Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.
DOI 10.1007/s40011-014-0360-5

RESEARCH ARTICLE

Association Analysis for Vegetative Propagation Traits in *Eucalyptus tereticornis* and *Eucalyptus camaldulensis* Using Simple Sequence Repeat Markers

Arumugasundaram Shanmugapriya · Vijaya Kumar Waman Bachpai · Marappan Ganesan · Ramasamy Yasodha

Received: 4 February 2014 / Revised: 3 March 2014 / Accepted: 25 April 2014

Abstract *Eucalyptus*, one of the most widely planted forestry species, is an introduced species to India which is mainly exploited for its pulpwood. Presently, the largest clonal forestry programs are in practice with species of *Eucalyptus* and the variable rooting potential among the selections are considered to be a hindrance to the success of clonal propagation. Many breeding programs target intra- and inter-specific hybridization for the transfer of vegetative propagation traits and hence SSR markers linked with vegetative propagation traits gained importance for practicing marker assisted selection. *Eucalyptus* species show high synteny and marker correspondence across genome of different species favoring use of simple sequence repeats (SSRs) linked to quantitative trait loci (QTLs) for association analysis. In this study, 43 accessions of *E. tereticornis* and 40 accessions of *E. camaldulensis* were examined for their rooting parameters and subjected to association analysis. The rooting percentage of *Eucalyptus* accessions showed continuous variation (0–100 %). Association analysis with 62 loci showed that two SSR loci (Embra40 and Embra7) were associated with rooting and mortality percent and shoot length in *E. tereticornis*. Two SSR loci (Embra167 and Embra39) were associated with shoot length and root length in *E. camaldulensis*. This study validated the presence of generic genomic regions through SSR markers, which enabled the identification of orthologous QTL regions for vegetative propagation properties in *E. tereticornis* and *E. camaldulensis*.

Keywords *Eucalyptus* · Vegetative propagation · SSR · Association analysis · Adventitious rooting

Introduction

Eucalyptus is one of the most widely planted pulp wood species comprising of around 700 species. They are introduced species to India which occupy an area of 3.943 Mha (http://git-forestry.com/Global_Eucalyptus_Map.htm), predominantly of *Eucalyptus tereticornis* and *E. camaldulensis*. During 1996, domestication program of *E. tereticornis* and *E. camaldulensis* was systematically implemented in India and provenance cum progeny trials, seed production areas, half pedigreed seedling seed orchards (SSO) and clonal trials were established [1, 2]. Clonal forestry programs are extensively practiced in *Eucalyptus* due its amenability to vegetative propagation thus capturing both additive and non-additive genetic variations. Although physiological and environmental factors play major role in the success of vegetative propagation, there are evidences that these quantitative traits have moderate to high heritability [3–5]. Considering the importance of cloning as a tool for establishment of clonal forestry, genetic control on vegetative propagation traits including adventitious rooting were initiated since 1990's [3, 6, 7]. Easy and difficult to root species were identified and high within species variability was reported in eucalypts [7]. Many breeding programs target intra- and inter-specific hybridization for the transfer of vegetative propagation traits. In Brazil and South Africa,

Electronic supplementary material The online version of this article (doi:10.1007/s40011-014-0360-5) contains supplementary material, which is available to authorized users.

A. Shanmugapriya
R & D Center, Bharathiar University, Coimbatore 641046, India

V. K. W. Bachpai · M. Ganesan · R. Yasodha (✉)
Division of Plant Biotechnology, Institute of Forest Genetics and Tree Breeding, Coimbatore 641 002, India
e-mail: yasodha@icfre.org

Journal : Large_Springer-India40011 Dispatch : 22-5-2014 Pages : 6
Article No. : 360 LE TYPESET
MS Code : NASB-D-14-00041 CP DISK

hybrids of *E. grandis* X *E. urophylla* are quite common with the involvement of *E. grandis* to form stem, while *E. urophylla* contributes most of the ability in rooting. However, in India the potential of hybrid eucalypt forestry is yet to take off with the commonly grown drought tolerant species like *E. tereticornis* and *E. camaldulensis*. Quantitative trait loci (QTLs) controlling adventitious rooting and other vegetative propagation traits have been tagged with DNA markers such as RAPD [6], AFLP [7, 8] and SSR [9, 10]. Genetic control and architecture of adventitious rooting in forest trees and congruence in QTL locations across the related species was reviewed by Shepherd et al. [11]. In eucalypts, QTL mapping strategy essentially depends on inter-specific hybrid generation, pseudotest cross strategy based linkage mapping and localization of QTLs on the consensus map [12]. However, in the recent years, linkage disequilibrium (LD) based association mapping has been successfully applied in crop species for the identification of QTLs and genes controlling the phenotypic traits. LD based association mapping was reported in conifers like pines, douglas fir and in hardwoods like *Eucalyptus* and *Populus* [13].

