ARTICLE; AGRICULTURE AND ENVIRONMENTAL BIOTECHNOLOGY

Development of a core collection for ramie by heuristic search based on SSR markers

Ming-Bao Luan\textsuperscript{a}, Zi-Zheng Zou\textsuperscript{b}, Juan-Juan Zhu\textsuperscript{a}, Xiao-Fei Wang\textsuperscript{a}, Ying Xu\textsuperscript{a}, Qing-Hua Ma\textsuperscript{a}, Zhi-Min Sun\textsuperscript{a} and Jian-Hua Chen\textsuperscript{*}

\textsuperscript{a}Chinese Academy of Agricultural Sciences, Institute of Bast Fiber Crops, Key Laboratory of Stem-fiber Biomass and Engineering Microbiology (Ministry of Agriculture), Changsha, Hunan, P. R. China; \textsuperscript{b}Department of Basic Medicine, Yiyang Medical School, Yiyang, Hunan, P. R. China; \textsuperscript{*}Department of Plant Nutrition, China Agricultural University, Beijing, P. R. China

(Received 21 January 2014; accepted 7 May 2014)

There are more than 2000 ramie germplasms in the National Ramie Germplasm Nursery affiliated with the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Science, China. As it is difficult to perform effective conservation, management, evaluation, and utilization of redundant genetic resources, it is necessary to construct a core collection by using molecular markers. In this study, a core collection of ramie consisting of 22 germplasms was constructed from 108 accessions by heuristic search based on 21 Simple Sequence Repeat (SSR) marker combinations. The results showed that there is a poor relationship between the core collection and the geographic distribution. The number of amplification bands for the core collection was the same as that for the entire collection. Shannon’s index for three of the SSR primers (14%) and Nei’s index for nine of the SSR primers (19%) were lower in the core collection than in the entire collection. The true core collection had wider genetic diversity compared with the random core collection. Collectively, the core collection constructed in this study is reliable and represents the genetic diversity of all the 108 accessions.

Keywords: ramie; core collection; SSR

Introduction

Germplasm collections exist to conserve the genetic diversity of crop species and their wild relatives. Redundant genetic resources result in difficulties in the effective conservation, management, evaluation, and utilization of germplasms. To solve these challenges caused by the large amount of germplasm collections, a limited number of genetically diverse accessions within a collection are usually selected as a core collection, i.e. a representative subset of the entire germplasm collection, consisting of introduced accessions with minimum genetic redundancy and retaining most of the initial collection [1] and given priority in evaluation and hybridization.[2] The core collection provides preliminary information on the diversity available in the larger collection.[3] At present, core collections have been developed not only in annual plants like rice,[4] maize,[5] cotton [6] and wheat,[7] but also in perennial plants such as \textit{Prunus mume} Sieb.[8] robusta coffee,[9] peach,[10] Chinese tea,[11] \textit{Betula platyphylla} [12] and persimmon.[13]

Ramie (\textit{Boehmeria nivea} L.), also called Chinese grass, is a popular perennial plant native to eastern Asia, a centre of origin for ramie, having the largest amount of genetic resources in the world. Ramie is thought to have been used for at least six thousand years, which makes it one of the oldest fibre crops. It is used principally for fabric production and the part used is the bark (phloem) of the vegetative stalks, from which bast fibre is obtained. A series of ramie products such as shirts, underwear, and health-care socks has been developed and is sold on the home markets. In recent years, ramie is used to conserve soil and water in Yangtze regions, in the south of China [14]; and is also widely cultivated as a forage crop in China.[15,16] Nowadays, more than 2000 accessions of ramie cultivars are preserved in the National Ramie Germplasm Nursery affiliated with the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Science (CAAS), China. Due to the large number of cultivars and redundant genetic resources, it is difficult to determine precisely and rapidly useful resources for plant breeders. It is, therefore, important to construct a core collection of ramie.

