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Evaluation of genetic diversity in Chinese kale (*Brassica oleracea* L. var. *alboglabra* Bailey) by using rapid amplified polymorphic DNA and sequence-related amplified polymorphism markers

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ABSTRACT. Chinese kale is an original Chinese vegetable of the Cruciferae family. To select suitable parents for hybrid breeding, we thoroughly analyzed the genetic diversity of Chinese kale. Random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) molecular markers were used to evaluate the genetic diversity across 21 Chinese kale accessions from AVRDC and Guangzhou in China. A total of 104 bands were detected by 11 RAPD primers, of which 66 (63.5%) were polymorphic, and 229 polymorphic bands (68.4%) were observed in 335 bands amplified by 17 SRAP primer combinations. The dendrogram showed the grouping of the

21 accessions into 4 main clusters based on RAPD data, and into 6 clusters based on SRAP and combined data (RAPD + SRAP). The clustering of accessions based on SRAP data was consistent with petal colors. The Mantel test indicated a poor fit for the RAPD and SRAP data (r = 0.16). These results have an important implication for Chinese kale germplasm characterization and improvement.

Key words: Chinese kale; Genetic diversity; RAPD markers; SRAP markers

INTRODUCTION

Chinese kale (*Brassica oleracea* L. var. *alboglabra* Bailey) is an original Chinese vegetable of the Cruciferae family. It is cultivated widely in South China and Southeast Asia and is present in relatively small quantities in Japan, Europe, and America. Chinese kale is usually planted for its flower stalks as a common edible part. The flower stalk is tender and crispy with good flavor. In recent years, tender rosette leaves and sprouts of Chinese kale are also consumed by Chinese people. Therefore, it has become one of the most important *Brassica* vegetables in South China, and its cultivated area is increasing along with the market demand.

DNA-based molecular markers are useful tools that provide a relatively unbiased estimation of genetic diversity and establish genetic relationship more precisely than morphological and biochemical markers (Soller and Beckmann, 1983). Among them, random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) are more commonly used markers. Molecular markers such as RAPD were previously used to assess genetic diversity among the species of Chinese kale (Matsui et al., 2002), cabbage (Cansian and Echeverrigaray, 2000; Koutita et al., 2005), kale (Okumus and Balkaya, 2007), cauliflower (Astarini et al., 2005, 2006), broccoli (Lu et al., 2009), mustard (Rabbani et al., 1998; Teklewold and Becker, 2006; Khan et al., 2008, 2011), non-heading Chinese cabbage (Han and Cheng, 2010), and rape (Riaz et al., 2001; Hu et al., 2003; Gecaite et al., 2009; Leonte et al., 2010). Besides, SRAP markers have been used for genetic diversity analysis of different species of *Brassica* (Li and Quiros, 2001; Wu et al., 2009; Han and Cheng, 2010; Jing et al., 2011; Yu et al., 2011).

In recent years, Chinese kale hybrid seeds are mainly imported from Japan to China. They are expensive. Therefore, breeding Chinese kale hybrid varieties with high quality, high yield, and strong resistance is essential. For hybrid breeding, the key is to select the parents and hybrid combinations. In order to select suitable parents, the genetic background needs to be thoroughly analyzed. Therefore, the objective of this study was to assess the genetic diversity of 21 Chinese kale accessions by using RAPD and SRAP molecular markers.

MATERIAL AND METHODS

Plant materials and DNA extraction

Twenty-one accessions of Chinese kale, including 20 accessions imported from Asian Vegetable Research and Development Center (AVRDC) and 1 commercial cultivar from

Guangzhou in China, were analyzed in this study (Table 1). Total genomic DNA was extracted from young leaves of 3 plants per accession by using the CTAB method (Doyle and Doyle, 1987). The quality of DNA was measured using agarose gel electrophoresis and ultraviolet spectrophotometry (NanoDrop 2000C; Thermo Scientific, Wilmington, MA, USA). The DNA samples were diluted to 50 ng/μL for future use.

Table 1. Accessions of Chinese kale used in the study.

