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Evaluation of Cultivated and Wild Brinjal Germplasm against Bacterial Wilt Disease with Tollinterleukin-1 Receptors (TIR)-NBS-LRR Type R-gene Specific Degenerate Primer

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Abstract

The experiment was conducted at C-Block Farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, India during 2017–18 to screen eight brinjal germplasm lines against BW disease using tollinterleukin-1 receptors (TIR)-NBS-LRR type R-gene specific degenerate primer. The study showed that wild genotype *S. torvum* was highly resistant to bacterial wilt incidence with no wilting symptom whereas two cultivated genotypes (Utkal Anushree and Utkal Madhuri) and one wild genotype *S. sisymbriifolium* were found to be resistant to BW disease. Out of the 7 germplasm sequences, 2 had no match with R-genes whereas the remaining 5 sequences have 70-93% homology with R-genes of other plant species submitted in Gene Bank sequence database. Nearly 90% sequence identity of brinjal NBS-LRR RGA was found by analyzing through BLASTn with NBS-LRR RGAs of other solanaceous crops. Two cultivated resistant genotypes (Utkal Madhuri and Utkal Tarini) were similar to the wild resistant type *S. sisymbriifloium*, while cultivable resistant genotype Utkal Anushree was highly different at sequence level. Two cultivable susceptible genotypes (BCB-30 and Garia) showed high level of similarity among them and they were strongly associated with the wild susceptible genotype *S. macrocarpum*. Two cultivable genotypes Utkal Anushree and Utkal Madhuri could be utilized in future breeding programme and two wild genotypes *S. torvum* and *S. sisymbriifolium* could be used as resistant rootstocks in brinjal grafting.

Keywords: Bacterial wilt (BW), brinjal, resistant genotype, screening wilt resistant

1. Introduction

Brinjal (*Solanum melongana* L.; 2n=24) is a one of the most important vegetable crops among the solanaceous family that grown in tropical and subtropical region. In India it is popularly known 'brinjal' as well as aubergine in Europe. Eggplant is generally grown all parts of India all around the years except some higher altitude. In India brinjal occupied about 668.7 ha land with production of 12399.9 million tones, and productivity of 18.5 mt ha^{-1} during 2016-17 (Anonymous, 2017) where West Bengal is the leading producer among states of India with production of 3019 MT from 162.93 ha area.

Bacterial wilt caused by *Ralstonia solanacerum* is a destructive causal

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agent of more than 200 plant species belong to 50 different botanical families including potato, tomato, brinjal, banana, pepper and tobacco. The disease was first reported in Kangra valley in 1981 on solanaceous texas, remained sporadic in nature till 1985 (Sood and Singh, 1992). Now it is widely distributed in different parts of India. In West Bengal most of brinjal or eggplant grown during winter season is susceptible to Bacterial wilt.

India is considered as the Centre of Origin of brinjal (Vavilov, 1931), although the primary diversification of brinjal took place in South-east Asia regions. Brinjal faces many biotic challenges, but nearly 90% crop loss may be happened due to bacterial wilt infection (Nishat et al., 2015) caused by *Ralstonia solanacerarum* (Yabuuchi et al., 1995). Previous study revealed that some wild brinjal species were recorded as resistant against wilt disease (Kubota et al., 2008; Lee et al., 2010; Gisbert et al., 2011; Ashok Kumar et al., 2017; Manickam et al., 2021). The response of cultivated varieties against this pathogen is variable in different parts of India (Yadav et al., 2017; Biswas and Ghosh, 2018; Sharma et al., 2018; Kumar et al., 2019). Obviously, screening of both cultivable and wild genetic resources against BW pathogen for future utilization in breeding programme is necessary.

