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Phenotypic Diversity of Non-heading Chinese Cabbage Germplasm Resources in Zhejiang Province

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Abstract In this study, 48 non-heading Chinese cabbage germplasms collected in the Third National Survey and Collection Action on Crop Germplasm Resources in Zhejiang Province were used as materials for phenotypic identification and genetic diversity analysis. The results showed that the variation coefficient of 10 quantitative characters ranged from 23.33% to 50.29%, and the genetic diversity index ranged from 1.26 to 2.01. And the variation coefficients of 9 quality traits ranged from 14.57% to 65.77%, and the genetic diversity index ranged from 0.29 to 1.61. There was a high correlation between different characters, and a significant positive correlation between plant height, plant width, leaf length and petiole length, besides, the number of lotus leaves was negatively correlated with other characters. The cumulative contribution rate of first 5 principal components is 76.587%, which contains most of the information. The tested materials were classified into 5 groups by cluster analysis. The first group is Wuta-tsai, the second group is mainly erect-type leafy cabbage, the third group is Pak-choi, the fourth group is mainly milk Chinese cabbage and the fifth group is a hybrid group. This study provides theoretical basis for efficient utilization and breeding of non-heading Chinese cabbage germplasm resources in Zhejiang Province.

Keywords Non-heading Chinese cabbage; Germplasm; Phenotypic diversity; Correlation analysis; Principal component analysis

Non-heading Chinese cabbage (*Brassica campestris* ssp., *Chinensis* Makino) is one of the *Brassica campestris* varieties of *Brassica* genus in the family of Brassicaceae. It is native to southern China and is also one of the main leaf vegetables in China (Zhang, 2011, China Agricultural Science and Technology Press, pp.979). There are six varieties of *Chinensis* Makino, var. *communis* Tsen et Lee, var. *rosularis* Tsen et Lee, var. *tsai-tai* Hort, var. *Tai-tsai* Hort, var. *Muticeps* Hort, and var. *Utilis* Tsen et Lee (Feng et al., 2019). Common cabbage, also known as “Qingcai”, “Qinggengcai”, “Xiaoyoucai”, “Xiaobaicai” in Chinese, is the most widely distributed, especially in the middle and lower reaches of the Yangtze River, accounting for 30% to 40% of the total area of vegetable multiple planting (Hou and Song, 2012).

Zhejiang is a coastal province in southeast China, with complex and diverse terrain, including plain, basin, hill, mountain and island, mainly are hill and mountain, with obvious stereoscopic weather, so it has strong biodiversity (Wang, 1988). Local varieties have high adaptability to local climate conditions and are rich in types, which are the original materials for breed selection. Since the launch of the Third National Survey and Collection Action on Crop Germplasm Resources in Zhejiang Province, a total of 48 local non-heading Chinese cabbage varieties that have been continuously planted in different areas of Zhejiang have been collected. In order to systematically evaluate the diversity and understand the types and characteristics of local non-heading Chinese cabbage varieties in Zhejiang province, we conducted phenotypic evaluation and diversity analysis on 19 agronomic traits of 48 local cabbage varieties in 2019. This study will provide reference for the exploration of excellent agronomic traits and efficient application in breeding of non-heading Chinese cabbage germplasm resources in Zhejiang Province.

1 Results and Analysis

1.1 Distribution of germplasm resources

From June 2017 to June 2019, germplasm resources investigation group of Vegetable Institute, Zhejiang Academy of Agricultural Sciences collected 48 non-heading Chinese cabbage germplasm resources through systematic

investigation in Zhejiang Province (Table 1), which were distributed in 10 prefecture-level cities and 23 counties and administrative regions (cities, districts, counties) in Zhejiang Province. Among them, 9 in Jiaxing, 8 in Quzhou, 7 in Hangzhou, 5 in Huzhou, 5 in Lishui, 4 in Zhoushan, 4 in Shaoxing, 3 in Ningbo, 2 in Jinhua, and 1 in Taizhou. In terms of counties, Tongxiang County, Jiaxing city have the most, a total of 8.

