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GENETIC DIVERSITY OF ADVANCED GENERATION BREEDING POPULATION OF *EUCALYPTUS UROPHYLLA* IN CHINA

Lu WH[1,] *, Qi J[1], Lan J[2] & Luo JZ[1]

[1]China Eucalypt Research Centre, Chinese Academy of Forestry, 30 Renmin Dadao, Zhanjiang, Guangdong 524022, China
[2]Dongmen State Forest Farm, Chongzuo, Guangxi 532108, China

**luwanhong@outlook.com*

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As a theoretical basis for improvement of *Eucalyptus urophylla* in China, the genetic diversity and structure for advanced generation breeding populations were evaluated, and the changes of genetic diversity between improved generation and their parental generation were compared. The genotyping was performed on families sampled from advanced generation with 16 neutral microsatellite loci. The testing of Hardy-Weinberg equilibrium showed significant heterozygote deficits in two improved generation breeding populations. The expected heterozygosity of the 3rd generation was very close to that of the 2nd generation breeding population, indicating no heterozygosity had been lost, and the genetic diversity was consistent in two improved populations supported by Shannon's information index. Wright's fixed index of two advanced generations also indicated significant heterozygote in advanced generation breeding populations, compared with their parental generation. Most of the molecular variation was explained by the source of the families, both in two advanced generations, using analysis of molecular variances (AMOVA). The advanced generation breeding population of *E. urophylla* had a high level of genetic diversity after heavy artificial selection, consistently, and with no significant difference among generations.

Keywords: Microsatellite locus, heterozygosity, genetic structure, genetic differentiation

INTRODUCTION

Eucalyptus urophylla is one of the economically important tree species in global forest plantations. It is native to the Lesser Sunda Islands, and has a massive distribution pattern, due to isolation by seawater, among islands where it originates. *Eucalyptus urophylla* and *E. deglupta* are the only two *Eucalyptus* species naturally distributed outside of mainland Australia (Eldridge et al. 1994). The first introductions of *E. urophylla* were carried out by researchers from Australia and France with focus on estimating genetic parameters of growth traits (Turnbull & Brooker 1978, Gunn & McDonald 1991, Maid & Bhumibhamon 2009, Souza et al. 2011). The data suggested that the growth performance of Mt Egon and Mt Lewotobi provenances in Flores Island were much better than those from other islands in Lesser Sunda Island. The Timor Island provenances, for example, have high elevation and the poorest growth (Tripiana et al. 2007). A previous assessment of the natural stands of *E. urophylla* indicated that there was inconsistent growth performance among provenances under different conditions, with the exception of the poor-performing Timor provenance (Hodge & Dvorak 2015).

Genetic research on diversity and structured tree breeding population will provide a better understanding of the source of phenotypic variation of interested traits. An analysis combining microsatellite locus and phenotypic data of a *E. urophylla* provenance test in Congo studied the genetic diversity of natural provenances, genetic and phenotypic differentiation and the variation in quantitative traits, providing comprehensive insight for genetic resources management in situ of *E. urophylla* (Tripiana et al. 2007). A study on the provenance trial of *E. urophylla* in Vietnam demonstrated low genetic differentiation among provenances (F_{ST} = 0.023) compared with the study in Congo, as well as phenotypic differentiation (Q_{ST} < 0.21) based on diameter at breast height (dbh) and tree height (Quang et al. 2013).

Other studies on natural *E. urophylla* stands have shown that generally there was high

genetic diversity and low genetic differentiation among provenances. Genetic diversity in natural *E. urophylla* provenances in Indonesia indicated high genetic diversity and low phenotypic differentiation among all natural provenances. Genetic differentiation increased with increasing geographical distance, suggesting that gene migration (pollen flow) might explain the low genetic differentiation among pairwise provenances (Payn et al. 2008). Meanwhile, Payn et al. (2007) examined molecular variation in the chloroplast genome of *E urophylla* and found high genetic differentiation (G_{ST} = 0.581). The genetic diversity of chloroplast genome demonstrated a decreasing trend from east to west in Less Sunda Island, and a clear geographical structure (N_{ST} = 0.724). These findings verified the *E. urophylla* seed colonisation route in Less Sunda Island (Payn et al. 2007).

