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Short Communication. Transferability of microsatellite markers located in candidate genes for wood properties between *Eucalyptus* species

Cintia V. Acuña[1], Pamela Villalba[1], H. Esteban Hopp[1, 2] and Susana N. Marcucci Poltri[1]*

[1] *Instituto de Biotecnología. CICVyA. Instituto Nacional de Tecnología Agropecuaria (INTA Castelar). Castelar, Argentina.* [2] *Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Buenos Aires, Argentina*

Abstract

Aim of study: To analyze the feasibility of extrapolating conclusions on wood quality genetic control between different *Eucalyptus* species, particularly from species with better genomic information, to those less characterized. For this purpose, the first step is to analyze the conservation and cross-transferability of microsatellites markers (SSRs) located in candidate genes.

Area of study: *Eucalyptus* species implanted in Argentina coming from different Australian origins.

Materials and methods: Twelve validated and polymorphic SSRs in candidate genes (SSR-CGs) for wood quality in *E. globulus* were selected for cross species amplification in six species: *E. grandis, E. saligna, E. dunnii, E. viminalis, E. camaldulensis* and *E. tereticornis*.

Main results: High cross-species transferability (92% to 100%) was found for the 12 polymorphic SSRs detected in *E. globulus*. These markers revealed allelic diversity in nine important candidate genes: cinnamoyl CoA reductase (CCR), cellulose synthase 3 (CesA3), the transcription factor LIM1, homocysteine S-methyltransferase (HMT), shikimate kinase (SK), xyloglucan endotransglycosylase 2 (XTH2), glutathione S-transferase (GST), glutamate decarboxylase (GAD) and peroxidase (PER).

Research highlights: The markers described are potentially suitable for comparative QTL mapping, molecular marker assisted breeding (MAB) and for population genetic studies across different species within the subgenus *Symphyomyrtus*.

Key words: validation; cross-transferability; SSR; functional markers; eucalypts; *Symphyomyrtus*.

Introduction

Recently, the increase of public genomic sequences and Expressed Sequence Tag (EST) data has allowed an effective approach for the identification of functional polymorphic DNA markers, such as microsatellites (SSRs). The high allelic diversity and abundance of SSRs in the forest tree species make these co-dominant molecular markers particularly useful for genetic mapping, diversity analysis and genotyping (Brondani *et al.,* 2002; Cupertino *et al.,* 2011). However, genomic sequence characterization including molecular marker development and application has been mainly studied in few *Eucalyptus* species. In accordance with the expected sequence conservation of transcribed regions of the genome, a significant portion of the primer pairs designed on SSR flanking regions is expected to possess a high potential for cross-transferability in related species. Furthermore, transferability of SSR markers across species of *Symphyomyrtus* has been proved (Glaubitz *et al.,* 2001; Brondani *et al.,* 2002; Faria *et al.,* 2010, 2011), even in the case of the more distantly related *Corymbia* subgenera (Jones *et al*., 2001).

Many genomic studies have reported the analysis of genes expressed during wood formation and xylogenesis (reviewed by Foucart *et al.,* 2006) and some important metabolic pathways are now well known. In *Eucalyptus,* several structural and regulatory candidate genes (CGs) involved in lignin biosynthesis were also identified (Paux *et al*., 2004). Among these genes, there are those encoding components of the common phenylpropanoid pathway, those of the monolignol specific

* Corresponding author: marcuccipoltri.s@inta.gob.ar
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pathway as well as lignin regulatory genes (Paux *et al*., 2004). However, only few studies in most widely cultivated species of *Eucalyptus* have analyzed CGs underlying wood quality traits using QTL approaches (Freeman *et al.,* 2009; Thumma *et al.,* 2010; Gion *et al.,* 2000, 2011). The use of already well-established variable markers present in CGs for wood quality is an interesting approach for mapping purposes and functional diversity studies. In addition, they may be particularly useful to demonstrate if a given QTL trait such as wood quality is governed by the same genes in different species (comparative QTL or association mapping). Furthermore, cross species amplification of SSR-CGs loci is considered a cost-effective approach for developing microsatellite markers for new species with no or little DNA sequence information.

The present study reports the cross-transferability analysis of SSR-CG for wood quality between *Eucalyptus* species.

