Plant Prod. Sci. 12(1) : 88—96 (2009)

Genetic Diversity among Wild Resources of the Genus *Boehmeria* Jacq. from West China Determined Using Inter-simple Sequence Repeat and Rapid Amplification of Polymorphic DNA Markers

Li-jun Liu[1], Zu-Qing Meng[2], Bo Wang[1], Xu-xia Wang[1], Jin-Yu Yang[1] and Ding-xiang Peng[1]

([1]*College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China;*
[2]*Tibet Agricultural and Animal Husbandry College, Linzhi 860000, China)*

Abstract : Ramie (*Boehmeria nivea* L.Gaud) is planted as an important fiber crop in China. Randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers were used for the first time for the detection of genetic polymorphism of 37 ramie accessions (29 wild genotypes and 8 commercial varieties) collected from various geographical regions of West China. The objectives of this study were: 1) to examine the genetic diversity of four species in genus *Boehmeria* Jacq.: *B. clidemioides var. diffusa*, *B. nivea* L. Gaud, *B. longispica* Steud, and *B. macrophylla* Hornem; and 2) to determine the genetic distance of the four species by these methods. *Laportea cuspidata* (Wedd.) Friis was used as an outgroup species. The results showed that 375 (17 monomorphic fragments) strips were amplified by 31 RAPD primers, while 266 (10 monomorphic fragments) strips by 18 ISSR primers. On the average, each RAPD and ISSR primer amplified 12.1 and 14.8 strips, respectively. Based on the appearance of the markers, the genetic relationships were analyzed using unweighted pair-group method of arithmetic average cluster analysis (UPGMA) and the genetic Dice coefficients were calculated. Clustering analysis indicated that the 37 accessions were classified into four clusters which belong to 3 sections (including Section *Phyllostachys*, Section *Tilocnide* and Section *Duretia*). The overall grouping pattern of clustering corresponded well with traditional botanical taxonomy. Principal component analysis (PCA) confirmed the patterns of genetic diversity observed among the species. These results suggested that RAPD and ISSR were efficient approaches suitable for taxonomic analysis of ramie wild materials. The results provided valid guidelines for collection, conservation, and characterization of *Boehmeria* genetic resources.

Key words : *Boehmeria*, ISSR marker, RAPD marker, Taxonomic analysis, UPGMA.

Ramie (*Boehmeria nivea* L. Gaud.), also called China grass, is a hardy perennial herbaceous plant of the Urticaceae family, which can be harvested up to six times a year. It is mainly planted in China and other Asian countries including Philippines, India, South Korea, and Thailand (Liu et al., 2001). China, the native home of ramie, possesses many wild species in the genus *Boehmeria*, including some rare species with stress resistance and good fiber qualities that can be used for biological engineering, genetics and breeding research (Liu et al., 2003; Jiang and Jie, 2005).

China possesses many precious wild ramie resources including 32 species and 11 varieties (Wang and Cheng, 1995), among which 16 species and 7 varieties were used as materials for comparative study on botanical morphology in *Boehmeria* (Zhang et al., 1998). With the development of market demand and fiber textile industry, fiber quality of ramie needs to be improved and elite cultivars are urgently required for ramie production.

The major constraints in achieving higher fiber yield of this crop are lack of genetic variability, absence of suitable ideotypes for collecting materials, poor harvest index and sensitivity to diseases (Lai et al., 2000). Current knowledge of the genetic basis of ramie wild germplasm agronomic characteristics and molecular markers that are applicable to its genetic improvement is limited (Liu et al., 2003). Research on this species has lagged behind that of jute (Roy et al., 2006) and other fiber crops. Therefore, improvement of this crop through utilization of available genetic diversity is needed.

The evaluation of genetic diversity and construction of linkage maps would promote the efficient use of genetic variations in the breeding program (Souframanien and Gopalakrishna, 2004). DNA markers provide an opportunity to characterize

Received 17 July 2008. Accepted 16 August 2008. Corresponding author: Ding-xiang Peng (pdxiang@mail.hzau.edu.cn; fax+86-27-87280796).
This research was supported by 948 Project of the Ministry of Agriculture of China (2006-G18(03)) and project of Hubei province science-technology attack of China (2007AA201C49).
Abbreviations : ISSR, Inter-simple sequence repeat; PCA, principal component analysis; RAPD, Randomly amplified polymorphic DNA; UPGMA, unweighted pair-group method of arithmetic average cluster analysis.

genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983).

DNA fingerprinting techniques are very useful in identification of taxa, and molecular markers are used in genetic diversity studies. Multi-locus markers are more advantageous than single-locus markers, as they have a high multiplex ratio (Sarwat et al., 2008). Rapid amplification of polymorphic DNA (RAPD) markers have been extensively used for the assessment of genetic diversity in a variety of plants such as *Zea mays* (Garcia et al., 2004), *Panax quinquefolius* (Lim et al., 2007), *Phaseolus vulgaris* L. (Marotti et al., 2007). ISSR markers are much more informative than RAPDs and have been used for the analysis of genetic diversity in *Corchorus species* (Roy et al., 2006), *Tribulus terrestris* (Sarwat et al., 2008) and others. Though there are many methods to measure genetic diversity, few studies focused on the relationships among wild ramie materials, such as, chromosomal karyotype (Guo et al., 2005), RAPD (Jie et al., 2002; Guo et al., 2003; Zhou et al., 2004; Li et al., 2006), ISSR (Liu et al., 2006b; Hou et al., 2006), the simple sequence repeat (SSR) (Zhou et al., 2005) and random amplified microsatellite polymorphism (RAMP) (Zhou et al., 2004). Zhou et al. (2004) used SSR, inter-simple sequence repeat (ISSR) and RAMP to determine the genetic relationships among 20 cultivars in ramie. All of the approaches were able to uniquely fingerprint each of the cultivars. Zhou et al. (2005) reported that

