Genetic linkage map and QTL identification for adventitious rooting traits in red gum eucalypts

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Abstract

The eucalypt species, *Eucalyptus tereticornis* and *Eucalyptus camaldulensis*, show tolerance to drought and salinity conditions, respectively, and are widely cultivated in arid and semiarid regions of tropical countries. In this study, genetic linkage map was developed for interspecific cross *E. tereticornis* × *E. camaldulensis* using pseudo-testcross strategy with simple sequence repeats (SSRs), intersimple sequence repeats (ISSRs), and sequence-related amplified polymorphism (SRAP) markers. The consensus genetic map comprised totally 283 markers with 84 SSRs, 94 ISSRs, and 105 SRAP markers on 11 linkage groups spanning 1163.4 cM genetic distance. Blasting the SSR sequences against *E. grandis* sequences allowed an alignment of 64% and the average ratio of genetic-to-physical distance was 1.7 Mbp/cM, which strengthens the evidence that high amount of synteny and colinearity exists among eucalypts genome. Blast searches also revealed that 37% of SSRs had homologies with genes, which could potentially be used in the variety of downstream applications including candidate gene polymorphism. Quantitative trait loci (QTL) analysis for adventitious rooting traits revealed six QTL for rooting percent and root length on five chromosomes with interval and composite interval mapping. All the QTL explained 12.0–14.7% of the phenotypic variance, showing the involvement of major effect QTL on adventitious rooting traits. Increasing the density of markers would facilitate the detection of more number of small-effect QTL and also underpinning the genes involved in rooting process.

Keywords *Eucalyptus* · Genetic map · Colinearity · Quantitative trait loci · Adventitious rooting traits

Introduction

Eucalypts, native to Australia and neighbouring islands, are one of the most important forest tree species cultivated globally, after pines and poplars. Majority of the eucalypt plantations are destined for paper manufacturing and it meets about 17.5% of world's paper pulp requirement (Hart and Santos 2016). Eucalypt fibre exhibits key papermaking properties such as bulk, opacity, formation, softness, porosity, smoothness, absorbency, and dimensional stability (Foelkel

2009). Therefore, the use of eucalypt pulp for paper making has received considerable attention in many countries. Although more than 900 species are available, about ten species are used in industrial plantations. In tropical countries like India, *Eucalyptus camaldulensis* (river red gum) and *Eucalyptus tereticornis* (forest red gum) are extensively cultivated. Both species have $2n = 22$ chromosomes, hybridises naturally. Interspecific hybrid generation through controlled pollination of these two species results in hybrid vigour (Venkatesh and Sharma 1977). Compared with other eucalypt species, there has been a relatively little progress in hybrid breeding of red gums particularly, *E. camaldulensis* and *E. tereticornis*.

Hybrid forestry in eucalypts is largely successful because of the clonal plantations raised through vegetative propagation of stem cuttings. Clonal propagation helps in rapid deployment of selected individuals into industrial plantations. However, the ability to produce adventitious roots from stem cuttings, an essential process in clonal

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propagation, has been shown to vary within and among eucalypt species and their hybrid progenies (Shepherd et al. 2008). DNA markers combined with genetic linkage mapping and quantitative trait loci (QTL) analysis are employed to understand the pattern of genetic inheritance and genetic architecture of various economically important traits in plants. The advances in DNA sequencing technologies have offered an alternative approach to the traditional breeding methods and eucalypt breeders are now giving more attention to the use of molecular tools to improve productive traits including fibre yield and quality (Henry and Kole 2014).

