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ARTICLE

Development of a mulberry core collection originated in China to enhance germplasm conservation

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Abstract: *China, origin of mulberry, has rich genetic resources usually. High expense, limited space and wavering environment of usually conservation in vivo poses dangerous situation for mulberry. The concept of core collection could takes priority for conservation of mulberry. In this study, 560 accessions were used with 40 morphological descriptors and stratified sampling strategies for a core collection. The core collection consisted of 28 accessions, accounting for 5% of the whole collection. The core collection included seven accessions belonging to Morus alba, one accession belonging to M. alba var. macrophylla, four accessions belonging to M. atropurprea, one accession belonging to M. nigra, three accessions belonging to M. australis, seven accessions belonging to M. multicaulis, two accessions belonging to M. wittorum and three accessions belonging to M. bombycis. The quality of core collection exceeded the evaluation criteria and could be a prioritized collection for high efficient and long-term conservation for mulberry.*

Keywords: *Morus L., genetic resources, morphological marker, germplasm conservation*

INTRODUCTION

Natural silk fiber, known as 'Queen of textile', is treated as a type of luxurious asset produced by silkworm. Crude silk fiber exported from China accounted for over 80% of worldwide production. Mulberry, the sole food source for silkworm, plays an important role in sericulture industry. Increasing demand for natural silk fiber all over the world needs more silkworms, leading to the lack of mulberry leaves in China. Propagation of mulberry vegetatively, natural and artificial crossing have produced abundant genetic resources. Over 3000 genotypes in China were documented (Pan 2000). Mulberry is usually conserved *in vivo*, exposed to environmental degradation and disadvantageous climatic conditions, resulting in the loss of genetic resources easily. Moreover, conservation *in vivo* of all germplasms is unpractical and highly expensive in human and financial resources. Therefore, it has been an urgent and challenging task for mulberry germplasm conservation, causing a serious threat to sustainable sericulture industry.

Frankel (1984) and Brown (1989) proposed the concept of core collection, a limited set of accessions of whole collection with minimum repetitiveness and maximum genetic diversity of a species and its relatives. Owing to representative

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number, core collection is a promising and efficient method for conservation of crops to reduce the expense and space. Core collections of many crops such as persimmon (Zhang et al. 2009), flax (Diederichsen et al. 2013), mungbean (Schafleitner et al. 2015), peach palm (Cristo-Araújo et al. 2015), apple (Liang et al. 2015), etc. have been developed. Core collection has been a preferential collection for high efficient and low cost conservation such as cassava (Escobar et al. 2000), European elms (Harvengt et al. 2004), and garlic (Keller et al. 2012).

An appropriate construction strategy is prerequisite to develop a core collection. van Hintum (2000) described a general procedure for developing a core collection in the following sections: i) identify the total sampling ratio; ii) divide all accessions in whole collection into distinct groups; iii) decide the sampling proportion within group and iv) select entries from each group. Many researchers proposed other methods for development of a core collection such as PowerCore (Kim et al. 2007), Mstrat (Gouesnard et al. 2001), stepwise clustering (Hu et al. 2000), least distance stepwise clustering (Wang et al. 2007). These different methods depend on some factors such as genetic diversity of species, the size of the whole collection, grouping of the whole collection and data type (i.e. phenotypic or molecular data). For example, in Brazil the cultivated area of soybean increased drastically, the level of genetic diversity in the soybean collection was low (Gwinner et al. 2017). Nevertheless, these methods could lay foundation on the study by van Hintum (2000).

Chen et al. (2008) selected 11 accessions as core collection of *Morus multicaulis* Perr from 46 accessions originated in Shandong and Hebei province, China. Zhang et al. (2011) defined a core collection of 16 entries from 73 Gelu ecotype mulberry accessions in China. Guruprasad et al. (2014) analyzed 850 mulberry accessions assembled from 23 countries with molecular and phenotypic markers, resulting in a core collection including 122 entries (about 14.4% of total sampling ration). These limited studies showed the small size of whole collection from China. In present study, we firstly used 560 accessions from *Mulberry Genetic Resources Catalog* (Sericultural Research Institute 1986) and *Mulberry Varieties Records in China* (Sericultural Research Institute 1993) as whole collection in order to obtain an appropriate mulberry core collection based on morphological descriptors in order to enhance germplasm conservation.