It was more than 30 years since *E. urophylla* was introduced into China. For much of this time, genetic improvement of this species has mainly been based on the analysis of genetic variation of quantitative characters in China, with little research on the molecular genetic of *E. urophylla* breeding population. More efforts should be dedicated to the genetic diversity and structure of breeding population, mating system and even detection of genes associated with interested traits, contributing to the improvement of *E. urophylla* generation. The current generation breeding population of *E. urophylla* in China has experienced a high intensity of artificial selection, and the genetic background and genetic structure have changed greatly compared with previous generation.

The aim of this study was to analyse the genetic diversity and structure of the current advanced generation breeding population using the same microsatellite loci that were used to assess genetic diversity of the 1st generation (i.e. the natural stands introduced) breeding population of *E. urophylla* in China. The study investigated changes in genetic diversity among different generations of *E. urophylla* breeding population in China. It is expected that the findings of this study can provide a theoretical basis for the improvement of *E. urophylla* breeding population for the selection of parents in the development of excellent *E. urophylla* hybrids in China.

MATERIALS AND METHODS

Plant material

The advanced generation breeding populations of *E. urophylla* studied included 2nd and 3rd generation breeding populations. The genetic materials comprising 2nd generation were open pollination progenies of the 1st generation breeding population, introduced from Less Sunda Islands. The genetic materials comprising 3rd generation breeding population were mainly composed of open pollinations progenies of the 1st generation, and some of the 2nd generation breeding populations of *E. urophylla,* improved in China. The original trials of genetic materials comprising 2nd (Trial 135, T135) and 3rd (Trial 164, T164) generation breeding populations of *E. urophylla* are listed in Table 1. The sampling number was determined based on the number of provenances tested in a trial (Table1). Individual trees were chosen randomly, from which the fresh leaves were collected, placed into plastic self-sealing bags immediately and the bag was filled with approximately 50 g silica gel. The samples were stored in -30 °C refrigerator for later use.

Genomic DNA extraction

A modified Cetyltrimethyl Ammonium Bromide (CTAB) method developed for microsatellite analysis was used in the extraction of genomic DNA from *E. urophylla* leaf samples (Gan et al. 2003). The concentration and quality of the genomic DNA extracted were evaluated using a NanoDrop 2000 and 1% agarose gel electrophoresis.

Microsatellite loci PCR amplification

A total of 608 microsatellite loci published for *Eucalyptus* were screened for *E. urophylla*, including neutral markers from the whole genome and expressed sequence tags, to check for specificity and polymorphism (Brondani et al. 2006, Zhou et al. 2014, He et al. 2012). The specificity was assessed by the PCR product of a marker, having a single clear band on an agarose gel. The polymorphism was checked by the method described by Wang et al. (2012). A total of 16 microsatellite loci were chosen to analyse

Table 1 Original trials of genetic materials comprising advanced generation breeding population of *Eucalyptus urophylla* and sampling number

Generation	Parental trials	Constituted provenances in parental trial	samples
2nd (T135)	T46	Mt Egon, Upper Ulanu River, Dongmen[a]	17
	T77	Mandiri, Mt Lewotobi, Mt Egon, Bangat, Wairteban, Iling Gele, Andalan, Jawaghar, Dongmen	28
	T54	Mt Egon, Soe, Congo[b], Seed Production Area, Aracruz	14
3rd (T164)	T46	Mt Egon, Mt Lewotobi, Upper Ulanu River, Dongmen	20
	T135	T46, T77, T54	14
	T77	Mandiri, Mt Lewotobi, Mt Egon, Wairteban, Iling Gele, Andalan, Dongmen	43
	T94	Wai Kui, Arnau, Uhak, Apui, Piritumas, Ilwaki, Telemar, Carbubu, Old Uhak, Mt Dalaki, Dongmen	32
	TJijia	Wai Kui, Arnau, Uhak, Apui, Piritumas, Ilwaki, Telemar, Carbubu, Dongmen	16

[a]Dongmen: material comprises of families from plus-trees selected in earlier trials at Dongmen Forest Farm, which consisted of Mt Egon, Mt Mandiri, Mt Wuko, Upper Ulanu River and Mt Lewotobi, [b]Congo: materials in Congo was introduced from natural stands of *E. urophylla*

genetic diversity and structure of advanced generation breeding populations of *E. urophylla*.

