Genomic regions linked to reniform nematode (*Rotylenchulus reniformis*) resistance in castor

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(Received: February 18, 2022; Revised: March 3, 2022; Accepted: March 7, 2022)

ABSTRACT

Reniform nematode (*Rotylenchulus reniformis*) infects castor and makes it vulnerable for vascular wilt and root rot diseases. In this study, a population consisting of 92 recombinant inbred lines (RILs) derived from the cross between reniform nematode resistant line JC-12 and susceptible line 48-1 was used to identify the genomic regions linked to reniform nematode resistance. The parents, F_1 and RILs were screened against reniform nematode in pot culture with artificial inoculation of nematodes. The scoring for nematode resistance was done on the basis of number of nematodes extracted from the soil at 60 days after inoculation. The mean nematode count in 48-1 and JC-12 was 215.0 ± 9.1 and 77.8±4.8 nematodes/ml of soil wash, respectively. The F_1 reaction (217.3±13.2 nematodes/ml of soil wash) was similar to the susceptible parent indicating that nematode resistance in JC-12 is recessive in nature. The nematode count in RILs ranged from 43.8 to 327.3. QTL mapping using a linkage map consisting of 1,090 SNP markers resulted in the identification one QTL each on chromosome-6 and chromosome-8, linked to resistance. This is the first report on mapping of genomic regions linked to reniform nematode resistance in castor, which form the basis for furthering the research on genetic and molecular biology of nematode resistance in castor.

Keywords: Castor, Genome mapping, Inheritance, Reniform nematode resistance

Castor is an important non-edible oilseeds crop having multifarious industrial applications. Castor seed oil and its derivatives are used in manufacturing of several industrial products including paints, coatings, inks and lubricants (Ogunniyi *et al.*, 2006). Castor is good candidate for biodiesel production owing to its ability to grow as annual crop in marginal soils and shorter growing duration compared to other non-edible oilseeds like Jatropha (Shrirame *et al.*, 2011). In India, castor is cultivated in an area of 0.75 million hectares, which accounts for 65% of the world's castor acreage. India produces 1.2 million tonnes of castor seed per annum, which contributes to more than 85% of the world's castor production (FAOSTAT, 2019).

Castor is known to be infected by many nematode species among which, reniform nematode (*Rotylenchulus reniformis*) is considered as an economically important pest (Seshadri and Shivakumar, 1963). Rotylenchulus reniformis is an obligate, sedentary semi-endoparasite. The vermiform juvenile females penetrate the host root system where they establish a feeding site. It has a wide host range affecting more than 300 plant species including important crops like cotton, cowpea, grapes, papaya etc. Symptoms are not specific and in general not very apparent. Die-back, stunting and growth reduction have been reported in castor fields heavily infested with reiniform nematodes (Seshadri and Sivakumar, 1963; Verma and Prasad, 1969). In addition, leaf shedding, early flowering, malformed and discoloured seeds, decreased yield and inferior quality of oil have also been reported as the consequences of reniform nematode infestation in castor (Sivakumar and Seshadri, 1971). The estimated yield loss due to reniform nematode was 13.9% (Jain *et al.*, 2007).

Moreover, reinform nematodes were found to be associated with diseases like Fusarium wilt and *Macrophomina* root rot in castor (Chattopadhyay and Reddy, 1995). In a study on interaction between *Rotylenchulus reniformis* and *Fusarium oxysporum* f. sp. *ricini* in castor, *Rotylenchulus reniformis* alone and in combination with *F. oxysporum* f. sp. *ricini* found to reduce plant growth of both wilt susceptible and resistant hybrids. Fusarium wilt - Reniform nematode interaction was found to be synergistic when the two pathogens were inoculated together and wilt resistant cv. GCH-4 became susceptible in the presence of reniform nematode and wilt disease appeared earlier in different combinations of nematode and fungus, than fungus alone (Patel *et al.*, 2000a). In a study on interaction between reniform nematode and *Macrophomina*, early appearance of disease and high mortality was observed when the plants were inoculated with *Rotylenchulus reniformis* and *Macrophomina phaseolina* together compared to inoculation of *Macrophomina* alone or *Macrophomina* followed by the nematode (Patel *et al.*, 2000b).

