Establishment of a core collection for maize germplasm preserved in Chinese National Genebank using geographic distribution and characterization data

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Abstract

Since the 1980s, a large collection of maize has been preserved in the China National Genebank. To facilitate the evaluation and utilization of these valuable germplasm resources, a core collection was established. The collection was firstly divided into two groups, i.e. the landraces and inbred lines. The percentage of the original collection to be included in the core was given as 7% based on a previous study of sampling strategies in maize. Each group was sequentially stratified based on administrative provinces or regions and kernel types, and a clustering method was applied in further stratification. A logarithmic strategy was used to determine the number of entries in the core at each step. The process resulted in the maize core comprising 951 landraces and 242 inbred lines. Shannon–Weaver diversity index and means were used to validate the core. The core can be used effectively in further in-depth research and maize improvement.

Introduction

In the past decades the size of germplasm collections in the world has increased at an unbelievable pace. This greatly discourages effective management, evaluation and utilization of available germplasm. To solve the problems caused by the huge size of germplasm collections, Frankel and Brown (1984) proposed to establish core collection to represent as much of the genetic diversity of the original collections as possible.

Brown et al. (1987) developed the first core collection from the Australian collections of perennial *Glycine* spp. Since then, a number of core collections have been established for different crops, including mungbean (Bisht et al. 1998), barley (Igartua et al. 1998), sweet potato (Huaman et al. 1999), sorghum (Grenier et al. 2001), chickpea (Upadhyaya et al. 2001), sugarcane (Tai and Miller 2001) and groundnut (Upadhyaya et al. 2003). Most of these core collections were established based on the original collections with less than 10,000 accessions and mainly according to passport data and characterization data. Since 1998, a key project aiming at establishing core collections and genetic diversity analysis for rice, wheat and soybean with accessions of huge size has been initiated in China and great progress has been made.

Maize is one of the most important crops in the world, as well as in China. Researchers in various countries, therefore, have paid great attention to collecting and preservation of maize germplasm and innumerable accessions have been stored in genebanks throughout the world. A few researchers have attempted to establish cores for maize germplasm from specific regions. For example, Taba et al. (1998) developed a core subset of Caribbean maize accession from the CIMMYT maize germplasm bank and evaluated the agronomic and morphological traits of these accessions

in the core. Malosetti and Abadie (2001) also established a core collection for Uruguayan maize landraces based on morphological traits. Currently, China preserves nearly 17,000 accessions of maize germplasm, mainly including maize landraces collected in China and inbred lines. The objective of this study is to develop a core collection for maize germplasm collection preserved in China National Genebank to facilitate the evaluation and use of these valuable germplasm. Stratified sampling method and clustering method were used in the process of entry selection for the core collection (Yozezawa et al. 1995; Charmet and Balfourier 1995).

Materials and methods

There are in total 13,521 maize landraces and 3258 inbred lines preserved in China National Genebank at present. All passport data and evaluation data used in the present study are stored in the Chinese Crop Germplasm Resources Information System (CGRIS).

Sampling strategy used in choosing entries of core collections

In the present study, we treated maize landraces and inbred lines separately. The percentage of the original collection to be included in the core was given to 7% based on the study of sampling strategies (Li et al. 2002a). In that study, 25 methods of sampling a core collection of maize landraces collected in Yunnan Province of China were compared. Core collections sampled with a stratified strategy better represented the germplasm collection than core collections assembled based solely on a simple random strategy. Based on the criteria of Shannon–Weaver diversity indices and overall distances, the core sampled based on a logarithmic strategy and 7% sample size was the optimal core representing the original core. This sampling method was applied in the establishment of the core collection for the whole maize collection preserved in China.

Malosetti and Abadie (2001) compared three classification strategies in establishing a core collection of Uruguayan maize landraces and concluded that a combination of kernel type and geographic origin was the best classification rule since it took into account two points which were closely related to the distribution of diversity: genotypic composition and geographic origin. Therefore, the same method was used in sampling entries of the core collection in the present study. When the core entries were chosen, stratified sampling strategy, specifically the logarithmic strategy (L) in which the representation of each group in the core was in proportion to the logarithm of the number of accessions in that group, was used. By using this strategy, the maize landraces and the inbreds were firstly divided into 34 and 28 groups respectively, according to administrative regions that are the sources of maize germplasm, respectively. Then the landraces and inbreds were divided into eight sub-groups in terms of kernel type (dent, flint, intermediate, waxy, pop, sweet, floury and tunicate). The number of entries in each group and sub-group was determined by the logarithmic strategy. Under each sub-group, cluster analysis using Ward's method was used to further divide that group into suitable clusters, using the data of eight quantitative traits, namely ear type, kernel type, kernel color, cob color, plant height, ear height, number of leaves, days from seedling emergence to maturity ('maturity' in Table 3), ear length, ear width, number of rows, and 1000-kernel weight. The cluster analysis was carried out using S-PLUS 6.0 software (Krause and Olson 2000). And then, core entries from each cluster were chosen at random.

