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ORIGINAL ARTICLE

# Quantitative trait loci (QTL) for salinity tolerance traits in interspecific hybrids of *Eucalyptus*

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**Abstract** Soil salinity is one of the major limiting factors in productivity of plants. Cultivation of industrially important fast growing saline tolerant tree species is one of the options to reclaim the saline soils. Some of the *Eucalyptus* species are salt tolerant and production of interspecific hybrids of these species would enhance productivity in saline environments. In this study, phenotypic parameters for growth, physiology and mineral nutrition were estimated in *Eucalyptus camaldulensis* × *E. tereticornis* F1 hybrids to understand the mechanism of salinity tolerance and localize quantitative trait loci (QTL) involved in sodium chloride (NaCl) stress. Salt injury scoring and plasma membrane damage showed a significant difference between tolerant and susceptible individuals, which was correlated with the gas exchange measurements and $Na^+$, $K^+$ and $K^+/Na^+$ ratio. Under salinity, correlation of gas exchange measurements showed strong positive correlations between the traits, *A*net, *g*s, *C*i and *E* indicated the role of stomatal function. It was inferred that sequestration of NaCl by the salt tolerant individuals was through compartmentalization of $Na^+$ and its detoxification by maintenance of $K^+/Na^+$ ratio. Totally, 33 QTL were identified under salinity and control conditions. Co-localization of QTL regulating $Na^+$ and $K^+$ transport substantiated their influence in salinity tolerance which could be due to the closely linked genes or by pleiotropic effect of same genes on these traits. Fine mapping with more molecular markers will locate the QTL precisely and validating with field trails could hasten the traditional methods for salinity breeding.

**Keywords** *Eucalyptus* · NaCl stress · SSR markers · Quantitative trait loci

## Introduction

Abiotic stresses affect the plant growth, among which salinity is the major stress limiting plant productivity. Salinity and sodicity pose threat to about 800 Mha land area globally (FAO 2005) and 6.74 Mha in India (Mandal et al. 2010). Reclamation of salt affected soils can be achieved through various practices including soil amendments and cultivation of salt tolerant plants. Establishment of fast growing forest tree plantations in saline soils may facilitate wood security and sustainable tree cover. The genus *Eucalyptus* consists of many industrially important tree species with potential to grow in saline conditions. Various species of *Eucalyptus* show a wide range of gradations to salinity tolerance. *E. camaldulensis*, a resource, providing raw material for paper industries is grown widely in semi arid regions of India shows higher tolerance to salinity (Allen et al. 1994). Experimental trials under controlled and field conditions revealed the existence of an intraspecific variability for salinity tolerance (Cha-um et al. 2013; Marcar 2016). Although inter and intraspecific hybridization has potential for salinity tolerance breeding in *E. camaldulensis* (Dale and Dieters 2007), very little progress has been made in developing salt tolerant eucalypts using traditional breeding approaches.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s40502-018-0403-7) contains supplementary material, which is available to authorized users.

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Physiological, biochemical and molecular functions in a plant system determines the level of its tolerance to soil salinity (Gupta and Huang 2014). Many of the plants have naturally evolved mechanisms to overcome salinity stress conditions through diverse biochemical and physiological processes. Progress in understanding the physiology and biochemical mechanisms underpinning tolerance has been made in different eucalypt species. It was observed that in *E. camaldulensis* maintenance of relatively low leaf $Na^+$ and $Cl^-$ concentrations by avoidance of excess ions in expanding and expanded leaves contributed to salinity tolerance (Marcar 1993). Whereas, other salt tolerant species such as *E. sargentii*, *E. spathulata* and *E. loxophleba* had increase in net selectivity for $K^+$ over $Na^+$ (Adams et al. 2005). Stomatal conductance and water use efficiency was assessed among the clones of *E. camaldulensis* and hypothesised that tolerant clones have the ability to control uptake of ions by roots or limit ion transport to leaf tissues (Farrell et al. 1996). Functional characterization of HKT gene from *E. camaldulensis* (EcHKT) in *Xenopus laevis* oocytes showed its ability to sense changes in the external osmolarity in the environment (Liu et al. 2001) which was not observed in other HKT genes (Waters et al. 2013). Anatomical parameters were correlated with salt tolerance of *E. largiflorens*, a putative hybrid species, where individuals with lower xylem vessel diameter showed higher salt tolerance and enhanced productivity over other individuals with larger xylem vessels (Zubrinich et al. 2000). Similarly, chloride toxicity in eucalypts was overcome by the compartmentalization of chloride in stem bark tissue, which was regularly shed (Feikema et al. 2012). In tissue culture raised plants of *E. camaldulensis* and *E. camaldulensis* × *E. urophylla*, susceptible individuals had higher accumulation of proline, reduced net photosynthetic rate and growth performance, whereas the salt tolerant individuals showed reduction in proline content and better physiological and growth performance (Cha-um et al. 2013). Salt tolerance potential of *E. camaldulensis* was manifested through better stomatal control, water use efficiency, dilution of toxic ions by decreasing the specific leaf area and higher root/aerial biomass ratio (Sixto et al. 2016). In the same study, gene expression analysis were conducted and found that the salt tolerant *E. camaldulensis* had lesser modifications of genes linked to wood formation. Recently, *E. camaldulensis* was advocated for phytostabilization of trace elements and leaves displayed the potential for biomonitoring soil extractability of Cd, Mn and Zn (Madejón et al. 2017).

In agricultural crops, genetic variations for salt tolerance between and within species are exploited to introduce salt tolerant genes in high productive varieties through traditional breeding (Munns 2005). Similarly, interspecific hybrids of eucalypts exhibit faster growth than either of their pure species parents, and highly preferred for biomass and pulping properties. Likewise, breeding for salt tolerance through novel hybrids between *E. camaldulensis* × *E. grandis* and *E. camaldulensis* × *E. globulus* were developed in Australia for the combination of salt tolerance of *E. camaldulensis* with the growth rate and wood property of *E. grandis* and *E. globulus* (Dale and Dieters 2007; Feikema et al. 2012). Random amplified polymorphic DNA (RAPD) markers derived genetic map was used to identify QTL for salt tolerance traits in *E. camaldulensis* × *E. grandis* and three QTL with 3–5% effects were observed (Dale et al. 2000).