Linkage maps are one of the tools used in genetic improvement of tree species. They provide basis for genome studies including dissection of polygenic traits, map based cloning of genomic regions, assembly of physical maps, identification of recombination hot spots, and evolutionary comparison of genome across species. High-density genetic maps have been developed in eucalypt pure species and hybrids of *E. globules*, *E. grandis*, and *E. urophylla*, and are used to reveal genome composition for selection of industrially valuable traits (Bartholomé et al. 2015). Availability of whole genome sequence of *E. grandis* increases the opportunity of application of molecular markers and serves as reference across species. Consequently, various genetic and genomic efforts are in progress across the world to increase productivity and enhance the pulp properties of eucalypts. Linkage maps are available for *E. camaldulensis* (Agrama et al. 2002), *E. tereticornis* × *E. globulus* (Marques et al. 1998), *E. tereticornis* × *E. urophylla* (Gan et al. 2003; Yu et al. 2012; Li et al. 2015), and *E. camaldulensis* × *E. urophylla* (Zarpelon et al. 2015). Many eucalypt genetic linkage maps have been developed over the past decade as summarized by Freeman (2014); however, there is no map available for *E. camaldulensis* × *E. tereticornis*.

Similarly, numerous reports of QTL detection are available in eucalypts on several economically important traits (Henry and Kole 2014). Limited studies are focusing the vegetative propagation traits including adventitious rooting potential of shoot cuttings. Randomly amplified polymorphic DNA (RAPD) markers were used for QTL analysis for rooting ability in *E. grandis* × *E. urophylla* (Grattapaglia et al. 1995). Amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) markers were employed to detect vegetative propagation related QTL in *E. tereticornis* × *E. globules* and *E. grandis* × *E. urophylla*, and synteny of these traits across eucalypt species was verified (Marques et al. 1998, 2002, 2005). Linkage analysis of single-nucleotide polymorphism (SNP) markers in full-sib families of *E. nitens* also revealed the presence of QTL for vegetative propagation traits (Thumma et al. 2010). However, there are no studies available on the interspecific cross *E. camaldulensis* × *E. tereticornis*, known for exhibiting

hybrid vigour for growth performance with the potential to grow in arid regions of India.

Accordingly, in this study, SSR markers along with sequence-related amplified polymorphism (SRAP) and intersimple sequence repeat (ISSR) markers were utilized to construct a linkage map in *E. camaldulensis* × *E. tereticornis* and to identify QTL related to adventitious rooting traits. SRAP markers are randomly distributed in the genome, both inside and between genes, and used widely in constructing linkage map of crops (Li and Quiros 2001). Similarly, ISSR markers have been used for genetic linkage map development in wide variety of species due to the cost-effective analysis and are relatively easy to generate good amount of polymorphism per assay (Chen et al. 2014). Furthermore, owing to the synteny and colinearity of genome across eucalypt species, efforts were made in this study to compare the genetic map of *E. camaldulensis* × *E. tereticornis* with physical map of *E. grandis*.

Materials and methods

Plant materials

Eucalyptus clones showing contrasting phenotypic characters for adventitious rooting traits were selected as parents for hybridization. Interspecific cross between *E. tereticornis* (clone 217) × *E. camaldulensis* (clone 17) or Et217 × Ec17 was generated, where clones 217 and 17 had poor (20%) and high (72%) rooting potential, respectively. These clones form the part of high yielding selections derived from the genetic improvement program of the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore. The hybridization experiments were carried out following the conventional-controlled pollination procedures. The flowers were emasculated after removing the operculum and then bagged to avoid pollinator visit. 3–5 days after the removal of the operculum, flowers were pollinated using a glass slide and the flowers were again bagged. After about 2 weeks of hand pollination, flowers were debagged and allowed to set the fruits. Hybrid seeds were germinated in nursery beds and 4–5-month-old seedlings were field planted as mapping population. Mapping population was established in IFGTB field research station at Panampally (10°47'N; 76°45'), Kerala. One hundred F₁ hybrid individuals along with their parents were used for genetic linkage map construction and phenotyping of rooting parameters for QTL studies.

