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THE GENETIC DIVERSITY OF THE MANGROVE *KANDELIA OBOVATA* IN CHINA REVEALED BY ISSR ANALYSIS

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Abstract

The genetic diversity of 7 populations of *Kandelia obovata* in China was characterized using inter simple sequence repeats (ISSR) technique. A total of 50 primers were screened, of which 9 polymorphic and informative patterns were selected to determine genetic relationships. ISSR amplification was conducted on 140 individuals from 7 populations, and 88 polymorphic loci were detected from 106 total loci. The total percentage of polymorphic loci (*PPL*) was 83.02%. The percentage of *PPL* at the population level ranged from 32.08% to 47.17%, with an average of 39.89%. Nei's gene diversity (*H*) and Shannon's information index (*I*) of *K. obovata* at the species level were 0.3631 and 0.5203, respectively. The genetic differentiation coefficient (*G*st) among populations was 0.5548. Among populations component accounted for 55.48% of the total variation, whereas the within populations component accounted for 44.52%, suggesting that genetic differentiation among *K. obovata* populations was relatively high. The gene flow among populations was 0.4012, indicating that gene flow was low among geographically diverse populations of *K. obovata*. The results of the genetic diversity and cluster analysis suggest that geographical isolation of *K. obovata* populations mainly results in low gene flow and random genetic drift, leading to genetic differentiation.

Introduction

Kandelia obovata (L.) Druce is a typical tropical and subtropical constituent of mangrove communities, and it is naturally found in the Hainan, Guangdong, Guangxi, Fujian and Taiwan provinces of China. In the 1950s, *K. obovata* was successfully introduced from Fuding, Fujian Province to Ximen Island Special Protected Area, Zhejiang Province, making it the northernmost mangrove in China (He *et al.*, 2007).

As a widespread mangrove plant with individual forests and the advantage of strong cold-resistance, *K. obovata* plays an important role in stopping wind and waves, protecting coastlines, making land, and improving the environment (Zhao & Lin, 2000; Fan, 2005). However, with global warming, environmental change and human activities (He *et al.*, 2009), mangroves are being destroyed at an alarming rate, leading to loss of genetic diversity (Field, 1998). On the other hand, global warming also brings new opportunities for the transplantation of mangroves further north and the development of *K. obovata* in Zhejiang (Gong *et al.*, 2009). Studying the genetic diversity of *K. obovata* is important in taking effective measures to protect this species.

There have been numerous studies on *K. obovata* in many fields, including ecology (Chen *et al.*, 1995; Nabiul Islam Khan& Rempei Suwa, 2005), physiology (Ye *et al.*, 2003; Chen *et al.*, 2005), biochemistry (Nabiul Islam Khan *et al.*, 2004), pharmacology (Bandaranayake, 1998), tolerance mechanism (Wang & Lin, 2003) and molecular biology

(Huang *et al*., 2003). Recently, a newly developed modification of the SSR-based marker systems (Zietkiewicz *et a1*., 1994), ISSR (inter simple sequence repeat), has been used in studies of *Sonneratia caseolaris* (Li & Chen, 2008), *Excoecaria agallocha* (Zhang *et al*., 2008), *Ceriops tagal* (Tan *et al*., 2008), and *Lumnitzera racemosa* (Su *et al*., 2006), but has not been reported in the study of *K. obovata* in populations throughout China. To clarify genetic diversity, genetic structure, the relationship between genetic distance and geographical distance, and to provide basic data and scientific basis for effective protection, ISSR molecular marker technology was used to study the genetic diversity of *K. obovata*.

Materials and Methods

Plant material: One hundred and forty samples representing 7 populations of *K. obovata* were collected from Hainan, Guangdong, Guangxi, Fujian and Zhejiang provinces. Each population was represented by 20 individuals. Latitude and longitude were recorded using a GPS locator (Table 1), and young leaves from selected trees were collected and stored with silica gel in zip-lock plastic bags before DNA extraction. Sample codes and the sampling locations are shown in Fig. 1.

DNA extraction and PCR amplification: Total DNA was extracted from silica gel-dried young leaves using the modified CTAB method (Doyle, 1991). Fifty ISSR primers from the Biotechnology Laboratory, University of British Columbia, were initially screened to amplify genomic DNA in order to identify potential primers that produced a higher number of polymorphic and repeatable fragments.

