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Cross-species amplification of eucalyptus SSR markers in Casuarinaceae

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Cross-species amplification of Simple Sequence Repeats (SSRs) loci is considered as a cost-effective approach for developing microsatellite markers for new species. We examined the transferability of eucalyptus SSR loci to the economically important members of the Casuarinaceae family. Ten primer pairs targeting (GA)n, (CTT)n, (TGA)n and (GAA)n motifs were screened in *Allocasuarina littoralis, A. luehmannii, Casuarina glauca* and *C. equisetifolia.* Up to 30% of the eucalyptus primer pairs amplified SSRs within Casuarinaceae. Nevertheless, in *Allocasuarina* no amplification products were observed. In *Casuarina* species, all the locus specific products were monomorphic while few nonspecific bands also amplified.

Key words: Microsatellite, markers, sequence, transferability, eucalyptus, *Casuarina*, *Allocasuarina*

Introduction

Microsatellites or Simple Sequence Repeats (SSRs) are the genetic markers of choice for plant and animal systems due to their abundance, co-dominance, hypervariablity, and transportability between species (ROEDER et al. 1995, POWELL et al. 1996, GUPTA and VARSHNEY 2000). In forestry tree species, SSRs are found to be powerful markers for genetic mapping, diversity analysis and genotyping (DAYANANDAN et al. 1997, BRONDANI et al. 1998, BUTCHER et al. 2000). Development of a microsatellite marker system for a new species requires isolation, cloning, sequencing, and characterization of microsatellite loci. Several procedures are available for the enrichment of microsatellites in a genomic library to improve the efficiency of microsatellite isolation (ZANE et al. 2002). The development of microsatellite markers through the laboratory based screening of SSR libraries is highly time consuming and expensive. Recently, publicly available collections of Expressed Sequence Tags (ESTs) were analyzed and utilized for SSR development (KANTETY et al. 2002). However, this approach is applicable only for well-studied species where abundant sequence data is available. An alternative approach that could be used in a genome with no or little DNA sequence information is the sourcing of SSR primers developed for other spe-

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cies. This approach offers a potential for low cost development of SSR markers for species with very little or no information on sequence, through the screening of primers from different sources. The level of polymorphism detected by such markers is yet to be fully tested. Potential transferability of SSR primers across species of the same genus (CIPRIANI et al. 1999, BUTCHER et al. 2000) and across genera of the same family was reported in Leguminaceae (PEAKALL et al. 1998), Myrtaceae (ZUCCHI et al. 2002) and Fagaceae (ALDRICH et al. 2003). ROSSETTO (2001) reviewed the status of transferability of SSR markers to related taxa including many tree species. Eucalyptus SSR primer pairs used for *Eugenia dysenterica* were found to be highly sensitive for the detection of population structure, genetic diversity and gene flow estimation (ZUCCHI et al. 2003). In *Quercus*, the cross-species amplification provided the basis for the construction of a phylogenetic relationship between the species and its wild relatives (ALDRICH et al. 2003). Markers developed by this approach present a valuable resource for subsequent comparison between related species. In the present study, an attempt was made to explore the transferability of eucalyptus SSR primers to an unrelated taxa belonging to the family *Casuarinaceae*. Species like *Casuarina equisetifolia, C. glauca, Allocasuarina littoralis* and *A. luehmannii* are some of the economically important species widely grown in the tropical regions. Systematic tree breeding activities are in progress particularly for *C. equisetifolia,* an industrial plantation species for pulp, paper and rayon production.

Materials and Methods

Plant material and DNA isolation

Seeds for the species of the genus *Allocasuarina* and *Casuarina* used in this study (Tab. 1) were obtained from Australian Tree Seed Center (ATSC), CSIRO, Australia. Seedlings were raised and field planted. The extreme growing tips of the needles were collected from one-year-old individuals, quickly washed in double distilled water and dried on paper towel for 1–2 min and stored at –20 °C until use. DNA was isolated from five different plants for each species using Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) and individual PCR amplifications were carried out for each DNA sample.