Phenotyping of mapping population

Adventitious rooting experiments were conducted in March–April (in the years 2013, 2014, and 2015), which are considered to be the suitable period for rooting. Four

independent settings were carried out to assess the rooting potential of F_1 hybrids of *E. tereticornis* × *E. camaldulensis*. Total of ten cuttings per clone were assessed for rooting parameters in each setting. One-year-old trees of the mapping population were cut back to stimulate coppice shoots, and after 45 days, shoot cuttings were harvested. The basal tip of two node cutting was dipped in a 2000 ppm indole-3-butyric acid (IBA) in talc and placed into a vermiculite medium. The shoot cuttings were kept for 30 days in polytunnels (70–90% humidity) under mist irrigation and transferred to shade net house for acclimatization. After 15 days of growth in shade net house, the plants were phenotyped for percent rooting (number of cuttings rooted/total number cuttings placed for rooting), average root length (length of all the roots/total number of roots), and number of roots.

Genotyping of mapping population

Screening experiments for SSR primer polymorphism detection was carried out using the two parents and 4 F_1 individuals. Three hundred and twenty SSRs (269 genomic SSRs and 51 EST-SSRs) developed from different species of *Eucalyptus* were cross amplified. SSR-PCR amplifications were carried out and separated on 5% denaturing polyacrylamide gel electrophoresis (PAGE) followed by silver staining and documentation. Forty six ISSR primers were screened, and PCR amplification, agarose gel electrophoresis, and documentation of results were carried out as reported by Balasaravanan et al. (2005). A total of 24 combinations were made from seven forward and seven reverse primers (Alghamdi et al. 2012; Li and Quiros 2001) to assess the SRAP polymorphism in parental clones. SRAP amplification was carried out as reported by Li and Quiros (2001) and the products were separated on 5% denaturing PAGE. The gel was run at 220 V constant power for 2 h and bands visualised by silver staining. SSR, ISSR, and SRAP primers showed polymorphism was assayed in the mapping population to generate the genotypic data. The details of successful primers are provided as Supplementary material in the Table S1, S2, and S3.

Genotypic data analysis and linkage map generation

Microsatellite markers were scored for their allele size in bp. ISSR and SRAP markers were scored as '1' for presence and '0' for absence of a specific band amplified over the parents and F_1 individuals. ISSR and SRAP markers were named after the primer serial number and the approximate fragment size. Chi-square (χ^2) test was performed to identify alleles that deviated from Mendelian segregation ratios. Markers that deviated from the theoretical expected ratios were considered as distorted with different significance

levels ($P < 0.01$, $P < 0.001$, and $P < 0.0001$) and used for further analysis.

Linkage analysis was performed using the JoinMap 4.0 software (Van Ooijen 2011) with the cross-pollination (CP) option. The data set was separated into a maternal and a paternal data set for map construction using the “two-way pseudo-testcross” (PTC) approach (Grattapaglia and Sederoff 1994). Linkage groups were estimated by applying independence LOD (logarithm of odds) threshold ranges from 1 to 10. Genetic distances were calculated based on recombination frequencies according to Kosambi mapping function. The SSR markers were assigned to the linkage groups based on the information in the published literature (Grattapaglia et al. 2015). Marker ordering was performed with regression mapping option with the following parameters: recombination frequency ≤ 0.4 , LOD ≥ 2.0 , goodness-of-fit jump threshold for removal of loci = 5, ripple value 1, Kosambi's mapping function, and third round = yes. Finally, using the Mapchart 2.1 software (Voorrips 2002), male and female maps were generated.

The parent-specific maps generated in JoinMap were integrated using the “Combine groups for map integration” function of JoinMap to produce the consensus map for each mapping population. This method uses mean recombination frequencies and combined LOD scores for mapping calculations. Linkage group marker orders of consensus maps were compared to individual parental maps, any marker order disagreements were investigated, and poorly fitting markers and highly distanced markers were removed from the group.