Luan et al. [17] published the first data on constructing a core collection of ramie based on agronomic traits. This involved a large project for collecting data of all resources over a multitude of years and sites. To characterize and effectively evaluate ramie in China, molecular markers such as Random Amplified Polymorphic DNA (RAPD), [18–20] Simple Sequence Repeats (SSR),[21–24] Inter-Simple Sequence Repeats (ISSR),[25–27] Sequence-Related Amplified Polymorphism (SRAP),[24,28,29] Restriction Site Amplification Polymorphism (RSAP)
and Random Amplified Microsatellite Polymorphism (RAMP) [23] have been employed. Among these markers, SSRs are highly polymorphic, informative, codominant, technically simple, and reproducible, and have become common in constructing core collections.[30] In 2011, our group [31] constructed a primary core collection consisting of 158 germplasms, based on data for 25 agronomic traits from 790 ramie germplasms of the National Ramie Germplasm Nursery. The size of the primary core collection is still quite large and redundancy of some accessions may occur because ramie is clonally propagated. This leads to limitations making its use still time-consuming and inconvenient due to low polymorphism on agronomic traits in used the field. Therefore, it is necessary to develop a core collection with the same genetic diversity as the whole collection, but smaller in size.

There are several advantages in constructing a core collection based on SSR markers as follows. First, there is high polymorphism in SSR markers, which produce a number of amplification bands. Second, the SSR test is simple and without seasonal restrictions. Finally, the statistical analysis of the SSR amplification bands is relatively easy and shows high repeatability, i.e. the method is reliable. All this makes it much less time- and labour-consuming for researchers to construct core collections using the SSR marker approach.

The objective of this study was to develop a core collection from a primary core collection by SSR markers, providing the basis for constructing a core collection by further using of a large number of accessions.

Materials and methods

Materials and DNA isolation

A total of 108 ramie accessions (Table 1) grown in the National Ramie Germplasm Nursery affiliated with the Institute of Bast Fiber Crops, CAAS, China were used in this study. DNA was isolated from young leaves collected from each ramie accession. The DNeasy plant mini prep kit (Qiagen, Germany) was used for DNA isolation.

Table 1. List of 108 germplasms.

| Code | Entry         | Origin  | Code | Entry         | Origin  |
|------|---------------|---------|------|---------------|---------|
| 1    | Wayaozhuma    | Guangxi | 55   | Xinningjingma | Guizhou |
| 2    | Ganzaerhao    | Jiangxi | 56   | Youqima       | Hunan   |
| 3    | Miaobazhuma   | Sichuan | 57   | Baiyema       | Jiangxi |
| 4    | Simaozhuma    | Yunnan  | 58   | Lichuanhoupizhuma | Jiangxi |
| 5    | Shuiqingzhuma | Chongqing | 59 | Boyanghuangyema | Jiangxi |
| 6    | Yichuntongpiqing | Jiangxi | 60 | Xiaopinggan     | Sichuan |
| 7    | Yachibaima    | Guizhou | 61   | Yihuangjiama   | Jiangxi |
| 8    | Rongchangzhuma | Chongqing | 62 | Xiangingdagelv | Hubei   |
| 9    | Guangdonghuangpidouerhao | Guangdong | 63 | Yedouzi     | Jiangxi |
| 10   | Qingyexzhuma  | Unkown  | 64   | Tiantaitiema   | Zhejiang |
| 11   | Hexianjiama   | Guangxi | 65   | Dayehongzhameng | Jiangxi |
| 12   | Guangpima     | Jiangxi | 66   | Jiangkousipingzuma | Guizhou |
| 13   | Juandongtuma  | Chongqing | 67 | Longtangbaima  | Chongqing |
| 14   | Limuqingma    | Guizhou | 68   | Wuchangshanpozhumayihao | Hubei |
| 15   | Dadaoma       | Guizhou | 69   | Jinpingqingma  | Guizhou |
| 16   | Lipingqingma  | Guizhou | 70   | Qingpigan      | Jiangxi |
| 17   | Lidazhuma     | Yunnan  | 71   | Feniyuangguangdou | Jiangxi |
| 18   | Gebuqingma    | Guangxi | 72   | Huangjiuma     | Hunan   |
| 19   | Tongmuqingma  | Guangxi | 73   | Guangpima      | Jiangxi |
| 20   | Qingpidamayihao | Chongqing | 74 | Qianzhuangyao | Guizhou |
| 21   | Gaoanma       | Jiangxi | 75   | Loushanhuangpima | Guizhou |
| 22   | Yujingma      | Jiangxi | 76   | Anlongzhumaerhao | Sichuan |
| 23   | Fulisima      | Chongqing | 77 | Linggongqingpima | Guizhou |
| 24   | Wuchuanbaima  | Guizhou | 78   | Japanzhumaqihao | Japan   |
| 25   | Huannanconma  | Sichuan | 79   | Xielingqima    | Chongqing |
| 26   | Nanconghzhuma | Sichuan | 80   | Kuguaqing      | Hunan   |
| 27   | Xinbingxiama  | Chongqing | 81 | Longhuibaimayihao | Hunan |
| 28   | Puqidayelvyihao | Hubei | 82   | Yongshanzhuma   | Yunnan |
| 29   | Wulonghonggan | Chongqing | 83 | Honggujin    | Hubei   |