DNA amplifications were performed in a 25 μL reaction volume containing 2.5 μL 10×PCR Buffer (100 mM KCl, 80 mM $(NH_4)_2SO_4$, 100 mM Tris-HCl, pH 9.0, 0.5% NP-40) 2.0 μL dNTP mixture (2.5 mmol/L), 1.5 μL $MgCl_2$ (25 mmol/L), 2.0 μL primer (10 μmol/L), 1.0 μL DNA (30~50 ng), and 1 U TaKaRa Taq (5 U/μL). Amplification was performed in a 2720 thermocycler under the following conditions: 5 min at 94°C, followed by 45 cycles of 45 s at 94°C, 45 s annealing at 52°C (depending on primers used; Table 2), and 1.5 min extension at 72°C, followed by 7 min at 72°C for the final extension step.

The amplification products were separated by electrophoresis in 1.5% agarose gels buffered with 0.5× TBE. Gels were stained with ethidium bromide, and band detection was performed using a BIO-RAD Gel Doc. Band size was estimated from a DL2000 DNA Marker.

Data analysis: ISSR was the dominant marker, and all bands amplified by the same primer pair with identical electrophoretic mobility were homologous. ISSR bands were used to assign loci for each primer and scored for presence (1) or absence (0). Assuming Hardy–Weinberg equilibrium, the binary data matrix was input into POPGENE (Yeh *et al*., 1997; Sun *et al*., 2009). The following indices were used to quantify the amount of genetic diversity within and among the populations examined:

The percentage of polymorphic loci (*PPL*), observed number of alleles (*N*a), effective number of alleles (*N*e) (Kimura & Crow, 1964) and Shannon's Information index (Lewontin, 1972). Genetic differentiation among the populations was estimated by Nei's gene diversity statistics (Nei, 1973). The total gene diversity (*H*t), the gene diversity within populations (*H*s) and the genetic differentiation coefficient (*G*st= (*H*t-*H*s)/ *H*t) were also calculated. The level of gene flow among these populations was estimated as *N*m= $(1/G_{ST} - 1)/4$ (Slatkin & Barton, 1989).

Table 1 Latitude and longitude were recorded using GPS Locator.

Assigned code	Sample collection location	Latitude	Longitude
Pop.A	Ximen Island Special Protected Area, Zhejiang, China	N28°20.912′	E121°10.712′
Pop.B	Fuding, Fujian, China	N27°13.391′	E120°17.680′
Pop.C	Zhangjiangkou Mangrove National Nature Reserve, Yunxiao, Fujian, China	N23°55.808′	E117°24.800′
Pop.D	Neilingding Futian Nature Reserve, Guangdong, China	N22°31.434′	E114°00.870′
Pop.E	Zhanjiang Mangrove National Nature Reserve, Guangdong, China	N21°34.054′	E109°45.508′
Pop.F	Qinglan'gang Nature Reserve, Hainan, China	N19°37.538′	E109°45.589′
Pop.G	Beilunhekou Marine Nature Reserve, Guangxi, China	N21°37.063′	E108°13.908′

Note: the "populations" assigned in this paper were identified as the codes in this table.

Table 2. Primers screened for ISSR amplification.

Primer	Sequence 5'→3'	Annealing temperature (^{o}C)
BW09461HAP	5'- ACACACACACACACACT-3'	50^{o}C
BW09462HAP	5'- ACACACACACACACACC-3'	52^{o}C
BW09477HAP	5'- GAGAGAGAGAGAGAGAYC	52^{o}C
BW09480HAP	5'- CTCTCTCTCTCTCTCTRC-3'	52^{o}C
BW09481HAP	5'- CTCTCTCTCTCTCTCTRG-3'	52^{o}C
BW09491HAP	5'- ACACACACACACACACYT-3	50^{o}C
BW09492HAP	5'- ACACACACACACACACYA-3	50^{o}C
BW09497HAP	5'- ACCACCACCACCACC-3	50^{o}C
BW09498HAP	5'- AGCAGCAGCAGCAGC-3	50^{o}C

To illustrate the genetic relationships among the populations, a dendrogram was constructed based on the similarity matrix using unweighted pair group method with arithmetic average (UPGMA) cluster analysis.

