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Conservation Genetics Resources
https://doi.org/10.1007/s12686-017-0928-5

TECHNICAL NOTE

Development of a set of SNP markers for population genetics studies of Ipe (*Handroanthus* sp.), a valuable tree genus from Latin America

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Received: 9 November 2017 / Accepted: 16 November 2017

Abstract

A combination of restriction associated DNA sequencing (RADSeq) and low coverage MiSeq genome sequencing was used for the development of single nucleotide polymorphisms (SNP) and INDEL (insertion/deletions) genetic markers for Ipe (*Handroanthus* sp.). Of the 402 putative loci identified, 389 SNPs and INDELs (315 nuclear SPNs, six chloroplast INDELs, 15 chloroplast SNPs, 12 mitochondrial INDELs and 41 mitochondrial SNPs) were successfully genotyped at 93 individuals from Brazil, Bolivia and French Guiana using a MassARRAY® iPLEX™ platform. This set of markers will be invaluable for population genetics, phylogeography and DNA fingerprinting studies.

Keywords *Handroanthus* sp. · Single nucleotide polymorphism · MassARRAY

Introduction

The genus *Handroanthus* comprises 30 tree species, belongs to the botanical family Bignoniacaea and presents one of the most valuable timber from South America, which is threatened because of its overexploitation (Schulze et al. 2008). This genus has a complex and long taxonomic history due to its anatomical and morphological variety. Grose and Olmstead (2007) reclassified part of the *Tabebuia* species under the *Handroanthus* genus, as a result of molecular and phylogenetic studies using sequences of the chloroplast regions trnL-F and ndhF. Of the 21 microsatellite loci developed for *Tabebuia aurea*, nine loci were successfully amplified in four other *Handroanthus* species (Braga et al. 2007). However, the amplification and scoring of microsatellite loci from timber material is problematic due to the low quality and quantity of DNA which can be recovered, which motived the development of SNP markers for timber tracking purposes in other economically important tropical species (Blanc-Jolivet et al. 2017a, b; Jardine et al. 2016; Pakull et al. 2016). Here we therefore describe a set of SNP markers for genetic studies in *Handroanthus* sp. We present a set of 101 SNP/Indel markers which were analyzed using MassARRAY® iPLEX™ genotyping (Agena Bioscience, Hamburg, Germany), which can be used for effective analysis of molecular population structure within the genus.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12686-017-0928-5) contains supplementary material, which is available to authorized users.

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Published online: 28 November 2017

Materials and methods

Marker development and marker screening

Dried *Handroanthus* sp. tissue material (leaf, cambium or bark) were collected from 28 trees in Bolivia, 50 trees in Brazil and 15 trees in French Guiana, totalling 93 individuals (Table 1). DNA was isolated from leaf, cambium or bark according to Dumolin et al. (1995), either at the Thünen Institute (Germany) or at the São Paulo State University (UNESP). Among the collected individual samples, five individuals confidently identified as *H. serratifolius* were selected for SNP/INDELs locus identification (Table 2). Restriction associated DNA sequencing (RADseq) was used for nuclear SNPs (nSNPs) discovery (Miller et al. 2007) and low coverage MiSeq genome sequencing was conducted for the detection of chloroplast and mitochondrial SNPs (cpSNPs and mtSNPs) and INDELs (Straub et al. 2012). The suitability of all identified genetic makers for a MassARRAY® iPLEX™ design (Assay Design Suite v2.0 [Agena Bioscience™, San Diego, USA]) was evaluated according to sequencing data quality and different allele distribution patterns among the five individuals. Once a set of SNPs/INDELs markers was identified, genotyping of all individuals was conducted using the MassARRAY® iPLEX™ platform (Agena Bioscience™, San Diego, USA) and the iPLEX™ GOLD chemistry (Table 1). Allele calling was processed with Typer Viewer v.4.0.24.71 (Agena Bioscience™, San Diego, USA).

Table 1 Country, species, number of individuals (*n*) and geographic coordinates of individuals used for the initial SNP selection on the MassARRAY® iPLEX™ platform (Agena Bioscience™)