Table 1 Forty-eight collected non-heading Chinese cabbage germplasm resources

Ordinal	Code	Varieties name	Collection data	Origin
1	2017332064	Non-heading Chinese cabbage	20170831	Hangzhou Jiande
2	2017333083	Savoying pak-choi	20170919	Ningbo Ninghai
3	2017334089	Zhongjiaobai	20170929	Ningbo Fenghua
4	2018331414	Non-heading Chinese cabbage	20180831	Jiaxing Tongxiang
5	2018331433	Changgeng Non-heading Chinese cabbage	20180831	Jiaxing Tongxiang
6	2018331435	Changgeng Non-heading Chinese cabbage	20180831	Jiaxing Tongxiang
7	2018332060	Laobaicai	20180615	Lishui Jingning
8	2018332083	Zisong	20180615	Lishui Jingning
9	2018332235	Non-heading Chinese cabbage	20180829	Lishui Qingyuan
10	2018332414	Yuangeng Non-heading Chinese cabbage	20180829	Quzhou Kaihua
11	2018332425	Non-heading Chinese cabbage	20180829	Quzhou Kaihua
12	2018332459	Black Non-heading Chinese cabbage	20180829	Quzhou Kaihua
13	2018333252	Non-heading Chinese cabbage	20180816	Quzhou Qujiang
14	2018334168	Youdonger	20181121	Shaoxing Zhuji
15	2018335022	Siyueqing	20180820	Zhoushan Dinghai
16	p330127053	Lao Savoying pak-choi	20180806	Hangzhou Chunan
17	p330127054	Lao Non-heading Chinese cabbage	20180806	Hangzhou Chunan
18	p330127069	Dongdongqing	20170922	Hangzhou Chunan
19	p330226003	Zhong Non-heading Chinese cabbage	20171113	Ningbo Ninghai
20	p330683026	Youdonger	20180612	Shaoxing Shengzhou
21	p330683035	Gaojiaobai	20180620	Shaoxing Shengzhou
22	p330781019	Lanxi Gaojiaobai	20180702	Jinhua Lanxi
23	p330783003	Non-heading Chinese cabbage	20170606	Jinhua Dongyang
24	2018331442	Changgeng Non-heading Chinese cabbage	20180831	Jiaxing Tongxiang
25	p330111055	Changgeng Non-heading Chinese cabbage	20180530	Hangzhou Fuyang
26	p330127091	Sanyueqing	20180806	Hangzhou Chunan
27	p330424016	Black Non-heading Chinese cabbage	20180620	Jiaxing Haiyan
28	p330822007	Gaogan Non-heading Chinese cabbage	20180115	Qushan Changshan
29	p330822020	Changshan Savoying pak-choi	20180619	Qushan Changshan
30	p330483024	Changgeng Non-heading Chinese cabbage	20180801	Jiaxing Tongxiang
31	2018331282	Zhouwencai	20180930	Huzhou Changxing
32	2018331283	Longxucai	20180930	Huzhou Changxing
33	2018331272	Siyueqing	20180930	Huzhou Changxing
34	2018331273	Aijiaobai	20180930	Huzhou Changxing
35	2018331445	Changgeng Non-heading Chinese cabbage	20181026	Jiaxing Tongxiang
36	2018331464	Aijiao Non-heading Chinese cabbage	20181026	Jiaxing Tongxiang
37	2018335006	Heiyoutong	20180925	Zhoushan
38	p331125014	Shenzhuang Non-heading Chinese cabbage	20181130	Lishui Yunhe
39	p330824025	Savoying pak-choi	20181122	Quzhou Kaihua
40	2018334183	Pak-choi rape	20181113	Shaoxing Zhuji
41	2018332415	Can Non-heading Chinese cabbage	20180829	Quzhou Kaihua
42	2018334475	Yangshetou Non-heading Chinese cabbage	20181130	Hangzhou Linan
43	2018332079	Xiaobaye	20180615	Lishui Jingning
44	2019335015	Eryueqing	20190106	Zhoushan Shengsi
45	2019335013	Siyueman	20190106	Zhoushan Shengsi
46	2019333672	Non-heading Chinese cabbage	20190612	Taizhou Huangyan
47	p330502014	Xiuhua jin	20190614	Huzhou Wuxing
48	2018331449	Chaozhoucai	20190618	Jiaxing Tongxiang

