Industrial Crops and Products 89 (2016) 103–108

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

The first genetic linkage map of *Ricinus communis* L. based on genome-SSR markers

Shuai Liu, Xuegui Yin*, Jiannong Lu**, Chen Liu, Chuan Bi, Hongbo Zhu, Yuzhen Shi, Dan Zhang, Danyou Wen, Jun Zheng, Yue Cui, Weijin Li

Guangdong Ocean University, Zhanjiang, 524088, China,

ARTICLE INFO

Article history:
Received 31 January 2016
Received in revised form 9 April 2016
Accepted 22 April 2016
Available online 12 May 2016

Keywords:
Ricinus communis L.
SSR marker
Genetic linkage map

ABSTRACT

Castor (*Ricinus communis* L.) is an important oil crop in the spurge family. The seeds contain an oil with unique chemical properties for industrial uses. With the development of economy the demand of castor bean and castor oil is increasing rapidly, but the castor bean production is suffering a lot from the low-yield variety because of the lack of new breeding method. Genomic simple sequence repeat (SSR) marker is particularly valuable and convenient in studies of genetic diversity, evolution, QTL mapping. because of its co-dominance, reproducibility, abundance and extensive genome coverage, but few were exploited in castor. So far, there is no genetic map of castor to be used, which severely hampered the genetic researches in castor. In this study, a total of 2, 719 castor SSR markers were developed from the genome sequencing information and a SSR genetic linkage map was constructed with 3 different F_2 populations derived from the cross combinations YC2 × YF1 and YC2 × YF2 respectively. It was revealed that the SSR density in castor genome was approximately 15.81 SSR/Mbp and the frequency of SSR motifs decreased with the increased repeat unit size, the dinucleotide and trinucleotide repeats, with (AT)n and (AAT)n as the most common repeat unit respectively, dominated the SSR types. After integration, the linkage map consisted of 331 markers, including 317 SSR markers, 7 SRAP markers, 3 SSRAP markers, 3 morphological markers and 1 ISSR marker, distributed on 10 linkage groups, encompassing 1164.73 cM of genome, with an average marker interval of 3.63 cM. This is the first genetic linkage map of castor which is expected to facilitate the researches on genetics and breeding in castor as well as the comparative genomics research in the spurge family.

1. Introduction

Castor (*Ricinus communis* L., 2n = 2x = 20) is a tropical and sub-tropical perennial shrub or temperate annual herbage that can be grown on marginal lands and varying weather conditions usually unsuitable for crops (Berman et al., 2011). The main economic value of castor lies in castor oil, which is considered as the only commercial source of ricinoleic acid, a hydroxylated fatty acid (Severino et al., 2012). The high content of ricinoleic acid in castor oil (approximately 80–90%) determines it a vital raw material in industrial production, including high-quality lubricants, paints, coatings, plastics, soaps, medications for skin affections (Ogunniyi, 2006; Rivarola et al., 2011). With the development of economy, the demand of castor oil has been increasing. However, the castor industry has also been sufferring a lot from the shortage of raw materials during the past decades. Although the castor has been cultivated for over 1400 years, the output per unit area of variety remains low due to the lack of effective improvement. Besides the poverty of genetic diversity within the species (Allan et al., 2008), the lagging behind in genetic research contributed to it.

Compared with AFLP (Amplified fragement length polymorphism), SRAP (Sequence-related amplified polymorphism) and RAPD (Random amplified polymorphism DNA) markers, SSR (Simple sequence repeats) marker is co-dominant and present high levels of reproducibility, abundance and genome coverage, so it is widely used in genetic evaluation of various characteristics, genetic map construction and quantitative trait loci mapping. Great achievements have been made by means of plenty SSR markers and furtherly high-density SSR genetic maps in more and more crops such as rice (McCouch et al., 2002), wheat (Gupta et al., 2002; Song et al., 2005), maize (Sharopova et al., 2002), potato (Milbourne et al., 1998), sesame (Rallo et al., 2000), but only a few SSR markers were exploited in castor and seldom applied in practice, not to mention

* Corresponding author.
** Co-corresponding author.
E-mail addresses: yinxuegui@126.com, 765267265@qq.com (X. Yin).

http://dx.doi.org/10.1016/j.indcrop.2016.04.063
0926-6690/

the genetic map, which severely hampered the genetic studies in castor. Allan (Allan et al., 2008) first reported 9 genome-SSR markers with which 41 accessions from five continents were identified to find low genetic diversity (heterozygosity, *He* = 0.188) in castor. Bajay (Bajay et al., 2009; Bajay et al., 2011) developed 23 genome-SSR markers from microsatellite-enriched libraries and identified 38 genotype accessions from the castor germplasm of the Brazilian Agricultural Research Company (EMBRAPA). Seo (Seo et al., 2011) published 28 genome-SSR markers from microsatellite-enriched libraries with expected heterozygosity of 0.04–0.54 (mean = 0.31) in 72 accessions. Machado and Silva (2013) developed microsatellite primers using bioinformatic tools Websat and Net Primer from sequences deposited at the GenBank/NCBI and found 9 were polymorphic. Qiu et al. (2010) develpoed 118 polymorphic EST-SSRs which displayed moderate gene diversity with an average heterozygosity of 0.41 by screening 24 castor bean samples collected from different countries and found that the trinucleotides were the dominant motifs in EST-SSRs. Pranavi et al. (2011) reported 92 polymorphic novel EST-SSRs and used in genetic purity assessment of hybrids. Although the exploited EST-SSR markers were more than genomic ones in castor, they revealed much less diversity in biparent mapping populations as well as in natural populations in our experiments before.

