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**Inheritance and Molecular Mapping of Fusarium Wilt  
Resistance Gene in Brinjal (*Solanum melongena*)**

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**Abstract**

The inheritance of resistance to fusarium wilt derived from AB-2 XAB-52 conferring resistance to *Fusarium oxysporum* has been determined. The F<sub>2</sub> individuals of the cross AB-2 (resistance) XAB-52 (susceptible) were screened against Fusarium wilt in combined tests which indicated dominant control by single locus of tightly linked gene(s). Bulk segregant analysis (BSA) was carried out on F<sub>2</sub> individuals with a set of 250 DNA markers that resulted in the identification and mapped the *FMI* locus between the two DNA markers bNBS-LRR-45 and bRGA-25 located ~ 2.2 cM apart from each other. These closely linked markers to the wilt resistance gene(s) provide a valuable basis for marker-assisted selection in castor breeding programs.

**Keywords:** Brinjal, Fusarium Wilt resistance, F<sub>2</sub> cross, locus, marker-assisted selection, Genic marker.

**Introduction**

Brinjal (*Solanum melongena*) which has probably originated in the Old World, mainly from India (Daunay 2008) is one of the few cultivated species in the family solanaceae. It has become an economically important vegetable crop in tropical and warm regions (Kashyap et al. 2003) with a global production of 49 million tons in 2013, increasing by 50 % from 2004 to 2013 (FAO 2004, 2013). Fusarium wilt, caused by the soil borne plant pathogen *Fusarium oxysporum* f. sp. *melongenae*, is one of the most serious diseases of brinjal. The fungus spreads widely and can persist for many years in the soil. This makes management practices like field idling and cultural control ineffective to eradicate this pathogen (Steekelenburg 1976; Altinok 2005; Daunay 2008; Safikhani et al. 2013). Under such conditions, an approach to develop wilt resistant brinjal cultivars offers a logical way to control this disease. The advancements in the recent past in the area of molecular marker analyses can aid efficient breeding. Susceptibility of brinjal plants to variety of soil borne diseases is one of the major factors affecting eggplant production. Further, Fusarium wilt is one of the most devastating diseases in eggplant cultivation (Rotino et al. 2004).

*Fusarium oxysporum melongenae* induces vascular wilt disease in eggplant is responsible for heavy yield losses in this vegetable crop particularly in Asian countries (Altinok 2005).

Fusarium wilt is one of the most investigated subject in plant disease management with extensive reports available in several horticultural crops. For instance in important solanaceous vegetable crop tomato, four resistance genes, I, I1, I2, and I3, derived from wild species (*Solanum pennellii* and *Solanum pimpinellifolium*) have been identified through molecular mapping studies (Bournival et al. 1990; Hemming et al. 2004; Lim et al. 2008; Ori et al. 1997; Sarfatti et al. 1989; 1991; Segal et al. 1992; Simons et al. 1998). I2 gene from tomato and melon Fom-1 and Fom-2 were subjected to map-based cloning (Brotman et al. 2012; Joobeur et al. 2004; Simons et al. 1998). This revealed that all three genes contain nucleotide-binding site and a leucine-rich repeat (NBS-LRR) domain. Publication of first report on Fusarium wilt in eggplant by Matsuo and Ishigami (1958) was rapidly followed by research efforts to identify resistant eggplant allies. One such prominent study was undertaken by Monma et al. (1996) who identified large number of wilt resistant germplasm in Ghana. Following this, Rizza et al. (2002) developed interspecific hybrids between eggplant and its allies (*Solanum aethiopicum*) which were found to be completely resistant to Fusarium wilt. Previous studies have reported Rfo-sal (a dominant single

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locus), to be responsible for Fusarium resistance in a somatic hybrid line mapped on chromosome 2 (Barchi et al. 2010, 2012; Portis et al. 2014) and SCAR markers linked to a Fusarium resistance locus in an eggplant line, LS2436, based on bulked segregant analysis (Mutlu et al. 2008). However, due to existence of low level of nucleotide polymorphism among eggplant lines, mapping studies on resistance loci in eggplant (*S. melongena*) have not been reported (Nunome et al. 2001). This creates difficulty in using intraspecific mapping populations for this purpose. Nunome et al. (2003a, b, 2009) and Fukuoka et al. (2012) have developed a large number of informative simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, which have allowed detection of polymorphisms among genetically close lines. These studies resolved to a great extent, the problem of genetic polymorphism and made markers available for genetic mapping for fruit shape and colour (Nunome et al. 2001) and parthenocarpy (Miyatake et al. 2012).

