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Full Length Article

Phenotypic Variation and Diversity of Cauliflower (*Brassica oleracea* Var. *Botrytis*) Inbred Lines

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Abstract

Inbred lines are important germplasm in cauliflower breeding programs. Knowledge on genetic variation and diversity of cauliflower inbred lines may be important to decide on breeding strategies for parent's selection during hybridization. In this study, the phenotypic diversity of 165 cauliflower inbred lines was evaluated based on ten quantitative traits and twenty qualitative traits. The experiment was conducted in field (sandy clay loam having pH of 6.8) at the Experimental Farm of Wenzhou Academy of Agricultural Science, Wenzhou, Zhejiang Province of China. Plants were arranged as randomized complete block with two replicates. Data were recorded during July 2015 to January 2016. Extensive variability was observed for most quantitative traits and qualitative traits with coefficients of variation (CV=13.7-42.6%) and Shannon–Weaver diversity index (H'=0.50-1.57) among the assayed genotypes, respectively. In which, the curd weight and stem color in curd showed highest degree of genetic variation and diversity. Phenotypic traits were categorized by principal components analysis (PCA) into 10 components which explained 71.64% of the total variation. Days to curd appearing, plant breadth, plant height, length of leaf, width of leaf, lengthways diameter of curd, curd weight, solidity of curd, stem color in curd were main optimized phenotypic traits for evaluation during breeding programs. These inbred lines were grouped into six different clusters. Based on cluster-mean, desirable recombination may be obtained by crossing inbred lines belong to different clusters. There were no association between morphological traits and geographical origin indicated that both genetic variation and geographical locations of the genotypes should be considered comprehensively in breeding programs for parent's selection. These findings of genotypic variation and diversity in phenotypic traits expressed usefulness of these inbred lines as parent materials for further utilization in cauliflower breeding programs. © 2018 Friends Science Publishers

Keywords: Morphological trait; Genetic diversity; Genetic variance; Principal component analysis; Cluster analysis; Cauliflower breeding

Introduction

Cauliflower *(Brassica oleracea* L. Var. *botrytis*, 2n=18, CC genome) is one of the major cultivated horticultural crops with an annual production of over 25.23 million tons in the world (Yousef *et al.,* 2015; FAO, 2016). It is popularly consumed for its healthy nutrition compounds and anti-cancerogenic properties (Izzah *et al.,* 2013; Yousef *et al.,* 2015). In China, due to the high nutritional and commercial value of cauliflower curd and its favorable effects on human health, the cauliflower is cultivated more than 520 thousand hectares with an annual production of more than 10.26 million tons (FAO, 2016).

Genetic diversity studies can provide potential germplasm resources by analyzing genetic information and relationships among individuals or populations for genetic improvement and utilization of germplasm resources (Izzah *et al.,* 2013; Roein *et al.,* 2014). Some previous studies have dealt with genetic diversity and relationships in cauliflower using molecular markers including RAPD, ISSR, SRAP, AFLP and SSR (Dos Santos *et al.,* 1994; Divaret *et al.,* 1999; Leroy *et al.,* 2000; Sun *et al.,* 2002; Astarini *et al.,* 2005; Astarini *et al.,* 2006; Louarn *et al.,* 2007; Wang *et al.,* 2011; Izzah *et al.,* 2013; Zhao *et al.,* 2014). Phenotypic variability and genetic diversity in cauliflower has also been evaluated using morphological traits in previous studies (Quamruzzaman *et al.,* 2007; Kumar *et al.,* 2011; Santhosha *et al.,* 2011; Lin *et al.,* 2012; Zhu *et al.,* 2012; Singh *et al.,* 2013; Chittora and Singh, 2015; Yousef *et al.,* 2015; Verma and Kalia, 2016). However most have investigated a limited range of genotypes. Santhosha *et al.* (2011) grouped 51 genotypes of cauliflower into 14 clusters according 16 quantitative characters. Singh *et al.* (2013) assessed genetic variability of 16 genotypes of cauliflower using 14 quantitative characters. Chittora and Singh (2015) investigated genetic variability for 8 quantitative characters and 5 qualitative characters in 40 genotypes of early cauliflower. Verma and Kalia (2016) analyzed genetic diversity and its relation to heterosis in early and mid-maturity Indian cauliflower.

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We have engaged in cauliflower breeding for nearly 20 years, and obtained many inbred lines by self-selection. However, these inbred lines are rarely effective utilized in breeding programs. Therefore, we aim to estimate the genetic diversity and relationships among 165 cauliflower inbred lines by using morphological traits, to provide useful information in cauliflower breeding programs.