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Though soil solarisation and application of carbofuran at the rate of 2 kg a.i/ha were found to reduce losses from reniform nematode infection, the use of genetic resistance is a better environmental friendly option to counter the reniform nematode infection (Schrimsher *et al.,* 2014). The cultivation of resistant cultivars helps in bringing down the nematode population in the soil over years, which might have greater impact on other more susceptible crops such as cotton and cowpea. Many castor cultivars and hybrids were identified as moderately resistant to reniform nematodes (Barre *et al.,* 2013). A castor inbred line JC12 was found to harbour less reniform nematodes in the repeated screenings at ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, India. The present study was aimed at genetic characterization and QTL mapping of reniform nematode resistance in castor inbred line 'JC-12'.

MATERIALS AND METHODS

Plant materials: JC-12 is an elite inbred line developed by Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India. It was found to be resistant in the in-house screenings (Fig 1). JC-12 was crossed with a susceptible line 48-1. 48-1 (Jwala) is a notified commercial variety developed by ICAR-IIOR. JC-12, 48-1, (JC12 × 48-1) F_1 and a sub-set (92 lines) of recombinant inbred line (RIL) population derived from the cross JC12 × 48-1 were used in this study.

Screening against reniform nematode: The seeds of parents, F_1 and RILs were sown in plastic pots containing 250 g of sterilized sand and soil mixture (1:1). Single plant was maintained per pot and 10 pots were maintained for each line. The culture of *Rotylenchulus reniformis* maintained on susceptible castor plants at ICAR- IIOR was used for screening. Ten days after sowing, 500 reniform nematodes (mixed life stages) suspended in 1 ml water were added to the soil in each pot. Pots were placed in a glass house at a constant temperature of ~30°C. Pots were watered time to time so as to maintain the soil moisture (Fig 2). Sixty days after inoculations, nematodes were extracted from the soil following the modified Cobb's sieving and decanting technique (Christie and Perry, 1951). The nematodes were counted under stereo-bionocular microscope and the count was expressed as 'number of nematodes/ml of soil suspension'.

