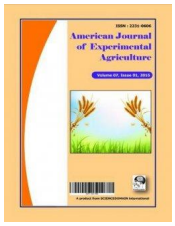

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Molecular Characterization of Selected Mutant Lines of Onion (*Allium cepa* L.) against Purple Leaf Blotch Disease Using SSR Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MC, MAKA and MAH conceptualized and designed the experiment. Author MC conducted the lab work and authors TQ, AH and MKS helped in conducting the lab work, collecting the data and analyzing the data, interpreting the results, preparing the tables and images. Authors MRH and SR prepared the manuscript. Author MAH supervised the entire work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purple leaf blotch (PLB) disease, for which there is no released resistant variety in Bangladesh, causes loss in production of onion in every year. The mutants of a released variety, BARI Piaz-2 has shown to possess resistance against PLB which is yet to be characterized at molecular level. An easy and simple molecular detection technique of the gene responsible for this disease will be of great use in future. The present study was thus carried out to molecularly characterize four mutant lines of onion using Simple Sequence Repeat (SSR) marker to detect the presence or absence of *PLB* gene conferring resistance against purple blotch disease in onion.
Place and Duration of Study: The research was carried out during the period from March, 2013 to

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April, 2014 in the Biotechnology Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA) in Mymensingh, Bangladesh.

Methodology: DNA was extracted from the vigorously growing fresh leaf samples of 25 days old seedlings of four mutant lines namely, BP_2-75/2, BP_2 -100/1, BP_2-100/2 and BP_2-100/12 of onion using CTAB method. The molecular characterization was done using two sets of SSR markers, namely MatK-1RKIM-f/MatK-3FKIM-r and rbcLa-F/rbcLa-R.

Results: All the four mutant lines showed clear band for the primer MatK-1RKIM-f/MatK-3FKIM-r which indicates the presence of *PLB* gene inferring resistance against purple blotch. Clear band was also observed with the marker rbcLa-F/rbcLa-R in all mutant lines except BP_2-100/12 indicating absence of *PLB* gene in BP_2 -100/12 which inferred susceptibility against purple blotch of onion. An unknown allele was also detected in this experiment which may be either linked with the *PLB* gene or a candidate gene which triggers the *PLB* gene responsible for purple blotch of onion.

Conclusion: Both the primers seemed to be effective in detecting the presence or absence of PLB gene in the studied mutant lines of onion variety BP_2. However, more number of primers should be tested for effective screening of diverse germplams that will be helpful in designing any future breeding programs.

Keywords: Onion; mutant lines; purple blotch; bari piaz-2; molecular markers.

1. INTRODUCTION

Onion (*Allium cepa* L. 2n=16), belonging to the family Alliaceae, is one of the most economically important and familiar vegetable and spice crops worldwide including Bangladesh [1]. The crop was originated in area, which includes Iran, Pakistan and specially their mountainous regions situated in the north of these countries [2-6]. Besides being used as salad and vegetable, onion is generally used as spice in most of the Asian countries. Onion has great economic importance due to it's medicinal and dietetic values [7-10]. Onion suffers from many diseases of which purple lear blotch (PLB) caused by *Alternaria porri* (Ellis) is a major one [11-14]. This disease caused substantial loss in both bulb and seed yield of onion in most onion growing countries including Bangladesh [15-16]. Control of this disease by using disease free seeds and fungicidal sprays are successful but have their disadvantages, such as the occurrence of fungicide resistance [17-19]. Due to these disadvantages, there is a constant requirement for inducing resistance to plants against the disease [20-23]. The recent development in the field of molecular markers analysis allows the rapid and accurate identification of genotypes that contain gene(s) responsible for resistance against purple blotch [7,24-26]. Identification of molecular markers and marker assisted selection for purple blotch disease resistance in onion has been studied before [25-27]; but no report of such studies with the genotypes of Bangladesh is recorded so far. Although a good number of local varieties of onion are available in Bangladesh, no recommended or released mutant lines resistant to purple blotch disease are available till now [28-29]. However, very recently, the mutant lines of BARI Piaz-2, a biennial type summer onion variety released by Bangladesh Agricultural Research Institute (BARI), had shown to possess resistance against purple leaf blotch (PLB) disease (unpublished data). The mutant lines had been developed by Bangladesh Institute of Nuclear Agriculture (BINA) by irradiating the dry seeds of BARI Piaz-2 with 75, 100, 125 and 150 Gy doses of gamma rays using the ^{60}Co source of Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERES), Bangladesh Atomic Energy Commission (BAEC). Thirteen promising mutant lines in M4 and subsequent generations had been selected based upon resistance to PLB, bulb and seed yield. The Karyological studies had revealed greater chromosomal length in these mutant lines which prompted to speculate that these modifications were taken place due to the increased gene dose effect through duplication of the chromosomal region and believed to be carrying the gene (s) responsible for resistance to PLB (unpublished data). The present piece of research program has used four of the most promising onion mutant lines to characterize the presence or absence of PLB gene conferring resistance against purple blotch using SSR markers.