SSR markers linked with important QTLs, identified through conventional mapping strategy have increased the power of association mapping in many economically important crops [14]. Several evidences in tree species were reported on association of SSRs with phenotypic traits such *Populus* [15] and peach [16]. The association of *Puccinia psidii* rust resistance QTLs with SSR loci in eucalypts species was confirmed by several studies [17–19]. Similarly in *Eucalyptus*, QTLs linked with vegetative propagation traits were identified through SSRs markers and putative QTLs influencing vegetative propagation traits were located on homeologous linkage groups of a few species in *Symphyomyrtus* subgenus [9]. The repeatable detection and collocation of QTL for propagation traits in inter-specific F1s of *Eucalyptus* spp. [9] and closely related *Corymbia torelliana* × *Corymbia citriodora* subspecies *variegata* [10] supported a common genetic basis for propagation traits. Hence, the present study explored the possibilities of identifying the SSR markers linked to vegetative propagation QTLs in *E. tereticornis* and *E. camaldulensis* following the association analysis strategy.

Material and Methods

Plant Material

The association population consisted of the accessions of *E. tereticornis* and *E. camaldulensis* clones. The germplasm for analysis was selected from one to few individual per provenance to enable a diverse population with contrasting phenotypic traits for association studies. Selected population was already assessed for population structure and LD and the details of the accessions were provided by Arumugasundaram et al. [20].

Phenotypic Measurements

The single nodal cuttings of *E. tereticornis* and *E. camaldulensis* were taken from the culled trees in SSO and clonal seed orchard (CSO) for conducting the adventitious rooting experiments through established vegetative propagation methods followed in the Vegetative Propagation Complex, Institute of Forest Genetics and Tree Breeding, Coimbatore, India [21]. In brief, the basal tip of each cutting was dipped in 4,000 ppm indole 3 butyric acid and placed on vermiculite in 150 cc root trainers. The cuttings were kept for 30 days in polytunnels with regular misting in the shade house. Every year the stock plants in the field were cut back to induce coppice shoots for cutting preparation and they were watered when required. Three settings across three seasons were conducted for the selected accessions to measure the phenotypic traits.

Vegetative propagation traits were measured on individual cuttings after 30 days. The selected accessions of *Eucalyptus* were monitored for the following phenotypic measurements: (1) rooting percent (rooted/surviving cuttings), (2) mortality (dead/total cuttings), (3) number of roots, (4) root length, (5) length of longest main root, (6) shoot length. The conditions in the rooting tunnels were homogeneous and data from all available cuttings per clone were averaged (25 cuttings per clone). The observations were averaged for each individual and for rooting percent the highest percentage recorded was considered. The populations were considered as poor rooters when the rooting percentage is less than or equal to 30, while the intermediate ranged from 31 to 69 and the best rooters had the rooting percentage equal to or above 70.

Microsatellite Amplification

The details of the microsatellites used in the study and methods for PCR amplification and genotyping were described previously [20]. SSR markers developed from *E. grandis* [22], *E. nitens* [23], *E. tereticornis* [24] and 4 SSRs (EMBRA 40, EMBRA195, EMBRA 207 and EMCRC 47) used for mapping adventitious rooting traits in *C. citriodora* subsp. *variegata* by Shepherd et al. [10] were cross amplified in all *Eucalyptus* accessions. Among the SSR markers used for cross amplification 23 were identified as sequence tagged sites (STS) markers linked with vegetative propagation traits by Marques et al. [9].

Journal : Large_Springer-India40011 Dispatch : 22-5-2014 Pages : 6
Article No. : 360 LE TYPESET
MS Code : NASB-D-14-00041 CP DISK

Data Analysis

Statistical analyses for the observed traits were done using one-way analysis of variance implemented in SPSS V.11.0 (http://www-01.ibm.com/software/analytics/spss/downloads.html). Correlations between each pair of traits were estimated using Pearson's correlation coefficient. All the observed effects were considered for statistical significance at $P \leq 0.05$ or $P \leq 0.01$. Association between microsatellite allele polymorphisms and mean phenotypic values were performed by the general linear model analyses in TASSEL (http://www.maizegenetics.net). It requires three data sets primarily for the analysis: (1) Phenotypic data (2) Genotypic data and (3) Ancestry coefficient data (Q matrix). The Q matrix produced by STRUCTURE was included as covariate in the analysis to control for populations structure. The polymorphisms were determined as significant for p-adj_Marker (based on 3000 permutations) equal to 0.05 or less. p-adj_Marker is a permutation based experiment-wise error rate which controls the error rate over all the markers tested. It was finally considered for interpretation marker-trait associations.

