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MOLECULAR CHARACTERIZATIONS OF BRINJAL MAPPING POPULATION USING SSR MARKERS AGAINST LITTLE LEAF RESISTANCE

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ABSTRACT

The experimental material for present investigation comprising of F_2 segregating population of cross resistant parent AB 15-06 and susceptible parent GRB 5 against little leaf disease. Total 168 mapping population were developed from F_1 seeds derived from crosses of above referred parents. F_1 hybrid seeds were collected in the year 2020-21. Total 168 F_2 mapping population were sown in the year 2021-22. Little leaf disease evaluation in 168 F_2 mapping population revealed that 128 individuals showed resistance and 40 individuals found susceptible reaction which followed 3:1 of segregation ratio suggested single dominant gene as per Mendelian genetics. Genotyping of parents and F_2 mapping populations were carried out through 305 SSR markers. Total 305 SSR markers used for screening of F_2 population along with parents of which 48 SSR markers resulted in polymorphic banding patterns. Out of 48 polymorphic markers, 28 followed the Mendelian segregation ratio in the F_2 mapping population.
Keywords **:** Brinjal, SSR markers, DNA Extraction, Mapping population, Little leaf disease.

Introduction

Solanum melongena L., or brinjal, is a widely produced vegetable crop in tropical and subtropical regions that is valued for its tasty fruit, which is categorized as a berry by nature. In *Solanaceae* family, brinjal is the third most important crop after potato and tomato. In 2022-23, 59,312,600 metric tonnes of eggplants were produced worldwide, an increase of 1.0% from 58,705,398 tons in 2021 (Anon., 2017). Brinjal output in India is anticipated to have reached 12.61 million metric tons in the fiscal year 2023. The major brinjal growing districts in Gujarat are Ahmedabad, Vadodara, Surat, Junagadh, Kheda, Anand, Bhavnagar and Navsari.

In India, little leaf disease is common because of overlapping crop cycles, weeds, and leafhopper populations that serve as phytoplasma reservoirs. Plants that harbor leafhoppers that carry phytoplasma can become infected at any point throughout their growth cycle. The disease can potentially spread through grafting. Numerous yellow-type diseases were earlier thought to be caused by viruses in view of their infective spread, their symptomatology and the fact that they were transmitted by insects. This yellow diseases causing microorganism were first time observed by Japanese workers Maejima & Oshima (2014) as pleomorphic cells in ultra-thin sections of leaves of mulberry infected with dwarf disease.

For numerous fundamental and practical uses in crop research, including the *Solanaceae* family, molecular markers have been widely employed to create genetic and physical genome maps (Tanksley *et al.*, 1992). The identification of polymorphisms between lines and cultivars is essential for breeding. Nonetheless, it has been noted that there is little variability between cultivars and intraspecific lines in *solanaceous* plants (Nunome *et al.*, 2003a; Smulders *et al.*, 1997; Stàgel *et al.*, 2008). Because SSR markers are extremely variable, simple to utilize, and readily

suited to high throughput in the majority of laboratories, they are especially valuable. There are several SSR markers found in the *Solanaceae*, however most of them haven't been used with eggplant. Despite the strong evolutionary relationships between these species, it is ineffective to analyze eggplant using SSR markers from other *Solanaceae* species (Nunome *et al.,* 2003b; Stàgel *et al.,* 2008).

The offspring resulting from selfing or sibmating F_1 individuals in a cross between the chosen parents is referred to as an F_2 mapping population. The F_1 offspring would exhibit heterozygosity for any locus where their parents diverge. The premise for detecting linkage between pairs of loci is the expectation that each F_2 individual will have a distinct combination of linkage blocks from the two parents. Recombination between any two loci can only happen once because F_2 generation is the result of a single meiotic cycle (in the F_1 plants). As a result, recombination frequency estimates between locus pairs derived from F_2 populations are used as a benchmark. The anticipated ratios for dominant and codominant markers in an F_2 population are 3:1 and 1:2:1, respectively. The ideal populations for first marker and oligogene mapping are F_2 mapping population.

Simple Sequence Repeats (SSR), also known as microsatellites, are the most extensively utilized and maybe the most informative molecular marker among all those that are accessible. They also require a little amount of DNA and are stable, locus specific, co-dominant, and highly polymorphic even within closely related lines. Because SSR markers are multi-allelic, they are a valuable marker system for marker-assisted selection and can detect higher levels of diversity (Bhattacharjee *et al.*, 2022).

Breeding for phytoplasma like resistance is the best approaches for combating little leaf. Interspecific hybridization with wild species which is resistance to little leaf that cross with susceptible species which produce F_1 hybrid that is given resistance against little leaf disease in brinjal (Warade *et al.,* 2004). By selfing the F_2 population for a single generation and collecting the seeds from each F_2 plant independently, each F_2 plant is represented as an individual plant offspring, resulting in an F_2-derived F_3 or $F_{2:3}$ population. The main aim to this study to developed F_2 mapping population by made a cross between resistance and susceptible parents after screening of F_2 mapping population through SSR markers to identify the genetic basis of symptoms that further helps to development of linkage map and identification of QTLs linked with little leaf resistance in brinjal.

Materials and Methods

Development of mapping population

Experimental Materials

The experimental material for present investigation comprised of F_2 segregating generation originating from a cross between a little leaf resistance parent of brinjal AB 15-06 (*Solanum melongena*) and a susceptible parent GRB 5 (*Solanum melongena*).

Experimental details

Experimental details for the field layout and additional information about the experiment are represented in Table 1.

