Genetic Diversity of *Casuarina equisetifolia* Provenances

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Abstract *Casuarina equisetifolia* is an important tree species in the tropical and subtropical zones of Asia, the Pacific and Africa. In this study, 220 individuals of *C. equisetifolia* from seven native provenances and eleven introduced provenances (land races) were analyzed to assess the genetic variation and structure using amplified fragment length polymorphism (AFLP) markers. A total of 465 bands were obtained by eight primer pairs, among which 153 were polymorphic. The mean NEI's gene diversity $H = 0.2113$ calculated for the 18 provenances and the total gene diversity $H_T = 0.4065$ calculated for native provenances suggested abundant variation within provenances and species. The genetic information provided important implications for future conservation and breeding programmes of *C. equisetifolia*.

1 Introduction

Casuarina equisetifolia (L. Johnson) has two subspecies: subsp. *incana* and subsp. *equisetifolia* (Wilson and Johnson, 1989). The former occurs exclusively along the coast of Queensland and northern New South Wales of Australia and in Vanuatu. The latter has a wide distribution from throughout Malesia to northern Australia, Melanesia, and Polynesia. As a fast-growing and nitrogen-fixing tree of social, economic and environmental importance, it is commonly used in agroforestry systems, for soil stabilization, in coastal protection and rehabilitation. Though a littoral species in natural habitats it is well adapted to inland area, and has been introduced into Asia (especially China, India and Vietnam), East and West Africa, the United States of America, the Caribbean and some Middle East countries for more than a century. Provenances of *C. equisetifolia* exhibit considerable variation in growth characteristics, and grow on a variety of sites with very different annual precipitation and climatic conditions. Marked variation in growth and branching habit between different provenances, as well as individual trees within provenances, has been observed (Pinyopusarerk and House, 1993; Pinyopusarerk and Williams, 2000; Pinyopusarerk *et al*., 2004). However, very little information is currently available on the genetic variation of this species based on molecular assessment.

Information on the magnitude of genetic variation within a species is an integral part of conservation and improvement of genetic resources. Amplified fragment length polymorphism (AFLP), which can be used to analyze large numbers of loci distributed throughout the genome, has proven a powerful tool in the assessment of genetic variation both within and among plant provenances without detailed prior knowledge of DNA sequences (Brown *et al*., 1990; Vos *et al*., 1995; Jones *et al*., 1998). AFLP markers have been successfully used for analyzing genetic variation and genetic structure (Janssen *et al*., 1996; Barrett *et al*., 1998; Xue *et al*., 2005; Stefenon *et al*., 2006).

The main goal of this study was to survey the levels and patterns of genetic variation of *C. equisetifolia* provenances using AFLP markers. Furthermore, we compared the genetic diversity between native and introduced provenances. This work provides valuable information for further genetic conservation and breeding programme for *C. equisetifolia*.

2 Materials and Methods

2.1 Plant materials and DNA extraction

A total of 220 individual trees belonging to 18 provenances and land races were collected from an international provenance trial of *C. equisetifolia* subsp. *equisetifolia* established at Zhangzhou in Fujian province of China. Introduced provenances (land races) were included in the samples in order to determine the variation and genetic information for future management of these provenances since they have been identified as potential provenances in the south of China (Zhong *et al*., 2001). The geographic and ecological parameters of provenances are shown in Table 1. Genomic DNA of each tree was extracted from fresh branchlets using a modified CTAB extraction (Doyle, 1991).

Table 1 Information of 18 provenances of *Casuarina equisetifolia* subsp. *equisetifolia* investigated for AFLP variation