Acc. No.	Cultivar name	Source	Petal color	Acc. No.	Cultivar name	Source	Petal color
1	Ba05	AVRDC	Yellow	12	Ba17	AVRDC	White
2	Ba41	AVRDC	White	13	Ba27	AVRDC	Yellow
3	Ba35	AVRDC	White	14	Ba11	AVRDC	White
4	Ba25	AVRDC	White	15	Ba09	AVRDC	Yellow
5	Ba34	AVRDC	White	16	Ba18	AVRDC	White
6	Ba43	AVRDC	Yellow	17	Ba44	Guangzhou	White
7	Ba38	AVRDC	Yellow	18	Ba20	AVRDC	White
8	Ba19	AVRDC	White	19	Ba33	AVRDC	White
9	Ba04	AVRDC	White	20	Ba24	AVRDC	White
10	Ba39	AVRDC	White	21	Ba08	AVRDC	White
11	Ba32	AVRDC	White				

AVRDC = Asian Vegetable Research and Development Center.

RAPD fingerprinting

Eleven selected random 10-mer primers (Sangon, Shanghai) were used for analyzing polymorphism (Table 2). Amplification reactions were performed in a 25-μL reaction volume containing 10X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 μM RAPD primer, 50 ng total genomic DNA, and 1 U Taq DNA polymerase (Takara, Japan). PCR was performed in a PTC-200 thermocycler (Bio-Rad, USA) with the following program: 4 min of denaturizing at 94°C, followed by 45 cycles of 1 min of denaturizing at 94°C, 1 min of annealing at 36°C and 90 s of extension at 72°C, and the last cycle was 10 min of extension at 72°C. PCR products were analyzed on 2% (w/v) agarose gel.

Table 2. RAPD primer sequences and the polymorphism of their products.

Primer order	Sequence	Total bands	Polymorphic bands	PPB (%)
S1218	CTACCGGCAC	11	8	72.7
S1271	CTTCTCGGTC	7	5	71.4
S1276	TCTTAGGCGG	10	7	70.0
S1295	GGCAGCAGGT	9	3	33.3
S1299	CTCGATCACC	13	9	69.2
S1307	AGCCCCCAAG	13	8	61.5
S1310	GGTGTTTGCC	9	7	77.8
S1342	TGCGAAGGCT	9	4	44.4
S1360	TCATTCGCCC	7	5	71.4
S1379	ACACTCTCGG	8	4	50.0
S1393	GAGCGTCGCT	8	6	75.0
Total		104	66	63.5

PPB = percentage of polymorphic bands.

SRAP fingerprinting

Seventeen SRAP primer combinations were used for detecting polymorphism in open reading frames (ORFs; Table 3). Amplification reactions were carried out in a 25-μL reac-

tion volume containing 10X PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.12 µM each primer, 50 ng genomic DNA, and 1 U Taq DNA polymerase. Amplification was performed in a PTC-200 thermocycler (Bio-Rad) as per the following procedure: 5 min at 94°C for initial denaturing; 6 cycles of 1 min at 94°C, 1 min at 35°C, and 1 min at 72°C; 36 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. The last cycle had a 10-min extension at 72°C. PCR products were separated on 6% polyacrylamide gels, and silver staining was used to view the bands.

Table 3. SRAP primer sequences and the polymorphism of their products.

Primer order	Sequence	Total bands	Polymorphic bands	PPB(%)
me1em11	me1 TGAGTCCAAACCGGATA em11 GACTGCGTACGAATTCCA	14	8	57.1
me2em5	me2 TGAGTCCAAACCGGAGC em5 GACTGCGTACGAATTAAC	14	9	64.3
me2em9	me2 TGAGTCCAAACCGGAGC em9 GACTGCGTACGAATTCGA	16	7	43.8
me2em11	me2 TGAGTCCAAACCGGAGC em11 GACTGCGTACGAATTCCA	21	16	76.2
me3em11	me3 TGAGTCCAAACCGGAAT em11 GACTGCGTACGAATTCCA	18	10	55.6
me5em3	me5 TGAGTCCAAACCGGGAG em3 GACTGCGTACGAATTGAC	21	17	81.0
me5em4	me5 TGAGTCCAAACCGGGAG em4 GACTGCGTACGAATTTGA	21	11	52.4
me5em5	me5 TGAGTCCAAACCGGGAG em5 GACTGCGTACGAATTAAC	36	30	83.3
me5em9	me5 TGAGTCCAAACCGGGAG em9 GACTGCGTACGAATTCGA	15	11	73.3
me6em3	me6 TGAGTCCAAACCGGTAA em3 GACTGCGTACGAATTGAC	21	16	76.2
me6em4	me6 TGAGTCCAAACCGGTAA em4 GACTGCGTACGAATTTGA	26	16	61.5
me6em5	me6 TGAGTCCAAACCGGTAA em5 GACTGCGTACGAATTAAC	16	8	50.0
me6em9	me6 TGAGTCCAAACCGGTAA em9 GACTGCGTACGAATTCGA	21	14	66.7
me7em3	me7 TGAGTCCAAACCGGTCC em3 GACTGCGTACGAATTGAC	23	16	69.6
me7em4	me7 TGAGTCCAAACCGGTCC em4 GACTGCGTACGAATTTGA	17	13	76.5
me7em5	me7 TGAGTCCAAACCGGTCC em5 GACTGCGTACGAATTAAC	13	10	76.9
me7em11	me7 TGAGTCCAAACCGGTCC em11 GACTGCGTACGAATTCCA	22	17	77.3
Total		335	229	68.4