Genotyping of mapping population using molecular markers

Genomic DNA Extraction

The experiment was conducted at Department of Agricultural Biotechnology, A.A.U., Anand. DNA was extracted from the fresh leaves by Cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) as follows: To create a fine powder, one gram of the sample was weighed, cut into small pieces, and ground in a mortar and pestle with liquid N_2. This powder was extracted into a 2 ml sterile eppendorf tube, to which 1 ml of pre-wash buffer was added and thoroughly mixed. The sample was incubated at 65 ˚C for 20 minutes in Eppendorf tubes. After incubation, the samples were centrifuged at 4000 rpm for 5 min. After cautiously discarding the supernatant, 800 µl of prewarmed CTAB solution and 2 µl of Proteinase-K were added. Tube was incubated initially at 45 ºC for 15 min. followed by incubation at 65 ˚C for 60 min. 800 µl of a Phenol: Chloroform: Isoamyl Alcohol (25:24:1) solution was added to the tube and gentle inverting for 5–10 minutes. After that, it was centrifuged for 15 minutes at 4 ºC at 12,000 rpm. The supernatant was carefully decanted and transferred to a new tube. The above steps, beginning with adding chloroform: iso amyl alcohol (24:1) and ending with decanting of supernatant, were repeated twice. In order to precipitate DNA, supernatant was collected into fresh tubes and an equal volume of pre-chilled isopropanol was added. The samples were stored overnight at -20 ºC for precipitation. Centrifuging the precipitated solution for ten minutes at 10,000 rpm and 10 ºC. After discarding the supernatant, 100µl of 70% ethanol was added to wash the palletes. The pellets were air dried and resuspended in 100 µl of TE buffer. 1 µl RNAase was added and incubated at 65 ºC for 10 min for remove RNA contamination from samples. The DNA stock was then utilized to create working

stock (30ng/µl) for PCR amplification and kept at -20 ºC. For quantification of DNA, separate the DNA on 0.8% agarose gel. Gel electrophoresis tank was filled with 1X TBE buffer. A current applied for 75 volts for 1-2 hours. Gel was observed under Bio RAD Gel Doc gel documentation system. DNA concentration and absorbance at 260 and 280 nm were measured, and Nano Drop-1000, Software V.3.3.0 was used to evaluate the results. DNA samples were pure if their 260/280 nm ratio was between 1.8 and 2.0.

Parental Polymorphism and Screening of Mapping Population

To find polymorphic markers, AB 15-06 and GRB 5, the parents, were screened using a total of 305 SSR primers. Among these markers, a subset of SSR markers demonstrating parental polymorphism was employed to screen each line of mapping population. The consistency of the band was verified and the reproducible bands were scored independently for each sample for each primer.

PCR amplification using SSR primers

The genomic DNA extracted from parents, and F2 brinjal population were subjected to PCR amplification using SSR primer. Total PCR volume taken 10µl comprising, 1 µl PCR assay buffer (10x), 1 µl Primers (0.5 µl of each forward & reverse), 0.2 µl dNTPs (2.5mM), 0.15 µl *Taq* DNA polymerase (5U/µl), 0.7 µl MgCl2 (25 mM), 1.0 µl genomic DNA and 5.95 µl Nuclease free water.

A thermal cycler (Bio Rad and Applied Biosystems) setup for PCR amplification: An Initial denaturation step at 94 ˚C for 5 min followed by 1 cycle, Denaturation step at 94 ˚C, Annealing step at ΔT 55˚C -65˚C and Initial Extension step at 72 ˚C for 45 sec followed by 35 cycle, Final extension step at 72 ˚C for 7 min followed by 1 cycle and then hold at 4 ˚C. To separate the amplified bands (Parental and F_2 mapping population), a 10 µl PCR product was loaded and run on a 2.5% agarose gel at 180 volts of continuous current. The samples were also analyzed using the Takara 100 bp standard DNA ladder. The separated bands were photographed using the Gel documentation (BIO RAD) system.

Disease scoring of mapping population for little leaf infection

Random observations of little leaf disease scoring in brinjal was carried out at weekly interval after 30 days of transplanting from September 2022 to February 2023. Little leaf disease incidence data were recorded from randomly selected five plants of the F_2 segregating population and their parents lines. Based on per cent disease incidence, the brinjal mapping population was classified into five categories (Venkataravanappa *et al.*, 2022).

1. Immune (0%)
2. Resistant (0.1-10%)
3. Moderately resistant (10.1-20%)
4. Susceptible (20.1-50%), and
5. Highly susceptible (> 50%)

Statistical analysis

Chi-Square Analysis

When utilizing a cross tabulation, the Chi-Square statistic is most frequently employed to assess Tests of Independence (also known as a bivariate Table). The Test of Independence evaluates whether an association exists between the two variables by closely examining the pattern of responses in the cells (Campbell, 1989).

$$\chi^2 = \sum_{i=1}^{n} \frac{(Oi - Ei)^2}{Ei}$$

Where, O*i* is the observed number of cases in category *i*, and E*i* is the expected number of cases in category *i*.

It is considered poor when one or more observed frequencies do not match the assumed hypotheses if the calculated values of $\chi 2$ are significant at the 5% level of significance, and vice versa. Thus, goodness of fit is another name for it. The degree of freedom (df) in χ^2 test is (n-1). Where n = number of classes.

Results and Discussion

Development of mapping population

The present study employed an initial screening of two parental genotypes of brinjal known to be resistant and susceptible to little leaf disease. The two parents used in the study were AB 15-06 and GRB 5 maintained at Main Vegetable Research Station, Anand Agricultural University, Anand. Based on their physiological characterizations AB 15-06 resistant to little leaf disease and GRB 5 susceptible to little leaf disease were selected. These parental genotypes were further used to development of F_2 segregating population for identification of a marker linked with little leaf disease resistance. F_1 hybrid was developed through crossing between both of the parents. Seeds of F_1 were used to development of F_2 mapping population. Total 224 F_2 mapping population sown during the year *kharif*, 2021-22 along with their parents. Out of 224 plants 56 could not survive due to a little leaf disease infection. Remaining 168 F_2 segregating population were tested by using SSR markers with F_1 hybridity test.

Toppino *et al.* (2016) developed F_2 intraspecific mapping population of 156 individuals, obtained by crossing the eggplant breeding lines “305E40” × “67/3” for the identification of some QTLs associated for the fruit metabolic content in eggplant. Similarly, Barchi *et al.* (2018) to developed 156 individuals of the F_2 intraspecific population derived from the cross between "305E40" x "67/3" to identified resistance locus against fungal disease *fusarium* and *verticillium wilt* in brinjal. Wei *et al.* (2020) also carried out cross between the domesticated eggplant variety "1836" and its wild relatives *S. linnaeanum* "1809" produced an interspecific F_2 population comprising 121 individuals for identification of major QTLs related to morphological traits.

