Genetic Diversity and Structure Analysis of Masson Pine Clonal Seed Orchard

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Abstract— *An experiment stand of clonal orchard of masson pine, which included the 123 plus trees of 8 provenances collected from 8 provinces of Southern China, was founded at Jingshan County of Hubei province. Randomly amplified polymorphic DNA (RAPD) technique was applied to assess genetic diversity and structure for this clonal seed orchard. Total genomic DNA was extracted from fresh needle tissue with Plant Genomic DNA Extraction Miniprep System made by Viotechnology Corporation The results indicated that the clonal seed orchard of masson pine had higher genetic diversity. The average genetic diversity of the clonal seed orchard was 0.3169, the Shannon's information index was 0.4813 respectively, and the percentage of polymorphic loci was 71.0%. Observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (H), Shannon's information index (I) and percentage of polymorphic loci (P) within population of Jiangxi, Hunan and Zhejiang were bigger than those of Guangdong, Guangxi, Anhui and Sichuan. Genetic distances among 8 populations were range from 0.0225 to 0.2175, whereas genetic identities were range from 0.8045 to 0.9777. 8 populations were clustered into 7 clusters, which showed that populations with similar latitude were clustered together and the clustering had nothing to do with geographic distributing. There was not significant correlation between genetic distance and geographic distance, while the correlation between genetic distance and latitude was more significant.*

Keywords— Genetic diversity, Genetic distance, Shannon's information index, Masson Pine, Clonal Seed Orchard.

I. INTRODUCTION

Since 1940s, forest tree genetic improvement has been performed principally for the purpose of seed orchard establishment, which is the bridge between breeding and afforestation. At present, seed orchards of more than 80 species of coniferous and broad-leaved trees have been established, including Pinaceae, Taxodiaceae Cupressaceae, Araucariaceae, Myrtaceae, Fagaceae, Verbenaceae, Lauraceae, Juglandaceae, Betulaceae, Rosaceae, Leguminosae, Aceraceae, Chenopodiaceae etc (Lai Huanlin and Wang Zhangrong, 1997). Seed orchards are production populations which produce high quality seeds for afforestation within a certain fixed range, and from which the genetic quality of seeds is transferred to the generation. First-generation seed orchards were established after phenotypic selection of plus trees from natural or artificial forests. Their genetic structure relationship among clones in seed orchard was not known It is important to know their genetic structure and genetic diversity for afforestation and forest management .The results could help to make sure the genetic quality of seed, improvement direction of seed orchard. But until now there is very few report about these.

DNA markers are able to reflect the variation degree directly on the DNA level and are more sensitive than protein markers. DNA markers are generally used to study the genetic variation because they could detect much more DNA sites and are not affected by development stages, physiological status and environments, etc (Lin and Sui 2010, Cai and Shao 2014). Under controlled condition, Random Amplified Polymorphic DNA (RAPD) markers can be used to identify taxons (Williams 1990, Wachira 1995) with a close relationship and to clarify the genetic variation of population for its higher sensitivity (Lynch 1994, Gillies 1997, Schierenbeck 1997), genetic diversity within and among populations of shortleaf pine and loblolly pine (Lynch 1994, Gillies 1997; Schierenbeck 1997), Characterization of the genetic diversity of the Tall coconut (Reina & Baudouin 2010). Genetic diversity within and among populations of shortleaf pine and loblolly pine (Shiqin & Tauer 2008), the genetic diversity and introgression of Juglans regia and Juglans sigillata in Tibet (Hua & Gang, 2015), patterns of genetic diversity of Prunus africana in Ethiopia (Mihreti & Schueler,2015) and genetic diversity and inbreeding in natural and managed populations of Scots pine (Rosario & Valentina, 2015) were also reported recently. To know the genetic diversity and variation within and among populations is very important to tree breeding and forest improvement.

Masson Pine (*Pinus massoniana* Lamb.), one of the most ecologically and economically important coniferous species for forest products, is widely distributed in 13 provinces of Southern China. It is also provide habitat for wildlife and other environmental amenities, including soil stabilization, clean water and air, and carbon sequestration. Because Masson Pine is widely distributed over most of the central and southern China, it is suggested that it possess a large amount of genetic

This paper is a part of project supported by National Natural Science Foundation of China (31570551)

variation due to adaptation to a variety of environments. In this paper, the genetic diversity and structure of the clonal seed orchard of Masson Pine have been studied by RAPD marker. Although, to date, molecular marker-based studies have been limited to genetic diversity and population structure for Masson Pine, the genetic analysis and structure of its clonal seed orchard has not been studied.