Touch-down PCR amplification procedure was performed as: (1) incubate at 94 °C for 1 min, (2) incubate at 94 °C for 20 sec, (3) incubate at 66 °C for 30 sec, decrease by 0.5 °C every cycle, (4) incubate at 72 °C for 30 sec, (5) cycle to step 2 for 19 more times, (6) incubate at 94 °C for 20 sec, (7) incubate at 56 °C for 30 sec, (8) incubate at 72 °C for 30 sec, (9) cycle to step 6 for 25 times, (10) incubate at 72 °C for 15 min, (11) hold at 4 °C. The amplification reaction contained: 1µl 10 × buffer, 0.025 µl dNTP, 0.01µl dUTP, 0.1µl Taq, 0.5µl Primer (equal mixture of forward and reverse), 7.365µl H_2O, 1µl DNA template. Deoxyuridine triphosphate (dUTP) was added into the PCR system to facilitate genotyping of PCR products by Applied Biosystems (ABI) 3130xl. Before genotyping, 0.18 µl internal standard GeneScan 500LIZ and 9.34 µl HiDi formamide were added into 1.5 µl PCR product and mixed, denatured at 95 °C for 5 min and cooled at 4 °C for 5 min (Li and Gan 2011). The PCR products were analysed by GeneMapper v 4.0.

Data analysis

Unbiased estimation probability of Hardy-Weinberg for every locus was estimated by Genepop v 4.2, based on Markov chain model (Rousset 2008). The significance level was set at $p \leq 0.01$, tested 1000 times randomly. The unbiased estimation probability included $P_{(HWE)}$ when the population was in genetic equilibrium, $P_{(ht.exc)}$ when the population was in heterozygote excess and $P_{(ht.\ def)}$ when the population was in heterozygote deficit.

PowerMarker v 3.25 was performed to evaluate the polymorphism information content (PIC) of every microsatellite locus. Number of alleles (Na), number of effective alleles, expected heterozygosity (He), observed heterozygosity (Ho), Wrights fixation index (F) and Shannon's Information Index (I) for every locus and provenance were estimated by the program GenAlEx v 6.4.1 (Peakall & Smouse 2006). The Nei's genetic distance pairwise provenances were calculated by GenAlEx v 6.4.1.

Allelic richness (A_R) (based on the number sampled in minimum population), between-population differentiation (F_{ST}), inbreeding coefficients of individuals relative to the sub-population (F_{IS}), and inbreeding coefficients of individuals relative to the total population (F_{IT}) for every locus and provenance were evaluated by online program FSTAT v 2.9.3.2. The number of migrants (Nm) was calculated using the formula:

$$Nm = [(1/F_{ST})-1]/4 \quad (1)$$

Analysis of molecular variances (AMOVA) of advanced generation population of *E. urophylla* was executed with program GenAlEx v 6.4.1,

number of permutation was 999 times. Structure analysis can cluster genetically homogeneous individuals into different groups. The number of groups was estimated by Program Structure v 2.3.4 based on correlated allele frequencies among provenances with clustering method of Bayesian model (Pritchard et al. 2000). The structure parameters were set as recommended by the user manual, the *K* value was set from 2 to 16, with ten replications each iteration. A burn-in period of 50,000 iterations was followed by 100,000 iterations of the Markov Chain Monte Carlo (MCMC). The optimal *K* value was determined with the highest Δ*K* value, which was calculated by online Program Structure Harvester v 0.6 (Evanno et al. 2005, Earl et al. 2012). The individuals were classified into different clusters on member coefficient (Q).

RESULTS

Test of Hardy-Weinberg equilibrium

Test of Hardy-Weinberg equilibrium with 16 microsatellite loci based on two advanced generations demonstrated that $P_{(HWE)}$ was 0 in two advanced generations on all loci (Table 2) suggesting that these two advanced populations deviated from Hardy-Weinberg equilibrium.

The $P_{(ht.def)}$ values on most of the loci were less than 0.01, and the $P_{(ht.exc)}$ values were much higher than 0.01 in two advanced generations (Table 2), indicating a strong heterozygote deficit in both the breeding populations of *E. urophylla*.

The polymorphism of microsatellite loci studied

Based on all the individuals from the two advanced generations, the overall mean number of alleles (Na) of all microsatellite loci were 19.6 and 26.8, the overall number of effective alleles were 8.7 and 10.4, and the overall mean of allelic richness (A_R) were 10 and 8.8 in 2nd and 3rd generation breeding populations respectively (Table 3).

