African Journal of Agricultural Research Vol. 6(25), pp. 5664-5671, 5 November, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR10.864
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

ISSR-based genetic diversity of *Casuarina* spp. in coastal windbreaks of Taiwan

Kuen-yih Ho[1]* and Shih-chieh Lee[2]

[1]Department of Forestry and Nature Resources, National Chiayi University, Chiayi, 60004, Taiwan.
[2]Department of BioIndustry Technology, Da-Yeh University, Changhua, 51591, Taiwan.

Accepted 2 November, 2011

The Genus *Casuarina* is a coastal plant distributed throughout South East Asia, Australia, and the Pacific islands. In Taiwan, more than 10 species of *Casuarina* have been introduced based on the proposition that these drought tolerant, fast growing trees would be suitable for windbreaks and would help stabilize coastal sand. The genetic diversity of three *Casuarina* species grown in Taiwan, *Casuarina equisetifolia*, *C. glauca,* and *C. cunninghamiana* was determined and compared to those of native populations grown in Australasia as part of an international provenance test using the Inter-Simple Sequence Repeat (ISSR) marker system. Based on our results, the average Nei gene diversity of *Casuarina* grown in Taiwan (0.1940) is considerably higher ($p < 0.001$) than that of native populations of *C. equisetifolia* (0.1288), *C. cunninghamiana* (0.0922), and *C. glauca* (0.0577). Cluster and principal component analyses indicated that the Taiwan-grown trees are more closely related to *C. equisetifolia* than either *C. cunninghamiana* or *C. glauca*. By integrated reasoning of genetic variation and branchlet morphology, we may conclude that coastal *Casuarina* plants currently survived and grown in Taiwan are the products of introgressive hybridization involving *C. equisetifolia*, *C. glauca*, and possibly *C. cunninghamiana*.

Key words: *Casuarina*, genetic diversity, hybridization, inter simple sequence repeat (ISSR).

INTRODUCTION

Casuarinaceae is a family of plants distributed throughout Southern Asia, tropical-subtropical coastal Australia, and the Pacific islands (Wilson and Johnson, 1989). These species are widely cultivated for a number of purposes, including the extraction of raw materials (pulp, timber, wood chips) and medicine, landscaping, and for controlling sand dunes in coastal areas (Doran and Hall, 1983). In Taiwan, more than 10 species of *Casuarina* have been introduced based on the proposition that these drought tolerant, fast growing trees would be suitable windbreakers and would help stabilize coastal sand soil; however, very few species have survived (Wang et al., 1984). Interestingly, based on morphological characteristics and the anatomical structure of the branchlets of surviving *Casuarina* species, Chen (1980) discovered that multiple hybridization events had occurred between *Casuarina equisetifolia* and *Casuarina glauca*. Further characterization of specific traits in 103 Taiwanese specimens supports this discovery with the generation of three taxa, including *C. equisetifolia* subsp. *equisetifolia* × *C. glauca* (Wang et al., 1984). In a similar study, Hwang and Hsiao (1985) also identified a subgroup of morphologically distinct plants that could have arisen from hybridization or introgressive hybridization between *C. equisetifolia* and *C. glauca*. Additionally, a close relationship between *C. glauca* and *Casuarina cunninghamiana* was identified (Hwang and Hsiaso, 1985). Consequently, the identification of Taiwanese *Casuarina* species has been difficult due to hybridization events that have occurred as a result of mixed cultivation and similar flowering periods that would enable hybridization to occur.

As part of a global initiative designed to evaluate the phenotypic response and genetic variation of *Casuarina* species in different environmental conditions, the

*Corresponding author. E-mail: kyho@mail.ncyu.edu.tw. Tel: +886-5-2717463. Fax: +886-5-2717467.

