

Original Research Article

Molecular characterization of selected mutant lines of Onion against Purple Blotch Disease using SSR markers

ABSTRACT

Aims: The purple blotch disease causes loss in production of onion in every year. 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 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 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, BP₂-75/2, BP₂-100/1, BP₂-100/2 and BP₂-100/12 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 BP₂-100/12 indicating absence of *PLB* gene in BP₂-100/12 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 BP₂. However, more number of primers should be tested for effective screening of diverse germplasm that will be helpful in designing any future breeding programs.

Keywords: *Onion, Purple Blotch, SSR markers, BP₂, PLB and CTAB*

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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 its medicinal and dietetic values [7-10, 35]. Onion suffers from many diseases [34], of which purple blotch caused by *Alternaria porri* (Ellis) is a major one [11-14]. This disease caused substantial loss of 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]. The present piece of research program has thus been undertaken to characterize and identify the mutant lines of onions of Bangladesh that harbours PLB gene conferring durable resistance against purple blotch using SSR markers.

2. MATERIAL AND METHODS

2.1 Experimental site and plant materials

The present work was conducted at biotechnology laboratory, Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Four mutant accessions namely, BP2 -75/2, BP2 -100/1, BP2 -100/2 and BP2-100/12 of the variety BARI Piaz-2 (BP2) released by Bangladesh Agricultural Research Institute (BARI) were used as plant materials for the present research work.

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 on the basis of intensity of bands, presence of smearing, consistency within individuals and potential for population discrimination. The primers are shown in Table 1.

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	Annealing Temperature	Product Size
MATK-1RKIM-F/MATK-3FKIM-R	F: ACCCAGTCCATCTGGAAATCTTGGTTC R: CGTACAGTACTTTTGTGTTTACGAG	55°C	214
RBCLA-F/RBCLA-R	F: ATGTCACCACAAACAGAGACTAAAGC R: GTAAAATCAAGTCCACCRCG	55°C	67

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

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 94°C for 5 minutes followed by 36 cycles of 5 minutes denaturation at 94°C, 1 minute annealing at 55°C and elongation or extension at 72°C for 2 minutes. After the last cycle, a final step for 7 minutes at 72°C to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 40°C.

3. RESULTS AND DISCUSSION

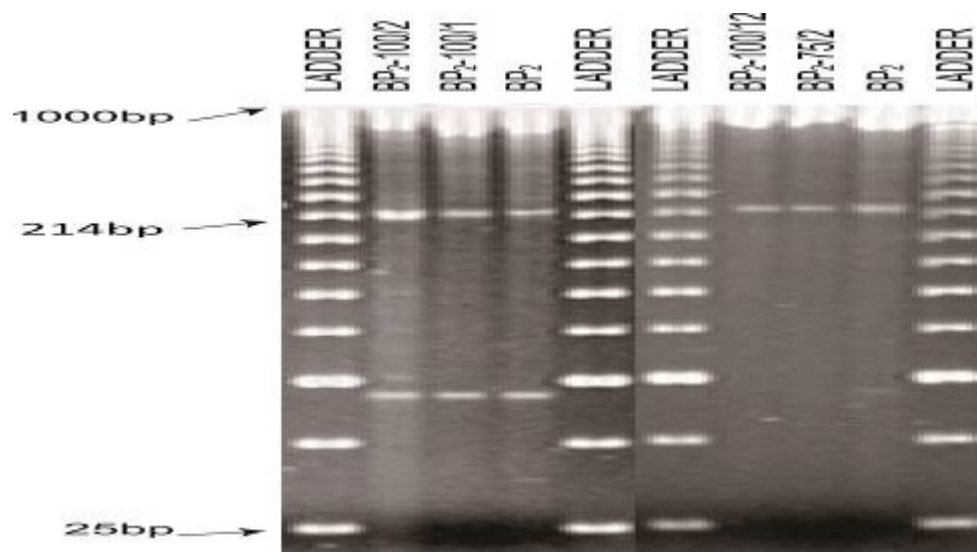
3.1 Selection of SSR markers for detecting the presence of PLB gene

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.cddb.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.cddb.ca/CCDB_DOCSCCDB_PrimerSets-Plants.pdf) as shown in table 1.

