Development of core collection using morphological descriptors in Sweet osmanthus (*Osmanthus fragrans* Lour.) germplasm[☆]

Wangjun Yuan, Jinsheng Lei, Yuanji Han, Xueyan Yan, Fude Shang[*]

College of Life Sciences & Institute of Agricultural Biotechnology, Henan University, Kaifeng, Henan 475001, China

---

Abstract

Sweet osmanthus (*Osmanthus fragrans* Lour.) is one of the most valuable and ornamental plants of China. *O. fragrans* in medicinal uses with an anti-tussive of its flowers and its essential oil used as a flavouring. The development of a core collection could facilitate easier access to sweet osmanthus genetic resources for their use and simplify the gene-bank management. The present study was initiated to develop a core subset of sweet osmanthus based on 12 qualitative traits and 5 quantitative traits on 122 sweet osmanhus accessions. The accessions were stratified by group, and data on 12 qualitative traits and 5 quantitative traits were used for clustering following Ward's method. About 30 % of the accessions were randomly selected from each cluster to constitute a core subset of 38 accessions. Mean comparisons using t-test, frequency distribution using χ^2-test, and Shannon-Weaver diversity index of 17 descriptors indicated that the genetic variation available for these traits in the entire collection has been preserved in the core subset. There was a fair degree of similarity in phenotypic correlation coefficients among traits in the entire collection and core subset, suggesting that this core subset has preserved most of the co-adapted gene complexes controlling these associations. [Life Science Journal. 2009; 6(2): 17 – 22] (ISSN: 1097 – 8135).

Keywords: sweet osmanthus; core collection; genetic resources; Shannon-Weaver diversity index

---

1 Introduction

Sweet osmanthus belongs to the genus *Osmanthus* in the Oleaceae family. Descriptions of this plant appear in 2500 years old documents, suggesting a long history of domestication[1]. It is certain that sweet osmanthus is a plant originated from a China-Himalayan region, in the southwest China, from the eastern Himalayan Mountains extending to northern Thailand[2]. Sweet osmanthus is one of the most valuable and ornamental plants of China. Appreciating osmanthus flowers in golden autumn is a traditional habitude in China. In order to develop and expand osmanthus culture, Osmanthus festivals are solemnly and grandly held every year in China to promote their local tourism[1]. It is especially valued as an additive for tea and other beverages and is native to

Asia which extends from the Himalayas east through China to Japan. *Osmanthus fragrans* Lour. (*O. fragrans*) is used in cosmetics for the hair and skin, but is mostly used for aromatic therapy. In addition, *O. fragrans* in medicinal uses with an anti-tussive of its flowers and its essential oil used as a flavouring[3].

Long-standing cultivation and the process of history has enabled us to boast of rich cultivars of sweet osmanthus, but due to the limitations of history, there had never been any detailed sweet osmanthus investigation, or theoretical and systematical classification research on sweet osmanthus and its cultivars had never been conducted until the 1980s. At that time a group of scientists devoted themselves to research into sweet osmanthus and its cultivars, and into its classification system (Chen, 1983; Liu, 1985; Lu, 1986; Zhu, 1992)[1]. At present, there are about 166 cultivars which could be categorized into 4 cultivars groups (Asiaticus Group, Albus Group, Luteus Group, and Aurantiacus Group) according to morphological and physiological characteristics[4]. The detailed characters of 122 cultivars of sweet osmantus were described in Xiang's an

Illustrated Monograph of the sweet Osmanthus Cultivars in China (2008). Accordintg to the flowing time and corolla color all the cultivars also are divided into 4 groups: Asiaticus Group, Albus Group, Luteus Group, and Aurantiacus Group.