Results and Discussion

Several studies have explicitly revealed the interest of tree breeders on the use of DNA markers for precise breeding in eucalypts for commercial trait improvement [25]. Particularly, in the countries where the species is introduced, limited seed sources form the breeding population and therefore integration of DNA markers in the genetic improvement program of these species will have a major impact on productivity. DNA markers such as SSRs have been closely associated with various quantitative traits including rust resistance in *Eucalyptus grandis* [26] phloroglucinol compounds in *E. globulus* [18] and wood properties [12, 27].

Phenotyping for vegetative propagation conducted in the present study showed high variations among rooting parameters and rooting percentage varies from 0 to 100 % among the individuals. The details on rooting parameters for 43 accessions of *E. tereticornis* and 40 accessions of *E. camaldulensis* were given in supplementary Table 1 and 2. The summary statistics of the rooting traits for the accessions of *E. tereticornis* and *E. camaldulensis* are provided in Table 1. The rooting percentage of *E. tereticornis* varied from 0 to 100, where 72 % of the individuals fall under poor rooters, but only 15 % of the *E. camaldulensis* individuals were below 30 % rooting. Similarly the average number of roots was found to be higher for *E. camaldulensis* (6.9 cm) as compared to *E. tereticornis* (2.8 cm). The root length and shoot length were found to be high for *E. camaldulensis* (11.0 and 11.0 cm) as compared to *E. tereticornis* (9.4 and 4.9 cm).

The correlation coefficients presented in Table 2 and 3, show the correlation between the rooting related traits in *E. tereticornis* and *E. camaldulensis* respectively. In *E. tereticornis*, highly significant correlation was found between the root length and the length of the longest main root ($r = 0.972$, $P < 0.01$) and moderate but significant correlation was found between root length and shoot length ($r = 0.755$, $P < 0.01$). The number of roots were found to be correlated with root length and shoot length ($r = 0.642$, $P < 0.01$ and $r = 0.677$, $P < 0.01$). However, the rooting percent and mortality percent were not significantly correlated with other rooting traits.

Table 1 Summary statistics of the vegetative propagation traits for *Eucalyptus tereticornis* and *E.camaldulensis* accessions

S. no	Name of the Species	Rooting %		Mortality %		No. of roots (Mean ± S.D)	Length of longest main root (cm) (Mean ± S.D)	Root length (cm) (Mean ± S.D)	Shoot length (cm) (Mean ± S.D)
		Min	Max	Min	Max				
1	*E. tereticornis*	0	100	0	100	3.4 ± 1.5	16.5 ± 6.09	11.5 ± 4.8	6.5 ± 2.6
2	*E. camaldulensis*	7	98	2	93	7.1 ± 2.7	19.8 ± 4.9	11.2 ± 3.0	10.7 ± 3.5

Table 2 Phenotypic correlations (Pearson correlation) among vegetative propagation traits (3 replicates) estimated in 53 genotypes of *E. tereticornis*

Traits analyzed	Number of roots	Root length	Length of longest main root
Root length (cm)	0.642**	–	
Length of longest main root (cm)	0.744**	0.972**	–
Shoot length (cm)	0.677**	0.755**	0.754**

** Significant at the 0.01 level(2-tailed)

Journal : Large_Springer-India40011 Dispatch : 22-5-2014 Pages : 6
Article No. : 360 LE TYPESET
MS Code : NASB-D-14-00041 CP DISK

Table 3 Phenotypic correlations (Pearson correlation) among vegetative propagation traits (3 replicates) estimated in 40 genotypes of *E. camaldulensis*

Traits analyzed	Rooting percent	Mortality percent	Number of roots	Root length	Length of longest main root
Rooting percent	–				
Mortality percent	0.996**	–			
Number of roots	0.269**	−0.260**	–		
Root length (cm)	0.257**	−0.263**	0.689**	–	
Length of longest main root (cm)	0.289**	−0.288**	0.808**	0.955**	–
Shoot length (cm)	0.216*	−0.216*	0.775**	0.821**	0.847 **