Taiwanese Forestry Research Institute, along with the Forestry/Fuelwood Research and Development Project, established a *Casuarina* Provenance test garden on the southwestern coast of Taiwan (Yang et al. (1995) and Zietkiewicz et al. (1994) devised the technique of Inter Simple Sequence Repeat (ISSR) marker analysis, which amplifies regions between adjacent, inversely oriented microsatellites using a single simple sequence repeat-containing primer. This technique is most commonly used to study genetic variation among closely related plant species and to determine the parentage of interspecific hybrids (Adams et al., 2003; Barth et al., 2003; Christoph et al., 2003; Ge et al., 2003; Chuang et al., 2004; Ho et al., 2005; Li and Xia, 2005; Xie et al., 2005). In this study, we used ISSR marker analysis to compare the genetic diversity of *Casuarina* species grown in coastal areas of Taiwan to those located in the Provenance test garden.

MATERIALS AND METHODS

Sample collection

Leaf samples of 456 individuals were collected from variable habitats including 12 individuals from 15 coastal locations around Taiwan (Populations T1 to T15), 12 individuals from 12 provenances of *C. equisetifolia* (Populations E1 to E12), six provenances of *C. glauca* (Populations G1 to G6) and five provenances *of C. cunninghamiana* (Populations C1 to C5). All provenances of *Casuarina* were obtained from the Provenance test garden in Taiwan (Table 1). Each location and provenance was treated as an individual population. Fifteen mature leaves were sampled from each tree.

Morphological analysis

Phenotypes associated with Taiwan coastal *Casuarina* were examined including three leaf morphological traits, such as branchlet length, internode length, and the number of sheath teeth (Hwang and Hsiao, 1985). SPSS software was used to perform Nested ANOVA analysis.

ISSR molecular markers analysis

DNA extraction

Leaf samples were stored in paper bags and dried using silica gel before DNA extraction. Genomic DNA was extracted using the CTAB method described in Doyle and Doyle (1987). The extracted DNA was resuspended in 0.1 ml TE buffer.

ISSR amplification

PCR amplifications were performed in 10 mM Tris-HCl, 50 mM KCl, 1.0 mM $MgCl_2$, 0.1% (w/v) 1% gelatin (w/v) Triton X-100, 100 µM dNTPs, 0.2 µM primer, 0.5 units Taq polymerase (HT Biotechnology LTD , UK) and 10 ng DNA template per 25-µl reaction. Amplification was performed in a thermocycler (Perkin Elmer Geneamp PCR System 9700) with the following steps: initial denaturation at 94°C for 6 min followed by 39 cycles of 94°C for 6 min, 50-55°C for 50 s, and 72°C for 2 min. The method concluded with a final extension at 72°C for 7 min. PCR products were separated on a 1.5% agarose gel buffered with 1X TBE and detected by ethidium bromide staining.

Data analysis

Reproducible polymorphic bands generated by the ISSR method were scored in terms of presence (1) or absence (0) for each sample. Nei's gene diversity (Nei, 1973) POPGENE 3.2 software (Yeh et al., 1999) was used to calculate gene diversities and genetic distances. In order to test for correlations between genetic and geographical distances among populations, NTSYS-pc software was used (Rohlf, 1993).

RESULTS

Morphological analysis

Nested analysis of variance (ANOVA) was used to analyze the variables in leaf morphology. Significant differences in three quantitative traits were observed, including branchlet length, internode length, and the number of sheath teeth ($p < 0.0001$) (Table 4).

Genetic diversity

A total of 82 polymorphic bands were observed using 13 ISSR primers (Table 2) for three species across 456 individuals of 38 populations. The level of Nei's gene diversity (H) of each population varied among species and localities (Table 3). Population T2 was the most genetically diverse (0.3197), while G2 of *C. glauca* was the least (0.00495). The average diversities of Taiwan coastal *Casuarina*, *C. equisetifolia*, *C. cunninghamiana*, and *C. glauca* were 0.1940, 0.1288, 0.0922, and 0.0577, respectively; the average for *Casuarina* grown in Taiwan was found to be highly statistically significant ($p < 0.001$) when compared to *C. equisetifolia*, *C. glauca* and *C. cunninghamiana*.

