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Bacterial wilt resistant gene searching in Eggplant (*Solanum melongena*) and its two wild relatives

Fatema Tuz Zohura[1] and Md Ekramul Hoque[2*]

[1]MS Student, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.
Email: tamannafatema75@gmail.com
[2]*Professor, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.
Email: ekramul.sau@gmail.com

Abstract**—** *Eggplant is an important vegetable in all over the world. Bacterial wilt caused by Ralstonia solanacearum is a major disease of eggplant. Near about 32% crop loss occurred due to this disease. The wild relatives of eggplant viz. Solanum villosum and Solanum sisymbriifolium showed resistant against bacterial wilt disease. Hence, attempted was made to search the resistant gene of bacterial wilt in three Solanum spp. Some total of 16 different bacterial wilt resistant molecular markers RAPD (OPB-17, OPG-05, OPH-07) UBC (176, 205, 317) SSR (STM0007, emh01J23, emb01N07) SCAR (me1, me2, me4, me5) and SRAP (SCU176-534, SCU176-1190F1R1, SCU176-1190F2R2) were screened to identified the resistant DNA fragment. Only one primer UBC#176 showed amplification in target DNA. The primer UBC#176 gave DNA amplification in target position at 350 bp in the species of Solanum villosum. The obtained DNA fragment showed maximum 79% homology with Solanum lycopersicum cultivar I-3 chromosome 8 which has GeneBank Accession No. CP023764.1. Alternative approach was made to identify resistant gene of bacterial wilt disease. Total RNA was extracted and cDNA was synthesized. The synthesized cDNA was used as template to find out the resistant gene. But none of the gene specific primer was amplified using cDNA as template. Further study was needed to find out the bacterial wilt resistant DNA fragment in wild type.*

Keywords**— Bacterial wilt, eggplant, eggplant wild relatives, resistant gene, sequencing.**

I. INTRODUCTION

Eggplant or brinjal (*Solanum melongena* L. 2n = 2x = 24) is an important solanaceous crop grown widely in Asia, Africa, and the subtropical region including the southern USA. It is the second most important solanaceous fruit crop after tomato *(Solanum lycopersicum* L.) [1]. In respect of total acreage production eggplant is the second most important vegetable crop next to potato in Bangladesh [2]. A large number of eggplant cultivars are grown in Bangladesh. In Bangladesh it is cultivated in an area of about 1,25,860 acres with productivity of 5,07,432 MT (BBS 2017). Eggplant has a number of health benefits such as it has ayurvedic medicinal properties and is good for diabetic patients. It has been recommended as an excellent remedy for those suffering from liver complaints [3]. It is an important source of fiber, potassium, manganese, as well as vitamins C, K, and B6. Phenolic compounds in eggplant contain significant amounts of chlorogenic acid, one of the most powerful free radical scavengers found in plants. Chlorogenic acid has been shown to decrease low density lipid (LDL) levels, and also serves as an antimicrobial, antiviral, and anti-carcinogenic agent.

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 [4, 5]. Bacterial wilt is an important disease of many plant species especially solanaceae [6]; causing enormous economic losses, which limits eggplant production from 4.24 to 86.14 percent [7]. 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 [8]. Understanding the host and pathogen is a pre requisite for devising proper strategies for control of disease. Control of wilt diseases is also complicated by the scarcity of sources of disease-resistant host germplasm, and the soil and vascular habitats of the pathogen [4, 9]. Host resistance appears to be the main strategy for the control of bacterial wilt because of the

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resistant imposed by the lack of effective chemical controls, the soil-borne nature of the pathogens, and the wide range of hosts [10].

In the Solanaceae, most cultivated species (*Solanum melongena* L.) is highly susceptible to bacterial wilt. The wild species *Solanum phureja* [11, 12] and *Solanum stenotomum* [11] were identified as possible sources of resistance for potato (*Solanum tuberosum*), *Lycopersicon pimpinelifolium* for tomato (*L. esculentum*) [13] and *S. villosum, S. torvum, S. sisymbriifolium, S. gilo* and *S. aethiopicum* [14] for eggplant (*S. melongena*). *Solanum villosum* Mill. has been reported to be useful for introgression of disease resistance. *Solanum villosum* is (2n= 48) believed to have originated in Eurasia, and is sometimes considered to be resistant to bacterial wilt. Interestingly *Solanum villosum* has been identified to carry traits of resistance to most serious diseases of eggplant, particularly bacterial and fungal wilts [15].