Material and methods

Plant material and DNA extraction

Eight trees of *E. globulus* were used to check reliability amplification and polymorphic status of the 21 selected primers from Acuña *et al*. (2012a) (Online resource 1).

A total of 44 nonrelated individuals of six species of *Eucalyptus* from the subgenus *Symphyomyrtus* were sampled for the transferability analyses. These individuals came from three sections: *E. grandis*, *E. saligna* (section *Latoangulatae*); *E. dunnii*, *E. viminalis* (section *Maidenaria*); and *E. camaldulensis*, *E. tereticornis* (section *Exsertaria*)

Total DNA was extracted from young leaves using the CTAB method with modifications as described in Marcucci Poltri *et al.* (2003).

Microsatellite selection and PCR amplification

Microsatellites within CGs were selected from previous studies (Acuña *et al*., 2012a,b).

For validation and transferability studies, 21 SSR were identified in 19 CGs for wood quality (Acuña *et al*. 2012a, Online resource 1). The selection was made from the function assignment (BLASTX). To assess the putative localization of these loci, we performed a sequence BLASTN search (E-value ≤ 1e – 3) against the *Eucalyptus grandis* genome database (version 1.1 available in Phytozome 9.1, http://www.phytozome.net/eucalyptus.php).

In addition, 8 polymorphic SSR in 7 CGs were incorporated in the transferability study (Acuña *et al*., 2012b).

Primers were designed to amplify these 21 regions. PCR conditions were carried out in a final volume of 12 µl with 20 ng of genomic DNA, 0.25 µM of each primer (Alpha DNA, Canada), 2mM $MgCl_2$, 0.2 mM of each dNTP, 1X reaction buffer and 1U Platinum Taq polymerase (Invitrogen). A final concentration of 3 mM $MgCl_2$ was used for the transferability analyses.

Amplifications were performed following a denaturation step of 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min. at annealing temperature and 1 min at 72°C. The final extension step was of 10 min at 72°C. Details of primers sequences, SSR location, amplification conditions, and product sizes are described in Table 1. Fluorescent dye-labeled forward primers (Alpha DNA, Canada) were used and fragments were separated on an ABI3100 Genetic Analyzer (Applied Biosystems, USA). Allele assignments were made by size comparison with the standard allelic ladders, using the GenMapper ID software provided by Applied Biosystems (USA).

Genetic Analyses

The number and frequency of alleles, as well as the observed heterozygosity (H_o), were determined using the GenAlEx 6.4 program (Peakall & Smouse, 2006).

The polymorphism information content (PIC) of each marker was calculated according to Botstein *et al*. (1980).

Shared Allele Distance (DAS), defined as one minus half the average number of shared alleles per locus, was implemented between individuals using the software POPULATIONS 1.2.28 (Langella, 2002).

Cluster analysis was implemented based on distance matrices using the unweighted pair group method arithmetic average (UPGMA), and the corresponding phenogram was constructed.

Results and discussion

From the 21 SSR-CGs involved in phenylpropanoid biosynthesis, cellulose biosynthetic process and me-

Table 1. Description of validated SSR-CG for wood quality in *E. globulus*. Table includes primer names, putative homology BLASTX, sequence name, *Eucalyptus grandis* chromosome scaffold and position (bp), motif and number of repeat within the sequence, SSR location forward (F) and reverse (R), primers annealing temperature (AT), observed product size (bp) and polymorphism