Country	Location	Species	*n*	Latitude	Longitude
Bolivia	Bajo Paraguá	*H. serratifolius*	1	−15.4182	−61.4443
Bolivia	Bajo Paraguá	*Handroanthus* sp.	2	−16.2612	−61.0780
Bolivia	Bajo Paraguá	*H. impetiginosus*	2	−16.2564	−61.0724
Bolivia	Concepción	*H. impetiginosus*	5	−16.1674	−61.7679
Bolivia	Guarayos	*Handroanthus* sp.	2	−15.5183	−63.1966
Bolivia	Guarayos	*H. serratifolius*	3	−15.5125	−63.1974
Bolivia	Roboré	*Handroanthus* sp.	1	−18.3275	−59.5904
Bolivia	Roboré	*H. impetiginosus*	4	−18.4522	−59.4169
Bolivia	Rurrenabaque, El Paraiso	*Handroanthus* sp.	1	−14.5667	−67.3033
Bolivia	Yapacaní, Naranjal	*H. serratifolius*	7	−17.4293	−63.9151
Brazil	FLONA do Jamari	*H. incanus*	5	−9.3965	−62.9226
Brazil	FLONA do Jamari	*H. impetiginosus*	5	−9.3985	−62.9248
Brazil	FLONA Amapá	*Handroanthus* sp.	6	0.8668	−51.6012
Brazil	FLONA de Tapajós	*H. serratifolius*	8	−2.8641	−54.9269
Brazil	FLONA do Carajás	*Handroanthus* sp.	13	−6.1971	−49.0848
Brazil	RESEX Chico Mendes	*Handroanthus* sp.	3	−10.7751	−69.6563
Brazil	RESEX Tapajós - Arapins	*Handroanthus* sp.	5	−3.0783	−55.2841
Brazil	Piracicaba, Brazil	*H. rosea albus*	1	−22.725	−47.6476
Brazil	Piracicaba, Brazil	*H. ochraceus*	1	−22.725	−47.6476
Brazil	Piracicaba, Brazil	*H. heptaphyllus*	1	−22.725	−47.6476
Brazil	Piracicaba, Brazil	*H. chrysotrichus*	1	−22.725	−47.6476
Brazil	Piracicaba, Brazil	*H. impetiginosus*	1	−22.725	−47.6476
French Guiana	Belizon	*Handroanthus* sp.	5	4.2089	−52.5480
French Guiana	Paracou	*H. serratifolius*	2	5.2601	−52.9361
French Guiana	Kourou-Cayenne	*H. serratifolius*	8	5.0020	−52.4808

Table 2 Location and number of individuals used for SNP discovery from both RADseq and MiSeq sequencing approaches

Sample code	Sequencing technique	Country	Location	Latitude	Longitude
TASER_1	MiSeq	French Guiana	Paracou	5.26024	−52.93566
TASER_2	RADseq	French Guiana	Paracou	5.26001	−52.93647
TASER_40	MiSeq/RADseq	Bolivia	Yapacaní, Naranjal	−17.43223	−63.92747
TASER_63	MiSeq/RADseq	French Guiana	Kourou-Cayenne	5.06545	−52.55594
TASER_66	MiSeq	French Guiana	Kourou-Cayenne	4.98347	−52.44759

Statistical analysis

Amplification rates were determined per locus over all 93 samples. Using the plastidial markers, the total number of haplotypes were estimated per country. *H. serratifolius* samples were pooled together according to their country of origin for further analysis. Genetic diversity was estimated as the observed (H_o) and expected heterozygosity (H_e) for the nuclear markers. Inbreeding was inferred by the fixation index (F_{IS}) and its statistical significance was estimated by random permutations of alleles among individuals and using a Bonferroni correction ($\alpha = 0.05$). All analyses were carried out using FSTAT software (Goudet 2002).

Results

The RADseq and MiSeq sequencing resulted in a set of 402 SNPs/Indels markers (Table S1): 15 cpSNPs, 44 mtSNPs, 325 nSNPs, and 12 mitochondrial and six chloroplast INDEL markers. A total of 389 SNPs and INDELs loci could be successfully amplified and scored. The mean amplification rate per locus was 88% for nSNPs, 95% for chloroplast and mitochondrial markers among countries and the seven *Handroanthus* species analysed (Tables S2 and S3). The mean fixation index of nSNPs ranged among countries from 0.008 to 0.678, indicating a significant homozygote excess (Table S2). Twenty-one haplotypes were detected among Bolivian samples, 46 among Brazilian samples and nine among French Guianese samples.

Conclusion

The SNPs and INDELs markers presented high amplification and cross-amplification rates. This new SNP marker set will be useful for future studies on genetic diversity and phylogeography within the genus *Handroanthus*.

Acknowledgements This research was supported by the German Federal Ministry of Food and Agriculture in the frame of the "Large scale project on genetic timber verification". Genotyping was performed at the Genomic and Sequencing Facility of Bordeaux (grants from the Conseil Regional d'Aquitaine Nos 20030304002FA and 20040305003FA, the European Union, FEDER No 2003227 and Investissements d'avenir, Nos ANR-10-EQPX-16-01 and CEBA: ANR-10-LABX-25-01 to the UMR EcoFoG). We would like to thank the Conselho Nacional de Ciencia e Tecnologia (CAPES) (Mater scholarship at the UNESP/FEIS to B.R.V.M-S), the Chico Mendes Biodiversity Institute (authorizations Nos 47960 and 49369 for sample transportation), FLONA do Jamari, AMATA Company, FLONA Amapá, FLONA de Tapajós, FLONA do Carajás, RESEX Chico Mendes, RESEX Tapajós - Arapins in Brazil, the Museo de Historia Natural Noel Kempff Mercado in Bolivia, as well as the Office National des Forêts in French Guiana for their support in the field.

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