(continued)
SSR primers
Twenty-one SSR primer pairs (Table 2) were synthesized according to Chen et al.[32]

SSR analysis
SSR-primed polymerase chain reactions (PCRs) were carried out in 10 μL reaction volumes with 1×PCR buffer, 0.2 mmol/L dNTP, 1 U Taq DNA polymerase (Tiangen), 0.5 μL of forward primer (10 nmol/L), 0.5 μL of reverse primer (10 nmol/L), and 0.5 μL of DNA from each accession. PCR was performed under the following conditions, 94 °C for 5 min, followed by 30 cycles of 30 s at 95 °C, 30 s at the primer-specific annealing temperature, 30 s at 72 °C, and a final extension of 10 min at 72 °C.

The PCR products were separated in 8% polyacrylamide gels, and silver dyeing was conducted according to Zhang et al.[33] Molecular weights were estimated using a DNA marker (DNA Marker 2000, BioTeke Co., Beijing, China). Clear bands were recorded as 1 and the absence of bands as 0, and the electrophoresis results were represented as a binary matrix. Amplification band types were recorded. SSR analysis was repeated at least twice.

Data analysis
Observed heterozygosity (H₀) and expected heterozygosity (Hₑ) were determined by using Ppopgen 1.31 software.[34] Powercore software was used to construct the core collection.[35] It can select candidate entries by calculating the costs to reach the goal. Therefore, even if the selection of subsets is repeated using the same data, only one core size is generated.

The genetic similarity matrix was obtained using the SIMQUAL sub-routine of the NTSYS-pc software statistical package[36] based on Jaccard’s algorithms.

Evaluation criteria for the core collection
The representativeness of the core collection was validated according to the following criteria. The coincidence rate of the band types of SSR markers was defined as the percentage of bands of SSR markers amplified in the core collection versus that in the primary core collection; the coincidence rate of Shannon’s index was calculated as the ratio between Shannon’s index in the core collection and that in the primary core collection; the coincidence rate of
Nei’s index was calculated as the ratio between Nei’s index in the core collection and that in the primary core collection. The appropriate core collection should bear at least 70% of the genetic diversity of the entire collection.

**Results and discussion**

Ramie germplasm resources are of great importance in breeding, scientific research, teaching, and production. Traditionally, its germplasm resources are conserved in the field, but there is always damage due to the spread of diseases among germplasm resources and to natural disasters. For instance, the extraordinarily serious flood of 1996, which overflowed the nursery of the national ramie germplasm collection, resulted in the spread of bacterial wilt, which caused enormous losses of ramie germplasm resources.[37] It is necessary, therefore, to back-up the germplasm collection to reduce the risks of damage. However, a major difficulty is that ramie is a clonal crop and the germplasms cannot be conserved via seed propagation. The technology of conservation is more complex in clonal crops than in seed crops and the redundancy is larger in clonal crops than in seed crops.[30] All this makes the cost of management very high, causing severe restrictions. A core collection, being a subset of a large germplasm collection, allows the maximum possible genetic diversity of a crop species to be preserved with minimum redundancy. Some species have core collections constructed using SSR markers. For example, Zhang et al. [30] constructed the core collection of Japanese persimmon by using SSR markers. At present, to the best of our knowledge, there is no report related to core collection construction of ramie, using SSR markers. Thus, in this study, a core collection of ramie was constructed based on SSR markers in order to back-up the entire germplasm collection of ramie.