Results

Polymorphisms detected by ISSR: ISSR amplifications using 9 primers (Table 2) generated a total of 9997 reliable bands in the 140 *K. obovata* samples. Of the 106 DNA loci, 88 loci were polymorphic, with only 18 being present in all samples examined. The total percentage of polymorphic loci was 83.02% (Table 3). The number of DNA loci amplified for a given primer/sample combination varied from 10 to 13 depending on the primer used, with an average of 11.8 loci per primer. The size of the bands ranged from 150 to 2200 bp.

Fig.1 Diagram of sampling locations.

Table 3. Number of loci for ISSR primers.

Primer	Total loci	Polymorphic loci	Percentage of polymorphic loci
BW09461HAP	12	10	83.33%
BW09462HAP	12	9	75.00%
BW09477HAP	12	10	83.33%
BW09480HAP	12	11	91.67%
BW09481HAP	13	11	84.62%
BW09491HAP	13	11	84.62%
BW09492HAP	11	8	72.73%
BW09497HAP	11	10	90.91%
BW09498HAP	10	8	80.00%
Average	11.8	9.8	83.05%
All loci	106	88	83.02%

Fig. 2 shows the amplification patterns generated using primer BW09480HAP across the 20 samples from Zhanjiang Mangrove National Nature Reserve, Guangdong, China (Pop.E).

Fig. 3 shows the amplification patterns generated using primer BW09497HAP across the 20 samples from Qinglan'gang Nature Reserve, Hainan, China (Pop.F).

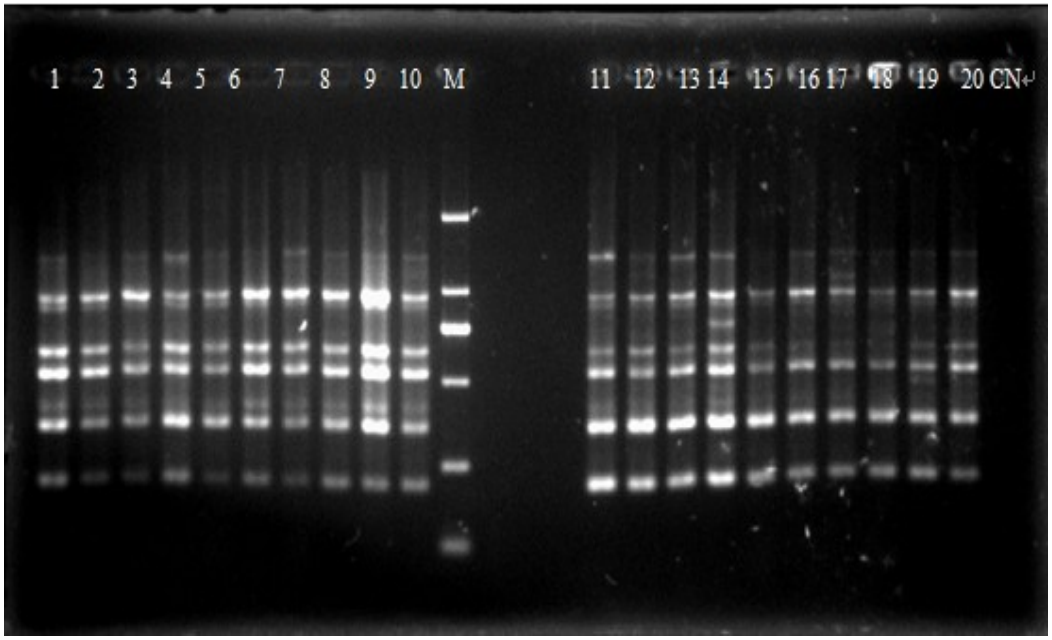

Fig. 2. PCR products of the twenty genomes of Pop. E using primer BW09480HAP (M: DL2000 DNA Marker; CN: Negative control)