MATERIAL AND METHODS

Materials and data identification

Five hundred and sixty Chinese mulberry accessions were used as genetic materials. Forty descriptors were recorded on *Mulberry Genetic Resources Catalog* (Sericultural Research Institute 1993a) and *Mulberry Varieties Records in China* (Sericultural Research Institute 1993b), which were standardized and numbered according to *Descriptors and Data Standard for Mulberry* issued by Ministry of Science and Technology (Table 1) for development of core collection.

All accessions have been grown in field at Shandong Institute of Sericulture, Yantai City, Shandong Province, China since the foundation of the institute in 1950's. Plants were distributed in a randomized complete design. For each accession, three plants were grown. Plants were spaced 70~80 cm between the rows and 70~80 cm within the row. All these data were recorded in detail for this study and were obtained from corresponding author.

Development procedure of core collection

The development procedures included grouping principle, the total sampling ratio, sampling proportion within group and sampling method within group (Figure 1). Grouping principle was carried out in term of traditional classification based on morphological characteristics. All

Table 1. Forty descriptors used in establishment of mulberry core collection

Code	Descriptors	Code	Descriptor
1	Branch shape	21	Leaf orientation
2	Branch length	22	Leaf color
3	Branch thickness	23	Leaf apex
4	Lateral branch number	24	Leaf margin
5	Branch color	25	Leaf base
6	Branching ability	26	Leaf brightness
7	Internode shape	27	Leaf smoothness
8	Internode length	28	Leaf crinkle
9	Lenticel shape	29	Leaf thickness
10	Lenticel size	30	Leaf length
11	Lenticel color	31	Leaf width
12	Lenticel number	32	Petiole length
13	Winter bud shape	33	Petiole thickness
14	Winter bud color	34	Floral sex
15	Shoot tip location form	35	Tassel length
16	Accessory bud number	36	Tassel number
17	Accessory bud size	37	Female style
18	Phyllotaxis	38	Fruit size
19	Leaf shape	39	Fruit number
20	Leaf surface	40	Fruit color

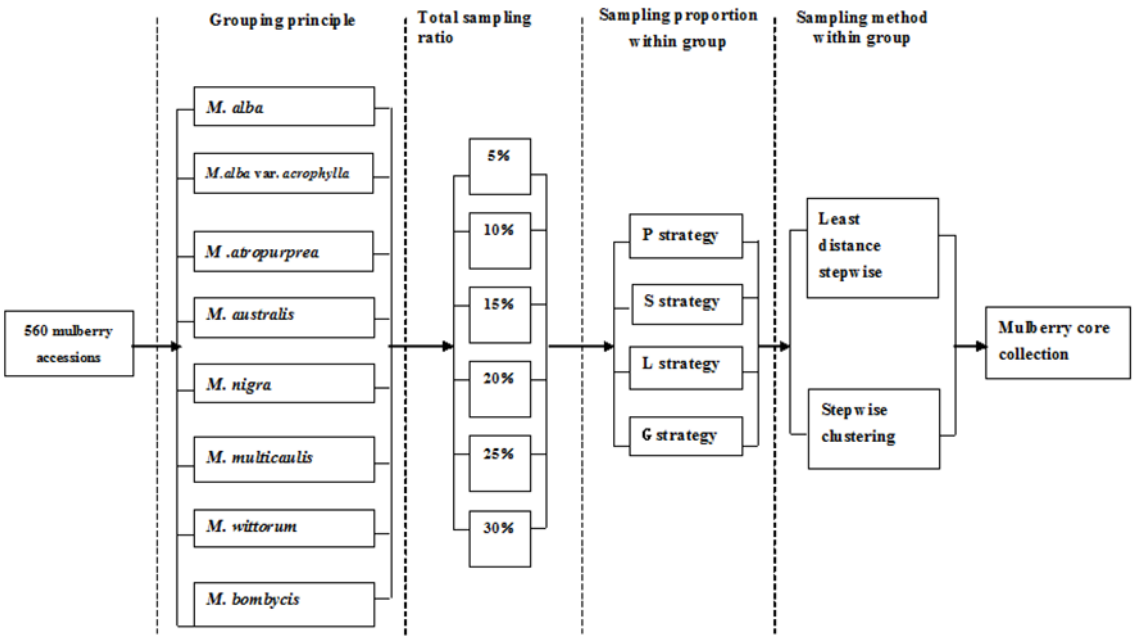

Figure 1. Formation of 48 candidate core collections.