Despite of its valuable potential for disease resistance, little is known about *S. villosum* regarding variability in morphology, fertility and levels of resistance to both bacterial and fungal wilts, as well as genetic diversity. The molecular technique is applied to identify disease resistant gene from wild relatives of eggplant. *Solanum villosum* and *Solanum sisymbriifolium* are two important wild relatives of eggplant. Sequence-related amplified polymorphism (SRAP) technology has been recognized as a new and useful molecular marker system for mapping and gene tagging in many crops plants [16]. Sequence characterized amplified region (SCAR) markers are more reproducible and easier to manipulate in marker-assisted selection (MAS) programs than other markers. Due to the co-dominant or dominant nature, SCAR marker can provide a valid tool for the accurate assessment of genotype at the linked locus. SCAR can be considered to be an ideal marker for plant breeding programs. It was screened that two SCAR markers linked to *Fusarium* wilt resistance gene in eggplant [16]. Hence, three *Solanum spp*. *viz. Solanum melongena, Solanum villosum* and *Solanum sisymbriifolium* were used for screening of bacterial wilt resistant gene through SCAR marker. In addition, other categories of molecular markers *viz.* RAPD, UBC, SSR and SRAP were used to identify the target amplification. Therefore, the research work was carried out to investigate the source of bacterial wilt resistant gene in three *Solanum spp*. including cultivated eggplant.

II. MATERIALS AND METHODS

One variety (BARI Begun-01) of eggplant (*Solanum melangena*) and two wild relatives *viz*. *Solanum villosum* and *Solanum sisymbriifolium* were used as experimental materials. DNA extractions from young leaves were performed according to a modified Doyle and Doyle (1990) [17] method by using CTAB protocol. On the basis of literature five different set of molecular markers were synthesized which showed linkage with bacterial wilt resistant in some other crops. The types of molecular markers are (a) RAPD (b) UBC (c) SSR (d) SCAR and (e) SRAP. Some total of 16 different primers were synthesized from all groups of primer. The list of primers is given in 'Table- 01'.

1.1. DNA isolation and purification

Genomic DNA was extracted using CTAB method from three species of *Solanum*. The RNase and proteinase treatment was given to the extracted DNA for purification from RNA and protein. The purified DNA was visualized by 2% agarose gel. DNA concentration was measured by DNA Nanodropper. Working DNA sample was prepared (20-25 nm/µl) for PCR reaction on the basis of concentration of main stock.

1.2. PCR amplification and elution of DNA fragment from gel

PCR amplification was carried out with purified genomic DNA of three *Solanum spp*. and the above gene specific primers. Ten microliter (10 µl) reaction mixture (Bio Basic, Canada, GeneON, Taiwan) was used for target amplification. The annealing temperature was adjusted on the basis of Tm of the primer. The PCR product was visualized on 2% agarose gel and save in gel documentation system. For further study the target fragment of DNA was cut from agarose gel with fine blade and taken in an eppondrof tube. Then the gel fragment was eluted by QIAquick Gel Extraction Kit. The eluted DNA fragment was stored at -20ºC and it was rechecked in 2% agarose gel.

1.3. DNA sequencing and alignment of nucleotide

The primer UBC- 176 produced approximate 350bp DNA fragment. The amplified 350bp fragment of DNA was sequenced by Applied Bio-system, DNA. The obtained DNA sequences were used in NCBI, BLAST program to carry out the sequence homology with other organism through computer software. Another molecular approach was carried out to findout the bacterial wilt resistant gene. The methodological steps are given below.