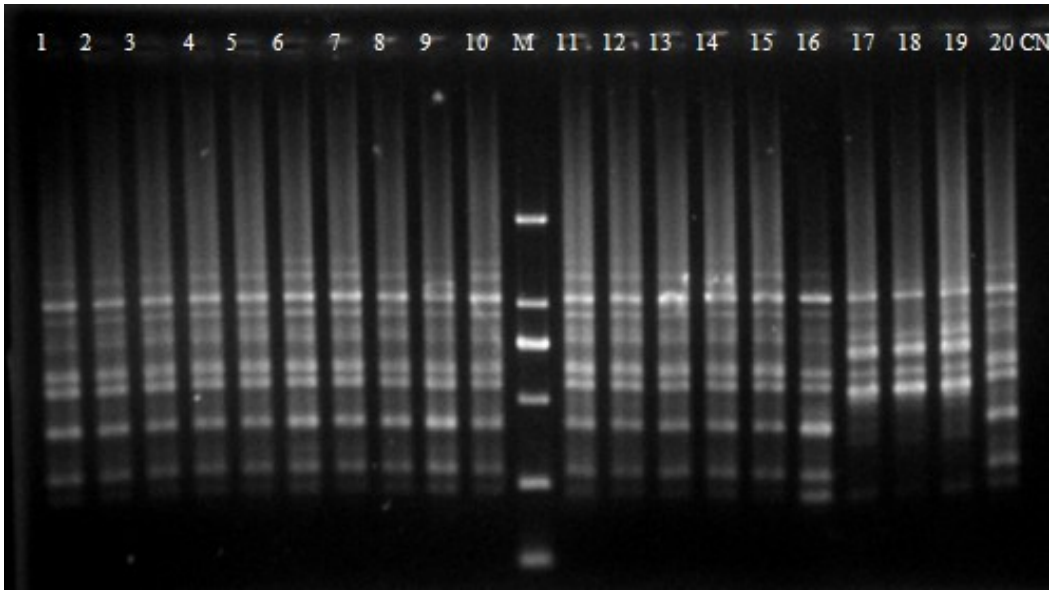

Fig. 3. PCR products of the twenty genomes of Pop. F using primer BW09497HAP (M: DL2000 DNA Marker; CN: Negative control).

Genetic diversity in seven populations of *K. Obovata*: Table 4 shows the calculated genetic variation in 7 populations of *K. obovata* using the software POPGEN. The percentage of polymorphic loci at the population level ranged from 32.08% to 47.17%, with an average of 39.89%. The average value of *N*e was 1.2844. As an index of the genetic diversity of populations, Nei's gene diversity (*H*) was measured, and ranged from 0.1328 to 0.1870, with an average of 0.1617, within populations and 0.3631 among populations. Shannon's information index (*I*) ranged from 0.1943 to 0.2741, with an average of 0.2359 within populations and 0.5203 among populations. Among the seven populations, Pop.F and Pop.G showed the highest genetic variation, while Pop. B and Pop. E showed the lowest.

Table 4. Genetic variation in seven populations of *K. obovata*.

Populations	Sample size	*N*a	*N*e	*H*	*I*	*PPL*
Pop.A	20	1.3396±0.4758	1.2445±0.3664	0.1386±0.2001	0.2021±0.2881	33.96%
Pop.B	20	1.3302±0.4725	1.2326±0.3573	0.1328±0.1963	0.1943±0.2834	33.02%
Pop.C	20	1.4151±0.4951	1.3081±0.3884	0.1733±0.2128	0.2510±0.3055	41.51%
Pop.D	20	1.4434±0.4991	1.3103±0.3834	0.1768±0.2088	0.2587±0.3004	44.34
Pop.E	20	1.3208±0.4690	1.2491±0.3787	0.1382±0.2050	0.1992±0.2935	32.08%
Pop.F	20	1.4717±0.5016	1.3256±0.3799	0.1870±0.2085	0.2741±0.3005	47.17%
Pop.G	20	1.4717±0.5016	1.3205±0.3766	0.1849±0.2067	0.2719±0.2982	47.17%
Average	20	1.3989±0.4878	1.2844±0.3758	0.1617±0.2055	0.2359±0.2957	39.89%
Total	140	1.8302±0.3772	1.6673±0.3554	0.3631±0.1784	0.5203±0.2479	83.02%

Abbreviation: *N*a = Observed number of alleles *N*e = Effective number of alleles [Kimura and Crow (1964)]
H = Nei's gene diversity (1973), *I* = Shannon's Information index [Lewontin (1972)]
PPL= The percentage of polymorphic loci

Genetic structure in seven populations of *K. Obovata*: The total gene diversity (*H*t) and the gene diversity within populations (*H*s) were 0.3631 and 0.1617 respectively, with the value of gene diversity 0.2014 among populations at the species level. The genetic differentiation coefficient (*G*st) among populations was 0.5548. The among populations component accounted for 55.48% of the total variation, while the within populations component accounted for 44.52%, showing that the genetic differentiation among populations was relatively high. The gene flow among populations was 0.4012, indicating that gene flow was low among geographically diverse populations of *K. obovata*.