Cell wall degrading enzyme and Type II secretion system (Huang and Allen., 2000; Denny et al., 1990; Liu et al., 2005; Peeters et al., 2013) are major pathogenicity factors of *R. solanacerum*. Resistant gene analogues (RGAs) is important tools for identification of disease resistant germplasm by way of cloning and sequencing Resistance gene (R-gene). Markers based on RGA are unique to other gene-targeted markers because it used specific genomic characteristics present in plant defense system (Flor, 1946 and 1947). RGAs contain special structural motifs, like NBS and LRR regions. Most of the sequences of these R-genes have conserved amino acid motifs consisting of NBS domain and hydrophobic domain (HD) with a consensus amino acid sequence, Gly-Leu-Pro-Leu (GLPL), downstream to NBS (Reddy et al., 2015). Several R-genes such as *N, M, RPF, Gpa2* and others have been cloned in different crops notably in brinjal (Reddy et al., 2015) and other crops. The degenerate primers were developed from the conserved motifs in these NBS domains. PCR amplification was made to isolate RGAs (Kanazin et al., 1996 and Leister et al., 1998). Sequencing of brinjal R-gene-related genomic region could provide valuable knowledge for the development of DNA markers linked to a specific disease resistant phenotypes. Therefore, the current research was carried out to screen both cultivable and wild genotypes of brinjal against bacterial wilt disease using tollinterleukin-1 receptors (TIR)-NBS-LRR type R-gene specific degenerate primer.

2. Materials and Methods

2.1. Experimental site

The experiment was conducted at C-Block Farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, India during 2017–18. Laboratory experiment was done in the Department of Agricultural Biotechnology and Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India.

2.2. *Isolation and preparation of Ralstonia suspension using TZC media*

Bacterial wilt disease infected brinjal plants were collected from nearby field. Small pieces of 4–5 mm in length were cut from the discolored stem. Small pieces were surface sterilized for disinfecting saprophytic organism. The presence of bacterial wilt disease pathogen was confirmed by ooze test (Acharya et al., 2018). Ooze from wilted shoots was collected in sterile distilled water. Suspended ooze was streaked on *R. solanacearum* specific medium consisting of casamino acid-peptone-glucose (CPG) amended with 0.005% 2,3,5-Triphenyl Tetrazolium Chloride (TZC) (Kelman, 1954). The plates with suspended ooze were incubated at 28 °C for 48 hours. Then plates were observed for development of both virulent and avirulent colonies of *R. solanacearum*. The virulent colonies having irregularly shaped, fluidal, dull white colonies with slight red center was cultured in nutrient broth (Difco, USA) with shaking at 28°C±1°C for 2 days. The pure culture of *R. solanacearum* was suspended in distilled water and maintained to 10^8 cfu ml^{-1} (OD_{600}=0.8) using spectrophotometer.

2.3. Evaluation of bacterial wilt resistance

Seedlings of eight brinjal genotypes including both cultivated wild types were transplanted in sick plot in replicated manner. After 14 DAT (Days after transplanting) roots were injured by inserting a blade at 1 cm from collar region. The suspended bacterial culture was injected in root with help of syringe. The bacterial suspension of 0.3 O.D. at 600 nm was injected per plant using a syringe. Wilting score of plants were recorded after 7 days of transplanting at 7 days intervals but effective wilt severity were observed from 14 days after first inoculation. Leaves of older plants showed first wilting symptoms than youngest leaves or one-sided stunting and wilting and finally death of plants (Agrios, 2005).

2.4. Bacterial wilt disease recording

Starting at 42 DAT, wilting symptom was evaluated for 6 weeks at 7 days intervals. Wilted plants generally died, therefore plants were recorded as wilted and healthy. Disease incidence was calculated on the basis of the last evaluation. Wilt symptoms and the number of wilted plants graded on the basis of 0–5 scale developed by Winstead and Kelman (1952) with some modifications (Table 1).

The disease index for evaluation of resistance and susceptible DI=(Sum of all disease rating/(Total no. of plant observed×maximum disease grade))×100

Scale of Disease Index (DI) for Resistance and susceptible genotypes were classified as highly resistant - 0%, Resistant - 1-20%, Moderately resistant - 21-40%, Moderately susceptible -41-60%, Susceptible - 61-80%, Highly susceptible - 81-100%.

