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RESEARCH ARTICLE

IDENTIFICATION OF RAPD MARKER LINKED TO SEX DETERMINATION IN SIMAROUBA

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ABSTRACT

Simarouba is an important oil yielding plant. Twenty decamer RAPD primers were tested on male and female plant to identify sex specific marker. Out of twenty markers fourteen RAPD primers produced polymorphic banding patterns. Out of fourteen polymorphic primers, primer OPU8 (5'GGCGAAGGTT3') produced a unique band of 1000bp only in male individual. The RAPD method successfully discriminates among male and female plant, therefore providing an easy and rapid tool for identification of gender in plants at early stage.

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INTRODUCTION

Simarouba belongs to Simaroubaceace family. It is native of Central America, grows well in waste land. The seeds contain 65 to 75% of oil. Simarouba is polygamodioecious consists of three types of plants showing male, female and bisexual flowers. The tree flowers after 4-5 years of planting. The ratio of planting male to female is 1:20 for effective pollination and good yield (Joshi 2000). Therefore it is important to maintain sex ratio. In recent years efforts have been made to understand basis of sex determination. Phenotypicaly it is not possible to discriminate male, female and hermaphrodite plant before the flowering stage. Genetic markers could be utilised to diagnose sex-linked DNA markers. The aim of the present research is to find male and female specific markers so that the gender of the plant can be identified at seedling stage. These markers aid in breeding programme and crop improvement.

MATERIALS AND METHODS

Plant material

Young green leaves from male and female Simarouba plants were collected from the plantations in the University of Agricultural Sciences, Bangalore and kept in liquid nitrogen.

Extraction of DNA

Genomic DNA was extracted from the sample by modified CATB (hexaadecyltrimethylammoniumbromide) method of Doyle and Doyle (1987). Five grams of young leaves of each male and female sample frozen in liquid nitrogen was ground to a fine powder and mixed with 20ml of CTAB buffer (10 mM Tris-HCl, pH=8.0, 20mM EDTA, 2% CTAB, 1.4 M NaCl,1% PVP, 0.2% β-mercaptoethanol, 0.1%$NaHSO_3$). The sample was incubated at 65^0c for 1 hour, mixed with an equal volume of chloroform- isoamyl alcohol (24:1) and centrifuged at 10.000 rpm for 10 minutes at 4^0c. The aqueous phase was recovered and mixed with 2/3 volume of isopropanol to precipitate DNA. The nucleic acid precipitate was recovered with glass hook washed with 70% ethanol and dried overnight and resuspended in 2ml of TE buffer (10 mM Tris-Hcl, 0.1 mM EDTA).

DNA purification and quantification

The extracted DNA was purified by using RNase, proteinase K, phenol: chloroform: isoamylalcohol (25:24:1) and sodium acetate to remove RNA, protein, polysaccharides and phenols respectively by the Murray and Thompson method. Quantification of DNA was done by Nano spectrophotometer. The extracted DNA was diluted to 25ng/µl and subjected to polymerase chain reaction (PCR) amplification.

PCR conditions

PCR amplification reactions were carried out following Williams *et al.* (1990) with minor modification. The 25µl reaction contained 50ng genomic DNA, 10mM Tris-HCl, 1.9 mM $Mgcl_2$ and 100 µM of each dATP, dGTP, dCTP and dTTP (Merk. Bangalore), 0.4 mm primer (Operon Technologies) and 0.5 units of Taq DNA polymerase (Merk. Bangalore). The reaction were performed in the master cycle gradient 5331 (Eppendorf, Germany) with initial denaturation at 94^0c for 4

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minute followed by 35 cycles at 94^0c for 1 minute, 37^0c for 1 minute, 72^0c for 2 minutes. The final extention step was at 72^0c for 10 minutes. The reactions were held at 4^0c. The PCR products were analysed by gel electrophoresis on 1.5% agarose gel stained with 0.01% ethedium bromide in 1.0 x TBE buffer (89 mM Tris HCl, 89 mM boric acid and 2mM EDTA, pH 8.0). The agarose gel was visualised using transilluminator and photographed on digital documentation system (SYNGENE). One kb λDNA ladder was run with the PCR reaction mixture to read the molecular weight of DNA banding pattern.