II. MATERIALS AND METHODS

2.1 Materials

The clonal seed orchard of Masson Pine is located at Jingshan County of Hubei province China (112°45′E, 30°57′N). It was established in 1993 and consisted of 123 clones derived from provinces of Jiangxi, Guizhou, Sichuan, Guangdong, Guangxi, Anhui, Hunan and Zhejing in China. Grafts were planted at a spacing of 6×6m and the total area was 12 hm^2. The needle leaves of each clone were collected in November, 2014 and saved at -80℃.

2.2 DNA extraction

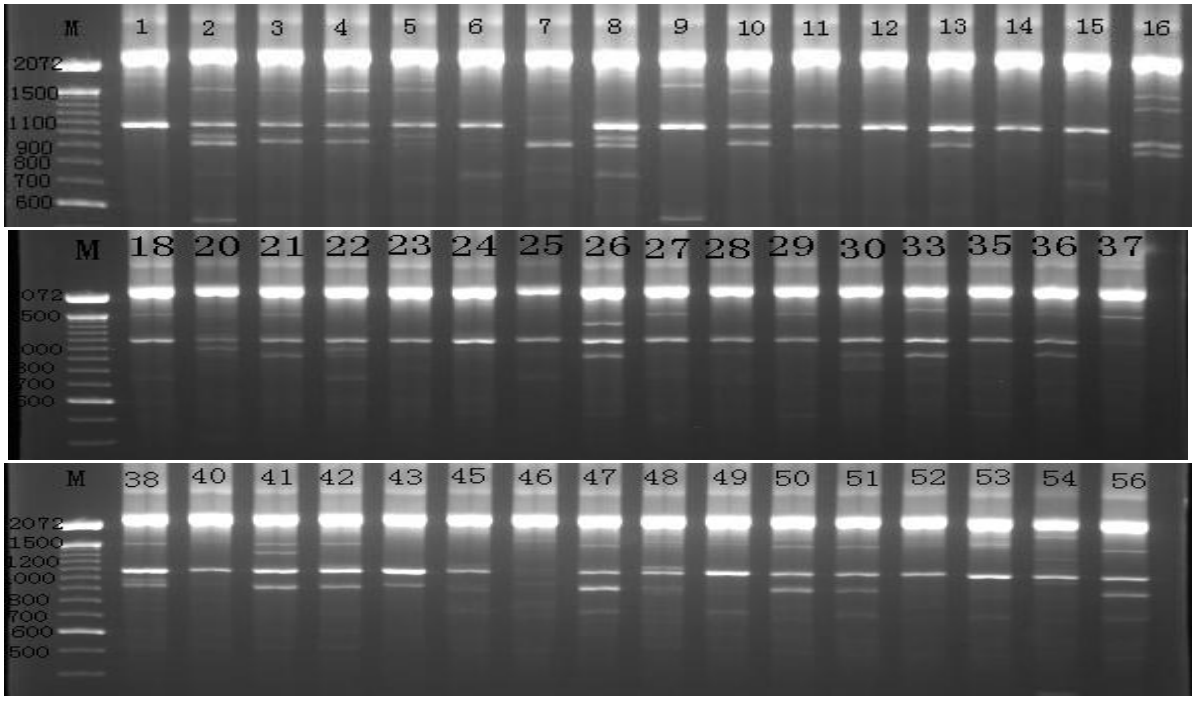
Total genomic DNA was extracted from fresh needle tissue with Plant Genomic DNA Extraction Miniprep System made by Viotechnology Corporation.

2.3 PCR reaction and primers selection

Genomic DNA from 123 individuals was amplified by using 10 mer-primers. Amplification reactions were conducted in 15μl reaction mixture containing 25 ng of template DNA, 0.45μmol of primer(Operon), 15μM of each of dNTP, 1.5μl of 10×PCR buffer($Mgcl_2$ free), and 0.5 U Platinum Taq DNA polymerase(Invitrogen). The PCR amplifications were carried out using PTC-2000 Thermo-cycler (MJ-Research). The PCR conditions were pre-denatured for 3 min at 94^0C followed by 40 cycles of 1 min at 94^0C, 45 sec at an annealing temperature of 37^0C and 90 sec at 72^0C. After the amplification, the programme allowed a final extension of 2 min at 72^0C before maintained at 4^0C. Amplified DNA fragments were separated electrophoretically in 2.0% agarose gels stained with ethidium bromide (0.5 μg/ml) in 1×TBE buffer. The banding patterns were compared with a DNA marker DL 2000 (Takara, China), which was used to know about the size of amplified DNA fragments. All reactions were repeated at least twice to test their repeatability. 8 samples from 8 provenances were used to select the polymorphic primers. 118 10-mer primers were screened.