**Genetic diversity in the primary collection**

The first step in our study was to analyse the genetic diversity in the primary collection. Table 3 shows that the number of alleles per locus ranged from two to three at the tested 21 loci. Fourteen primer pairs amplified two alleles, while the remaining seven primer pairs amplified three alleles. The observed heterozygosity (HO) over all tested loci ranged from 0.23 to 0.93 and the expected heterozygosity (HE) ranged from 0.21 to 0.64.

**Construction of the core collection**

In this study, the core collection of ramie was constructed by using of heuristic search based on SSR markers. Heuristic search is a method of state-space search that estimates each search node until finding the best one, and then searches sequentially from this best node until finding the goal. That is, state-space search is a problem-solving
process that aims to find the optimal path from an initial state to the goal state. Thus, the core collection constructed using heuristic search is unique.

The core collection included 22 ramie germplasms (Table 4), of which 7 germplasms were collected from Guizhou Province, 6 germplasms from Jiangxi Province, 4 germplasms from the municipality of Chongqing city, 2 germplasms from Guangxi Province, and the rest of the ramie germplasms from Yunnan, Sichuan, and Hubei Province, respectively. Their geographic distribution indicated that there was poor relationship between the core collection and the geographic distribution.

**Evaluation of the core collection**

Table 5 shows the number of amplification bands produced by the 21 SSR primer pairs in the entire collection and the core collection, respectively. The number of amplification bands in the core collection was the same as that in the entire collection, suggesting that all belt types of the entire accession were contained in the core collection. Shanon’s index and Nei’s index of the core collection and the entire collection were calculated, respectively. Of 21 SSR primer pairs, the Shanon Index for three SSR primers was lower in the core collection than in the entire collection, accounting for 14% of all the SSR primers used. Nei’s index for four SSR primers was

| Locus | Number of alleles | Observed heterozygocity | Expected heterozygocity |
|-------|-------------------|-------------------------|------------------------|
| b50   | 2                 | 0.23                    | 0.40                   |
| b35   | 3                 | 0.72                    | 0.53                   |
| b38   | 2                 | 0.48                    | 0.39                   |
| b40   | 3                 | 0.37                    | 0.45                   |
| b43   | 2                 | 0.40                    | 0.32                   |
| c03   | 2                 | 0.46                    | 0.50                   |
| c07   | 2                 | 0.93                    | 0.50                   |
| b27   | 2                 | 0.35                    | 0.45                   |
| b28   | 2                 | 0.29                    | 0.41                   |
| b11   | 2                 | 0.34                    | 0.50                   |
| b16   | 2                 | 0.46                    | 0.46                   |
| b24   | 2                 | 0.34                    | 0.47                   |
| b34   | 2                 | 0.28                    | 0.40                   |
| b64   | 2                 | 0.24                    | 0.21                   |
| c17   | 2                 | 0.58                    | 0.41                   |
| b57   | 2                 | 0.59                    | 0.50                   |
| b65   | 3                 | 0.37                    | 0.42                   |
| c18   | 3                 | 0.67                    | 0.64                   |
| b53   | 3                 | 0.65                    | 0.60                   |
| b56   | 3                 | 0.59                    | 0.61                   |
| c10   | 3                 | 0.51                    | 0.64                   |

**Table 4. List of core collection accessions.**

| Germplasm Origin | Germplasm |
|------------------|-----------|
| Ganzaerhao       | Jiangxi   |
| Shuiqingqingma   | Chongqing |
| Hexianjiangma    | Guangxi   |
| Dadama           | Guizhou   |
| Lipingqingma     | Guizhou   |
| Fulisima         | Chongqing |
| Wuhuanbaima      | Guizhou   |
| Tianhuangganzhuma| Yunnan    |
| Tianbaoma        | Jiangxi   |
| Chuanzhuerhao    | Sichuan   |
| Lvzhubai         | Jiangxi   |
| Zhuzibian        | Jiangxi   |
| Ximingqingma     | Guizhou   |
| Lichuanhoupizhuma| Jiangxi   |
| Jiangkouqingpizhuma| Guizhou |
| Qianzhuylao      | Guizhou   |
| Sichuangodibaima | Chongqing |
| Shanqingbaima    | Chongqing |
| Ximatuma         | Guangxi   |
| Shengbaxianma    | Hubei     |
| Tianbaoma        | Jiangxi   |
| Huangpihuangganma| Guizhou   |