Frankel (1984) proposed ''core collection'' which would represent, with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives[5]. Frankel and Brown (1984)[6] and Brown (1989)[7]outlined the procedure for the development of core collection by using information on the origin and characteristics of the accessions. The issues that should be taken into consideration while developing a core are the size, the sampling strategy, the grouping within the collection, and the number of accessions to be included in the core from each group. The core collection should be about 10% of the total collection that will retain over 70% of the alleles in the whole collection[7]. Using the stratified sampling, the collection is first divided into non-overlapping groups or strata, and then a simple random sampleis drawn from within each group. Passport and characterization data may be used to determine the groups within the germplasm collection. The hierarchy of groupings begins with the groupings suggested by taxonomy followed by assigning accessions to major geographic groups or agro-ecological regions. Clustering within the broad geographic group could be done based on information from available genetic diversity, cytological variation, marker loci or quantitative traits, and data on stress tolerances. Collection with abundant discriminating data of this type will require a multivariate clustering to discern groups of similar accessions[8]. The number of accessions selected from each cluster will depend on the strategy used. A good core collection should have maximum genetic diversity and no genotypically redundant entries, should represent the whole collection, and should be small enough to manage easily[9].

Core collection has been developed in many crops[10 −14]. Core collection has also been developed in some horticultral plants. Johnson *et al* developed a core subset (CS) of 210 safflower accessions based on branching pattern, flower color, flowering time, growth habit, head diameter, plant height, iodine number, lysine content, oil content, and spineless on 2000 accessions from 50 countries[15]. The present study was therefore aimed to develop a CS of sweet osmanthus based on four groups, 12 qualitative traits and 5 quantitative traits.

2 Materials and Methods

We used 122 accessions of four groups described by

Xiang (2008) to develop the CS for sweet osmanthus (Table 1). The accessions were first stratified by group. The data on 12 qualitative traits in each group was standardized using the range of each variable to eliminate scale differences[16]. The standardized data was subjected to the hierarchical cluster algorithm of Ward[17], using SPSS13.0. This method optimizes an objective functionbecause itminimizes the sum of the square within groups and maximizes the sum of squares among groups. The agglomerative procedure starts with n groups (i.e., one observation in one group; maximum among group sum of squares), and proceeds by merging observations in groups so that the between-groups sum of squares decreases and within-groups sum of squares increases. In certain cases the within-groups sum of square will remain the same, but it will never decrease[18]. From each cluster, 30% of the accessions were randomly selected for inclusion into the CS. At least one accession was included from those clusters that had less than 3 accessions.

Means of the entire collection (EC) and CS for the 12 morphological descriptors were compared using Newman-Keuls procedure[19]. The homogeneity of variances of the EC and CS was tested with the Levene's test[20]. The distribution homogeneity for each descriptor among the EC and the CS was analyzed by the χ^2-test. The phenotypic correlation among different traits in the EC and CS was estimated to know whether these associations, which may be under genetic control, were conserved in the CS.

3 Results

A CS of 38 accessions was established from 122 sweet osmantus accessions. These 38 accessions were arrayed into 4 distinct clusters (Table 2). Albus Group and Luteus Group accounted for 66.4% (81 accessions) of the accessions in the EC, and this predominance was also reflected in the CS that contain, 65.8% (25 accessions) of the accessions from the two groups. About 13.1% (5) of the accessions in the CS were from Asiaticus Group, 21.1% (8) from Aurantiacus Group.

The ranges, means, and variances of the 12 qualitative traits and 5 quantitative traits are given in Table 3. Differences among means of the EC and CS for the 12 qualitative traits used in developing the CS were not significant, and the variances of the EC and CS were homogeneous for all the traits. The CS captured 100% range variation for 10 qualitative traits and 90% for leaf margin and shape of spring leaf. The differences between means and variances of the EC and CS for the 5 quantita-

Table 1. List of morphological descriptors recorded on 122 sweet osmanthus accessions