The genetic differentiation index could only describe the extent of the variation, not the evolutionary distance among populations, while the genetic identity and the Nei's genetic distance provided this information.

Table 5 shows the Nei's genetic distance (*D*) and the genetic identity (*I*) among populations of *K. obovata*. The Nei's genetic distance (*D*) ranged from 0.1489 to 0.4593, with the nearest (0.1489) being between Pop.A and Pop.B, and the farthest (0.4593) being between Pop.B and Pop.F. An inverse relationship was found for genetic identity (*I*).

The clustering result indicated that all seven populations could be distinguished by ISSR markers. Populations with lower genetic distance were grouped together. Four major clades were revealed by the UPGMA dendrogram (Fig. 4). Pop.A from Ximen Island, Zhejiang, and its neighbor, Pop.B, from Fuding, Fujian, formed a clade. Pop.E from Zhanjiang, Guangdong, and Pop.G from Beilunhekou, Guangxi, formed another clade. Pop.C from Zhangjiaokou, Fujian, and Pop.D from Neilingting Futian, Guangdong, formed another clade, leaving Pop.F from Qinglan'gang, Hainan, in its own clade.

Discussion

Genetic diversity of *K. Obovata*: Genetic diversity at the species level, the product of long-term evolution, is a prerequisite for survival and development. Studying the genetic diversity and genetic structure of a species is the basis of exploring its adaptability and viability (Shen *et al.*, 2005). The genetic diversity is generated by errors in the replication process (such as inversion, translocation, deletion or transposition of DNA fragments etc.), which can be caused by both external and internal factors. Mutations, including point mutations, continue to occur spontaneously at very low frequencies, and along with those more complex mutations caused by DNA turnover mechanisms, they become fixed or disappear due to genetic drift and long-term selection, creating high genetic diversity within and among species (Xiao, 2005).

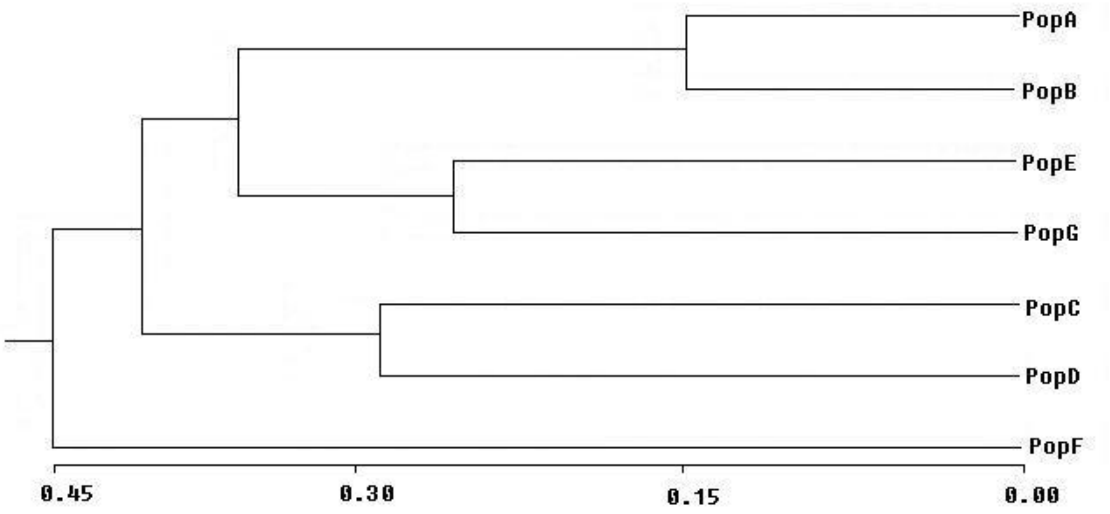

Fig. 4. Nei's Unbiased Measures of Biosystematics dendrogram of *K. obovata* populations.