Comparison of consensus map with eucalypt genome sequence

To search for physical locations of the mapped SSR loci *E. grandis*, genome sequence Phytozome v.11 (Version 2.0 available in Phytozome 11—<http://www.phytozome.net/> accessed on October, 2017) was used for alignment with sequences containing SSR markers. The parameters for BLASTN program were as follows: Expected (*E*) threshold = 10, comparison matrix = Blosum62, alignment to show = 100, allow gaps = yes, filter = yes. Only the final framework integrated microsatellite markers were evaluated, and other markers were omitted. To align microsatellite sequences onto the *E. grandis* genome, all the available microsatellite sequences were derived from NCBI. Functional annotation of the genomic region corresponding to the microsatellite was determined using BLASTX reporting the best hit with a cut-off *E*-value 10^{-5} . When more than one hit was observed, best hit was identified with lowest *E*-value.

The alignment of microsatellite loci onto the physically mapped *E. grandis* genome assembled into the 11 pseudomolecules was carried out. The ratio between physical sequence length in Mbp and genetic distance in cM was

calculated by dividing the average Mbp intermarker distance per pseudomolecule by the average cM intermarker distance per linkage group.

Quantitative trait loci (QTL) analysis

QTL analysis was carried out using interval mapping (IM) and composite interval mapping (CIM) procedure in Win-QTL Cartographer v 2.5 (Wang et al. 2007) to identify putative QTL locations and distances, maximum value of LOD score, the additive effect, and the percent of phenotypic variations explained by each QTL. Permutation analysis with 1000 repetitions was used to determine LOD threshold. The analysis window size was maintained at 10 cM with a walk speed (mapping resolution) of 1 cM and control marker number five. Stepwise regression (forward method) under model 6 and the LOD score (> 2.5) corresponding to $P = 0.05$ were used to identify significant QTL.

Results

Marker segregation

Among the 320 SSRs screened, 86 polymorphic primer pairs were selected for genotyping based on the segregation pattern and allele size of the loci (Supplementary material, Table S1) and details on SSR allele size of the mapping population are presented as Supplementary material in Table S2. Eighteen ISSR primers were selected for genotyping of Et217 × Ec17 and a total of 155 loci were amplified, with an average of 8.6 loci per primer. Of the 155 loci, 111 loci (72%) were polymorphic of which 51 were specific to *E. tereticornis* (Et217) and 60 were specific to *E. camaldulensis* (Ec17) (Supplementary material, Table S3). Fifty-one loci were maternally informative and 60 loci were paternally informative. Among the 24 SRAP primers, 14 detected 166 loci with an average of 11.9 loci per primer. Of the 166 loci, 112 loci (67.8%) were polymorphic of which 50 were specific to *E. tereticornis* (Et217) and 62 loci were specific to *E. camaldulensis* (Ec17) (Supplementary material, Table S4). The details on marker type, segregation pattern, male and female informativeness, and Chi-square significance are shown in Table 1.

Linkage map generation

To generate linkage maps, totally, 309 markers (86 SSR, 111 ISSR, and 112 SRAP) were used for analysis. Framework maps were generated with LOD 3.0 and 283 markers (84 SSR, 94 ISSR, and 105 SRAP) were found suitable to place on linkage map. A total of 149 markers were mapped in the female parent, *E. tereticornis* (Et217), and distributed among 11 linkage groups covering 1034.8 cM. The average distance between two markers was 7.3 cM and the linkage groups ranged from 69.0 to 125.8 cM in size (Supplementary material, Table S5). The male parent, *E. camaldulensis* (Ec17), consisted of eleven linkage groups covering 1100.9 cM with 170 markers (Supplementary material, Table S6). The average distance between two markers in male map was 6.7 cM and the linkage groups ranged from 75.2 to 135.9 cM in size. Consensus map spanned a total length of 1163.4 cM with 25.7 markers in average for each LG with the length range of 80.9–144.2 cM (Table 2; Fig. 1).