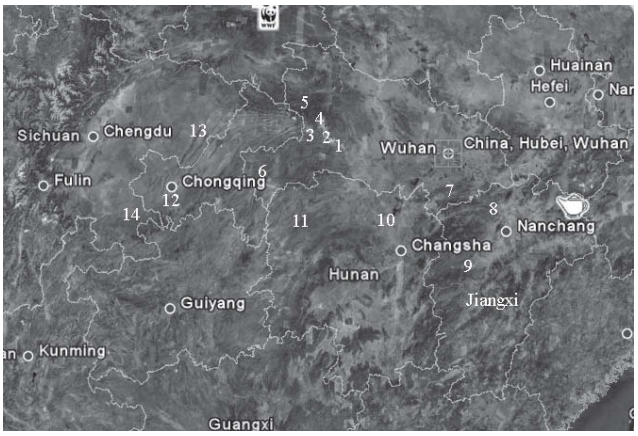

Fig. 1. Map of China showing locations of collecting areas for samples of *Boehmeria* used in this study. 1) Changyang, Hubei; 2) Zigui, Hubei; 3) Badong, Hubei; 4) Gaoyang, Hubei; 5) Shennongjia, Hubei; 6) Xianfeng, Hubei; 7) Xianning, Hubei; 8) Ruichang, Jiangxi; 9) Wanzai, Jiangxi; 10) Yuanjiang, Hunan; 11) Dayong, Hunan; 12) Chongqing; 13) Dazhu, Sichuan; 14) Luxian, Sichuan (source: Google Earth).

Table 1. Sample details of 4 ramie species detected in the study.

No.	Voucher	Sample site	No.	Voucher	Sample site
1	HR-20-03 ○	Gaoyang, Hubei N31°23′, E110°33′	20	HR-01-04 □	Gaoyang, Hubei N31°23′, E110°33′
2	HR-06 ◇	Shennongjia, Hubei N31°23′, E110°33′	21	HR-01-06 □	Changyang, Hubei N30°38′, E110°52′
3	HR-07 ○	Shennongjia, Hubei N31°14′, E110°45′	22	HR-01-07 □	Shennongjia, Hubei N31°27′, E110°16′
4	HR-08 ◇	Shennongjia, Hubei N31°23′, E110°33′	23	HR-01-10 □	Changyang, Hubei N30°37′, E110°50′
5	HR-09 ○	Shennongjia, Hubei N31°26′, E110°26′	24	HR-01-11 □	Changyang, Hubei N30°37′, E110°50′
6	HR-10 ○	Shennongjia, Hubei N31°28′, E110°18′	25	HR-01-12 □	Chongqing, China N29°32′, E106°30′
7	HR-11 ◇	Shennongjia, Hubei N31°27′, E110°16′	26	HR-01-05 □	Shennongjia, Hubei N31°14′, E110°45′
8	HR-12 ○	Shennongjia, Hubei N31°28′, E110°18′	27	HR-01-01 □	Badong, Hubei N31°01′, E110°24′
9	HR-13 ◇	Shennongjia, Hubei N31°26′, E110°17′	28	HR-01-02 □	Badong, Hubei N31°01′, E110°23′
10	HR-14 ○	Shennongjia, Hubei N31°26′, E110°26′	29	HR-03-03 △	Gaoyang, Hubei N31°23′, E110°33′
11	HR-15 ○	Shennongjia, Hubei N31°27′, E110°16′	30	HR-03-06 △	Gaoyang, Hubei N31°23′, E110°33′
12	HR-16 ○	Shennongjia, Hubei N31°23′, E110°33′	31	Xiyelv △	Ruichang, Jiangxi N29°48′, E115°34′
13	HR-17 ○	Xianfeng, Hubei N29°26′, E109°11′	32	Tongpiqing △	Wanzai, Jiangxi N28°06′, E114°26′
14	HR-18 ☆	Xianfeng, Hubei N31°25′, E110°17′	33	Huangkezao △	Yuanjiang, Hunan N28°50′, E112°22′
15	HR-02 □	Xianfeng, Hubei N29°26′, E109°11′	34	Dayongma △	Dayong, Hunan N29°03′, E110°29′
16	HR-21 ◇	Xianfeng, Hubei N29°28′, E109°22′	35	Dazhuxianma △	Dazhu, Sichuan N30°25′, E107°21′
17	HR-22 ◇	Shennongjia, Hubei N31°28′, E110°18′	36	Qingpidama △	Luxian, Sichuan N31°14′, E110°45′
18	HR-19 ○	Shennongjia, Hubei N31°14′, E110°45′	37	Xianningyema △	Xianning, Hubei N29°52′, E114°16′
19	HR-05-01 △	Changyang, Hubei N30°37′, E110°50′	38	Ziguiqingma △	Zigui, Hubei N31°00′, E110°40′

Note: All the vouchers are preserved in the Ramie Repository, Huazhong Agricultural University, Wuhan, China. ○, *B. clidemioides var. diffusa* (herb, alternate leaf, spike, dioecious flower); △, *B. nivea* (herb, alternate leaf, panicle, monoecious flower); ◇, *B. longispica*(herb, opposite leaf, spike, dioecious flower); □, *B. macrophylla* (undershrub, opposite leaf, spike, monoecious or dioecious flower); ☆, *Laportea cuspidata*(genus *Laportea Gaud.*, Urticaceae) used as an outgroup species.

Table 2. Nucleotide sequences of ISSR (P3-P33) and RAPD (S47-S329) primers used in this study.

