



## Molecular mapping of QTLs for resistance to northern corn leaf blight in maize

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### Abstract

The objectives of this study were to identify RAPD and ISSR markers linked to northern corn leaf blight (NCLB) resistant genes in F<sub>2</sub> population of maize using bulked segregant analysis and to map NCLB resistance genes in F<sub>2</sub> populations of maize. The F<sub>2</sub> white population of maize was developed from a cross between the resistant line Sd-63 and the susceptible line Sd-7. Bulked segregant analysis with RAPD and ISSR markers was conducted to identify markers that were linked to the Ht1 gene. The linkage relationship between the RAPD markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>) and northern corn leaf blight resistance (NCLB) Ht1 gene were estimated using F<sub>2</sub> population derived from the cross Sd63 x Sd7. The genetic distances between RAPD markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>) and NCLB resistance Ht1 gene were determined to be 25.4, 17.5 and 9.6 cM, respectively, with LOD scores of 38.9, 38.5 and 56.5, respectively. Therefore, RAPD markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>) were linked to the quantitative trait loci (QTL) for (NCLB) resistance Ht1 gene. The genetic distance between ISSR markers (AD1<sub>820bp</sub> and AD6<sub>650bp</sub>) and NCLB resistance Ht1 gene were determined to be 7.4 and 3.3 cM, respectively, with LOD scores of 49.1 and 51.8, respectively. Therefore, ISSR markers (AD1<sub>820bp</sub> and AD6<sub>650bp</sub>) were linked to the quantitative trait loci (QTL) for NCLB resistance Ht1 gene. The present study indicated that RAPD and ISSR markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to NCLB resistance gene in maize. Once these markers are identified, they can be used in maize breeding programs, as a selection tool in early generations.

**Key words:** Northern corn leaf blight, ISSR, QTLs mapping, RAPD.

### Introduction

Maize (*Zea mays* L.) is the third most important cereal crop in the world. In Africa, maize is increasingly becoming an important non-traditional agricultural export crop. In addition to strong demand for maize as a food crop, the demand for maize is projected to rise with increasing population growth and an expanding need for livestock feed. Production of maize grain is insufficient relative to the needs of food and feed consumption in many areas. Accordingly, increasing maize production is considered essential for food security in developing countries <sup>1</sup>. Maize is grown for food, feed, fodder and industrial purposes in Egypt. Egypt imports approximately 35% of its maize need. It is important to develop high-yielding and disease resistant hybrids to meet the country's demands.

During the past few decades, a major fungal foliar disease of maize (*Zea mays* L.) called northern corn leaf blight (NCLB) causes substantial yield losses worldwide. NCLB, caused by the fungus *Helminthosporium turcicum* (Pass) <sup>2</sup>, is one of the most serious maize diseases throughout the world, particularly in the humid mid-altitude and highland regions of Africa <sup>3,4</sup>. Symptoms of NCLB are elliptical leaf lesions, which are at first chlorotic and gray-green and later becoming necrotic, result in wilted leaves. Lesions may coalesce in susceptible plants and result in complete destruction of the foliage. Grain yield losses can exceed 50% in susceptible maize cultivars if infection occurs before flowering <sup>5</sup>.

NCLB is mainly controlled by resistant cultivars. The resistance is either qualitative or quantitative. Qualitative resistance is

typically race specific and inherited by single genes whereas quantitative resistance is race non-specific and oligogenic or polygenic. The categories, qualitative and quantitative, refer to the distribution of a trait in a population and not to its effectiveness<sup>6</sup>.

Molecular markers that are closely linked with target alleles present a useful tool in plant breeding since they can help to detect the resistant genes of interest without the need of carrying out a field disease test. Also, they allow for screening large number of breeding materials at early growth stages and in a short time. The Polymerase Chain Reaction (PCR) <sup>7</sup> offers the potential to lessen the time and expense of molecular mapping. In particular, Randomly Amplified Polymorphic DNAs (RAPDs), involving the use of single short DNA primer to direct amplification of discrete sequences <sup>8</sup>, have shown promise in cereals <sup>9-11</sup>. Recently, identification of molecular markers linked to northern corn leaf blight resistance in yellow population of maize have been reported<sup>12</sup>.