2. MATERIALS AND METHODS

2.1 Experimental Site and Plant Materials

The present research project was conducted at biotechnology laboratory, Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Four mutant lines (of the variety BARI Piaz-2) namely, BP2 -75/2, BP2 -100/1, BP2 -100/2 and BP2-100/12, developed by the same institute were used as plant materials.

2.2 Genomic DNA Isolation and Polymorphism Survey for Primer Selection

For the SSR analysis, young, vigorously growing fresh leaf samples were collected from 25 days old seedlings of each of the plant material which were used as the source to extract genomic DNA. The leaf samples were stored at -80ºc freezer. DNA was extracted from the leaves of each genotype using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method [30]. The quality of the isolated DNA was sufficient for PCR analysis (data not shown). Two sets of SSR markers associated with the PLB gene conferring resistance against purple blotch were selected based on their potentiality for population discrimination which was determined by preliminary experiment with three sets of primers (data not shown). The primers are shown in Table 1.

2.3 PCR Amplification Profile

For MatK-1RKIM-f/MatK-3FKIM-r and rbcLa-F/rbcL-R primers set, DNA amplification was performed in an oil-free thermal cycler. The reaction mix was preheated at 94ºC for 5 minutes followed by 36 cycles of 5 minutes denaturation at 94ºC, 1 minute annealing at 55ºC and elongation or extension at 72ºC for 2 minutes. After the last cycle, a final step for 7 minutes at 72ºC to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4ºC.

3. RESULTS AND DISCUSSION

3.1 Selection of SSR Markers for Detecting the Presence of PLB Gene

This study was conducted to identify the mutant lines of onions that possess the PLB gene which confers resistance against purple blotch disease using simple sequence repeats or microsatellites (SSRs) markers. The SSR markers based molecular characterization experiments on onion are limited and in *In vitro* condition no such reliable work is reported so far. Several co-dominant simple sequence repeats or microsatellites (SSRs) have been reported in onion [31], of which two sets of markers, namely rbcL and matK were used to detect the presence or absence of PLB gene present in the shorter arm of chromosome 8 in the s1/s2 locus which confers resistance to purple blotch of onion. The PCR products for rbcL and matK were obtained using modified standard CCDB protocols (http://www.ccdb.ca/bCCDB_DOCS/CCDB_AmplificationPlants.pdf) The 67 bp rbcL nucleotide was obtained with the primers rbcLa-F [32] and rbcLa-R [33], while the 214 bp matK nucleotide was obtained with the matK-KIM primers, MatK-1RKIM-f and MatK-3FKIM-r (http://www.ccdb.ca/CCDB_DOCSCCDB_PrimerSets-Plants.pdf) as shown in Table 1.