No.	sequence	No.	sequence	No.	sequence
P3	GAGAGAGAGAGAGAGAT	S47	TTGGCACGGG	S310	CCCTAGACTG
P5	AGAGAGAGAGAGAGAGG	S52	CACCGTATCC	S312	TCGCCAGCCA
P7	ACACACACACACACACT	S60	ACCCGGTCAC	S314	ACAGGTGCTG
P10	GAGAGAGAGAGAGAGT	S79	GTTGCCAGCC	S315	CAGACAAGCC
P12	GCTGCTGCTGCTGCTGCT	S92	CAGCTCACGA	S317	GACACGGACC
P13	GAGGAGGAGGAGGAGG	S101	GGTCGGAGAA	S320	CCCAGCTAGA
P15	CTCTCTCTCTCTCTCTG	S107	CTGCATCGTG	S321	TCTGTGCCAC
P17	TCTCTCTCTCTCTCTCG	S122	GAGGATCCCT	S323	CAGAACCGCA
P18	CACACACACACACACAG	S124	GGTGATCAGG	S324	AGGCTGTGCT
P19	TGTGTGTGTGTGTGTGA	S125	CCGAATTCCC	S325	TCCCATGCCTG
P20	TGTGTGTGTGTGTGTGRC	S132	ACGGTACCAG	S326	GTGCCGTTCA
P21	GAGAGAGAGAGAGAGYA	S134	TGCTGCAGGT	S327	CCAGGAGGAC
P22	GAGAGAGAGAGAGAGYC	S301	CTGGGCACGA	S329	CACCCCAGTC
P24	ACACACACACACACARC	S302	TTCCGCCACC		
P25	AGAGAGAGAGAGAGAYC	S305	CCTTTCCCTC		
P27	AGAGAGAGAGAGAGAYG	S307	GAGCGAGGCT		
P31	GAGAGAGAGAGAGAGYT	S308	CAGGGGTGGA		
P33	GAGAGAGAGAGAGAGAC	S309	GGTCTGGTTG		

ISSR were more efficient for fingerprinting ramie cultivars than SSR markers. In spite of having manifold industrial uses and world-wide distribution, there has been no report on the molecular reports of the genetic diversity prevalent in *Boehmeria* Jacq. to the best of our knowledge.

Many wild ramie species, sub-species or ecological types have been distributed widely in the past in China. However, the deterioration of ecological conditions caused by devegetation is threatening the existence of the wild ramie species; especially the tree and shrub types are in danger of extinction because of their low reproductive ability (Lai et al., 1999; 2000). The wild species in the genus *Boehmeria* are the important genetic resources that are not yet utilized by man. Breeding of the new cultivars with high yield, good quality and resistances to stresses and diseases are becoming more and more important and feasible, as the human society and economy progress, especially for the plant genetic and breeding techniques advance. The availability of plenty of plant germplasm is the key factor for creating a breakthrough achievement in plant breeding, so collecting and studying the wild ramie species to enrich the genetic resources of the crop are of great importance for new cultivars breeding (Liu et al., 2003). Therefore, collecting, researching and utilizing the wild ramie germplasms are necessary and absolutely reasonable. This work will help to breed new ramie cultivars with high yield, good quality and strong stress resistances, which are propitious to improve the ramie textile quality.

The aim of this study was to determine the genetic variability and relationships among 8 commercial cultivars and 29 accessions of *Boehmeria* spp. collected in West China, in order to contribute to germplasm bank management, conservation programs, and breeding. The relationships obtained by RAPD and ISSR marker systems are discussed.

Material and Methods

1. Plant material

A total of 29 ramie accessions belonging to four species (*B. clidemioides var. diffusa, B. nivea, B. longispica and B. macrphylla*) from West China, and *Laportea cuspidata* (Wedd.) Friis as an outgroup (Fig. 1, Table 1) were used. Furthermore, 8 commercial cultivars were included because of the importance of these cultivars on the Chinese market. All materials were obtained from the Ramie Repository, Huazhong Agricultural University, Wuhan, China.

2. DNA extraction

Genomic DNA was isolated from leaves using the modified CTAB method of Doyle and Doyle (1987). Integrity and quality of DNA were evaluated by electrophoresis on 1% agarose gel. Gel images were captured on a GENE GENIUS imaging system (Gene Co., Ltd., Hong Kong, China). The DNA was stored at –20°C.

3. RAPD and ISSR analysis

Primers used in this study were synthesized by

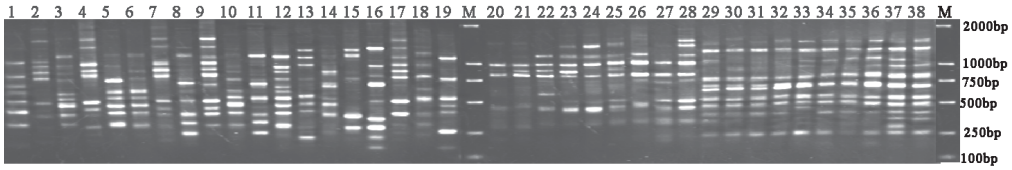

Fig. 2. The RAPD amplification profile of primer S47. M: DL2000 (2000bp, 1000bp, 750bp, 500bp, 250bp, 100bp). The numbers of ramie species were the same as those in Table1.

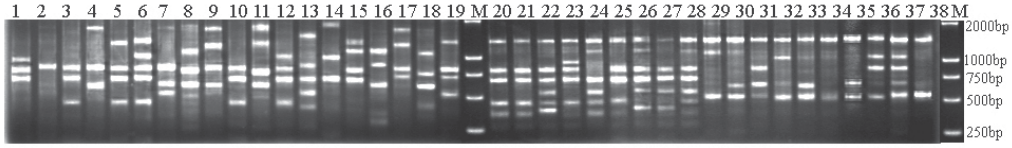

Fig. 3. The ISSR amplification profile of primer P33. M: DL2000 (2000bp, 1000bp, 750bp, 500bp, 250bp, 100bp). The numbers of ramie species were the same as those in Table1.