Morphological descriptor	Abbreviation	Classes and stage of evalution
Qualitative traits		
Growth habit	GH	shrub-1, tree-2, shrub or tree-3
Growth status	GS	vigorous-1, medium vigorous-2, slight vigorous-3
Shape of tree crown	STC	ellipsoidal-1, spheroidal-2, spheroidal or broad spheroidal-3, ovoid-4, ellipsoidal or spheroidal-5, ovoid or spheroidal-6, oblate spheroidal-7, long spheroidal-8, obovoid-9, irregular spheroidal-10
Leaf margin	LM	entire or nearly-1, entire-2, thin serrate-3, serrate-4, entire or sprase serrate-5, entire or serrate-6, always serrate-7, dense serrate-8, sprase serrate-9
Base of leaf	BL	cuneate-1, narrow cuneate-2, cuneate or narrow-3, broad cuneate-4, rounded-5
Shape of spring leaf	SPL	elliptic-1, obovate-elliptic-2, obovate-ellipticto broad elliptic-3, broad elliptic to rounded-4, elliptic to broad elliptic-5, obovate or obovate-ellipticto-6, elliptic to elliptic-lanceolate-7, elliptic-lanceolate-8, linear-lanceolate-9, oblanceolate-10, elliptic to ovate-elliptic-11, linear-12, oblong-lanceolate-13, lanceolate-14
No. buds per node	NBN	1 pair-1, 2 pairs-2, 1-2 pairs-3, 1-3 pairs-4, 2-3pairs-5, 3-4pairs-6
Corolla color	CC	Yellow white-1, milky white-2, silvery white-3, milky yellow-4, white-5, light orange-6, orange yellow-7, light yellow-8, golden-yellow-9, yellow-10, deep yellow-11, deep orange yellow-12, orange red-13
Expand mode of corolla	EMC	spreading-1, inclined-2, spreading or inclined-3, reflexed-4, unexpanding-5
Shape of corolla lobe	SCL	elliptic-1, ovate-2, obovate-3, oblong-4, linear-5, elliptic orrounded-6, obovate or elliptic-7
No. flowering of every year	NFE	1 time-1, 2 times-2, 1-2 times-3, 2-3 times-4, 3-4 times-5, time after time-6
Pistil	PI	fruit-1, sterile-2, abortive-3
Leaf length (cm)	LL	Average length
Length of petiole (mm)	LP	Average length
No.of leaf side venation (pair)	NLV	Average pair
Corolla diameter (mm)	CD	Average diameter
Length of pedicel (mm)	LPE	Average length

tive traits were also not significantly different. The CS captured over 90% range variation for three quantitative traits (Leaf length, Corolla diameter and Length of pedicel).

Table 2. Group representation of accessions in EC and CS in sweet osmanthus

Group	No. of accessions in EC	No. of accessions in CS
Asiaticus	14	5
Albus	46	15
Luteus	35	10
Aurantiacus	27	8

The analysis of frequency distribution of 12 morphological descriptors indicated the homogeneity of distribution among the EC and CS inferred from probability (> 0.05) (Table 4).

Ortiz *et al* emphasized the importance of proper and adequate sampling for the conservation of phenotypic associations arising from coadapted gene complexes in core collection[21]. There is a fair degree of similarity in phenotypic correlation coefficients among qualitative and quantitative traits, suggesting that this CS has preserved most of the co-adapted gene complexes controlling these associations. The correlation (*r*) values were low but significant (48 and 42 correlation coefficients in EC and CS, respectively in Table 5 and 7 and 6 correlation coefficients in EC and CS, respectively in Table 6), indicating that these correlation's did not explain a large fraction of the total variation.

The Shannon-Weaver diversity index (H′) was used to measure allelic richness and evenness in the EC and CS. A low H′ indicates an extremely unbalanced frequency of classes for an individual trait and a lack of genetic diversity. In the present study, H′ values for all the 12 qualitative traits and quantitative traits were similar in the EC and CS (Table 7), indicating that the diversity of the EC was represented in the CS.

Table 3. Range, mean, and variance of 12 qualitative traits and 5 quantitative traits recorded in the EC and CS of sweet osmanthus