Table 5. Nei's genetic distance below diagonal and genetic identity above diagonal.

Populations	Pop.A	Pop.B	Pop.C	Pop.D	Pop.E	Pop.F	Pop.G
Pop.A	****	0.8616	0.6906	0.6966	0.7545	0.6413	0.7375
Pop.B	0.1489	****	0.7526	0.7188	0.7375	0.6317	0.7065
Pop.C	0.3702	0.2842	****	0.7509	0.6856	0.6991	0.7266
Pop.D	0.3615	0.3302	0.2865	****	0.7018	0.6942	0.7289
Pop.E	0.2817	0.3045	0.3775	0.3541	****	0.6826	0.7851
Pop.F	0.4442	0.4593	0.3580	0.3650	0.3819	****	0.7261
Pop.G	0.3045	0.3475	0.3194	0.3163	0.2420	0.3201	****

Adaptability and evolution of a species are based on the level of genetic diversity present in populations, which reflect the richness of diverse genotypes in a specific environment.. The percentage of polymorphic loci can be used as indicator to measure the level of genetic diversity. In this study, the percentage of polymorphic loci of *K. obovata* ranged from 32.08% to 47.17%. The decreasing order of genetic diversity was Pop.F> Pop.G> Pop.D> Pop.C> Pop.A> Pop.B> Pop.E, indicating that Pop.F has a higher level of genetic diversity, richness of genes, and is very suited for the specific environment. From the genetic perspective, a higher level of genetic diversity results in a greater ability to adapt and evolve (Li & Chen, 2004). Low genetic diversity can result in reduced adaptability and increased occurrence of less beneficial genes, leading to eventual extinction of the species. The *PPL* among populations in this study is 83.02%, showing a high level of genetic diversity; however, the lower level of diversity within populations should be paid special attention.

Genetic structure of *K. obovata*: The level of genetic diversity can provide very important information about the status of a species, an assessment of its conservation value, and *ex situ* conservation. Different genotypes in different habitats have different fitness, which causes the same genotype to clump together into more suitable microhabitats, resulting in genetic differentiation (Chen & Song, 1998). The genetic differentiation coefficient (*G*st) shows the level of genetic variation. According to the level of genetic diversity within populations (*H*s) and among populations (*H*t - *H*s), the genetic differentiation coefficient (*G*st) was 0.5548. The among populations component accounted for 55.48% of the total variation, while the within populations component

accounted for 44.52%, showing that there was higher differentiation among the populations of *K. obovata* than within populations.

In a sense, the genetic structure of a population results from the interaction of gene flow and genetic drift. When the value of gene flow is greater than 1 it can cause homogenization, lowering genetic differentiation, which is caused by genetic drift among the populations (Wright, 1978, 1931). Alternately, when the value of gene flow is less than 1, homogenization does not occur, and genetic drift is recognized as a main cause of genetic differentiation. A low level of gene flow might cause populations to adapt to the local ecological environment and thus promote genetic isolation, leading to significant genetic differentiation among the populations (Li *et al.*, 2004; Cozzolino *et al.*, 2003; Hartl & Clark, 1989).

Gene flow among populations is communicated through media such as pollen, seeds, spores, individual plants and other objects carrying genetic information. Among these, pollen and seed are the main gene dispersal media in natural plant populations (Li & Chen, 2008). Geographical isolation is the main factor in preventing gene flow from the spread of pollen and seed. The gene flow reported in this study is 0.4012, which is less than 1. The long geographical distance involved, with populations spanning several provinces, is likely the main reason for the low gene flow among the populations. Moreover, the existing distribution area of *K. obovata* has been greatly reduced, leading to random genetic drift, and has likely brought about significant differentiation among the populations. To summarize, geographical isolation mainly caused low gene flow and random genetic drift, resulting in genetic differentiation.

The results of cluster analyses, corresponding to the latitude and longitude of the seven populations (Table 1), demonstrate that populations with lower geographical distance always group together. Further results show that there are obvious regional patterns among the populations and illustrates that the genetic relationships among the populations are closely related to geographic distribution.