Genotyping of mapping population using molecular markers

DNA Extraction

The integrity and purity of the DNA were examined from young leaves of parental population of AB 15-06 (P_1) and GRB 5 (P_2), 168 F_2 segregating generation. DNA extracted from P_1, P_2 and 168 mapping population and its purity and quality were verified on 0.8% agarose gel electrophoresis (Plate 1). DNA further was quantified measuring the ratio at A260/A280 nm on nanodrop spectrophotometer. The absorbance ratio of DNA at A260/A280 nm ranged from 1.68 to 2.12 of total 168 F_2 individuals. The pure DNA has an A260/A280 nm ratio of 1.8–2.0. Strong absorbance at 280 nm, resulting in a low A260/A280 nm ratio, indicates the presence of contaminants such as proteins. None of the DNA samples analysed in the present investigation had poor quality. The yield of DNA was in the range of 860.73 ng/µl to 4896.20 ng/µl. DNA thus extracted from F_2 mapping population along with parents was utilized for screening through SSR markers.

Screening of mapping population using Simple Sequence Repeat (SSR) markers

Screening of parental DNA samples *i.e.*, AB 15-06 and GRB 5 was carried out using 305 primers which were identified based on literature studied. Out of 305 primers, 152 primers resulted in amplifications. From the amplified 152 primers, 48 primers which observed parent polymorphism were further used for screening mapping population. Polymorphism was decided on the basis of difference in position of the bands. Primers which resulted to produce clear and distinct DNA bands were considered to be polymorphic. Amplified primers were used for genotyping of F_2 mapping population (Plate 2-3). The list of selected SSR primers and their amplification pattern have been presented in Table 2.

Stàgel *et al.* (2008), Nunome *et al.* (2009), Vilanova *et al.* (2012) developed SSR markers which is used in construction of linkage map in mapping population of brinjal. Similar SSR markers were used for screened mapping population in present investigation.

The predicted ratio of genotypes in the F_2 population produced by a biparental cross is AA: AB: BB (progeny homozygous for the first parent's allele, heterozygotes, and progeny homozygous for the second parent's allele). 1:2:1 in the absence of segregation distortion (second parent). The phenomenon known as segregation distortion occurs when alleles in the offspring of a cross between two kinds or species diverge from predicted Mendelian ratios. Microsoft Excel application was used to examine the Mendelian segregation ratio of SSR markers. When allele frequencies deviate from expected patterns, skewed loci tend to favor more common alleles, indicating potential advantages. Without segregation distortion, parental class frequencies are expected to be equal.

In Table 3. presents chi-square (χ^2) values for 48 genetic markers analyzed in a 168 F_2 mapping population, evaluated for their conformance to the expected 1:2:1 Mendelian segregation ratio. Out of 48 markers, 28 markers were followed the Mendelian segregation ratio of F_2 mapping population of cross AB 15-06 X GRB 5. SSR markers at a probability of $p>0.05$ to fit the null hypothesis of no difference between the expected and observed chi square ratio.

Markers such as emh11G21 (10.8182**), CSM47 (11.9747**), eme05B09 (9.0408*), EMB120 (11.8571**), eme03H10 (9.3292*), emf01O01 (9.1854*) and emd05F05 (11.1375**) among others, displayed highly significant deviations from the expected ratio, with chi-square values ranging from 9.0408 to 11.9747. These results suggested that these loci are under strong selective pressures or are affected by other genetic factors causing deviations from the expected segregation pattern.

In this F_2 population, 20 out of 48 markers show significant segregation distortion, with 4 markers highly significant at the 1% level (**) and 16 markers moderately significant at the 5% level (*). These significant markers account for approximately 41.67% of the total markers analyzed. The presence of segregation distortion in these markers suggests underlying genetic or environmental factors

influencing their inheritance patterns, which are crucial for genetic mapping and breeding programs. Further investigation into these markers may provide insights into the genetic architecture and the selective forces at play in this population (Xu *et al*., 1997).

The high proportion of markers with significant distortion approximately 41.67% indicates substantial segregation distortion in this F_2 population. This phenomenon could be due to various biological factors, including gametic selection, zygotic selection, or linkage to regions under selective pressure. Identifying these distorted markers is crucial for accurate genetic mapping and breeding programs. Understanding and addressing segregation distortion is essential for improving the accuracy of genetic studies and optimizing breeding strategies. Zhang *et al*. (2010) suggested that results of QTL mapping will not be significantly affected by segregation distortion if the distorted markers are not significantly associated with any of the QTL.

First report of segregation distortion in brinjal reported by Barchi *et al.*, 2010 discovered higher segregation distortion 68% a in eggplant populations that have been twice haploid. Toppino *et al.* (2020) developed a RIL population from the intraspecific cross '305E40', (androgenetic introgressed line carrying the locus Rfo-Sa1 conferring Fusarium resistance) x '67/3' (suceptible) from that total of 1744 markers showed segregation distortion with $p < 0.001$, covering about 24% of the total mapped markers. Wei *et al*. (2020) observed higher segregation distortion 70% in interspecific F_2 population of 121 individuals was developed from the cross between cultivated eggplant "1836" and the wild relative *Solanum linnaeanum* "1809".

Disease scoring of mapping population for little leaf infection

Parent GRB 5 was reported to be highly vulnerable to little leaf disease, whereas another parent AB 15-06 which did not exhibit any indications of the little leaf infection was found highly resistant. F_1 plants did not exhibit any signs of infection. Total 168 F_2 mapping population was used for screening of little leaf severity in field condition. After 30 days of transplantation, little leaf disease infection screening was initiated. Total 128 plants of F_2 population showed resistance against little leaf disease recorded disease incidence around 0%-20% while 40 plants showed observable disease incidence around 20.1%-50% (Table 4).

The chi square value (0.1269) has been founded to be lesser then the critical chi square value at df=1. With one degree of freedom, the computed value of $\chi 2$ at 0.05 and 0.01 probability was 3.841 and 6.634, respectively (Table 5). Consequently, the null hypothesis was disproved, and it was determined that the data agreed with a 3:1 ratio. Monogenically dominant single gene is responsible for expression of the trait. Initially, breeders might observe that when crossing certain varieties or lines of brinjal, approximately 75% of the progeny show resistance to the disease, while the remaining 25% are susceptible. This segregation follows Mendelian inheritance patterns, the observed ratio (75% resistant, 25% susceptible) corresponds to the classic Mendelian ratio of 3:1, which typically results from a monohybrid cross indicating that the resistance trait is governed by one major gene with a recessive allele responsible for susceptibility. As the plants grow and are exposed to the disease pressure, the susceptible individuals, which constitute around 25% of the population, start showing symptoms. This results in a rapid increase in disease scoring, forming the descending side of the U-shaped graph.