Estimation of Shannon–Weaver diversity indices

Four qualitative characteristics and eight quantitative traits were considered in this study, namely ear type, kernel type, kernel color, cob color, plant height, ear height, number of leaves, days from seedling emergence to maturity ('maturity' in Table 3), ear length, ear width, number of rows, 1000-kernel weight. For the quantitative traits examined in this study, the overall entry mean (X) and standard deviation (σ) were used to subdivide the accession values (x_i) into ten frequency classes ranging from class 1 (if $x_i < X - 2\sigma$) to 10 (if $x_i < X + 2\sigma$), the class interval being 0.5σ (Pecetti et al. 1992).

Phenotypic diversity for each province or region for each character and over all traits was estimated using the Shannon–Weaver diversity index as presented by Poole (1974), i.e.

$$H' = - \sum p_i \, \log_e p_i$$

where p_i is the frequency of the phenotypic class. The variance is given by the following expression:

$$\text{Var}(H') = \left[\sum (P_i \ln P_i)^2 - \left(\sum P_i \ln P_i \right)^2 \right]$$
$$/N + (n-1)/2N^2$$

H' values for different groups of entries or characters can be compared by a t-test:

$$t = (H'_1 - H'_2)/[\text{Var}(H'_1) + \text{Var}(H'_2)]^{1/2}$$

which has $(\text{Var}(H'_1) + \text{Var}(H'_2))^2/(\text{Var}^2(H'_1)/N + \text{Var}^2(H'_2)/N_2)$ denominator degrees of freedom, N_1 and N_2 are the number of entries in the two groups and H'_1 and H'_2 are the Shannon–Weaver diversity indices in the corresponding groups, respectively.

Results and discussion

The core for maize landraces preserved in China

The distribution of the 13521 maize landraces in the CNG is shown in Table 1. Briefly, most material comes from the provinces in southern China including Yunnan, Hubei, Sichuan, Guangxi and Guizhou and two provinces in the Loess Plateau including Shanxi and Shaanxi.

By the use of the logarithmic strategy, a total of 951 maize landraces were chosen as the entries of the core collection, accounting for about 7% of the original (Table 1).

Theoretically, the logarithmic strategy favors small groups, that is, compared with the proportional strategy, a higher percentage of entries will be selected from small groups compared to large groups (Brown et al. 1987). The extreme example in the present study is that all maize landraces (100%) from Tibet and Tianjin were chosen as the entries of the core but only 54 of 1957 accessions (2.8%) from Yunnan were selected as the core entries.

The same trend is reflected in the composition of the core in terms of kernel types (Table 1). Although relatively more entries of flint corn, dent corn and intermediate corn were chosen, compared with the proportional strategy, the logarithmic strategy favored the small groups including popcorn, sweet corn, floury corn, waxy corn and tunicate corn. In particular, three of four accessions of tunicate corn in the original collection were chosen as the entries of the core collection. These results from the logarithmic strategy can be accepted because currently speciality corns, especially waxy corn, sweet corn and popcorn, gain a great attention of breeders and the core collection should have a place of these germplasm of speciality corns.

When the Shannon–Weaver indices and the averages of the core of maize landraces were compared with the original collection, significant differences were detected for most characteristics (Table 3). The results showed that in the core collection the Shannon–Weaver indices of all traits except Kernel type were significantly higher than in the original. This phenomenon is associated with the natures of the logarithmic strategy and the Shannon–Weaver index. In addition, in the core collection the means of all quantitative traits except ear length and ear width were also significantly different from those in the original. This was also observed in other studies. For example, Grenier et al. (2000) found that the subset of a sorghum collection selected based on the logarithmic strategy showed differences for characters associated with the photoperiod reaction. When a faba bean core collection was established using random sampling by geographic regions, Scippa et al. (2001) also found that the estimates of the Shannon–Weaver index pooled across 10 descriptors for eight geographical regions in the whole subsets were significantly different from the respective indices for the core collection.