Table 1: Disease rating scale of bacterial wilt disease

Disease rating scale	Status of single plant
0	No symptoms found
1	Only leaf partially wilted found
2	Two and three leaves partially wilted found
3	All leaves wilted except two to three leaves of upward found
4	All leaves found wilted
5	Death of whole plant

2.5. Isolation of genomic DNA

Genomic DNA was extracted by modified CTAB method (Sambrook et al., 2001). Tender brinjal leaves were flash frozen in liquid nitrogen; 3 g of frozen leaves were ground in a precooled mortar and pestle with 50 mg of PVP (Polyvinyl pyrrolidone) and was transferred to a 50 ml falcon tube containing 15 ml of DNA extraction buffer (2% CTAB, 100 mMTris, 20 mMEDTA, 1.4 M NaCl, pH 8.0) preheated at 60˚C. The contents were mixed gently by swirling and inverting the tube and incubated at 65˚C in water bath for 45 min with occasional mixing at every 10 min. The tubes were taken out, cooled to room temperature, and an equal volume of Chloroform-Isoamyl alcohol (24:1) was added. The contents were mixed by inversion for 10 min and centrifuged at 12,000 rpm for 10 minutes. The clear aqueous upper layer was transferred to a new tube and re-extracted with an equal volume of Chloroform-Isoamyl alcohol (24:1). To the final aqueous solution, an equal volume of Isopropanol was added, mixed by inversion and placed at -20˚C for 15 min. Genomic DNA was pelleted by centrifuging at 10,000 rpm for 10 min and the pellet was washed with 70% ethanol. The DNA pellet was dried in DNA concentrator (Thermo) and dissolved in 1 ml of T50E1. About 20 µl of RNase A (5 mg ml^{-1}) was added and incubated at 37˚C for 3 hour. DNA was purified by adding an equal volume of Chloroform-Isoamyl alcohol (24:1) mixing briefly and spinning at 10,000 rpm for 10 min. To the top aqueous layer, $1/10^{th}$ volume of 3 M sodium acetate and twice the volume of Isopropanol was added, mixed by inversion and kept at -20°C for hour. DNA was then pelleted by centrifuging at 10,000 rpm for 10 min at 10 °C. The pellet was washed with 70% ethanol, dried and dissolved in 300 µl TE and stored at -20°C for further use.

2.6. Quantification of genomic DNA

Genomic DNA (5 µl) was mixed with 5 µl of 6X loading dye and loaded on a 1% agarose gel along with different concentrations of uncut λ DNA and electrophoresis was done at 80V for 40 min. The quantity of extracted DNA was estimated based on the intensity of uncut λ DNA. DNA quantification and purity was checked also by measuring the O.D at 260 nm and 280 nm using a UV visible spectrophotometer (Thermo). The purity of DNA was confirmed using the ratio of O.D recorded at 260/280 nm.

2.7. *Primers*

In the present study a set of tollinterleukin-1 receptors (TIR)-NBS-LRR type R-gene specific degenerate primer was used for identification and characterization of candidate resistance genes present among the studied brinjal genotypes. Ploop-F1 and GLPL-R1 primers were used as forward and reverse primers in the PCR (Table 2). In the primer sequence N stands for any nucleotide suggesting degeneracy of four fold in that region and I stand for Inosine.

Table 2: Forward and reverse primers used in the PCR

No.	Oligo name	Sequence (5'- 3')
1	Ploop-F1	GGNGGNRTNGGNAAGACGAC
	GLPL-R1	GAGGGCTAAAGGAAGGCC

2.8. *PCR analysis by using degenerate primers*

PCR Primers were diluted to required working concentrations. Each 20 µl reaction volume contained 2 µl eluted DNA, 2 µl of 10X PCR buffer, 2 mM MgCl2, 2 mMdNTPs (Sigma), 1 µl of 10 pmol of primer, 0.2 µl of 5 unit µl-1 Taq polymerase (Jonaki) and sterile millipore water. DNA amplification was performed in the Mastercycler Gradient (Eppendorf, USA) programmed according to Williams et al. (1990) with minor modifications. PCR was programmed for an initial denaturation step of 4 minutes at 94°C followed by 30 cycles of 45 sec denaturation at 94°C, 30 sec annealing at different temperatures as per set of degenerate primers (i.e. for PLoop/GLPLAL 52°C, for P1/P2at 50°C and K03/HD6 at 55°C), 1 min extension at 72 °C and final extension for 8 min and a hold temperature of 4 °C at the end. Electrophoresis was carried out with the amplified products using 1.5% (w/v) agarose gel, stained with ethidium bromide and visualised under UV illumination (Gene flash Syngenebioimaging). The 100 bp DNA ladder (Genei, India) was used as a molecular weight marker and and subjected to DNA sequencing by outsourcing.