PPB = percentage of polymorphic bands.

Data analysis

RAPD and SRAP amplifications were repeated twice, and only clear bands produced were scored as presence (1) or otherwise scored as absence (0) across 21 accessions for each primer (RAPD) or primer combination (SRAP). Cluster analysis was based on Jaccard similarity coefficient by using the unweighted pair group method with arithmetic average (UPGMA) with SAHN modules of numerical taxonomy and multivariate analysis system (NTSYS-pc) version 2.10 (Rholf, 2000). A principal coordinate analysis was performed to construct a two-dimensional array of eigenvectors by using the DCENTER module of the

NTSYS-pc program. Finally, a Mantel test was performed to determine the significance level between RAPD and SRAP, RAPD and RAPD + SRAP, and SRAP and RAPD + SRAP based on Jaccard's coefficient by using the NTSYS-pc 2.10 software.

RESULTS

RAPD analysis

The 11 selected RAPD primers generated 104 amplified products, with a mean of 9.5 bands per primer, ranging from 7 (S1271 and 1360) to 13 bands (S1299 and S1307) (Table 2). Of the bands, 66 were polymorphic (63.5%) with the size ranging from 100 to 2000 bp (Figure 1). The percentage of polymorphic bands (PPB) among the primers ranged from 33.3% (S1295) to 77.8% (S1310), with an average of 63.5% (Table 2).

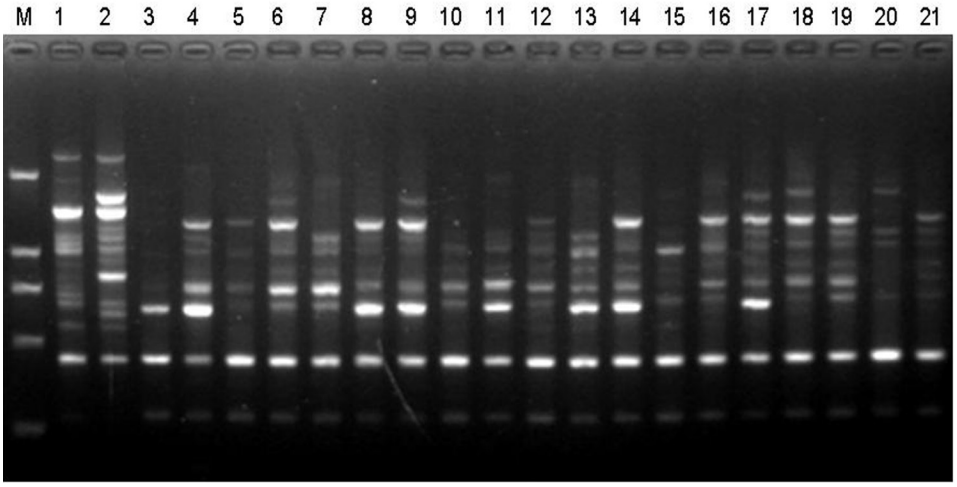

Figure 1. Gel electrophoresis of amplification products obtained with primer S1307. *Lane M* = molecular marker (DL2000); *lanes 1-21* = samples.

Genetic similarity coefficients were calculated using UPGMA algorithm based on Jaccard's coefficient. The similarity coefficient ranged from 0.34 to 0.71. The dendrogram grouped the 21 accessions into 4 main clusters at a coefficient of 0.440. Cluster I comprised 10 accessions. Accessions within cluster I were further divided into 4 subclusters. Subcluster I-1 comprised Ba05 and Ba41. Subcluster I-2 contained Ba35, Ba25, Ba34, Ba43, and Ba38. Subcluster I-3 included Ba19 and Ba04, and subcluster I-4 included Ba18. Within cluster I, Ba43 and Ba38 appeared to be closer to each other, with a 0.71 similarity coefficient. Nine accessions formed cluster II, which was further divided into 3 subclusters. Subcluster II-1 consisted of Ba39, Ba11, Ba44, Ba33, Ba24, Ba09, and Ba17. Ba20 and Ba32 formed subcluster II-2 and II-3, respectively. Ba08 and Ba27 formed groups III and IV, respectively, showing less similarity with other accessions studied (Figure 2).