Qualitative traits	Range		Mean*		Significance	Variance**			
	EC	CS	EC	CS		EC	CS	*F*-value	*P*-value
GH	1 – 3	1 – 3	2.2	2.3	NS	0.57	0.49	0.325	0.570
GS	1 – 3	1 – 3	1.5	1.4	NS	0.38	0.41	0.027	0.871
STC	1 – 10	1 – 10	3.4	3.5	NS	6.54	7.18	0.012	0.914
LM	1 – 9	1 – 8	4.0	4.1	NS	4.76	4.48	0.071	0.791
BL	1 – 5	1 – 5	2.9	2.9	NS	2.53	2.69	0.087	0.769
SPL	1 – 14	1 – 13	6.4	6.1	NS	14.53	14.75	0.019	0.891
NBN	1 – 6	1 – 6	3.7	3.4	NS	2.05	2.07	0.191	0.663
CC	1 – 13	1 – 13	6.1	6.2	NS	16.42	17.74	0.160	0.690
EMC	1 – 5	1 – 5	2.0	2.2	NS	0.62	0.86	1.513	0.220
SCL	1 – 7	1 – 7	3.5	3.4	NS	3.66	3.44	0.153	0.696
NFE	1 – 6	1 – 6	2.6	2.7	NS	2.84	3.22	0.703	0.403
PI	1 – 3	1 – 3	1.8	1.8	NS	0.22	0.19	2.434	0.121
LL (cm)	6 – 14.5	7 – 13	9.8	10.0	NS	2.93	2.27	0.342	0.559
LP (mm)	5 – 15	6 – 15	9.8	9.8	NS	4.68	5.67	0.474	0.492
NLV (pair)	6 – 12	6 – 12	8.5	8.5	NS	1.63	1.92	0.236	0.628
CD (mm)	4.5 – 12	6 – 11	7.7	7.6	NS	2.56	2.85	0.517	0.473
LPE (mm)	4 – 14.5	5 – 14.5	8.5	8.4	NS	5.31	5.18	0.135	0.714

*: NS (Non significant) at 0.05; **: Differences between mean of EC and CS were tested by Newman-Keuls test, and variance homogeniety was tested by Levene's test.

Table 4. Chi-square test and probability for comparison of frequency distribution of 12 qualitative traits between the CS and entire sweet osmanthus collection

Descriptor	No. of classes	χ^2	Probability
GH	3	0.831	0.660
GS	3	0.239	0.887
STC	8	3.057	0.962
LM	8	5.015	0.756
BL	5	1.015	0.908
SPL	13	4.494	0.985
NBN	6	2.701	0.746
CC	14	6.490	0.927
EMC	4	2.661	0.616
SCL	7	1.203	0.977
MFE	6	1.014	0.908
PI	3	0.888	0.641

4 Discussion

Based on the conception and the significance of the core collection, and the characteristics and the status of germplasmand core collection of horticultural plants, we should quickly set up some core collections of traditional famous and important horticultural plants aimed at making full use of the germplasm resources, promoting innovation ability of germplasm resources and propelling horticultural industry[22]. Sweet osmanthus, as a traditionally popular flower in china, should set up core collection and possess the condition by the efforts of the scientists of International Cultivars Regitration Center For Osmanthus. In the present study we developed core cellection of sweet osmanthus base on 12 qualitative traits and 5 quantitative traits described in Xiang's an Illustrated Monograph of the sweet Osmanthus Cultivars in China. We expect to provide some help for protecting of germplasm resources and breeding of sweet osmanthus.

Suresh and Balakrishnan compared the diversity of the core sample with that of the whole collection using six different sampling strategies. The pool diversity index based on 28 descriptors was close to the diversity of the whole collection. However, when accessions from different diversity groups were allocated with equal frequency or in proportion to the logarithm of the number of accessions in each group or in the proportion to the square root-proportion of the number of accessions in each group, the resultant core sampleshad higher levels of diversity than the whole collection[23]. Liu *et al* also reported that a core of 719 mungbean accessions, roughly

Table 5. Correlation coefficients between 12 morphological descriptors in the EC (above diagonal) and CS (below diagonal) of sweet osmanthus