SSR-PCR amplifications

Ten eucalyptus SSR primer pairs developed by CSIRO, Division of forestry and forest products, Canberra, Australia (http://www.ffp.csiro.au/tigr/molecular/eucmsps.html) targeting di- and tri- nucleotide repeats (Tab. 2) were selected for cross-species amplification of casuarina species.

PCR reactions were set up in a 25 µl reaction volume containing 50 ng of template DNA, 1.0 µM of each primer (forward and reverse primer), 100 µM of each dNTP, 1.5 mM $MgCl_2$, 50mM KCL, 20mM Tris-HCl pH 8.8, and 1 U of Taq polymerase. PCR was performed on an MJ Research DNA engine thermal cycler with an initial denaturation at 94 °C for 5 min, followed by 45 cycles at 94 °C for 1min, 56 °C or 50 °C for 1 min, 72 °C for 2 min, and the last step for extension at 72 °C for 7 min. A control reaction was included in the experiment with DNA isolated from *Eucalyptus nitens* and PCR amplification condition was same as described above except for the annealing temperature of 56 °C. The am-

plified fragments were separated in 8% acrylamide gel of size (10× 7 cm) in a run with 1× TBE at 100v for 1h, and stained with Electran silver staining kit (BDH Laboratory Supplies, Poole, UK) following the manufacturer's protocol. Amplification products were scored as positive only if a sharp and reproducible band was observed.

Tab. 1. List of Casuarinaceae members used for SSR

Name of the Species	Chromosome number (n)	CSIRO Seed lot number	Origin
Casuarina equisetifolia L.	9	19129	Lakei/sibur Bako, Malaysia
Casuarina glauca Sieb. Ex Spreng	9	15941	Burrum Heads QLD
Allocasuarin littoralis (Salisb.) L. Johnson	11	13876	Gordan and Chilicks QLD
Allocasuarina luehmannii (R. Baker) L. Johnson	14	13880	Mt. Molloy Mareeba QLD

Tab. 2. Eucalyptus primer sequences used in cross-species amplification of Casuarinaceae members

Locus	Primer 1(5'-3')	Primer 2 (5'-3')	Repeat size
En 6	GAGCTGGAAATGGAGCAGAC	TCAATTTTTGCCTCTCCCC	(GA)15
En 10	ATCAAATGGCTTTAGCTTTGTG	CCCAGAGACAAACCGCTC	(GA)10
En 12	CAGAACCCAGCGGAGGA	GGAAACGCCAATGTAGCTCT	(GA)15
En 13	GCCAAATTGATGGTAGGCAT	CCAGCAAATTCAAATTCACA	(GA)17
Eg 24	CGACGTCACAGTTATGTGGG	CCTGAGCTTTTGAATACGGG	(CT)10
Eg 8	AGGTTTATGAGATCAACCCAGG	GGTCGTTTATTCCTTCTCACTCTG	(CTT)8
Eg 16	AGCTTTCTTGATGTGGGACG	TCATGTAATCTGCTGAGGCG	(GA)2 (TGA)2
Eg 76	CGAGGACTCTTCCAGCTTTG	CACCAAAATCTGCACTGCC	(CTT)17
Eg 117	CGCTCAGTCTTTCTCAGTGG	CACCCTTCGCTGAGTTTCTC	(GAA)19
Eg 128	TTTTGGAGGTAGATGAGGGG	CCATGAAAACCAGCAAAATATG	(CTT)13

Results

Patterns of cross-species amplification in plants across genera are beginning to emerge as a source for developing SSRs in lesser-studied species, although only few studies that systematically explore microsatellite transferability beyond closely related genera are available. ROSSETTO (2001) reviewed cross-species transferability in several plant species. Most of the authors showed the possibility of using SSR primers to amplify heterology with members belonging to the same family (PEAKALL et al. 1998). In the present study, when all the primers were amplified with the annealing temperature of 56 °C, stutter bands were produced and therefore the annealing temperature was reduced to 50 °C. Such modifi-