Considerable variations displayed in salt tolerance of eucalypt species permits the application of DNA markers for genetic mapping and localization of quantitative trait loci (QTL). Although several interspecific hybrids of eucalypts were targeted for high density genetic maps (Freeman 2014; Grattapaglia et al. 2015), major traits considered for QTL identification were wood properties (Thumma et al. 2010) with popular hybrid combinations between *E. grandis*, *E. urophylla* and *E. globulus*. Despite *E. camaldulensis* being the economically important species for India, only very limited research has been attempted on salinity tolerance. Thus, the present study attempts to develop genetic map and identify QTL for traits related salt tolerance in the interspecific cross *E. camaldulensis* × *E. tereticornis*.

## Materials and methods

### Plant material

Salinity tolerance experiments were carried out in an interspecific cross, *E. camaldulensis* (clone Ec7) × *E. tereticornis* (clone Et88). Individuals used as parents for controlled hybridization are part of superior performing clonal selections of the ongoing breeding program of *E. camaldulensis* and *E. tereticornis* at Institute of Forest Genetics and Tree Breeding, Coimbatore, India. Originally, breeding populations of these species were established from the seeds obtained from CSIRO, Australia during the year 1996.

In the preliminary experiments, 25 clones of each species were assessed for salt tolerance and clones Ec7 and Et88 were selected as salt tolerant and salt susceptible respectively based on their survival percentage at 400 mM sodium chloride (NaCl) treatment. Controlled hybridization between clones Ec7 and Et88 was carried out at Karunya, Coimbatore, Tamil Nadu (11°00′N latitude, 76°58′E longitude) following the standard procedures. F1 hybrid seeds harvested were germinated and field planted in the vegetative multiplication garden at the Institute of

Forest Genetics and Tree Breeding, Coimbatore. After six months, plants were coppiced and stem cuttings were vegetatively propagated (Shanthi et al. 2015). Rooted cuttings were grown for 90 days with regular watering and used for the experiments.

### Fixing of critical NaCl concentration

Two hundred and fifteen F1 hybrid plants along with their parents (Ec7 and Et88) were subjected to NaCl treatments in plastic pots. Experiments were conducted in seven pots consisting of four plants each, where six pots were used for treatment and one pot was control. Before NaCl treatment, uniform sized rooted stem cuttings were grown with Hoagland's nutrient solution in pots for 14 days. Pots with nutrient medium were supplemented with NaCl by gradual increase of salt concentration from 100, 150, 200, 250, 300, 350, and 400 mM. Control plants were maintained in nutrient solution throughout the study. Treatment of each concentration was extended for 14 days. The nutrient solution was replaced once in 5 days and the pH of nutrient medium was maintained at 5.6 by adding 1.0 M NaOH or 1 N HCl throughout the period. The experiments were conducted on a nursery bench under shade house with the provision to keep rain out. At the end of the each NaCl treatment, survival percentage of the plants were calculated which determined their tolerance to salinity level. According to the survival percentage at 400 mM NaCl treatment, plants were ranked into four different categories viz. T—highly tolerant (> 70%); MT—moderately tolerant (50–69%); S—susceptible (26–49%); and SS—highly susceptible (< 25%). Beyond 300 mM NaCl, the number of plants available in each F1 hybrid individual was very limited making phenotyping difficult and hence 300 mM NaCl was fixed as critical concentration for phenotyping experiments. Hundred F1 individuals (25 individuals selected from each salt tolerance group) were selected randomly and used for phenotyping of traits.

### Trait measurements

Selected F1 hybrid individuals (100) belonging to different categories and parents (2) were vegetatively propagated from the original stock plants. Totally, 2448 plants propagated from 102 individuals were included in the experiment. Every individual was represented by 24 plants with 12 plants each for control and treatment. As described earlier, NaCl stress was imposed gradually from 100 mM until the desired critical concentration of 300 mM was reached. In total, 17 traits were measured after 14 days in 300 mM NaCl treatment and 15 traits (excluding salt injury scoring and leaf abscission rate) were measured in control plants. All measurements were made in triplicates under control and treatment conditions. Reduction in dry weight of leaves (LRDW), stem (SRDW) and roots (RRDW) were estimated by comparing treated and control samples. Salt injury scoring (SIS) was carried out on 1–9 scale to assess the salt damage to plants (Gregorio et al. 1997): 1—normal growth with no symptoms; 3—nearly normal growth but leaves become sluggish and juvenile leaves started to dry; 5—growth retarded and juvenile leaves completely dried; 7—complete cessation of growth and mature leaves started to dry and 9—plant dried completely.

To determine the leaf abscission rate (LAR), difference in total number of leaves present in each individual before the NaCl treatment (N0) and at the end of 300 mM NaCl treatment (Nt300) was recorded. LAR was calculated as (Number of abscised leaves/Number of leaves present initially) × 100 as recommended by Abbruzzese et al. (2009). Dry weight (DW) was assessed according to Ghoulam et al. (2002) for leaves, stem and root by drying in hot air oven at 70 °C for 3 days to achieve a constant weight. Reduction of dry weight in leaf (LRDW), stem (SRDW) and root (RRDW) were calculated in comparison to control, using the formula [(1 − (treated/control)] × 100. Relative water content (RWC) was estimated by collecting the youngest fully expanded leaves. Leaf sample of 2 × 2 cm size were weighed immediately to obtain fresh weight (FW) and samples were floated in deionized water for 12 h at room temperature under conditions without light. Turgid weight (TW) was obtained after removing the superficial droplets of water from leaf sample. The same sample was dried in a hot air oven at 70 °C for 3 to 4 days until a constant dry weight (DW) was achieved. Relative water content was estimated as described by Canavar et al. (2014) using the formula RWC (%) = [(FW − DW)/(TW − DW)] × 100.