This demonstrated that, in accordance with Mendelian genetics, the resistance attribute was determined by a single dominant gene (Bhattacharjee *et al*., 2022). Previous research has been investigated and demonstrated that the dominance of resistant traits over susceptible ones (Narayanswami *et al*., 2023) in brinjal.

Bainsla *et al*. (2016) carried out a chi square analysis based on the bacterial wilt disease trait of the F_2 mapping population in brinjal. In segregating population found only 22 plants was resistance against bacterial wilt out of 67 plants, based on these the chi square ratio was observed 3:1 in F_2 mapping population. This was similar to our finding results in F_2 mapping population.

Conclusion

Two brinjal parents, resistance AB 15-06 and susceptible GRB 5 against little leaf disease were selected for crossing programme and generated F_1 cross. F_2 mapping population was created by selfing of F_1 plants. For genotyping study, DNA extraction was carried out as per standard protocols and purified DNA A260/280 with ratio of 1.68-2.12. Genotyping of parents and F_2 mapping populations were carried out through molecular analysis using 305 SSR markers, 48 SSR markers resulted in polymorphic banding patterns. Out of 48 polymorphic markers, 28 markers resulted in 1:2:1 Mendelian segregation ratio with 41.67% segregation distortion. The outcome implied that the segregated group had an overexpression of the favorable alleles. F_2 mapping population and

phenotypic ratio found 3:1 out of 168 F_2 mapping population. 128 plants were phenotypically observed resistant and 40 plants were susceptible against little leaf disease. Present investigation proposed that screening of F_2 mapping population through SSR markers to identify the genetic basis of symptoms related to little leaf disease in brinjal mapping population that further helps in future to development of linkage map and identification of QTLs linked with little leaf resistance in brinjal.

Table 1 : Experimental details

(a)	**Location**	**:**	Main Vegetable Research Station, AAU, Anand
(b)	**Year & Season**	**:**	*kharif* 2022-23, Development of F_2 mapping population for genotyping
(c)	**Number of Entries**	**:**	Parents: AB 15-06, GRB 5 and their 168 F_2 mapping population
(d)	**Design**	**:**	RBD
(d)	**Spacing**	**:**	90 × 60 cm
(e)	**Package of practices**	**:**	The package of practices was followed as per the recommendations.

Table 2 : Polymorphic SSR primers in F_2 mapping population

Sr. No.	Name	Brinjal SSR markers	Tm (˚C)
1.	CSM12_F	CAATGGTATGTCTCCACTCGTC	60.25
	CSM12_R	AAGCTAAACATGAGATGCCGAT	56.53
2.	EM 133_F	GCGGATCACCTGCAGTTACATTAC	62.72
	EM 133_R	TCCTTTGACCTATAGTGGCACGTAGT	63.22
3.	smSSR01_F	GTGACTACGGTTTCACTGGT	57.30
	smSSR01_R	GATGACGACGACGATAATAGA	55.92
4.	EEMS17_F	TGACATGTAGCTGGGCAGAG	59.35
	EEMS17_R	TGGAGTGTGCATCCCAAATA	55.25
5.	EMB120_F	CAAAAGATAAAAAGCTGCCGGATG	59.30
	EMB120_R	CATGCGTGAGTTTTGGAGAGAGAG	62.72
6.	EM 120_F	GGATCAACTGAAGAGCTGGTGGTT	61.93
	EM 120_R	CAGAGCTTCAATGTTCCATTTCACA	61.65
7.	eme25D01_F	AGTCCCAACCAAAATCGTAGAGGC	62.72
	eme25D01_R	GTTTCACTGAAGGATGTGGAGTGTGA	63.22
8.	emg11A06_F	AGTGCTAATATGCAAGGGGAATGG	61.01
	emg11A06_R	GTTTACGGTGATCTTTCCGTATTCCAAA	62.19
9.	emd05F05_F	ACGGGGGTGTCTCATTACACTACTGG	66.38
	emd05F05_R	GTTTACCCGTTCCTCAGCTTATAGACCC	66.59
10.	emf01O01_F	AGGAATTGGATTTCCACTCATACG	59.30
	emf01O01_R	GTTTGGAAGATGAGATTCCTTTCTTGA	60.41
11.	EEMS44_F	CCTTCAAACCCTCTCCCTTC	59.35
	EEMS44_R	GTGAAACGTGGTGGAGGTCT	59.35
12.	emh11N11_F	ATTCAGTTCTTCGCTTTGGAGCTT	59.30
	emh11N11_R	GTTTCCAAACCCGACCCATCCTAAATAA	63.66
13.	emb01A21_F	TCATGGTAGGTGGAGACAGAACCA	62.72
	emb01A21_R	GTTTGGATTAGCATGTGGAGGACTGAA	63.45
14.	eme05B09_F	ATGAAAACTCCACTCTACTCTACTCCAC	63.66
	eme05B09_R	GTTTGCTAACGTACGCCTCAATTGCTCT	65.12
15.	eme09E09_F	ACGGTATCGAAGAGAGTGAATGCCT	62.98
	eme09E09_R	GTTTCCCCATTTCATCTGAAAAATCCAC	62.19
16.	emh11G21_F	ATGTGTGAACTCAAATGGAAGGGA	59.30
	emh11G21_R	GTTTCGAATTGCTTTTTGGTGCATGTAG	62.19
17.	EES021_F	AAAAATCCCCAAATCCATCT	51.15
	EES021_R	ACGCTCTCTCACAACAACAA	55.25
18.	emh05B02_F	ATACCAAAGACACGTTGGGATCAT	59.30
	emh05B02_R	GTTTCTAGGAGAGCATCTCCCTCCCT	66.38
19.	CSM63_F	CAGCCATGGACCACATTTTAC	57.87
	CSM63_R	ACATGCCACTCATGTTGGTG	57.30
20.	CSM16_F	ACGTGCCATTTCAAACTTGG	55.25
	CSM16_R	TCCTTTTCTTGAGCTGAATTTG	54.66