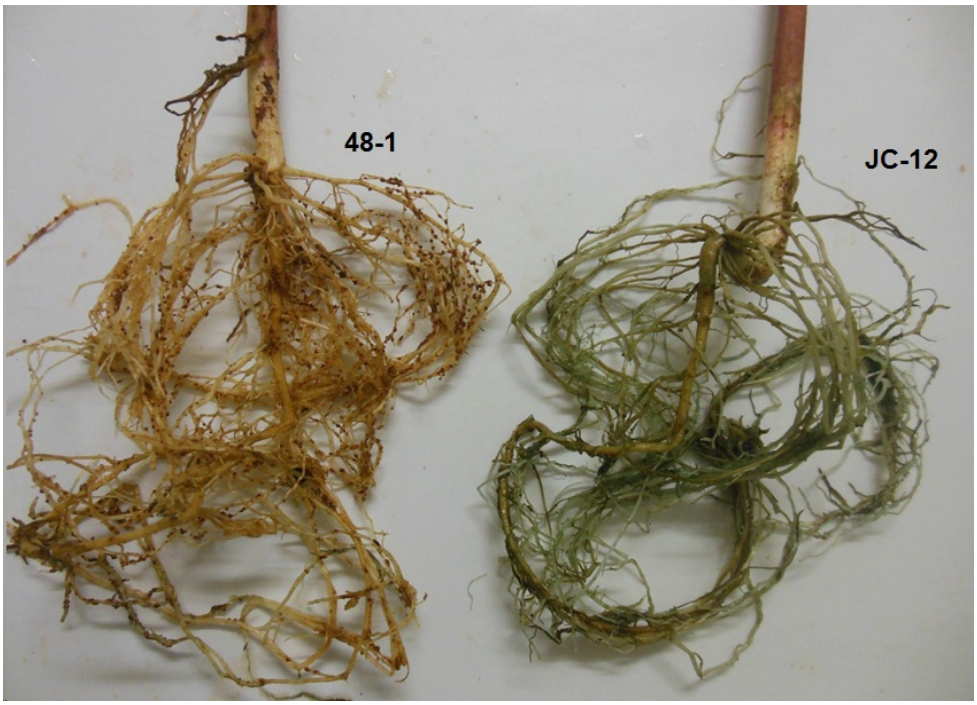

Fig. 1. Egg masses of reniform nematode on the roots of castor inbred lines 48-1 and JC-12

QTL mapping: The genotypic data for 1,090 SNP loci generated earlier was used for constructing the linkage map. Linkage map construction and QTL mapping were carried out using the QTL IciMapping software version 4.1.0.0 (Meng *et al.,* 2015). Anchor markers with known chromosomal locations as per Xu *et al.* (2021) were used for

identification of chromosomes. The markers were grouped into linkage groups representing 10 castor chromosomes at the logarithm of odds (LOD) threshold of >3. The markers within the linkage group were ordered using 'nnTwoOpt' algorithm. Map distances between markers were calculated using the Haldane mapping function. Refinement of marker order within each chromosome was done with rippling function 'SARF' along with window size of '5'. The QTL mapping was carried using inclusive interval mapping algorithm for additive effects (Li *et al.,* 2007). LOD threshold to identify significant QTL was set at >2.5.

Fig. 2. Screening of JC-12 × 48-1 RILs for reniform nematode resistance in glasshouse

RESULTS AND DISCUSSION

The present study was aimed at genetic characterization of reniform nematode resistance in an elite castor inbred line JC-12. The screening against the reniform nematodes through artificial inoculation has confirmed that JC-12 is resistant. The mean number of nematodes harboured by JC-12 was 77.75/ml of soil wash, which was significantly lesser compared to 48-1, which harboured 215.0/ml of soil wash (Table 1). The hybrid plants of JC-12 × 48-1 recorded on an average 215 nematodes/ml of soil wash similar to the susceptible parent. Therefore, it was inferred that resistance to reniform nematode in castor is as that of soybean (Harville *et al.,* 1985). The recombinant inbred line (RILs) population from the cross JC12 × 48-1 showed wide variation for nematode count. The mean nematode count/g of soil ranged from 43.75 to 309.75 (Fig 3). Positive and negative transgressive segregations were detected in the RIL population suggesting that alleles from both parental lines might have contributed to the resistance. Six RILs (P3-96, P3-108-NSp, P3-109NSp, P3-131Sp, P3-131NSp & P3-241) were found to harbour lesser nematodes than the susceptible parent JC12. These RILs being homozygous lines can be directly used for breeding for resistance to reniform nematode in castor.

Linkage analysis yielded 10 linkage groups representing the haploid chromosome number of castor. Inclusive composite interval mapping using SNP genotypic data and mean nematode count for the RILs identified one significant QTL (LOD >2.5) each on chromosome-6 and chromosome -8 (Fig 4). The details of QTL are given in Table 2. Previously, genomic regions linked to reniform nematodes have been identified in crops like Gossypium arboreum (Erpelding and Stetina, 2018), Gossypium barbadense (Gutiérrez *et al.,* 2011) and soybean (Ha *et al.,* 2007; Wilkes *et al.,* 2020). However, the present study is the first report on mapping of QTLs for reniform nematode resistance in castor to the best of our knowledge. This study has laid the foundation for further validation of putative regions associated with reniform nematode resistance and its use in marker assisted selection for developing reniform nematode resistance inbred lines in castor.