Genetic relationships among populations

Nei's genetic distance matrix among populations was used to conduct UPGMA cluster analysis. The cophenetic correlation coefficient of this cluster analysis was 0.9227, indicating agreement between the genetic distance matrix and the cophenetic matrix. A dendrogram (Figure 1) including all populations sampled clearly identified two groups separated by a genetic distance of 0.20. The first group comprised all populations of *C. equisetifolia* and most *Casuarina* grown in Taiwan except for populations T2 (Fangyuan) and T3 (Wuchi). This group contained two subgroups. One subgroup comprised loosely linked populations of *C. equisetifolia,* while the other contained more closely linked Taiwanese *Casuarina*. Within the subgroup of *C. equisetifolia*, population C1 (Cairns, Australia) was the most isolated group. Population C1

Table 1. Thirty-eight *Casuarina* OTUs studied.

Population	CSIRO seedlot no.	Location	Latitude
C1	15958	Wangetti Beach, Cairns, Australia	S16º 41´ E145º 41´
C2	18008	Darwin, Australia	S12º 25´ E130º 50´
C3	18121	Mariana Island, Gum	N14º 40´ E145º 0´
C4	18153	Ela Beach, Papua New Guinea	S 9º 15´ E148º 17´
C5	18117	Philippines	N12º 21´ E121º 2´
C6	18244	Bako Borneo, Sarawak, Malaysia	N 1º 44´ E111º 29´
C7	18157	Pantai Moyog, Sabah, Malaysia	N 5º 55´ E116º 5´
C8	18158	Tanjung Aru, Sabah, Malaysia	N 5º 55´ E116º 5´
C9	18270	Baravi Viti Levu, Fiji	S17º 0´ E177º 0´
C10	18271	Wainunu Vanua Levu, Fiji	S17º 0´ E177º 0´
C11	18154	Aklan, Panay Island, Philippines	N11º 31´ E122º 30´
C12	18298	Had Chab Hai Trakg, Thailand	N 7º 33´ E100º 37´
K1	13511	26km S of Mt. Morgan, Australia	S23º 49´ E150º 18´
K2	17186	Flagstone Ck Rd, Australia	S27º 38´ E152º 3´
K3	13518	8km S of Mt. Molloy, Australia	S16º 44´ E145º 21´
K4	14999	River Lett Hartley, Australia	S33º 33´ E150º 19´
K5	13519	9km N Rollingstone, Australia	S19º 1´ E146º 20´
S1	13139	8km N of Woolgoolga, Australia	S30º 3´ E153º 11´
S2	17200	Aberdare Colliery, Australia	S27º 37´ E152º 51´
S3	15218	Caloundra, Australia	S26º 48´ E153º 9´
S4	15939	Tukean Swamp, Australia	S28º 59´ E153º 23´
S5	15938	Yuragir, Australia	S29º 52´ E153º 15´
S6	15941	Burrum Heads, Australia	S25º 12´ E152º 37´
T1		Suhu, Yunlin, Taiwan	N23º 60´ E120º 12´
T2		Fangyuan, Changhua, Taiwan	N23º 97´ E120º 31´
T3		Wuchi, Taichung, Taiwan	N24º 25´ E120º 50´
T4		Houlung, Miauli, Taiwan	N24º 55´ E120º 80´
T5		Kuanyin, Tauyuan, Taiwan	N25º 10´ E121º 12´
T6		Shihmen, Taipei, Taiwan	N25º 28´ E121º 53´
T7		Kungliao, Taipei, Taiwan	N25º 2´ E121º 90´
T8		Nanao, Ilan, Taiwan	N24º 55´ E120º 80´
T9		Chian, Hualien, Taiwan	N23º 97´ E121º 55´
T10		Changpin, Taitung, Taiwan	N23º 33´ E121º 45´
T11		Taimali, Taitung, Taiwan	N22º 61´ E121º 0´
T12		Hengchun, Pingtung, Taiwan	N21º 85´ E120º 90´
T13		Linyuan, Kaoshiung, Taiwan	N22º 51´ E120º 35´
T14		Chiating, Kaoshiung, Taiwan	N22º 89´ E120º 14´
T15		Peimen, Tainan, Taiwan	N23º 25´ E120º 10´

C denotes native *Casuarina equisetifolia* populations; K denotes *Casuarina cunninghamiana*; S denotes *Casuarina glauca* and T denotes *Casuarina* cultivated in Taiwan.