Plasma membrane damage (PMD) is assessed to determine the membrane stability of plants (Lutts et al. 1996). Five leaf discs of 1 cm diameter were incubated in 20 ml deionised water on a rotary shaker at room temperature for three hours and electrical conductivity of the solution (C1) was determined. Same samples were then autoclaved at 120 °C for 20 min and electrical conductivity (C2) was determined and PMD was estimated as

$$(C1/C2) \times 100.$$

Gas exchange parameters such as photosynthesis rate (*A*net), stomatal conductance (*g*s), intercellular $CO_2$ concentration (*C*i), transpiration rate (*E*) were monitored on fully expanded 4th or 5th leaf of the plants using LI-6400XT portable photosynthesis system (LI-COR Biosciences, USA). $Na^+$ and $K^+$ ions in leaves (LNC and LKC), shoots (SNC and SKC) and roots (RNC and RKC) were estimated by triacid (nitric acid, perchloric acid and sulphuric acid in 9:2:1 ratio) digestion. Sample was diluted

50 times with double distilled water and analysed for $Na^+$ and $K^+$ contents using a flame photometer (Systronics, India) using NaCl and KCl as standard. $K^+/Na^+$ ratio was calculated separately for leaves (LKNR), shoots (SKNR) and roots (RKNR).

### Marker genotyping

Genomic DNA was extracted from the leaf tissues of Ec7, Et88 and F1 hybrid plants using Qiagen DNeasy plant mini kit (Qiagen, USA). Totally, 57 SSR markers were analysed in ABI 3500 genetic analyzer (Applied Biosystems, USA) (Subashini et al. 2014).

### Data Analysis

Trait measurements were analysed with SPSS version 16 (SPSS, Cary, NC, USA) for descriptive statistics. Under control and salinity treatment conditions, estimates of the parents were compared using *t* test. Correlations among the traits were assessed using Pearson correlation coefficient.

Genetic linkage map construction and QTL positioning were carried out using integrated genetic analysis software for clonal F1 and double cross populations Version 1 (GACD) (http://www.isbreeding.net; Zhang et al. 2015). Allelic data of the microsatellites were coded as per the allelic pattern in the parents i.e. male informative (A = B), female informative (C = D) and fully informative (ABCD). Chi square ($\chi^2$) test was performed to identify the marker distorted from the expected Mendalian segregation (1:1 and 1:1:1:1). Genetic linkage map of the parents and consensus map was developed using construction of genetic linkage maps in F1 and double cross population (CDM) functionality. Grouping of the markers was carried out based on anchored marker information as per the published linkage map of eucalypt species (Grattapaglia et al. 2015). Grouped markers were ordered with the algorithm, nnTwoOpt and fine tuning of the ordered chromosome was carried out using SARF (sum of adjacent recombinant frequencies) with the window size of 5. Male map was generated with fully informative and male informative microsatellites and female map was generated with fully informative and female informative microsatellites. Consensus map was generated with all the complete and partial informative microsatellites.

QTL mapping was carried out with inclusive composite interval mapping (ICIM) and mapping of additive and dominance genes using CDQ function and population type clonal F1 and double cross population. A logarithm of odds (LOD) threshold of $> 3$ was set to declare a significant QTL. All traits except reduction in dry weight of leaves, stem and root under control and NaCl treatment were analyzed for QTL and the significant minor and major QTL were positioned on the consensus linkage map. The linkage maps with QTL were displayed with MapChart 2.3 (Voorrips 2002).

## Results

### Phenotypic variation and correlations among traits

Two hundred and fifteen F1 hybrids and 2 parents at 400 mM NaCl treatment were categorized into 41 (19.1%) tolerant individuals, 48 (22.3%) moderately tolerant individuals, 45 (20.9%) susceptible individuals and 81 (37.7%) highly susceptible individuals (Fig. 1, Supplementary Table S1). Salinity tolerant parent, *E. camaldulensis* (Ec7) with survival percent of 75.0 was added to tolerant group and salinity susceptible parent, *E. tereticornis* (Et88) with survival percent of 20.8 was added to the highly susceptible group. Proprieties of the trait distribution were observed using frequency distribution and histograms reflected a wide variation among the F1 hybrids and their distribution showed nearly normal (Supplementary Fig S1). Trait mean values, skewness and kurtosis of F1 hybrids and their parents under control and salinity are provided in Table 1.

Pearson's correlation coefficient among the measured traits under control and salinity are shown in Supplementary Table S2. Under salinity, 26 significant correlations were observed among 17 measured traits. Salt injury score (SIS) was negatively correlated with *A*net, LNC, SNC and LKC and positively correlated with PMD and LAR. Gas exchange parameters (*A*net, *g*s, *C*i and *E*) were inter-related and showed positive correlations ($p \leq 0.01$). LNC showed a significant ($p \leq 0.05$) positive correlation with RNC whereas SNC significantly ($p \leq 0.01$) correlated with LKC and SKC. RNC correlated significantly ($p \leq 0.05$) with RKC, whereas LKC correlated with SKC. Under control conditions, 13 significant correlations were observed among the measured traits (15) indicating high level of correlation under NaCl stress.

### QTL analysis

A consensus linkage map of length 2230.7 cM generated in this study with 57 SSR markers was used to localise QTL for salt tolerance traits. Out of 57 loci, thirty (53%) segregated as per the expected ratio of 1:1 and twenty-seven (47%) distorted from the expected Mendelian ratio ($p \leq 0.01$ and $p \leq 0.001$). Consensus map constructed with all the 57 microsatellites mapped on 11 linkage groups spanning a total map length of 2230.7 cm (Fig. 2). QTL identified under control and salinity are summarised in the Table 2, where the name of the QTL contains the trait name followed by linkage number and control (C) or salinity (S)condition. With a threshold of LOD 3.0, 11 QTL