21.	CSM29_F	GGATGAAATGAAGGCTTAGGG	57.87
	CSM29_R	GCCATCCTCATCTTTGATGG	57.3
22.	CSM78_F	AGGGAGGAGCTCTCGTGTG	60.99
	CSM78_R	CAATAACGTAGCTTAATTACTCCCAAG	62.41
23.	emd04E10_F	ATGGTTATCCGGCCTCCTATACCTC	64.62
	emd04E10_R	GTTTAGGAATTGAAGGTCCAAAGCATGA	62.19
24.	emb01J19_F	GACAGGGATAGGGGTACGGATAGG	66.12
	emb01J19_R	ATCCATGTGATGCCTCGATTTTCT	59.30
25.	emf21P02_F	ATGAAGCAGATCTTTCGACTGCAC	61.01
	emf21P02_R	GTTTAGGCCAAGGATGTCAAACTGGT	63.22
26.	CSM44_F	CGTCGTTGTAACCCATCATC	57.30
	CSM44_R	TTGCCAAATTCCTTGTGTTC	53.20
27.	CSM47_F	CAACATTCTCAGCAAGCATAGC	58.39
	CSM47_R	GCAACTCCTGAAGACGGAAG	59.35
28.	emb01H07_F	GTAATGTCGGTGGCTTGATGACTG	62.72
	emb01H07_R	ACTCGGATTTCAGGAGCCACAATA	61.01
29.	emf11F07_F	ATTGGACCAAGTATGTGGTGTGGA	61.01
	emf11F07_R	GTTTGCTCTTCACCTGGCTTGATTT	61.34
30.	smSSR03_F	ATTGAAAGTTGCTCTGCTTC	53.20
	smSSR03_R	GATCGAACCCACATCATC	53.69
31.	CSM31_F	CAACCGATATGCTCAGATGC	57.30
	CSM31_R	GCCCTATGGTCATGTTTTGC	57.30
32.	Sm_781_F	AAAACTTGCACTATCTTTGA	47.05
	Sm_781_R	ACCATGAGCATAGTATTCAG	47.05
33.	BMS 31_F	TTTGGACAAGAACAAGGATGG	61.34
	BMS 31_R	GCAGAGCCTGAGTTTTCTCAT	59.30
34.	EM 120_F	GGATCAACTGAAGAGCTGGTGGTT	61.93
	EM 120_R	CAGAGCTTCAATGTTCCATTTCACA	61.65
35.	EEMS20_F	AACATCAGCCAGGGTGTTTC	61.35
	EEMS20_R	TGCTGAAAATTACAAGCCAAA	63.45
36.	emd05B11_F	ATTGCTTCAATTAAGGCTGAGAGGG	57.59
	emd05B11_R	GTTTATTTTTCGCTTGAGAGTGGTGGC	63.22
37.	eme03H10_F	ATGGAATTGTTCCCTGAAGTCCTGT	59.3
	eme03H10_R	GTTTCCCAAGCCTGCAATCTTTAACATC	61.65
38.	emg11M09_F	ATACATTGAAATTGGCTGAGCTTG	57.59
	emg11M09_R	GTTTGGATCTTCGCTAGAACTTTGGC	61.93
39.	emk03O04_F	ATGATTTGGGCAGCCACTTTTGTA	62.98
	emk03O04_R	GTTTGGAACCAACTAAACTTAGGGCA	62.44
40.	emf11D18_F	AGAGACAGGGAGAGTGCATTCTATG	67.59
	emf11D18_R	GTTTGCAGTTCATAAGGTTGCATCAATAC	61.93
41.	emf11H23_F	ATTCTGAAAACAAGAGCAGCCCTC	61.34
	emf11H23_R	GTTTCTCAACACCTCTGTGTCTGGCAT	64.97
42.	Sm_908_F	AAGTTTCCGATAACTCTGTT	50.19
	Sm_908_R	GAATTGTTATTAGAAGCCG	53.69
43.	embe08D09_F	ATGGATTAGCATGTGGAGGACTGAA	64.8
	embe08D09_R	GTTTCATGGTAGGTGGAGACAGAACCA	65.12
44.	emd03E08_F	ACGTACGCATGCTGTGTTTGTTAGGC	59.30
	emd03E08_R	GTTTGGACACCCGGAGACAATCTACTTT	58.87
45.	Sm_1002_F	GTTGATAGGGATGTAAAACA	55.25
	Sm_1002_R	CTTCAAGCTAACAAAAATTG	51.15
46.	emi04O04_F	ATTAAGGGCTTCCATCACTTTGGA	62.19
	emi04O04_R	GTTTGCACCATCTATCAATCCACGA	59.70
47.	emf11D19_F	ACCAGAGGAGCAAAGGGAAAAATA	62.72
	emf11D19_R	GTTTACGCTACTGGACCAAACCAACAAT	63.66
48.	ecm009_F	ATCTAGTACCATCAAGTCTAAGCAGCA	61.93
	ecm009_R	GTTTAACAACAGCTGAGGCCATGAAA	61.65

Table 3 : Chi square values of SSR markers in F_2 mapping population

Sr. No.	Marker	χ2 (1:2:1) Chi square	Sr. No.	Marker	χ2 (1:2:1) Chi square
1	emh11G21	10.8182**	25	Sm908	6.4969*
2	CSM12	6.3212*	26	CSM31	7.3241*
3	CSM47	11.9747**	27	CSM63	9.4027*
4	CSM44	4.2209	28	emg11M09	1.6795
5	eme05B09	9.0408*	29	EM133	2.1111
6	smSSR03	3.9359	30	emf01O01	9.1854*
7	emB01H07	4.1951	31	emd05F05	11.1375**
8	Sm_781	1.7152	32	emf11D19	6.1205*
9	emi04O04	2.6316	33	emh11G21	4.3704
10	EM120	7.1529*	34	emk03O04	4.5276
11	emf11F07	3.0822	35	CSM16	3.7818
12	EEMS20	6.2357*	36	emd05B11	7.5963*
13	emb01J19	4.6770	37	emb01A21	4.1699
14	smSSR03	3.7273	38	emh05B02	3.2927
15	EEMS44	3.3494	39	emf11H23	1.5316
16	EEMS17	8.9141*	40	eme09E09	1.2138
17	CSM29	3.6190	41	emf11D18	3.0318
18	EMB120	11.8571**	42	CSM78	4.5092
19	emb01O01	8.8929*	43	eme25D01	1.1198
20	emg11A06	3.8571	44	EES021	4.1500
21	embe08D09	3.2050	45	Sm_1002	8.9868*
22	eme03H10	9.3292*	46	emf21P02	3.7651
23	emd04E10	1.6098	47	emd03E08	6.1899*
24	BMS31	2.3375	48	ecm009	6.2765*

