Nonheading Chinese cabbage [Brassica rapa var. chinensis (Linnaeus) Kitamura], belonging to the genus Brassica in Brassicaceae, is an annual or biennial herb (Wu et al., 2001). It is widely cultivated and one of the most consumed vegetable crops in Asia. Nonheading Chinese cabbage originated in China, which has a long history of cultivation and includes numerous and complex agricultural crops.

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Abstract. ‘Suzhouqing’ is a unique landrace of nonheading Chinese cabbage [Brassica rapa var. chinensis (Linnaeus) Kitamura] with a long history of cultivation in Suzhou of Jiangsu Province, China. However, transitional and overlapped morphologic traits make it difficult to authenticate this accession from other nonheading Chinese cabbages. Genetic relationship between ‘Suzhouqing’ and the related 10 popular accessions in the Yangtze River Delta were analyzed using two well-studied single-copy nuclear genes—ARGONAUTES 7 (AGO7) and BcMIF15; the molecular identification of ‘Suzhouqing’ was determined based on the intersimple sequence repeat—sequence-characterized amplified region (ISSR-SCAR) marker. The results indicated that ‘Suzhouqing’ could be identified specifically from the other 10 accessions based on 21 specific nucleotide variations of the AGO7 gene. Sequence variations show a strong correlation with leaf morphology, suggestive of partial causal links between the two. Genetic relationship analysis showed that five accessions with close geographic locations had a very close genetic relationship, whereas the genetic relationship of the other five accessions was related to their morphologic similarity. One exception, ‘AJH’, might undergo a special evolutionary process. Furthermore, ISSR-880 was screened as the specific primer to identify accession ‘Suzhouqing’, and a specific discrimination ISSR-SCAR marker was explored, which amplified no target band in any other accessions. The development of molecular markers for the specific identification of ‘Suzhouqing’ in 11 popular accessions in the Yangtze River Delta could provide a theoretical basis for the protective identification of other agricultural crops.

Within the past two decades, several molecular markers have been developed and widely applied in genetic relationship analysis of nonheading Chinese cabbage (Han et al., 2007; Li et al., 2008; Ma et al., 2012). However, the identification results based on molecular evidence were not always consistent with those based on morphologic traits. For example, the genetic relationship revealed by the amplified fragment length among different accessions does not completely correspond to the differences in their morphologic characteristics (Guo et al., 2002; Sun et al., 2001). Furthermore, most of these studies have focused on the genetic diversity of germplasms, while markers that could discriminate one accession uniquely from the others, have not been developed yet. Only two exceptions have been reported. One is that based on amplified fragment length polymorphism molecular markers. Li et al. (2008) screened molecular markers with high efficiency in the identification of accession ‘Youdonger’ in Hangzhou. The other exception was by our research team. We developed a pair of ISSR-SCAR markers for the specific identification of Wujiang Fragrant Bok Choy (Shen et al., 2016).

Low- or single-copy nuclear genes among many species have been tested recently as effective phylogenetic markers. In Tildenia, a subgenus of Peperomia (Piperaceae), 25% of aligned characters from the agt1 intron sequence are parsimony informative, which could be developed for species identification (Naumann et al., 2011). Eight single-copy genes contain as many as 5.8% variable sites, and primers were developed for phylogenetic studies across the Melastomataceae (Reginato and Michelangeli, 2016). In addition, Zhao et al. (2016) used 36 whole genomes and 27 transcriptomes to identify 891 orthologous genes with one or low copies, and reconstructed the rosids phyllogeny. Huang et al. (2016) screened 113 low-copy orthologous nuclear genes from transcriptomes or genomes of 55 Brassicaceae species. Recent studies used transcriptome or genome data sets to reconstruct successfully phylogenies of various scales from genus to angiosperm-wide, which also shed light on research of species discrimination by low- or single-copy nuclear genes.