Materials and Methods

Plant Material

One hundred and sixty-five (165) cauliflower inbred lines were used to assess the level of phenotypic variation and diversity in breeding programs. These inbred lines were obtained by self-selection of different cauliflower cultivars from different regions (Table 1). The originally collection region of these cauliflower cultivars was variable: Fujian (66), Zhejiang (44), Taiwan (25), Shanghai (5), Hongkong (4), Jiangxi and Japan (3, respectively), Henan and Hunan (2, respectively), Chongqing, Nederland and Italia (1, respectively), unknown region (8).

Experimental Design and Data Collection

The experiment was conducted at the Experimental Farm of Wenzhou Academy of Agricultural Science (28°05'N, 120°31'E), Wenzhou, Zhejiang, China during July 2015 to January 2016. Seeds were sown with nursery in a greenhouse on July 30, 2015. Ten plants per accession were then transplanted to the field (sandy clay loam having pH of 6.8) on September 3, 2015 with plot spacing 50 cm × 50 cm. Common cultivated management was adopted. The experiment was arranged as randomized complete block with two replicates.

10 quantitative traits and 20 qualitative traits were recorded according to the standard cauliflower descriptors described by Li *et al*. (2008). Quantitative traits (Table 3) were measured based on 10 replicates except curd traits based on 5 replicates as follows: days to 50% curd appearing (number of days from translating to 50% curd appearing), days to 80% curd maturity (number of days from translating to 80% curd maturity), plant height (length in cm from the soil surface to tip of spikes at maturity), plant breadth (width of the plant at maturity), number of rosette leaves (number of leaves at maturity), length of leaf (length in cm from base to tip of the maximum leaf at maturity), width of leaf (blade width of the maximum leaf at maturity), curd weight (average weight of five curd), lengthways diameter of curd (average height of five curd) and transverse diameter of curd (average width of five curd). Plant size (height and breadth) and leaf size (length and width) were measured by ruler. The curd size (height and width) were measured by caliper. Curd weight was measured by an electronic scale. All the qualitative traits were also assessed based on 10 plants at maturity. These qualitative traits such as plant growth habit, leaf shape, leaf color, margin of outer leaf, division of outer leaf, leaf apex shape, leaf wax, leaf luster, leaf surface, auricle of outer leaf, petiole shape in transverse section, petiole color, curd shape, curd color, leaflet in curd, alabastrum size, amount of curd hair, hair color on curd, solidity of curd, and stem color in curd were determined based on rating and coding according to the follow descriptor (Table 2) described by Li *et al.* (2008).

Data Analysis

Range, mean, standard deviation and coefficient of variation were calculated to assess the extent of genetic diversity among the inbred lines using Microsoft Excel 2007 software. Shannon–Weaver information index were computed by the phenotypic frequencies of qualitative traits to estimate the diversity of different inbred lines. The index (H') was calculated as presented by Negassa (Assefa and Labuschagne, 2004; Shakhatreh *et al.*, 2010).

$$H' = -\sum_{i} pi \ln pi$$

Where, *pi* is the frequency of the *i*th morphotype. Relationships among genotypes were investigated by principal component analysis using DPS 7.50 statistics software. To better understand the patterns of variation among inbred lines, Euclidean distance matrices generated from morphological data was used for cluster analysis (UPGMA) by MEGA 4.0 software.

Results

Genetic Diversity Analysis of Quantitative Characters

All quantitative traits showed large differences between the inbred lines according the analysis of variance (ANOVA), indicating a high level of phenotypic variation. Range, mean, standard deviation (SD) and coefficients of variation (CV) calculated from the quantitative traits of the inbred lines were presented in Table 3. Coefficient of variation (CV) was the lowest for number of rosette leaves (CV=13.7%), while it was the highest for curd weight (CV=42.6%). In this study, coefficients of variation (CV) for 6 quantitative traits were more than 20% including curd weight, days to 50% curd appearing, days to 80% curd maturity, plant height, lengthways diameter of curd, and transverse diameter of curd, indicating high variation for these quantitative traits in the studied inbred lines.

For days to 50% curd appearing and days to 80% curd maturity, the highest values were 117.0 and 143.5 d, while the least values were 31.0 and 47.0 d, respectively. Plant height and plant breadth ranged 22.2–75.2 cm and 42.9–108.1 cm, respectively. Length of leaf ranged between 25.6 and 70.6 cm, and width of leaf ranged between 12.5 and 34.3 cm. The average curd weight was 466.0 g and a significant difference (114.5–1200.0 g) was observed among these inbred lines.