1.4. RNA extraction and cDNA synthesis

The total RNA was extracted from three *Solanum spp*. by QIAGEN RNA extraction kit. Near about 50-100 mg fresh leaf sample were collected and it was soaked in liquid

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nitrogen and then grind with mortar and pestle. In the successive step QIAGEN RNA extraction protocol was used to isolate total RNA from the leaf sample. Finally the RNeasy spin column was used to isolate the total RNA. Approximate 30-40 µl RNase-free water was added directly to the spin column membrane and the RNA elution was done by 10000 rpm for 1 min. The extracted RNA was visualized by 1% agarose gel.

The total isolated RNA was used for cDNA synthesis. cDNA Synthesis was done by QIAGEN Quantitect Reverse Transcription Kit. Template RNA was thaw on ice. gDNA Wipe out Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-free water were thaw at room temperature (15–25°C). Each solution mixed by flicking the tubes. It was centrifuged briefly to collect residual liquid from the sides of the tubes, and then stored on ice. The genomic DNA elimination reaction was prepared on ice according to QIAGEN Kit mixed and then stored on ice. Incubation was done for 2 min at 42°C. Then immediately placed on ice. The reverse-transcription master mix was prepared on ice according to same protocol mixed and then stored on ice. The reverse-transcription master mix contains all components required for first-strand cDNA synthesis except template RNA. Template RNA was added to each tube containing reverse-transcription master mix. Mixed and then stored on ice. Incubation was done for 15 min at 42°C. Incubation was done for 3 min at 95°C to inactivate Quantiscript Reverse Transcriptase. Reverse-transcription reactions were stored on ice and proceed directly for long-term storage, store reverse-transcription reactions at –20°C temperature.

1.5. PCR amplification using cDNA as a template

All five categories primers ('Table no.1') were used for amplification of target DNA band. Different thermal conditions were used for different primer to get specific DNA fragment.

III. RESULTS AND DISCUSSION

Solanum melangena (eggplant) is highly susceptible to bacterial wilt disease. *Solanum villosum* and *Solanum sisymbriifolium* are two wild relatives of *Solanum melangena* which showed considerable resistance to bacterial wilt. Hence, attempt was made to screen these two *spp*. with different molecular markers to identify any DNA fragments which are linked with bacterial wilt resistance gene. The major finding of these experiments was given in following sub-heading.

1.6. PCR amplification with different gene specific primers

The three species *viz. Solanum melangena* (BARI begun-01), *Solanum villosum, Solanum sisymriifolium* template DNA were used for amplification of DNA fragment which may linked with bacterial wilt resistant gene. Some total of 16 primers ('Table No. 1') were used for the same. It was showed that, only one primer UBC#176 was given amplification at 350 bp DNA fragment in the species *S. villosum* ('Fig. No.1'). It was not amplified in *S. melangena* (BARI begun- 01), *S. sisymriifolium*. It was reported that a total of 800 RAPD primers were screened and only six primers (UBC#176, 205, 287, 317, 350, and 676) showed polymorphism between resistant pool and susceptible pools of bacterial wilt in the Tomato Line Hawaii 7996 [19] . Of these, only two markers UBC#176 and 317 revealed a 100% linkage in the individual plants comprising the contrasting bulks. The marker UBC#176 was converted into a co-dominant SCAR marker and designated as SCU176-534. The marker SCU176-534 was confirmed by genotyping the individual of the resistant pool and susceptible pools and gave the same result as UBC#176 [19]. The above mentioned reference was a good evidence of our research finding. Hence, there is a probability to identify bacterial wilt resistance DNA sequence in this 350 bp DNA fragment. Hence investigation was carried out with the specific 350 bp fragment.