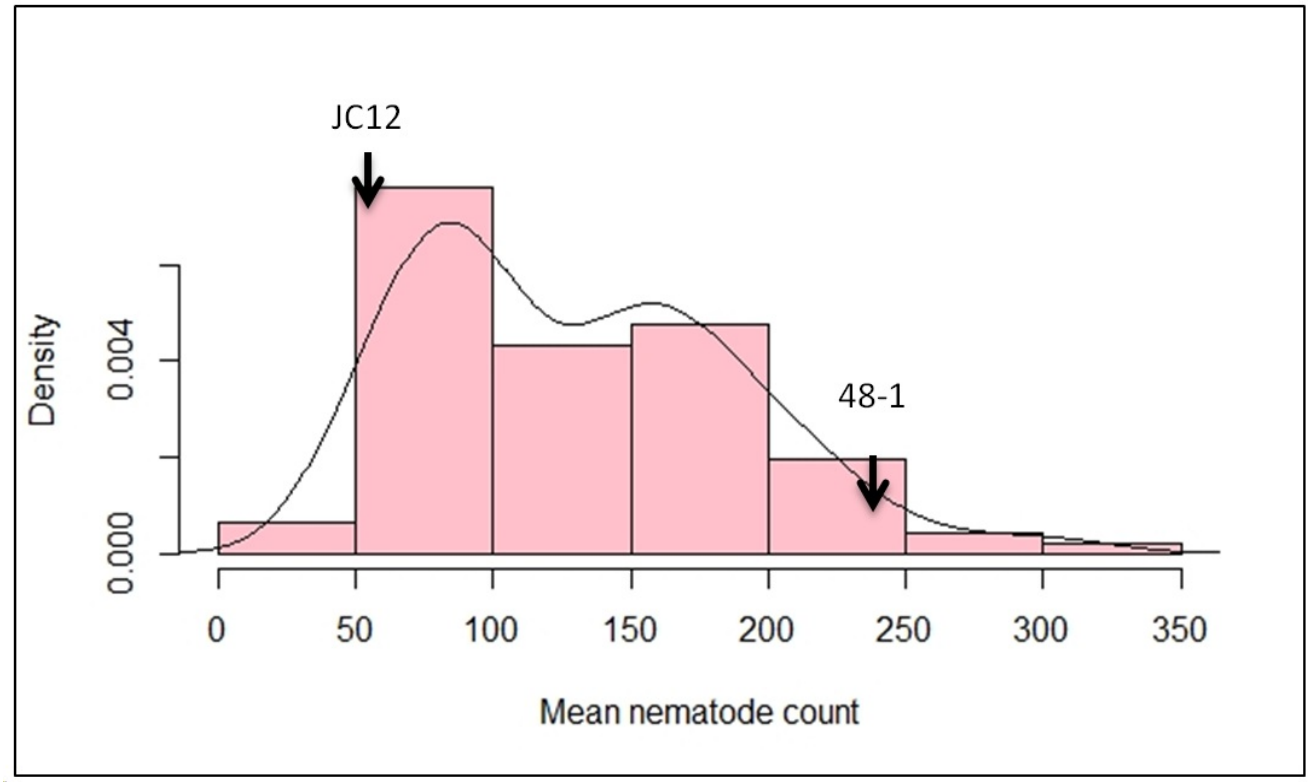

Fig. 3. Frequency distribution of nematode count in RIL population

Table 1 Reniform nematode count in JC-12, 48-1, F1 and RILs

Particulars	JC-12	48-1	(JC-12 × 48-1)F1	(JC-12 × 48-1)RILs
Mean	77.8	215	217.2	131.5
Range	69-87	199-237	189-251	44-327
Sd	9.6	18.3	26.4	61. 2
Se	4.8	9.1	13.2	6.3

Table 2 QTLs linked to reniform nematode resistance in JC-12 × 48-1 RIL population

Chromosome	Position	Left marker	Right marker	LOD	R2	LOD support interval	Additive effect	Allele source
6	79	Rc_29666-381712	Rc_29666-471509	5.04	17.91	76.5 – 81.5	-26.13	JC-12
8	66	Rc_28037-33296	Rc_28151-12413	3.62	13.23	64.5 – 67.5	-22.34	JC-12