The other genetic indices of all loci had no significant difference between the two improved generation breeding populations of *E. urophylla*. The PIC suggested high polymorphism in two advanced generations. The I suggested that the two advanced generations had high

Table 2 Test of Hardy-Weinberg equilibrium for 16 microsatellite loci based on 2nd and 3rd generation breeding populations of *E. urophylla*

Locus	$P_{(HWE)}$		$P_{(ht.def)}$		$P_{(ht.exc)}$	
	2nd	3rd	2nd	3rd	2nd	3rd
EUceSSR805	0.000	0.000	0.000	0.000	1.000	1.000
Embra226	0.000	0.000	0.000	0.000	1.000	1.000
Embra130	0.000	0.000	0.000	0.000	1.000	1.000
Embra78	0.000	0.000	0.000	0.000	1.000	1.000
Embra86	0.000	0.000	0.000	0.000	1.000	1.000
Embra192	0.000	0.000	0.000	0.000	1.000	1.000
Embra333	0.000	0.000	0.000	0.554	1.000	0.636
Embra198	0.000	0.000	0.000	0.000	1.000	1.000
Embra145	0.000	0.000	0.000	0.000	1.000	1.000
Embra94	0.000	0.000	0.006	0.000	0.993	1.000
Embra57	0.000	0.000	0.000	0.000	1.000	1.000
Embra368	0.000	0.000	0.002	0.000	0.998	1.000
Embra186	0.000	0.000	0.711	1.000	0.277	0.000
Embra119	0.000	0.000	0.024	0.074	0.985	0.747
Embra165	0.000	0.000	0.000	0.007	1.000	1.000
Embra377	0.000	0.000	0.000	0.000	1.000	1.000

$P_{(HWE)}$ = population in genetic equilibrium, $P_{(ht.def)}$ = population in heterozygote excess, $P_{(ht.exc)}$ = population in heterozygote deficit; ($p \leq 0.01$)

Table 3 The total mean genetic diversity indices of 16 microsatellite loci based on advanced generation breeding populations of *E. urophylla*

Generations	Na	Ne	A_R	PIC	I	He	Ho	F_{IS}	F_{IT}	F_{ST}
2nd	19.6	8.7	10.0	0.84	2.37	0.86	0.60	0.28	0.30	0.03
3rd	26.8	10.4	8.8	0.87	2.58	0.88	0.60	0.31	0.33	0.03

Na = number of alleles, Ne = number of effective alleles, $A_{R\ =}$ allelic richness, PIC = polymorphism information content, I = Shannon's information index, He = expected heterozygosity, Ho = observed heterozygosity, F_{IS} = inbreeding coefficients of individuals relative to the sub-population, F_{IT} = inbreeding coefficients of individuals relative to the total population, F_{ST} = between-population differentiation

genetic diversity. The observed heterozygosity values were less than expected heterozygosity, proposing a strong heterozygote deficit in both generations. The overall mean of inbreeding coefficients of individuals, relative to the sub-population (F_{IS}), showed heterozygote deficit in two advanced generations. The average of inbreeding coefficients of individuals, relative to the total population (F_{IT}), indicated inbreeding in two generations in some extent.

Genetic diversity of advanced generation breeding population of *E. urophylla*

The genetic materials constituting advanced generation breeding populations were progenies of open pollination from parental generations (trials). The 2nd generation was the progeny of 1st generation (progenies of natural provenances) and the 3rd generation was the progeny of 2nd and 1st generations. The subpopulations in two improved generations were named after the trials which the parental families belonged to. Thus, the subpopulations were T46, T54 and T77 in 2nd generation, and T46, T77, T94, T135 and TJijia in the 3rd generation.

Table 4 shows the genetic diversity indices of two advanced generations. The total mean of allele for all subpopulations were 11.8 and 14.2 and the number of effective alleles averaged were 6.5 and 8.4 in 2nd and 3rd generations, respectively. The average value of I was 2.09 and 2.27, and with no significant variance among subpopulations and generations, indicating that heterogeneity was similar among subpopulations of the two generations. The overall mean value of allelic richness (A_R) of all subpopulations were 9.3 and 8.5, with no obvious variance among subpopulations in the two generations, and the allelic diversity was higher in 2nd than 3rd generations.