In recent years, the eggplant draft genome sequence was published by Hirakawa et al. (2014).

In this study, we report the precise mapping position of a *Fusarium* wilt resistance locus with two alleles derived from cross **AB-2 (resistance) X AB-52 (susceptible)**, based on the mapping populations and linkage maps reported by Fukuoka et al. (2012) and Hirakawa et al. (2014); the positional relationships of resistance loci reported here and in previous studies (Barchi et al. 2010, 2012; Mutlu et al. 2008; Portis et al. 2014); and (3) the availability of resistance-linked markers that is suitable for marker-assisted selection (MAS).

**Material and Methods**

**Plant material and mapping populations**

The F2 mapping population used in the study was derived from **AB-2 (resistance) X AB-52 (susceptible)**. The F1 plants were selfed to obtain segregating F2 population. The morphological, Biochemical and pathological characters recorded are as given in table 1, 2 & 3.

**Table 1: - Morphological Characterization most prominent resistant and susceptible lines of brinjal**

Accession No.1	Plant height (cm)	No. primary branches/plant	Stem diameter (cm)	Days to 1st Flowering	Days to first fruiting	Harvesting period	No. of fruits in inflorescence/plant	Fruit stalk length	Fruit circumference	Fruits/plants	Yield per plant
AB-2	159	16.5	4.8	105	112.5	70	13.91	6.28	23.85	18.92	4588
AB-52	176	15.5	5.87	131.5	144	89.5	3.67	7.6	9.48	6.17	2523

**Table 2: - Pathological Characterization most prominent resistant and susceptible lines of brinjal**

Accession No.	Total plant population	% Infection after 50 Days	% Infection after 65 Days	% Infection after 80 Days	% Infection after 95 Days	% Infection after 50 Days	% Infection after 65 Days
AB-2	20	0	0	0	0	0	0
AB-52	25	22.22	33.33	55.56	77.78	22.22	33.33

**Table 3: - Biochemical Characterization most prominent resistant and susceptible lines of brinjal**

Accession No.	Peroxidase assay (units/g)	Phenol content (µg catechol/g tissue)
AB-2	2.01	1520.23
AB-52	0.241	1050.86

### Morphological and Pathological characterization of mapping population

The morphological observations of the parents of mapping population were observed in the field. These observations include Plant height (cm), No. Primary branches /plant, Stem diameter (cm), Days to 1st Flowering, Days to first fruiting, Harvesting period, No. of fruits in inflorescence/plant, Fruit stalk length, Fruit circumference, No. of Fruits/plants, and fruit Yield per plant Morphological observations were recorded as per the DUS procedure and other trait specific standard procedures reported. The pathological observations were taken after a fixed interval of days viz 50 days, 65 days, 80 days and 95 days) for parents and mapping population under natural conditions of field. The observation of each line recorded in two replications for quantitative and qualitative traits along with the contributing traits were used for calculating the mean performance. Data of three randomly selected plants from each replication were averaged. The mean performance, range (minimum and maximum), standard deviation (SD), coefficient of variance (CV %) and number of transgressive segregants for all the traits studied in F2 mapping population were worked out along with mean of parents. The results revealed that the mapping population has segregation between the lines for specific parameters. The mean sum of squares for population was highly significant for the traits related to Fusarium wilt resistance and reported to be highly contributing in Fusarium wilt resistance.

#### DNA isolation and quantification

The genomic DNA was extracted from the parents **AB-2 (resistance)**, **AB-52 (susceptible)**, and F2 plants derived from the cross according to the protocol Dellaporta et al. (1983). Leaf samples obtained from 30-35 days old plants were freeze dried (-80°C) and lyophilized were ground to a fine powder and used for DNA extraction. The isolated DNA was quantified using NanoDrop and diluted to a final concentration of 50 ng/μl for PCR analysis. Phenotypic data recorded for each trait was used to score individual F2 plants. 10 most resistant and 10 most susceptible plants were chosen for bulked segregant analysis *ie* BSA (Michelmore et al. 1991). After quantification, resistant and susceptible pools were prepared with equimolar amounts of DNA from the 10 F2 individuals. Bulk samples were then screened for polymorphism with over 250 Oligo-nucleotide primers.