**Table 5. Genetic diversity in core collection and entire collection.**

| Locus | Shannon | Nei | BT | Shannon | Nei | BT | Shannon | Nei | BT |
|-------|---------|-----|----|---------|-----|----|---------|-----|----|
| b50   | 1.151   | 0.64| 9  | 1.078   | 0.582| 9  | 1.07    | 1.10| 1.00|
| b35   | 1.491   | 0.756| 5 | 1.41    | 0.73 | 5  | 1.06    | 1.04| 1.00|
| b38   | 1.123   | 0.628| 4 | 0.908   | 0.552| 4  | 1.24    | 1.14| 1.00|
| b40   | 1.728   | 0.793| 7 | 1.35    | 0.67 | 7  | 1.28    | 1.18| 1.00|
| b43   | 0.837   | 0.533| 3 | 0.716   | 0.486| 3  | 1.17    | 1.10| 1.00|
| c03   | 1.448   | 0.731| 5 | 1.378   | 0.714| 5  | 1.05    | 1.02| 1.00|
| c07   | 0.663   | 0.318| 4 | 0.422   | 0.188| 4  | 1.57    | 1.69| 1.00|
| b27   | 1.024   | 0.566| 4 | 1.087   | 0.628| 4  | 0.49    | 0.90| 1.00|
| b28   | 1.503   | 0.736| 6 | 1.336   | 0.682| 6  | 1.13    | 1.08| 1.00|
| b11   | 1.227   | 0.69 | 4  | 1.167   | 0.676| 4  | 1.05    | 1.02| 1.00|
| b16   | 1.207   | 0.678| 4  | 1.145   | 0.642| 4  | 1.05    | 1.06| 1.00|
| b24   | 1.123   | 0.628| 4  | 1.102   | 0.648| 4  | 1.02    | 0.97| 1.00|
| b34   | 1.184   | 0.616| 5  | 1.167   | 0.615| 5  | 1.01    | 1.00| 1.00|
| b64   | 0.586   | 0.397| 2  | 0.541   | 0.356| 2  | 1.08    | 1.12| 1.00|
| c17   | 0.625   | 0.434| 2  | 0.679   | 0.486| 2  | 0.92    | 0.89| 1.00|
| b57   | 1.19    | 0.665| 4  | 1.25    | 0.672| 4  | 0.95    | 0.99| 1.00|
| b65   | 1.54    | 0.719| 7  | 1.243   | 0.624| 7  | 1.24    | 1.15| 1.00|
| c18   | 1.934   | 0.839| 8  | 1.778   | 0.776| 8  | 1.09    | 1.08| 1.00|
| b53   | 1.772   | 0.817| 5  | 1.568   | 0.728| 7  | 1.13    | 1.11| 1.00|
| b56   | 1.918   | 0.835| 8  | 1.798   | 0.811| 8  | 1.07    | 1.03| 1.00|
| c10   | 1.811   | 0.818| 7  | 1.815   | 0.821| 7  | 1.00    | 1.00| 1.00|

*BT: band type.*
also lower in the core collection than in the entire collection, accounting for 19% of all the SSR primers used. When the core collection retains at least 70% of the genetic diversity of the entire collection, it could be considered satisfactory. Thus, the core collection developed in this study could be considered to reliably represent the genetic diversity of the entire collection.

Additionally, a random core collection consisting of 22 ramie germplasms was selected and the calculations were repeated three times. The similarity coefficients between the random core collection and the true core collection in this study were compared. The results are shown in Table 6. The similarity coefficient between the entire collection and the random core collection or the true core collection ranged from 0.48 to 0.87 and from 0.32 to 0.78, respectively, suggesting that the true core collection had wider genetic diversity compared with the random core collection. These results further confirmed that the core collection constructed in this study was reliable.