*Significant at 5%, **Significant at 1%

Table 4 : Disease incidence in F_2 mapping population

Classes of plants Resistant/Susceptible	No. of F_2 plants	Percentage of Resistant and Susceptible plants in population	Ratio
Resistant plants	128	75	128: 40
Susceptible plants	40	25	(3 :1)

Table 5 : Chi square analysis for disease

	O	E	(O-E)	(O-E) ^2	chi square*
Resistant plants	128	126	2	4	0.0317
Susceptible plants	40	42	-2	4	0.0952
Total	168	168	0	8	0.1269
* χ2 non-significant at 1% and 5%					
O = Observed Frequency, E = Expected Frequency, χ2 = Symbol of Chi square					

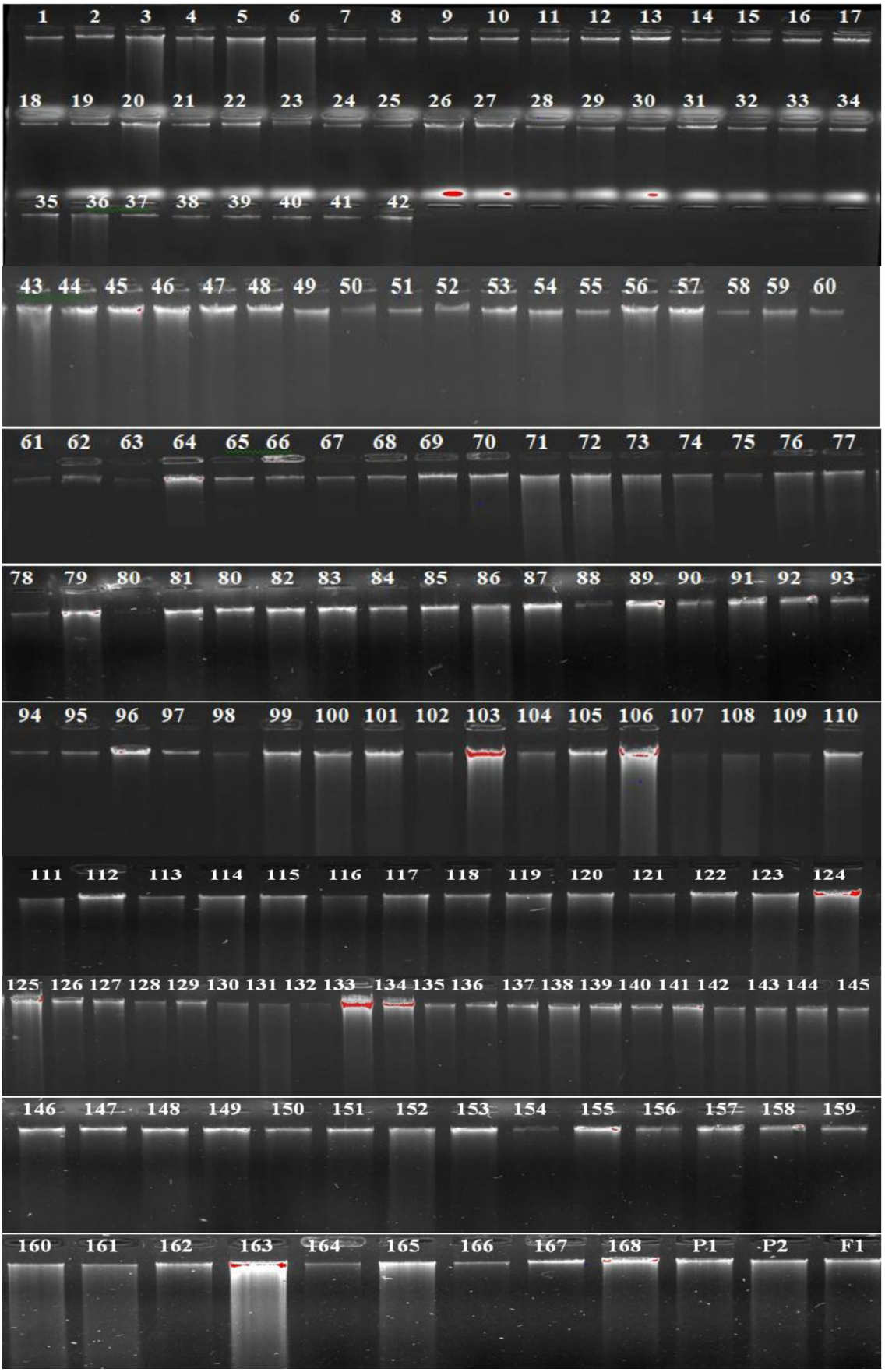

Plate 1 : Representation of genomic DNA banding pattern of 168 F_2 mapping population, parental line (AB-15-06 and GRB-5) and F_1 hybrid