Pro. No.	CSIRO Seedlot No.	Location	Sample size	Latitude	Longitude	Altitude (m)
Natural provenances						
1	18153	Ela Beach, Papua New Guinea	15	09°05′ S	148°17′ E	10
2	18008	Darwin, Northern Territory, Australia	10	12°25′ S	130°50′ E	20
3	18357	Narra, Palawan, Philippines	10	09°19′ N	118°29′ E	10
4	18154	Aklan, Panay Island, Philippines	10	11°31′ N	122°30′ E	30
5	18348	Kuantan, Pehang, Malaysia	15	06°30′ S	99°45′ E	30
6	18298	Had Chao Mai, Trang, Thailand	10	07°33′ N	100°37′ E	2
7	18297	Ban Kam Phuam, Ranong, Thailand	15	09°21′ N	98°27′ E	10
Introduced provenances						
8	Local	Dongshan, Fujian, China	12	23°40′ N	117°28′ E	4
9	18128	Non Nuoc, Vietnam	10	16°06′ N	106°20′ E	2
10	18086	Hai Thinh, Ha Nam Ninh, Vietnam	13	20°22′ N	106°21′ E	1
11	18152	Ninh Chu, Ninh Thuan, Vietnam	10	11°33′ N	108°59′ E	2
12	18015	Chandipur, Balasore, Orissa, India	15	21°30′ N	86°54′ E	2
13	18014	Balukhanda, Orissa, India	15	19°50′ N	85°53′ E	10
14	18120	Chengai Anna, Tamil Nadu, India	10	12°36′ N	79°48′ E	50
15	18119	Rameswaram, TamilNadu, India	10	09°15′ N	79°20′ E	5
16	18288	Madagama, Sri Lanka	15	08°06′ S	80°15′ E	80
17	18287	Hambantota, Sri Lanka	10	06°08′ N	81°07′ E	16
18	18355	Cotonou, Benin	15	06°23′ S	02°13′ E	8

2.2 AFLP analysis

AFLP analysis was carried out by the method of Vos *et al*. (1995) with some modifications. Approximately 300 ng of total genomic DNA per samples were digested with *Eco*RI and *Mse*I and then ligated with *Eco*RI adaptor and *Mse*I adaptor. Of the total 64 primer combinations with three selective bases screened in a preliminary test, eight primer combinations (Table 2) found to give good amplifications were selected for further study. Following the selective amplification, the reaction products were mixed with an equal volume of loading buffer (98% formamide, 10 mm EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). The mixtures were denatured for 5 min at 94℃ then directly placed on ice. About 6 μl of the mixtures were run in 6% (v/v) polyacrylamide gels with a DNA sequencer. The gels were silver stained using the protocol published by Streiff *et al*. (1998).

Table 2 Number of fragments, number of polymorphic fragments, percentage of polymorphic fragments, and size of fragments for each primer pair used for AFLP analysis in *Casuarina equisetifolia*

Primer pairs	Number of fragments	Number of polymorphic fragments	Percentage of polymorphic fragments	Size of fragments (bp)
M-CAC/E-ACT	45	19	42.22	75-600
M-CTA/E-AAG	80	24	30	50-620
M-CTA/E-ACA	65	23	35.38	115-820
M-CTC/E-AAC	60	25	41.67	40-530
M-CTC/E-ACT	50	20	40	40-375
M-CTG/E-AAG	48	22	45.83	100-400
M-CTT/E-ACC	64	9	14.06	40-680
M-CTT/E-AGC	53	11	20.75	102-410
Total	465	153		
Average	58.13	19.13	32.90	

2.3 Genetic analysis

Each AFLP band of unambiguous pattern was given a score of 1 for presence or 0 for absence across all the polymorphic loci to create a binary matrix. POPGENE, version 1.31 (Yeh *et al*., 1999) was used to calculate genetic parameters within the 18 sampled provenances, including the number of polymorphic loci (NP), percentage of polymorphic loci (P), observed number of alleles per locus (Na), effective number of alleles per locus (Ne), gene diversity (H) (Nei, 1973), Shannon's information index (I) (Lewontin, 1972). The total gene diversity (H_T) within species and within provenances (H_S) were calculated only for native provenances assuming Hardy-Weinberg equilibrium.

3 Results

The amplified results by each primer pair were shown in Table 2. Eight primer pairs revealed a total of 465 DNA fragments ranging from 40 bp to 820 bp, among which 153 (32.90%) of these bands were polymorphic loci either among or within provenances. Primer pairs were significantly different in their capacity to detect polymorphisms.

The genetic parameters for each of 18 *C. equisetifolia* provenances and land races summarized in Table 3 indicated a tendency for higher genetic variation of the Ela Beach native provenance in Papua New Guinea and Balukhanda, Orissa introduced provenance in India than other seed sources.