1.2 Genetic diversity analysis

The genetic diversity analysis of 19 traits of 48 non-heading Chinese cabbage germplasm resources showed that there were large differences among different materials, and the variation of each trait was abundant (Table 2). The variation coefficients of the 10 quantitative traits ranged from 23.33% to 50.29%, among which the rosette leaves number (RLN) and petiole length (PL) were the largest (more than 50%). The minimum is crown diameter (CD). From big to small sort of PL(50.29%)>RLN(50.15%)>SH(39.83%)>PH(39.79%)>PT(37.30%)>LL(32.32%)>PW(29.85%)>LW(26.98%)>SH(25.01%)>CD(23.33%). The genetic diversity index of 10 quantitative traits ranged from 1.26 to 2.01, in which the stem height (SH) was the highest and the RLN was the lowest.

Table 2 Variation and genetic diversity analysis of 10 quantitative traits of non-heading Chinese cabbage

Characters	Min.	Max.	Mean	SD	Variance	CV (%)	H
Plant height, PH	10.70	61.20	29.13	11.59	134.34	39.79	1.88
Crown diameter, CD	22.70	68.00	43.25	10.09	101.82	23.33	1.99
Rosette leaves number, RLN	6.00	54.00	13.29	6.67	44.42	50.15	1.26
Leaf length, LL	14.10	67.60	36.33	11.74	137.90	32.32	1.93
Leaf width, LW	5.80	33.70	15.67	4.23	17.88	26.98	1.79
Petiole length, PL	3.00	36.90	14.40	7.24	52.44	50.29	1.92
Petiole width, PW	1.50	5.50	3.35	1.00	1.00	29.85	1.98
Petiole thick, PT	0.40	1.40	0.74	0.28	0.08	37.30	1.82
Stem height, SH	1.00	5.90	3.19	1.27	1.61	39.83	2.01
Stem width, SW	1.30	4.80	2.39	0.60	0.36	25.01	1.83

The variation coefficients of 9 quality traits ranged from 14.57% to 65.77%, among which, leaf shape (LS) was the largest and leaf type (LT) was the smallest (Table 3). From big to small sort of LT(65.77%)>LM(61.49%)>LSS(57.17%)>PC(48.17%)>LTS(36.64%)>LC(25.99%)>PT(25.09%)>HS(22.91%)>LS(14.57%). The genetic diversity index of nine quality traits ranged from 0.29 to 1.61, with leaf shape being the highest and leaf type the lowest.

Table 3 Variation and genetic diversity analysis of 9 qualitative traits of non-heading Chinese cabbage.

Characters	Min.	Max.	Mean	SD	Variance	CV (%)	H
Plant type, PT	1	5	4.06	1.02	1.04	25.09	1.20
Hourglass sign, HS	1	2	1.79	0.41	0.17	22.91	0.51
Leaf type, LT	1	2	1.92	0.28	0.08	14.57	0.29
Leaf shape, LS	1	8	3.27	2.15	4.63	65.77	1.61
Leaf tip shape, LTS	1	4	2.92	1.07	1.14	36.64	1.31
Leaf margin, LM	1	4	1.42	0.87	0.76	61.49	0.78
Leaf surface smoothness, LSS	1	4	1.67	0.95	0.91	57.17	1.07
Leaf color, LC	2	7	3.67	0.95	0.91	25.99	1.27
Petiole color, PC	1	4	2.31	1.11	1.24	48.17	1.04

1.3 Correlation analysis of quantitative characters

Correlation analysis was conducted on 10 quantitative traits of non-heading Chinese cabbage resources, and the results showed that there were significant correlations among several traits (Table 4). Plant height, crown diameter, leaf length and petiole length were positively correlated with each other. Leaf width was significantly positively correlated with plant height, crown diameter and petiole thick, but significantly positively correlated with leaf length and petiole width. Rosette leaves number was negatively correlated with other traits, and was significantly different from leaf width, plant height, crown diameter and leaf length. Petiole width was significantly positively correlated with petiole thick and stem height, and positively correlated with leaf width and stem width.