	GH	GS	STC	LM	BL	SPL	NBN	CC	EMC	SCL	NFE	PI
GH	–	0.093	– 0.111	– 0.111	0.171*	0.027	– 0.030	– 0.224*	0.241*	– 0.161*	0.196*	– 0.274*
GS	– 0.095*	–	– 0.282*	– 0.077*	– 0.056	– 0.314*	– 0.082	0.124*	0.039	0.120*	– 0.082*	– 0.030
STC	0.032	– 0.110*	–	0.043	0.073	0.163*	0.090*	0.017	0.127*	0.103*	– 0.086	0.135*
LM	0.211**	– 0.170*	0.153*	–	0.142*	– 0.080*	0.065	– 0.022	– 0.074	0.057	– 0.131*	– 0.342*
BL	0.173*	– 0.022	0.005	0.134*	–	0.131*	0.279*	– 0.169*	0.209*	0.272*	0.376*	0.014
SPL	0.099*	– 0.188*	0.109*	0.153*	0.084*	–	0.233*	0.101*	0.382*	– 0.325*	– 0.078*	0.010
NBN	0.041	– 0.063*	0.112*	0.049	0.171*	0.153*	–	– 0.164*	0.256*	– 0.052	– 0.308*	0.002
CC	– 0.051*	– 0.110*	0.128*	0.075*	– 0.120*	0.057*	– 0.020	–	0.011	0.020	– 0.339*	0.092
EMC	0.088*	0.062*	– 0.001	0.116*	0.075*	0.066*	0.052*	– 0.151*	–	0.114*	0.079*	0.074*
SCL	– 0.050*	0.114*	– 0.063*	0.000	– 0.023	– 0.006	0.021	0.044	0.117*	–	0.097	0.213*
NFE	– 0.133*	0.181*	– 0.128*	– 0.115	0.117*	– 0.102*	0.113*	– 0.245*	0.120*	– 0.011	–	0.118*
PI	– 0.061*	– 0.049	– 0.072*	0.024	0.008	0.090*	0.237*	0.155*	– 0.029	0.074*	0.070*	–

*and **: Significant at 0.05 and 0.01 probability level, respectively.

Table 6. Correlation coefficients between 5 quantitative traits in the EC (above diagonal) and CS (below diagonal) of sweet osmanthus

	LL	LP	NLV	CD	LPE
LL	–	0.350*	0.342*	0.172	– 0.021
LP	0.320**	–	0.281*	0.068	0.222*
NLV	0.460**	0.223**	–	0.071	– 0.217*
CD	0.249**	0.086	0.198*	–	0.439**
LPE	0.102	0.203*	0.009	0.437**	–

*and **: Significant at 0.05 and 0.01 probability level, respectively.

Table 7. Shannon-Weaver diversity index for 12 morphological and 5 quantitative traits in the ES and CS of sweet osmanthus

Qualitative traits	EC	CS
GH	1.05	0.99
GS	0.85	0.85
STC	1.84	1.77
LM	1.46	1.43
BL	1.51	1.44
SPL	2.08	2.03
NBN	1.50	1.60
CC	2.32	2.28
EMC	1.10	1.12
SCL	1.57	1.66
NFE	1.41	1.49
PI	0.64	0.59
LL (cm)	2.54	2.35
LP (mm)	2.77	2.61
NLV (pair)	2.20	2.14
CD (mm)	2.49	2.43
LPE (mm)	2.75	2.51

14.2% of the 5072 accessions, represented the whole collection[24]. The accessions were stratified by group, and data on 12 qualitative traits and 5 quantitative traits were used for clustering following Ward's method. About 30% of the accessions were randomly selected from each cluster to constitute a CS of 38 accessions. Mean comparisons using t-test, frequency distribution using χ^2-test, and Shannon-Weaver diversity index of 17 descriptors indicated that the genetic variation available for these traits in the EC has been preserved in the CS. There was a fair degree of similarity in phenotypic correlation coefficients among traits in the EC and CS, suggesting that this CS has preserved most of the co-adapted gene complexes controlling these associations.

References

1. Xiang QB, Liu YL. An illustrated monograph of the sweet osmanthus cultivars in China. Zhejiang Science & Technology Press, 2008 (in chinese).
2. Green PS. A monographic revision of Osmanthus in eaet Asia and north America. Notes from the Royal Botanic Garden Edingurg 1958; 22: 435 – 542.
3. Deng C, Song G, Hu Y. Application of HS-SPME and GC-MS to characterization of volatile compounds emitted from Osmanthus flowers. Annali di chimica 2004; 94: 921 – 27.
4. Zang DK , Xiang QB. Studies on the cultivar classification of Chinese Sweet Osmanthus. Landscape Plants 2004; 11: 40 – 9 (in chinese).
5. Frankel OH. Genetic perspective of germplasm conservation. In: Arber W, Llimensee K, Peacock WJ, Starlinger P (Eds). Genetic Manipulations: Impact on Man and Society. Cambridge University Press, Cambridge, England, 1984; 161 – 70.
6. Frankel OH, Brown AHD. Current plant genetic resources – a critical appraisal. In: Chopra VL, Joshi BC, Sharma RP, Bansal HC (Eds). Genetics: New Frontiers, Vol IV. Oxford and IBH Publishing Company, New Delhi, India, 1984; 1 – 63
7. Brown AHD. Core collections: a practical approach to genetic re-

sources management. Genome 1989; 31: 818 – 24.