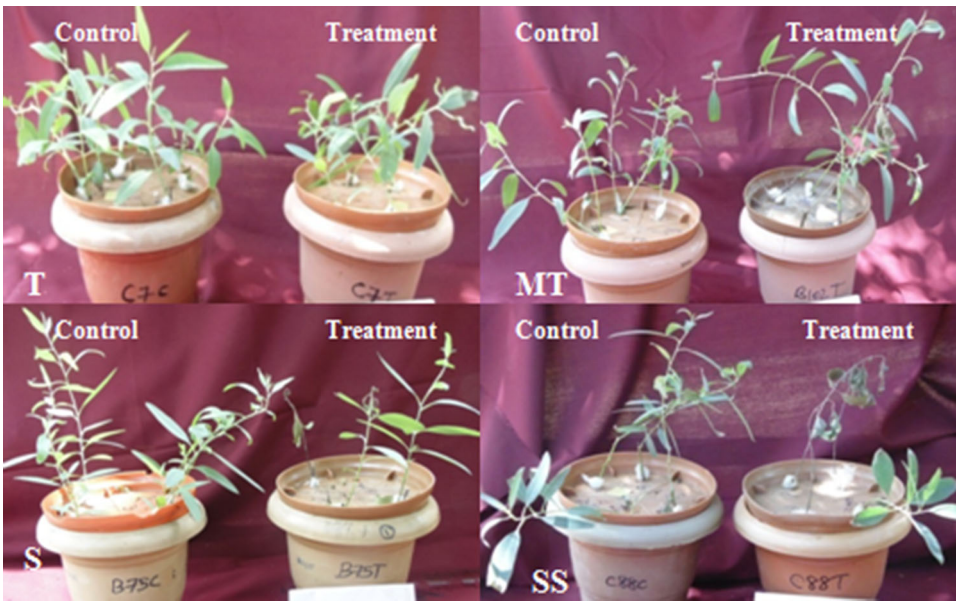


**Fig. 1** Grouping of *Eucalyptus* interspecific hybrids based on salinity tolerance levels

in control and 22 QTL in salinity treatment ($p = 0.01$) were located on 7 linkage groups (Fig. 2). Most of the QTL were localised in three major genomic regions of LG2, LG4 and LG6. The first genomic region between Embra126 and Embra43 on LG2 harboured QTL for LKC and RKC under control (*q*LKC2_C and *q*RKC2b_C) and QTL for SIS, PMD, LKNR and SKNR (*q*SIS2_S, *q*PMD2_S, *q*LKNR2_S and *q*SKNR2_S) under salinity. The second genomic region was between Embra213 and Embra186 on LG4, where QTL responsible for RNC, SKC, LKNR under control (*q*RNC4b_C, *q*SKC4_C and *q*LKNR4_C), and LNC and SKC (*q*LNC4b_S and *q*SKC4b_S) under salinity treatment were localised. Third genomic region between Embra50 and Embra81 had QTL for SNC and RKNR (*q*SNC6_C and *q*RKNR6_C) under control and SNC and LKC (*q*SNC6_S and *q*LKC6_S) under salinity.

Co-localization of QTL for SNC and SKC under control and salinity treatment was observed in LG6 and LG4 respectively. QTL influencing SNC under control (*q*SNC6_C) and treatment (*q*SNC6_S) were localized between Embra50 and Embra81 and QTL for SKC under control (*q*SKC4_C) and treatment (*q*SKC4b_S) localized between Embra213 and Embra186. In addition, QTL responsible for LKC under control (*q*LKC2_C) and SKNR treatment (*q*SKNR2_S) were co-localized at 60 cM between Embra126 and Embra43 on LG2 (Table 2).

## Discussion

Short rotation salt tolerant tree species are significant for reclamation of saline soils. Genetic improvement of trees for salinity tolerance had been attempted and individuals exhibiting salt tolerance were identified (Harfouche et al. 2014). Hybrid selections of eucalypts such as *E. camaldulensis* x *E. grandis* and *E. camaldulensis* x *E. globulus* were proved to produce higher biomass under saline environments (Dale and Dieters 2007). Several studies were conducted in food crops to elucidate the mechanism of salinity tolerance and to identify possible indicators to select tolerant individuals. Phenotypic traits such as SIS, $Na^+$ and $K^+$ concentrations and photosynthesis were considered to be the indicators for selection of tolerant individuals in rice (De Leon et al. 2015). In *Populus*, compartmentalization of $Cl^-$ in root cortex, diminished xylem loading of NaCl, $Na^+$ extrusion into soil and avoidance of $K^+$ loss had been found to be the key salt tolerance mechanisms (Chen and Polle 2009). Eucalypts being important genera with many salt tolerant species, identification of QTL for salt tolerance related traits would assist in targeted breeding for productivity.

This study explored the effect of salinity on the growth, physiology and mineral nutrients in the interspecific cross *E. camaldulensis* × *E. tereticornis*. At the critical concentration of 300 mM NaCl the hybrids showed mixed response to salinity tolerance. Traits estimated had wide variations, indicating transgressive segregation under control and treatment, a feature essential for accumulating QTL related to salinity tolerance (Ghomi et al. 2013).

Salt injury score (SIS) obtained visually through morphological parameters is an important indicator for selection and breeding of salt tolerant varieties (Puram et al. 2018). In rice, SIS was correlated with other traits such as electrolyte leakage, reduction in chlorophyll, reduction in shoot length, shoot $K^+$ concentration, and shoot $Na^+/K^+$ ratio (De Leon et al. 2015). Similarly, in this study under