** Significant at the 0.01 level (2-tailed)

* Significant at the 0.05 level (2-tailed)

Table 4 SSR markers significantly associated with vegetative propagation traits in *E. tereticornis* genotypes

Trait	Locus	LG	p_Marker	P_adj_Marker	Rsq_Marker
Mortality	E40*	10	0.0012	0.025	0.522
Rooting percent	E40*	10	0.0012	0.029	0.512
Shoot length	E7**	9	0.0084	0.017	0.730

* STS markers associated with adventitious rooting traits [9] and vegetative propagation traits [10]

** STS marker associated with adventitious rooting traits [9]

Table 5 SSR markers significantly associated with vegetative propagation traits in *E. camaldulensis* genotypes

Trait	Locus	LG	p_Marker	p_adj_Marker	Rsq_Marker
Shoot length	E167	7	0.0094	0.004	0.934
Root length	E39	11	0.0013	0.019	0.986

In *E. camaldulensis*, the root length was highly correlated with the length of the longest main root and shoot length ($r = 0.955$, $P < 0.01$ and $r = 0.821$, $P < 0.01$). The correlation between the number of roots and the root length and shoot length was moderate but highly significant ($r = 0.689$, $P < 0.01$ and $r = 0.775$, $P < 0.01$). Also highly significant, but low level of correlation was found between rooting percentage and root length ($r = 0.257$, $P < 0.01$). But significant and very low level of correlation was observed between the rooting percentage and shoot length ($r = 0.216$, $P < 0.05$).

An estimate of LD showed that between the species, the accessions from *E. tereticornis* showed more number of allele pairs in LD than *E. camaldulensis* accessions [20]. In the present study, all the 62 SSR markers including the 19 STS were linked with vegetative propagation QTLs of *E. grandis, E. urophylla, E. tereticornis* and *E. globulus* by Marques et al. [9] and 4 SSR markers linked with vegetative propagation QTLs of *Corymbia* sps by Shepherd et al. [10]. They were employed for association analysis. Two SSR loci Embra40 and Embra7 were associated with rooting percent and mortality and shoot length in *E. tereticornis* (Table 4). Two SSR loci Embra167 and Embra39 were associated with shoot length and root length in *E. camaldulensis* (Table 5). One of the SSR loci (Embra 40) linked with vegetative propagation traits was found to be common between eucalypt species and *Corymbia* species [9, 10] showing association with mortality trait in *E. tereticornis* (Table 4). These SSR containing DNA sequences available in NCBI did not show similarity with any gene sequences.

Heritability for adventitious rooting of stem cuttings in *E. globulus* was estimated and found to have high narrow sense heritability ($h^2 = 0.54$) suggesting that large gains can be achieved by direct selection for rooting ability [4]. QTL detection was carried out for propagation traits in a mapping pedigree of *E. tereticornis* x *E. globulus* wherein putative QTLs accounted for 2.6–17.0 % of the phenotypic variation. As the rooting success of *E. camaldulensis* is relatively high, a breeding program for this species using clonal deployment could focus on selection for growth, form and resistance traits. The importance of *E. camaldulensis* plantation for saline lands, due to their ease for propagation through vegetative means necessitates the molecular studies in the species. Large phenotypic variances were reported for rooting and other propagation traits, with significant proportions attributable to differences between clones (5). The same study identified one of the QTL explaining more than 60 % variation for percent rooting in *C. torelliana* × *C. citriodora* subspecies *variegata* hybrid family ($n = 18$) using the SSR markers. Similarly, SSR loci Embra125 and Embra1071 flank a rust

Journal : Large_Springer-India40011 Dispatch : 22-5-2014 Pages : 6
Article No. : 360 LE TYPESET
MS Code : NASB-D-14-00041 CP DISK

resistant gene (*Ppr*) and were found to be in LD in *E. grandis* population [26]. Further, in *E. grandis* hybrid population, Embra125 was in close association with rust resistance QTL explaining 42 % of the phenotypic variation [18]. It was again confirmed with the presence of rust resistance QTL between EST-SSR markers Embra1656 and Embra1071 [19]. In *E. nitens*, QTL analysis was performed and four QTLs were found for percentage of roots explaining 4.9–15.4 % variation [28].