Table 1: Code and origin of 165 cauliflower inbred lines

Genotype	Origin	Genotype	Origin	Genotype	Origin	Genotype	Origin	Genotype	Origin
XS 45	Taiwan, China	FZTX 120	Fujian, China	AYXF 60	Zhejiang, China	QX 90	Taiwan, China	XN 60	Fujian, China
YDJG 50	Zhejiang, China	YN 120	Fujian, China	YX 60	Fujian, China	Baiyu	Taiwan, China	CS 65F1	Taiwan, China
YDJG 55	Zhejiang, China	YDXG 50	Fujian, China	Shanghai 80	Shanghai, China	QN 90	Taiwan, China	QS 100	Zhejiang, China
SLTD 60	Hongkong, China	FZXQ 80	Fujian, China	RXTD 100	Zhejiang, China	MT 120	Fujian, China	TWQG	Taiwan, China
YDSL 78	Hongkong, China	SN 65	Fujian, China	RXTD 90	Zhejiang, China	SF 120	Hunan, China	XM 100	Fujian, China
YDJG 45	Zhejiang, China	SN 120	Fujian, China	14-524	unknown	FZXQ 120	Fujian, China	BY 100	Fujian, China
RXTZ 50	Zhejiang, China	TDXG 120	Jiangxi, China	BMWZ 80	Zhejiang, China	LN 120	Hongkong, China	YF 100	Fujian, China
RXTD 80	Zhejiang, China	Xuebao	Japan	TWQG 50	Taiwan, China	XS 90	Zhejiang, China	TS 100	Fujian, China
YY 70	Zhejiang, China	Xueyang	Henan, China	JM 50	Fujian, China	CS 80F1	Taiwan, China	FS 120	Fujian, China
CJBY 55	Hongkong, China	XLXQ 80	Shanghai, China	JM 60	Fujian, China	XM 85	Zhejiang, China	BY 120	Fujian, China
CJXW 68	Zhejiang, China	TSXL 100	Chongqing, China	CHW 60	Taiwan, China	TG 90	Zhejiang, China	CM 120	Fujian, China
12-23	unknown	CG No.2	Zhejiang, China	GM 65	Fujian, China	SHW 65	Zhejiang, China	R5	Zhejiang, China
YDZN 50	Fujian, China	YG 50F1	Shanghai, China	NB 65	Fujian, China	YDXM 65	Fujian, China	R111	Zhejiang, China
TB 80	Fujian, China	Xueliya	Henan, China	FZJY 65	Fujian, China	BS 80	Fujian, China	R112	Zhejiang, China
WX 80	Fujian, China	YGF1	Shanghai, China	MT 65	Fujian, China	BY 80	Fujian, China	R114	Zhejiang, China
JSTD 80	Fujian, China	CX 160	Zhejiang, China	XMSH 65	Fujian, China	XH 65	Taiwan, China	R131	Zhejiang, China
JZ 80	Fujian, China	QGSH 65	Fujian, China	Xinmei 65	Fujian, China	XSJ 65	Fujian, China	R132	Zhejiang, China
TDXG 100	Jiangxi, China	QGSH 90	Fujian, China	Xiumei 65	Taiwan, China	XLH 65	Taiwan, China	RA 70	Zhejiang, China
XG 108	Fujian, China	Xuebei	Japan	TW 65	Fujian, China	JM 70	Fujian, China	R4	Zhejiang, China
GF No.2	unknown	YG60F1	Shanghai, China	TH 65	Taiwan, China	ZQ 70	Fujian, China	R521	Zhejiang, China
YG 40A	Fujian, China	Xuelian	Nederland	TWQG 65	Taiwan, China	FZJY 80	Fujian, China	R522	Zhejiang, China
ZQ 50	Fujian, China	CJXW 100	Zhejiang, China	QX 65	Taiwan, China	GS 80	Fujian, China	R8	Zhejiang, China
JSTD 60	Fujian, China	YDJG 120	Zhejiang, China	SX 65	Taiwan, China	XMSH 80	Fujian, China	R9	Zhejiang, China
YDZN 70	Fujian, China	XL 85	Zhejiang, China	TM 65	Taiwan, China	TDBX 120	Hunan, China	Un1	unknown
YDXL 75	Fujian, China	ZS 45	Zhejiang, China	XGS 65	Taiwan, China	CJRH 100	Fujian, China	R133	Zhejiang, China
LM 80	Fujian, China	AYXF 70	Zhejiang, China	GH 70	Fujian, China	XMSH 120	Fujian, China	ZCY	Taiwan, China
ZH 80	Fujian, China	14-510	unknown	YDXM 80	Fujian, China	TWQG 60	Taiwan, China	9871	unknown
TDXG 80	Jiangxi, China	RGJP 80	Zhejiang, China	TWQG 80	Taiwan, China	NM 70	Taiwan, China	9872	unknown
AJ 90	Fujian, China	RGJP 100	Zhejiang, China	SM 80	Fujian, China	TWQG 90	Taiwan, China	ZIH	Italia
WX 90	Fujian, China	AYXF 90	Zhejiang, China	YB 80	Zhejiang, China	Taibao 80	Fujian, China	Un2	unknown
YN 100	Fujian, China	YF 139	Zhejiang, China	SH 80	Fujian, China	TM 75	Taiwan, China	XLYH	Japan
WX 100	Fujian, China	RG 42	Taiwan, China	YM 80	Fujian, China	HC 50	Zhejiang, China	BMWZ 60	Zhejiang, China
FZRX 100	Fujian, China	TZS 60	Zhejiang, China	XN 80	Fujian, China	SH 60	Fujian, China	SH 120	Fujian, China