**Table 1** Phenotyping of the parents and hybrid population in *Eucalyptus camaldulensis* (Ec7) × *E. tereticornis* (Et88) under control and salinity conditions

| S.no. | Traits | Treatment | Parents | | F1 hybrids | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ec7 (Mean ± SE) | Et88 (Mean ± SE) | Mean ± SE | Range | Skewness | Kurtosis |
| 1 | RWC (%) | Control | 86.95 ± 1.74 | 81.32 ± 1.80* | 85.08 ± 1.08 | 52.82–95.94 | − 1.19 | 2.88 |
| 2 | PMD (%) | | 16.18 ± 2.40 | 21.47 ± 0.64 | 29.02 ± 1.31 | 9.53–55.29 | 0.18 | − 0.18 |
| 3 | *A*net (μmol$CO_2$/$m^2$/s) | | 6.11 ± 0.35 | 6.02 ± 0.024 | 4.33 ± 0.30 | 1.12–9.28 | − 0.43 | − 0.36 |
| 4 | *g*s (mol$H_2O$/$m^2$/s) | | 0.460 ± 0.035 | 0.387 ± 0.033* | 0.312 ± 0.017 | 0.137–0.586 | 0.77 | 0.42 |
| 5 | *Ci* (μmol$CO_2$mol/air) | | 334.12 ± 1.99 | 302.4 ± 6.86** | 290.50 ± 13.48 | 207.06–342.71 | − 1.89 | 2.15 |
| 6 | *E* (mol$H_2O$/$m^2$/s) | | 7.68 ± 0.37 | 6.11 ± 0.47** | 5.46 ± 0.36 | 0.99–10.41 | − 0.20 | − 0.49 |
| 7 | LNC (mg/g dwt) | | 3.04 ± 0.07 | 3.03 ± 0.08 | 2.2 ± 0.09 | 1.25–4.99 | 1.31 | 3.64 |
| 8 | SNC (mg/g dwt) | | 2.34 ± 0.03 | 2.64 ± 0.03** | 2.28 ± 0.12 | 0.86–4.83 | 1.11 | 0.87 |
| 9 | RNC (mg/g dwt) | | 2.32 ± 0.10 | 1.41 ± 0.06** | 2.67 ± 0.18 | 1.06–6.52 | 0.80 | − 0.48 |
| 10 | LKC (mg/g dwt) | | 7.85 ± 0.16 | 6.00 ± 0.04** | 5.50 ± 0.25 | 3.00–10.37 | 1.00 | 0.64 |
| 11 | SKC (mg/g dwt) | | 5.20 ± 0.13 | 4.90 ± 0.10 | 5.40 ± 0.3 | 2.13–9.74 | 0.44 | 0.50 |
| 12 | RKC (mg/g dwt) | | 4.50 ± 0.02 | 2.45 ± 0.07** | 4.60 ± 0.49 | 1.90–10.30 | 1.16 | 0.65 |
| 13 | LKNR | | 2.61 ± 0.09 | 1.90 ± 0.01* | 2.66 ± 0.12 | 0.98–5.16 | 1.26 | 2.25 |
| 14 | SKNR | | 2.33 ± 0.07 | 1.80 ± 0.04* | 2.64 ± 0.14 | 0.92–6.00 | 0.77 | 1.80 |
| 15 | RKNR | | 1.93 ± 0.08 | 1.79 ± 0.06 | 1.59 ± 0.09 | 0.55–3.01 | 0.68 | 1.04 |
| 16 | SIS | NaCl | 2.50 ± 0.37 | 5.50 ± 0.62** | 3.95 ± 0.13 | 1.25–6.25 | − 0.07 | − 0.85 |
| 17 | LAR (%) | | 20.68 ± 2.39 | 75.69 ± 7.67** | 44.55 ± 2.85 | 9.38–86.32 | 0.22 | − 1.14 |
| 18 | RWC (%) | | 80.02 ± 0.98 | 61.34 ± 1.49** | 60.61 ± 2.53 | 8.51–92.84 | − 0.33 | − 0.53 |
| 19 | PMD (%) | | 34.62 ± 0.96 | 80.93 ± 1.41** | 56.88 ± 2.17 | 25.74–84.13 | − 0.20 | − 1.16 |
| 20 | *A*net (μmol$CO_2$/$m^2$/s) | | 3.27 ± 0.07 | 1.22 ± 0.24** | 1.93 ± 0.16 | 0.1–5.11 | 0.65 | − 0.15 |
| 21 | *g*s (mol$H_2O$/$m^2$/s) | | 0.057 ± 0.002 | 0.010 ± 0.003** | 0.04 ± 0.003 | 0.012–0.13 | 1.79 | 4.05 |
| 22 | *Ci* (μmol$CO_2$mol/air) | | 270.26 ± 0.76 | 198.55 ± 24.16* | 223.76 ± 14.50 | 156.8–315.73 | 0.08 | − 1.16 |
| 23 | *E* (mol$H_2O$/$m^2$/s) | | 2.10 ± 0.02 | 0.56 ± 0.08** | 1.8 ± 0.13 | 0.37–3.56 | 0.14 | − 1.81 |
| 24 | LNC (mg/g dwt) | | 10.18 ± 0.06 | 3.34 ± 0.08** | 6.18 ± 0.48 | 2.02–16.1 | 0.56 | − 0.68 |
| 25 | SNC (mg/g dwt) | | 7.68 ± 0.16 | 3.02 ± 0.13** | 5.66 ± 0.28 | 2.43–9.45 | 0.55 | − 1.10 |
| 26 | RNC (mg/g dwt) | | 4.59 ± 0.16 | 1.83 ± 0.12** | 3.93 ± 0.28 | 1.80–7.66 | 0.80 | − 4.06 |
| 27 | LKC (mg/g dwt) | | 26.00 ± 0.45 | 2.10 ± 0.11** | 10.53 ± 2.37 | 0.45–27.56 | 0.50 | − 1.18 |
| 28 | SKC (mg/g dwt) | | 16.30 ± 0.21 | 2.40 ± 0.05** | 9.78 ± 2.08 | 1.01–26.3 | 0.64 | − 0.45 |
| 29 | RKC (mg/g dwt) | | 5.12 ± 0.27 | 1.60 ± 0.11** | 3.77 ± 1.71 | 0.15–12.5 | 0.99 | 1.69 |
| 30 | LKNR | | 2.35 ± 0.04 | 0.61 ± 0.05** | 1.55 ± 0.19 | 0.15–2.76 | − 0.30 | − 0.98 |
| 31 | SKNR | | 2.10 ± 0.08 | 0.80 ± 0.03** | 1.89 ± 0.22 | 0.38–3.54 | − 0.06 | 0.33 |
| 32 | RKNR | | 1.11 ± 0.04 | 0.88 ± 0.07** | 0.94 ± 0.16 | 0.05–2.12 | 0.51 | − 0.59 |
| 33 | LRDW (%) | Control versus NaCl | 20.55 ± 1.91 | 57.00 ± 5.40** | 32.05 ± 1.76 | 0.79–77.01 | 0.37 | − 0.68 |
| 34 | SRDW (%) | | 11.70 ± 0.72 | 49.21 ± 1.28** | 38.77 ± 1.73 | 2.88–75.35 | − 0.06 | − 0.64 |
| 35 | RRDW (%) | | 40.42 ± 0.79 | 55.37 ± 4.85 | 36.68 ± 1.68 | 8.44–77.99 | 0.45 | − 0.34 |