cations in the PCR protocols especially annealing temperature are recommended for the successful transfer of microsatellite primer pairs between species (ROSOTTO 2001). Out of the four microsatellite primer pairs targeted to amplify $(GA)_n$ motifs, three primer pairs amplified one or both the species of *Casuarina* studied (Tab. 3). The remaining six primers targeted to amplify $(CT)_n$, $(CTT)_n$, $(GAA)_n$ and $[(GA)_n(TGA)_n]$ motifs did not show any band on 8% PAGE. In both the species of *Allocasuarina*, no banding profiles were observed with all the ten primer combinations. Three loci (En6, En12 and En13) amplified in all the individuals of *C. glauca* and only one locus amplified in *C. equisetifolia* (En6) (Fig. 1). The bands were sharp with high intensity and no stuttering was observed. The putative SSR loci of all the four primer combinations were monomorphic within the species but allelic variation was observed between the species. Moreover non-specific amplification products were observed in few of the individuals.

Tab. 3. Potential cross-species amplification of three eucalyptus SSR loci in *Casuarina* species

Locus	Allelic Size (bp)	
	C. glauca	*C. equisetifolia*
En 6	102	94
En 10	–	–
En 12	102	–
En 13	100	–
Eg 24	–	–
Eg 8	–	–
Eg 16	–	–
Eg 76	–	–
Eg 117	–	–
Eg 128	–	–

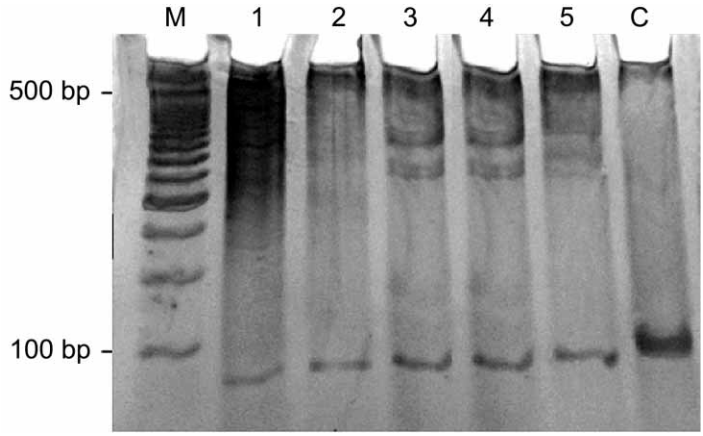

Fig. 1. Transferability of eucalyptus SSR targeted for the loci En6 across *C.equisetifolia*. M-Molecular weight marker; Lane 1–5 – five individuals of *C.equisetifolia*; C- *E.nitens*

Discussion

In plant genome, $(GA)_n$ microsatellites are in high abundance compared to other dinucleotide SSR as reported in *Oryza, Aegilops, Arabidopsis* or *Brassica* (GUPTA and VARSHNEY 2000; GUYOMARC et al. 2002; SUWABE et al. 2002; UZUNOVA and ECKE, 1999) and they are well distributed throughout the genome, thus ensuring good genome coverage. HILLE et al (2002) reported that monomorphic loci observed in cross amplifications could be due to null heterozygous condition.

Transferability of eucalyptus SSR markers across two species of *Casuarina* was confirmed in this study. Eucalyptus SSRs targeted for (En 6, En12 and En13) amplified across all the individuals of *C. glauca* and En 6 amplified in *C. equisetifolia*. This indicated that the sequences flanking the microsatellite regions in eucalyptus are highly conserved across genera. Though the studies on transferability of SSRs indicated low success rate among plant species, some sequences are associated with highly conserved regions (PEAKALL 1998; DIRLEWANGER et al. (2002) reported amplification of *Prunus persica* (Rosaceae) SSR in *Castanea sativa* (Fagaceae). Recently, ALDRICH et al (2003) showed the successful amplification of *Quercus rubra* SSR loci in *Castanea mollissima*, a distantly related species of Fagaceae. Similarly, eucalyptus SSRs amplified across its unrelated members belonging to the family Casuarinaceae. Hence, screening of SSR markers from different genera can lead to the development of SSR loci in *Casuarina* species.

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References

ALDRICH, P. R., JAGTAP, M., MICHLER, C. H., ROMERO-SEVERSON, J., 2003: Amplification of North American red oak microsatellite markers in European white oaks and Chinese chestnut. Silvae Genet. 52, 176–179.