Table 2: Codes and states of the qualitative variables for cauliflower inbred lines

Qualitative variable	Code and state					
	0	1	2	3	4	5
Plant growth habit		Erect	Semi-erect	Spreading		
Leaf shape		Elliptic	Long elliptic	Lanceolate	Broad lanceolate	
Leaf color		Light green	Green	Dark green	Greyish green	Grayish green
Margin of outer leaf		Entire	Undulate	Serrate		
Division of outer leaf	Entire	Sinuate	Lyrate			
Leaf apex shape		Acute	Blunt pointed	Round	Broad round	
Leaf wax	Absent	Little	Intermediate	Much		
Leaf luster	Absent	Present				
Leaf surface		Wrinkling	Smooth			
Auricle of outer leaf	Absent	Present				
Petiole shape in transverse section		Round	Semi-round	Flat		
Petiole color		Whitish green	Light green	green	Greyish green	
Curd shape		Flat spherical	Spherical	High spherical		
Curd color		Milky white	Yellowish white			
Leaflet in curd	Absent	Present				
Alabastrum size		Little	Intermediate	Large		
Amount of curd hair	Absent	Little	Medium	Much		
Hair color on curd	Absent	White	Light yellow	Light green	Green	Purple
Solidity of curd		Loose	Intermediate	Tight		
Stem color in curd		White	Yellowish white	Greenish white	Light green	Green

Lengthways diameter of curd and transverse diameter of curd showed significant differences with range of 5.6–20.5 cm and 6.5–22.4 cm, respectively. These quantitative data also showed that the studied inbred lines are diverse.

Genetic Diversity Analysis of Qualitative Characters

The shannon–Weaver index (H') of diversity estimated based on 20 qualitative traits of all inbred lines are shown in Table 4. The three highest values of H' were of

Table 3: Descriptive statistics for the quantitative traits among 165 cauliflower inbred lines

Quantitative variable	Unit	Min	MAX	Range	Mean	SD	CV/%
Days to 50% curd appearing	d	31.0	117.0	86.0	67.9	17.6	26.0
Days to 80% curd maturity	d	47.0	143.5	96.5	87.1	20.8	23.9
Plant height	cm	22.2	75.2	53.0	50.5	10.2	20.3
Plant breadth	cm	42.9	108.1	65.2	77.8	13.9	17.9
Number of rosette leaves	piece	15.2	30.8	15.6	22.1	3.0	13.7
Length of leaf	cm	25.6	70.6	45.0	48.0	8.7	18.1
Width of leaf	cm	12.5	34.3	21.8	19.9	3.6	18.2
Curd weight	g	114.5	1200.0	1085.5	466.0	198.7	42.6
Lengthways diameter of curd	cm	5.6	20.5	14.9	10.7	2.9	27.3
Transverse diameter of curd	cm	6.5	22.4	15.9	14.5	3.1	21.7

Table 4: Phenotypic diversity index (H') for 20 qualitative characters among 165 cauliflower inbred lines

Qualitative variable	Frequency of various grades of different traits (%)						Shannon–Weaver index (H')
	0	1	2	3	4	5	
Plant growth habit		18.79	52.12	29.09			1.01
Leaf shape		29.09	39.39	7.88	23.64		1.27
Leaf color		7.27	25.45	21.82	19.39	26.06	1.54
Margin of outer leaf		21.21	72.12	6.67			0.75
Division of outer leaf	84.85	12.12	3.03				0.50
Leaf apex shape		24.85	14.55	58.18	2.42		1.03
Leaf wax		18.79	40.00	41.21			1.05
Leaf luster	56.36	43.64					0.69
Leaf surface		64.85	35.15				0.65
Auricle of outer leaf	26.67	73.33					0.58
Petiole shape in transverse section		6.06	47.88	46.06			0.88
Petiole color		15.76	30.91	25.45	27.88		1.36
Curd shape		44.24	43.64	12.12			0.98
Curd color		50.30	49.70				0.69
Leaflet in curd	94.55	5.45					0.21
Alabastrum size		24.24	41.21	34.55			1.08
Amount of curd hair	58.79	18.79	13.94	8.48			1.12
Hair color on curd	58.79	12.12	10.30	3.64	3.03	12.12	1.29
Solidity of curd		32.12	26.67	41.21			1.08
Stem color in curd		23.64	10.30	26.06	21.82	18.18	1.57