accessions could be divided into eight groups i.e. eight ecotypes, including *M. alba* L., *M. alba* var. *macrophylla* Loud., *M. atropurprea* Roxb., *M. nigra* L., *M. australis* Poir., *M. multicaulis* Perr., *M. wittorum* Handelb-Mazett. and *M. bombycis* Koidz. Six total sampling ratios, 5, 10, 15, 20, 25 and 30%, were compared. There were four sampling proportion within group including constant proportion (P strategy), logarithm proportion (S strategy), square root proportion (L strategy) and genetic diversity proportion (G strategy) (van Hintum 2000). The clustering distance was Euclidean distance, and the clustering method was Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Mohammadi and Prasanna 2003). Two sampling method within group were stepwise clustering (Hu et al. 2000) and least distance stepwise clustering (Wang et al. 2007). Thus, 48 candidate core collections were formed to define the most suitable core collection. At least one accession should be chosen from each group.

Evaluation of candidate core collections

In order to evaluate the efficiency of candidate core collections, eight parameters were selected including coefficient of variation (CV), ratio of phenotype retained (RPR), variance of phenotypic value (VPV), variance of phenotypic frequency (VPF), index of diversity (I), deviation of phenotypic mean (D_{mean}), deviation of phenotypic maximum (D_{max}) and deviation of phenotypic minimum (D_{min}) (Li et al. 2002). Significant differences of the above parameters were calculated by SPSS16.0 (P=0.05). Duncan's multiple range test was used to compare the differences of these parameters among total sampling ratios, among sampling proportions within group and among sampling methods within group. Based on the result of multiple comparisons, each of parameters was allotted a rank. The results were expressed by average ranks of the different parameters (Reviewer: 3 Show how the "average rank" is calculated.). The same value of average rank showed no difference while lower value of average rank showed better efficiency by multiple comparison of total sampling ratio, grouping of all accessions, sampling proportion within group and sampling method within group (Li et al. 2002).

A homogeneity test (F-test) for variances and a t-test for means (P=0.05) were performed to determine the ultimate core collection. The mean difference percentage (MD%), variance difference percentage (VD%), variable rate (VR%), coincidence rate (CR%) and coverage (%) were used for validation of core collection. The criteria were listed as follows: (1) no more than 20% of the traits have different means significantly (at P=0.05) between the core collection and the whole collection; (2) the CR% retained by the core collection is no less than 80%; (3) MD% should lower while VD%, CR% and coverage (%) showed higher (Hu et al. 2000, Kim et al. 2007).

Table 2. Variance analysis of eight parameters for 48 candidate core collections

Parameters[1]	SV	df	SS	MS	F value	P
Means coefficient of variation (CV)	Total sampling ratio	5	1673.773	334.755	654.958	0
	Sampling proportion within group	3	17.553	5.851	11.448	0
	Sampling method within group	1	13.893	13.893	27.183	0
	Interaction	1	9942.298	9942.298	1.95E+04	0
	Error	38	19.422	0.511		
	Total	48	11666.94			
Means ratio of phenotype retained (RPR)	Total sampling ratio	5	0.099	0.02	248.145	0
	Sampling proportion within group	3	0.001	0	2.122	0.114
	Sampling method within group	1	0.004	0.004	51.277	0
	Interaction	1	41.402	41.402	5.18E+05	0
	Error	38	0.003	7.99E-05		
	Total	48	41.509			
Means variance of phenotypic value (VPV)	Total sampling ratio	5	6.45E-06	1.29E-06	0.911	0.484
	Sampling proportion within group	3	4.43E-06	1.48E-06	1.042	0.385
	Sampling method within group	1	1.45E-06	1.45E-06	1.022	0.319
	Interaction	1	47.985	47.985	3.39E+07	0
	Error	38	5.39E-05	1.42E-06		
	Total	48	47.985			
Means variance of phenotypic frequency (VPF)	Total sampling ratio	5	3.03E+07	6067688	160.327	0
	Sampling proportion within group	3	1210761	403587.2	10.664	0
	Sampling method within group	1	154643.8	154643.8	4.086	0.05
	Interaction	1	4.68E+07	4.68E+07	1.24E+03	0
	Error	38	1438141	37845.81		
	Total	48	8.00E+07			
Means index of diversity (I)	Total sampling ratio	5	0.002	0	2.979	0.023
	Sampling proportion within group	3	0.001	0	3.224	0.033
	Sampling method within group	1	0.018	0.018	148.411	0
	Interaction	1	43.944	43.944	3.61E+05	0
	Error	38	0.005	0		
	Total	48	43.97			
Means deviation of phenotypic mean (D_{mean})	Total sampling ratio	5	0	6.77E-05	0.959	0.455
	Sampling proportion within group	3	0	0	1.66	0.192
	Sampling method within group	1	0	0	1.833	0.184
	Interaction	1	0	0	2.869	0.099
	Error	38	0.003	7.06E-05		
	Total	48	0.004			
Means deviation of phenotypic maximum (D_{max})	Total sampling ratio	5	1.113	0.223	15.853	0
	Sampling proportion within group	3	0.036	0.012	0.85	0.475
	Sampling method within group	1	0.266	0.266	18.952	0
	Interaction	1	11.028	11.028	785.583	0
	Error	38	0.533	0.014		
	Total	48	12.976			
Means deviation of phenotypic minimum (D_{min})	Total sampling ratio	5	0.92	0.184	48.18	0
	Sampling proportion within group	3	0.006	0.002	0.519	0.672
	Sampling method within group	1	0.076	0.076	19.977	0
	Interaction	1	4.338	4.338	1.14E+03	0
	Error	38	0.145	0.004		
	Total	48	5.485			