Primer name	Sequence description	Sequence name	Scaffold	Motif	Primers F and R (5′-3′)	AT	Amplicon size	Polymorphism
Cellulose biosynthesis process								
CesA5	cellulose synthase 5	ES593659	Scaffold 3 53,198,448- 53,198,558	$(tat)_4$	F:TTCACAGTTGGCTCCTGTTG R:ACTGTTAAATGCCGGACTCG	60-50	292	monomorphic
PME	pectin methylesterase	ES594767	Scaffold 8 13,309,313- 13,308,591	$(cct)_6$	F:GCTCAAACCAGAAAACCGAG R:GAGTGCCTTGGCAGGTAGAG	60-50	159	monomorphic
Phenylpropanoid biosynthesis								
PAL	phenylalanine ammonia-lyase	ES591880	Scaffold 7 46,739,431- 46,739,980	$(cgct)_3$	F:GGGACTTGACAGTTTCGGTC R:GCCTGAGAAGAGGAGTCGAA	60-50	147	monomorphic
COMT	caffeic acid o-methyltransferase	Contig675	Scaffold 11 870,379-870,805	$(gagag)_3$	F:TCTCGCGGAAGAGAGAGAAG R:GCCATGATCTCGAGGAGGT	60-50	184	monomorphic
PER	peroxidase atp19a	ES588966	Scaffold 3 52,638,684- 52,639,176	$(attt)_3$	F:CGTTTCGTGTGCTGACATCT R:ATGATACGTGTGGCCAATCA	60-50	262-270	polymorphic
Methionine metabolism								
HMTe	homocysteine S-methyltransferase	ES588789	Scaffold 8 50,171,470- 50,171,798	$(ctc)_5$	F:TTCTTCCGCGTACAAAATCC R:GGGTGAAGTGATGAGGCACT	60-50	229	polymorphic
Pentose phosphate pathway								
SMT	24-sterol c-methyltransferase (SMT1)	Contig334	Scaffold 7 51,714,466- 51,714,397	$(ct)_7$	F:GATTCCCGGGATCTTTGACT R:ACGTCGCTCTTGTCCATCTT	60-50	172	monomorphic
Plant development and stress responses								
GAD_a	glutamate decarboxylase	Contig557	Scaffold 8 8,127,112- 8,126,841	$(caag)_3$	F:TAAAGCGAAAGCAAAACCGT R:AATTGCGGACGTATCTGGAG	60-50	248-257	polymorphic
GAD_b	glutamate decarboxylase	Contig557	Scaffold 8 8,127,112- 8,126,841	$(aaga)_3$	F:AAAGCCAACTTCCCCTGTCT R:AGAATTGCGGACGTATCTGG	60-50	175-189	polymorphic

thionine metabolism that has not been previously validated, nine markers (43%) generated reproducible and reliable amplicon patterns in the *E. globulus* sample tested. From these nine markers, five markers were monomorphic and four polymorphic in this small sample. Furthermore, these loci were physically aligned to a unique position on the chromosome scaffolds 3, 7, 8 and 11 (Table 1). The polymorphism rate was similar to those described by Torales *et al*. (2012), in *Nothofagus* (19%) but lower than those described by Faria *et al*. (2011), (39%) and Acuña *et al*. (2012a), (46%) in *Eucalyptus*.

In this work, we also assessed the cross transferability of 12 SSR-CG by using new and previously validated primers: four polymorphic primers validated here and other eight primers previously validated in Acuña *et al*., (2012b). A high cross transferability (92% to 100%) was found in *E. grandis*, *E. saligna, E. dunnii*, *E. viminalis, E. tereticornis* and *E. camaldulensis*. As the primers were designed from genic regions, it is reasonable that they have a high potential for cross-transferability in related species. In fact, in a recent study, similar cross-species transferability of SSR markers was already reported for species of the subgenus *Symphyomyrtus* (Zhou *et al*., 2013).

Nine out of 12 loci showed polymorphic patterns within all species analyzed, while XTH2, HMTe and HMTi loci were monomorphic in some species (Table 2). However, their classification as monomorphic loci is still preliminary because of the relatively limited number of species and trees that were tested per species. Furthermore, average values of number of alleles

Table 2. Descriptive statistics of 12 polymorphic SSR-CGs markers. Estimates are reported for each *Eucalyptus* species separately including: tree sample sizes (N); number of alleles (N_a); allele size range in base pairs; Observed Heterozygosity (H_o) and Polymorphism Information Content (PIC); Averages are presented for each marker across the seven species and for each species averaging for all markers. (*) published in Acuña *et al.* 2012b. M, NA and NC stand for Monomorphic, No Amplification and Not Calculated respectively