Shanghai Sangon Biological Engineering Technology and Service Co., Ltd., China (Table 2). Primers were selected on the basis of the number and intensity of polymorphic amplified bands. Duplicate amplifications were conducted for each sample to ensure reproducible results and only bands present in both runs were scored for analysis. Details of the RAPD system and program (Liu et al., 2006a) and the ISSR system and program (Liu et al., 2006b) were as reported previously. Separation of the amplified fragments was performed on 2.0% (w/v) agarose gels stained with ethidium bromide.

4. Data analysis

RAPD and ISSR profiles were visually scored considering the presence of a band (1), and its absence (0). Only distinct, reproducible, well-resolved fragments, in the size range between 2000 bp and 100 bp were scored.

The genetic similarity among accessions was estimated using the Dice coefficient (Dice, 1945) of the NTSYS package (Numerical Taxonomy and Multivariate Analysis for personal computer) version 2.1 (Rohlf, 2000). Dendrograms were constructed using the UPGMA method. The matrices of genetic similarity were also used to obtain a principal component analysis (PCA) plot to resolve the patterns of variation among genotypes. The bootstrap method was employed to evaluate the reliability of tree topology. The calculations were performed with the WinBoot program (Yap and Nelson, 1996). The cophenetic coefficient between the matrix of genetic similarity and the dendrogram were computed using an appropriate routine of the NTSYS-pc software. The significance of the cophenetic correlation was tested by using the Mantel correspondence test (Mantel, 1967).

Results

1. RAPD analysis

The analysis of the 37 ramie accessions with 31 RAPD primers identified a total of 375 reproducible fragments. Among them, 358 were polymorphic (95.5%) with an average of 12.1 bands per primer. Fig. 2 shows an example of a agarose gel with polymorphic RAPD fragments.

A cluster analysis was performed using the Nei and Li distances (Nei and Li, 1979) and the UPGMA method. The range of Dice coefficients varied between 0.21 (between HR-01-04 and HR-07) and 0.88 (between two *B. macrophylla* accessions from Changyang, Hubei). The cophenetic coefficient was 0.945 indicating a good fit. The dendrogram showed a clear separation between the 4 species (Fig. 4a). Cluster I included 6 *B. clidemioides var. diffusa* accessions (HR-07, 09, 10, 14, 16 and HR-20-03) from the Shennongjia areas. Within this cluster, the accessions from different areas were clustered independently. Cluster II consisted of the other *B. clidemioides var. diffusa* accessions (HR-12, 15, 17 and 19), except HR-02 (*B. macrophylla*), from Xianfeng county Hubei which clustered intermingled. Cluster III included all the *B. nivea* accessions from Hubei, Hunan, Jiangxi and Sichuan provinces. Within this cluster, in two cases, the accessions had a common geographical origin: Hubei Island (Xianningyema and Ziguiqingma) and Sichuan Island (Dazhuxianma and Qingpidama). In the other two cases, the accessions pairs did not have the same geographical origin. All 8 commercial cultivars clustered together, halfway between HR-03-03 and HR-03-06. Cluster IV included all the *B. longispica* accessions (HR-06, 08, 11, 13 and 22) from Shennongjia Hubei, except HR-21, from Xianfeng county Hubei, which clustered independently. Cluster V included the other 9 *B. macrophylla* accessions, and HR-05-01 (*B. nivea*) was also in this cluster.