stem color in curd (1.57), leaf color (1.54) and petiole color (1.36). While the three lowest values of H' were found in leaflet in curd (0.21), division of outer leaf (0.50) and auricle of outer leaf (0.58). The H' values of the rest qualitative traits such as leaf surface, curd color, leaf luster, *etc*. ranged from 0.65 to 1.29. In this study, the H' for 11 qualitative traits such as stem color in curd, leaf color, petiole color, hair color on curd, leaf shape, amount of curd hair, solidity of curd, alabastrum size, leaf wax, leaf apex shape and plant growth habit were more than 1.0, suggesting relatively wide variation for these qualitative traits among the studied inbred lines.

Principal Component Analysis

Principal component analysis (PCA) was used to characterize accessions according to the 30 phenotypic traits. The first 10 PCs eigen values >1 explained 71.64% variation (Table 5), indicating that these attributes have high variation among the inbred lines. Days to 50% curd appearing and plant breadth were influential in the first PC1 (13.77%). In PC2 (11.80%) four traits had a stronger influence: plant height, length of leaf, width of leaf, and lengthways diameter of curd. PC3 with 10.21% contribution was associated with curd weight, solidity of curd and stem color in curd. The remaining components (PC4-PC10) explained less variability (35.85% of the total variance) and included other variables such as leaf shape, leaf apex shape, transverse diameter of curd, leaf color, leaf wax, margin of outer leaf, leaf surface, auricle of outer leaf, plant growth habit, leaflet in curd, number of rosette leaves, petiole shape in transverse section, curd color, and alabastrum size.

Cluster Analysis

Cluster analysis grouped the 165 inbred lines into 6 clusters based on Euclidian distance matrices among the phenotypic differences. Differences in cluster-means (Table 6) existed for almost all traits. Highest mean value for days to 50% curd appearing (106.6 d), days to 80% curd maturity (137.3 d), number of rosette leaves (25.2), width of leaf (22.3 cm), curd weight (591.5 g), leaf color (5.0), leaf wax (3.0) and curd shape (2.3) was observed in cluster 4. Cluster 3 recorded maximum plant height (58.0 cm), plant breadth (85.5 cm) and length of leaf (54.2 cm) while cluster 6 recorded maximum lengthways diameter of curd (17.4 cm),

Table 5: Principal component analysis (PCA) of 30 morphological characters showing their contributions to the total variation among 165 cauliflower inbred lines

Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Days to 50% curd appearing	0.305	-0.199	0.188	-0.144	0.183	0.021	0.037	-0.061	-0.086	-0.208
Days to 80% curd maturity	0.288	-0.211	0.233	-0.100	0.238	0.032	0.050	-0.049	-0.063	-0.143
Plant height	0.302	0.337	0.078	-0.052	-0.011	-0.152	0.021	-0.012	-0.023	0.057
Plant breadth	0.338	0.226	0.187	-0.025	-0.052	-0.122	0.067	0.000	-0.060	-0.046
Number of rosette leaves	0.136	0.053	0.026	-0.081	0.164	-0.087	0.065	0.172	-0.593	0.119
Length of leaf	0.303	0.329	0.125	-0.047	0.003	-0.206	0.002	-0.019	-0.003	0.055
Width of leaf	0.066	0.320	0.230	-0.191	0.002	-0.193	0.020	0.036	0.162	0.138
Curd weight	-0.116	0.175	0.413	0.165	-0.094	0.169	0.054	0.037	0.121	-0.013
Lengthways diameter of curd	-0.081	0.288	0.215	0.234	-0.099	0.269	-0.140	0.027	-0.066	0.035
Transverse diameter of curd	-0.050	0.262	0.166	0.162	-0.324	0.234	0.124	0.138	0.121	-0.230
Plant growth habit	0.144	-0.098	-0.001	0.058	-0.254	0.127	0.202	-0.347	-0.078	-0.177
Leaf shape	0.200	-0.056	-0.028	0.423	-0.086	-0.106	-0.109	-0.289	0.006	0.157
Leaf color	-0.039	0.171	-0.162	0.194	0.438	0.103	0.266	0.051	0.168	0.024
Margin of outer leaf	0.175	-0.026	0.002	-0.077	-0.071	0.511	-0.043	-0.113	-0.135	0.047
Division of outer leaf	0.073	0.184	-0.248	-0.030	0.073	0.188	-0.465	-0.085	0.059	-0.175
Leaf apex shape	-0.186	0.079	0.007	-0.438	0.089	0.182	0.180	0.221	-0.020	-0.017
Leaf wax	0.062	0.104	-0.100	0.271	0.413	0.133	0.276	0.069	0.103	0.109
Leaf luster	-0.115	-0.003	0.211	-0.192	0.042	-0.006	0.005	0.239	0.236	0.091
Leaf surface	-0.156	0.125	-0.155	0.156	-0.120	-0.414	0.006	-0.007	-0.200	-0.086
Auricle of outer leaf	0.011	-0.141	0.094	0.136	-0.146	0.024	0.577	-0.130	-0.032	0.023
Petiole shape in transverse section	-0.013	-0.069	-0.007	0.295	-0.049	0.067	-0.037	0.315	-0.193	0.500
Petiole color	0.091	0.169	-0.185	0.140	0.313	-0.025	0.058	-0.116	0.220	-0.175
Curd shape	-0.127	0.029	0.294	0.096	0.268	0.103	-0.270	-0.145	-0.259	0.163
Curd color	0.077	-0.056	-0.119	-0.172	-0.197	-0.100	0.160	-0.064	0.176	0.425
Leaflet in curd	0.180	-0.165	0.020	0.146	-0.003	0.112	0.067	0.383	-0.142	-0.142
Alabastrum size	0.097	0.012	0.000	-0.213	0.050	0.253	-0.006	-0.366	0.102	0.439
Amount of curd hair	0.267	-0.246	0.097	0.111	-0.075	0.010	-0.148	0.278	0.209	-0.009
Hair color on curd	0.274	-0.218	0.024	0.086	-0.015	-0.063	-0.182	0.206	0.375	0.110
Solidity of curd	-0.238	-0.167	0.327	0.056	0.160	-0.162	-0.035	-0.158	0.081	0.054
Stem color in curd	0.183	0.162	-0.358	-0.081	-0.151	0.167	0.050	0.160	-0.071	0.087
Eigen value	4.132	3.54	3.064	2.465	1.933	1.598	1.416	1.193	1.126	1.024
% of variance	13.77	11.80	10.21	8.22	6.44	5.33	4.72	3.98	3.75	3.41
Cumulative % of variance	13.77	25.58	35.79	44.01	50.45	55.77	60.49	64.47	68.22	71.64

transverse diameter of curd (16.7 cm), leaf shape (3.7), petiole shape in transverse section (3.0), petiole color (4.0) and stem color in curd (4.3). Cluster 1 showed highest mean value for leaf apex shape (2.9), alabastrum size (2.4) and solidity of curd (2.7). While cluster 5 recorded highest mean values for plant growth habit (2.5), amount of curd hair (2.1) and hair color on curd (3.4).

Cluster 5 ranked lowest in curd weight (393.8 g), lengthways diameter of curd (9.8 cm), width of leaf (17.8 cm), leaf apex shape (1.6), leaf luster (0.2) and curd shape (1.4). Cluster 6 ranked lowest for days to 50% curd appearing (48.2 d), days to 80% curd maturity (64.3 d), plant growth habit (1.7), curd color (1.0) and solidity of curd (1.0). Cluster 2 showed lowest for plant height (39.7 cm), plant breadth (62.6 cm), number of rosette leaves (20.7), length of leaf (38.7 cm), margin of outer leaf (1.5) and alabastrum size (1.5). Cluster 1 showed lowest in leaf shape (1.7), leaf wax (1.9), petiole color (2.0) and stem color in curd (1.9) while cluster 4 had the lowest transverse diameter of curd (12.6 cm).

We also found that the clustering patterns of majority of inbred lines did not clearly agree with their geographic locations from which the sources derived (Fig. 1). Cluster 1 included 38 lines originating from Zhejiang, Fujian, Taiwan, Shanghai, Hong Kong, Nederland and Japan. Cluster 2 included the 33 lines deriving from Zhejiang, Fujian, Shanghai, Taiwan and Hongkong. Cluster 3 consisted of 55 lines originating from Zhejiang, Fujian, Jiangxi and Taiwan. Cluster 4 consisted of 6 lines originating from Zhejiang, Fujian, Chongqing, Taiwan and Italia. Cluster 5 comprised of 30 lines mainly deriving from Zhejiang, Fujian and Taiwan. Cluster 6 consisted only of Xinmei 65, XMSH 65 and BY 80 deriving from Fujian.