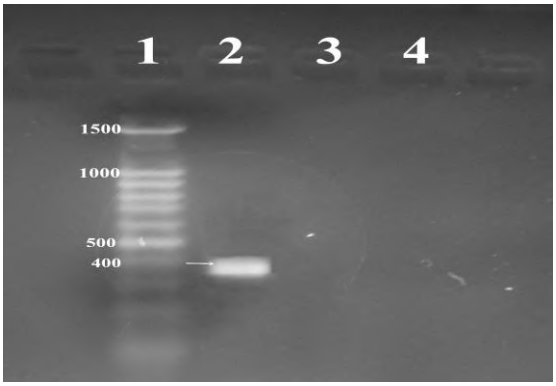

Fig. 1: DNA amplification in Solanum villosum at 350 bp position, Line 1: Ladder, lane 2: Solanum villosum, lane 3: Solanum sisymriifolium,lane 4: Solanum melangena

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Table 1: List of molecular markers which are tightly linked or linked with bacterial wilt resistant gene

Sl No.	Types of Marker	Marker Name	Forward Primer(5′-3′)	Reverse Primer (5′-3′)	Reference List
1.	RAPD	OPB-17	AGGGAACGAG	----	[19]
2.		OPG-05	CTGAGACGGA	----	
3.		OPH-07	CTGCATCGTG	----	
4.	UBC	UBC#176	CAAGGGAGGT		[20]
5.		UBC#205	CGGTTTGGAA		
6.		UBC#317	CTAGGGGCTG		
7.	SSR	STM0007	GACAAGCTGTGAAGTTTATT	AATTGAGAAAGAGTGTGTGTG	[19]
8.		emh01J23	ATGCAGCTCCCATAAACCCTAAAA	GTTTCCAAGACCAGCACTCCAAAC	[21]
9.		emb01N07	TGATAAGAAGGGCAAGCTCAGTCC	GTTTCGAGCTTATGGCTACACTGGACCT	
10.	SRAP	me1	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT	[22]
11.		me2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC	
12.		me4	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTGA	
13.		me5	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCGA	
14.	SCAR	SCU176-534:	TTGAACCAAGAATCTATTCG	GAACTTGAATGCCTACCAAA	[20]
15.		SCU176-1190F1R1	TGCGGATACTATCGGAAATA	CAACTCATTTCAGTCCGATT	
16.		SCU176-1190F2R2	TCACTCGGTGAGTCAATAGAT	TTTGCCGATGTTATCATGT	

1.7. Gel elution, DNA purification and sequencing of target DNA band

The species *Solanum villosum* produced 350 bp DNA fragment with UBC#176 primer. The amplified fragment was eluted from agarose gel and taken into eppendorf tube. It was purified by QIAGEN DNA purification Kit. The purified DNA fragment was again loaded in the 2% agarose gel for confirmation of purification. It showed exact, DNA band in the same 350 bp position. Hence, it proved that, purification of DNA was successfully completed. The concentration of purified DNA was done by DNA nanodropper. It was 20-25 ng/µl. This amount is sufficient for sequencing works. The required amount of DNA was sent to abroad for sequencing of nucleotide. The sequence of nucleotide was given below. It was 341 bp fragments.

GCAGGAAAAAATGCGGGAATTCCTATTGGCGCCA
GCTCGTTCACGCCGGAAAACCCCTTTTTCAATCGT
GGGAGCTTGACGTACACCTCCCTTGAAAAGTCTG
ATCTATTTTGCTATTGTCCTTACGAATTTTATCGG
AAAATTGATGAAATATGATCGAAGAACCATCCCC
AAAAAAATTATGAAAACTGGGATAATTCCTCCTG
CACCGGGTTGGTTACACTCGAAAACCCCTCCTTAT
ATCGTGTATGCTTGTCCTTTGCCTCCCTTGGGGGG
GTCCGGCCCAGTTTTTGCGAACTCCAACAAATTCC
CCGGAATACCTGACCCCTCCCTGGACAATGGTGT
GGT = 341 bp

1.8. Alignment and homology searching

The obtained sequenced DNA was used for NCBI, BLAST to identify homology with other gene of interest. *Solanum villosum* sequence get 78% homology with *Solanum lycopersicum* cultivar I-3 chromosome 5 which has GeneBank Accession No. CP023761.1. It also showed 78% homology with *Solanum pennellii* chromosome ch05, complete genome which has GeneBank Accession No. HG975444.1 and 79% homology with *Solanum*

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lycopersicum cultivar I-3 chromosome 8 which has GeneBank Accession No. CP023764.1.