‘Suzhouqing’, which has a wonderful taste, is a unique landrace of nonheading Chinese cabbage with a long history of cultivation in Suzhou, Jiangsu Province. Based on the morphologic traits, it is difficult to discriminate ‘Suzhouqing’ from the other popular accessions of nonheading Chinese
cabbage in the Yangtze River Delta. For example, there only exist slight differences in plant height, petiole length, and leaf color between ‘Suzhouqing’ and ‘Shanghaiaqing’. In this study, 11 major accessions of nonheading Chinese cabbage in the Yangtze River Delta were collected. Combined with ISSR and two well-studied single-copy nuclear genes—ARGONAUTES 7 (AGO7) and BeMF15—the genetic relationships of the major accessions of nonheading Chinese cabbage were investigated, and an authentic marker for ‘Suzhouqing’ discrimination was explored. AGO7 has been suggested to function in leaf-shape formation, harboring high polymorphism in Chinese cabbage (Liang et al., 2016). BeMF15 was suggested to involve in microspore development and might be male sterile related in nonheading Chinese cabbage (Tian et al., 2009). Therefore, these two genes may have the potential to be applied for identification and genetic relationship analysis of nonheading Chinese cabbage.

Materials and Methods

Plant materials. ‘Suzhouqing’ and 10 other accessions of nonheading Chinese cabbage (Table 1; the acronyms included therein are used hereafter) that mainly cultivated in the Yangtze River Delta were sampled in this study. The plant materials were provided by the Taihu base of Seed Administrative Station of Suzhou. For each cultivar, 8 to 10 healthy individuals were selected, from which tender leaves were collected and stored in silica gel until use.

Total DNA extraction. Total genomic DNA was extracted from silica-gel dried leaf tissues. One hundred-milligrams of leaves were disrupted by a tissue disrupter (Tissue-Lyser LT; Qiagen, Germany) and total DNA was disrupted by a tissue disrupter (TissueLyser LT; Qiagen, Germany) and total DNA was isolated using the Easy Pure Plant Genomic DNA Kit (TransGen Biotechnology Co., Ltd., Beijing, China). The extracted DNA was diluted to a final concentration of 20 ng·μL⁻¹. Concentration and purity of DNA were detected using a nucleic acid and protein detector (Eppendorf; Saxony, Germany). DNA samples were stored at –20°C for future use.

Isolation of AGO7 and BeMF15 genes. The sequences of AGO7 (Gene ID: Bna003999.1PACid:22700727) and BeMF15 (Genbank no.: EF600901) were downloaded from an online database (https://phytozome.jgi.doe.gov/pz/portal.html) and Genbank, respectively, for primer designing. AGO7 was amplified with primers AGO7F (5’-ATGGAGAAGAAAAACCAGAATC-3’) and AGO7AS (5’-TCAAGGTAATTTATGATGATT-3’). BeMF15 was amplified with primers LTPS (5’-ATGAAGTTAATTTTATGATTG-3’) and LTPAS (5’-TTGTGTGATACGTCAAGC-3’). These primers were all designed using Primer Premier 5.0 (Premier Biosoft, CA).

Amplifications were performed using GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT). The polymerase chain reaction (PCR) mixtures contained the following components: 1.0 μL DNA template, 25.0 μL 2× Reaction Mix (Dongsheng PCR Kit, including 20 mmol·L⁻¹ Tris-HCl, 100 mmol·L⁻¹ KCl, 3 mmol·L⁻¹ MgCl₂, 400 μmol·L⁻¹ deoxyribonucleoside-5’-triphosphate (dNTPs), and bromophenol blue), 2.0 μL each of forward and reverse primers (10 μmol·L⁻¹), 1.0 μL Taq DNA polymerase (2.5 U·μL⁻¹), and double-distilled water to a total volume of 50 μL. The reaction was carried out as follows: predenaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 53 to 55°C for 45 s, and extension at 72°C for 1 to 3 min, then a final extension step at 72°C for 10 min. PCR products were subjected to electrophoresis on 0.8% agarose gel containing ethidium bromide, then visualized under ultraviolet transilluminator and imaged by a gel documentation system (Bio-Rad, Hercules, CA). The amplicons with correct length were submitted to BGI Gene Technology Co., Ltd. (Shanghai, China) for sequencing, and the bidirectional results were assembled using Sequencher 4.5 software (GeneCodes, Ann Arbor, MI). DNA matrices were aligned in MEGA6 (Tamura et al., 2013) and used to build a maximum likelihood tree to analyze the genetic relationships among these accessions.