Anchoring of consensus map to physical map of *E. grandis*

To anchor the SSR markers mapped on the consensus map of eucalypts to the genome assembly of *E. grandis*, marker position and order of microsatellites were evaluated. Comparison of consensus map with the physical map of *E. grandis* and alignment of linkage groupwise SSR markers are given as Supplementary material (Fig. S1). All the SSR markers (84) except one were physically assigned to the sequenced genome of *E. grandis* by matching the chromosome scaffold number and linkage group number of the consensus map. The location of the loci, Embra147, on the genome scaffolds of *E. grandis* could not be determined, as they did not return any hit. Details on the linkage groupwise information on number of markers, total size, intermarker distance in cM, number of physically mapped markers, physical distance covered, intermarker distance in Mbp, and ratio between physical sequence distance (Mbp) are given in Table 2. About 64% of the mapped SSR markers used in alignment were colinear with the sequence assembly, indicating that the genetic map could achieve adequate fidelity with *E. grandis* genome sequence. Thirty-one SSR

Table 1 Details of marker types, segregation pattern, informativeness, and Chi-square significance in *E. tereticornis* (Et217) × *E. camaldulensis* (Ec17)

Marker	Segregation type		Parent informative-ness		Chi-square significance			
	1:1	1:1:1:1	Female	Male	Non-deviated	$P < 0.01$	$P < 0.001$	$P < 0.0001$
SSR	50	36	24	26	61	14	12	27
ISSR	111	0	51	60	17	5	7	82
SRAP	112	0	50	62	19	6	8	79

Table 2 Mapping statistics of consensus map and its alignment to the 11 pseudomolecules of the *E. grandis* reference genome sequence

LG/chromosome	SSR	ISSR	SRAP	Total	Total size (cM)	Intermarker distance (cM)	Physically mapped markers	Physical distance covered (Mbp)	Intermarker distance (Mbp)	Ratio (Mbp/cM)
1	13	5	14	32	88.7	2.8	12	39.7	3.3	1.1
2	10	6	9	25	123.1	4.9	10	46.9	4.6	0.9
3	3	9	14	26	80.9	3.1	3	56.1	18.6	5.9
4	9	9	11	29	81.2	2.8	9	38.1	4.2	1.5
5	9	12	10	31	107.4	3.5	10	68.7	6.8	1.9
6	8	10	8	26	90.5	3.5	8	48.8	6	1.7
7	10	8	3	21	100.2	4.8	10	43.8	4.3	0.9
8	8	6	7	21	144.2	6.8	8	69.6	8.7	1.2
9	4	13	11	28	133.4	4.8	4	32.1	8	1.6
10	5	9	8	22	82.5	3.8	5	13.7	2.7	0.7
11	5	7	10	22	131.3	5.9	5	39.8	7.9	1.3
Total	84	94	105	283	1163.4	—	84	497.3	—	—
Mean	7.6	8.5	9.5	25.7	105.7	4.2	105.8	—	105.9	1.7

loci showed homology with genes having varied functions (Supplementary material, Table S7).

QTL mapping of rooting traits

QTL localization on genetic linkage map is carried out to identify regions of a genome associated with variations in a quantitative trait under study. In this study, a total of six putative additive QTL that underlie rooting percent and root length were identified and mapped on five different chromosomes of the eucalypt genome (Table 3; Fig. 1). Five QTL were identified in IM and one in CIM analysis. Phenotypic variations explained by QTL governing rooting percent were ranging from 12.1 to 14.9%. A QTL (M1E9-463) with higher LOD value of 3.7 was associated with root length. The additive effects of all QTL except one (ISSR50_475) were negative for both the traits in the Et217×Ec17 population. No QTL was detected for the number of adventitious roots produced from shoot cuttings.