Table 6. Similarity coefficients of the true and random core collection.

| Collection             | Max | Min |
|------------------------|-----|-----|
| True core collection   | 0.32| 0.78|
| Random core collection | 0.48| 0.87|

Final remarks
Ramie, being a cash crop in China, plays an important role in natural fibre production. In recent years, with the expansion of its field of applications, ramie is used in medicine,[38] feedstuffs,[15,16] soil and water conservation,[14] industrial raw materials, and bio-fuels.[15] For example, there are significant achievements on the medicinal value of its leaves and roots.[38] Its leaves can also be used as animal feed and the trials have entered the stage of pilot scale.[15] Moreover, the Chinese Ministry of Water Resources has demonstrated that ramie is one of the most effective crops for conserving soil and water in the south hilly region.[14] As the germplasm resource basis for scientific research, it is essential to expand the knowledge on the genetic effects and to locate genes of multiple-use ramie. However, it is rather complicated and time-consuming to screen for genes associated with a specific use of ramie because of the very large number of germplasms. A core collection representing the entire genetic diversity of germplasm resources can improve the identification efficiency. For example, Holbrook and Anderson [39] identified leaf-spot resistance in peanut, using a core collection, which took twice as less time than screening the whole germplasm. Jiang et al. [40] also used a core collection as a less time-consuming approach to identify peanut germplasm with resistance to Aspergillus flavus. Therefore, building a ramie core collection can lay a solid foundation for exploring new applications of ramie.

In this study, only 108 accessions were used because SSR molecular markers were first employed in constructing a core collection of ramie. The next step will be to widen the amount of ramie germplasms. The results of this study provide the basis for further improvement of the core collection.

Conclusions
To the best of our knowledge, this study is the first to use SSR molecular markers to construct a core collection of ramie. The core collection constructed on the basis of SSR markers showed the same number of amplification bands as the entire collection, suggesting that it represents the genetic diversity of the entire collection. The constructed core collection is of great significance for ramie germplasm conservation, evaluation, and utilization. The next step will be to widen the amount of ramie germplasms. The results of this study provide the basis for further improvement of the core collection.

Acknowledgements
We specially thank Dr. Yan-fang Zhang of Fujian Academy of agricultural science, who provided the Power Core software and guidance.

Funding
This study was financially supported by the National Natural Science Foundation of China (Youth Program) [grant number 30900913]; Hunan Provincial Natural Science Foundation of China [grant number 13J4116]; Science and Technology Funds Program of CAAS (2009).

References
[1] Frankel OH. Genetic perspective of germplasm conservation. In: Arber W, Llimensee K, Peacock WJ, Starlinger P, editors. Genetic manipulation: impact on man and society. Cambridge: Cambridge University Press; 1984. p. 161—170.
[2] Brown AHD. The case for core collections. In: Brown AHD, Frankel OH, Marshall RD, Williams JT, editors. The use of plant genetic resources. Cambridge: Cambridge University Press; 1989. p. 136—156.
[3] Marita JM, Rodriguez JM, Nienhuis J. Development of an algorithm identifying maximally diverse core collections. Genet Resour Crop Evol. 2000;47:515—526.
[4] Tereza CD, Rosana PV, Paulo HN, Claudio B. Microsatellite marker-mediated analysis of the EMBRAPA rice core collection genetic diversity. Genetica. 2009;137:293—304.
[5] Ronaldo RC, Glauco VM, Cosme DC. Development of a Brazilian maize core collection. Genet Mol Biol. 2009;32: 538—545.
[6] Xu HM, Mei YJ, Hu J. Sampling a core collection of Island cotton (Gossypium barbadense L.) based on the genotypic
values of fiber traits. Genet Resour Crop Evol. 2006;53: 515–521.

[7] Francois B, Valérie R, Pjotr S. A worldwide bread wheat core collection arrayed in a 384-well plate. Theor Appl Genet. 2007;114:1265–1275.

[8] Ming J, Zhang QX, Lan YP. Core collection of Prunus mume Sieb. et Zucc. J Beijing For Univ. 2005;27:65–69.

[9] Prakash NS, Combes MC, Dussert S, Naveen S, Lashermes P. Analysis of genetic diversity in Indian robusta coffee gene pool (Coffea canephora) in comparison with a representative core collection using SSRs and AFLPs. Genet Resour Crop Evol. 2005;52:333–343.

[10] Li YX, An LJ, Jiang Q. Establishment and evaluation of the core collection of peach (Prunus persica L. Bat sch.) cultivars. J China Agric Univ. 2007;12:22–28.

[11] Wang XC, Liu Z, Yao MZ. Sampling strategy to establish a primary core collection of Chinese tea germplasms. J Tea Sci. 2009;29:159–167.