The objectives of this investigation were to identify RAPD and ISSR markers linked to NCLB resistance genes in F<sub>2</sub> populations of maize, using bulked segregant analysis and to map NCLB resistance genes in F<sub>2</sub> populations of maize.

### Materials and Methods

**Plant materials and disease evaluation:** Identification of RAPD and ISSR markers linked to northern corn leaf blight (NCLB)

disease resistance were carried out on a segregating F2 population derived from a cross between two white lines, the resistant line Sids-63 (Sd-63) and the susceptible line Sids-7 (Sd-7). The cross was made during the season of 2005 and was selfed in 2006 to produce the F2 population.

For evaluating against NCLB, F2 population (259 individual plants) and their parents were planted under field conditions, in the late summer of 2007 at the Experimental Farm Station, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, where environmental conditions allow for a uniform disease infection.

Artificial infection was done to enhance the natural infection, using an isolate of *Helminthosporium turcicum* cod T-13AS which was a single spore culture grown in Petri dishes containing potato dextrose agar medium for ten days at 25 ± 2°C. Spore suspensions were prepared by adding sterilized distilled water over fungal growth, which was scraped off, using a sterilized needle. The suspensions were then strained through a sterilized cheese-cloth. Spore concentration was adjusted to 2.5 × 10<sup>3</sup> spore/ml, using sterilized distilled water. Plants were inoculated at the three to five leaf stage of growth in the evening using a spore suspension. Severity of NCLB, as a percentage of infected leaf area (percentage average lesion size), was assessed after flowering growth stage around eight weeks after the inoculation and readings were classified, according to Elliot and Jenkins<sup>13</sup> as follows:

Rating scale	Leaf area infected (%)	Resistance level
0.5	<5	Highly resistant (HR)
1.0	6-10	Resistant (R)
2.0	11-25	Moderately resistant (MR)
3.0	26-50	Moderately susceptible (MS)
4.0	51-75	Susceptible (S)
5.0	>75	Highly susceptible (HS)

**DNA extraction:** Genomic DNA was extracted from fresh leaves of individual F2 plants and their parents, using CTAB<sup>14</sup>. RNA was removed from the DNA preparation by adding 10µl of RNAase (10 mg/ml) and incubating for 30 min at 37°C. DNA sample concentration was quantified by using a spectrophotometer (Beckman Du-65).

**PCR amplification:** Thirty-eight primers (Table 1) were used in the present investigation to amplify the template DNA. Each amplification reaction was performed in a 25-µl vol., containing 50 ng of genomic DNA, 1 x PCR buffer MgCl<sub>2</sub> (60 mM KCl, 10 mM Tris- HCl (pH 9.0), 2 mM MgCl<sub>2</sub> and 1% Triton X-100), 200 mM each of dATP, dCTP, dGTP and dTTP (Promega), 50 pM primer, 50 ng template DNA and 1.5µ of Taq DNA polymerase. Amplifications were carried out in an MJ Research PTC-100 thermal cycler with amplification conditions adopted from Williams *et al.*<sup>8</sup>, DNA denaturation at 94°C for three minutes and 45 cycles of melting at 94°C for one min, annealing at 36°C for one min and extending at 72°C for two min, This was followed by a seven min final extension step at 72°C, and the reactions were kept at 10°C. RAPD fragments were size-fractionated in 2% agarose gel in 0.5 x TBE buffer, with a 1-kb ladder molecular weight marker. Gels were stained in ethidium bromide solution and photographed.

**Bulked segregant analysis:** Bulkied-segregant analysis (BSA) was used, in conjunction with RAPD and ISSR analysis<sup>15</sup>, to find markers linked to genes of interest. Resistant and susceptible

bulks were prepared from F2 individuals by pooling aliquots, containing equivalent amounts of total DNA, approximately, 50 ng/µl from each of fourteen susceptible, and fourteen resistant F2 plants were selected based on phenotypic assessments. RAPD and ISSR primers were then screened on the parents and the two bulk DNA samples, from which some primer combinations revealed bands that were polymorphic, not only between parental genotypes, but also between the pair of the bulk DNA. Based on the evaluations of DNA bulks, individual F2 plants were analyzed with cosegregating primers to confirm RAPD and ISSR markers linkage to the NCLB resistance genes .