Discussion

Eucalyptus being an industrially important tree species, application of molecular marker-assisted tree improvement could help the paper industry to develop improved and superior germplasm to grow trees in marginal lands. Implementation of marker-assisted selection (MAS) necessitates the development of genetic map to identify markers associated with genomic regions governing traits of interest. In the present study, attempts were made to construct the genetic linkage map in the interspecific cross employing red gum eucalypts, *E. tereticornis* and

E. camaldulensis, cultivated in larger areas of South East Asia. Several linkage maps including a few high-density linkage maps developed in eucalypts mainly targeted *E. globulus*, *E. grandis*, and *E. urophylla*, the species predominantly grown in Brazil, Australia, and South Africa (Freeman 2014). These maps were constructed using different types of markers like RAPD, AFLP, SSR, DArT, and SNP. In this study, both non-anonymous (SSR) and anonymous (ISSR and SRAP) markers were used. Like any other dominant markers, SRAP has frequently been employed for the construction of linkage maps and identification of quantitative trait loci (Guo et al. 2014). SRAP markers reveal comparable levels of variation to AFLP markers with significantly less technical input and cost for similar levels of polymorphism and reproducibility (Li and Quiros 2001). Development of SRAP and SSR marker-based genetic linkage maps of *Psidium guajava* belonging to Myrtaceae was reported with the purpose to map QTL (Padmakara et al. 2015). Dominant markers such as RAPD are continuously used in *Eucalyptus* for mapping purposes (Barthalomé et al. 2013). Till date, no reports are available on the use of SRAP markers in eucalypts. Compared to RAPD, the use of ISSR markers are also limited in eucalypts except for few studies including genetic diversity analysis (Balasaravanan et al. 2005; Chezhian et al. 2010).

This study exploited the SSRs developed for other species of eucalypts and the cross transferability recorded was 76%. Comparable levels of cross amplification were recorded across different species of eucalypts (Brondani et al. 2006). Eighty-four SSR markers were mapped as the segregation distortion (22.8%) had a little impact on map order or length. Similarly, in *E. tereticornis*×*E. urophylla*, about 48% of the SSR markers showed polymorphism and useful for mapping

Fig. 1 Consensus linkage map of *Eucalyptus tereticornis* (Et217) × *E. camaldulensis* (Ec17) showing QTL positions. The consensus linkage map was constructed with 283 markers (84 SSR markers, 94 ISSR and 105 SRAP) covering 11 linkage groups with a total length of 1163.4 cM (1049.5 cM). Markers in red colour are SSR markers, those

in black colour are ISSR markers, those in blue colour are SRAP markers, and those in underlined markers are common between maternal and paternal maps. Green boxes are QTL for rooting percent (identified by interval mapping and pink box is QTL for root length identified by composite interval mapping)

of which 25.6% of the SSRs showed segregation distortion (Li et al. 2015).

Segregation deviation (SD) is a frequent phenomenon encountered in interspecific mapping populations (Liu et al.

2012; Zhou et al. 2015) and is also common in eucalypts (Brondani et al. 2006; Li et al. 2015). Segregation-deviated markers are generally assumed to form separate clusters on the linkage groups or present through the entire length of

Table 3 QTL analysis related to rooting percent and root length traits in *E. tereticornis* × *E. camaldulensis*

Trait	Linkage group	Locus name	Left marker	Right marker	LOD	PV (%)	Additive
Interval mapping							
Rooting percent	2	ISSR50_475	M14E10-356	ISSR50_700	3.0	14.8	11.2
	5	ISSR27_900	ISSR48_1000	M13E13-278	2.7	12.2	– 10.9
	5	M14E12-296	M13E13-278	ISSR48_750	2.9	14.9	– 11.3
	10	M1E10-388	EMBRA194	ISSR16_625	2.7	12.8	– 10.9
	11	ISSR13_1800	M12E10-338	ISSR13_1700	2.65	12.1	– 10.2
Composite interval mapping							
Root length	9	M1E9-463	ISSR1_800	M1E10-475	3.75	16.7	– 1.5