8. Spagnoletti Zeuli PL, Qualset CO. Geographical diversity for quantitative spike characters in a world collection of durum wheat. Crop Science 1987; 27: 235 – 41.

9. Brown AHD. The case for core collections: a practical approach to genetic resources management. In: Brown AHD, Frankel OH, Marshall DR, Williams JT (Eds). The Use of Plant Genetic Resources. Cambridge University Press, Cambridge, England, 1989; 136 – 55.

10. Erskine W, Muehlbauer FJ. Allozyme and morphological variability, outcrossing rate and core collection formation in lentil germplasm. Theoretical and Applied Genetics 1991; 83: 119 – 25.

11. Cordeiro CMT, Morales EAV, Ferreira P, Rocha DMS, Costa IRS, Valois ACC, Silva S. Towards a Brazilian core collection of cassava. In: Hodkin T, Brown AHD, van Hintum ThJL, Morales BAV (Eds). Core Collection of Plant Genetic Resources, International Plant Genetic Resources Institute (IPGRI). John Wiley and Sons, New York, USA, 1995; 155 – 68.

12. Upadhyaya HD, Bramel PJ, Singh S. Development of a chickpea core subset using geographic distribution and quantitative traits. Crop Science 2001; 41: 206 – 10.

13. Upadhyaya HD, Bramel PJ, Ortiz R, Singh S. Developing a mini core of peanut for utilization of genetic resources. Crop Science 2002; 42: 2150 – 6.

14. Upadhyaya HD, Bramel PJ, Ortiz R, Singh S. Development of a groundnut core collection using taxonomical, geographical and morphological descriptors. Genetic Resources and Crop Evolution 2003; 50: 139 – 48.

15. Johnson RC, Stout DM, Bradley VL. The US Collection: a rich source for safflower germplasm. In: Dajue L, Yuanzhou H (Eds). Proceedings of the Third International Safflower Conference, Beijing Botanical Garden, Institute of Botany. Chinese Academy of Sciences,

1993; 202 – 8.

16. Milligan GW, Cooper MC. An examination of procedures for determining the number of clusters in a data set. Psychometrika 1985; 50: 159 – 79.

17. Ward J. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 1963; 38: 236 – 44.

18. Dwivedi SL, Upadhyaya HD, Hegde DM. Development of core collection using geographic information and morphological descriptors in safflower (*Carthamus tinctorius* L.) germplasm. Genetic Resources and Crop Evolution 2005; 52: 821 – 30.

19. Newman D. The distribution of range in samples from a normal population expressed in terms of an independent estimate of standard deviation. Biometrika 1939; 31: 20 – 30.

20. Levene H. Robust tests for equality of variances. In: Olkin I (Ed). in Honour of Harold Hotelling. Stanford University Press, Stanford, 1960; 278 – 92.

21. Ortiz R, Ruiz-Tapia EN, Mujica-Sanchez A. Sampling strategy for a core collection of Peruvian quinoa germplasm. Theoretical and Applied Genetics 1998; 96: 475 – 83.

22. Li BY, Zhang QX. Reviewof the studieson core collection for horticultural plants. Journal of Fruit Science 2007; 24(2): 204 – 9 (in chinese).

23. Suresh KK, Balakrishnan R. Strategies for developing core collection of safflower (*Carthamus tinctorius* L.) germplasm – Part 1. Sampling from diversity groups of quantitative morphological descriptors. Indian J Plant Genet Resour 2001; 14: 22 – 31.

24. Liu CY, Wang SH, Wang LX, Sun L, Mei L, Xu N, Cheng XZ. Establishment of candidate core collection in Chinese Mungbean germplasm resources. Acta Agromomica Sinica 2008; 34(4): 700 – 5 (in chinese).