Table 2. Nucleotide sequence, GC content, number of fragments (nb), and number of polymorphic fragments (np) of each primer used.

Primer used	Sequence(5'→3')	GC content (%)	nb	np
AM15	ACACACACACACACACT	47.1	9	7
AM16	ACACACACACACACACCTA	47.2	9	6
AM19	TCTCTCTCTCTCTCTCC	52.9	9	6
AM24	GGAGAGGAGAGGAGA	60.0	12	6
IS29	TCTCTCTCTCTCTCTCA	47.1	8	6
IS30	TCTCTCTCTCTCTCTCG	52.9	8	6
IS37	ACTAGCACTCACACACACACACATACTAGCACT	46.2	13	7
IS42	CACACACACAC	36.8	11	8
IS60	AAGAAGAAGAAGAAGAAGG	52.9	13	7
IS62	CACACACACACACACAG	52.8	12	6
IS63	CACACACACACACACA(AG)G	47.2	10	6
IS65	CACACACACACACACA(AG)T	63.2	13	6
IS81	CCTCCTCCTCCTCCTCCTT	47.1	9	5
Total			136	82

Table 3. Nei's gene diversity (H) of the populations studied.

Population	H	Population	H	Population	H	Population	H
T1	0.1778	C1	0.0721	K1	0.0968	S1	0.0692
T2	0.3197	C2	0.1228	K2	0.0944	S2	0.0495
T3	0.1901	C3	0.1325	K3	0.1024	S3	0.0699
T4	0.1998	C4	0.1215	K4	0.0703	S4	0.0508
T5	0.1210	C5	0.1146	K5	0.0972	S5	0.0551
T6	0.2008	C6	0.1212			S6	0.0514
T7	0.2791	C7	0.1164				
T8	0.1449	C8	0.1193				
T9	0.2529	C9	0.1992				
T10	0.1968	C10	0.1789				
T11	0.1855	C11	0.1148				
T12	0.1793	C12	0.1326				
T13	0.1497						
T14	0.1837						
T15	0.1293						
Average	0.1940		0.1288		0.0922		0.0577

C denotes native *C. equisetifolia* populations ; K denotes *C. cunninghamiana*; S denotes *C. glauca, and* T denotes *Casuarina* cultivated in Taiwan.

Table 4. Nested ANOVA of branchlet length, internode length, and number of sheath teeth.

Characters	Level	df	Fs	Var. comp.	Total variation (%)	p-val
Branchlet length	Among species	3	7.90 ***	21.26	28.89	<0.001
	Variance among populations within specie	34	11.22*	24.57	33.40	<0.05
	Individual s	400		27.73	37.70	
Internode length	Among species	3	1.18	0.0003	1.15	>0.05
	Variance among populations within specie	34	11.38*	0.0131	46.81	<0.05

	Individual	400		0.0146	52.03	

Table 4. Contd.

Number of sheath teeth	Among species	3	26.62***	5.25	61.05	<0.001
	Variance among populations within specie	34	12.2*	1.65	19.19	<0.05
	Individual	400		1.7	19.76	

Asterisks denote significant differences according to a Duncan's multiple test range (* = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$).

