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Mapping of Genomic Region linked to Fusarium wilt Resistance in AP-42 inbred line of Castor (*Ricinus communis* L.)

Kumbha Divya Sravanthi, J Venkata Ramana, Lal Ahamed M, Y Satish, Manmode Darpan Mohanrao, R Satish Kumar , M Santha Lakshmi Prasad and S Senthilvel
ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad

ABSTRACT

The study was aimed at mapping the genomic region linked to Fusarium wilt resistance in a castor inbred line AP-42. A set of 95 F_2 plants derived from the cross between AP-42 and a susceptible line JI-35 was raised in wilt sick plot during *kharif* 2019 and their reactions to *Fusarium oxysporum* f.sp. *ricini* infection were scored. All the plants were genotyped using 110 SNP markers. Using the SNP genotypic data, a genetic linkage map of 1296.4 cM was constructed with an average marker distance of 11.8 cM. The length of individual linkage group ranged from 77.0 cM (LG-10) to 182.1 cM (LG 1). QTL analysis was performed following composite interval mapping approach. A major QTL linked to Fusarium wilt resistance was mapped on to the chromosome-6 flanked by the SNP markers Rc 43141-440 and Rc 29609-144169. The QTL identified in this study is novel comparing the other mapped QTLs. As the wilt resistance in AP-42 inherits as dominance, it is a highly desirable source for developing resistant parental lines and hybrids in castor. Markers linked to wilt resistance in AP-42 will assist in faster and efficient deployment of resistance genes into elite genetic background.

Key Words: *Castor, Fusarium wilt, Linkage map, QTL and SNP markers*

Castor (*Ricinus communis* L.) is an important non-edible oil seed crop having widespread industrial application. India contributes to approximately 80 per cent of the world castor production and earns sizeable foreign exchange through export of castor seed and oil. Though castor production is constrained by several biotic stresses, Fusarium wilt caused by the fungus *Fusarium oxysporum* f.sp. *ricini*. is the most destructive disease. The disease can infect at any stage of the crop growth, starting from seedling to maturity, resulting in loss of plant population. As the fungus is primarily soil borne, it is difficult to manage through chemical or cultural methods. Cultivation of resistant cultivars is the only viable option.

To develop resistant cultivars, reliable resistant sources and effective screening techniques are crucial. Screening against Fusarium wilt disease in castor is done at both field and glasshouse conditions. Under field conditions, artificially created sick plots are used for raising the test entries to test their reaction to Fusarium wilt. Glasshouse based screening include seed soaking, soil drenching, root dip and sick pot methods (Raoof and Rao, 1996; Desai *et al*., 2003; Prasad *et al*. 2008; Shaw *et al*., 2016). For the traits like disease resistance, which cannot be reliably scored and the screening process is cumbersome, marker assisted selection is the most useful approach. Finding molecular markers strongly associated with resistance genes helps in speeding up the pyramiding of important genes into the elite genetic background. The selection of resistant plants in segregating generations is made simple, if the resistance genes are marked with molecular markers.

QTL mapping has been successfully used to identify genomic regions responsible for traits of importance in several crops. QTL mapping experiment requires few basic things among which mapping population and genetic markers are most important. Choice of population depends on the objective of experiment and nature of the trait. Among different molecular markers available for genetic mapping, SNP markers have several advantages, including abundance, stability, amenability for high-throughput genotyping, and relatively low mutation

rates. A large number of SNPs can be identified within a species *via* high-throughput next generation sequencing (NGS) technologies which is a very promising approach to construct high-density maps for QTL mapping and gene cloning (Davey *et al.,* 2011). In recent years, a draft genome sequence of castor and chromosome-level assembly have been published (Chan *et al*., 2010; Lu *et al*., 2021). A high-density linkage map has been developed in castor using genome-wide SNP markers (Senthilvel *et al.,* 2019). Availability of reference genome sequences and high-density linkage maps provide an opportunity for QTL identification and fine mapping of trait of interest in castor. In this background, the present study was undertaken to identify the QTL linked to Fusarium wilt resistance in a castor inbred line AP-42.