The total mean values of expected heterozygosity were 0.83 and 0.86, and the average values of observed heterozygosity were 0.60 and 0.59 in the two advanced generations. There was no significant difference of the two indices, between the two generations, indicating a high and similar genetic diversity in both the advanced generations. The value of He was greater than Ho in both the generations, proposing heterozygote deficit in both advanced generations.

The overall mean value of F were 0.28 and 0.31 in 2nd and 3rd generations, and the values of F of all subpopulations were positive, indicating an obvious heterozygote deficit and inbreeding in both generations. Besides, the values of F also showed a weak genetic differentiation in both advanced generation breeding populations of *E. urophylla*.

Genetic structure

The AMOVA on both advanced generation breeding populations of *E. urophylla* showed 70% molecular variation among all individuals (Table 5). Approximately 30% molecular variation was explained by the source of individuals within subpopulation, and 1% came from the subpopulations. The results of AMOVA also indicated a fairly low molecular variation in both the advanced generation breeding populations of *E. urophylla*.

Structure analysis speculated the number of groups, based on correlation of alleles frequency among individuals. Individuals could be classified into different groups according to membership coefficient. In 2nd generation, individuals of 16.9, 20.3, 20.3, 18.6 and 23.7% were divided into five clusters (Figure 1a). In 3rd generation, individuals of 32.8, 28.8 and 38.4% were divided into three clusters (Figure 1b). Comparison of

Table 4 Genetic diversity indices for advanced generation breeding populations of *E. urophylla*

Generations	Subpopulations	Na	Ne	I	A_R	He	Ho	F
2nd	T46	11.1	6.9	2.08	9.1	0.84	0.63	0.24
	T54	10.2	5.9	1.99	9.0	0.81	0.59	0.28
	T77	14.2	6.6	2.21	9.9	0.84	0.58	0.32
	Mean	**11.8**	**6.5**	**2.09**	**9.3**	**0.83**	**0.60**	**0.28**
3rd	T135	11.6	7.8	2.20	8.9	0.86	0.57	0.34
	T46	12.2	7.9	2.18	8.0	0.85	0.54	0.37
	T77	18.2	8.8	2.34	8.4	0.85	0.63	0.26
	T94	16.0	8.7	2.32	8.3	0.86	0.63	0.27
	TJijia	12.9	8.6	2.29	9.0	0.87	0.59	0.33
	Mean	**14.2**	**8.4**	**2.27**	**8.5**	**0.86**	**0.59**	**0.31**

Na = alleles averaged for each subpopulation over all loci, Ne = number of effective alleles averaged for each subpopulation over all loci, I = Shannon's information index averaged for each subpopulation over all loci, A_R = allelic richness per subpopulation, He = expected heterozygosity per subpopulation, Ho = observed heterozygosity per subpopulation, F = Wright's fixation index per subpopulation

Table 5 AMOVA based on 16 microsatellite loci and advanced generation breeding population of *E. urophylla*

Variations Source	Generation	df	Sum of squares	Variance components	Percentage of variation (%)
Among subpopulations	2nd	2	21.50	0.05***	1.0
	3rd	4	42.86	0.03***	0.4
Among individuals within subpopulation	2nd	56	504.73	2.12***	31.0
	3rd	120	1106.18	2.19***	31.1
Within individuals	2nd	59	281.50	4.77***	69.0
	3rd	125	604.00	4.83***	68.5
Total	2nd	117	807.74	6.94	100
	3rd	249	1753.04	7.06	100

Significance levels were based on 999 permutations, (***$p < 0.001$), df = degree of freedom

classifications with real subpopulations in the two advanced generations showed no clear threshold for inferring individuals into different clusters. The individuals from a subpopulation and a specific parental provenance were randomly divided into different clusters, based on genetic distance among individuals.

Additionally, the genetic structural map indicated that there was more complex heterogeneity in 2nd generation than 3rd generation due to more clusters in 2nd generation compared with 3rd generation breeding population of *E.urophylla.*

Genetic differentiation and gene flow

The genetic differentiation coefficient (F_{ST}) between pairwise subpopulations were fairly low in both the two advanced generation breeding populations (Table 6 and Table 7) suggesting a weak heterogeneity of genetic materials from advanced generation breeding populations of *E. urophylla.* This may be explained by the origins of genetic materials of the advanced generation populations that experienced high intensity artificial selection.