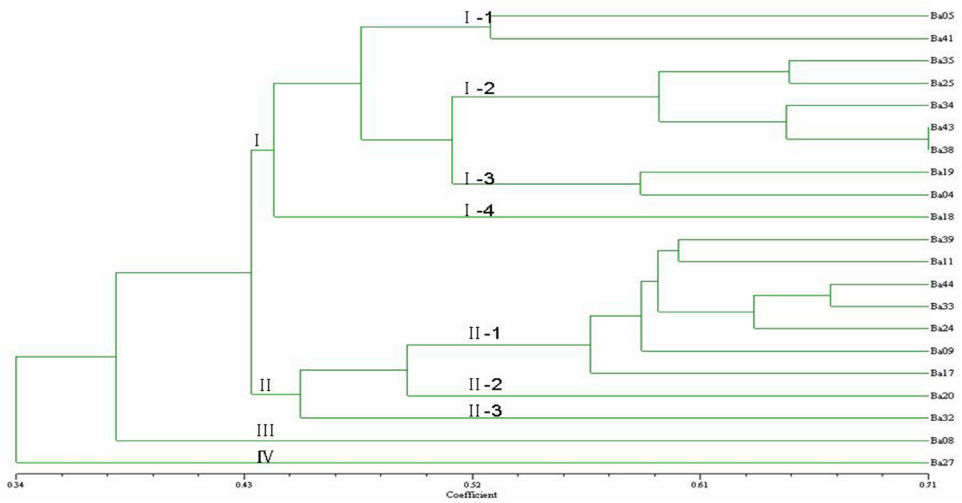

Figure 2. Dendrograms of genetic similarity relationships in 21 Chinese kales produced by UPGMA clustering method based on the Jaccard similarity coefficient matrix derived from 66 RAPD markers.

SRAP analysis

Analysis of the 21 Chinese kale accessions with 17 SRAP primer combinations identified 335 repeatable fragments, of which 229 were polymorphic (68.4%) with the size ranging from 50 to 2000 bp (Figure 3). The number of fragments detected by an individual primer combination ranged from 14 (me1em11 and me2em5) to 36 (me5em5), with an average of 19.7. The number of polymorphic fragments per primer combination varied from 7 (me2em9) to 30 (me5em5), with a mean of 13.5. PPB ranged from 43.8% (me2em9) to 83.3% (me5em5), with an average polymorphism of 68.4% across all the accessions (Table 3).

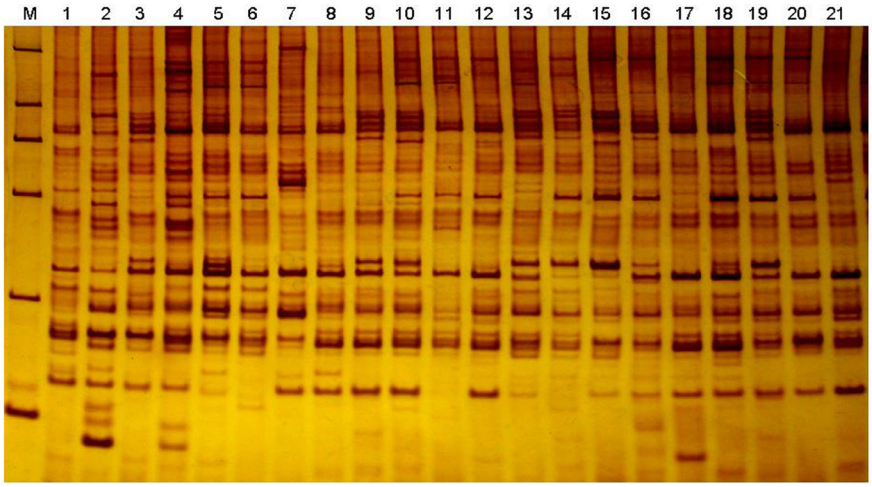

Figure 3. Gel electrophoresis of amplification products obtained with the primer combination me5em5. *Lane M* = molecular marker (DL2000); *lanes 1-21* = samples.