MATERIAL AND METHODS

Plant materials and disease screening

The resistant line AP-42 was crossed with the susceptible line JI-35. AP-42 is an inbred derived from the germplasm accession RG-999 through single seed decent method. The wilt resistance in AP-42 is reported to be inherited as dominance (Shaw *et al*., 2018) making it a desirable source for resistance breeding. The susceptible parent is the standard check used for screening against Fusarium wilt in castor. The F_1 of the cross between JI-35 x AP-42 was advanced through selfing. The F_2 seeds collected from a single F_1 plant was used in this study.

The F_2 seeds were sown during *kharif* 2019 in the permanent wilt sick plot maintained at ICAR-IIOR, Hyderabad. The wilt sick plot was prepared by repeated incorporation of fungal inoculum, wilt affected plant debris and continuous cultivation of wilt susceptible cultivars. The seeds were sown in 4.5 m long rows with a spacing of 90 cm (between rows) x 45 cm (between plants). Susceptible (JI-35) and resistant checks (48-1) were sown after every five rows of test material (F_2 plants). The individual F_2 plants were observed for disease reaction up to 150 days and scored as resistant or susceptible based on the disease symptoms.

Genotyping

The young leaves from parents and F_2 plants were collected before the disease appearance and stored at -80°C. The genomic DNA was extracted from the stored leaf tissues using NucleoSpin Plant II, Mini kit (Macherey-Nagel) following the manufacturer's instructions. The quality of DNA samples was tested through 0.8% agarose gel electrophoresis. DNA samples were normalized to a final concentration of 4-5ng/ul. A total of 110 SNP loci polymorphic between JI-35 and AP-42 were selected from the catalogue of SNPs reported by Senthilvel *et al*. (2019). SNP genotyping was done through KASP assays, a competitive allele specific PCR based assay as described in Mohanrao *et al*. (2022).

Mapping of Fusarium wilt resistance

Genotypic data generated from a total of 110 polymorphic SNP marker loci were used to construct linkage map of F_2 population of the cross JI-35 and AP-42. Mapping was carried out through IciMapping QTL software using Kosambi mapping function (Meng *et al*., 2015). The threshold LOD was set to 3.0 with recombination frequency of 0.3. Markers within the linkage groups were ordered using 'nnTwoop' algorithm while rippling of markers was carried out with window size 5. QTL analysis was carried out using R/qtl package by following the binary traits model (Broman *et al*., 2003). The LOD threshold for QTL was predicted with permutation test involving 1000 iterations.

RESULTS AND DISCUSSION

In the present study, 95 F_2 plants derived from the cross JI-35 (susceptible) and AP-42 (resistant) were phenotyped for wilt resistance by screening them in sick plot. Out of 95 F_2- plants, 71 plants showed resistant reaction and 24 plants showed susceptible reaction. Thus, the F_2 population segregated in the ratio of 3:1 (resistant : susceptible) indicating that wilt resistance in AP-42 inherit as single dominant gene as reported by Shaw *et al*. (2018).

The same set of F_2 plants was genotyped using 110 SNP markers. Out of 110 markers 105 markers showed the expected mendelian pattern of inheritance (1:2:1) and remaining five markers showed segregation distortion. The genotypic data for all 105 markers were used for linkage map construction (Fig:1). A total of 10 linkage groups (LG) corresponding to haploid chromosomes number in castor were obtained. The number of markers within linkage group ranged from 7 (LG-2, 8, 10) to 20 (LG-6). The map distance of individual LG ranged

from 77.0 cM (LG-10) to 182.1 cM (LG-1). The total length of the genetic map was 1296.4 cM with an average marker interval of 11.8 cM. The average inter-marker distance between two adjacent markers ranged from 8.1 cM (LG-6) to 15.2 cM (LG-9) as given in Table:1.

The linkage map constructed in this study was further used along with the phenotypic data (reaction to wilt) for detecting QTLs. The details of the QTL linked to Fusarium wilt resistance is given in Table 2. In our study, QTL was detected on chromosome-6, whereas a previous study has reported QTL for Fusarium wilt was on chromosome-7. Thus, the present study has confirmed the postulation on existence of gene diversity for Fusarium wilt resistance in castor and underscoring the significance of understanding the molecular basis of castor wilt resistance (Shaw *et al*., 2022). The identified QTL and flanking markers have potential applications in marker-assisted selection for wilt resistance in castor.

Identifying tightly linked DNA markers to the disease resistance and pyramiding these multiple resistance genes into a single genotype provides durable resistance as earlier reported in rice pyramiding for three major bacterial blight resistance genes exhibiting high level of resistance (Pradhan *et al*., 2015). The results from the present study have laid the foundation for fine mapping the QTL region on chromosome-6, which may lead to map-based cloning of the resistance gene.