[12] Wei ZG, Gao YC, Yang CP. Sampling method for constructing germplasm core collections of Betula platyphylла. J Northeast For Univ. 2009;37:1–4.

[13] Zhang YF, Zhang QL, Yang Y, Luo ZR. Development of primary core collection for Japanese persimmon originated in China (Diospyros kaki Thunb.) by stepwise clustering. Acta Hortic. 2007;760:69–76.

[14] Tu XN, Chen SC. Ramie—an effective plant for soil and water conservation in the southern sloped farmlands. Global Seabuckthorn Res Dev. 2007;5:45–48.

[15] Xiong HP. The production status and policy suggestion of bast and leaf fiber crops in China. Plant Fiber Sci China. 2010;32:301–304.

[16] Yu CM, Chen JR, Wang YZ. Research progresses of molecular breeding in ramie and forage ramie. Plant Fiber Sci China. 2007;29:389–392.

[17] Luan MB, Chen JH, Xu Y, Wang XF, Sun ZM. Method of establishing ramie core collection. Acta Agron Sin. 2010;36:2099–2106.

[18] Hou SM, Duan QJ, Liang XN, Ding XW, Liu FH. Detection for mtDNA of cytoplasmic male sterile (CMS) line and maintainer line of ramie (Boehmeria nivea (L.) Gaud.) by ISSR. Plant Physiol Commun. 2006;42:705–707.

[19] Liu LJ, Sun ZX, Peng DX. Optimization for ISSR reaction system in ramie (Boehmeria nivea L. Gaud.). Chinese Agric Sci Bull. 2006;22:64–68.

[20] Ding MZ, Pan GT, Zhang ZH. Genetic relation analysis of EST-SSRs in the ramie. African J Microbiol Res. 2011;5:3504–3508.

[21] Zhang J, Wu YT, Guo WZ. Isolation and characterization of EST-SSRs in the ramie. African J Microbiol Res. 2011;5:3504–3508.

[22] Zang GG, Zhao LN, Chen JH, Li YJ. Medical health effects and prospect in ramie. Plant Fiber Prod. 2002;24:27–29.

[23] Holbrook CC, Anderson WF. Evaluation of a core collection to identify resistance to late leaf spot in peanut. Crop Sci. 1995;35:1700–1702.

[24] Zou ZZ, Chen JH, Luan MB. Evaluation of genetic relationship in ramie based on RAPD, SRAP, and SSR. Acta Agronomica Sinica. 2012;38:840–847.

[25] Hou SM, Duan QJ, Liang XN, Ding XW, Liu FH. Detection for mtDNA of cytoplasmic male sterile (CMS) line and maintainer line of ramie (Boehmeria nivea (L.) Gaud.) by ISSR. Plant Physiol Commun. 2006;42:705–707.

[26] Liu LJ, Sun ZX, Peng DX. Optimization for ISSR reaction system in ramie (Boehmeria nivea L. Gaud.). Chinese Agric Sci Bull. 2006;22:64–68.

[27] Chen JH, Luan MB. Construction of core germplasm in ramie. Plant Fiber Sci China. 2011;33:59–64.

[28] Chen JH, Luan MB, Song SF. Isolation and characterization of EST-SSRs in the ramie. African J Microbiol Res. 2011;5:3504–3508.

[29] Zhang J, Wu YT, Guo WZ. Fast screening of microsatellite markers in cotton with page/silver staining. Acta Gossypii Sin. 2000;12:267–269.

[30] Yeh FC, Yang RC, Boyle T. POPGENE version 1.31 quick user guide. Canada: University of Alberta; 1999.

[31] Kim KW, Chung HK, Cho GT, Ma H, Chandrabalan D. PowerCore: a program applying the advanced M strategy with a heuristic search for establishing core sets. Bioinformatics. 2007;23:2155–2162.

[32] Rohlf FJ. NTSYS – pc v 2.1: numerical taxonomy and multivariate analysis system. New York: Applied Biostatistics Inc., Exeter Software; 2000.

[33] Institute of Bast Fiber Crops CAAS. The institute of bast fiber crops, CAAS (1958). Beijing: Agricultural Science and Technology Press; 2008.