RESULTS AND DISCUSSION

Screening of efficient evaluation parameters for mulberry core collection originated in China

For total sampling ratio, CV, RPR, VPF, I, D_{max} and D_{min} had lower *P* value than 0.05, showing significant difference of core collections by different total sampling ratio; for sampling proportion ratio, CV and VPF had lower *P* value than 0.05, showing significant difference of core collections by different sampling proportion ratios. For sampling method within group, CV, RPR, I, D_{max} and D_{min} had lower *P* value than 0.05, showing significant difference of core collections by different sampling methods within group (Table 2). Therefore, we selected six other parameters for testing efficiency of sampling strategies including CV, RPR, VPF, I, D_{max} and D_{min}.

Development and evaluation of mulberry core collection originated in China

A core collection of *Morus multicaulis* Perr. had developed by Chen et al. (2008), containing 11 entries selected from 46 accessions originated in Shandong and Hebei province, China. Zhang et al. (2011) defined a core collection of 16 entries from 73 Gelu ecotype mulberry accessions in China. Guruprasad et al. (2014) developed a core collection of 122 entries by analyzed 850 mulberry accessions assembled from 23 countries. These core collections could not represent genetic diversity of mulberry in China. In our study, we used 560 characterized accessions originated in China as whole collection for higher representatives of core collection.

Given the total sampling ratio, 10% - 30% of whole collection was suggested (Brown 1989, van Hintum 2000). Various total sampling ratios were compared for suitable one because of genetic diversity of one crop, accession number of base collection, available management of genetic resources and data type (i.e. phenotypic or molecular data), etc (Balas et al. 2014, Leroy et al. 2014, Taniguchi et al. 2014). In previous studies, there was no information of effects of total sampling ratio on development of mulberry core collection (Chen et al. 2008, Zhang et al. 2011, Guruprasad et al. 2014). In our study, according to comparison of average ranks of six total sampling ratios, the lowest value of average rank was 13.73 when total sampling ratio was 5%, showing significant difference at *P*=0.05 (Table 3). Therefore, 5% was taken as suitable total sampling ratio.

Comparison of average ranks of four sampling proportions within group showed that the average rank was lowest and different significantly, when logarithm proportion was used as sampling proportion within group (Table 3). Logarithm