2.9. *Data analysis*

The sequences were put in VecScreen software (https://www.ncbi.nlm.nih.gov/tools/vecscreen/) to trim vector and primer sequences. Homology search was performed using BLASTn algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch),with default settings through National Centre for Biotechnology Information (NCBI), a non-redundant Gen Bank database to identify R-genes as well as homologous sequences. Multiple sequence alignment was done using CLUSTAL 2.1 software (https://www.genome.jp/tools-bin/clustalw). A phylogenetic tree was constructed using UPGMA (https://pubmlst.org/software/analysis/start/manual/upgma.shtml) software. Data were analyzed statistically in randomized block design (RBD) using OPSTAT (CCS Haryana Agricultural University).

3. Results and Discussion

3.1. Bacterial wilt incidence

The reaction of different eggplants genotypes to bacterial

wilt caused by *Ralstonia solanacerum* differed significantly at different day after transplanting (DAT) (Table 3). First incidence of bacterial wilt disease was found at 42 DAT in BCB-30, Utkal Tarini and *S. macrocarpum.* The highest bacterial wilt incidence was recorded from cultivar *S.macrocarpum* (44.44%) followed by BCB-30 (23.33%) at 49 DAT. The lowest disease incidence was recorded in case of *S. torvum* (0.00%) followed by Utkal Anushree (4.44%), *S. sisymbriifolium* (4.44%), Utkal Madhuri (8.88%) and Utkal Tarini (11.11%) at 49 DAT. After 56 days of transplanting, variation to bacterial wilt incidence among the eggplant cultivars was recorded (Table 3). The highest bacterial wilt incidence was recorded from *S. macrocarpum* (53.33%) which was statistically identical with BCB-30 (40.00%). The lowest bacterial wilt incidence was recorded in case of *S. torvum* (0.00%), Utkal Anushree (6.66%) and *S. sisymbriifolium* (6.66%) followed by Utkal Madhuri (11.11%) and Utkal Tarini (17.77%). Statistically significant variation was recorded at 63 DAT in respect to bacterial wilt incidence (Table 1). The highest bacterial wilt incidence was recorded from *S. macrocarpum* (62.22%) which identically similar with BCB-30 (60.00%).The lowest bacterial wilt incidence was recorded in *S. torvum* (0.00%), Utkal Anushree (6.66%) which was closely related to *S. sisymbriifolium* (11.11%) and Utkal Madhuri (15.55%). Statistically significant variation was recorded at 70 DAT in term of bacterial wilt incidence (Table 3). The highest incidence of bacterial wilt was recorded in BCB-30 (73.33%)

Table 3: Bacterial wilt incidence at different stages of plant growth on different germplasm of brinjal

Germplasm	Bacterial wilt incidence (%)						
	Source	42 DAT	49 DAT	56 DAT	63 DAT	70 DAT	77 DAT
BCB-30	BCKV, Kalyani	13.33	23.33	40.00	60.00	73.33	86.66
Garia	BCKV, Kalyani	0.00	10.00	30.00	40.00	56.66	73.33
Utkal Tarini	OUAT, Orissa	6.66	11.11	17.77	31.11	37.77	35.55
Utkal Madhuri	OUAT, Orissa	0.00	8.88	11.11	15.55	17.77	17.77
Utkal Anushree	OUAT, Orissa	0.00	4.44	6.66	6.66	6.66	6.66
S. torvum	Kalyani, West Bengal	0.00	0.00	0.00	0.00	0.00	0.00
S. macrocarpum	IARI, New Delhi	22.22	44.44	53.33	62.22	66.66	66.66
S. sisymbriifolium	Jhargram, West Bengal	0.00	4.44	6.66	11.11	15.55	15.55
SEm±	0.90	1.33	0.61	0.63	0.88	0.71	
CD (*p*=0.05)	2.71	4.00	1.84	1.86	2.68	2.16	

whereas lowest incidence of bacterial wilt was recorded in *S. torvum* (0.00%), Utkal Anushree (6.66%) followed by *S. sisymbriifolium* (15.55%) and Utkal Madhuri (17.77%). The statistically significant variation among different cultivar of eggplant was recorded under present experiment at 77 DAT in respect of bacterial wilt incidence (Table 3). Except *S. torvum* (0.00%), Utkal Anushree (6.66%), S*. sisymbriifolium* (15.55%) and Utkal Madhuri (17.77%), all cultivar under study showed comparatively high level of wilt incidence at 77 DAT.