2.4 Data analysis

For clarity, only clear and reproducible bands were scored as present (1) or absent (0) in each pattern， as showed in figure 1. Data spreadsheet was prepared with binary data of RAPD markers, which were used to estimate genetic parameters and to construct the dendrogram by using POPEGENE version 1.32 (Yeh et al. 1999).



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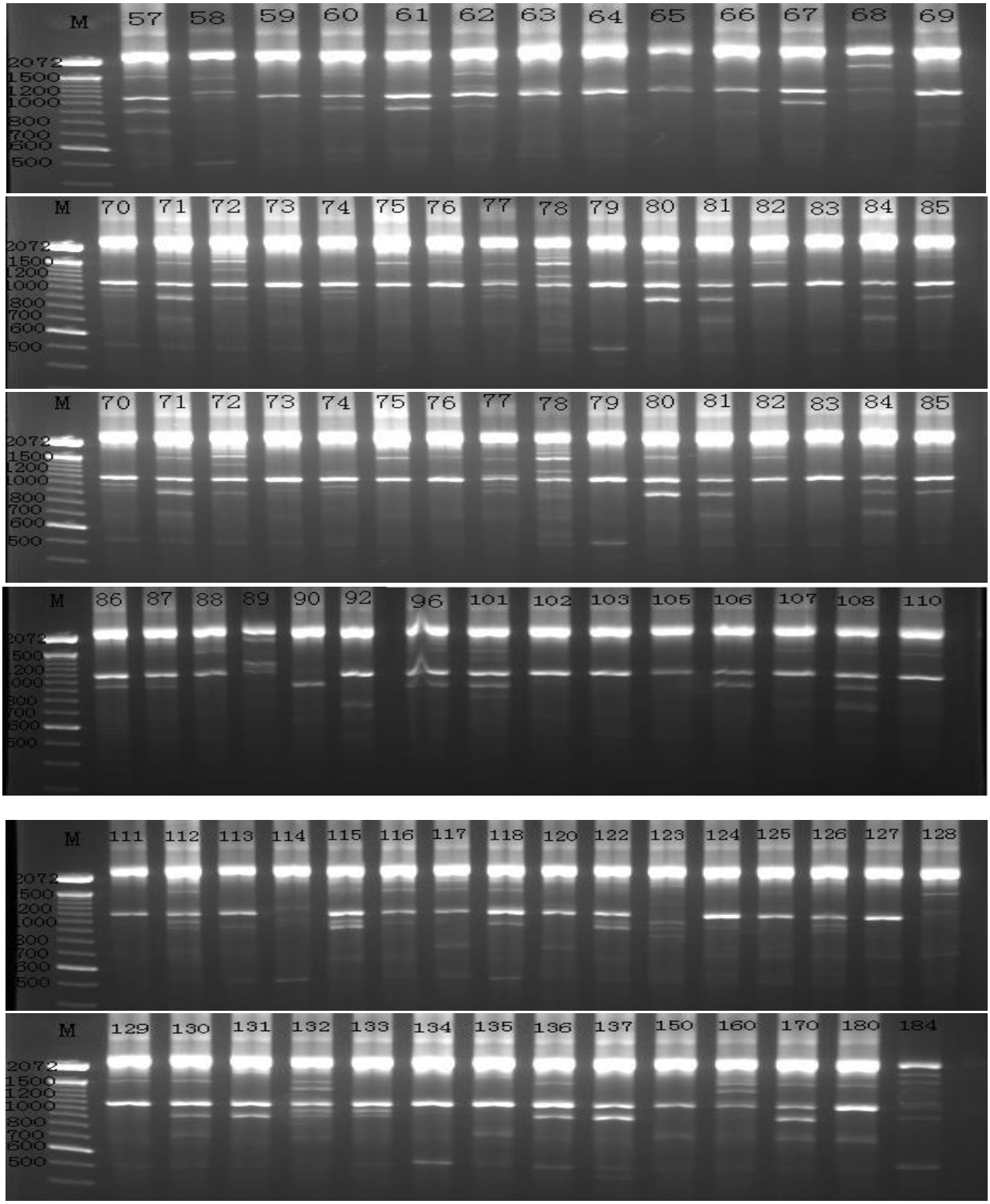