3.2 Banding Pattern by the SSR Markers and PLB Gene Detection

Using the primer MatK-1RKIM-f/MatK-3FKIM-r, clear bands were observed for all the mutant lines, BP2-100/1, BP2-100/2, BP2-75/2 and BP2-100/12 of onion variety BP2 (Fig. 1) indicating the presence of PLB gene inferring the resistance against purple blotch of onion (Table 2).

Table 1. SSR primers used in the present study to detect the presence or absence of *PLB* gene conferring resistance against purple blotch disease in onion

SSR primers	Sequence of primer ('5—3')	Annealing temperature	Product size
MATK-1RKIM-F/MATK-3FKIM-R	F: ACCCAGTCCATCTGGAAATCTTGGTTC R: CGTACAGTACTTTTGTGTTTACGAG	55ºC	214
RBCLA-F/RBCLA-R	F: ATGTCACCACAAACAGAGACTAAAGC R: GTAAAATCAAGTCCACCRCG	55ºC	67

F: = sequence for forward primer (5'-3'); R: = sequence for reverse primer ('5-3')

By using rbcLa-F/rbcLa-R marker clear bands were also observed in BP2variety, BP2-100/1, BP2-100/2 and BP2-75/2 mutant lines mutant lines (Fig. 2) indicating the presence of PLB gene inferring the resistance. But no band was identified in BP2-100/12 mutant line which indicates the absence of PLB gene inferring susceptibility against purple blotch (Table 3). The reason behind the failure of rbcLa-F/rbcLa-R primer sets to identify any specific band in BP2-100/12 mutant is unclear. But a possibility behind this failure could be that the predicted high amount of gene duplications in the mutants has reduced segmental homology which restricted the primers to amplify the target genes in onion.

In this experiment, another unknown allele was detected between 50-75bp by the primer set MatK-1RKIM-f/MatK-3FKIM-r in BP2, BP2-100/1 and BP2-100/2 mutant lines (Fig. 1) which may be related with the PLB gene conferring resistance against purple blotch of onion or this also may be a candidate gene which trigger the PLB gene responsible for the purple blotch of onion. However, not much is known about this allele and further investigation will be needed to detect this unknown allele.

Table 2. Resistance pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -100/1, BP2 -100/2 and BP2-100/12 against purple blotch disease as determined by using MatK-1RKIM-f/MatK-3FKIM-r primer

Genotypes	Banding pattern		Resistant (R)	Susceptible (S)
	Presence (P)	Absence (A)		
BP_2	P	-	R	-
BP_2-75/2	P	-	R	-
BP_2-100/1	P	-	R	-
BP_2-100/2	P	-	R	-
BP_2-100/12	P	-	R	-