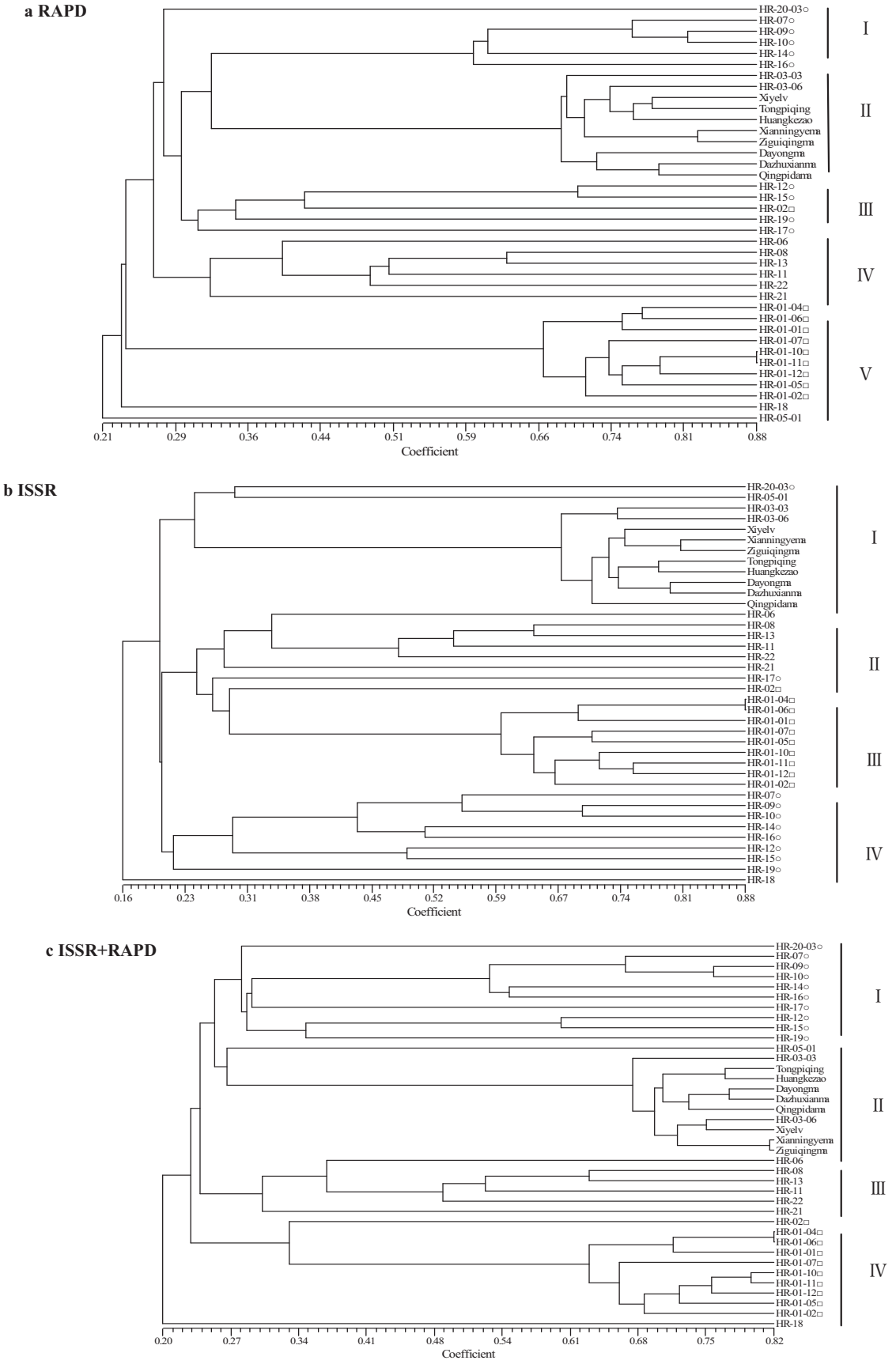

Fig. 4. UPGMA dendrograms using Dice genetic distances of 4 wild species. (a) tree built using RAPD data. (b) tree built using ISSR data. (c) tree built using ISSR+RAPD data.

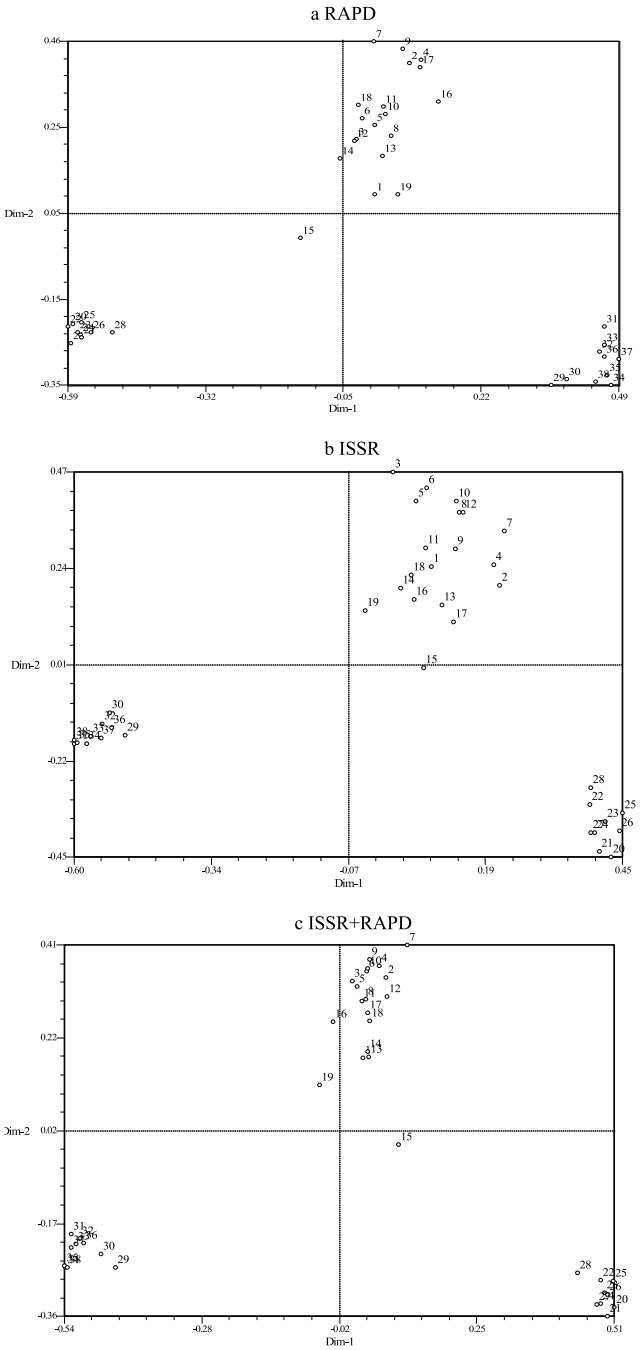

Fig. 5. Two-dimensional plot of principal component analysis of elite *Boehmeria* genotypes using RAPD, ISSR, and both combined analysis. The numbers plotted represent individual accessions and correspond to the ones listed in Table 1.

Fig. 5a shows the distribution of the different accessions according to the two principal axes of variation using PCA. On the basis of the first coordinate, which accounted for 19.61% of the total variation, and on the basis of the second coordinate, which accounted for 12.11% of the total variation, the accessions were clearly classified into three groups.

2. ISSR analysis

All 18 ISSR primers produced well-defined and scorable amplification products and showed polymorphisms in all 37 investigated ramie accessions. Fig. 3 shows the amplification profile of ISSR primer P33 which indicated that all the genotypes could be clearly distinguished by this single primer. The percentage of polymorphic ISSR fragments was 96.3% with an average of 14.2 bands per primer (data not shown).