The similarity coefficient based on SRAP data varied from 0.42 to 0.73. The 21 Chinese kale accessions were grouped into 6 clusters at a coefficient of 0.470. Cluster I comprised 14 accessions. Accessions within cluster I were further divided into 4 subclusters. Subcluster I-1 comprised Ba05, Ba43, Ba38, and Ba09, and their petals were yellow. Subcluster I-2 consisted of Ba25, Ba04, Ba39, Ba19, Ba34, Ba18, and Ba44, and the petal colors of the 7 accessions were white. Subcluster I-3 contained Ba33 and Ba08, and they had white petals. Ba24 that had white petals formed subcluster I-4. Within cluster I, Ba43 and Ba38 appeared to be closer to each other, with a 0.73 similarity coefficient. Ba41 and Ba20 formed cluster II, and they had white petals. Ba17 (white petals), Ba27 (yellow petals), and Ba32 (white petals) formed clusters III, IV, and V, respectively, which indicated that they have less similarity with other accessions studied. Cluster VI comprised Ba35 and Ba11, which had white petals (Figure 4).

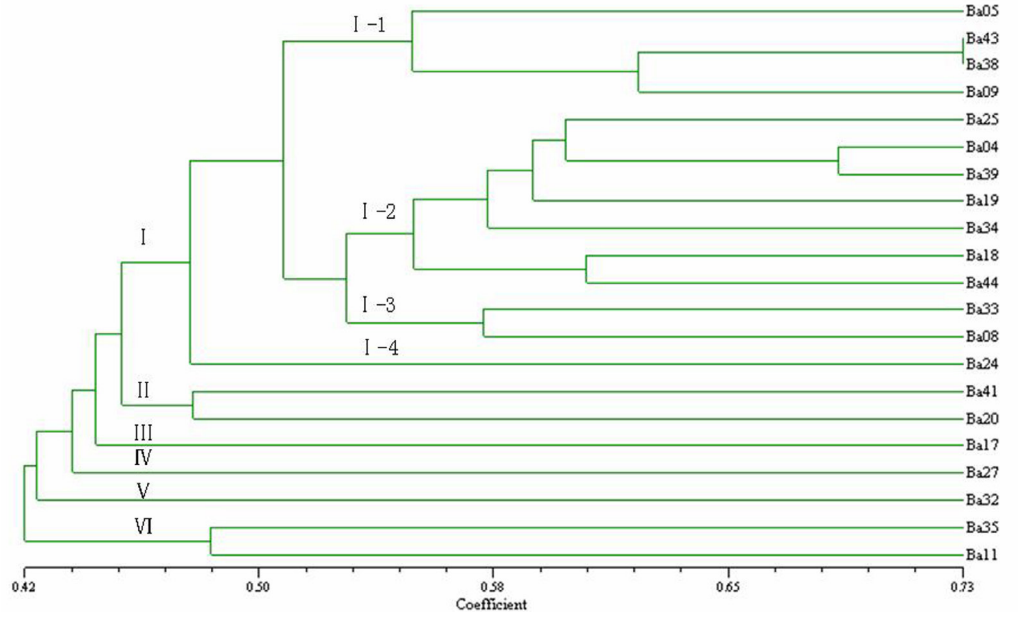

Figure 4. Dendrograms of genetic similarity relationships in 21 Chinese kales produced by UPGMA clustering method based on the Jaccard similarity coefficient matrix derived from 229 SRAP markers.

Combined RAPD and SRAP data analysis

Further accurate genetic estimates were obtained by combining the RAPD and SRAP data for cluster analysis. Six clusters similar to SRAP clusters were formed with Jaccard's coefficient ranging from 0.42 to 0.72 (Figure 5). The pattern of clustering of the accessions remained almost the same in SRAP and combined (SRAP and RAPD) data, whereas the dendrogram based on RAPD data showed some variation in the clustering of accessions. The matrices for RAPD and SRAP markers were also compared using the Mantel test (Mantel, 1967) for matrix correspondence. The correlation between the matrices of cophenetic correlation values for the dendrogram based on RAPD and SRAP data was low (r = 0.16). Twenty-one accessions were grouped in 6 clusters in SRAP and combined (SRAP and RAPD) data, whereas

a dendrogram based on RAPD data showed only 4 clusters. In all the 3 dendrograms, Ba27 was represented as an independent cluster.