The gene flow between pairwise subpopulations increased with generations (Table 6 and Table 7). The genetic materials comprising advanced generations were the progenies of open pollination from their parental generation breeding populations, thus, this could be one of the reason for the increase of Nm values in improving generations. It was worth noting that the value of Nm between subpopulation T77

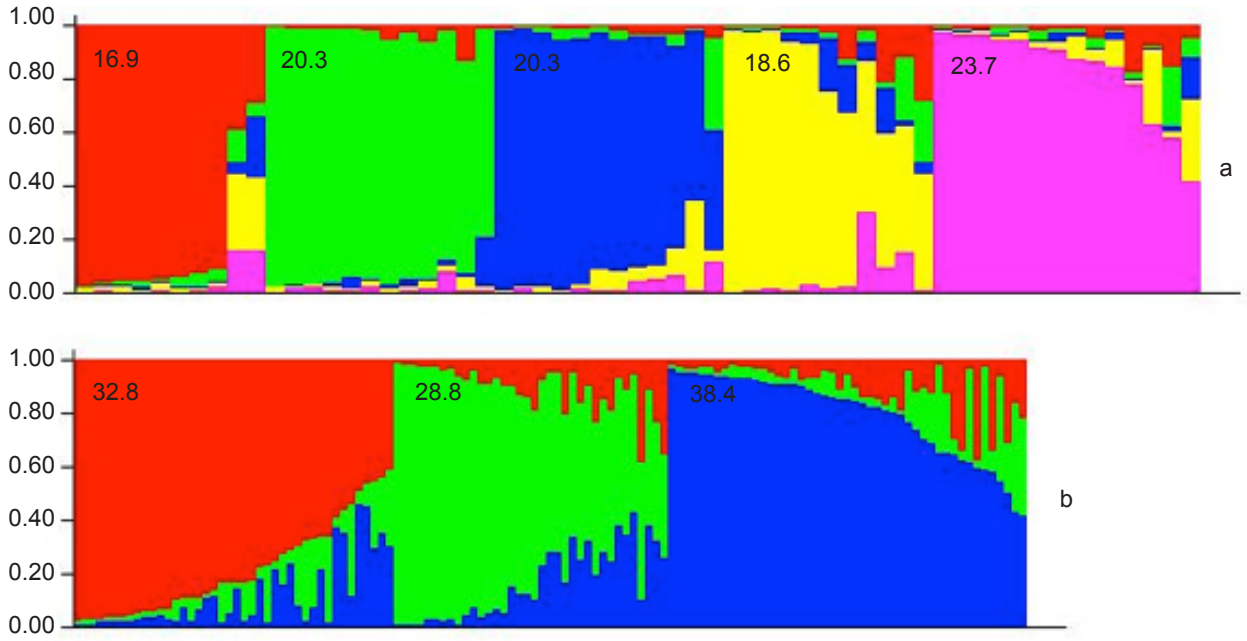

Figure 1 Genetic structural map estimated by Structure Program on individuals sampled from 2nd (a) and 3rd (b) generation breeding populations of *E. urophylla;* clusters were defined by optimal delta K value and individuals were classified into different clusters based on their membership coefficient Q values

Table 6 Pairwise subpopulation gene flow (Nm, above diagonal) and genetic differentiation index (F_{ST}, below diagonal) for 2nd generation breeding population of *E. urophylla*

Subpopulations	T46	T54	T77
T46		11.10	12.51
T54	0.02		10.91
T77	0.02	0.02	

Table 7 Pairwise subpopulation gene flow (Nm, above diagonal) and genetic differentiation index (F_{ST}, below diagonal) for 3rd generation breeding population of *E. urophylla*

subpopulations	T135	T46	T77	T94	TJijia
T135		12.06	14.19	12.35	10.22
T46	0.02		15.53	15.28	12.48
T77	0.02	0.02		24.98	13.62
T94	0.02	0.02	0.01		13.23
TJijia	0.02	0.02	0.02	0.02	

and T94 was 24.98, which was much higher than that of other pairwise subpopulations in 3rd generation, proposing that many of the genetic recombination (hybridising) probably occurred between families from Trial 77 and Trial 94 in 2nd generation.