Table 4 The correlation coefficient between the quantitative traits of non-heading Chinese cabbage.

Traits	PH	CD	RLN	LL	LW	PL	PW	PT	SH	SW
PH	1.00									
CD	0.83**	1.00								
RLN	-0.31*	-0.33*	1.00							
LL	0.91**	0.88**	-0.33*	1.00						
LW	0.37**	0.48**	-0.52**	0.30*	1.00					
PL	0.72**	0.59**	-0.08	0.77**	0.04	1.00				
PW	0.08	0.11	-0.14	-0.08	0.29*	0.08	1.00			
PT	-0.09	0.03	-0.20	-0.22	0.40**	-0.23	0.67**	1.00		
SH	-0.11	-0.06	-0.03	-0.21	0.15	-0.32*	0.41**	0.36*	1.00	
SW	0.10	0.15	-0.20	-0.06	0.32*	-0.31*	0.52*	0.21	0.58**	1.00

1.4 Principal component analysis

Principal component analysis was conducted on 19 traits of 48 non-heading Chinese cabbage germplasm resources, and the results showed that the cumulative contribution rate of the first five principal components was 76.587%, containing most of the information of 19 traits (Table 5). Among them, the numerical value of the first principal component was 5.454, and the contribution rate was 28.707. The traits with higher absolute value of characteristic vector were LL (0.935), PH (0.893), CD (0.884), PL (0.724), LC (0.737) and HS (-0.719). The numerical value of the second principal component was 3.432, and the contribution rate was 18.065. The traits with higher absolute value of characteristic vector were SW (0.808), PW (0.791) and PT (0.694). The numerical value of the third principal component was 2.816, and the contribution rate was 14.823%. The traits with higher absolute value of characteristic vector were LM (-0.713), LT (0.668) and plant type (0.625). The numerical value of the fourth principal component was 1.737, contribution rate was 9.141%, and LW (0.670) was higher. The numerical value of the fifth principal component was 1.112, and the contribution rate was 5.852%. The eigenvector value of RLN (0.677) was higher.

Table 5 Factor loading matrix and contribution rate of traits of test non-heading Chinese cabbage.

Traits	Factor				
	1	2	3	4	5
PH	0.893	0.001	0.020	0.177	0.085
CD	0.884	0.076	0.098	0.187	0.123
RLN	-0.392	-0.364	-0.084	-0.342	0.677
LL	0.935	-0.138	-0.058	0.152	0.016
LW	0.338	0.536	0.051	0.670	0.006
PL	0.724	-0.416	0.178	0.120	0.207
PW	0.092	0.545	0.542	-0.006	0.276
PT	-0.094	0.791	0.313	0.061	0.264
SH	-0.073	0.694	0.216	-0.436	0.034
SW	0.051	0.808	0.235	-0.132	0.071
PT	0.212	-0.183	0.625	-0.253	-0.435
HS	-0.719	0.288	-0.263	0.191	-0.262
LT	-0.202	-0.505	0.668	0.271	0.129
LS	0.528	0.034	-0.534	-0.467	0.186
LTS	-0.587	-0.112	0.467	0.443	0.104
LM	0.249	0.390	-0.713	0.194	-0.143
LSS	-0.330	-0.227	-0.502	0.447	0.261
LC	-0.737	-0.190	0.027	-0.008	-0.010
PC	-0.493	0.363	-0.314	0.174	0.166
Numerical value	5.454	3.432	2.816	1.737	1.112
Contribution rate	28.707	18.065	14.823	9.141	5.852
Total account	28.707	46.771	61.594	70.736	76.587