Protection of *K. Obovata*: Currently there are very limited resources of natural *K. obovata* populations in China, and the most important aspect in protecting these resources is maintaining their genetic diversity. The richness of genetic diversity determines the ability to adapt and evolve, and it also provides very important information on the status of a species and conservation value. When selecting the best provenance for introduction and transplantation, quality groups, and individuals for genetic improvement, special attention should be paid to the populations and individuals that come from special habitats or demonstrate irreplaceable uniqueness. As an example from this study, the population from the Qinglan'gang Nature Reserve, Hainan (Pop.F), has a genetic diversity greater than that of the other populations, and it has diverse genotypes within the population. Thus, it should be a priority to protect its germplasm resources. Also, greater efforts should be made in taking its provenance when screening germplasm, improving genetics, and introducing and transplanting. Naturally occurring *K. obovata* exist in Hainan Island, Guangdong, Guangxi and Fujian, with introduced and transplanted *K. obovata* existing in Zhejiang. In addition, the population currently in Ximen Island Special Protected Area, Zhejiang (Pop.A), and described in this paper was introduced from Fuding, Fujian. This knowledge aligns with the results that Pop.A and Pop.B had high genetic identity. Currently, *K. obovata* is introduced into new habitats mainly from Fujian, but this practice is detrimental to the protection of genetic diversity. Therefore, the species should be introduced from populations other than that in Fujian to better protect the genetic diversity of *K. obovata*.

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References

Bandaranayake, W.M. 1998. Traditional and medicinal uses of mangroves. *Mangroves and Salt Marshes*, 2: 133-148.

Chen, G.Z., S.Y. Mao, N.F.Y. Tame, Y.S. Wong, S.H. Li and C.Y. Lan. 1995. Effect of synthetic wastewater on young *Kandelia candel* plants growing under greenhouse conditions. *Hydrobiologia*, 295: 263-273.

Chen, L.Z., W.Q. Wang and P. Lin. 2005. Influence of waterlogging time on the growth of *Kandelia candel* seedlings. *Acta Oceanologica Sinica*, 27(2): 141-147.

Chen, X.Y. and Y.C. Song. 1998. Microgeographic differentiation in a Cyclobalanopsis glauea population in western Huangshan, Anhui Provinc. *Journal of Plant Resurces and Environmen*, 7(1): 10-14.

Cozzolino, S., D. Cafasso, G. Pellegrino, A. Musacchio and A. Widmer. 2003. Fine-scale phylogeographical analysis of Mediterranean Anacamptis palustrisr Orchidaceae populations based on chloroplast minisatellite and microsatellite variation. *Molecular Ecology*, 12(2): 783-792.

Doyle, J.J. 1991. DNA protocols for plants-CTAB total DNA isolation. In: *Molecular Techniques in Taxonomy*. (Eds.): G.M. Hewitt, A. Johnston, Springer-Verlag, Berlin, Germany, pp. 283-293.

Fan, H.Q. 2005. Protection and development of Mangrove in China—Inspiration of tsunami and storm surge. *Land and Resources*, 10: 16-18.

Field, C.D. 1998. Rehabilitation of mangrove ecosystems: an overview. *Marine Pollution Bulletin*, 37: 383-392.

Gong, J., Y.Q. Song and S.B. Chen. 2009. Effects of Global Climate Change on Mangrove in Zhejiang Coast. *Journal of Anhui Agricultural Science,* 37(20): 9742-9744, 9784.

Hartl, D.L., Clark, A.G. 1989. *Principles of population genetics*. (3rd Ed). Sinauer Associates, Sunderland, MA.

He, B.Y., H.Q. Fan, M. Wang, T.H. Lai and W.Q. Wang. 2007. Species diversity in mangrove wetlands of China and its causation analyses. *Acta Ecologica Sinica*, 27(11): 4859-4870.

He, Q.F., H.Q. Fan and Zh. Ch. Mo. 2009. Impacts of Human Disturbance on Coastal Mangrove Wetlands. *Wetlands Science & Management*, 5(3): 44-46.

Huang, W., X.D. Fang, G.Y. Li, Q.F. Lin and W.M. Zhao. 2003. Cloning and expression analysis of salt responsive gene from *Kandelia candel*. *Biologia Plantarum*, 47(4): 501-507.

Kimura, M. and J.C. Crow. 1964. The number of alleles that can be maintained in a finite population. *Genetics*, 49: 725-38.