PV phenotypic variation

LGs (Kullan et al. 2012). As reported by earlier researchers, all the distorted markers were used for mapping to avoid larger gaps on the LGs (Brondani et al. 2006; Kullan et al. 2012). These markers can be included in linkage map and QTL studies of tree species unless they distort the map structure (Bodénès et al. 2016). Segregation deviation might be linked to several factors, such as viability selection (Myburg et al. 2014; Zhou et al. 2015), difference in genomic structure between parents of mapping populations (Truco et al. 2007), distortion of the transmission between genetically divergent genome (Fishman et al. 2001), finite sampling, sampling errors, and genotyping errors (Alheit et al. 2011).

Total number of linkage groups, total map length, and the average distance between markers obtained in this study were comparable with the previous reports in eucalypts. The individual parent-specific map generated in this study for *E. tereticornis* was 1035.8 cM. The map size reported in this study is higher than *E. tereticornis* map generated only with AFLP markers, wherein 919 cM was obtained with 14 LGs (Marques et al. 1998). However, in the same species using RAPD markers and JoinMap software, Gan et al. (2003) reported 1306 cM size across 23 linkage groups. In another study by Yu et al. (2012), a linkage map of size 1488 cM for *E. tereticornis* using EST-CAPS and RAPD markers with 21 linkage groups was reported. Recently, Li et al. (2015) generated linkage map of 1241 cM length with 585 DArT, SSR, and EST-CAPS with JoinMap. Such inconsistency in length of linkage maps are attributable to type of markers, recombination rate variations in the mapping population, and mapping algorithms used. In full-sib family of *E. camaldulensis*, 1236 cM genome coverage was observed with RAPD, AFLP, and SSR markers (Agrama et al. 2002), whereas, in the present study, the map size was 1101 cM. A study by Zarpelon et al. (2015) reported 1045 cM for *E. camaldulensis* using only SSR markers. Similarly, in *E. grandis*, more than 15 linkage maps were generated using AFLP, RAPD, SSR, and DArT markers individually or in combination, with the linkage map length ranging from around 1000 cM (Kullan et al. 2012; Bartholomé et al. 2015) to 1815 cM

(Brondani et al. 2006). The present study also observed the map length of 1047 cM with 172 markers belonging to SSR, ISSR, and SRAP, which is in accordance with the earlier reports.

The pseudo-testcross mapping strategy employed in this study was facilitated by the microsatellite markers for the generation of consensus and map. The consensus linkage map spanned a total length of 1163.4 cM with the mean distance between the markers of 4.24 cM and the total number of markers was 283 (84 SSRs, 94 ISSRs and 105 SRAP markers). In eucalypts, several consensus maps have been reported with the size variation from 560 cM in *E. gunnii* × *E. globulus* (Vaillancourt et al. 1995) to 1462 cM in *E. nitens* (Byrne et al. 1995). SSR only consensus map with 437 microsatellites (295 genomic SSRs and 142 EST-SSRs) was constructed, covering 1065 cM with an average distance between adjacent markers equal to 2.6 cM (Grattapaglia et al. 2015). In the current consensus map, the number of markers placed in each LG was lesser than the DArT and SSR-based linkage maps reported earlier (Hudson et al. 2012a; Kullan et al. 2012; Petroli et al. 2012). The presence of a few gaps larger than 10 cM on the LGs of 7, 8, 10, and 11 observed in the consensus map represents low-degree polymorphism in those regions and similar observations were reported from other tree species like *Hevea brasiliensis* (Pootakham et al. 2015). Addition of more number of sequence known markers such as SNPs, DArTs, InDels, and SSR would further benefit QTL analysis.