1.5 Cluster analysis of agronomic traits

The 48 non-heading Chinese cabbage samples were analyzed by systematic clustering method, and the tested materials could be divided into 5 categories (Figure 1). The first group has one resource, which is Wuta-tsai. There were 16 resources in the second group, which were mainly Pak-choi with broad and light green petioles, small plant height and width, thick heads, green leaves and purple leaves. The leaves of code 2018332083 were purple. There were four resources in group III, all of which were erect-type leafy cabbages, with tall plants, large plant width, long leaves, long petioles and white petioles. There were 12 resources in group IV, which were mainly milk cabbage, with shorter plants, larger plant width, thick heads and wide leaves. There were 15 resources in group V, and the classification was mixed, mainly was erect-type leafy cabbage, but also 1 milk Chinese cabbage (P330226003) and 1 Pak-choi (P330127053). And some hard-to-divide materials formed by hybridization (P330127054, P330822020, P330502014) (Figure 2).

Figure 1 Clustering dendrogram of non-heading Chinese cabbage germplasm resources.

Figure 2 Representative plants of different types of non-heading Chinese cabbage germplasm resources

Note: type I: 2018332079; type II: P330783003; type III: P330483024; type IV: 2017334089; type V: P330127054, P330822020, P330502014

2 Discussion

The genetic diversity of crop germplasm resources is evolved to adapt to the environment, and abundant variation is the genetic basis of variety selection. Analyzing the genetic diversity of crop germplasm resources can provide

material basis for breeding. Rapid and simple identification of agronomic traits is the most basic and effective method to study germplasm resources (Xiang et al., 2018). Indicators such as coefficient of variation and genetic diversity index are commonly used to study population characteristic diversity (Han and Jiang, 2014). It has become a common practice to evaluate the diversity of crop germplasm resources based on phenotypic or genotypic data by principal component analysis, correlation analysis and cluster analysis, and has been widely used in genetic diversity research, classification and variety breeding of various crops (Nie et al., 2015; Wan et al., 2017).

The coefficient of variation reflects the degree of dispersion of characters. Previous studies believed that when the coefficient of variation was greater than 10%, there was a large difference between samples for this trait (Lv et al., 2019). In this study, the variation coefficients of 19 phenotypic traits of 48 non-heading Chinese cabbage ranged from 14.57% to 65.77%, all of which were higher than 10%, indicating that there were abundant germplasm resources of non-heading Chinese cabbage in Zhejiang Province. Characters with coefficient of variation greater than 50 % are petiole length, rosette leaves number, leaf margin and leaf shape. Petiole length showed high coefficient of variation in the studies of Sun (2014), Han (2010), Ma et al. (2015) and Xie et al. (2018). Leaf margin showed high coefficient of variation in the studies of Ma et al. (2015). Rosette leaves number and leaf type showed high coefficient of variation only in this study, which was not high in previous studies. However, there are also some traits with high coefficient of variation in previous studies, which are not particularly high in this study. These results indicated that the non-heading Chinese cabbage resources in Zhejiang had certain special characteristics, which could effectively broaden the genetic background of the non-heading Chinese cabbage population and provide material basis for its variety breeding.