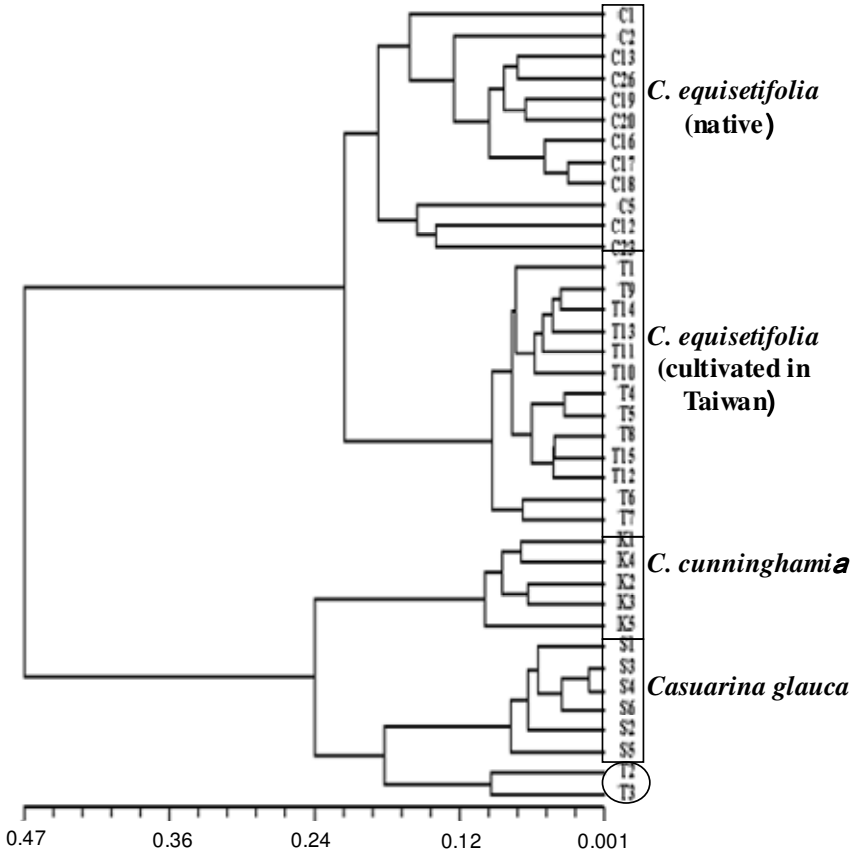

Figure 1. Dendrogram generated by UPGMA clustering of ISSR genetic distances.

belongs to *C. equisetifolia* subsp. *incana* (Benth) L. Johnson, while populations C2 to C12 belong to *C. equisetifolia* subsp. *equisetifolia*. The second group contained provenances of *C. cunninghamiana*, *C. glauca*, and T2 and T3 of Taiwan. Two further population distinctions could be identified within this subgroup of *C. equisetifolia*. The first of which included five populations of *C. cunninghamiana,* while the other group included populations T2, T3, and six provenances of *C. glauca*. This out-group clustering was consistent with current taxonomic classifications of the species. Furthermore, the clustering also indicated that *C. cunninghamiana* and *C. glauca* are closely related, while *C. equisetifolia* is the most isolated among these three species.

The genetic distance matrix among populations was also used to conduct a principal coordinates analysis (Figure 2). A three dimensional diagram was constructed based on the first three coordinates, which explained 92.62% of the total variation. The relationships among all populations identified in this method correlated with the genetic relationships observed by cluster analysis. The populations of *C. equisetifolia* and most of the *Casuarina* grown in Taiwan formed a generally coherent clade with *C. cunninghamiana* and *C. glauca*, with T3 forming a separate group. Interestingly, T2 was located between these clades.

Furthermore, a two-dimensional diagram constructed using the first and second coordinates, accounting for

95.67% of the total variation, clearly identified a population of *Casuarina* grown in Taiwan located between *C. equisetifolia* and a major clade comprising *C. cunninghamiana* and *C. glauca*.