*SE* standard error, $h^2$ heritability, *RWC* relative water content, *PMD* plasma membrane damage, *A*net photosynthesis rate, *g*s stomatal conductance, *Ci* intercellular $CO_2$ concentration, *E* transpiration rate, *LNC* leaf $Na^+$ concentration, *SNC* stem $Na^+$ concentration, *RNC* Root $Na^+$ concentration, *LKC* leaf $K^+$ concentration, *SKC* stem $K^+$ concentration, *RKC* root $K^+$ concentration, *LKNR* leaf $K^+/Na^+$ ratio, *SKNR* stem $K^+/Na^+$ ratio, *RKNR* root $K^+/Na^+$ ratio, *SIS* salt injury score, *LAR* leaf abscission rate, *LRDW* leaf reduction in dry weight, *SRDW* stem reduction in dry weight, *RRDW* root reduction in dry weight

**Significant at 0.01 probability level and *Significant at 0.05 probability level

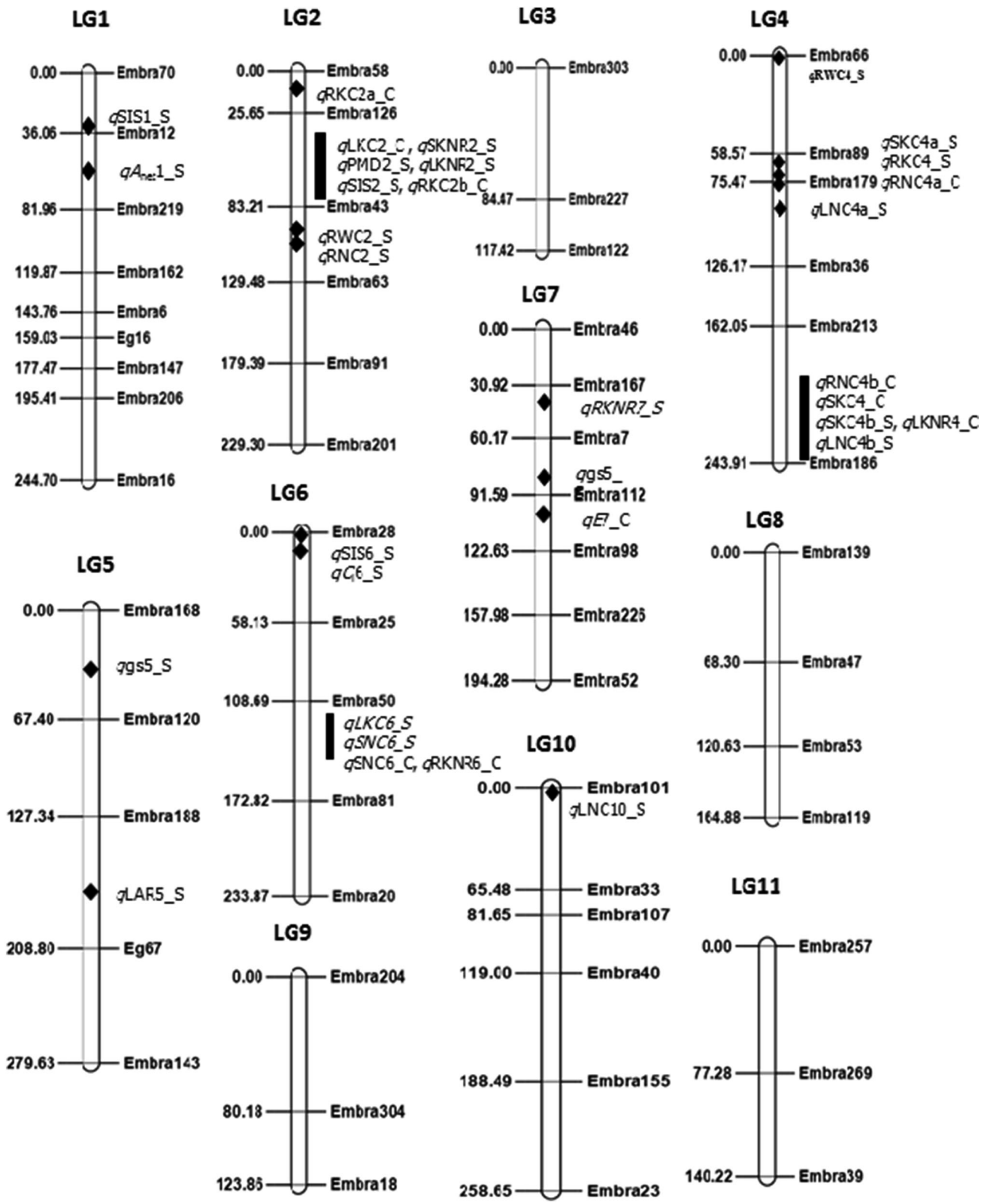


**Fig. 2** Genetic linkage map showing QTL position for measured traits in *Eucalyptus camaldulensis* (Ec7) × *E. tereticornis* (Et88) under salinity and control conditions. (Black square and bar in each LG represents the approximate position of QTL)

