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Molecular mapping and identification of QTLs responsible for charcoal rot resistance in Castor (*Ricinus communis* L.)

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ABSTRACT

Charcoal rot caused by *Macrophomina phaseolina* in castor is one of the major pathogenic determinants for extreme yield loss. The charcoal rot resistance trait is complex and controlled by quantitative trait locis (QTLs). This study was aimed to develop linkage map and to identify novel QTLs associated with charcoal rot resistance in castor. A mapping population ($F_{2:3}$) derived from the cross between two castor inbred lines JI 357 (Resistant genotype) and SKI 338 (Susceptible genotype) was developed and screened in the sick plot using randomised block design. After screening 920 (520 RAPD, 100 ISSR, 300 SSR) primers among the parental lines, 336 polymorphic markers were identified to be used for genotyping the mapping population to develop genetic linkage map. The genetic map consisting of 10 linkage group covering a total map length of 1833.4 centimorgan (cM) with average marker interval of 6.93 cM was developed. The length of linkage groups varied from 121.5 cM for linkage group 10 (C10) to 278.7 cM for linkage group 9 (C9). The average marker interval was maximum in C3 (8.23 cM) while it was lowest in C10 (5.78 cM). Analysing genotypic data along with phenotypic data collected from mapping population against charcoal rot in sick plot, identified three novel QTLs explaining 11.3–71.2% of phenotypic variation. One major QTL with LOD score of 6.5 was identified on linkage group 2 explaining 71.2% of phenotypic variation, is a most promising QTL for molecular breeding. This is the first study reporting novel QTLs for charcoal rot resistance in castor and thus future studies will be conducted to refine these QTLs.

1. Introduction

Castor (*Ricinus communis* L.) is one of the ancient and important industrial and non-edible oil crop of the world with a total global production of 18.54 million tonnes from 15.02 million ha of which India contributes 16.4 million tonnes from 10.9 million ha (FAO, 2013) followed by China and Brazil. It is most suited crop for dry land farming as it has the ability to grow under low rainfall and fertility conditions. The oil content of the seeds varies from 50 to 55% in different varieties which contains an unusual hydroxyl fatty acid, ricinoleic acid in high amount (85–95%). The castor oil is the only vegetable oil which is soluble in alcohol, has high viscosity and requiring less heating for production of biodiesel in comparison with others oils (Jeong and Park, 2009) and hence widely used as a lubricant in high speed engines and aeroplanes, in manufacture of soaps, printing inks, linoleum, varnishes, transparent paper and plasticizers. It also has many medicinal values as well as is used for lighting purposes. After the extraction of oil, the remaining cake is usually used as organic manures as it contains 6.4% N, 2.5% phosphoric acid, 1% K and some micronutrients, while the plant stalks are used as fuel or in the paper industry as thatching material for the preparation of paper pulp. The average yield of castor is 1234.6 kg/ha, which is heavily affected by three major diseases namely charcoal rot (*Macrophomina phaseolina*), vascular wilt (*Fusarium oxysporum* f. sp. *ricini*) and gray mold (*Botryotinia ricini*). Among these diseases, in most castor growing countries charcoal rot is a major disease (Rajani and Parakhia, 2009). Management of charcoal rot is primarily based on cultivar resistance, although resistant genotype has been identified, but transfer of resistant gene into the cultivar is a time-consuming process especially in

Abbreviations: RAPD, randomly amplified polymorphic DNA; SSR, simple sequence repeats; ISSR, inter-simple sequence repeats; PCR, polymerase chain reaction; LOD, logarithm of odds.

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the absence of accurate screening procedure and linked molecular markers. Integration of genomic tools with conventional breeding has been successful in some crops but castor lagged behind in the absence of genomic data required for this approach. However on the other hand, with the development of understanding towards molecular markers and DNA sequencing along with their application, the time may be reduced by identifying the DNA based markers from QTLs and linkage maps (Reddy et al., 2011). With the help of QTL mapping, loci of specific disease resistance genes and interactions between resistant genes in parental lines can be analyzed (Wuirschum, 2012). With the development of different molecular marker technology and its use in linkage mapping has made it possible to study the effects of the individual loci that is involved in the control of quantitatively inherited trait (quantitative trait loci, or QTLs). These QTLs can be further utilised for development of resistance towards biotic stress. Identification of major genes and QTLs for many important agricultural traits in different crops through genetic linkage mapping has reduced the time required for conventional breeding process and has merged the field of biotechnology with conventional breeding (Zhang et al., 2014). However very few studies have been carried out to detect association between molecular marker and QTLs in castor plants, moreover, there have been no reports of QTLs associated with charcoal rot in castor. With the availability of draft genome of castor, identification and mapping of disease resistance genes or quantitative trait locis (QTLs) will provide valuable information and tools for marker assisted selection as well as will be useful in development of disease resistant castor line in the absence of sick plot or sufficient microbial load.