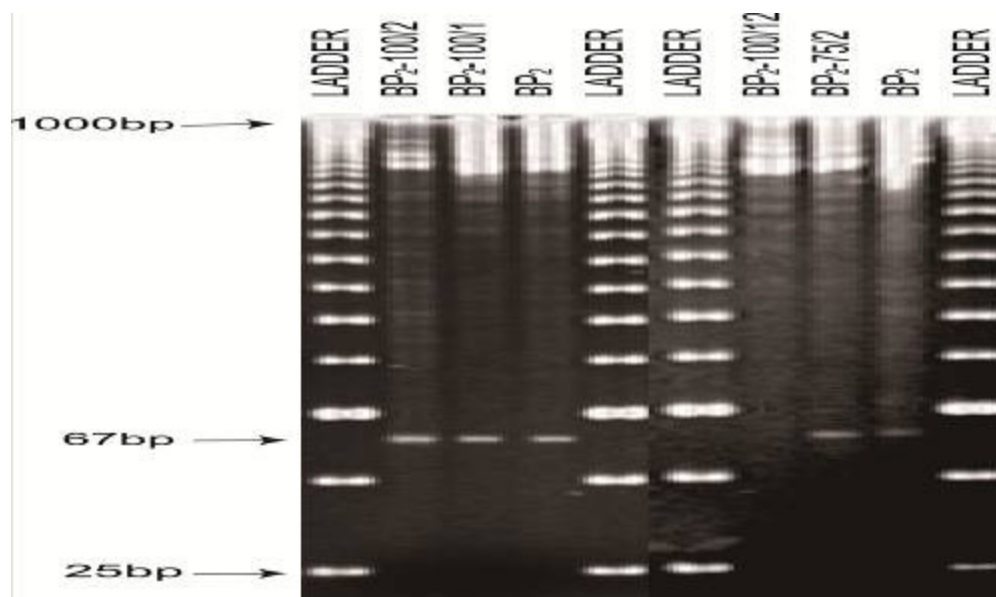
3.2 Banding pattern by the SSR markers and PLB gene detection

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). By using rbcLa-F/rbcLa-R marker clear bands were also observed in BP2 variety, BP2-100/1, BP2-100/2 and BP2-75/2 mutant lines (Fig 2) indicating the presence of PLB gene inferring the resistance. But no band was identified in BP2-100/12

95 mutant line which indicates the absence of *PLB* gene inferring susceptibility against purple
 96 blotch (Table 3).
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99
 100 **Figure 1. Banding pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -**
 101 **100/1, BP2 -100/2 and BP2-100/12 using MatK-1RKIM-f/MatK-3FKIM-r primer**
 102 **confirming the presence of *PLB* gene conferring resistance against purple blotch**
 103 **disease.**



104
 105 **Figure 2. Banding pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -**
 106 **100/1, BP2 -100/2 and BP2-100/12 using rbcLa-F/rbcLa-R primer to detect the**
 107 **presence/absence of *PLB* gene conferring resistance against purple blotch disease.**

108 **Table 2. Resistance pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -**
 109 **100/1, BP2 -100/2 and BP2-100/12 against purple blotch disease as determined by**
 110 **using MatK-1RKIM-f/MatK-3FKIM-r primer**

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

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 112
 113
 114 **Table 3. Resistance pattern of onion variety BP2 and it's mutant lines, BP2 -75/2, BP2 -**
 115 **100/1, BP2 -100/2 and BP2-100/12 against purple blotch disease as determined by**
 116 **using rbcLa-F/rbcLa-R primer**

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

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

123 124 125 **4. CONCLUSION**

126
 127 The presence of the PLB gene conferring resistance to purple leaf blotch in onion was
 128 successfully detected in the mutant lines of BP2 variety using two sets of SSR markers. The

129 clear banding pattern and the easy detection of the presence of this gene shows the
130 potentiality of using this technique for screening of wider germplasm preferably using more
131 number of related primers that can be very helpful in selecting the parents for any breeding
132 programs or biotechnological manipulations for improving the resistance for this disease in
133 onion in future.

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