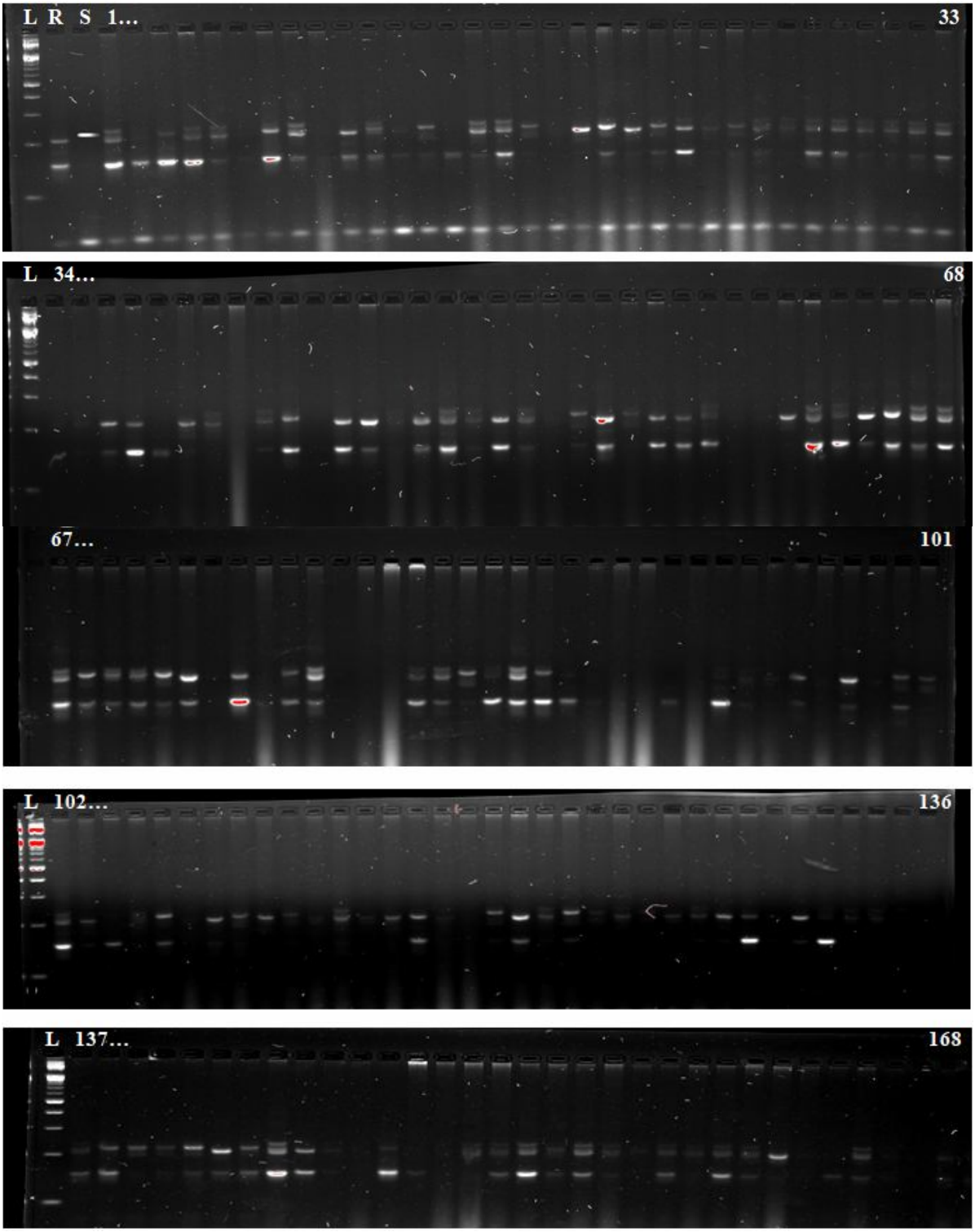

Plate 2 : Segregation pattern of 168 F_2 mapping population of cross AB 15-06 X GRB 5 with CSM12 marker and 100 bp ladder

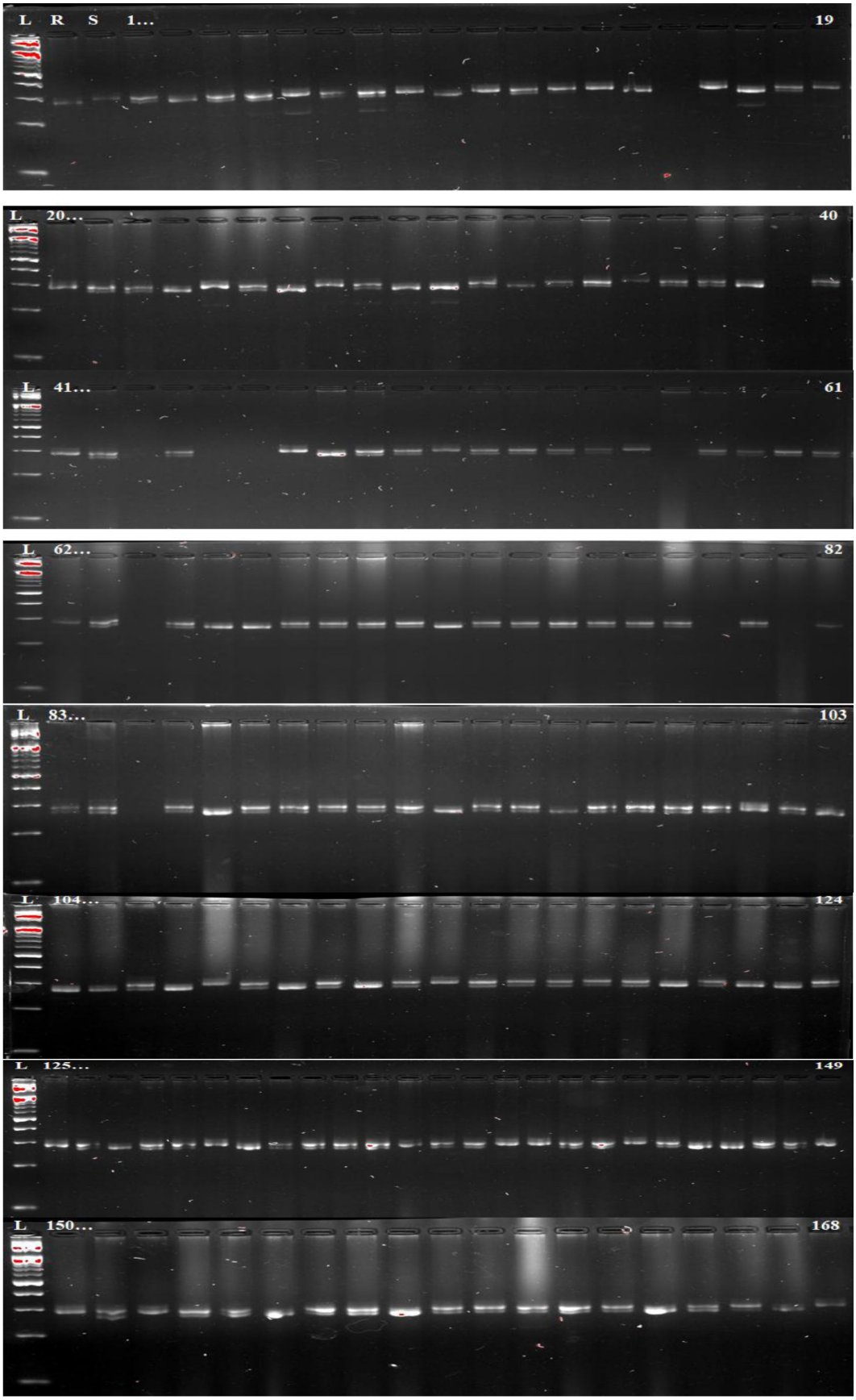

Plate 3: Segregation pattern of 168 F_2 mapping population of cross AB 15-06 X GRB 5 with EM 133 marker and 100 bp ladder

References

Anonymous, (2017). Food and agriculture organization corporate statistical database. Available from:http:// faostat.fao.org.