**Data analysis:** Goodness of fit to a 3:1 ratio was calculated for RAPD and ISSR markers by chi-square test. The association between molecular markers and resistance to NCLB trait was assessed with correlation and simple regression analysis, using PROC REG of SAS version 9.1 software packages<sup>16</sup>. Magnitude of the marker associated phenotypic effect was described by the coefficient of determination (r<sup>2</sup>) and simple correlation (r), which represented the fraction of variance explained by the polymorphism of the marker.

**Linkage analysis:** Map manager QTX Version 0.22<sup>17</sup> was used to analyze the linkage relationship of RAPD and ISSR markers detected from bulked segregant analysis. Linkage was detected when a log of the likelihood ratio (LOD) threshold of 3.0 and maximum distance was 50 cM. The Kosambi's mapping function was used.

## Results and Discussion

**RAPD markers analysis:** Out of 38 arbitrary primers (Table 1), which were screened for polymorphisms between the two tested inbred lines: Sd63 (resistant) and Sd7 (susceptible) parents, 27 RAPD primers (71.1%) that gave polymorphic bands suitable to differentiate between the two parents, were identified. A total of 201 bands were amplified, using 38 RAPD primers, produced an average of 5.3 bands per primer. The number of RAPD fragments, that were amplified, ranged from two to thirteen and the size ranged from about 130 to 1200 bp. Of these 27 RAPD primers, Pr11 primer (5' CAATCGCCGT 3') produced two strong polymorphic bands at 180 and 300 bp that were present only in the susceptible parent (Sd7), as shown in Fig. 1. The Pr11 primer was selected for screening DNA bulks and their parental DNA. The Pr11 primer, which generated two polymorphic fragments at 180 and 300 bp, were present only in NCLB-susceptible bulk and Sd7 (susceptible parent) and were missing in NCLB-resistant bulk and Sd63 (resistant parent). In addition, the OPB08 (5' GTCCACACGG 3') primer produced a strong polymorphic band at 290 bp that was present in only the susceptible bulked DNA but not in the resistant bulked DNA. These RAPD markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>) were regarded as candidate markers, linked to NCLB susceptible gene in F2 population of maize .

These polymorphic markers Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub> were further used to check their linkage to the NCLB susceptible gene, using a segregating F2 population derived from the cross between the resistant parent (Sd63) and the susceptible parent (Sd7). When analyzing the individual plants of F2 population, the Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub> fragments were amplified only in the DNA, obtained from susceptible F2 plants. The PCR

**Table 1.** Number of amplifications and polymorphic products of 38 primers used to screen the polymorphism between the two inbred lines (Sd63 and Sd7).