Lewontin, R.C. 1974. *The Genetic Basis of Evolutionary Change*. (3rd Ed) Columbia University Press, New York.

Li, H.S. and G.Z. Chen. 2004. Genetic diversity of mangrove plant Sonneratia caseolaris in Hainan Island based on ISSR analysis. *Acta Ecologica Sinica*, 24(8): 1657-1663.

Li, H.S. and G.Z. Chen. 2008. Genetic relationship among species in the genus Sonneratia in China as revealed by inter-simple sequence repeat (ISSR) markers. *Biochemical Systematics and Ecology*, 36: 392-398.

Li, J.M., Z.X. Jin and Z.C. Zhong. 2004. RAPD analysis of genetic diversity of Sargentodoxa cuneata at different altitude and the influence of environm ental factors. *Acta Ecologica Sinica*, 24(3): 567-573.

Nabiul Islam Khan. Md., Suwa Rempei and Hagibara Akio. 2005. Allometric relationships for estimating the aboveground phytomass and leaf area of mangrove *Kandelia candel* (L.) Druce trees in the Manko Wetland, Okinawa Island, Japan. *Trees*, 19: 266-272.

Nabiul Islam Khan. Md., Suwa Rempei, Hagihara Akio and Ogawa Kazuharu. 2004. Interception of photosynthetic photon flux density in a mangrove stand of *Kandelia candel* (L.) Druce. *J For Res.*, 9: 205-210.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *The Proceedings of the National Academy of Sciences of the United States of America,* 70: 3321-3323.

Shen, X.B., G.B. Xu, J.H. Chen and X.P. Wang. 2005. RAPD in genetic resources study of plant. *Hunan Forestry Science*, 32(1): 25-28.

Slatkin, M. and N.H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution*, 43: 1349-1368.

Su, G.H., Y.L. Huang, F.X. Tan, X.W. Ni, T. Tang and S.H. Shi. 2006. Genetic variation in Lumnitzera racemosa, a mangrove species from the Indo-West Pacific. *Aquatic Botany*, 84: 341-346.

Sun, F., M.S. Yang, J., Zhang.. 2009. ISSR Analysis of Genetic Diversity of Robinia pseudoacacia Populations. *Journal of Plant Genetic Resource*, 10(1): 91-96.

Tan, F.X., Y.L. Huang, X.J. Ge, G.H. Su, X.W. Ni, and S.H. Shi. 2005. Population genetic structure and conservation implications of Ceriops decandra in Malay Peninsula and North Australia. *Aquatic Botany*, 81: 175-188.

Wang, W.Q. and P. Lin. 2003. Element distribution in Mangroves and salt-tolerant mechanism. *Scientia Silvae Sinicae*, 39(4): 30-36.

Wright, S. 1931. Evolution in Mendelian population. *Genetics*, 16: 97-159.

Wright, S. 1978. *Evolution and the Genetics of Populations*. (3rd Ed) University of Chicago Press, Chicago.

Xiao, Y. 2005. *Genetic Diversity and Population Structure of Camellia Nitidissima as Revealed by ISSR,RAPD and AFLP Markers*. (3rd Ed) Master degree thesis of Guangxi Normal University, Guangxi.

Ye, Q.H., H.Z. Wu, X.Y. Peng and W.Q. Wang. 2003. Study on the relationship between cell size and chlorophyll content of *kandelia candel* leaves and seaw ater salinity by fcm. *Marine Science*, 27(8): 67-71.

Yeh, F.C., R.C. Yang, T. Boyle, Z.H. Ye and Mao. 1997. POPGENE, the User Friendly Shareware for Population Genetic Analysis. (3rd Ed) Molecular Biology and Biotechnology Center, University of Alberta, Edmonton, Canada.

Zhang, Z.H., R.C. Zhou, T. Tang, Y.L. Huang, Y. Zhong and S.H. Shi. 2008. Genetic variation in central and peripheral populations of *Excoecaria agallocha* from Indo-West Pacific. *Aquatic Botany*, 89: 57-62.

Zhao, M.L. and P. Lin. 2000. Progress in studies of mangroves diversity. *Chinese Biodiversity*, 8(2): 192-197.

Zietkiewicz, E., A. Ratalski and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics*, 20: l76-l83.

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