BRONDANI, R. P. V., BRONDANI, C., TARCHINI, R., GRATTAPAGLIA, D., 1998: Development, characterization and mapping of microsatellite markers in *Eucalypts grandis* and *E. urophylla*. Theor. Appl. Genet. 97, 816–827.

BUTCHER, P. A., DECROOCQ, S., GRAY, Y., MORAN, G. F., 2000: Development, inheritance and cross-species amplification of microsatellite markers from *Acacia mangium*. Theor. Appl. Genet. 101, 1282–1290.

CIPRIANI, G., LOT, G., HUANG, W. G., MARRAZZO, M. T., PETERLUNGER, E., TESTOLIN, R., 1999: AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterization and cross-species amplification in *Prunus*. Theor. Appl. Genet. 99, 65–72.

DAYANANDAN, S., BAWA, K. S., KESSELI, R., 1997: Conservation of microsatellites among tropical trees (Leguminosae). Am. J. Bot. 84,
1658–1663.

DIRLEWANGER, E., COSSON, P., TAVAUD, M., ARANZANA, M. J., POIZAT, C., ZANETTO, A., ARUS, P., LAIGRET, F., 2002: Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor. Appl. Genet. 105, 127–138.

GUPTA, P. K., VARSHNEY, R. K., 2000: The development and use of microsatellite markers for genetic analysis and plant breeding with special emphasis on bread wheat. Euphytica 113, 163–185.

GUYOMARC, H., SOURDILLE, P., CHARMET, G., EDWARDS, K. J., BERNARD, M., 2002: Characterization of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor. Appl. Genet. 104,1164–1172.

HILLE, A., JANSSEN, I. A. W., MENKEN, S. B. J., SCHLEGEL, M., THORPE, R. S., 2002: Heterologous amplification of microsatellite markers from colubroid snakes in European Natricines (Serpentes: Natricinae). J. Hered. 93, 63–66.

KANTETY, R. V., ROTA, M. L., MATTHEWS, D. E., SORRELLS, M. E., 2002: Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. Plant Mol. Biol. 48, 501–510.

PEAKALL, R., GILMORE, S., KEYS, W., MORGANTE, M., RAFALSKI, A., 1998: Cross-species amplification of Soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: Implications for the transferability of SSRs in plants. Mol. Boil. Evol. 15, 1275–1287.

POWELL, W., MORGANTE, M., DOYLE, J. J., MCMICOL, J. W., TINGEY, S. V., RAFALSKI, A. J., 1996: Gene pool variation in genus *Glycine* subgenus *Soja* revealed by polymorphic nuclear and chloroplast microsatellites. Genetics 144, 793– 803.

ROEDER, M. S., PLASCHKE, J., KONIG, S. U., BORNER, A., SORRELLS, M. E., TANKSLEY, S. D., GANAL, M. W., 1995: Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246, 327–333.

ROSSETTO, M., 2001: Sourcing of SSR Markers from related plant species. In: HENRY, R. J. (ed), Plant genotyping: The DNA fingerprinting of plants, 210–224. CAB International, Oxford.

SUWABE, K., IKETANI, H., NUMONE, T., KAGE, T., HIRAI, M., 2002: Isolation and characterization of microsatellites in *Bassica rapa* L. Theor. Appl. Genet. 104,1092–1098.

UZUNOVA, M. I., ECKE, W., 1999: Abundance, polymorphism and genetic mapping of microsatellites in oilseed rape (*Brassica napus* L.). Plant Breeding 118, 323–326.

ZANE, L., BARGELLONI, L., PATARNELLO, T., 2002: Strategies for microsatellite isolation: a review. Mol. Ecol. 11,1–16.

ZUCCHI, M. I., BRONDANI, R. P. V., PINHEIRO, J. B., BRONDANI, C., VENCOVSKY, R., 2002: Transferability of microsatellite markers from Eucalyptus spp. to *Eugenia dysenterica* (Myrtaceae family). Mol. Ecol. Notes 2, 512.

ZUCCHI, M. I., BRONDANI, R. P. V., PINHEIRO, J. B., 2003: Genetic structure and gene flow in *Eugenia dysenterica* DC in the Brazilian Cerrado utilizing SSR markers. Genet. Mol. Biol. 26, 449–457.