversity analysis of eggplant (*Solanum melongena* l.) and related wild species in the Philippines using morphological and SSR markers. *SABRAO Journal of Breeding and Genetics* **46**: 183–201.

Cao B H, Lei J, Wang Y and Chen G. 2009. Inheritance and identification of SCAR marker linked to bacterial wilt-resistance in eggplant. *African Journal of Biotechnology* **8**: 5201–7.

Chaudhary D R and Sharma S D. 2000. Screening of some eggplant cultivars against bacterial wilt and fruit borer. *Agricultural Science Digest* **20**: 129–30.

Chourey S K, Solanki S, Gaikwad A B, Pandey C D and Archak S. 2017. SSR marker analysis points to population admixture and continuum of genetic variation among Indian landraces of eggplant (*Solanum melongena* L.). *Scientia Horticulturae* **224**: 68–73.

Das C R and Chattopadhyay SB 1953. Bacterial wilt on eggplant. *Indian Phytopathology* **8**: 130–5.

Demir K, Bakir M, Sarıkamis G and Acunalp S. 2010. Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. *Genetics and Molecular Research* **9**: 1568–76.

Doyle J J and Doyle J L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–5.

Frary A, Doganlar S and Daunay M C 2007. Eggplant. (*In*) Vegetables SE - 9, pp 287–313. *Genome Mapping and Molecular Breeding in Plants,* Kole C (Ed). Springer, Berlin.

Fukuoka H, Miyatake K, Nunome T, Negoro S, Shirasawa K, Isobe S, Asamizu E, Yamaguchi H and Ohyama A. 2012. Development of gene-based markers and construction of an integrated linkage map in eggplant by using Solanum orthologous (SOL) gene sets. *Theoretical Applied Genetics* **125**: 47–56.

Gopalakrishnan C, Singh T H and Artal R B. 2014. Evaluation of eggplant accessions for resistance to bacterial wilt caused by *Ralstonia solanacearum* (E.F. Smith) Yabuuchi et al. *Journal Horticultural Sciences* **9**: 202–5.

Gopalan C, Rama Sastri B V and Balasubramanian S. 2007. *Nutritive Value of Indian Foods*. National Institute of Nutrition (NIN), ICMR.

Hayward A C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum. Annual Review of Phytopathology* **29**: 65–87.

Hussain Z M, Rahman M A and Bashar M A. 2005. Screening of eggplant accessions for bacterial wilt caused by *Ralstonia solanacearum*. *Bangladesh Journal of Botany* **34**: 53–8.

Kalia R K, Rai M K, Kalia S, Singh R and Dhawan A K. 2011. Microsatellite markers: an overview of the recent progress in plants. *Euphytica* **177**: 309–34.

Kalinowski S T, Taper M L and Marshall T C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**: 1099–106.

Khapte P S, Singh T H, Sadashiva A T and Reddy K M. 2012. Performance of parents and hybrids for yield and yield attributing characters in manjarigota type of brinjal (*Solanum melongena* L.). *Madras Agricultural Journal* **99**: 438–41.

Kumar Arun M B, Dadlani M, Kumar R and Jacob S R 2014. Identification and validation of informative SSR markers suitable for ensuring the genetic purity of eggplant (*Solanum melongena* L.) hybrid seeds. *Scientia Horticulturae* **171**: 95–100.

Lakshmana Reddy D C, Khandagale K, Srinivas Reddy S H, Kanupriya C, Chennareddy A and Singh T H. 2012. SSR-Based DNA barcodes as a tool for identification of eggplant genotypes. *International Journal of Vegetable Science* **18**: 260–71.

Liu T, Yu Y, Cai X, Tu W, Xie C and Liu J. 2016. Introgression of bacterial wilt resistance from *Solanum melongena* to *S. tuberosum* through asymmetric protoplast fusion. *Plant Cell Tissue and Organ Culture* **125**: 433–43.

Mangal M, Upadhyay P and Kalia P. 2016 Characterization of cultivated and wild genotypes of eggplant (*Solanum melongena* L.) and confirmation of hybridity using microsatellite markers. *Vegetos* **29**: 27–34.

Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P, Dow M, Verdier V, Beer S V, Machado M A, Toth I, Salmond G and Foster G D. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology* **13**: 614–29.

Martin F W and Rhodes A M. 1979. Sub-specific grouping of eggplant cultivars. *Euphytica* **28**: 367–83.

Mondal B, Bhattacharya I, Sarkar A and Khatua D S. 2013. Evaluation of local eggplant (*Solanummelongena* L.) germplasm for bacterial resistance. *International Journal of Agricultural and Statistical Sciences* **9**: 709–16.

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A and Fukuoka H. 2009. Development of SSR markers derived from SSR-enriched genomic library of eggplnt (*Solanum melongena* L.). *Theoretical Applied Genetics* **119**: 1143–53.

Nunome T, Suwabe K, Iketani H and Hirai M. 2003. Identification and characterization of microsatellites in eggplant. *Plant Breeding* **122**: 256–62.

Oliveira I T, Lopes C A and Moura A B. 2014. Fruit yield and bacterial wilt symptoms on eggplant genotypes grown in soil infested with *Ralstonia solanacearum. Horticultura Brasileira* **32**: 453–7.

Prohens J, Munoz-Falcon J E, Vilanova S and Nuez F. 2011.

Comparison of morphological, AFLP and SSR markers for the protection of eggplant germplasm. *Acta Horticulturae* **898**: 123–31.

Rai P V, Shivappa Setty K K A and Vasantha Setty K P. 1975. Bacterial wilt of petunia and its source of inoculum. *Current Research* **4**: 173–4.

Rao M V B, Sohi H S and Vijay O P. 1976. Reaction of some varieties of eggplant to *Pseudomonas solanacearum*. *Vegetable Science* **3**: 61–4.

Sarkar S and Chaudhuri S. 2016. Bacterial wilt and its management. *Current Science* **110**: 1439–45.

Shukla V and Naik L B. 1993. Agro-techniques of solanaceous vegetables. (*In*) *Advances in Horticulture,*Vol 5. Vegetable Crops, Part 1, p 365. Chadha K L and Kalloo G (Eds). Malhotra Pub House, New Delhi.

Stagel A, Portis E, Toppino L, Rotino G L and Lanteri S. 2008. Gene based microsatellite development for mapping and phylogeny studies in eggplant. *BMC Genomics* **9**: 357–70.

This P, Jung A, Boccacci P, Borrego, Botta J, Constantini L, Crespan M, Dangl G S, Eisenheld C, Ferreira-Monteiro F, Grando S, Ibanez J, Lacombe T, Laucou V, Magalhaes R, Meredith C P, Milani N, Peterlunger E, Regner F, Zulini L and Maul E. 2004. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theoretical Applied Genetics* **109**: 1448–58.

Verma M, Rathi S, Munshi A D, Kumar A, Arya L, Bhat K V and Kumar R. 2012. Genetic diversity of Indian eggplant revealed by RAPD and SSR markers. *Indian Journal Horticulture* **694**: 517–22.

Yang X, Cheng Y, Deng C, Ma Y, Wang Z, Chen X and Xue L. 2014. Comparative transcriptome analysis of eggplant (*Solanum melongena* L.) and turkey berry (*Solanum torvum* Sw.): phylogenomics and disease resistance analysis. *BMC Genomics* **15**: 412.