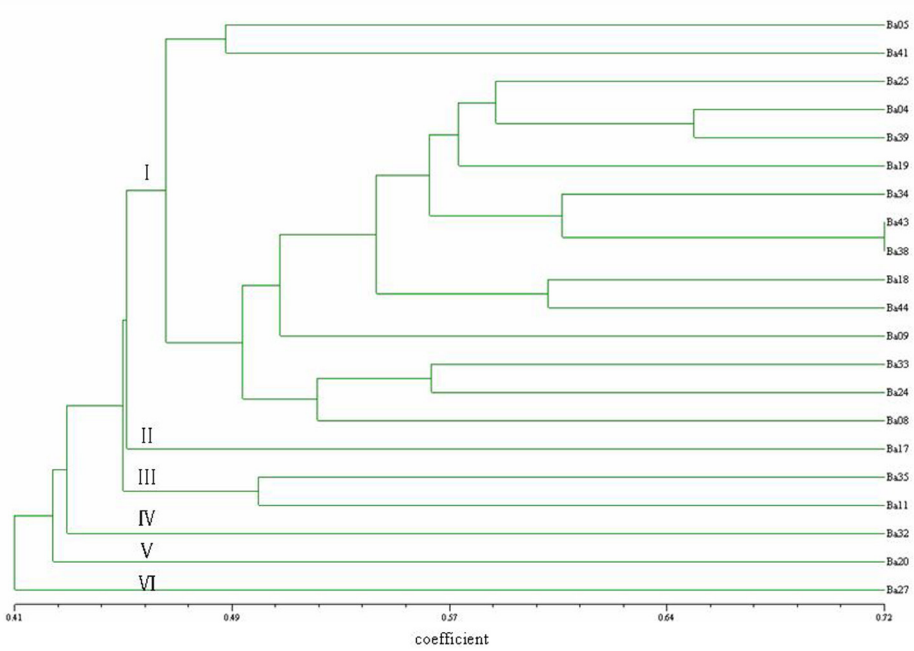

Figure 5. Dendrograms of genetic similarity relationships in 21 Chinese kales produced by UPGMA clustering method based on the Jaccard similarity coefficient matrix derived from 295 markers (RAPD+SRAP).

DISCUSSION

Analysis of diversity is very important in plant breeding and conservation of genetic resources, and molecular markers offer an approach to unveil the genetic diversity among accessions based on nucleic acid polymorphisms. In this study, 2 molecular markers, RAPD and SRAP, were simultaneously used to investigate the genetic diversity of 21 Chinese kale accessions. Wide genetic variability among different accessions of Chinese kale was observed in the study. This high level of morphological polymorphism in Chinese kale has been reported previously (Zhang and Zhang, 2008). Unlike in other *B. oleracea* species, the genetic diversity of Chinese kale was higher than that of kale (RAPD, PPB = 54.5%; Okumus and Balkaya, 2007) and broccoli (RAPD, PPB = 56.03% and SRAP, PPB = 47.3%; Lu et al., 2009; Yu et al., 2011).

The applicability of RAPD and SRAP as genetic markers to characterize the genetic diversity of Chinese kale was compared. RAPD markers showed lower PPB (63.5%) than the SRAP markers (68.4%). Similar results were also observed in non-heading Chinese cabbage (Han and Cheng, 2010).

The present study showed that the clustering of accessions based on RAPD and SRAP markers was inconsistent. A possible reason is that the 2 marker techniques targeted different portions of the genome. The RAPD technique involves the amplification of random segments of genomic DNA by using random primers and a low annealing temperature, which allows

non-specific binding to the DNA. On the other hand, SRAP markers only amplify ORFs (Li and Quiros, 2001), which might include coding regions of the genome involved in morphological and agronomic traits, and likely reflect differences in coding sequences, which are thought to be relatively conserved across species (Ferriol et al., 2003). In this study, the clustering of accessions based on SRAP markers was consistent with the petal colors. The correlation between 2 Jaccard similarity coefficient values was low between RAPD and SRAP data (r = 0.283), high between RAPD and RAPD + SRAP (r = 0.571), and higher between SRAP and RAPD + SRAP (r = 0.949). This suggests that SRAP data are obviously closer to RAPD + SRAP combined data.

Our results indicate the presence of remarkable genetic variability among the 21 Chinese kale accessions. Genetic variation among Chinese kale accessions based on RAPD and SRAP analyses could be useful to select parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes.

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