Discussion

Knowledge on the genotypic variation and diversity of germplasm resources is important to decide on breeding strategies (Faruq *et al.,* 2015; Khadivi-Khub and Etemadi-Khah, 2015; Naheed *et al.,* 2016; Alghamdi *et al.,* 2017). The coefficient of variation (CV) and Shannon-Weaver diversity index (H') were used as measurements of phenotypic diversity to identify species with the maximum diversity (Shakhatreh *et al.,* 2010; Khadivi-Khub *et al.,* 2015). In this study, the mean value of CV on quantitative traits and H' of qualitative traits were 22.97% and 0.97, respectively.

Table 6: Cluster means for 30 characteristics in the studied cauliflower inbred lines

Character	Unit	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Days to 50% curd appearing	d	73.8	50.6	63.4	106.6	81.9	48.2
Days to 80% curd maturity	d	95.9	67.5	79.6	137.3	103.4	64.3
Plant height	cm	47.9	39.7	58.0	54.7	50.2	57.7
Plant breadth	cm	77.0	62.6	85.5	83.2	81.2	68.2
Number of rosette leaves	piece	21.8	20.7	22.5	25.2	22.5	21.9
Length of leaf	cm	46.4	38.7	54.2	52.4	47.3	53.3
Width of leaf	cm	20.8	18.0	21.3	22.3	17.8	19.5
Curd weight	g	548.1	468.9	430.7	591.5	393.8	514.6
Lengthways diameter of curd	cm	10.7	10.4	11.1	10.5	9.8	17.4
Transverse diameter of curd	cm	14.0	14.6	15.0	12.6	14.2	16.7
Plant growth habit	code	2.0	1.8	2.2	1.8	2.5	1.7
Leaf shape	code	1.7	2.1	2.3	2.2	3.0	3.7
Leaf color	code	2.7	3.5	3.7	5.0	2.7	4.7
Margin of outer leaf	code	1.9	1.5	1.9	1.7	2.2	2.0
Division of outer leaf	code	0.1	0.1	0.3	0.0	0.1	2.0
Leaf apex shape	code	2.9	2.3	2.5	2.5	1.6	1.7
Leaf wax	code	1.9	2.1	2.4	3.0	2.2	3.0
Leaf luster	code	0.8	0.5	0.3	0.3	0.2	0.3
Leaf surface	code	1.1	1.7	1.5	1.2	1.1	1.3
Auricle of outer leaf	code	0.7	0.7	0.7	0.8	0.9	0.0
Petiole shape in transverse section	code	2.1	2.5	2.2	2.8	2.8	3.0
Petiole color	code	2.0	2.6	3.0	3.7	2.5	4.0
Curd shape	code	2.1	1.6	1.5	2.3	1.4	2.0
Curd color	code	1.4	1.5	1.5	1.5	1.6	1.0
Leaflet in curd	code	0.0	0.0	0.0	0.0	0.3	0.0
Alabastrum size	code	2.4	1.5	2.3	1.8	2.0	2.3
Amount of curd hair	code	0.9	0.0	0.3	0.5	2.1	0.7
Hair color on curd	code	1.2	0.0	0.6	1.0	3.4	2.0
Solidity of curd	code	2.7	2.6	1.6	2.5	1.7	1.0
Stem color in curd	code	1.9	2.4	3.9	2.0	3.5	4.3

It suggested that phenotypic traits revealed considerable phenotypic variances among the 165 cauliflower inbred lines. Higher CV and H' means that the genotypes *per se* have a wide variability in each trait (Upadhyaya *et al.,* 2011). The highest curd weight variation (CV=42.6%) and stem color in curd diversity index (H'=1.57) were seen in this study indicating high degree of genetic diversity among all the parameters studied. This suggested that the curd weight and stem color in curd are important phenotypically breeding objectives. Similar result has been reported by Zhu *et al.* (2012) who found that curd weight showing the highest variability (CV=35.37%) among 54 cauliflower inbred lines.

The morphological traits of cauliflower are complex and diverse, which brings difficulties to select phenotypic traits in cauliflower breeding programs. Principal component analysis (PCA) can simplify several phenotypic traits into several principal components with fewer phenotypic traits, to improve the efficiency of parent's selection in breeding (Upadhyaya, 2003; Zhu et *al.*, 2012; Khadivi-Khub and Etemadi-Khah, 2015). In this study, principal component analysis revealed that, days to curd appearing, plant breadth, plant height, length of leaf, width of leaf, lengthways diameter of curd, curd weight, solidity of curd, stem color in curd were prevalent in the first 3 PCs and contributed 35.79% variation. It indicated that these attributes have the highest variation among the studied inbred lines, and days to curd appearing, plant breadth, plant height, length of leaf, width of leaf, lengthways diameter of curd, curd weight, solidity of curd, stem color in curd were main optimized phenotypic traits for germplasm evaluation in cauliflower breeding programs. Similar observations have been made in our previous work analyzing among 54 inbred lines (Zhu *et al.,* 2012).