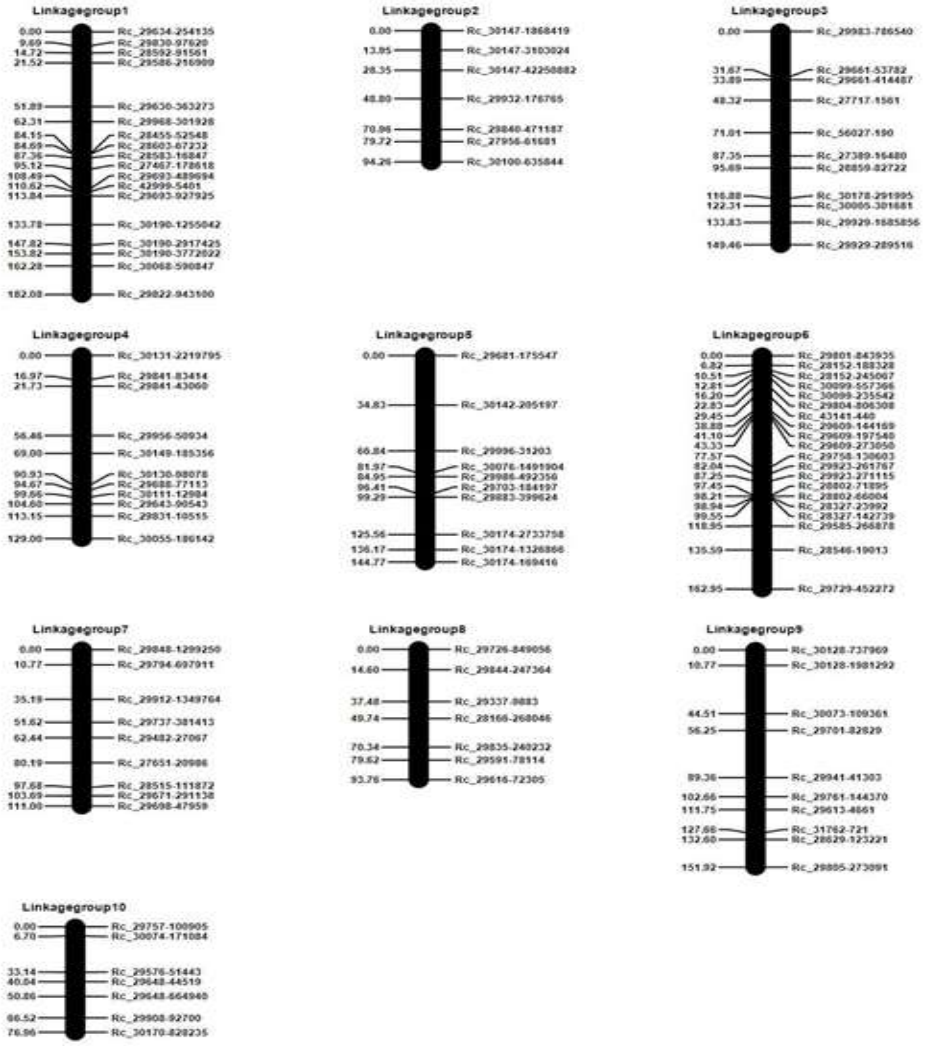

Fig 1. SNP marker-based linkage map of (JI-35 × AP-42) F_2 population

Table 1. Details of linkage map of JI-35 × AP-42 F_2 population

Linkage group (Chromosome)	Map length	No. of markers	Average inter-marker distance (cM)
LG-1	182.1	18	10.1
LG-2	94.3	7	13.5
LG-3	149.5	11	13.6
LG-4	129.0	11	11.7
LG-5	144.8	10	14.5
LG-6	163.0	20	8.1
LG-7	111.0	9	12.3
LG-8	93.8	7	13.4
LG-9	151.9	10	15.2
LG-10	77.0	7	11.0
Total	1296.4	110	11.8

Table 2. Details of QTL linked to Fusarium wilt resistance in AP-42

QTL	Chromosome	Position	Left Marker	Right Marker	LOD
For6.1	LG-6	29.5 - 38.9	Rc_43141-440	Rc_29609-144169	6.47

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