Till date, there are no reports on linkage maps and QTL mapping for charcoal rot in castor, however Dhingani et al. (2012) had carried out genetic diversity study on castor genotype resistant to charcoal rot using RAPD, ISSR and SSR markers. Therefore, with an objective to develop linkage map and identification of QTLs responsible for charcoal rot resistance in castor, this study was designed.

2. Materials and methods

2.1. Plant material

For the identification of parental lines, 35 promising genotypes were collected from Main Mustard and Castor Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat and Main Oilseed Research Station, Junagadh Agricultural University, Junagadh, Gujarat. The collected genotypes were screened to identify resistant genotype for the development of mapping population by sowing them in the sick plot developed for *Macrophomina phaseolina* at Junagadh Agricultural University, Junagadh.

2.2. Mapping population

The F_2 mapping population was developed by hybridizing JI 357 as highly resistant female parent after screening and SKI 338 as highly susceptible male parent. The F_1 produced from the cross of parental line was selfed to produce F_2 mapping population. Young leaf tissues from parents and F_2 plants were harvested, immersed in liquid nitrogen and stored at −80 °C before DNA extraction.

2.3. Phenotypic evaluation

The $F_{2:3}$ families were sown in the charcoal rot-sick plot developed at Main Oil Seed Research Station, JAU, Junagadh against *Macrophomina phaseolina* in three replications using randomised block design (RBD). The experimental units were two row plots of 9 m length with 60 cm spacing between plants and 90 cm between rows. Susceptible check 'SKI 338' was planted after every 6 rows to provide a constant disease pressure for the charcoal rot. Observations for disease incidence were recorded at 85 days after sowing (DAS). The charcoal rot incidence was measured on the basis of a formula (% charcoal rot incidence = number of plants showing charcoal rot symptoms/total number of plants × 100).

2.4. DNA isolation

Initially the total genomic DNA of parental lines and F_2 population consisting of 190 individuals was isolated from young leaves using CTAB method as previously reported by Tomar et al. (2014) in castor. The DNA concentrations were estimated spectrophotomatically by Picodrop (Picodrop Ltd. Cambridge, UK.), while purity of DNA was estimated through electrophoresis on 0.8% agarose gels containing ethidium bromide-staine and appropriate molecular weight standards.

2.5. SSR primer development and screening

SSR primers to be used in linkage mapping were designed using the castor genome submitted by J. Craig Venter Institute (GenBank Accession: AASG00000000). Available scaffolds were scanned using the MISA software package to identify all SSRs within a set of sequences (Thiel et al., 2003). For SSR development, following parameters were used to mine motifs with a relatively high minimum numbers of repeats (1) ten for dinucleotide motifs; (2) eight for trinucleotide motifs; (3) six for tetranucleotide motifs; (4) four for pentanucleotide motifs and (5) three for hexanucleotide motifs. For primer designing, Primer3 software through batch mode via the p3_in.pl and p3_out.pl Perl5 scripts (Rozen and Skaletsky, 2000; Koressaar and Remm, 2007; Untergasser et al., 2012) within the MISA package was used. The parameters for primers design were: (1) primer length from 18 to 22 bp with 20 bp as the optimum; (2) annealing temperature from 55 °C to 65 °C with an optimum of 60 °C; (3) PCR product size from 100 to 300 bp; (4) GC contents from 45% to 55%, with 50% as optimum. The primer pairs were synthesized by Sigma Aldrich (Banglore, India) with the prefix CST which stands for castor.

2.6. Polymorphism and genotyping

Parental polymorphism screening was conducted using 920 primers belonging to RAPD, ISSR and SSR markers. For marker genotyping, PCR amplification was carried out in a 25 μl total reaction volume containing 2.5 μl 10× reaction buffer (Invitrogen; 100 mM Tris–HCl pH 9.0, 15 mM $MgCl_2$ and 500 mM KCl), 0.6 μl of 10 mM dNTPs, 1.2 U of Taq DNA polymerase (Invitrogen), 2 μl of 10 pmol primer for RAPD and ISSR while 1 μl of each primer in case of SSR and 40–50 ng of genomic DNA. PCR amplification was carried out in Veriti thermo cycler (Life Technology) as the protocol described by Tomar et al. (2014). The PCR products generated using different markers were analyzed in capillary electrophoresis (LCGX, Caliper).

2.7. Linkage analysis and map construction

The segregation of each marker and linkage analysis based on 190 $F_{2:3}$ plant for charcoal rot was done using Mapdisto software version 1.7.2.4 (Lorieux, 2007). In mapdisto, the logarithm of odd (LOD) score for the test of linkage between marker pairs was set at 6.0 and recombination fraction at 0.30 were used to assign segregating markers to linkage groups. Markers found unlinked at LOD 6.0 were tried at decreasing LOD, down to LOD 3.0 and the markers that were attributed to linkage group at a LOD grouping threshold of 3.0 were only included. WinQTL Cartographer 2.5 software (Wang et al., 2007) was further used to construct the linkage map from the