ASL 1: Towards developing a genetic linkage map of isabgol (*Plantago ovata* Forsk.), a medicinal plant with potent laxative properties

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Abstract

Genetic linkage maps facilitate the genetic dissection of complex traits and comparative analyses of genome structure, as well as molecular breeding in species of economic importance. Isabgol [*Plantago ovata* (Forsk.)], a medicinal plant with potent laxative properties is used in several traditional systems of Medicines and cultivated in India. We explored the DNA sequences of Isabgol in the Genbank (NCBI) and developed over 1500 simple sequence repeats (SSR) markers. Some of them were validated through DNA amplification. Transferability of SSRs from wild *Plantago* species viz., *P. major*, *P. coronopus*, *P. lancelolata*, *P. maritina* and *P. intermida* into *Plantago ovata* was studied. We developed a genetic linkage map using recombinant inbred lines (RILs) population which comprises of 30 random amplified polymorphic DNA (RAPD) markers spreading across 11 linkage groups (PO-1 to PO-11) with a total map distance of 75.6 cM. The SSR markers developed will have applications in assessing the functional diversity, comparative mapping and other applications in isabgol.

Keywords: Isabgol, *Plantago*, Genetic linakge map, simple sequence repeats (SSR) markers, medicinal plant

Introduction

Isabgol also known as Blond psyllium [*Plantago ovata* (Forsk.)] belonging to the family-Plantaginaceae is an important medicinal plant cultivated in India. The husk and seeds are economically important parts used in traditional medicine. Enhancing the yield and quality are the important objectives of isabgol breeding. Attempts to increase the seed yield and husk quality through conventional breeding methods by exploiting natural variation were made. However, not much progress has been realized till today. The lack of clear-cut segregation and small undetectable effects of individual minor genes make it impossible to monitor these traits in traditional breeding programs. However, the advent of molecular markers has facilitated the identification of hidden genetic variability, which can be exploited for the construction of high-resolution genetic linkage maps and detection of QTL governing these traits (Collard et al., 2005). The DNA markers flanking the major QTL can be pyramided into the genetic backgrounds of adaptable and agriculturally desirable genotypes. Nonetheless, a few reports are available on the use of molecular markers including random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and inter-simple sequence repeat (ISSR) for assessment of genetic diversity and population stratification in *Plantago* species (Wolff and Richards, 1998; Squirrell and Wolff, 2001; Koorevaar et al., 2002; Marie and Wolff, 2003; Vahabi et al., 2008; Singh et al., 2009; Vala et al., 2011; Rohilla et al., 2012).

The main objectives of study are to (1) develop Simple sequence repeats (SSR) markers for genomics applications and (2) to develop genetic map using random amplified polymorphic DNA (RAPD) markers.

Materials and Methods

Development of simple sequence repeats (SSR) markers

The raw reads of Isabgol from NCBI-Short Read Archive deposited by Jensen et al. (2013) was used for SSR marker development. The high quality data was assembly using CLC genomics workbench

on default parameters. For identification of SSRs, all the Isabgol transcript contigs were searched with Perl script MISA (http://pgrc.ipk-gatersleben.de/misa/_) with a minimum repeat length of 12bp. Primers were designed using primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) software with the default parameters. The genomic DNA was extracted using the CTAB method as given in Wolff et al. (2009). PCR reactions were set up as described by Wolff et al. (2008). Further, 62 SSR markers reported by SQUIRRELL AND WOLFF (2001); KOOREVAAR et al. (2002); HALE AND WOLFF (2003); Nilsson et al. (2006) and WOLFF et al. (2008) for wild *Plantago* species viz., *P. major, P. coronopus, P. lancelolata, P. maritina* and *P. intermida* were tested for amplification in *Plantago ovata*.

Genetic mapping

The pure seed of DPO-185 and DPO-14 were grown at the research farm of the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand during the year 2010 and the F_1 hybrid seed of DPO-185 x DPO-14 was produced. The F_1 seeds were grown during the year 2011 and F_2 seeds were produced selfing and futher advanced to F_5 through single seed decent method (GOULDEN, 1941). The genomic DNA of parents and F_5 RILs were extracted as above and were amplified using RAPD markers (WOLFF AND MORGAN RICHARDS, 1998). PCR products were scored as describe by REDDY et al. (2014) and was used for linkage map construction. The Kosambi mapping function was used to convert recombination into genetic distance.