ISSR analysis. DNA samples were mixed to construct DNA pools for each of the 11 accessions of nonheading Chinese cabbage. Forty-eight ISSR primers from the literature (Song et al., 2012) were synthesized by Realgene Bio-Technologies, Inc. (Nanjing, China). Each PCR had a total volume of 20 μL and contained 1.0 μL DNA template (20 ng·μL⁻¹), 10.0 μL 2× Reaction Mix (Dongsheng PCR Kit, including 20 mmol·L⁻¹ Tris-HCl, 100 mmol·L⁻¹ KCl, 3 mmol·L⁻¹ MgCl₂, 400 μmol·L⁻¹ dNTPs, and bromophenol blue), 1.5 μL of each primer (10 μmol·L⁻¹), and 0.4 μL of Taq DNA polymerase (2.5 U·μL⁻¹). PCR was performed on a BioMetra T1 PCR thermocycler (Analytik Jena, Germany) as follows: initial denaturation at 94°C for 7 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C (slightly altered for different primers) for 45 s, extension at 72°C for 1 min, and the final extension at 72°C for 10 min. PCR products were separated by electrophoresis in 3% agarose gel and stained with ethidium bromide. The gel was run at a constant voltage of 100 V for 1.5 h and was visualized under a gel documentation system. The 2000-base pair (bp) DNA ladder (Dongsheng Bi-Technology Co., Ltd., Guangzhou, China) was used to determine the size of the amplified fragments.

The primers that can generate clear DNA bands with abundant polymorphic profiles were selected from the 48 ISSR primers set. They were used to amplify the DNA pools of the 11 accessions of nonheading Chinese cabbage. The DNA bands exhibiting divergence among different samples were picked out. Each amplification product at a specific position with the same migration rate was considered a homologous locus. The presence or absence of an allele at a particular locus was scored as 1 and 0, respectively, to generate a binary matrix for further analysis. The POPGENE 32 software (Yeh et al., 1999) were used to calculate Nei’s gene diversity index (H), the effective number of alleles (NE), Shannon’s information index (I), the genetic differentiation coefficient (GST), and gene flow (Nm). The genetic similarity coefficients and genetic distances between samples were estimated by NTSYS2.12 software (Rohlf, 2000). The obtained distance matrices were used to cluster the strains by the unweighted pair group method using arithmetic means (UPGMA) method, and a dendrogram was plotted.

Development and validation of specific ISSR-SCAR bands. A unique DNA band specific to ‘Shanghaiqing’ was excised from the agarose gel and purified by the QiAquick gel extraction kit (Qiagen, Shanghai, China). The purified DNA fragments were ligated to the pMD19-T vector (TaKaRa, Dalian, China) and transformed into Escherichia coli DH5ά competent cells by a heat-shock method. Positive clones were selected through PCR and then sequenced by Realgene Bio-Technologies (Nanjing, China). A pair of SCAR primers was designed based on the obtained sequence.

A total of 88 individual DNA samples from 11 nonheading Chinese cabbage accessions (eight individuals for each accession) were amplified with the newly designed SCAR primers. Each ISSR reaction was carried out in a final volume of 20 μL. The components and their concentration of PCR mixture were as follows: 1.0 μL DNA template (20 ng·μL⁻¹), 10.0 μL 2× Reaction Mix (Dongsheng PCR Kit, including 20 mmol·L⁻¹ Tris-HCl, 100 mmol·L⁻¹ KCl, 3 mmol·L⁻¹

| Accessions       | Acronyms         | Variety          | Provenance       | Characteristics                  |
|------------------|------------------|------------------|------------------|----------------------------------|
| Suzhouqing       | SZQ              | var. communis   | Suzhou           | Plant type: Smooth               |
| Zhongqibai       | ZQB              | var. communis   | Changzhou        | Leaf type: Smooth                |
| Wuuyeman         | WYM              | var. communis   | Shanghai         | Smooth                           |
| Aijiiaohanhuang  | AJH              | var. communis   | Nanjing          | Smooth                           |
| Siyueman         | SYM              | var. communis   | Shanghai         | Smooth                           |
| Shanghaiaqing    | SHQ              | var. communis   | Shanghai         | Smooth                           |
| Huangximou       | HXW              | var. tai-tai    | Hefei            | Prostrate                       |
| Xiaobaye         | XBY              | var. tai-tai    | Shanghai         | Prostrate                       |
| Huangzhangxiongqiai | QXCH          | var. communis   | Suzhou           | Semi-prostrate                  |
| Heizhongxiongqiai | QCQ              | var. communis   | Suzhou           | Semi-prostrate                  |
| Xishuaqin        | XJH              | var. communis   | Suzhou           | Prostrate                       |