Primer	Nucleotide sequence (5' → 3')	No. of amplification products	No. of polymorphic products
Pr <sub>1</sub>	CAGGCCCTTC	3	1
Pr <sub>2</sub>	TGCCGAGCTG	3	0
Pr <sub>3</sub>	AGTCAGCCAC	4	1
Pr <sub>4</sub>	AATCGGGCTG	7	3
Pr <sub>5</sub>	AGGGGTCTTG	4	0
Pr <sub>6</sub>	GGTCCCTGAC	6	2
Pr <sub>7</sub>	GAAACGGGTG	6	2
Pr <sub>8</sub>	GTGACGTAGG	4	3
Pr <sub>9</sub>	GGGTAACGCC	4	3
Pr <sub>10</sub>	GTGATCGCAG	5	2
Pr <sub>11</sub>	CAATCGCCGT	5	3
Pr <sub>12</sub>	TCGGCGATAG	4	1
Pr <sub>13</sub>	CAGCACCCAC	12	3
Pr <sub>14</sub>	TCTGTGCTGG	5	1
Pr <sub>15</sub>	TTCCGAACCC	3	1
Pr <sub>16</sub>	AGCCAGCGAA	6	0
Pr <sub>17</sub>	GACCGTGTGT	3	0
Pr <sub>18</sub>	AGGTGACCGT	5	2
Pr <sub>19</sub>	CAAACGTCGG	8	3
Pr <sub>20</sub>	GTTGCGATCC	13	2
UBC321	ATCTAGGGAC	0	0
UBC475	CCAGCGTATT	2	1
UBC532	TTGAGACAGC	6	3
OPA02	TGCCGAGCTG	6	4
OPA06	GGTCCCTGAC	0	0
OPA07	GAAACGGGTG	4	0
OPB08	GTCCACACGG	9	4
OPB09	TGGGGGACTC	0	0
OPB13	TTCCCCGCT	4	1
OPC04	CCGCATCTAC	6	1
OPC15	GACGGATCAG	7	3
OPE20	AACGGTGACC	7	4
OPF15	CCAGTACTCC	0	0
OPH13	GACGCCACAC	8	1
OPJ04	CCGAACACGG	8	2
OPJ10	AAGCCCGAGG	6	0
OPU06	ACCTTTGCGG	10	0
OPZ03	CAGCACCGCA	8	1

amplification of resistant parent (Sd63), susceptible parent (Sd7), resistant bulk, susceptible bulk and five F2 resistant and five F2 susceptible individuals, using Pr<sub>11</sub> and OPB08 primers, are shown in Fig. 1A and B, respectively.

For the RAPD markers Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>, 76 of 259 (29.3%) individuals in the F2 population exhibited the amplified polymorphic fragments (180, 300 and 290 bp) while the remaining did not. The ratio fitted the expected Mendelian ratio 3:1 ( $\chi^2 = 2.6$ ,  $P < 0.1$ ) (Table 3).

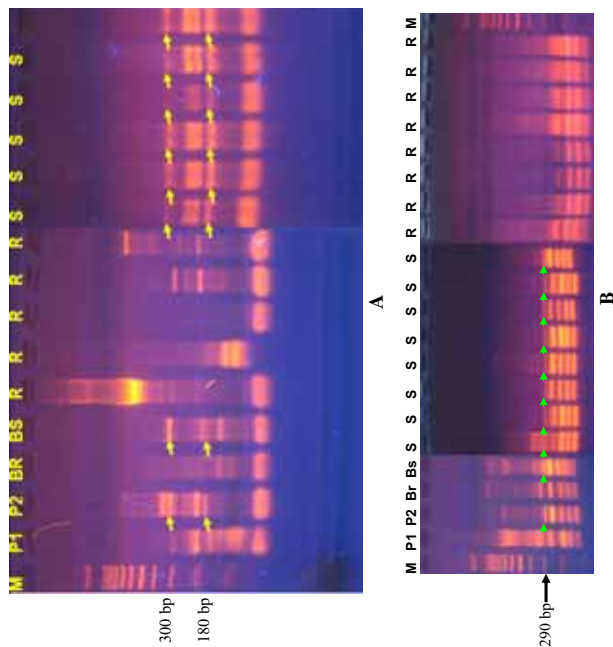
Correlation and simple regression analysis were carried out in

order to confirm an association between the Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub> markers and resistance to NCLB in all 259 F2 progenies. The results showed that the correlation and regression analysis for the relationship between the three markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>) and the phenotypes of F2 individuals were significant and they recorded  $r = -0.77$ ,  $-0.73$  and  $-0.78$ , respectively, and  $r^2 = 0.60$ ,  $0.54$  and  $0.61$ , respectively (Table 2). This indicates that the three markers were linked with the NCLB resistance gene.