The genome synteny and colinearity among the eucalypt species belonging to different sections were compared and found that high levels of colinearity and synteny exists among the genomes of eucalypts (Marques et al. 2002; Hudson et al. 2012b; Petroli et al. 2012; Grattapaglia et al. 2015). The *E. grandis* genome organized into 11 pseudomolecules and 4941 smaller unanchored scaffolds covering 640 Mb of sequences (Myburg et al. 2014). Physical alignment of the consensus map of *E. tereticornis* × *E. camaldulensis* showed 64% marker ordering, whereas Grattapaglia et al. (2015) showed 84% of

overall colinearity between SSR-based genetic map and physical positions. Similarly, recent study on synteny and colinearity of *Eucalyptus* and *Corymbia* showed the significant correlation of marker order within the species and intrachromosomal rearrangements (Butler et al. 2017). Comparatively lesser amount of colinearity observed in this study could be due to relatively small mapping population used, wherein the genomic region having too little recombination did not allow precise placement or orientation. Furthermore, inconsistency among the linkage maps could arise due to recombination heterogeneity between different populations and different mapping algorithms and thresholds statistics used for constructing the maps (Gustafson et al. 2009).

Several reports are available on the genes associated with rooting-related traits in agricultural (Ye et al. 2018) and horticultural crops (Moriya et al. 2015; Knäbel et al. 2017), whereas information on genomic regions controlling rooting traits in forest trees is very limited. QTL controlling adventitious rooting-related traits have been identified for pine (Shepherd et al. 2006), poplar (Zhang et al. 2009) and *Quercus* (Scotti-Saintagne et al. 2005). In eucalypts and *Corymbia*, QTL for rooting traits have been identified in different species and the results indicated that in interspecific hybrids, a large proportion of the variation is controlled by a relatively small number (2–7) of major effect QTL explaining phenotypic variations of 17 to 73.2% (Grattapaglia et al. 1995; Marques et al. 1999; Shepherd et al. 2008). In pure species population such as *E. nitens*, the phenotypic variation for rooting traits was 3.1–6.4% (Thumma et al. 2010). In the present study, *E. tereticornis* × *E. camaldulensis* population revealed five QTL for rooting percent and 12.0–14.8% phenotypic variation (Table 3), which was in agreement with the previous reports on interspecific hybrid populations which was due to between-species effects and possibilities of oligogenic effects (Shepherd et al. 2006). Although several other factors such as size of mapping populations, marker density and type of marker play a role in QTL identification (Sexton et al. 2014). Small-effect QTL were identified when SNP markers were used (Thumma et al. 2010), whereas RAPD, AFLP, and SSR markers could detect the major effect QTL (Grattapaglia et al. 1995; Marques et al. 1999); furthermore, these DNA markers influenced the density of map. The negative additive effect observed in this study indicated the contribution of alleles related to rooting percent and root length by *E. camaldulensis*, a best rooting phenotype. Similar observations were reported on contribution of favourable alleles from male parent in several interspecific crosses (Grattapaglia et al. 1995; Shepherd et al. 2008; Hussain et al. 2017). These results substantiate that adventitious rooting in eucalypts is governed by both larger and smaller effect QTL, and genetic dissection of major effect QTL will pave the way for the identification of major genes involved in

rooting process as well as effective strategies for practicing marker-assisted selection.

Conclusion

In conclusion, a genetic linkage map was constructed for red gum eucalypts, a forest tree species of high relevance to India. A few QTL with major effects were localized for rooting percent and root length in the interspecific cross *E. tereticornis* × *E. camaldulensis*. Although several studies were completed in locating genes with relevance to cellulose and lignin content, fibre parameters, and resistances to various biotic stress factors, new research on adventitious rooting characteristics is highly essential. Furthermore, many of the SSR regions have homologies with sequences showing gene function facilitating gene-based analysis. This would activate the research on hybrid breeding and marker-assisted selection in eucalypts. Emphasis needs to be given to exploit the hybrid vigour of interspecific crosses to improve productivity and ensure sustainability of *Eucalyptus* plantations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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