		pCCR*	CesA3_A*	CesA3_B*	LIM*	HMTi*	HMTe	SK*	XTH2*	GST*	GAD_A	GAD_B	PER	Species averages
	SSR position	promoter	promoter	promoter	promoter	intron	exon	exon	exon	exon	exon	exon	exon	
E. camaldulensis	N	7	7	7	7	6	7	NA	7	7	7	7	7	6.9
	N_a	7	8	8	8	9	M	NA	2	7	4	4	5	6.2
	size	170-231	282-312	334-352	157-196	411-457	221	NA	129-130	86-137	248-255	185-191	259-267	
	H_o	0.86	0.57	1	0.71	0.67	NC	NA	0.14	0.29	0.57	0.71	0.57	0.61
	PIC	0.77	0.79	0.82	0.82	0.85	NC	NA	0.12	0.73	0.55	0.55	0.59	0.66
E. dunnii	N	6	5	5	5	5	6	7	7	7	6	6	5	5.8
	N_a	4	5	6	5	4	3	7	3	6	5	3	3	4.5
	size	169-200	290-300	346-371	159-179	431-437	221-224	333-380	129-139	90-116	249-264	186-190	264-270	
	H_o	0.5	0.8	1	0.2	0.6	0.67	0.71	0.29	0.29	1	0.5	0.8	0.61
	PIC	0.65	0.79	0.77	0.7	0.68	0.54	0.82	0.24	0.72	0.74	0.36	0.59	0.63
E. grandis	N	7	7	7	8	8	6	7	8	8	8	8	8	7.5
	N_a	6	2	6	5	M	2	5	M	7	4	3	3	4.3
	size	169-231	289-290	339-356	161-174	411	221-222	341-369	129	90-124	245-258	186-188	263-265	
	H_o	0.86	0.14	0.71	0.62	NC	0.17	0.57	NC	0.5	0.75	0.63	0.37	0.53
	PIC	0.71	0.35	0.7	0.72	NC	0.14	0.62	NC	0.76	0.58	0.46	0.37	0.54
E. saligna	N	6	6	6	6	4	2	5	6	6	6	6	6	5.4
	N_a	6	3	9	8	4	M	2	M	5	6	5	4	5.2
	size	168-231	289-293	336-373	161-180	411-448	221	353-367	129	92-113	248-260	185-189	263-266	
	H_o	0.33	0.83	1	1	0	NC	0	NC	0.17	0.67	0.83	0.33	0.52
	PIC	0.746	0.535	0.862	0.83	0.703	NC	0.269	NC	0.643	0.763	0.726	0.639	0.67
E. tereticornis	N	7	8	8	8	6	8	3	8	8	7	8	8	7.3
	N_a	7	7	10	9	8	3	4	M	9	6	6	7	6.9
	size	168-231	289-305	336-363	153-205	413-445	208-222	325-371	129	75-140	248-260	185-191	259-267	
	H_o	0.57	0.37	1	0.88	0.5	0.25	0.33	NC	0.62	0.71	0.75	0.88	0.62
	PIC	0.73	0.81	0.84	0.82	0.85	0.37	0.67	NC	0.83	0.66	0.76	0.77	0.74
E. viminalis	N	8	7	8	8	7	8	8	7	8	7	8	6	7.5
	N_a	11	11	8	8	10	3	6	M	8	2	4	3	6.7
	size	167-214	274-314	343-363	155-194	398-454	208-224	340-364	129	99-128	252-257	185-190	263-265	
	H_o	1	0.71	0.87	0.75	0.86	0.25	0.88	NC	0.62	0.29	0.5	0.83	0.69
	PIC	0.88	0.86	0.83	0.82	0.88	0.22	0.73	NC	0.8	0.21	0.39	0.58	0.65
E. globulus	N	7	7	8	8	8	8	8	8	8	8	8	8	7.8
	N_a	5	5	6	5	7	2	7	3	6	2	2	6	4.7
	size	182-216	288-306	346-365	159-177	417-439	221-224	331-359	122-134	83-116	248-256	185-189	264-269	
	H_o	0.29	0.57	0.87	0.75	0.87	0.12	0.62	0.5	0.62	0.12	0.25	0.5	0.51
	PIC	0.45	0.66	0.76	0.67	0.84	0.11	0.77	0.35	0.73	0.34	0.3	0.75	0.56
Microsatellite average	N_a	6.6	5.90	7.6	6.9	7.0	2.6	5.2	2.7	6.9	4.1	3.9	4.4	5.5
	H_o	0.63	0.57	0.92	0.70	0.58	0.29	0.52	0.31	0.44	0.59	0.60	0.61	0.58
	PIC	0.71	0.69	0.80	0.77	0.80	0.28	0.65	0.24	0.74	0.55	0.51	0.61	0.64

(N_a), Heterozygosity (H_o) and PIC were found across the seven species. The 12 microsatellite loci revealed 202 alleles and the averages of N_a, H_o and PICs for each combination specie/SSR were 5.5, 0.58 and 0.64, respectively (Table 2). These values are similar to the polymorphic patterns described in an study of EST-SSRs in *E. urophylla* and *E. tereticornis* (He *et al*., 2012) and other in *E. grandis* (Zhou *et al*., 2013).