The highest bacterial wilt incidence was recorded from BCB-30 (86.66%) which was followed by Garia (73.33%) and *S. macrocarpum* (66.66%). The result showed that three genotypes Utkal Anushree, S*. sisymbriifolium* and Utkal Madhuri were resistant to bacterial wilt whereas, *S. torvum* was highly resistant to bacterial wilt incidence with no wilting symptoms, and Utkal Tarini was moderately resistant (Table 3). Mondal et al. (2012) also found similar result when seven genotypes of brinjal viz., Utkal Anushree (BB 45C), Utkal Madhuri (BB 44), Utkal Jyoti (BB 13), BCB 64, Ayeb 2, Soila and Muktakeshi (susceptible check) were screened against bacterial wilt in laboratory conditions during 2006-07. Only three genotypes of brinjal viz, Utkal Madhuri, Ayeb 2 and Soila were found to be resistant. Satyaprakash et al. (2020) reviewed the sources of tolerance of brinjal genotype against this pathogen and they reported the wild species *S. torvum* and the cultivated variety Utkal Anushree were found to be resistant. Ramesh et al. (2016) also identified *S. torvum* as a resistant rootstock in brinjal grafting. Secondary metabolism of polyphenols, and the higher concentration of steroidal glycoalkaloids present in resistant plants may imparts the resistance exhibited by some genotypes. Thereby these metabolites may prevent the bacterial movement into the vicinity of the plant system (Vasse et al*.,* 2005). Other hypothesis is that tyloses and gums in resistant plants may act as filters resulting it prevent bacterial movement within a plant system (Prior et al*.,* 1994). In the vascular system, cell walls of xylem vessels was dissolved by the bacterial wilt pathogen. It produces highly polymerized polysaccharides which increase the viscosity of the xylem resulting plugging xylem vessels. Blocking of xylem vessels by bacteria is the major cause of wilting and yellowing of plant (Hayward, 1991). In heavily colonized vessels, bacterial cells and extracellular polymeric substances (EPSs) can occlude xylem vessels (Caldwell et al., 2017). Vessels can also be blocked by tyloses, which are balloon-like out-growths of the living parenchyma cells adjacent to vessels (Yadeta and Thomma, 2013).

On the basis of reaction of bacterial wilt, brinjal cultivars were categories into different groups (Table 4). The wild species *Solanum torvum* was categorized as highly resistant; Utkal Anushree and *S. sisymbriifolium* as resistant; Utkal Madhuri and Utkal Tarini as moderately resistant to bacterial wilt. The genotype BCB-30 was highly susceptible, whereas Garia and *S. macrocarpum* were susceptible to this pathogen.

Table 4: Reaction of eggplant cultivar to bacterial wilt at 77 DAT

Treatment	Bacterial wilt incidence (%)	Reaction
BCB-30	86.66	Highly susceptible (HS)
Garia	73.33	Susceptible (S)
Utkal Tarini	35.55	Moderate Resistant (MR)
Utkal Madhuri	17.77	Resistant (R)
Utkal Anushree	6.66	Resistant (R)
S. torvum	0.00	Highly resistant (HR)
S. macrocarpum	66.66	Susceptible (S)
S. sisymbriifolium	15.55	Resistant (R)

Scale: Highly Resistance- 0%, Resistance-1-20%, Moderately Resistance-21-40%, Moderately Susceptible-41-60%, Susceptible-61-80%, Highly susceptible-81-100%

3.2. *Identification of eggplant NBS-LRRs and BLASTn*

PCR amplification of NBS encoding brinjal genomic DNA (Figure 1) using a set of degenerate primers (Figure 2), generated fragments with an expected size of 900-1000 bp appeared as a single band on 1.5% agarose gel. Fragments generated were sent for sequencing by out sourcing. Among the 7 acquired sequences, 2 had no match with R-genes whereas the remaining 5 sequences have 70–93% homology with R-genes of other plant species submitted in Gene Bank sequence database and highest identity to different NBS-LRR