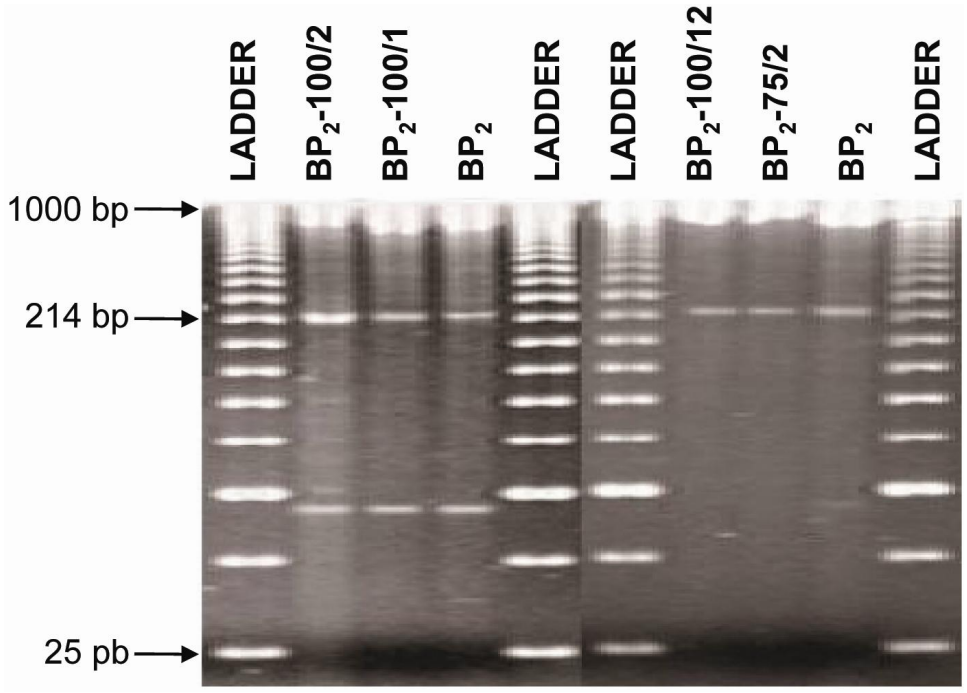

Fig. 1. Banding pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -100/1, BP2 -100/2 and BP2-100/12 using MatK-1RKIM-f/MatK-3FKIM-r primer confirming the presence of *PLB* gene conferring resistance against purple blotch disease

Table 3. Resistance pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -100/1, BP2 -100/2 and BP2-100/12 against purple blotch disease as determined by using rbcLa-F/rbcLa-R primer

Genotypes	Banding pattern		Resistant (R)	Susceptible (S)
	Presence (P)	Absence (A)		
BP2	P	-	R	-
BP2-75/2	P	-	R	-
BP2-100/1	P	-	R	-
BP2-100/2	P	-	R	-
BP2-100/12	-	A	-	S

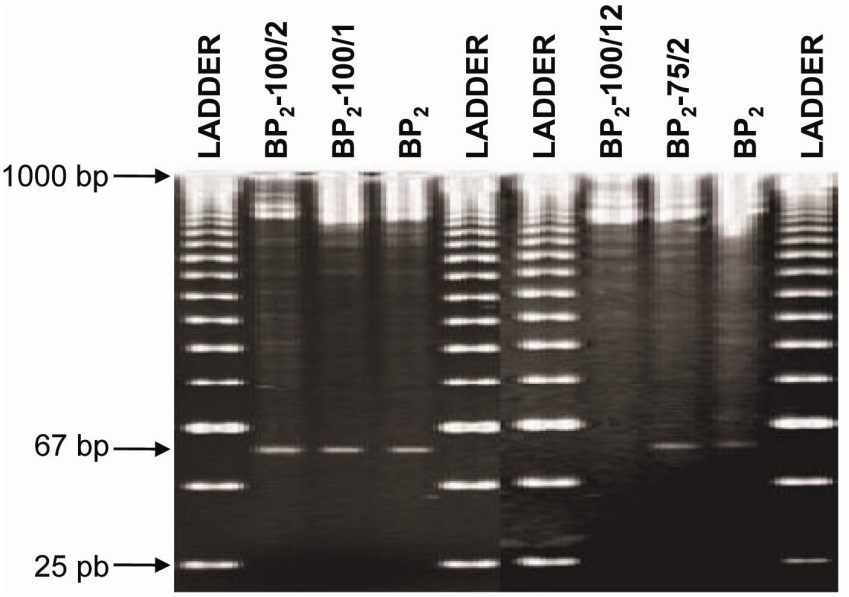

Fig. 2. Banding pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -100/1, BP2 -100/2 and BP2-100/12 using rbcLa-F/rbcLa-R primer to detect the presence/absence of *PLB* gene conferring resistance against purple blotch disease

4. CONCLUSION

The presence of the PLB gene conferring resistance to purple leaf blotch in onion was successfully detected in most of the mutant lines using two sets of SSR markers. The clear banding pattern and the easy detection of the presence of this gene shows the potentiality of using this technique for screening of wider germplasm preferably using more number of related primers that can be very helpful in selecting the parents for any breeding programs or biotechnological manipulations for improving the resistance for this disease in onion in future.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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