FIG. 1 SEGREGATION OF RAPD BANDS AMPLIFIED WITH PRIMER OPB11

III. RESULTS AND DISCUSSION

3.1 Marker analysis and individual identification

9 polymorphic primers were proved useful for producing valuable RAPD bands (Table 1). A total of 69 bands were detected from these RAPD markers, out of which 49 bands were found polymorphic. The percentage of polymorphic loci was 71.0%, which was similar to the results of natural population of Masson pine indicated by allozyme (Wu Ruojing, 2002). 123 individuals could be divided by these polymorphic markers, and each one had its own profile.

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TABLE 1
SEQUENCE AND NUMBER OF TOTAL AMPLIFICATION BANDS IN MASSON PINE BY 9 RANDOM PRIMERS

No.	Primer	Sequence	Total bands
1	OPB11	GTAGACCCGT	8
2	OPB12	CCTTGACGCA	3
3	OPC13	AAGCCTCGTC	9
4	OPF03	CCTGATCACC	13
5	OPF05	CCGAATTCCC	10
6	OPF16	GGAGTACTGG	6
7	OPG09	CTGACGTCAC	7
8	OPG12	CAGCTCACGA	5
9	OPG13	CTCTCCGCCA	8
Total			69

3.2 Genetic diversity analysis of clonal seed orchard

Nei's gene diversity（h）and Shannon's information index were 0.3169 and 0.4687, respectively. The averaged total genetic diversity (H_T) among the populations was higher (0.292) than that within population (H_S=0.220). The mean coefficient of population differentiation (G_{ST}) among the populations was 0.247, showing that 75.3% of the variation was present within populations. The estimated gene flow (N_m) from the estimated G_{ST} was high (1.528).

3.3 Genetic diversity comparison among populations

Observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (H), Shannon's information index (I) and percentage of polymorphic loci (P) within population of Jiangxi, the results indicated that Hunan and Zhejiang were bigger than that of Guangdong, Guangxi, Anhui and Sichuan, which maybe was related to the number of population samples (Table 2). The number of population samples of Jiangxi, Hunan, and Zhejiang were much greater than that of Guangdong, Guangxi, Anhui and Sichuan.

TABLE 2
GENETIC DIVERSITY WITHIN POPULATION OF MASSON PINE

Population	No. of sample	Observed number of alleles（Na）	Effective number of alleles（Ne）	Nei's gene diversity（H）	Shannon information index（I）	Percentage of polymorphic loci（p）
Jiangxi	18	1.8980	1.5007	0.2985	0.4512	89.80%
Guizhou	9	1.7143	1.4711	0.2642	0.3885	71.43%
Sichuan	5	1.5918	1.3260	0.1961	0.2988	59.18%
Guangdong	2	1.2245	1.1587	0.0930	0.1358	22.45%
Guangxi	2	1.3265	1.2309	0.1353	0.1975	32.65%
Anhui	5	1.4490	1.3159	0.1759	0.2573	44.90%
Hunan	42	2.0000	1.5472	0.3246	0.4896	100.00%
Zhejiang	40	1.8776	1.4586	0.2718	0.4124	87.76%

Nei's genetic distance and genetic identity among populations in this study were present in table 3. Genetic distances among 8 populations were range from 0.0225 to 0.2175. The lowest genetic distance recorded between population Hunan and Jiangxi was 0.0225, and the highest genetic distance recorded between population Guangdong and Jiangxi with the value of 0.2175. On the contrary, genetic identities among 8 populations were range from 0.8045 to 0.9777. The lowest genetic recorded between population Guangdong and Jiangxi was 0.8045, and the highest genetic distance recorded between population Hunan and Jiangxi with the value of 0.9777.