The results showed that there were significant or extremely significant correlations among most of the characters of non-heading Chinese cabbage, indicating that different characters of non-heading Chinese cabbage were correlated and influenced each other. In this study, leaf length was significantly positively correlated with plant height, plant width and petiole length, and significantly negatively correlated with leaf number, which was basically consistent with the conclusion of Xie et al. (2018). However, Sun et al. (2014) believed that leaf length was significantly positively correlated not only with petiole length but also with leaf number, while Liu et al. (2014) believed that leaf length was not significantly correlated with leaf number. This may be related to the different test materials. Principal component analysis showed that the information content of the first five principal components accounted for 76.587% of the total information, which could effectively reflect the information content of many original trait variables. The first principal component included leaf length, plant height, plant width, petiole length, leaf color, hourglass sign and other traits, which were the target traits of breeding improvement. Cluster analysis could classify all materials according to phenotypic similarity. In this study, Wuta-tsai was a separate category, and Pak-choi, erect-type leafy cabbage and milk Chinese cabbage could also be clearly distinguished, but there was a hybrid category. In the previous clustering analysis of non-heading Chinese cabbage based on phenotypic data or molecular data, there were mixed groups (Han et al., 2010; Qin et al., 2013; Ma et al., 2015; Xie et al., 2018) showed high coefficient of variation, indicating high similarity of genetic background and phenotypic traits among different types of non-heading Chinese cabbage, with differences only in some key traits.

3 Materials and Methods

3.1 Experimental material

The test materials were non-heading Chinese cabbage collected from the Third National Survey and Collection Action on Crop Germplasm Resources in Zhejiang Province, which was collected and sorted by Vegetable Institute, Zhejiang Academy of Agricultural Sciences.

3.2 Experiment design

The experiment was carried out in Qiaosi Farm (Hangzhou), Zhejiang Academy of Agricultural Sciences. The materials were raised in mid-September 2019, transplanted in early October, harvested in late November, and routinely managed in the field. Phenotypic data were collected according to "Descriptors and Data Standard for Non-heading Chinese Cabbage". The investigated traits included plant height, crown diameter, rosette leaves

number, leaf length, leaf width, petiole length, petiole width, petiole thick, stem height, stem width, per plant weight, plant type, hourglass sign, leaf type, leaf shape, leaf tip shape, leaf surface, leaf color, and petiole color. Three replicates were set for each material, and 5 plants were selected from each replicate for trait measurement.

3.3 Data analysis

The phenotypic data were collated by Microsoft Excel 2016. After statistics, the nine traits of non-heading Chinese cabbage, including plant type, hourglass sign, leaf type, leaf shape, leaf tip shape, leaf margin, leaf surface smoothness, leaf color and petiole color, were quantitatively processed according to "Descriptors and Data Standard for Non-heading Chinese Cabbage" (Table 6). Ten quantitative traits (PH, CD, RLN, LL, LW, PL, PW, PT, SH and SW) were divided into 10 grades and assigned values. Grade 1 was defined as less than or equal to $X-2\delta$, and grade 10 was defined as greater than or equal to $X+2\delta$, with a difference of 0.5δ (X was the mean value and δ was the standard deviation). Shannon's diversity index was calculated using the relative frequency (P_i) of each level (He and Zong, 2011; Wang et al., 2014; Lv et al., 2020). SPSS 16.0 software was used for principal component and correlation analysis of traits, and R language program system clustering Ward method was used for cluster analysis.

Table 6 Classifying and assignment of quality traits

Traits	Assignment							
	1	2	3	4	5	6	7	8
PT	Erect	Semi-erect	Spreading	Semi-prostrate	Prostrate			
HS	Waist-shaped	Non-waistshaped						
LT	Leaf with lobes	Leaf without lobes						
LS	Nearly orbicular	Ovate	Lone Ovate	Obovate	Lone obovate	Elliptic	Oblong	Lanceolate
LTS	Acute	Blunt point	Round	Broad round				
LM	Absent	Small	Intermediate	Large				
LSS	Smooth	Slightly wrinkling	Wrinkling	Very wrinkling				
LC	Yellowish green	Light green	Green	Dark green	Blackish green	Purplish green	Purple	
PC	White	Greenish white	Light green	Green	Dark green	Purplish Green	Purple	

Authors' contributions

HHJ and WJL are the main executors of this study. HTH, WWH and WQZ were involved in data collection of phenotypic traits. BCL was the architect and principal of the project, directing experimental design, data analysis, paper writing and revision. All authors read and approved the final manuscript.

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