Bulked segregant analysis (BSA)<sup>15</sup>, combined with several types of molecular markers, has been extensively used to find markers linked to disease resistance genes in a number of species<sup>18-20</sup>. In several previous studies, RAPD, combined with BSA, have been successfully used to identify DNA marker(s) linked to many important traits. For example, Poulsen *et al.*<sup>21</sup> found RAPD marker (OPU022<sub>700</sub>) linked to leaf rust resistance in barley. The OPU022<sub>700</sub> marker was shown to be useful in the identification of the individual F2 plants, originally misclassified as having susceptible infection types. Motawei *et al.*<sup>22</sup> reported the presence of RAPD marker (Pr7<sub>700</sub>) linked to the leaf rust resistance gene, Lr29, in wheat. Moreover, Motawei *et al.*<sup>23</sup> found two RAPD markers (Pr8<sub>450</sub> and Pr18<sub>700</sub>) linked to stripe rust resistance gene in wheat. Recently, RAPD markers, combined with bulk segregant analysis, have been used to identify molecular markers, linked to leaf and stripe rust resistance genes in wheat<sup>24-26</sup>.

**ISSR markers analysis:** Out of 25 ISSR primers (Table 3) which were screened for polymorphisms between the two tested inbred lines Sd63 (resistant) and Sd7 (susceptible) parents, 18 ISSR primers (72.0%) that gave polymorphic bands suitable to differentiate between the two parents were identified. An average of 5.32 bands was produced per primer. The number of ISSR fragments that were amplified ranged from four to ten and the sizes ranged from about 180 to 1600 bp. Of these 18 ISSR primers, AD1 and AD6 primers (5' (GAG)3GC 3' and GT(CAC)7, respectively) produced two strong polymorphic bands at 820 and 650 bp, respectively, that were present only in the susceptible parent (Sd7). These ISSR primers were selected for screening DNA bulks and their parental DNA. The primers, AD1 and AD6, generated the two polymorphic fragments at 820 and 650 bp, which were present only in NCLB-susceptible bulk and Sd7 (susceptible parent) and were missing in NCLB-resistant bulk and Sd63 (resistant parent). These ISSR markers (AD1<sub>820bp</sub> and AD6<sub>650bp</sub>) were regarded as candidate markers, linked to NCLB resistant gene in maize.

These polymorphic markers, AD1<sub>820bp</sub> and AD6<sub>650bp</sub>, were further used to check their linkage to the NCLB resistant gene, using a segregating F2 population derived from the cross between the resistant parent (Sd63) and the susceptible one (Sd7). When analyzing the individual plants of F2 population, the AD1<sub>820bp</sub> and AD6<sub>650bp</sub> fragments were amplified in the DNA, obtained only in F2 susceptible ones. The PCR amplification of resistant parent (Sd63), susceptible parent (Sd7), resistant bulk, susceptible bulk



**Figure 1.** RAPD fragments, produced by Primer 11 (5' CAATCGCCGT'3) at (A) and Primer OPB08 (5' GTCCACACGG'3) at (B). M: Molecular weight followed by P<sub>1</sub> and P<sub>2</sub> parents Sd63 and Sd7, respectively. Br: bulk resistance; Bs: bulk susceptible, F2 individuals in the cross, Sd63 x Sd7 (R: resistant; S: susceptible).

**Table 2.** Significant association between NCLB resistance and markers (RAPD and ISSR) in the 259 F2 plants population of maize detected, using chi-square ( $\chi^2$ ), correlation (r) and determination coefficient ( $r^2$ ) analysis.

Tool	Markers	Tested of plant		Expected ratio	$\chi^2$	r	$r^2$
		Total	R S				
RAPD	Pr 11 <sub>300bp</sub>	259	183	76	3 : 1	2.6 <sup>ns</sup>	0.6 <sup>**</sup>
	Pr 11 <sub>180bp</sub>	259	183	76	3 : 1	2.6 <sup>ns</sup>	0.54 <sup>**</sup>
	OPB08 <sub>290bp</sub>	259	183	76	3 : 1	2.6 <sup>ns</sup>	0.61 <sup>**</sup>
ISSR	AD1 <sub>820bp</sub>	259	183	76	3 : 1	2.6 <sup>ns</sup>	0.6 <sup>**</sup>
	AD6 <sub>650bp</sub>	259	183	76	3 : 1	2.6 <sup>ns</sup>	0.59 <sup>**</sup>

R = Resistant plants, S = susceptible plants  
ns: non significant and \*\*: significant at 0.01 level of probability.