In the past, the cost of developing SSR markers was high due to microsatellite-enriched libraries. The release of whole-genome sequence of castor bean (Chan et al., 2010) made SSR development become relatively easier to do. Meilian Tan (Tan et al., 2014) developed 1435 SSR primer pairs by mining data from whole-genome sequences, among which 670 (46.7%) SSR markers were polymorphic between six accessions. Of course, it was impractical to construct a genetic map with practical value just using existing SSR markers, taking into account the number of polymorphic markers and inevitable gap. Recent years, with the rapid development of high-throughput sequencing platform, SNP (single nucleotide polymorphisms) markers have become an increasingly valuable marker. Foster (Foster et al., 2010) reported 232 high quality SNPs and used them to genotype a worldwide castor collection of 488 germplasm samples described in five genetic clusters. In this study, it was intended to exploit more genome-SSR markers and furtherly to construct the first genetic map of castor.

2. Materials and methods

2.1. Castor SSR-containing data retrieval, SSR detection and SSR primer development

The source of SSR-containing sequences was genomic DNA Sequence released by the institute for genomic research (TIGR) (http://castorbean.jcvi.org/downloads.php), which contains 25,828 assemblies of castor genome. The downloaded data was sumbitted to Microsatellite Identification Tool Software (MISA) (http://pgrc.ipk-gatersleben.de/misa/) to mine SSR motifs including di-nucleotide repeats (DNRs), tri-nucleotide repeats (TNRs), tetra-nucleotide repeats (TTRs), penta-nucleotide repeats (PNRs), hexa-nucleotide repeats (HNRs) and hepta-nucleotide repeats (HTRs) with numbers of repeat units of 20, 10, 7, 7, 7 and 7, respectively.

Primer pairs were designed based on the flanking sequences of the detected SSR motifs and the parameters were set as follows, primer length of 15–27 bp, amplification product size of 100–400 bp, melting temperature at 55–60 °C and GC content of 40–60%.

In order to reduce the cost for map construction, some SRAP, SSRAP, ISSR (Inter-simple sequence repeat) and 3 morphological markers were also used. Among them, the SSRAP marker was a kind of novel marker randomly combining primer pairs between SSR and SRAP markers to be proved a economic and efficient method developing markers massively using existing markers.

2.2. Plant materials and DNA extraction

3 different populations were used for the construction of the genetic linkage map. They were derived from the cross combinations between the pistillate inbred line YC2 and the monoecious inbred lines YF1 and YF2 respectively, named F_2-1, F_2-2and F_2-3 with 162,182 and 161 individuals respectively. Populations F_2-1 and F_2-2 came from the only pistillate F_1 individual and one of the monoecious F_1 individuals of the same cross YC2 × YF1 respectively. The third F_2 population, F_2-3, was from the cross of YC2 × YF2 which produced no pistillate F_1 individual. The pistillate line YC2 was created from the self progeny of hybrid Jinbi No. 2, bred in Shanxi, China, and the monoecious lines YF1 and YF2 were siblines developped from the self progeny of the hybrid CSR6.181, bred in Costa Rica. The molecular genetic distance between YC2 and YF1,YF2 was 14.88, 15.12 respectively, which was medium to large among 24 alternative key parents the genetic distance between which ranged from 7.49 to 17.36, which was considered benefit to heterosis. Moreover, the phenotypic characters between the parents was extremely sharp distinction, including sex type (pistillate/monecious), plant height (220.4 cm/81.5 cm/80.6 cm), the color of stem and petiole (green/red), resistance to castor blight (resistance/susceptible), leaf style (smooth and flat/shrinkage and triangle-cup), panicle type (column/pyrimid), seed coat color (all black/decorative pattern), wax on capsule and main stem (present/absent), covering a wide range of variation in many important agronomic and morphological traits.

Genomic DNA was extracted from fresh leaves using CTAB method (Murray and Thompson, 1980) and checked on 1% (w/v) agarose gels.

2.3. Marker analysis

SSR and other kinds of markers were surveyed for polymorphism using DNA template from both parental lines. The polymorphic markers were used for genotyping F_2 mapping populations. SSR PCR amplification was performed in 10 μL reaction system consisting of 10 ng of genomic DNA, 1 μL10 × PCR Buffer, 0.8 μL Mg^{2+} (25 mM), 0.1 μL dNTP Mix (10 mM each), 0.1 μL DNA Taq Polymerase (5U/μL), 0.1 μL of each primer (10 μM). After an initial denaturation of 5 min at 94 °C, 35 cycles for 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C were performed, followed by a final extension of 7 min at 72 °C. DNA fragments were separated by electrophoresis on a 6% non-denaturing polyacrylamide gel running at 250 V for 2 h in 1 × TBE buffer and visualizing by silver staining. The molecular weights of amplicon were determined according to DL500 DNA Marker (TAKARA, Japan).

2.4. Data analysis and map construction

All of the markers used in scanning populations were tested for segregation ratios using Chi-square (χ^2) test. The markers that did not fit the segregation ratios ($p < 0.05$) were deemed distorted and were assigned a "*" suffix. Mapping was performed using the software JoinMap 4.0 with a population type code F_2 (Van Ooijen, 2006), linkage groups of three populations were determined respectively with LOD score above 3.0, then the groups with the same markers were integrated by Combine Groups for Map Integration function, the markers were ordered by regression mapping algorithm and the Kosambi function was used to convert reconbination units into genetic distance for all linkage groups. The genetic linkage map was drawn by software MapChart V2.3