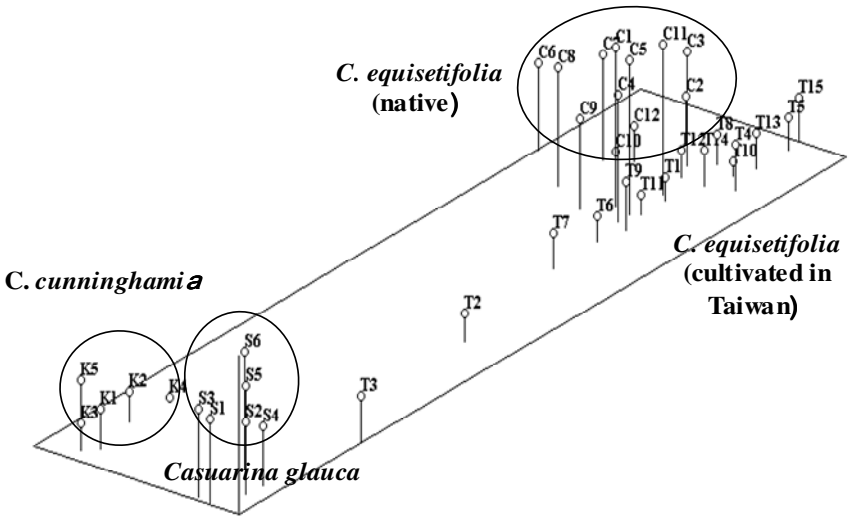

Figure 2. Principal coordinate diagram of OTUs based on ISSR genetic distances.

Table 5. Phenotypes associated with coastal Taiwanese *Casuarina* spp.

Provenance code	Branchlet length (cm)		Internode length(cm)		Number of sheath teeth	
	Variance of range	Mean	Variance of range	Mean	Variance of range	Mean
T1	13.9~26.5	19.23	0.51~0.90	0.72	7~8	7
T2	17.5~53.8	33.37	0.48~1.14	0.78	7~18	10
T3	32.6~49.9	40.39	0.86~1.35	1.14	12~15	13
T4	12.4~28.9	19.99	0.49~0.72	0.62	6~8	7
T5	11.2~18.7	15.03	0.47~0.65	0.55	6~8	7
T6	13.8-38.9	23.52	0.51~0.99	0.80	7~12	9
T7	13.1~54.6	23.60	0.48~1.23	0.67	6~11	8
T8	12.7~49.5	29.27	0.44~1.14	0.75	6~12	9
T9	13.3~60.6	21.25	0.56~1.14	0.73	6~15	8
T10	11.1~20.5	15.20	0.47~0.75	0.61	6~7	7
T11	9.9~19.9	15.67	0.51~0.84	0.64	6~7	7
T12	10.1~23.6	17.29	0.50~0.85	0.70	6~7	7
T13	10.0~16.7	13.77	0.40~0.68	0.58	6~8	7
T14	11.3~22.3	15.30	0.40~0.75	0.58	6~7	7
T15	13.2~29.2	17.61	0.50~0.80	0.63	7~8	7

DISCUSSION

Genetic diversity

Our results indicate that the genetic diversity of *Casuarina* grown in Taiwan is greater than other populations endemic to Australasia. The average genetic diversity of *Casuarina* grown in Taiwan (0.1940) is statistically significant ($p < 0.001$) when compared to native populations of *C. equisetifolia* (0.1288), *C. cunninghamiana* (0.0922), and *C. glauca* (0.0577). Ho et al. (2004) observed low levels of genetic diversity for *Casuarina junghuhniana* Miq isolated from native populations when compared to Taiwanese populations. These differences may be attributable to geographical isolation (Ho et al., 2004). In contrast, cultivated populations had higher genetic diversity, which would suggest inter-species hybridization.

Documentation of hybridization

The relationships among branchlet morphology, genetic variation, and speciation were also investigated (Table 4). This study found that the branchlet morphology of most

Taiwanese *Casuarina* resemble the population of *C. equisetifolia* introduced from Australia. Interestingly, a subgroup of populations, T2 and T3, closely resembled native *C. glauca* and not *C. equisetifolia* (Table 5).