A cluster analysis was performed using the Nei and Li distances (Nei and Li, 1979) and the UPGMA method. Dice coefficients varied from 0.16 (between HR-18 and HR-10) to 0.88 (between HR-01-10 and HR-01-11). The cophenetic coeffcient was 0.956 indicating a good fit. The dendrogram showed a clear separation between the 4 species (Fig. 4b). All the 11 *B. nivea* accessions except for HR-20-03 (*B. clidemioides var. diffusa*) belonged to Cluster I. Cluster II included 6 *B. longispica* accessions (HR-06, 08, 11, 13, 21 and 22). Cluster III consisted of 10 *B. macrophylla* accessions and HR-17 (*B. clidemioides var. diffusa*). Cluster IV included 8 *B. clidemioides var. diffusa* accessions.

3. RAPD and ISSR analysis

The RAPD and ISSR data were combined for UPGMA cluster analysis giving Dice genetic distances ranging from 0.20 to 0.82 (Fig. 4c). The pattern of clustering of the genotypes remained more or less the same in ISSR and RAPD data (Fig. 4), whereas the dendrogram based on RAPD showed some variation in the clustering of accessions (Dice coefficient ranging from 0.21 to 0.88, Fig. 4a). The correlation between the matrices of cophenetic correlation values for the dendrogram based on RAPD and ISSR data was 0.956 indicating a good fit (p=1.00). The dendrogram showed a clear separation between the Hubei, Hunan, Jiangxi, Sichuan and Chongqing accessions (Fig. 4c). Among the Hubei landraces, the Shennongjia partial accessions clustered separately from the Badong and Gaoyang ones. Cluster I included 10 accessions which were collected from the crest Banbiyan of Shennongjia area and consisted of HR-07, 09, 10, 12, 14, 15, 16 and 19 and HR-20-03 from Gaoyang, Hubei, and HR-17 from Xianfeng county Hubei. According to the botanical characterization (Wang and Cheng, 1995), all 10 accessions were *B. clidemioides var. diffusa* belonging to section *Phyllostachys*. The cluster analysis demonstrated that the wild ramie materials from different regions were distinctly separated from each other. HR-05-01 and HR-03-03 clustered mainly with cultivars planted in China such as Tongpiqing, Huangkezao, Ziguiqingma, which were grouped in cluster II (section *Tilocnide*). Cluster III (mainly *B. longispica*, section *Duretia*) included the 5 accessions from Shennongjia (HR-02, 06, 08, 11, 13, 21 and 22), except 'HR-02-21' from Xianfeng county Hubei, which

clustered independently. Cluster IV included many *B. macrophylla* (HR-01-01, 02, 04, 05, 06, 07, 10, 11 and 12) collected from Shennongjia (HR-01-05 and HR-01-07), and Changyang (HR-01-06, 10 and 11).

Discussion

Wild ramie accessions are very important breeding materials and over 100 wild ramie materials have been collected since 2000 from Hubei, Jiangxi, Yunnan, Sichuan, Guangxi Zhuang Autonomous region, which would enhance the effectiveness of the breeding program. Identification and use of PCR-based molecular markers, such as RAPD and ISSR can be used in marker assisted selection (MAS) and genetic resource conservation. Advanced technologies are in progress by sequencing the amplified fragments to shed light on the genetic relationship between genotype and phenotype. In addition, it would be necessary to enlarge the number of varieties either to deepen the insight of the genetic diversity within ramie or to detect molecular markers that allow discrimination between genotypes. Comparing results obtained by different genetic diversity methods could identify the best method for parental selection for plant breeders, thus increasing breeding efficiency.

Every genotype has its unique band type which help in their differentiation. Thirty-oneRAPD markers produced 358 amplified fragments with an average genetic diversity range from 0.21 to 0.88. While 18 ISSR markers produced 256 amplified fragments with an average genetic diversity range from 0.16 to 0.88. The Mantel test between the two Dice similarity matrices gave r=0.956, showing high correlation between RAPD- and ISSR-based similarities. Clustering of genotypes within groups was not similar when RAPD and ISSR-derived dendrogram were compared, whereas the pattern of clustering of the genotypes remained more or less the same in ISSR and combined data of RAPD and ISSR. The ISSR markers provide more conservative estimates of genetic diversity than RAPD markers (Souframanien and Gopalakrishna, 2004). ISSR marker-based clustering was noticeable for its differences from the RAPD clusters, again indicating that it may be measuring a different aspect of genetic diversity. For example, using the RAPD data cluster I (HR-07, 09, 10, 14 and 16) belong to *B. clidemioides var. diffusa* and the commercial cultivars grouped together. These results indicated that RAPD markers have potential for genetic diversity and genotypic identification, but will give clustering patterns different from ISSR clusters (Sarwat et al., 2008). Most clusters formed from clustering using all molecular markers were largely based upon the contributions of ISSR markers, which sustained the classified method by Zhang et al. (1998) and Bahattin (2003).

Lai et al. (1999; 2000) and Liu et al. (2003) reported that the collected species in China could be classified according to the morphology and the previous references into the 5 sections *Boehmeria, Tilocnide, Phyllostachys, Duretia* and *Zollingerianae*. They may also be classified into hygric type, moderately hygric type, semi-shade-hygrophyte and xeromorphy, based on their ecological adaptability and primitive growing circumstances. This study suggested that the collected 37 accessions belong to 3 of the 5 sections *Phyllostachys, Tilocnide* and *Duretia*, which also agreed with Zhang et al. (1998) and Lai et al. (2000).