**Table 3.** Number of amplifications and polymorphic products of 25 ISSR primers used to screen the polymorphism between the two inbred lines (Sd63 and Sd7).

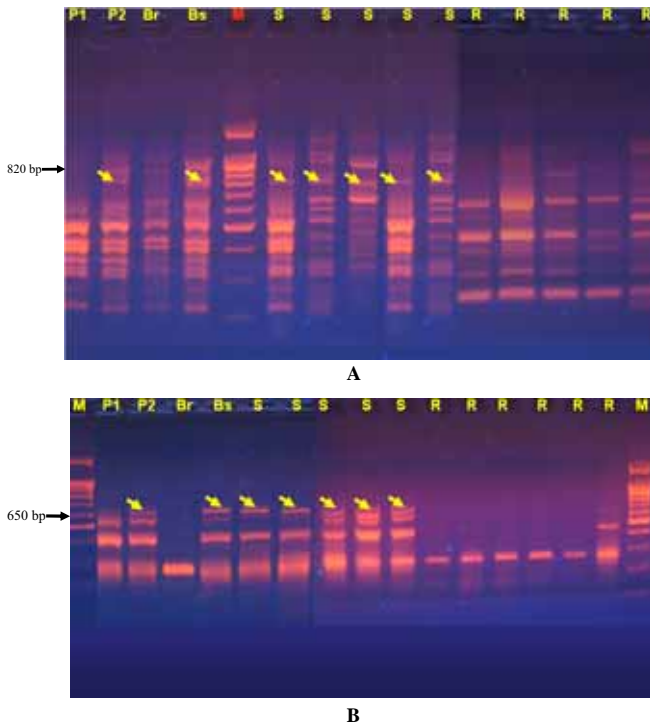
Primer	(5' → 3')	No. of amplification products		No. of polymorphic products	
		products	products	products	products
AD1	(GA)9C	9	9	3	3
AD2	(AGC)6G	6	6	1	1
AD3	(ACC)6G	4	4	1	1
AD4	(AGC)6C	0	0	0	0
AD5	(CA)10C	7	7	3	3
AD6	GT(CAC)7	4	4	1	1
AD7	(AG)9C	7	7	2	2
AD8	(AGC)6G	6	6	1	1
AD9	(AC)9G	9	9	1	1
M-1	(AC)8CG	6	6	3	3
M-2	(AC)8CC-T	7	7	4	4
M-6	(CAC)5	5	5	1	1
M-7	(CAG)5	10	10	2	2
M-8	(GTG)5	8	8	10	10
M-9	(GACAC)4	0	0	0	0
M-10	(CA)6A/GG	10	10	2	2
M-11	(CA)6A/G	6	6	2	2
M-12	(CA)6RY	6	6	1	1
M-13	(AGC)3Y	0	0	0	0
HB13	(GAG)3GC	6	6	0	0
HB14	(CTC)3CG	0	0	0	0
HB15	(GTG)3GC	7	7	4	4
P02	AT(CGAT)3CG	0	0	0	0
D12	(GAGA)3CG	0	0	0	0
D14	(CAC)3GC	10	10	2	2

and F2 resistant and F2 susceptible individuals, using AD1 and AD6 primer are shown in Fig. 2 (A) and (B), respectively.

For the ISSR markers, AD1<sub>820bp</sub> and AD6<sub>650bp</sub>, 76 of 259 (29.3%) individuals in the F2 population exhibited the amplified polymorphic fragments (820 and 650 bp) while the remaining did not (Fig. 2 A-B). The ratio fitted the expected Mendelian ratio 3:1 ( $\chi^2=2.6$ ,  $P<0.1$ ) (Table 3).