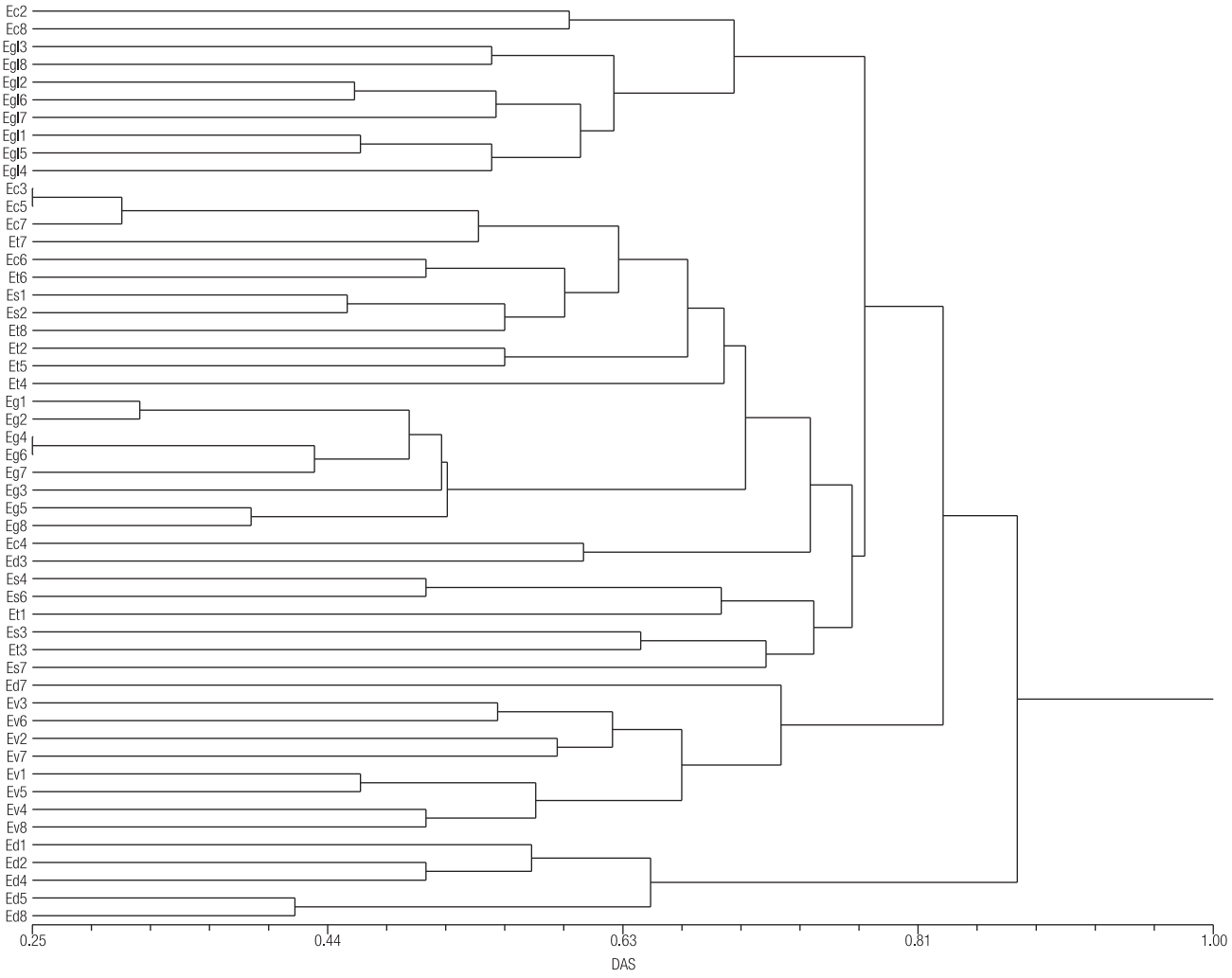

Figure 1. Cluster analysis. Phenogram of *Eucalyptus* spp. sample obtained by UPGMA cluster analyses based on shared allele distance (DAS) calculated with the 12 SSR-CG. E.g.: *E. grandis*, Es: *E. saligna*, Ed: *E. dunnii*, Ev: *E. viminalis*, Ec: *E. camaldulensis*, Et: *E. tereticornis*, Egl: *E. globulus.*

However, the values found in the present study are lower than those described by Faria *et al*., (2010), also in *E. grandis*. These lower results could be explained because of the difference in selection criteria. While Farias *et al*., (2010) based their selection on polymorphism level, we selected the loci according to their putative function in wood quality development.