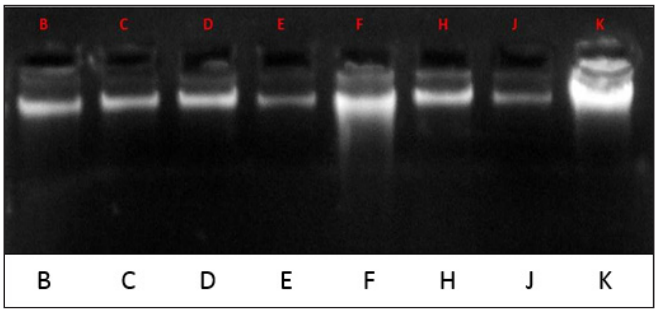

Figure 1: Gel picture of brinjal genomic DNA, Lane 1-8: (B= Utkal Tarini, C= UtkalMadhuri, D= UtkalAnushree, E= S. sisymbriifolium, F= *S. macrocarpum*, H=*S. torvum*, J=BCB 30, K=Garia)

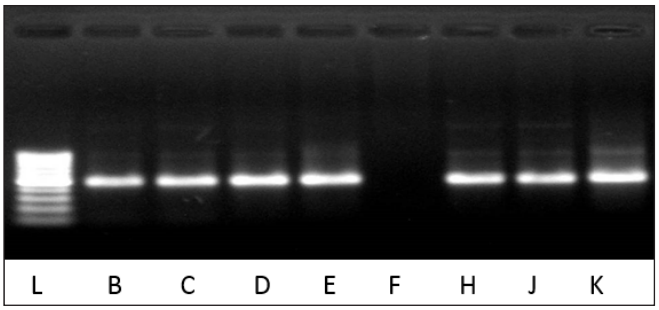

Figure 2: Amplification products of obtain by PCR using one set of degenerated primers combination and template of genomic DNA of eight germplasms. Lane L: 1000bp DNA ladder, Lane 2-8: bringal germplasms (B= Utkal Tarini, C= Utkal Madhuri, D= Utkal Anushree, E= *S. sisymbriifolium*, F= *S. macrocarpum*, H=*S. torvum*, J=BCB 30, K=Garia)

RGAs analyzed through BLASTn are tabulated also named as C-RGA, D-RGA, F-RGA, J-RGA and K-RGA (Table 5).

3.5. *Phylogenetic analysis of brinjal RGAs*

Multiple sequence alignment of RGAs isolated in this study was done using CLUSTAL 2.1 software. This analysis revealed that green colored regions are unique for wild germplasms whereas the yellow colored shows specialty of wild resistance genotype

Table 5: Similarity search between eggplant RGAs and genbank accessions carried out using the BLASTn algorithm

Brinjal RGAs	Function and GenBank accession number	Plant species	Identity (%)
Utkal Madhuri-RGA (C-RGA)	PREDICTED: *Capsicum annuum* TMV resistance protein N-like (LOC107851268), transcript variant X2, mRNA (XM_016696220.1)	*Capsicum annuum*	90%
Utkal Anushree-RGA (B-RGA)	*Solanum tuberosum* TMV resistance protein N-like (LOC102594024), mRNA (XM_015311740.1)	Solanum tuberosum	90%
S. macrocarpum-RGA (F-RGA)	*Solanum lycopersicum* TMV resistance protein N-like (LOC101259277), mRNA (XM_004228451.3)	*Solanum lycopersicum*	70%
BCB 30-RGA (J-RGA)	*Solanum tuberosum* TMV resistance protein N-like (LOC102594024), mRNA (XM_015311740.1)	*Solanum tuberosum*	93%
Garia-RGA (K-RGA)	*Solanum tuberosum* TMV resistance protein N-like (LOC102594024), mRNA (XM_015311740.1)	*Solanum tuberosum*	92%

E. Red coloured region having Thiamine are present in all cultivated germplasm whereas green region having Adenosine are present only in wild brinjal germplasm (Figure 3).