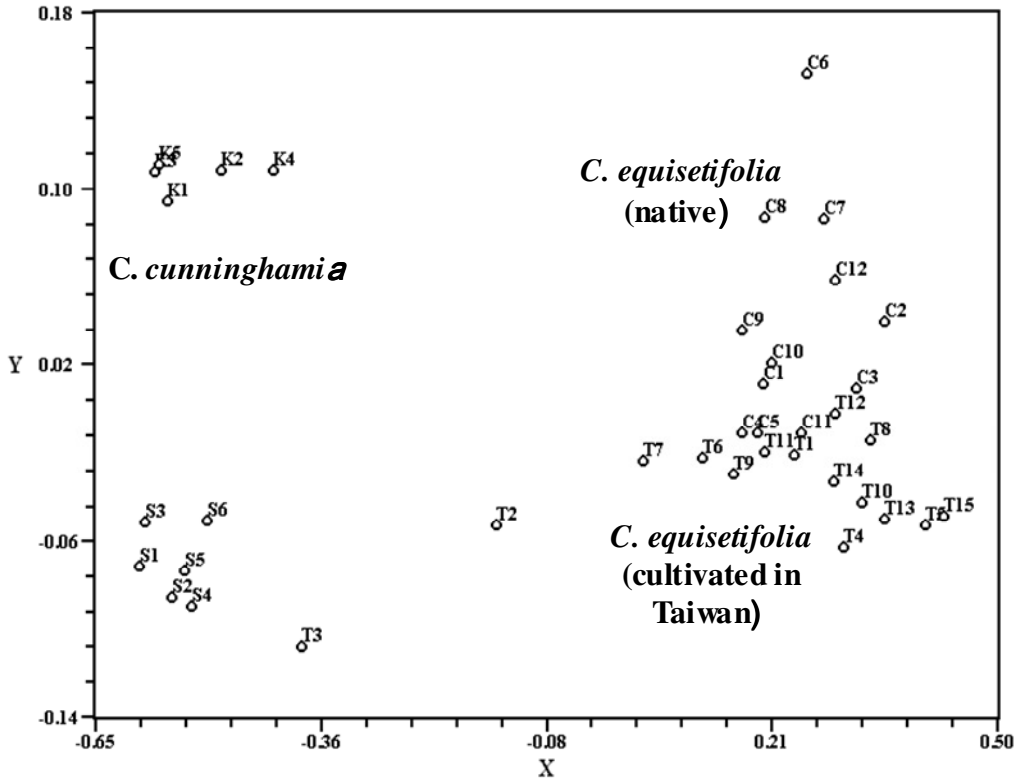

Figure 3. Two-dimensional principal coordinate diagram of OTUs based on ISSR genetic distances.

The results of cluster analysis and principal coordinate analysis support the hypothesis of hybridization. Cluster analysis demonstrates that most sampling sites of *Casuarina* grown in Taiwan form a clade that is linked to native populations of *C. equisetifolia*. This correlation indicates that most plants in Taiwan are derived, albeit distantly, from the native populations of *C. equisetifolia* imported from Australasia.

In addition, results of principal coordinates analyses (Figures 2 and 3) indicate that Taiwanese plants are more closely related to *C. equisetifolia* than either *C. cunninghamiana* or *C. glauca,* but they contain traits apparent in *C. cunninghamiana* and *C. glauca.* The integrated reasoning of genetic variation and branchlet morphology suggests that some *Casuarina* plants currently grown in Taiwan are products of introgressive hybridization involving *C. equisetifolia*, *C. glauca*, and possibly *C. cunninghamiana*.

The analytical results indicate that *Casuarina* survived in coastal land of Taiwan have high genetic differentiation, and the introduction of populations from other countries has increased gene flow and enabled *Casuarina* to inhabit even more environmentally diverse coastal areas.

REFERENCES

Adams RP, Schwarzbach AE, Pandey RN (2003) The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes:an example from Juniperus. Biochem. System. Ecol., 31: 375-387.