In general, in the current study the average number of alleles (N_a) as well as the average observed heterozygosities (H_o) and PICs were higher in SSRs located within predicted promoter or intron regions (6.8, 0.68 and 0.75, respectively) than in exons (4.3, 0.48 and 0.51, respectively). Analyzing across species, we found the largest number of alleles for *E. tereticornis, E. viminalis* and *E. camaldulensis*. The SSRs characterized in the current study may be useful for population genetic studies in these species. This is particularly useful, since a few SSR markers have been reported until now for these species (Yasodha *et al*., 2008; Acuña *et al*., 2012a).

The 12 SSR-CG markers distinctively fingerprinted the 52 genotypes analyzed, allowing the construction of individual molecular identification patterns. This was corroborated with a genetic distance analysis between all 52 individuals that showed a mean of 0.78 and only 4 pairs of trees with distances lower than 0.25. These results reflect the high resolution capacity of microsatellites.

Estimates of average shared allele distance (DAS) among individuals within species ranged from 0.48 to

0.71 (*E. camaldulensis* (0.62), *E. dunnii* (0.67), *E. grandis* (0.48), *E. saligna* (0.68), *E. tereticornis* (0.71), *E. viminalis* (0.62) and *E. globulus* (0.59)), similar to those found in Faria *et al.* (2011). As expected, these values increased when considering DAS between individuals of different species (82% of pairwise data were higher than 0.7).

Finally, the cluster analysis showed that this set of markers allowed a clear-cut discrimination among all individuals, reflecting a high resolution capacity of microsatellites (Fig. 1). For *E. globulus, E. viminalis* and *E. grandis*, the individuals of the same species grouped together. Other clusters observed were congruent with taxonomic relationships described by Steane *et al*. (2011): individuals of *E. tereticornis* and *E. camaldulensis* (section *Exsertaria*) grouped together with *E. saligna* and E. *grandis* (section *Latoangulatae*).

Conclusion

High cross species transferability (92% to 100%) of this polymorphic SSR-CGs was demonstrated. Potentially, these markers may contribute to the verification of synteny and collinearity between different *Eucalyptus* maps. Furthermore, they would also allow the validation of gene and QTL positions in multiple pedigrees in the botanical sections to which most of the commercially planted eucalypt species belong: *Maidenaria*, *Exsertaria*, and *Latoangulatae*.

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References

Acuña C, Fernández P, Villalba P, García M, Hopp E, Marcucci Poltri S, 2012a. Discovery, validation and in silico functional characterization of EST-SSR markers in *Eucalyptus globulus*. Tree Genetics and Genomes 8: 289-301.

Acuña C, Villalba P, Pathauer P, Hopp, Marcucci Poltri S, 2012b. Characterization of novel microsatellite markers in candidate genes for wood properties for application in functional diversity assessment in *Eucalyptus globulus*. Electronic Journal of Biotechnology 15(2): 12-28.

Botstein D, White RL, Skolnick M, Davis RW, 1980. Construction of genetic linkage map in man using restriction length polymorphisms. The American Journal of Human Genetics 32(3): 314-331.

Brondani RP, Brondani C, Grattapaglia D, 2002. Towards a genus-wide reference linkage map for *Eucalyptus* based exclusively on highly informative microsatellite markers. Molecular Genetics and Genomics 267(3): 338-347.

Cupertino FB, Leal JB, Correa RX, Gaiotto FA, 2011. Genetic diversity of *Eucalyptus* hybrids estimated by genomic and EST microsatellite markers. Biologia Plantarum 55(2): 379-382.

Faria DA, Mamani EMC, Pappas MR, Pappas GJ Jr, Grattapaglia D, 2010. A selected set of EST-derived microsatellites, polymorphic and transferable across 6 species of *Eucalyptus*. J Hered 10: 512-520.