The local Dongshan, Fujian provenance showed very low level of variation (P = 13.07% and H = 0.0472). The overall mean gene diversity (H) was 0.2113 and Shannon information index was 0.3109. The POPGENE analyses for native provenances showed an abundant diversity (H_T = 0.4065 ± 0.0128) at the species level, partitioning which H_S = 0.2139 ± 0.0093 distributed within provenances and the rest among provenances.

Table 3 Genetic variation parameters of *Casuarina equisetifolia* provenances detected by AFLP markers

Pro. No.	*NP*	*P*	*Na*	*Ne*	*H*	*I*
1	117	76.47	1.7647	1.4569	0.2669	0.3990
2	62	40.52	1.4052	1.2651	0.1533	0.2263
3	87	56.86	1.5686	1.3701	0.2125	0.3140
4	91	59.48	1.5948	1.4481	0.2466	0.3568
5	87	56.86	1.5686	1.4328	0.2369	0.3411
6	72	47.06	1.4706	1.3066	0.1737	0.2561
7	104	67.97	1.6797	1.3515	0.2077	0.3175
8	20	13.07	1.1307	1.0817	0.0472	0.0701
9	85	55.56	1.5556	1.4030	0.2248	0.3266
10	92	60.13	1.6013	1.3307	0.1972	0.2986
11	91	59.48	1.5948	1.4258	0.2359	0.3430
12	99	64.71	1.6471	1.4026	0.2328	0.3458
13	105	68.63	1.6863	1.5186	0.2854	0.4121
14	81	52.94	1.5294	1.3909	0.2186	0.3173
15	80	52.29	1.5229	1.3261	0.1871	0.2774
16	93	60.78	1.6078	1.4113	0.2355	0.3460
17	97	63.40	1.6340	1.4316	0.2457	0.3609
18	78	50.89	1.5098	1.3475	0.1963	0.2878
Mean	85.61	55.95	1.5596	1.3723	0.2113	0.3109

The mean values were calculated across provenances. *NP*, number of polymorphic loci; *P*, percentage of polymorphic loci; *Na*, observed number of alleles per locus; *Ne*, effective number of alleles per locus; *H*, NEI's gene diversity; *I*, Shannon's information index.

4 Discussion

This study revealed that a large amount of genetic variation in *C. equisetifolia* at provenance level (both native and introduced sources) and at species level. The only exception was local land race from Dongshan, Fujian. The mean gene diversity (0.2113) was higher than 0.1473 reported by Ho *et al.* (2002) for 12 *C. equisetifolia* provenances assessed by RAPD. This may be attributed to the different provenances used in the two studies and more gene loci be detected by AFLP markers. The average Shannon's information index (0.3109) and the total gene diversity (0.4065) were also higher than many other plants such as *Picea asperata* (0.227 and 0.237, respectively), based on AFLP (Xue *et al.*, 2005).

The present results showed genetic variation among provenances (46.07%) was a little higher than Ho's study (39.28%) for 12 *C. equisetifolia* provenances. However, there was a similar tendency of high proportion of total variation among provenances tested by the two different markers. Such results are in contrast with expectations for woody, perennial, predominantly outcrossed species, which maintain most variations within provenances (Hamrick and Godt, 1990). However, this may be due to the small sample size in the study. High variation among provenances was also found in another tropical tree, e.g. 43% in *Tectona grandis* (Shrestha *et al.*, 2005) by AFLP. Higher

variations among provenances (51.53%) have been detected in *Syringa oblate* (Ming and Gu, 2006), *Moringa oleifera* Lam. (59%, Muluvi *et al*., 1999) and *Euterpe edulis* Mart. (57%, Cardoso *et al*., 2000) based on AFLP. The presence of such a high genetic variation among provenances could be attributed to the features of the wide distribution range of this species and the habitats where the sampled provenances occur. Its wide distribution is scattered through many islands, and therefore geographical isolation may occur.

It is interesting to find out that an introduced provenance Balukhanda, Orissa, India recorded the highest in genetic variation, reflecting the important implications on the introductions into this site. Multiple seed sources could have been collected to establish this plantation. Similarly, presumption that introduced provenances may have been derived from multiple seed sources can be seen from the higher variation (two Orissa provenances in India, two Sri Lanka provenances) than some native provenances.

5 Conclusions

Using AFLP markers this study showed abundant variation in *C. equisetifolia* within provenances and species. The genetic information provided important implications for future conservation and breeding programmes of *C. equisetifolia*.

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