**Table 2** Map positions and genetic effect of QTL detected for measured traits in *Eucalyptus camaldulensis* (Ec7) × *E. tereticornis* (Et88) under control and salinity

| S.no. | TraitName | QTL | Linkage group | LOD | Position | Flanking microsatellites | aF | aM | d | PVE (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $A_{net}$_Control | $qA_{net}$7_C | 7 | 3.8 | 81 | Embra7–Embra112 | − 0.35 | 0.58 | − 0.50 | 27.15 |
| 2 | *E*_ Control | *qE*7_C | 7 | 4.3 | 105 | Embra112–Embra98 | − 0.67 | 0.76 | − 0.52 | 27.71 |
| 3 | SNC_ Control | *q*SNC6_C | 6 | 9.5 | 130 | Embra50–Embra81 | − 0.53 | 0.43 | − 0.52 | 54.06 |
| 4 | RNC_ Control | *q*RNC4a_C | 4 | 4.9 | 75 | Embra89–Embra179 | 0.80 | 0.26 | 0.14 | 15.40 |
| 5 | | *q*RNC4b_C | 4 | 7.1 | 202 | Embra213–Embra186 | − 0.35 | − 0.64 | 0.68 | 50.14 |
| 6 | LKC_ Control | *q*LKC2_C | 2 | 6.0 | 60 | Embra126–Embra43 | − 0.95 | − 0.76 | − 0.42 | 77.45 |
| 7 | SKC_ Control | *q*SKC4_C | 4 | 3.8 | 211 | Embra213–Embra186 | − 1.83 | 0.44 | − 0.25 | 69.34 |
| 8 | RKC_ Control | *q*RKC2a_C | 2 | 3.7 | 14 | Embra58–Embra126 | − 0.98 | − 0.56 | 0.96 | 17.42 |
| 9 | | *q*RKC2b_C | 2 | 7.5 | 74 | Embra126–Embra43 | − 0.95 | − 1.55 | 0.98 | 33.55 |
| 10 | LKNR_ Control | *q*LKNR4_C | 4 | 5.6 | 226 | Embra213–Embra186 | − 0.27 | 0.25 | − 0.65 | 61.40 |
| 11 | RKNR_ Control | *q*RKNR6_C | 6 | 5.6 | 130 | Embra50–Embra81 | − 0.34 | − 0.55 | 0.62 | 58.04 |
| 12 | SIS_Salt | *q*SIS1_S | 1 | 3.0 | 36 | Embra70–Embra12 | 0.09 | − 0.46 | 0.08 | 9.58 |
| 13 | | *q*SIS2_S | 2 | 4.0 | 70 | Embra126–Embra43 | 0.27 | 0.63 | − 0.27 | 27.53 |
| 14 | | *q*SIS6_S | 6 | 3.3 | 0 | Embra28–Embra25 | − 0.37 | − 0.16 | − 0.05 | 10.54 |
| 15 | LAR_Salt | *q*LAR5_S | 5 | 4.7 | 158 | Embra188–Eg67 | − 7.71 | 3.69 | − 15.59 | 63.16 |
| 16 | RWC_Salt | *q*RWC2_S | 2 | 5.8 | 95 | Embra43–Embra63 | 1.37 | − 10.30 | 2.59 | 37.49 |
| 17 | | *q*RWC4_S | 4 | 3.6 | 0 | Embra66–Embra89 | 1.85 | − 5.22 | − 3.12 | 11.82 |
| 18 | PMD_Salt | *q*PMD2_S | 2 | 7.3 | 63 | Embra126–Embra43 | 2.12 | 12.36 | − 0.63 | 53.10 |
| 19 | $A_{net}$_Salt | $qA_{net}$1_S | 1 | 4.8 | 62 | Embra12–Embra219 | 0.20 | − 0.05 | − 0.86 | 63.58 |
| 20 | $g_s$_Salt | $qg_s$5_S | 5 | 3.3 | 30 | Embra168–Embra120 | − 0.01 | 0.01 | − 0.02 | 46.93 |
| 21 | $C_i$_Salt | $qC_i$6_S | 6 | 3.3 | 33 | Embra28–Embra25 | − 22.02 | 31.59 | 10.61 | 41.61 |
| 22 | LNC_Salt | *q*LNC4a_S | 4 | 3.6 | 92 | Embra179–Embra36 | 2.04 | 0.79 | 0.51 | 25.67 |
| 23 | | *q*LNC4b_S | 4 | 6.8 | 231 | Embra213 –Embra186 | 0.98 | − 1.79 | 0.14 | 32.91 |
| 24 | | *q*LNC10_S | 10 | 3.6 | 0 | Embra101–Embra33 | 0.37 | 1.10 | − 0.08 | 10.33 |
| 25 | SNC_Salt | *q*SNC6_S | 6 | 4.7 | 127 | Embra50–Embra81 | − 0.08 | 0.24 | − 1.50 | 59.61 |
| 26 | RNC_Salt | *q*RNC2_S | 2 | 6.0 | 96 | Embra43–Embra63 | − 0.32 | − 1.44 | 0.66 | 40.16 |
| 27 | LKC_Salt | *q*LKC6_S | 6 | 5.1 | 123 | Embra50–Embra81 | − 0.70 | 4.36 | − 10.6 | 56.12 |
| 28 | SKC_Salt | *q*SKC4a_S | 4 | 3.3 | 68 | Embra89–Embra179 | 7.24 | 2.26 | 0.24 | 16.39 |
| 29 | | *q*SKC4b_S | 4 | 4.6 | 204 | Embra213–Embra186 | − 3.11 | − 10.01 | 2.66 | 58.02 |
| 30 | RKC_Salt | *q*RKC4_S | 4 | 4.9 | 71 | Embra89–Embra179 | 7.49 | 0.49 | 0.61 | 20.23 |
| 31 | LKNR_Salt | *q*LKNR2_S | 2 | 6.5 | 66 | Embra126–Embra43 | − 0.51 | − 0.85 | 0.33 | 59.31 |
| 32 | SKNR_Salt | *q*SKNR2_S | 2 | 6.3 | 60 | Embra126–Embra43 | − 0.28 | − 1.06 | 0.13 | 39.79 |
| 33 | RKNR_Salt | *q*RKNR7_S | 7 | 3.3 | 40 | Embra167–Embra7 | 0.49 | 0.37 | 0.06 | 18.97 |

*LOD* logarithm of odds, *aF* additive effect of female parent, *aM* additive effect of male parent, *d* dominant effect, *PVE* phenotypic variation explained

salinity treatment, SIS correlated with LAR and PMD. SIS showed a significant negative correlation with *A*net, LNC, SNC and LKC, suggesting that salinity tolerant eucalypts plants with lesser SIS could survive due to higher photosynthesis rate, $Na^+$ and $K^+$ concentration and with lesser PMD and LAR. Lesser PMD observed in these individuals supported the view that cell integrity is maintained as a strategy to enhance salinity tolerance (Stevens et al. 2006).