The distribution and adaptability of the wild *Boehmeria* species are formed under the pressure of natural selection. Understanding of the differences in ecological adaptability of the species is helpful for successful another place conservation of the species that are potentially useful for breeding or direct utilization (Lai et al., 1999). In this study, 10 *B. macrophylla* accessions belong to moderately hygric type, which should be planted homologous conditions.

Genetic marker technology designed to detect naturally occurring polymorphisms at the DNA level has become an invaluable and revolutionary tool for both applied and basic studies of ramie. A higher proportion of polymorphic bands were observed using the ISSR (96.3%) method than RAPD (95.5%) method, as suggested by some authors (Bahattin, 2003; Budak et al., 2004; Marotti et al., 2007).

Clustering of genotypes within groups was not similar when RAPD- and ISSR-derived dendrograms were compared. These differences may be attributed to marker sampling error and/or the level of polymorphism detected, reinforcing again the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al., 1996). The putatively similar bands for RAPDs in different individuals are not necessarily homologous, although they may share the same size in base pairs. This situation may lead to mistakes when calculating genetic relationships (Fernandez et al., 2002).

As expected, both ISSR and RAPD markers were highly correlated with materials developed regionally, which are assumed to be neutral under selection (as shown in Fig. 4c). This result may indicate that many wild ramie materials are retained by zone and others, especially some varieties categorized, were neutral in selection similar to commixed analysis by ISSR and RAPD markers.

The combined analysis may have avoided the underestimates or overestimates of genetic distances based on single molecular markers (Lim et al., 2007). The fact that combined diversity estimate based on several molecular markers cover more genetic regions than a single marker alone, genetic distance estimate based on all molecular markers most likely gave the most unbiased distance estimate.

In the previous study, Zhou et al. (2004) used SSR,

ISSR and RAMP markers to determine the genetic relationships among 20 cultivars of ramie but none of the approaches could uniquely fingerprint each cultivar. Jie et al. (2002) used 25 RAPD primers to investigate the genetic diversity of 6 drought-resistant and 6 sensitive ramie cultivars and the result corresponded to their geographical distribution, but drought-resistant genotypes were clustered in different type-groups. Li et al. (2006) also used RAPD to analyze the lignin content of 21 ramie varieties and reported that the genetic relationship among cultivars was correlated with the regions of origin of cultivars. However, there are few reports on the use of RAPD and ISSR markers in analyzing the genetic relationships among wild ramie materials. A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner (Souframanien and Gopalakrishna, 2004). This limited the use of morphological characters and isozymes, which are few or lack of adequate level of discrimination in ramie. Molecular genetic markers have been developed into a powerful tool to analyze genetic relationships and genetic diversity of ramie.

In this study, the RAPD and ISSR markers have been successfully used. Wild ramie materials were classified into 4 clusters using this method, which supplied molecular evidence for the exploitation of this rich genetic resource in China. The overall grouping pattern of clustering corresponded well with that obtained by the method of traditional botanical taxonomy and principal component analysis (PCA) confirming the patterns of genetic diversity observed among the species. RAPD and ISSR markers may have various applications for genetic studies and practical breeding programs in ramie. Among these wild ramie materials, we obtained good amplification and easily found polymorphism. Therefore, RAPD and ISSR markers were useful for the molecular characterization and the investigation of phylogenic relationships in ramie (Jie et al., 2002; Zhou et al., 2004). These results showed that the RAPD and ISSR markers can be used to examine the genetic diversity in wild ramie materials.

Classification of wild *Boehmeria* genotypes based on phenotypic variations or isozyme patterns should be reconsidered in the context of the results obtained from molecular analyses with RAPD and ISSR. There were no significant differences in isozyme patterns among *Boehmeria* (unpublished data). Thus, RAPD and ISSR markers were useful to distinguish different genotypes which were indistinguishable by isozyme markers. Additional phylogenetic studies using chloroplast or mitochondrial gene sequences or appropriate nuclear genes like internal transcribed spacer (ITS) of nrDNA sequences can be helpful to reevaluate the systematic positions of different *Boehmeria* species.

In ramie, the quality of resistance to the drought, coldness, diseases and pests is the basis for normal growth and a high yield. Current ramie fiber is prone to break due to the vertical grooves, crannys at the nodes in the fiber. It was observed that there are fewer nodes in the fiber cells of *B. clidemioides var. diffusa* and *B. pseudotricuspis* (Zhang et al., 1998). This is a very useful trait for overcoming the mentioned imperfections of the current ramie fibers.

In this study, we used molecular markers to investigate the genetic relationships of 4 *Boehmeria* species under different agroecological conditions. Our results indicated the presence of great genetic variability among elite genotypes of ramie. ISSR markers are useful in the assessment of ramie diversity, the detection of duplicate sample in germplasm collection, and the selection of a core collection to enhance the efficiency of germplasm management for use in ramie breeding and conservation programs.

Acknowledgements

This research was supported by 948 Project of the Ministry of Agriculture of China (2006-G18(03)) and project of Hubei province science-technology attack (2007AA201C49) of China. The authors are grateful to Dr. Ilaria Marotti of the University of Bologna, Italy and Prof. Cong-hua Xie, Dr. Li-xiao Nie of Huazhong Agricultural University for their valuable advices and generous help on the research.

References

Bahattin, T. 2003. Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum. vulgare subsp. spontaneum*) populations from west Turkey. Genet. Resour. Crop Evol. 50 : 611-614.

Budak, H., Shearman, R.C., Parmaksiz, I. and Dweikat, I. 2004. Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs and SRAPs. Theor. Appl. Genet. 109 : 280-288.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 6 : 297-302.

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19 : 11-15.

Fernandez, M.E., Figueiras, A.M. and Benito, C. 2002. The use of ISSR and RAPD marker for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet. 104 : 845-851.