Table 3. Comparison of average ranks of evaluation parameters

Parameters		CV[1]	RPR[1]	VPF[1]	I[1]	D_{max} [1]	D_{min} [1]	Average[2]
Total sampling ratio (%)	5	4.50	4.50	4.50	19.25	5.75	43.88	13.73 c
	10	12.50	13.75	12.5	24.50	17.50	35.62	19.40 b
	15	20.50	21.69	20.5	26.88	22.62	25.50	22.95 ab
	20	28.75	29.06	28.75	27.88	28.12	17.25	26.64 ab
	25	36.75	36.62	37.12	26.25	33.12	12.25	30.35 ab
	30	44.00	41.38	43.62	22.25	39.88	12.50	33.94 a
Sampling proportion within group	Constant proportion	26.25	26.42	27.33	26.33	27.00	23.67	26.17 a
	Logarithm proportion	22.25	22.50	22.17	19.58	24.42	23.58	22.42 c
	Square root proportion	24.75	25.25	24.58	25.25	23.42	25.92	24.86 ab
	Diversity proportion	24.75	23.83	23.92	26.83	23.17	24.83	24.56 b
Sampling method within groups	Stepwise clustering	22.96	28.48	23.33	12.58	30.75	19.71	22.97 a
	Least distance stepwise clustering	26.04	20.52	25.67	36.42	18.25	29.29	26.03 a

[1] See code in Table 2; [2] Different lowercase letters represented significant difference (P<0.05)

Table 4. Comparison of core collections and whole collection

	VD (%)[1]	MD (%)[2]	CR (%)[3]	VR (%)[4]	Coverage (%)
Core collection by stepwise clustering	30.0 a	10.0 a	90.3 a	110.1 a	85.1 a
Core collection by least distance stepwise clustering	32.5 a	2.5 b	91.8 a	114.1 a	85.3 a

[1] Variance difference percentage; [2] Mean difference percentage; [3] Coincidence rate; [4] variable rate. Different lowercase letters represented significant difference (P<0.05)

proportion could be suitable for sampling within group.

Comparison of two sampling methods within group showed that least distance stepwise clustering had lower value of average rank (26.03), but there was no significant different average rank between stepwise clustering and least distance stepwise clustering (Table 3). It showed that there was no difference of effects on efficiency of development of mulberry core collection between two methods of sampling within group. It was not in accordance with the results of Wang et al. (2007). Our results showed similarity between lowest hierarchical level and least distance because of distinct grouping and details of characterization of the whole collection. Previous studies also indicated that rational grouping could enhance the efficient sampling for core collections (van Hintum 1995, Zhang et al. 2000, Wang et al. 2011).

Stepwise clustering and least distance stepwise clustering were compared for selection of the ultimate core collection based on VD%, MD%, CR%, VR% and Coverage (%) (Table 4). The two core collections by clustering meet all validation criteria with MD % lower than 20% and CR% higher than 80%. Moreover, showed that least distance stepwise clustering showed higher MD% than stepwise clustering methods. Therefore, we confirmed that the ultimate core collection was established by least distance stepwise clustering.

The most suitable core collection could be one by least distance stepwise clustering when total sampling ratio was 5% and sampling proportion within group was logarithm proportion. All accessions of the ultimate core collection were listed in Table 5. There were 28 accessions from all eight ecotypes including *M. alba* L., *M. alba* var. *macrophylla* Loud., *M. atropurprea* Roxb., *M. nigra* L., *M. australis* Poir., *M. multicaulis* Perr., *M. wittorum* Handelb-Mazett. and *M. bombycis* Koidz.

Table 5. The list of mulberry core collection

Number	Accession name	Group
1	Jianyeqingsang	*M. alba*
2	Jiesang	
3	li'ersang	
4	Santaiyaosang	
5	Xiangbaitiaosang	
6	Yunsang No.2	
7	Zhensang	
8	Husang No.199	*M.alba* var. *macrophylla*
9	Beiqu No.7	*M .atropurprea*
10	E'jian No.13	
11	E'sang No.2	
12	Yongxinsang	
13	Yaosang	*M. nigra*
14	Chasang	*M. australis*
15	Shuyasang	
16	Yazhousang	
17	Baiqingsang	*M. multicaulis*
18	Haiyanmianqing	
19	Koutouheilu	
20	Miyanqing	
21	Qingpisang	
22	Shuaisang No.4	
23	Zitengsang	
24	Baiyuwang	*M. wittorum*
25	Zhenonghuosang	
26	Pingshan No.9	*M. bombycis*
27	Wo'ersang	
28	Zhaiyesang	

In conclusion, a systematic and suitable mulberry core collection originated in China is firstly developed. Compared with previous mulberry core collections, the larger size of whole collection, more scientific and systematic development strategies were defined. In addition, Mulberry core collection originated in China in our study took advantages of more ecotypes. The core collection can be considered as a preferential collection for conservation and characterization of mulberry.

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