Bainsla, N. K., Singh, S., Singh, P. K., Kumar, K., Singh, A. K. & Gautam, R. K. (2016). Genetic behaviour of bacterial wilt resistance in brinjal (*Solanum melongena* L.) in tropics of Andaman and Nicobar Islands of India. *American Journal of Plant Sciences*, **7**(02), 333. DOI:10.4236/ajps.2016.72033

Barchi, L., Lanteri, S., Portis, E., Stàgel, A., Valè, G., Toppino, L. & Rotino, G. L. (2018). QTL analysis reveals new eggplant loci involved in resistance to fungal wilts. *Euphytica*, **214**, 20. https://link.springer.com/article/ 10.1007/s10681-017-2102-2

Barchi, L., Lanteri, S., Portis, E., Stàgel, A., Valè, G., Toppino, L. & Rotino, G. L. (2010). Segregation distortion and linkage analysis in eggplant (*Solanum melongena* L.). *Genome*, **53**(10), 805-815. https://doi.org/10.1139/ G10-073

Bhattacharjee, T., Maurya, P. K., Banerjee, S., Mukherjee, D., Kundu, S., Islam, S. M. & Chattopadhyay, A. (2022). Genetics of tolerance to bacterial wilt disease in eggplant. *International Journal of Vegetable Science*, **28**(6), 603-617.

Campbell, J. C. (1989). A test of two explanatory models of women's responses to battering. *Nursing Research* **38**(1), 18-24. https://doi.org/10.1097/00006199-198901000-00004

Doyle, J. J. & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, **19**, 11-15.

Maejima & Oshima (2014). Exploring the phytoplasmas, plant pathogenic bacteria. *Journal of General Plant Pathology*, *80*, 210–221. DOI:10.1007/s10327-014-0512-8

Narayanswami, B., Tomar, B. S., Akhtar, J., Behera, T. K., Ellur, R. K., Sevanthi, R. M. V., & Saha, P. (2023). Genetic analysis and identification of SSR marker linked to Phomopsis blight resistance in eggplant (*Solanum melongena* L.). *Plant Breeding*, **142**(5), 700-710. DOI: 10.1111/pbr.13133

Nunome, T., Suwabe, K., Iketani, H. & Hirai, M. (2003a). Identification and characterization of microsatellites in eggplant. *Plant Breeding*, **122**, 256-262. DOI:10.1046 /j.1439-0523.2003.00816.x

Nunome, T., Suwabe, K., Ohyama, A. & Fukuoka, H. (2003b). Characterization of trinucleotide microsatellites in eggplant. *Breed Science*, **53**, 77-83. DOI:10.1270/jsbbs .53.77

Smulders, M. J. M., Bredemeijer, G., Rus-Kortekaas, W. & Aren, P. (1997). Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessons of other *Lycopersicon* species. *Theoretical and Applied Genetic*, **97**, 264-272. DOI:10.1007/s001220050409

Stàgel, A., Portis, E., Toppino, L., Rotino, G. L. & Lanteri, S. (2008). Genebased microsatellite development for mapping and phylogeny studies in eggplant. *BMC Genomics*, **9**, 357-370. DOI:10.1186/1471-2164-9-357

Tanksley, S. D., Ganal, M. W., Prince, J. P., de Vicente, M. C., Bonierbale, M. W., Broun, P., Fulton, T. M. & Young, N. D. (1992). High density molecular linkage maps of the tomato and potato genomes. *Genetics*, **132**, 1141-1160. DOI: 10.1093/genetics/132.4.1141

Toppino, L., Barchi, L., LoScalzo R., Palazzolo, E., Francese, G., Fibiani, M. & Rotino, G. (2016). Mapping quantitative trait loci affecting biochemical and morphological fruit properties in eggplant (*Solanum melongena* L.) *Frontiers in Plant Science*, **7**, 256. DOI: 10.3389/fpls.2016.00256

Venkataravanappa, V., Kodandaram, M. H., Manjunath, M., Chauhan, N. S., Nagendran, K., Tiwari, S. K. & Rao, G. P. (2022). Molecular characterization of phytoplasma strains associated with brinjal little leaf and screening of cultivated and wild relatives of eggplant cultivars for disease resistance. *European Journal of Plant Pathology*, (*pp 1-21*). DOI:10.1007/s10658-021-02414-5

Vilanova, S., Manzur, J. P. & Prohes, J. (2012). Development and characterization of genomic simple sequence repeat markers in eggplant and their application to the study of diversity and relationships in a collection of different cultivar types and origins. *Molecular Breeding*, **30**, 647-660. DOI: 10.1007/s11032-011-9650-2

Warade, S. D., Sanap, P. B., Sawant, D. M. & Gupta, N. S. (2004). Studies on interspecific hybridization in eggplant (*Solanum melongena* L.) for little leaf disease resistance. *Wageningen Plant Research International Netherlands*, (*pp 79-83*). https://www.cabidigitallibrary.org/doi/full/ 10.5555/20063220181

Wei, Q., Wang, W., Hu, T., Hu, H., Wang, J. & Bao, C. (2020). Construction of a SNP-based genetic map using SLAF-Seq and QTL analysis of morphological traits in eggplant. *Frontiers in Genetics*, **11**, 178. https://doi.org/10.3389/ fgene.2020.00178

Xu, Y., Zhu, L., Xiao, J., Huang, N. & McCouch, S. R. (1997). Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). *Molecular and General Genetics*, **253**(5), 535-545. DOI: 10.1007/s004380050355

Zhang, L., Wang, S., Li, H., Deng, Q., Zheng, A., Li, S. & Wang, J. (2010). Effects of missing marker and segregation distortion on QTL mapping in F_2 populations. *Theoretical and Applied Genetics*, **121**, 1071-1082. DOI: 10.1007/s00122-010-1372-z