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TABLE 3
GENETIC IDENTITY (BELOW DIAGONAL) AND GENETIC DISTANCE (ABOVE DIAGONAL) AMONG8 POPULATIONS

Pop ID	Jiangxi	Guizhong	Sichuan	Guangdong	Guangxi	Anhui	Hunan	Zhejiang
Jiangxi		0.9594	0.9221	0.8045	0.8368	0.8905	0.9777	0.9771
Guizhou	0.0414		0.9244	0.8584	0.8493	0.9128	0.9539	0.9740
Sichuan	0.0811	0.0787		0.8308	0.8537	0.9279	0.9467	0.9461
Guangdong	0.2175	0.1527	0.1854		0.8347	0.8815	0.8477	0.8614
Guangxi	0.1781	0.1634	0.1582	0.1807		0.8605	0.8460	0.8766
Anhui	0.1160	0.0912	0.0748	0.1261	0.1502		0.9205	0.9397
Hunan	0.0225	0.0472	0.0548	0.1653	0.1672	0.0829		0.9756
Zhejiang	0.0231	0.0263	0.0554	0.1492	0.1317	0.0622	0.0247	

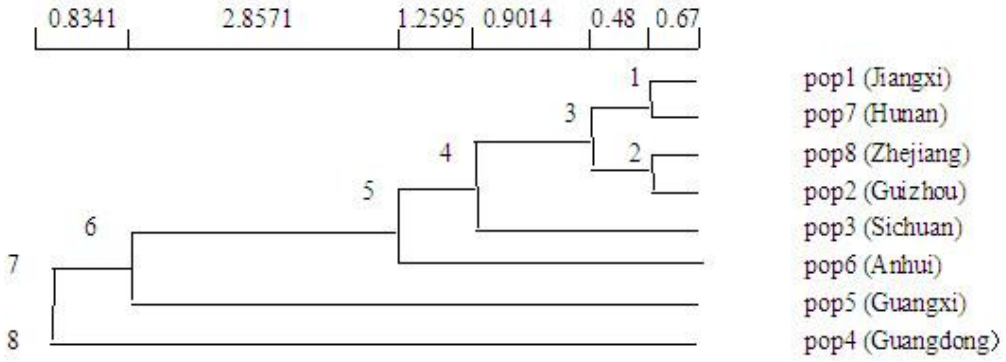

FIG.2 DENDROGRAM DEVELOPED USING UPGMA BASED ON NEI'S GENETIC DISTANCE, SHOWING RELATIONSHIPS AMONG 8 POPULATIONS

As in the dendrogram (Fig. 2), there were 7 clusters. To be specific, Jiangxi population and Hunan population were classified into the first sub-cluster 1; Zhejiang population and Guizhou population were classified into the second sub-cluster 2; sub-cluster 3 consisted of sub-cluster 1 and sub-cluster 2, and the 2 sub-clusters were the same close to Sichuan population, which clustered into the sub-cluster 4; sub-cluster 5 contained sub-cluster 4 and Anhui population; sub-cluster 6 contained sub-cluster 5 and Guangxi population; finally, sub-cluster 6 and Guangdong population formed the sub-cluster 7. Based on the cluster analysis, it was indicated that population with similar latitude were clustered together and the clustering had nothing with geographic distributing. Other researches also indicated there was no relation between growth characteristics, longitude, geographic distance but associated with latitude. Thus it could be speculated that the primary factors affecting genetic differentiation of Masson pine most likely were temperature and moisture.

IV. CONCLUSION

This study showed that the clonal seed orchard of Masson Pine had a higher level of genetic diversity, which was similar to the results of Lai's study. As a kind of explanation verified by Wheeler & Jech, natural forests differentiated geographically and genetically, from which plus trees were selected, then, the higher genetic diversity was transferred to the seed orchards. Genetic structure between filial and parental generations had a close correlation, which was proved by a lot of studies. Szmidt also had verified that the genetic diversity of the offspring of seed orchard did not decline obviously compared with that of the offspring of the natural forests (Szmidt 1984, Reina 2010, Mihreti 2015, Hua 2015). Lai drew a conclusion that there was no significant change of frequency between seed orchard and its progeny forest.

Genetic diversity among forest population had been revealed in this study, perhaps, which was connected with the different population samples. Providing that raising the number of population samples of Gongdong, Guangxi, Anhui, Sichuan, the genetic diversity of this seed orchard would be much higher. Therefore, quantifying the total number of the seed orchard and the number of each provenance is the most concern in seed orchard establishment, which is related both to the level of genetic diversity and genetic gain obtained from afforestation. As for the next step for seed orchard management, mating

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ability and inbreeding depression should be estimated via controlled pollination, based on which and the genetic structure clarified above, the seed orchard should be improved, or the second generation seed orchard should be established.

ACKNOWLEDGEMENTS

The stand of clonal orchard for masson pine was founded under the supports of China–Japan Cooperation of Forest Tree Improvement Project and Taizishan Forest Farm. This experiment was a part of project supported by National Natural Science Foundation of China (31570551).

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