To check for potential co-segregation of DNA fragments and NCLB resistant phenotypes, correlation and simple regression analysis were carried out in order to confirm an association between the AD1<sub>820bp</sub> and AD6<sub>650bp</sub> markers and the resistance to NCLB in all 259 F2 progenies. The results showed that the correlation and regression analysis for the relationship between the presence of the two markers AD1<sub>820bp</sub> and AD6<sub>650bp</sub> and the phenotypes of F2 individuals were significant and recorded  $r = -0.78$  and  $0.76$ , respectively, and  $r^2 = 0.60$  and  $0.59$ , respectively (Table 3). This indicates that the two markers were linked to the NCLB resistant gene.

**Mapping of gene resistance to NCLB:** The linkage relationship between the RAPD markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>) and northern corn leaf blight (NCLB) *Ht1* resistance gene was estimated using F2 population derived from the cross Sd63 x Sd7. The genetic distance between RAPD markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub>) and NCLB *Ht1* resistance gene was determined to be 25.4, 17.5 and 9.6 cM, respectively, with LOD scores of 38.9, 38.5 and 56.5, respectively (Table 4 and Fig. 3). Therefore, RAPD markers (Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub> primers) were linked to the quantitative trait loci (QTL) for (NCLB) *Ht1* resistance gene.



**Figure 2.** ISSR fragments, produced by AD1 primer (5' (GAG)3GC 3') at (A) and AD6 primer (5' GT(CAC)7 '3) at (B). M molecular weight followed by P<sub>1</sub> and P<sub>2</sub> parents, Sd63 and Sd7, respectively. Br bulk resistance; Bs bulk susceptible, F2 individuals in the cross, Sd63 x Sd7 (R resistant, S susceptible).

After mapmaker linkage analysis on the F2 population, the genetic distance between ISSR markers (AD1 and AD6) and NCLB resistance *Ht1* gene were determined to be 7.4 and 3.3 cM, respectively, with LOD scores of 49.1 and 51.8, respectively (Table 4 and Figure 3). Therefore, ISSR markers (AD1<sub>820bp</sub> and AD6<sub>650bp</sub>) were linked to the quantitative trait loci (QTL) for NCLB resistance *Ht1* gene.

In several previous studies, quantitative trait loci (QTLs) associated with general resistance to NCLB, were identified on all 10 maize chromosomes<sup>27-30</sup>. All of these studies reported that molecular markers associated to NCLB resistance are potentially useful for the identification of genotyped individuals carrying NCLB resistant traits in breeding programs. The use of molecular markers can increase the efficiency of conventional plant breeding by identifying markers linked to the trait of interest, which are difficult to evaluate and/or are largely affected by the environment<sup>31</sup>. Hence, there is a need to develop a rapid screening method to select for resistance to NCLB. Tight linkage between molecular markers and genes for disease resistance can be of great benefit to disease resistance breeding programs by allowing the investigator to follow the DNA markers (PCR-based markers) through early generation rather than waiting for phenotypic expression of the resistance genes<sup>12</sup>. Molecular markers that are closely linked with target alleles present a useful tool in plant breeding since they can help to detect the resistant genes of interest without the need of carrying out field disease test. Also, it allows for screening big number of breeding materials at early growth stages and in short time.



**Figure 3.** RAPD markers (RAPD Pr11<sub>180bp</sub>, Pr11<sub>300bp</sub> and OPB08<sub>290bp</sub> primers) and ISSR markers (ISSR AD1<sub>820bp</sub> and AD6<sub>650bp</sub> primers) were located through the MAPMAKER-QTL analysis. All distances are given in centi-Morgan, using Kosambi's mapping function.

**Table 4.** Location of QTL's affecting host-plant response to *E. turcicum* in the F2 population of maize.

Locus <i>Ht1</i>	No. of F2 plants	Map (cM)	SE (+/-)	LOD
D6	259	3.3	1.1	51.8
D1	259	4.1	1.3	49.1
OPB08	259	2.2	0.9	56.5
Pr11 <sub>300bp</sub>	259	7.9	1.8	38.5
Pr11 <sub>180bp</sub>	259	7.9	1.8	38.9

The present study indicated that RAPD and ISSR markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to northern corn leaf blight resistance gene in maize. Once these markers are identified, they can be used in maize breeding programs as a selection tool in early generations.

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