The core for maize inbred lines preserved in China

The distribution of the 3258 maize inbred lines in the CNG is shown in Table 2. Briefly, many maize inbreds were introduced from Europe (230) and the Americas (742), including mainly the USA and CIMMYT. In addition, a considerable number of

Table 1. Number of accessions selected in the core collection of maize landraces in terms of geographic regions and kernel types.

Province or region	No. of accessions in the orginal	No. of accessions in the core	No. of entries in the core in terms of kernel typ							
			Intermediate	Pop	Sweet	Floury	Dent	Waxy	Flint	Tunicate
Anhui	40	20	9				4		7	
Beijing	137	27	7	1	3		7	2	7	
Fujian	38	19	6				3		10	
Gansu	183	29	9	2	1		5		12	
Guangdong	91	24	6	2			3	4	9	
Guangxi	1195	47	9	5		2	6	9	12	
Guizhou	958	38	8	5			7	8	10	
Hainan	104	21					1		20	
Hebei	390	33	10	2	1		8	1	11	
Henan	285	31	10				8		13	
Heilongjiang	407	33	6	4	2	1	5	5	10	
Hubei	1250	45	9	5			7	5	13	2
Hunan	44	20	7				2	1	10	
Jilin	657	36	9	3	3	2	8	4	7	
Jiangsu	111	24	8				7	3	6	
Jiangxi	71	21	6				4	1	10	
Liaoning	272	31	8	3			11	2	7	
Neimenggu	218	30	8	2	1	1	5	1	12	
Qinghai	49	20					1		19	
Shandong	507	35	8	1	1		8	5	11	1
Shanxi	1054	42	9	3	2	4	8	3	11	
Shaanxi	761	37	10	5			7	5	10	
Shanghai	19	15	6		1		4	3	1	
Sichuan	1232	45	11	6			6	6	12	
Tianjin	15	15	5				6		4	
Tibet	7	7	1						6	
Xinjiang	289	30	8	1	1		6		14	
Yunnan	1957	54	11	4	1		8	10	16	
Zhejiang	118	25	10				5		10	
America*	447	34	8	4	6		8		8	
Europe	432	34	8	2	2		12		10	
Others	183	29	7	2	5		8		7	
Total	13521	951	242	62	30	10	191	81	332	3

Note: * 'America' includes Canada, USA, Mexico, Guatemala, Peru, Brazil, Chile, Argentine, Puerto Rico; 'Europe' includes Albania, Romania, Yugoslavia, Hungary, Bulgaria, Poland, former Czechoslovakia, Germany, Austria, Italy, Spain, Portugal, France, Netherlands, Belgium, Britain, former USSR; 'Others' includes Mongolia, Korea, Japan, Vietnam, Thailand, Malaysia, Indonesia, Philippines, Pakistan, India, Iran, Turkey, Yemen, Israel, Australia, Egypt, Nigeria, Somalia, Zaire, South Africa.

maize inbred lines came from those provinces of China that are major maize producers, including Liaoning, Jilin, Heilongjiang and Neimenggu.

Again by the use of the logarithmic strategy, 242 maize inbreds were chosen as the entries of the core collection, accounting for about 7% of the original (Table 2). As in the case of maize landraces, the logarithmic strategy also favored the provinces with few maize inbreds (e.g. Anhui, Guangxi, Zhejiang, Yunnan, Ningxia, Jiangsu, Hunan, Hubei and Gansu) and the kernel type groups with relatively fewer accessions (e.g. pop, sweet, floury and waxy).

When the Shannon–Weaver indices and the averages of the core of maize inbreds were compared with the original collection, interestingly, significant differences were detected only for a few characteristics, unlike the case of maize landraces (Table 4). The results showed that in the core collection the Shannon–Weaver indices of all traits except ear height, ear color and 1000-kernel weight were not significantly different from those in the original. In addition, in the core collection the means of all quantitative traits except plant height, maturity (i.e. the days from seedling to maturity) and content of crude fat were also not significantly

Table 2. Number of accessions selected in the core collection of maize inbred lines in terms of geographic regions and kernel types.