Garcia, A.A.F., Benchimol, L.L., Barbosa, A.M.M., Geraldi, I.O., Souza, C.L. Jr. and Souza, A.P. 2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genet. Mol. Biol. 27 : 579-588.

Guo, A.P., Zhou, P., Li, X.Y., Li, Z.D. and Zheng, X.Q. 2003. RAPD analyses of 17 ramie (*Boehmeria nivea*) cultivars. J. Agric. Biotechnol. 11 : 318-320*.

Guo, Y. L., Liu, E.P., He, L.K. and Guo, A.P. 2005. Studies on chromosomal karyotype in ramie. Chinese J. Trop. Agric. 25 :

8-10*.

Hou, S.M., Duan ¸J.Q., Liang, X.N., Ding, X.W. and Liu, F.H. 2006. Detection for mtDNA of cytoplasmic male sterile (CMS) line and maintainer line of ramie *Boehmeria nivea* (L.) Gaud. by ISSR. Plant Physiol. Commun. 42 : 705-707*.

Jiang, Y.B. and Jie, Y.C. 2005. Advances in research on the genetic relationships of *Boehmeria* in China. J. Plant Genet. Resour. 6 : 114-118*.

Jie, Y.C., Zhou, Q.W. and Chen, P.D. 2002. Genetic relation analysis of ramie cultivars with RAPD marker. Acta Agron. Sinica 28 : 254-259*.

Lai, Z.J., Pan, Q.H., Sun, X.B., Lai, X.P. and Huang., X.Y. 1999. Collection, systemization and research of China wild ramie germplasm. Acta Agric. Jiangxi 11 : 1-8*.

Lai, Z.J., Pan, Q.H. and Sun, X.B. 2000. Characteristics and utilization of the wild *Boehmeria* germplasms in China. Acta Agric. Jiangxi 12 : 11-16*.

Li, J.J., Guo, Q.Q. and Chen, J.R. 2006. RAPD analysis of lignin content for 21 ramie varieties. Plant Fibers Products 28 : 120-124*.

Lim, W., Mudge, K.W. and Weston, L.A. 2007. Utilization of RAPD markers to assess genetic diversity of wild populations of North American Ginseng (*Panax quinquefolius*). Planta Med. 73 : 71-76.

Liu, F.H., Liang, X.N., Zhang, N.G., Huang, Y.S. and Zhang, S.W. 2001. Effect of growth regulators on yield and fiber quality in ramie (*Boemheria nivea* L. Gaud.), China grass. Field Crops Res. 69 : 41-46.

Liu, F.H., Li, Z.J., Liu, Q.Y., He, H., Liang, X.N. and Lai, Z.J. 2003. Introduction to the wild resources of the genus *Boehmeria* Jacq. in China. Genet. Resour. Crop Evol. 50: 793-797.

Liu, L.J., Peng, D.X. and Meng, Z.Q. 2006a. Optimization for RAPD reaction system in ramie (*Boehmeria nivea* L.Gaud). Chinese Agric. Sci. Bull. 22 : 35-39*.

Liu, L.J., Sun, Z.X. and Peng, D.X. 2006b. Optimization for ISSR reaction system in ramie (*Boehmeria nivea* L.Guad). Chinese Agric. Sci. Bull. 22 : 64-68*.

Loarce, Y., Gallego, R. and Ferrer, E. 1996. A comparative analysis of genetic relationships between rye cultivars using RFLP and RAPD markers. Euphytica 88 : 107-115.

Mantel, N. 1967. The detecion of disease clustering a generalized regression approach. Cancer Res. 27 : 209-220.

Marotti, I., Bonetti, A., Minelli, M., Catizone, P. and Dinelli, G. 2007. Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semi-random and ISSR molecular markers. Genet. Resour. Crop Evol. 54 : 175-188.

Nei, M. and Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction edonucleases. Proc. Natl. Acad. Sci. USA. 79 : 5269-5273.

Rohlf, F. J. 2000. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1. Exeter Publications, New York, USA.

Roy, A., Bandyopadhyay, A., Mahapatra, A.K., Ghosh, S.K., Singh, N.K., Bansal, K.C., Koundal, K.R., and Mohapatra, T. 2006. Evaluation of genetic diversity in jute (*Corchorus species*) using STMS, ISSR and RAPD markers. Plant Breed. 125 : 292-297.

Sarwat, M., Das, S. and Srivastava, P. S. 2008. Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. Plant Cell Rep. 27 : 519-528.

Soller, M. and Beckmann, JS. 1983. Genetic polymorphism in varietal identification and genetic improvement. Theor. Appl. Genet. 67 : 25-33.

Souframanien, J. and Gopalakrishna, T. 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor. Appl. Genet. 109: 1687-1693.

Wang, W.TS and Cheng, J.R. 1995. China Flora (vol 23, Sect 2). Science Press of China, Beijing 320-355**.

Yap, IV. and Nelson, RJ. 1996. Winboot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Discuss. Pap. Ser. 14.

Zhang, B., Zheng, X.Q., Zang, G.G. and Zha,o L.N. 1998. The comparative Morphology of Boehmeria in China. Sicentia Agric. Sincia 31 : 36-40*.

Zhou, J.L., Jie, Y.C., Jiang, Y.B., Hu, X. and Zhang, J. 2004. Genetic relation analysis on ramie cultivars with microsatellite markers. Acta Agron. Sinica 30 : 289-292.

Zhou, J.L., Jie, Y.C., Jiang, Y.B., Zhong, Y.L., Liu, Y.H. and Zhang, J. 2005. Development of simple sequence repeats (SSR) markers of ramie and comparison of SSR and inter-SSR marker systems. Prog. Nat. Sci. 15 : 136-142.

* In Chinese with English abstract.

** In Chinese.