RESULTS AND DISCUSSION

A total of 20 oligonucleotide primers were screened to amplify the genomic DNA of male and female individual (Table 1) of which 14 primers showed reproducible results. Out of which primer OPU8 (GGCGAAGGTT) primer showed a unique band of 1000kb which was present only in male plant and absent in female plant (Fig. 1).

Table 1. Sequences or RAPD primers used in the study

primers	Sequence(5'-3')
OPA6	GGTCCCTGAC
OPA7	GAAACGGGTG
OPA8	GTGACGTAGG
OPD18	GAGAGCCAAC
OPD20	ACCCGGTCAC
OPR8	CCCGTTGCCT
OPR9	TGACACGAG
OPR10	CCATTCCCCA
OPS1	CTACTGCGCT
OPS2	CCTCTGACTG
OPS4	CACCCCCTTG
OPS6	GATACCTCGG
OPT5	GGGTTTGGCA
OPT6	CAAGGGCAGA
OPT7	GGCAGGCTGT
OPU8	GGCGAAGGTT
OPU9	CCACATCGGT
OPU11	AGACCCAGAG
OPU13	GGCTGGTTCC
OPU16	CTGCGCTGGA

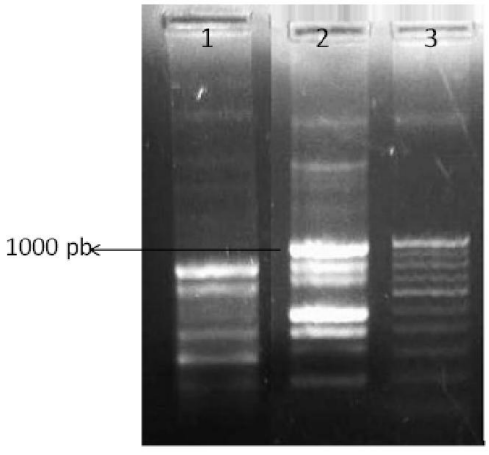

Fig.1. Amplification patterns of male and female individuals with OPU8 primer. Lane1; female banding pattern, Lane 2; male banding pattern, Lane 3; 1kb molecular marker(λ DNA). Arrow indicates unique band of 1000 bp present only in male plant

Here we can say that such a band is linked to sex controlling gene in Simarouba and can be considered as genetic marker for sex determination in *Simarouba species*. In order to examine the application of marker as indicator of sex the OPU8 primer was used to screen the other male plants of Simarouba. The study showed that the marker produced unique bands in all male plants and was absent in female plants. These observations indicate that the marker is closely linked to sex determination. PCR amplification technology is a reliable resource for identification of sex associated markers in dioecious and bisexual plants. RAPD is the one of the cheapest molecular technique used for figure printing of many plants (Williams *et al.,* 1990). Previous study have shown male and hermaphrodite specific primers OPB01 in Carica papaya (Gangopadhyay *et al.,* 2007), OPN16 primer in Commiphora wightii (Sanghamitra *et al.,* 2010), OPA08 primers in *Simarouba glauca* (Prasanthi *et al.,* 2010), OPG05 primer in *Simmondsia chinensis* (Agrawal *et al.,* 2007). OPU10 primer is male specific in *Simarouba gluaca* (Gayatri Vaidya *et al.,* 2014) were the male plant sample was collected from University of Agricultural Sciences Darwar. In the present study plant samples were collected from plantations at University of Agricultural Sciences Bangalore. Thus different agro-climatic conditions may differ the DNA banding patterns in the plant species. The development of sequence characterised amplified regions **(**SCAR) markers could be advantageous because the amplification is less sensitive to reaction condition (Paran and Michelmore, 1993). SCAR were developed from RAPD marker for distinguishing sex specificity (Benerji *et al.,* 1999; Urasaki *et al.,* 2002). The markers obtained could be sequenced to convert into SCAR marker and can be used in marker assisted selection. In the present study we report the RAPD marker OPU8 associated with male plant that was absent in female plant.

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