In forest trees, tremendous efforts have been made on QTL mapping using the LD generated through the interspecific hybrids (F1 and F2) for several economically important traits [29]. Such QTL identification process is time intensive where the researcher has to wait for many years to assess the phenotypic variations expressed at intermittent stages of growth period. In association mapping approach, LD present in the extant population of interest is exploited and hence it is highly attractive for tree species. Integrating both QTL and association mapping methods, Thumma et al. [30] have established the potential of informative functional polymorphisms underlying quantitative traits. Conserved QTLs have been located on homeologous linkage groups of the taxonomically related species with SSRs [10, 13] and several candidate genes co-located to QTL positions [27]. In addition, collocation of QTL for different rooting traits was identified in a single region on linkage group [11]. In *Eucalyptus*, the estimated genetic distance of 1.0 cm was about 385 kb [22] and hence the SSRs surrounding the genes/QTLs would be a perfect target for LD estimation and association studies. Low LD in eucalypts promises a higher resolution in genome-wide association mapping however, many more markers are required for covering the whole genome. Given the genome size (~650 Mb) and availability of whole genome sequence of eucalypt species, it should be possible to develop high-density SSR and SNP markers.

Conclusion

The aim of the present work was to investigate the potential use of SSR markers linked to vegetative propagation QTLs in *E. tereticornis* and *E. camaldulensis* and SSR loci Embra40 and Embra7 were the potential candidates to be used in eucalypts breeding. The results validated the presence of generic genomic regions, which enabled the identification of orthologous QTL regions for vegetative propagation traits in eucalypts.

Acknowledgments The first author acknowledges the Senior Research Fellowship from the Indian Council of Forestry Research & Education (ICFRE), Dehradun, India.

References

1. Varghese M, Harwood CE, Hegde R, Ravi N (2008) Evaluation of provenances of *Eucalyptus camaldulensis* and clones of *E. camaldulensis* and *E. tereticornis* at contrasting sites in Southern India. Silvae Genet 57:170–179
2. Varghese M, Kamalakannan R, Harwood CE, Lindgren D, McDonald MW (2009) Changes in growth performance and fecundity of *Eucalyptus camaldulensis* and *E.tereticornis* during domestication in southern India. Tree Genet Genomes 5:629–640
3. Borralho NMG, Wilson Ph (1994) Inheritance of initial survival and rooting ability in *Eucalyptus* globulus Labill. stem cuttings. Silvae Genet 43:238–242
4. Lemos L, Carvalho A, Araújo JA, Borralho NMG (1998) Importance of additive and specific combining ability effects for rooting ability of stem cuttings in *Eucalyptus globulus*. Silvae Genet 46:307–308
5. Shepherd M, Pomroy P, Dieters MJ, Lee DJ (2007) Genetic control of propagation traits in a single *Corymbia torelliana* × *C. citriodora* spp *variegata* family. Can J For Res 37:2563–2574
6. Grattapaglia D, Bertolucci FL, Sederoff RR (1995) Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross strategy and RAPD markers. Theor Appl Genet 90:933–947
7. Marques CM, Vasquez-Kool J, Carocha VJ, Ferreira JG, O'Malley DM, Liu B-H, Sederoff R (1999) Genetic dissection of vegetative propagation traits in *Eucalyptus tereticornis* and *E. globulus*. Theor Appl Genet 99:936–946
8. Marques CM, Carocha VJ, de Sa ARP, Oliveira MR, Pires AM, Sederoff R, Borralho NMG (2005) Verification of QTL linked markers for propagation traits in *Eucalyptus*. Tree Genet Genomes 1:103–108
9. Marques CM, Brondani RPV, Grattapaglia D, Sederoff R (2002) Conservation and synteny of SSR loci and QTLs for vegetative propagation in four *Eucalyptus* species. Theor Appl Genet 105:474–478
10. Shepherd M, Kasem S, Lee D, Henry R (2008) Mapping species differences for adventitious rooting in a *Corymbia torelliana* × *C. citriodora* subspecies *variegata* hybrid. Tree Genet Genomes 4:715–725
11. Shepherd M, Dieters MJ, Baltunis BS (2009) Genetic control and architecture of adventitious rooting in forest trees. In: Niemi K, Scagel C (eds) Adventitious root formation of forest trees and horticultural woody plant—from genes to applications. Research Signpost, Kerala, pp 51–84
12. Thumma BR, Southerton SG, Bell JC, Owen JV, Henery ML, Moran GF (2010) Quantitative trait locus (QTL) analysis of wood quality traits in *Eucalyptus nitens*. Tree Genet Genomes 6:305–317
13. Khan MA, Korban SS (2012) Association mapping in forest trees and fruit crops. J Exp Bot 63:4045–4060
14. Morrell PL, Buckler ES, Ross-Ibarra J (2011) Crop genomics: advances and applications. Nat Rev Genet 13:85–96
15. Du Q, Gong C, Pan W, Zhang D (2013) Development and application of microsatellites in candidate genes related to wood properties in the Chinese white poplar (*Populus tomentosa* Carr.). DNA Res 20:31–44
16. Picañol R, Eduardo I, Aranzana MJ, Howad W, Batlle I, Iglesias I, Alonso JM, Arús P (2013) Combining linkage and association mapping to search for markers linked to the flat fruit character in peach. Euphytica 190:279–288
17. Freeman JS, O'Reilly-Wapstra M, Vaillancourt RE, Wiggins N, Potts BM (2008) Quantitative trait loci for key defensive