Province or region	No. of accessions in the original	No. of accessions in the core	No. of entries in the core in terms of kernel type						
			Intermediate	Pop	Sweet	Floury	Dent	Waxy	Flint
Anhui	2	1	1						
Beijing	167	11	4	1			2	1	3
Fujian	66	8	4				1		3
Gansu	30	7	3				3		1
Guangdong	57	8	3				2		3
Guangxi	7	4	1				1		2
Guizhou	72	9	2	1			1	1	4
Hebei	124	10	3		2		2		3
Henan	56	8	3				2		3
Heilongjiang	321	13	4				5		4
Hubei	25	6	2				1		3
Hunan	23	6	2				1		3
Jilin	251	12	5				4		3
Jiangsu	23	6	2		1		1	1	1
Liaoning	295	12	4				5		3
Neimenggu	185	11	3			1	4		3
Ningxia	28	7	3				3		1
Shandong	92	9	2				2	2	3
Shanxi	140	10	3	1		1	3		2
Shaanxi	126	10	3				3		4
Shanghai	16	5	1	1	1		1		1
Sichuan	114	10	4				2		4
Yunnan	13	5	2				1		2
Zhejiang	9	4	2				2		1
America	742	32	11	2	2		10		7
Europe	230	11	3				5		3
Others	44	7	2	1	1		2		1
Total	3258	242	82	7	7	2	68	5	71

different from those in the original. Considering the diversity profiles of maize landraces and inbred lines, we postulate that the reason for these differences between the landraces and inbreds is that the maize landraces had much higher phenotypic diversity than the maize inbred lines (Li et al. 2002b).

A few issues involving in the establishment of the maize core collection

Choice of a suitable sampling strategy is critical in the establishment of core collections. As a general rule, the logarithmic algorithm might be the first sampling strategy to consider, since it gives intermediate results when compared with a constant strategy (C) and a proportional strategy (P) (Van Hintum et al. 2000). In the present study, a combination of the logarithmic strategy and the random strategy was used. That is, when determining the number of entries in the groups according to the geographic regions and in the sub-groups according to the kernel types, the logarithmic strategy was applied twice; when choosing entries under the sub-groups, the random method was applied after the cluster analysis was carried out. The use of 'double logarithmic strategy' can be explained by that some specific kernel types were expected to be more represented in the core because specialty corns including sweet corn, pop corn and waxy corn etc. have developed quickly in recent years and waxy corn possibly originated in China and is favored by Chinese people (Hallauer 2001). If the proportional strategy or the random strategy rather than the logarithmic strategy would be used in determining the number of entries for each sub-group, much fewer accessions would have been allocated to these specific kernel types.

As pointed out by Van Hintum et al. (2000), the effectiveness of sampling strategies depends on a

Table 3. Comparisons of diversity between the core collection and the original collection for maize landraces.

Character	Shannon index		Mean		Variance		CV (%)	
	Original	Core	Original	Core	Original	Core	Original	Core
Plant height (cm)	2.05	2.54**	224.9	214.6**	2349	2537	23.47	22.41
Ear height (cm)	2.02	2.53**	97.9	92.4**	1264	1395	38.99	40.39
No. of leaves	2.05	2.18**	18.7	18.4**	8.43	9.88	15.52	17.10
Maturity (days)	2.05	2.57**	113.9	112.4**	209	224	12.70	13.32
Ear length (cm)	2.08	2.64**	15.0	15.0	7.86	9.31	18.75	20.35
Ear width (cm)	2.00	2.64**	3.9	3.9	0.25	0.35	12.88	15.14
1000-kernel weight (g)	2.07	2.72**	250.2	242.4**	3960	5626	25.15	30.96
Content of crude protein (%)			11.9	12.1**	1.55	2.58	10.42	13.28
No. of kernel rows	2.01	2.57**	12.8	13.2**				
Content of crude fat (%)			4.9	5.0**				
Ear shape	0.89	1.31**						
Kernel type	1.30	1.35						
Kernel color	1.21	1.73**						
Ear color	0.61	1.32**						

Note: * significant at 5% level; ** significant at 1% level.

Table 4. Comparisons of diversity between the core collection and the original collection for maize inbred lines.

Character	Shannon index		Mean		Variance		CV (%)	
	Original	Core	Original	Core	Original	Core	Original	Core
Plant height (cm)	1.57	1.60	169.0	173.9*	972	863	17.93	17.35
Ear height (cm)	1.54	1.42*	62.5	62.0	367	520	30.86	36.55
No. of leaves	2.05	2.02	18.4	18.2	6.47	6.94	13.95	14.35
Maturity (days)	2.11	2.09	118.4	121.2**	251	266	13.07	13.79
Ear length (cm)	2.01	2.07	14.6	14.8	7.90	7.19	18.98	18.38
Ear width (cm)	2.09	2.03	3.9	4.0	0.27	0.31	13.04	14.32
1000-kernel weight (g)	2.57	1.97**	231.9	238.7	3167	3928	23.63	27.10
Content of crude protein (%)			11.8	11.9	2.01	2.25	11.93	12.78
No. of kernel rows	2.01	1.99	14.0	14.3				
Content of crude fat (%)			4.3	4.1*				
Ear shape	0.77	0.74						
Kernel color	0.61	0.51						
Ear color	0.72	0.80*						