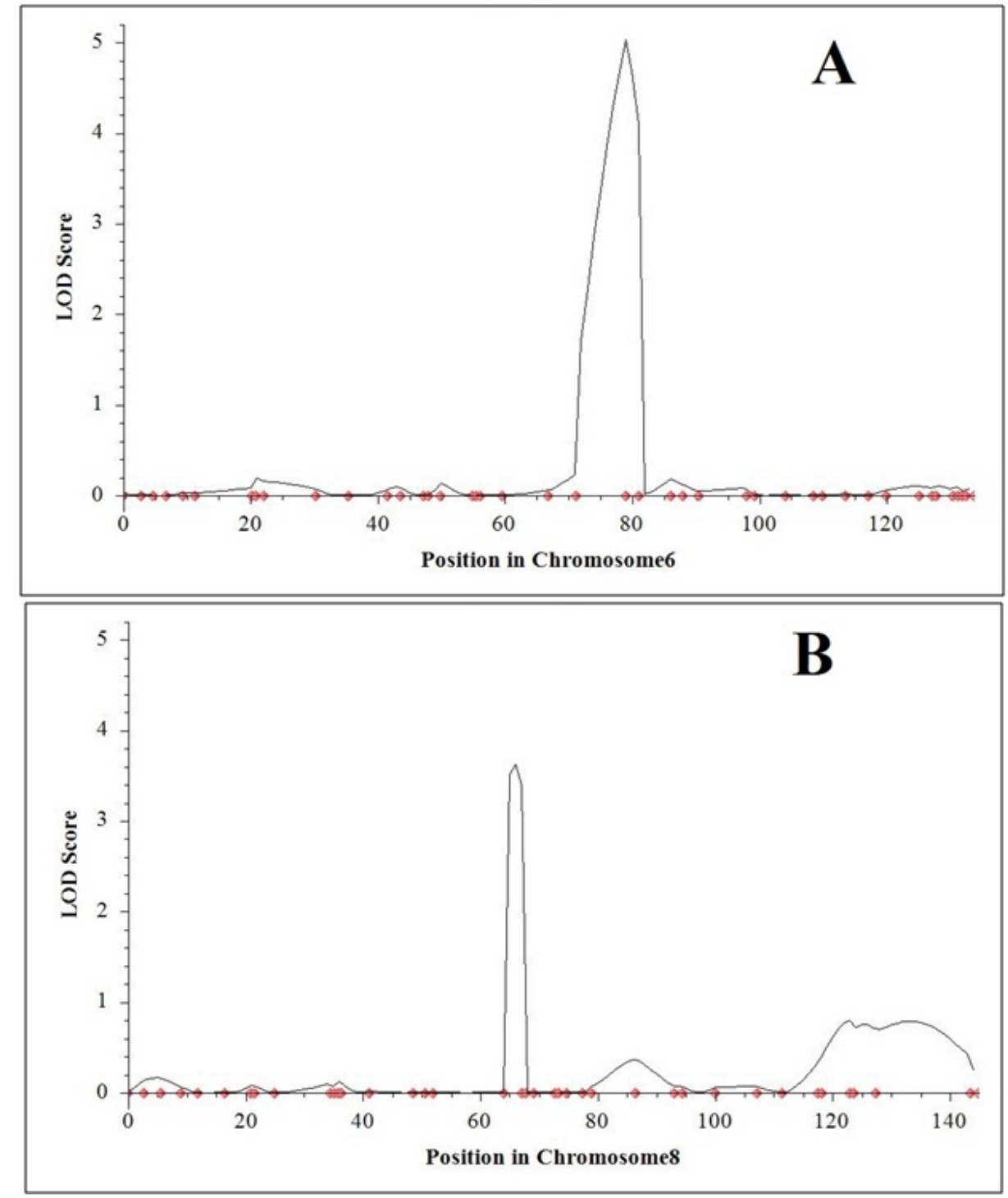

Fig. 4. QTL positions on chromosome 6 (A) and chromosome 8 (B)

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