UPGMA software was used to analyze evolutionary relationship among eggplant RGAs of the present study. Phylogenetic study by UPGMA depicted that wild resistance was clearly different

```
D       --AGCGTTAATTATTTTGAACCTCTC--CCTCATTTGATGG-TGCTTGTTTTCTTGCCGA
K      -GAACTTCGCTTTTTTAGAAACTTCTTACTTCATTTGATGG-TGCTTGTTTTCTTGCCGA
J       AGAGCCTC---CTTTTGAATCCCTCTCACCTCATTTGATGG-TGCTTGTTTTCTTGCCGA
C    --GGACCTTTAAAAAAAGCTAACTCTCACCTCATTTGATGG-TGCTTGTTTTCTTGCCGA
F     --GACCCTGGTTTTAAAAAAAATTCTCACTTCATTTGAAGGGTGCTTGTTTTCTTGCCAA
B      --GGCCCCTTATCCATTTGTCACTCTCACTCAATTTGATGG-TGCTTGTTTTCTTGCCGA
E      CAAGAGTCTTTCTTTTGAACCCCTCT--ATCATTTTGAAGG-TGCTTGTTTTCTTGCGGA
****** ** *************** *

D      CGTCAAAGAAAACA-AAGGTGGA-----TTGCATTCTTTGCAAAATATCCTTCTCTCAGA
K      CATCAAAGAAAACA-AAGGTGGA-----TTGCATTCTTTGCAAAATATCCTTCTCTCAGA
J      CATCAAAGAAAACA-A-GGTGGA-----TTGCATTCTTTGCAAA-TATCCTTCTCTCAGA
C      CGTCAAAGAAA-CA-AAGGTGGA-----TTGCACTCTTTGCTTA-TATCCTTCTCTCACA
F      CATCAA-GAAA-AA-AAGGTGGA-----ATACATTCTTTGCAAAATATCCTTCTCTCAGA
B      CGTCAAAGAAA-CA-AAGGTGGA-----TTGCATTCTTTGCTAA-TATCCTTCTCTCAGA
E      TGTTAAAGAAAATACAAGAAAGAAGCAGCTGCATTCTTATCTCAACACCCTTCTCTCTGA
* ** **** * * *  **    * ** **** * * * * ********* *

D      ATTGTTGAGGAAAAAAAATGATTACGTGCATAATAAGGAGGATGGAAAGCACATGATAGC
K      ATTGTTGAGGAGAAAAA-TGATTACGTGCATAATAAGGAGGATGGAAAGCACATGATAGC
J      ATTGTTGAGGAAAAAAGATGATTACGTGCATAATAAGGAGGATGGAAAGCACATGATAGC
C      ATTGTTGAGGAAAAAGAATGATTACGTGCATAATAAGGAGGATGGAAAGCCCATGATAGC
F      ATTGTTGAGGAAAAAGAATGATTACGTGCATAATAAGGAGGATGGAAAGCACATGATAGC
B      ATTGTTGAGGAAAAA-AATAATTACGTGCATGATAAGGAGGATGGAAAGCGCATGATCCC
E      ACTTTTG-GAAGAAAATTTGATGACATCAATAATAAGG---ATGGGCACAACATGGGTCC
* * *** * * ***  * ** ** * ** ******  **** *  ****  *

D      TCG--TAGACTTCGATTTAAGAAGGTTTTAATTGTTCTTGATGACATAGATCACCGTGAT
K      TCG--TAGACTTCGATTTAAGAAGGTTTTAATTGTTCTTGATGACATAGATCACCGTGAT
J      TCG--TAGACTTCGATTTAAGAAGGTTTTAATTGTTCTTGATGACATAGATCACCGTGAT
C      TCG--TAAACTTCAATTTAAGAAGGTTTTAATTGTGCTTGATGACATAGATCACCGTGAT
F      TCG--TAA-CTTCCATTTAAAAAGGTTTTAATTGTTCTTGATGACATAAATCACCGTGAT
B      TCG--TAGACTTCGATTTAAGAAGGTTTTAATTGTTCTTGATGACATAGATCACCGTGAT
E      TAGACTAGACTTTTTTCGAAGAAGGTGCTTATGGTTGATGACGACAAAAATCATGGTTAT
* *  **  ***  *  ** ***** * ** **  *** **** * **** ** **

D      CATTTGGATTACCTGGCAGGGGATCTTTGTTGGTTTGACAAGGGCAGTAGGATTATTGC
K      CATTTGGATTACCTGGCAGGGGATCTTTGTTGGTTTGACAAGGGCAGTAGGATTATTGC
J      CATTTGGATTACCTGGCAGGGGATCTTTGTTGGTTTGACAAGGGCAGTAGGATTATTGC