Cluster analysis is helpful for understanding the trend of evolution and choosing genetically diverse parents for obtaining desirable recombination (Govindaraj *et al*., 2014; Malik *et al.,* 2014; Naheed *et al.,* 2016; Bakhsh *et al.,* 2017). In this study, the 165 inbred lines were grouped into 6 clusters. Different clusters showed various in curd maturity, curd weight, leaf color, leaf wax, plant breadth and solidity of curd, etc. (Fig. 1 and Table 6). Cluster 1 showed mid-late maturity, heavier curd weight, and tighter curd. Cluster 2 showed earlier curd maturing, having lowest plant height, plant breadth, number of rosette leaves with compact curd. Cluster 3 had characters of middle maturity, highest plant height, plant breadth with semi-loose curd. Cluster 4 exhibited latest maturity, highest curd weight, leaf color and leaf wax while cluster 5 was the type of late curd maturity, having the lowest curd weight, width of leaf and lengthways diameter of curd. Finally, cluster 6 showed earliest maturity, highest lengthways diameter of curd, transverse diameter of curd, and loose curd with light green stem. This suggested that improvement genetic of cauliflower will be easier to succeed by parent materials selection from different clusters.

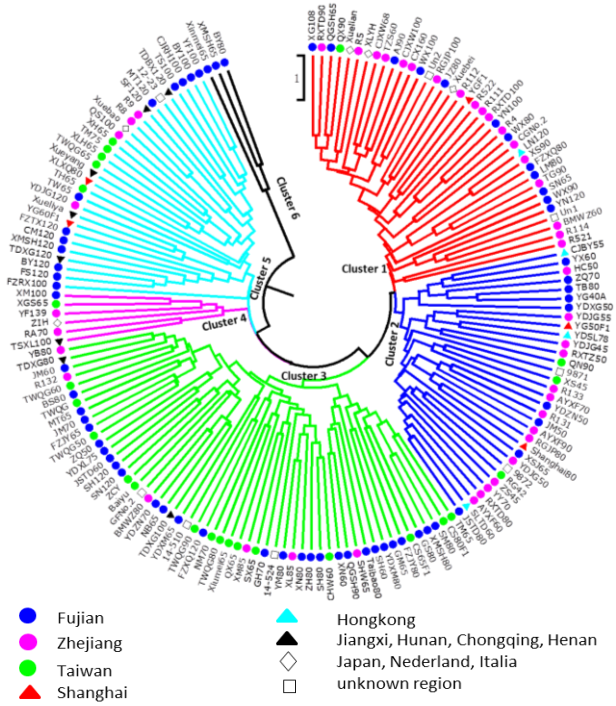

Fig. 1: Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) tree based on Euclidean distance matrix constructed from 30 phenotypic traits data of 165 cauliflowers lines

For example, based on cluster-mean, cross between genotypes of cluster 4 with genotypes of cluster 6 might result in highly transgressive segregants for curd-weight and short maturity contributing traits. The selection of parents for hybridization should be done from different clusters having higher inter-cluster distance, to aim for higher variability (Santhosha *et al.*, 2011; Zhu *et al.*, 2012)

We also found that the cluster results of the accessions based on phenotypic similarity did not reflect their geographic region of origin (Fig. 1). The reason of which might be high frequency of interchange of cultivars or artificial oriented selection making genetic difference more than geographical distance in cauliflower. The obtained results correspond with previous work in other species (Elameen *et al.*, 2011; Yıldız *et al.*, 2016). Cauliflower is known to have narrow genetic diversity as reported in previous studies (Sun *et al.*, 2002; Tonguç and Griffiths, 2004; Louarn *et al.*, 2007; Zhao *et al.*, 2014), so that we should comprehensively consider both geographical location and genetic variation of the genotypes in future breeding for parent's selection.

Conclusion

The 165 cauliflower inbred lines exhibited a wide degree of variability for most phenotypic traits, and were grouped into 6 clusters based on the phenotypic data. Different clusters displayed various in curd maturity, curd weight, leaf color, leaf wax, plant breadth and solidity of curd, *etc*. However, the clustering patterns of majority of inbred lines presented disagree with their geographic locations from which the sources derived. It indicated that both genetic variation and geographical location of the genotypes should be considered comprehensively for parent's selection in future cauliflower breeding programs.

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