Factors causing reduction of gas exchange parameters in plant under stress conditions could be due to stomatal and non-stomatal functions (Rangani et al. 2016). In this study, under salinity, correlation of gas exchange measurements showed a strong positive relationships among them (*A*net, *g*s, *C*i and *E*). Decrease in $CO_2$ diffusion through the stomata to the intercellular space and limitation of metabolic or biochemical ability of leaves to fix $CO_2$ would lead to photosynthesis inhibition (Fan et al. 2011). Lesser availability of $CO_2$ levels in the fixation site may be the consequence of reduced *g*s that led to decrease in *A*net (Yu et al. 2011). A large reduction in *g*s under salinity (Table 1)

could be due to stomatal closure, which is an important stress avoidance mechanism in plants (Wang et al. 2013). This reduction in gas exchange measurements in the present study could be due to stomata related functions, however, further studies on stomatal functions will substantiate these results. In previous studies under salinity conditions, decrease of *A*net and *E* was observed in *E. camaldulensis* (Van der Moezel et al. 1989; Poss et al. 2000). Similar observations on stomata controlled functions like *E*, *C*i, *g*s and reduction of chlorophyll synthesis was recorded in *Zea mays* under salinity treatment (Rattan et al. 2014). In species like *Populus* (Sixto et al. 2005) and citrus (Lopez-Climent et al. 2008) greater reduction in *gs* contributed to higher reduction in *A*net of the salt sensitive plants.

In the present study, LNC showed a significant negative correlation with the LAR and SIS (Supplementary Table S2) and this could be due to detoxification by intracellular compartmentalization and reduced leaf senescence as observed in potato salt tolerant cultivars (Jaarsma et al. 2013). Tolerance of $Na^+$ in tolerant cultivar was due to their $Na^+$ sequestration mechanisms into vacuoles of leaf cells (Yamaguchi and Blumwald 2005). This sequestered $Na^+$ in the vacuoles promotes water uptake in saline environments which acts as an osmoticum could maintain osmotic driving force (Horie et al. 2009). It was reported that salt tolerant species of eucalypts like *E. spathulata, E. loxophleba* and *E. sargentii* were able to maintain well regulated leaf $Na^+$ concentrations even at 300 mM NaCl (Adams et al. 2005). Further, under high salinity, ion transporters (EcHKT) of *E. camaldulensis*, mediate mainly $Na^+$ transport (Liu et al. 2001). Excessive $Na^+$ inhibited various important cellular processes, many of which were directly correlated with $K^+$ transport and essential functions of $K^+$. However, $K^+$ alleviates the toxic effects of $Na^+$ and maintains a high $K^+/Na^+$ ratio especially in leaves (Hauser and Horie 2010). In the present study, under salinity treatment, correlation existed between SNC and SKC and also between RNC and RKC. Similar observation was reported in rice genotypes and it was postulated that the major pathways of $Na^+$ and $K^+$ uptake occur in parallel (Wang et al. 2012). Various reports have indicated that increasing cytosolic $K^+$ levels relative to $Na^+$ ($K^+/Na^+$ ratio) is crucial for $Na^+$ tolerance in plants, and maintaining high $K^+/Na^+$ ratio in shoots which is highly correlated with salinity tolerance in glycophytes (Zhu et al. 2015; Chunthaburee et al. 2016). During salt stress the observed variability among the hybrid individuals enabled us to understand salt stress response by mapping QTL regions.

Salinity related QTL provided an insight on complex genetic nature of salt response in eucalypts hybrids. Most of the traits localised are related to $Na^+$ and $K^+$ concentrations, providing that the importance of ion regulation for the plant survival during stress conditions. Three genomic regions were associated with a significant number of QTL on LG2, LG4 and LG6. In chickpea, enhanced yield during salinity was controlled by one QTL responsible for pod number and seed number (Pushpavalli et al. 2015). Co-localization of the QTL in control and salt treatment observed in this study could be due to the presence of closely linked genes or by pleiotropic effect from the same genes on these traits (Azadi et al. 2015) and these QTL were important in regulating salinity stress response in eucalypts.

The population size and number of markers used in QTL detection may determine the number of QTL and their effects (Vales et al. 2005; Zhang et al. 2010). The large phenotypic effects for many traits with threshold LOD value of 3.0 could be due to limited population size and number of SSRs. Increasing the density of markers on the selected linkage groups could increase the resolution of map to locate the loci closer to markers flanking the trait. Validation of the QTL position with the different mapping population or with same mapping population in field conditions will confirm salt tolerance nature of hybrid eucalypts.

In conclusion, the present study demonstrated the significant contribution of growth, physiological and mineral nutrient ($Na^+$, $K^+$ and $K^+/Na^+$ ratio) parameters in *E. camaldulensis* × *E. tereticornis* under salinity. Alleles from salinity tolerant *E. camaldulensis* (Ec7) could potentially be selected in future breeding programs to incorporate into the salinity sensitive genotypes for improving their tolerance. Fine mapping of selected QTL regions would provide insights on the genes responsible for salt tolerance. The salinity tolerant hybrids could be used for large scale field tests. This study provided valuable information for future investigations on genetics and breeding of salinity tolerance in eucalypts.

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**Authors' contributions** VSU conducted salt tolerance experiments, SSR genotyping, data analysis and drafted the manuscript. VKWB conducted field establishment and vegetative propagation, AM, BN and VSI carried out the controlled pollination and hybrid establishment, VSI participated in data analysis, RY conceived, organized and planned the research and finalised the manuscript.

**Compliance with ethical standards**

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

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