Note: * significant at 5% level; ** significant at 1% level.

successful classification that gives genetically meaningful groups. An optimal grouping procedure should ensure that the major components of adaptation across all habitats or separate areas are properly represented. In the present study, two stratification steps dividing the whole collection into the groups according to the geographic regions and in the sub-groups according to the kernel types were implemented. A combination of geographic origin and kernel type was also used in the studies of Malosetti and Abadie (2001) in which the core collection of Uruguayan maize landraces was established.

It should be pointed out that the logarithmic strategy is highly dependent of stratification methods used. For example, America was regarded as one region although it comprises two continents in the present study. If similar geographic groups for America (also for Europe and other regions other than China) would be created as did for China, much more accessions would have been chosen from the region. Although this is true theoretically,

because the emphasis of establishing this core was placed on Chinese maize germplasm and tropical maize germplasm are of relatively little interest to Chinese breeders, the adjustment by combining more foreign countries together into three categories, i.e. 'America', 'Europe' and 'Others', was made to decrease the size of the groups with introduced maize germplasm. Since different maize core collections have been, or are being, established in the USA, Europe and other countries/regions and at CIMMYT as well, coordination and cooperation in exchanging those germplasm of the maize core collections will facilitate the use of 'hidden' genetic diversity in maize germplasm worldwide.

The representativeness of the maize core collection can be verified and validated by some measures of the diversity, including variance and Shannon–Weaver diversity index due to their familiarity and simplicity (Galwey 1995). Theoretically, the diversity of a core collection will not exceed that of an original collection from which the core is established in terms of alleles containing. However, if a logarithmic strategy is used in choosing entries and measures of variance, coefficient of variance and Shannon–Weaver diversity index are used when the core collection and the original collection is compared, the diversity measures of the core for most traits will often be higher than those of the original because of the nature of Shannon–Weaver indices (Poole 1974) and because the logarithmic strategy favors small groups compared with the proportional strategy (Brown 1989). This was confirmed by the present study in which the variance, the mean, the coefficient of variance and the Shannon–Weaver diversity index for the continuous descriptors were calculated: for the maize landraces, the core collection had higher variances, coefficients of variance and Shannon–Weaver diversity indices for all traits than those of the origin collection; for the maize inbreds, there existed the same trends for all traits except ear length and plant height. It implies that a core collection should be more diverse than the original collection if traditional measures of diversity are used, and a core collection may favor rare alleles with lower frequencies if the utility of the core (such as in breeding) is emphasized.

The core collection has to be a dynamic, rather than a static, set of accessions (Jaradat 1995). Particularly, change of the contents and composition of the core should be made when new accessions are received. For instance, the present core collection does not contain any materials of wild relatives (mainly including teosintes and *Tripascum*). Some typical accessions containing different functional alleles of these sources can be added to the core. Additionally, more representative accessions newly introduced from other countries (e.g. Mexico, the center of origin for maize) or collected from distinct or new areas can also be considered to be members of the core collection in the future.

In addition, much more seeds of the maize accessions in the core collection should be multiplied to provide to various users, including breeders and researchers in different disciplines. These accessions of the core for distribution will be stored at the medium-term genebank where further regenerations should be followed if necessary. Moreover, molecular fingerprinting and characterization of the accessions in the core collection are needed to clarify molecular genetic diversity profile and genetic relationships among those accessions. This will provide clues of the introduction, dispersion and selection of maize in China (Rebourg et al. 2003).

To understand the potential of use of these maize accessions, racial classification and heterotic grouping should be carried out in the near future. These have seldom been done in China, although racial classification of Latin American maize landraces has been quite clear (Goodman and Brown 1988). Heterotic grouping of these germplasm, especially Chinese maize landraces, will help establish new basic breeding populations adapted to local ecological environments. Furthermore, systemic characterization and evaluation of these accessions in the core collection for agronomic characters of importance should be emphasized. These agronomic characters not only include morphological traits such as plant height, ear height and number of tillers but also yield components, disease/pest resistance and abiotic tolerance.

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