C      CATTTGGATTACCTGGCAGGGGATCTTTGTTGATTTTACAAGGGCCGTACGATTATTTC
F      CATTTGGATTACCTGGCAGGGGATCTTTGTTGGGTTGACACTCGGCGAAACAATTATTC
B      CATTTGGATTACCTGGCAGGGGATCTTTGTTGGTTTGACAAGGGCAGTAGGATTATTGC
E      CTTTAGGATTATTGAGCTGGTGATGGTTGTTGGTTTGGCA------GTAGAATAATTGT
* ** ******   ** ** *** ****** ** **    * ** ** *
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Figure 3: Multiple sequence alignment of NBS-LRR RGAs from different brinjal cultivars using CLUSTAL 2.1 (D= utkal Anushree, K= Garia, J= BCB-30, C= Utkal Madhuri, F= *S. macrocarpum*, B= Utkal Tarini, E= *S. sisymbriifloium*)

from rest of all (Figure 4). Two cultivated resistant lines (Utkal Madhuri and Utkal Tarini) were close to the wild resistance *S. sisymbriifloium*, while cultivable resistance Utkal Anushree was highly different at sequence level. Cultivable susceptible lines (BCB-30 and Garia) showed high level of similarity among them and they showed strong associationship with the line *S. macrocarpum*, i.e. wild susceptible genotype. Further research is needed to identify the full length gene sequence to understand the involvement of this particular gene.

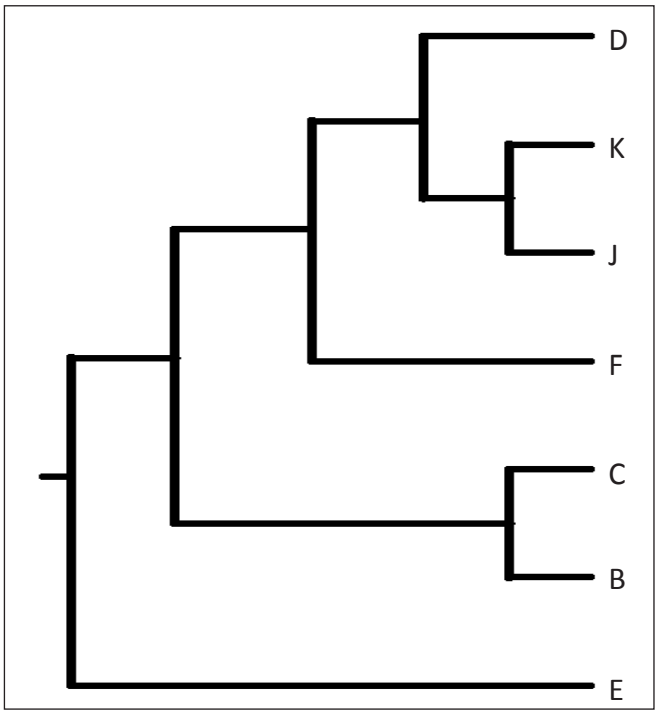

Figure 4: Phylogenetic analysis of Brinjal RGAs (D: Utkal Anushree, K: Garia, J: BCB-30, C: Utkal Madhuri, F: *S. macrocarpum*, B: Utkal Tarini, E: *S. sisymbriifloium*)

Degenerative primers approach were used for isolating RGAs through PCR amplification from resistant germplasm of the target region of nucleotide binding site-leucine rich repeat (NBS-LRR) which helpful in understanding and developing cultivated varieties resistant to bacterial wilt. This approach has been successful in many plant like citrus (Deng et al., 2000), grapevine (Donald et al., 2002), chestnut (Xu et al., 2005) and related crop like chilli (Naresh et al., 2017).

4. Conclusion

The cultivated resistant genotypes (Utkal Madhuri and Utkal Tarini) were similar to wild resistant genotype *S. sisymbriifloium*, while other cultivable resistant genotype Utkal Anushree was highly different at sequence level. Two cultivated susceptible genotypes (BCB-30 and Garia) showed high level of similarity with the wild susceptible genotype *S. macrocarpum*. Cultivable genotypes Utkal Anushree and Utkal Madhuri could be utilized in future breeding programmes and wild genotypes *S. torvum* and S. *sisymbriifolium* could be used as resistant rootstocks in brinjal grafting.

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