#### PCR analysis

A set of 250 Oligo-nucleotide primers synthesized from Operon Technologies Inc. (Alameda, CA,

USA) were used for genotyping study. These markers were initially tested on the parents AB-2 (resistant) and AB-52 (susceptible) for the detection of polymorphism between them. Out of these 250 markers, 68 markers were found to be polymorphic between the contrasting parents. The primers generating reproducible polymorphism among individual plants were selected for genotyping the F2 individuals.

PCR reactions were performed in the Perkin-Elmer 9600 thermal cycler (Barnstead Thermolyne Corp., Iowa, USA). The reactions were performed in a 20 μl reaction mix, containing 25 ng/ul of genomic DNA as template, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl<sub>2</sub>, 2 mM of each dNTP, 5 moles/μl of primer, and 0.3 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd.). The PCR program was set as Initial denaturation at 94°C for 3 min followed by denaturation at 94°C for 1 min, annealing at 60°C for 2 min and extension at 72°C for 2 min and these steps were repeated for 35 cycles with a final extension at 72°C for 5 min and hold at 4°C. The PCR products were resolved on 1.8% agarose gels. Ethidium bromide stained gels were visualized and documented by gel documentation system (BioRad).

#### Statistical analysis

Segregation ratios of F2 individuals were checked using Chi-square tests. Linkage analysis was performed using a tool, MAP-MAKER (Lander et al. 1987). This tool applies the maximum likelihood method for determination of recombination values and the Kosambi's mapping function (Kosambi 1944) for correcting the recombination values to map distances in cM. The logarithm of odds (LOD) score of 3.0 was used as a linkage threshold in this study. MAPMAKER is capable of performing both two-point and multipoint linkage analysis. Two-point linkage analysis is used to work out pair-wise linkage distances between any two segregating loci taken while the multipoint linkage analysis derives the best possible locus order along with a map based on the data on all segregating loci at one time.

#### Results and Discussion

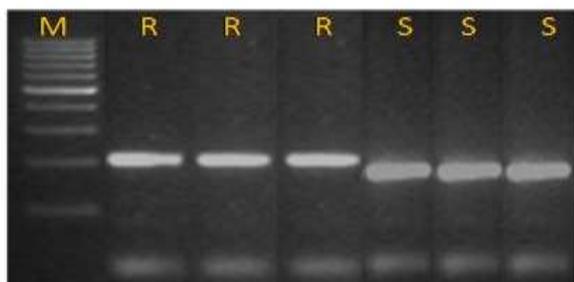
In field screening, 70 % of the accessions analyzed were observed as resistant while 30 % of them were found to be susceptible to *Fusarium* wilt (Table 5.8). As per the disease rating scale, the experimental material was categorized into resistant (R), with wilt incidence (%) less than 30%, resistant (R), with wilt incidence (%) 21.4, tolerant (T) with 30 to 58% wilt incidence (%) and susceptible (SS) group for which wilt incidence (%) was observed to be more than 60 % under field condition after 95 days of



The DNA from the resistant AB-2 and susceptible analogue AB-52 cultivars and their F<sub>1</sub> was subjected to generic markers screening. A total of 260 oligonucleotide primers were tested, of which 68 primers generated polymorphism between the parents in the cross respectively. The identified polymorphic primers were used to screen the F<sub>2</sub> mapping populations which resulted in the identification of two markers associated with the *Fusarium* wilt resistant phenotype of these, bNBS-LRR45 and bRGA-25. The segregation ratio of the two linked markers was in agreement with Mendelian segregation ratios of 3: 1 and 1: for dominant markers.

#### Efficacy of Markers Linked to Fusarium Wilt Resistance and Their Validation

The first markers of the two markers identified for fusarium wilt resistance in this study were selected for the validation of linked markers. These markers included bNBS-LRR-45 and bRGA-25 and were validated in a set of 8 germplasm lines consisting of 4 resistant and 4 susceptible genotypes. The markers could effectively distinguish the resistant and susceptible genotypes. Marker bNBS-LRR-45 and bRGA-25 produced resistant type allele in the all resistant genotypes while no alleles were amplified in susceptible genotypes as shown in the Figure 3.



**Fig. 3: Validation of the linked markers bNBS-LRR-45 in resistant genotypes.**

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