Journal : Large_Springer-India40011 Dispatch : 22-5-2014 Pages : 6
Article No. : 360 LE TYPESET
MS Code : NASB-D-14-00041 CP DISK

compounds affecting herbivory of eucalypts in Australia. New Phytol 178:846–851
18. Rosado TB, Tomaz RS, Junior MFR, Rosado AM, Guimarães LMS, Araújo EF, Alfenas AC, Cruz CD (2010) Detection of QTL associated with rust resistance using IBD-based methodologies in exogamic *Eucalyptus* spp. Populations. Crop Breed App Biot 10:321–328
19. Alves A, Rosado C, Faria D, Guimarães L, Lau D, Brommonschenkel S, Grattapaglia D, Alfenas A (2012) Genetic mapping provides evidence for the role of additive and non-additive QTLs in the response of inter-specific hybrids of *Eucalyptus* to *Puccinia psidii* rust infection. Euphytica 183:27–38
20. Arumugasundaram S, Ghosh M, Veerasamy S, Ramasamy Y (2011) Species Discrimination, Population Structure and Linkage Disequilibrium in *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* Using SSR Markers. PLoS One 6(12):e28252. doi: 10.1371/journal.pone.0028252
21. Vivekanandan K, Gurumurthi K, Jayaraj RSC (1997) Clonal multiplication of eucalypts. IFGTB/97/001 Field Document No.1, 65 pages
22. Brondani RPV, Williams ER, Brondani C, Grattapaglia D (2006) A microsatellite-based consensus linkage map for species of *Eucalyptus* and a novel set of 230 microsatellite markers for the genus. BMC Plant Biol 6:1–16
23. Thamarus K, Groom K, Murrell J, Byrne M, Moran G (2002) A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood, fibre and floral traits. Theor Appl Genet 104:379–387
24. Yasodha R, Sumathi R, Chezhian P, Kavitha P, Ghosh M (2008) *Eucalyptus* microsatellites mined in silico: survey and evaluation. J Genet 87:21–25
25. Grattapaglia D, Vaillancourt R, Shepherd M, Thumma B, Foley W, Külheim C, Potts B, Myburg A (2012) Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. Tree Genet Genomes 8:463–508
26. Mamani EMC, Bueno NW, Faria DA, Guimarães LMS, Lau D, Alfenas ACA, Grattapaglia D (2010) Positioning of the major locus for *Puccinia psidii* rust resistance (*Ppr1*) on the *Eucalyptus* reference map and its validation across unrelated pedigrees. Tree Genet Genomes 6:953–962
27. Freeman JS, Whittock SP, Potts BM, Vaillancourt RE (2009) QTL influencing growth and wood properties in *Eucalyptus globulus*. Tree Genet Genomes 5:713–722
28. Thumma BR, Baltunis BS, Bell JC, Emebiri LC, Moran GF et al (2010) Quantitative trait locus (QTL) analysis of growth and vegetative propagation traits in *Eucalyptus nitens* full-sib families. Tree Genet Genomes 6:877–889
29. Thavamanikumar S, Southerton SG, Bossinger G, Thumma BR (2013) Dissection of complex traits in forest trees—opportunities for marker-assisted selection. Tree Genet Genomes 9:627–639
30. Thumma BR, Matheson BA, Zhang DQ, Meeske C, Meder R, Downes GM, Southerton SG (2009) Identification of a cis-acting regulatory polymorphism in a eucalypt cobra-like gene affecting cellulose content. Genetics 183:1153–1164

Journal : Large_Springer-India40011 Dispatch : 22-5-2014 Pages : 6
Article No. : 360 LE TYPESET
MS Code : NASB-D-14-00041 CP DISK