Results

Development of SSR markers

A total of 23,586 transcript contigs having more than 200bp size were obtained after the assembly. The details of the assembly statistics are given in Table 1. Analysis using MISA software identified 16,375 simple sequence repeats (SSRs) having more than 12bp motif length in 10,308 transcript contigs (43.7 % of 23,586 transcript contigs), indicating an average frequency of 1 SSR per 1.2 kb. This frequency is consistent to the frequency range of 2.65 to 16.82 % which has been reported in 49 dicot species (KUMPATLA AND MUKHOPADHYAY, 2005). SSR frequency is dependent on several factors such as genome structure, arithmetical method for SSR detection, and the parameters for exploration of microsatellites (TOTH et al., 2000). Of which 352 (2.15 %) were dimers, 2808 (17.15 %) were trimers, 1127 (6.88) were tetramers, 345 (2.11 %) were pentamers and 11743 (71.71 %) were hexamers. This is consistent with the SSRs distributions reported in the transcriptome of soybean (LI et al., 2010) and sorghum (REDDY et al., 2012). Primers were designed for one thousand five hundred SSR markers. Of the 300 markers tested, 280 (93.3 %) markers showed amplification. The majority of SSRs generated high-quality amplicons, suggesting that ESTs are suitable for specific primer design. These results suggest that the assembled transcripts were of high quality and that the SSRs identified in our dataset could be used in the future.

Tab1. Details of the assembly statistics of Isabgol transcriptome

Description	Plantago ovata
Number of transcript contigs	23586
Transcriptome length(bp)	19686649
Max transcript contigs size(bp)	9806
Min transcript contigs size(bp)	200
Mean transcript contigs size(bp)	835
N50 value (bases)	1075

Transferability SSR markers

SSRs derived from the expressed sequence tags (EST-SSRs) are popular markers due to their rapid *in silico* development and high cross-species and genera transferability (REDDY et al., 2011). Inter-

specific transferability of SSRs from wild *Plantago* species viz., *P. major, P. coronopus, P. lancelolata, P. maritina* and *P. intermida* in to *Plantago ovata* was studied. Of the 60 SSRs tested from the wild species, 43 (71.6 %) SSRs showed transferability into *Plantago ovata*. The markers with high Inter-specific transferability will be highly useful for assessing the functional diversity, comparative mapping and other applications.

Genetic mapping

One hundred RAPD markers (OPA1-OPA20, OPB1-OPB20, OPC1-OPC20, OPD1-OPD20 and OPE1-OPE19) were tested for parental polymorphism between mapping parents (DPO-185 and DPO-14). Eighteen RAPD markers were found polymorphic and subjected to genotyping of 160 F_5 DPO-185 and DPO-14 recombinemnet inbred lines. The genetic map comprises of 33 markers spreading across 12 linkage groups (PO-1 to PO-12) (Figure 1.) with a total map distance of 13.7 cM. The linkage groups PO-06 and PO-10 were having maximum distance. The staturation of present may with more marker is being carried out.The genetic linkage map will have the potential to facilitate the genetic dissection of complex traits and comparative analyses of genome structure, as well as molecular breeding efforts in species of agronomic importance in isabgol.

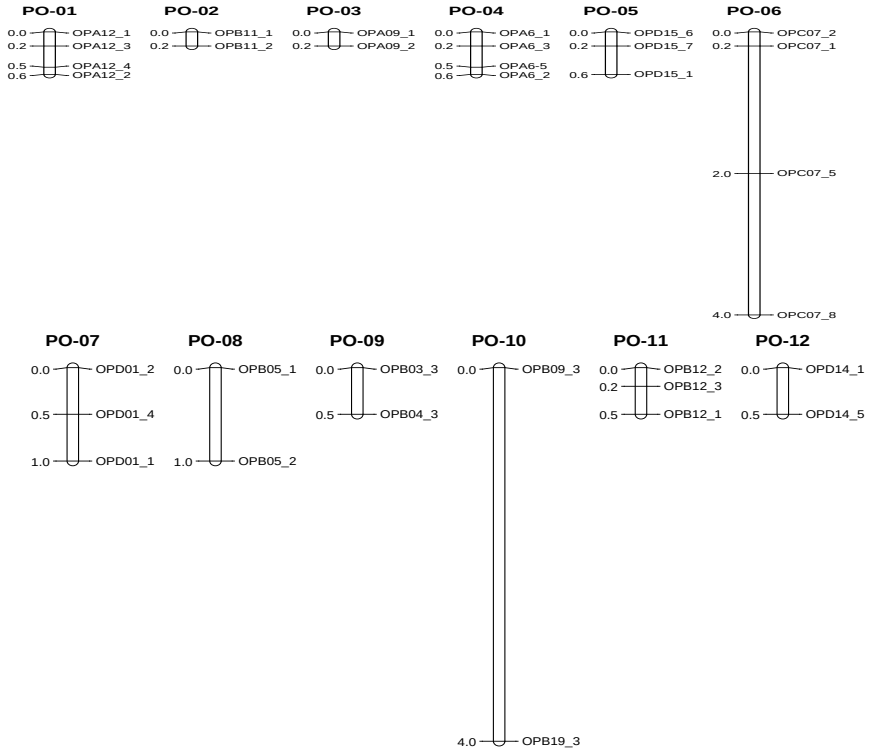

Fig1. Genetic linkage map of Isabgol (*Plantago ovata*) with 33 RAPD markers.

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