Another alternative approach was made to search the bacterial wilt resistance gene in *S. melangena, S. villosum* and *S. sisymriifolium*. The assumption is that, from total RNA we will synthesis cDNA and this cDNA will be used as template for amplification of target bacterial wilt resistant DNA fragment. Previous synthesized all linked gene specific primer will be used for the same. On the basis of this principle the following activities were done.

1.9. Total RNA extraction and cDNA synthesis

Total RNA was extracted from three species *viz. S. melangena* (BARI begun-01), *S. villosum, S. sisymriifolium* were used to check the RNA molecule. It was done on the basis of QIAGEN RNA purification kit. The 2% agarose gel ('Fig. 2'). The high quality RNA band was noticed from the three species. Near about 400-500 µg/µl RNA was generated from leaf tissue. Those total RNA used as a template for cDNA synthesis. QIAgen cDNA synthesis kit was used for the same. The reaction mixture and other protocol were mentioned in the methodology. The produced cDNA was stored at -20°C for further use.

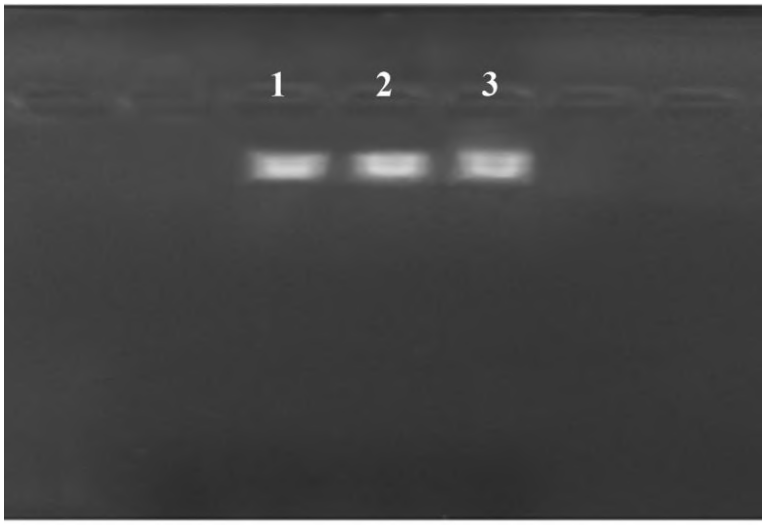

Fig. 2: RNA amplification in Lane 1- BARI Begun 1, Lane 3-Solanum villosum, Lane 4- Solanum sisymbriifolium

1.10. PCR amplification with cDNA as template

The previously synthesized all tightly linked gene specific primers were used for PCR amplification to identify bacterial wilt resistant gene. It was sorry to say that, none of the 16 primers gave amplification in any one of the three *Solanum spp.*

IV. CONCLUSION

Eggplant is an important vegetable in our country. It has multipurpose used in our daily consumption. It's production is seriously hampered due to different biotic diseases. Among them bacterial wilt disease is major one. Wild relatives of eggplants are resistant to bacterial wilt. *Solanum villosum* and *Solanum sisymriifolium* are two common wild relatives of eggplant which showed resistant to bacterial wilt. Hence, attempt was made to identify DNA fragment from wild species which will be resistant to bacterial wilt. Two different approaches was carried out to fulfill the objectives. Some total of 16 different bacterial wilt resistant molecular markers were used for the same. Out of them only one primer UBC#176 was gave DNA amplification in target position (350 bp) in the species *Solanum villosum.* The obtained DNA fragment was sequenced and homology was detected through NCBI, BLAST. It was showed maximum 79% homology with *Solanum lycopersicum* cultivar I-3 chromosome 8 which has GeneBank Accession No. CP023764.1.

Alternatively, Total RNA and cDNA was used to amplify the target link DNA fragment by using of previous primer. None of the primer was able to regenerate any DNA fragment which was linked to bacterial wilt. Hence further CODEHOP method, genome editing or any advanced molecular technique may be applied for searching of target bacterial wilt resistant gene.

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