Barth S, Melchinger AE, Lubberstedt T (2003). Genetic diversity in *Arabidopsis thaliana* L. Heynh. Investigated by cleaved amplified polymorphic sequence (CAPS) and inter-simple sequence repeat (ISSR) markers. Mol. Ecol., 11(3): 495-505.

Chen ZZ (1980). Identification, introduction of new species and comparative study of the salt tolerance in certain *Casuarina* spp. in Taiwan. Taipei, Taiwan. National Taiwan University. (in Chinese with English summary)

Christoph R, Poschlod P, Wingender R (2003) Genetic variation of *Saxifraga paniculata* Mill. (Saxifragaceae): molecular evidence for glacial relict endemism in central Europe. Bio. J. Lin. Soc., 80: 11-21.

Chuang HY, Tsao SJ, Lin JN, Chen KS, Liou TD, Chung M (2004) Genetic diversity and relationship of non-heading Chinese cabbage in Taiwan. Bot Bull Acad Sin., 45: 331-337.

Doran JC, Lubberstedt HN (1983). Notes on fifteen Australian *Casuarina* species. In: Midgley SJ, Turnbull JW, Johnson RD. editors. *Casuarina* ecology, management and utilization, Melbourne: CSIRO, pp.19-25

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19: 11-15.

Ge XJ, Yu Y, Zhao NX, Chen HS, Qi WQ (2003). Genetic variation in the endangered Inner Mongolia endemic shrub *Teteaena mongolica* Maxim. (Zygophyllaceae). Biol. Conserv., 111: 427-434.

Ho KY, Hsiao JY, Ou CH, Deng SH (2004). Branchlet variation of *Casuariana* spp. In Taiwan Q. J. For. Res., 26(1): 15-26.

Ho KY, Yang JC, Deng SL, Chen TH (2004) Assessment of genetic variation and relationships of international provenances of *Casuarina*

junghuhniana using ISSR. Taiwan J. For. Sci., 19(1): 79-88.
Ho KY, Deng SL, Chang YH, Tsai CS, Kao MF, Hsiao JY (2005) Genetic variation of endangered *Scaevola hainanensis* (Goodeniaceae) in the Jiangjun Stream mouth, Taiwan. Taiwan J. For. Sci., 20(3): 193-202.
Hwang YH, Hsiao JY (1985) A taxonomic study on Casuarinaceae in Taiwan. Proc. Nat. Sci. Counc. B ROC, 9: 13-19.

Li F, Xia N (2005) Population structure and genetic diversity of an endangered species, *Glyptostrobus pensilis* (Cupressaceae). Bot. Bull. Acad. Sin., 46: 155-162.
Nei M (1972) Genetic distance between populations. Am. Nat., 106: 283-292.
Nei M (1973) Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA, 70: 3321-3323.
Rohlf FJ (1993) NTSYS-pc Numerical Taxonomy and Multivariate Analysis System New York. Applied Biostatistics Inc.
Wang TT, Yang JC, Chen ZZ (1984) Identification of hybridity of *Casuarinas* grown in Taiwan. Silvae Genet., 33: 128-133.
Wilson KL, Johnson LAS (1989). Casuarinaceae. In: Flora of Australia, volume 3, Hamamelidales to Casuarinales, Australian Government Publishing Service, Canberra.
Xie GW, Wang DL, Yuan YT, Ge XJ (2005). Population genetic structure of *Monimopetalum chinense* (Celastraceae), an endangered endemic species of eastern China. Ann. Bot., 95(5): 773-777
Yang JC, Chang TY, Chen TH, Chen ZZ (1995). Provenance trial of *Casuarina equisetifolia* in Taiwan. 1. Seed weight and seedling growth. Bull Taiwan For Res Inst New Series 10(2): 197-207. (in Chinese with English summary) .
Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1999) POPGENE 3.2, The user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton.
Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)- anchored polymerase chain reaction amplication. Genome, 20: 176-183. Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplication. Genome, 20: 176-183.