DISCUSSION

Test of Hardy-Weinberg equilibrium

Tests of Hardy-Weinberg equilibrium on the two advanced generations suggested that both

advanced populations deviated from Hardy-Weinberg equilibrium, contributed by several factors, i.e. heavy intensity artificial selection in the establishing of advanced generation breeding populations of *E. urophylla* in China. An analysis of genetic diversity on *Larix principis-rupprechtii* demonstrated that three improved generation breeding populations deviated from Hardy-Weinberg equilibrium (Yu et al. 2014). Another reason might be flowering asynchronism, which affects the free combination of alleles. Some studies proposed that, in some years reduced flowering will induce low outcrossing rates, increase selfing, and ultimately affected the genetic diversity of seed (Swain et al. 2013, Hodge & Dvorak 2015). Lade et al. (1996) reported that the main reason for the deviation from Hardy-Weinberg equilibrium were Wahlund effect and inbreeding, which led to the heterozygote deficit (Wahlund 1928).

Eucalyptus have mixed mating patterns, thus, it can be speculated that heterozygote excess and heterozygote deficit in populations of *Eucalyptus* should be in a status of equilibrium in open pollinated or natural populations (Elliott & Byrne 2003). However, test of Hardy-Weinberg equilibrium in this study indicated that there was a significant heterozygote deficit in both advanced generations, similar to findings of heterozygote deficiencies in *E. globulus*, *E. populnea* and *E. marginata* (Jones et al. 2002, Holman et al. 2003, Wheeler et al. 2003).

Genetic diversity

The genetic materials comprising advanced generation breeding populations of *E. urophylla* in China were open pollination progenies of their parental generation, with strong artificial selection and reduced genetic diversity. However, in the current study, the genetic diversity evaluated by He and I was almost consistent, and no heterozygosity assessed by Ho had been lost between the two improved generation breeding populations. The findings were similar with the genetic diversity on *E. globulus* among breeding and native population, in which the He of breeding population was consistent with that in native population, but with higher Ho in breeding samples (Jones et al. 2006). There were also some converse examples, such as the genetic diversity of *Pinus massoniana* breeding population which was higher than the natural population (Lai & Wang 1997). Studies on *E. pellita* and *E. scias*, *A. aulacocarpa* and *Picea glauca* demonstrated little reduction in genetic diversity assessed by He in breeding populations, compared with natural population (House & Bell 1996, McGranahan et al. 1997, Rajora 1999).

Genetic structure

The results of AMOVA showed that 70% of the genetic variation occurred among all individuals, and 30% within subpopulations, and the smallest component of genetic variation was among subpopulations. Many studies on AMOVA of populations demonstrated similar conclusions, such as on *E. cladocalyx* and *E. camaldulensis* (Ballesta et al. 2015, Dillon et al. 2015, Mora et al. 2016).

The structure analysis with Bayesian discriminant models suggested that there were five and three groups in 2nd and 3rd generations respectively based on the correlation of alleles frequency among individuals. The genetic structural map indicated that the heterogeneity in 2nd generation might be higher than that in 3rd generation, but it could not explain the level of genetic diversity in advanced generation breeding populations of *E. urophylla* in China.

Genetic differentiation

The F_{ST} between populations were generally defined as none, moderate, significant or high: $F_{ST} < 0.05$, $0.05 < F_{ST} < 0.15$, $0.15 < F_{ST} < 0.25$ and $F_{ST} > 0.25$ respectively. In the current study, the F_{ST} of the two advanced generations suggested a fairly low genetic differentiation among subpopulations in 2nd and 3rd generation breeding populations. The analysis of genetic diversity on native population of *E. urophylla* by isozyme also showed a low genetic differentiation among populations studied (House & Bell 1994), and the genetic diversity of *E. urophylla* mainly existed within the population, but not among populations. Some studies on other species of *Eucalyptus*, such as *E. camaldulensis, E. marginata* and *E. cladocalyx* also proposed a low genetic differentiation in the population, analysed by molecular markers. (Butcher et al. 2002, Wheeler et al. 2003, Ballesta et al. 2015, Dillon et al. 2015).

CONCLUSIONS

The genetic diversity assessed by He in advanced generation breeding population of *E. urophylla* in China was consistent, with no significant heterozygosity loss, compared with parental generation, suggesting successful improvement programs of *E. urophylla* executed in China. The genetic differentiation evaluated with F revealed a low genetic differentiation among subpopulations and improved generations. The findings of this study highlighted the importance of molecular analysis on breeding populations and its continuous improvement by generations.

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