Faria DA, Mamani E, Pappas GJ, Grattapaglia D, 2011. Genotyping systems for *Eucalyptus* based on tetra- penta- and hexanucleotide repeat EST microsatellites and their use for individual fingerprinting and assignment tests. Tree Genetics & Genomes 7(1): 63-77.

Foucart C, Paux E, Ladouce N, San-Clemente H, Grima-Pettenati J, Sivadon P, 2006. Transcript profiling of a xylem vs phloem cDNA subtractive library identifies new genes expressed during xylogenesis in *Eucalyptus*. New Phytology 170(4): 739-752.

Freeman JS, Whittock SP, Potts BM, Vaillancourt RE, 2009. QTL influencing growth and wood properties in *Eucalyptus globulus*. Tree Genetics & Genomes 5(4): 713-722.

Gion J, Rech P, Grima-pettenati J, Verhaegen D, Plomion C, 2000. Mapping candidate genes in *Eucalyptus* with emphasis on lignification genes. Molecular Breeding 6: 441-449.

Gion JM, Carouche A, Deweer S, Bedon F, Pichavant F, Charpentier JP, Bailleres H, Rozenberg P, Carocha V, Ognouabi N, Verhaegen D, Grima-Pettenati J, Vigneron P, Plomion C, 2011. Comprehensive genetic dissection of wood properties in a widely-grown tropical tree: *Eucalyptus*. BMC Genomics 12: 301.

Glaubitz JC, Emebiri LC, Moran GF, 2001. Dinucleotide microsatellites from *Eucalyptus sieberi*: inheritance diversity and improved scoring of single-base differences. Genome 44(6): 1041-1045.

He X, Wang Y, Li F, Weng Q, Li M, Xu L, Shi J, Gan S, 2012. Development of 198 novel EST-derived microsatellites in *Eucalyptus* (Myrtaceae). American journal of botany 99: 134-148.

Jones ME, Stokoe RL, Cross MJ, Scott LJ, Maguire TL, Shepherd M, 2001. Isolation of microsatellite loci from spotted gum (*Corymbia variegata*), and cross-species amplification in *Corymbia* and *Eucalyptus*. Molecular Ecology Notes 1(4): 276-278.

Langella O, 2002. Populations 1.2.28. Logiciel de génétique des populations. Available at URL: http://www.legs.cnrs-gif.fr.

Marcucci Poltri SN, Zelener N, Rodriguez Traverso J, Gelid P, Hopp HE, 2003. Selection of a seed orchard of *Eucalyptus dunnii* based on genetic diversity criteria calculated using molecular markers. Tree Physiology 23(9): 625-632.

Paux E, Tamasloukht MB, Ladouce N, Sivadon P, Grima-pettenati J, 2004. Identification of genes preferentially expressed during wood formation in Eucalyptus. Plant Molecular Biology 55: 263-280.

Peakall R, Smouse PE, 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

Steane D, Nicolle D, Sansaloni CP, Petroli CD, Carling J, Kilian A, Myburg A, Grattapaglia D, Vaillancourt RE, 2011. Population genetic analysis and phylogeny reconstruction in *Eucalyptus* (*Myrtaceae*) using high-throughput, genome-wide genotyping. Molecular phylogenetics and evolution 59: 206-24.

Thumma BR, Southerton SG, Bell J.C, Owen JV, Henery ML, Moran GF, 2010. Quantitative trait locus (QTL) analysis of wood quality traits in *Eucalyptus nitens*. Tree Genetics & Genomes 6: 305-317.

Torales SL, Rivarola M, Pomponio MF, Fernández P, Acuña CV, Marchelli P, Gonzalez S, Azpilicueta MM, Hopp HE, Gallo L, Paniego NB, Poltri SNM, 2012. Transcriptome survey of Patagonian southern beech Nothofagus nervosa (= N. Alpina): assembly, annotation and molecular marker discovery. BMC genomics 13: 291.

Yasodha R, Sumathi R, Chezhian P, Kavitha S, Ghosh M, 2008. *Eucalyptus* microsatellites mined in silico: survey and evaluation. Journal of Genetics 87(1): 21-25.

Zhou C, He X, Li F, Weng Q, Yu X, Wang Y, Li M, Shi J, Gan S, 2013. Development of 240 novel EST-SSRs in *Eucalyptus* L'Hérit. Molecular Breeding 1-5.