Table 1. List of nonheading Chinese cabbage accessions used in this study, and their sources.
MgCl₂, 400 μmol·L⁻¹ dNTPs, and bromophenol blue), 0.8 μL of each primer (10 μmol·L⁻¹), 0.4 μL of Taq DNA polymerase (2.5 U·μL⁻¹) and 7 μL double-distilled water. The PCR program consisted of predenaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and elongation at 72 °C for 1 min; and a final elongation at 72 °C for 8 min. The PCR products were visualized on 1% agarose gel in an electrophoresis system run at a constant voltage of 80V. After 0.5 h of electrophoresis, the PCR products were examined and imaged using a gel imaging system. A 2000-bp DNA ladder was used as a molecular weight marker.

**Results**

Identification of the 11 nonheading Chinese cabbage accessions based on AG07 and BcMF15. AG07 and BcMF15 were amplified successfully in 11 nonheading Chinese cabbage accessions. The length of AG07 was 3419 to 3433 bp, and the length of BcMF15 was 604 to 614 bp. The aligned AG07 gene matrix was 3442 bp in length (Supplemental Table 1), within which 102 single nucleotide polymorphisms (SNPs) (90 located in exons, including 51 synonymous mutations and 37 nonsynonymous mutations) and seven indels (two of which were located in exons, resulting in one aa and three aa indels, respectively) were detected in the 11 accessions. All of the 11 nonheading Chinese cabbage accessions exhibited distinct and specific characters. For example, 21 specific characters were identified in accession ‘SZQ’, including the specific bases C, T, and T at positions 230 bp, 233 bp, and 239 bp, respectively (vs. T, C, and C, respectively, in other accessions).

The aligned BcMF15 gene matrix was 617 bp in length (Supplemental Table 2). Seven SNPs (four nonsynonymous mutations located in exons) were detected and three indels (two located in exons, resulting in four aa indels and one aa indel, respectively). The variations in the BcMF15 gene could divide the 11 accessions into two groups. One group contained ‘SYM’, ‘WYM’, and ‘SHQ’; the other group consisted of the remaining eight accessions (Supplemental Table 2). In the first group, ‘SHQ’ was differentiated from other accessions based on C at position 408 bp, compared with T in other accessions. ‘XBY’ in the second group was differentiated by A at position 585 bp, compared with T in other accessions.

Analysis of the genetic relationship of the 11 nonheading Chinese cabbage accessions based on AG07 and BcMF15. The results of genetic distance and the genetic similarity coefficient revealed that the genetic similarity of AG07 and BcMF15 gene sequences in the 11 nonheading Chinese cabbage accessions was 97.4% to 100%, and the genetic distance was 0 to 0.016. The phylogenetic tree of the 11 nonheading Chinese cabbage accessions was based on the maximum likelihood method (Fig. 1). As shown in the Fig. 1, the 11 nonheading Chinese cabbage accessions could be divided mainly into two clusters. Cluster I contained ‘SZQ’, ‘XYM’, ‘WYM’, ‘SHQ’, and ‘ZQB’, whereas the remaining six accessions were clustered in cluster II. Within cluster I, ‘SYM’ and ‘WYM’ were more closely related, as was ‘SHQ’ and ‘ZQB’, whereas ‘SZQ’ was obviously independent from the other four accessions. Within cluster II, ‘HXW’ and ‘AJH’ were closer together, obviously independent from the subcluster of ‘XBY’, ‘XQCB’, ‘XQCH’, and ‘XJH’.

Identification of the 11 nonheading Chinese cabbage accessions using ISSR molecular markers. PCR amplification of the 11 nonheading Chinese cabbage accessions was performed using 48 ISSR primers. A total of 12 primers with clear bands, high polymorphism, and good repeatability were screened. A total of 96 clear bands were obtained, with 8.0 for each primer on average. Of these bands, 55 were polymorphic bands, with 4.6 for each primer on average. The percentage of polymorphic bands was as much as 57.3%. Among the 12 primers, ISSR-807 and ISSR-834 amplified the most bands at 11 for each primer, whereas ISSR-827 amplified the fewest at five. ISSR-891 amplified the greatest percentage of polymorphic bands, which was up to 100.0%.

The genetic diversity analysis showed that the average number of effective alleles of the 11 nonheading Chinese cabbage accessions was 1.2884. H was 0.1773 and I was 0.2738, falling in between the self-pollinated species and the outcrossed species. The GST of the 11 accessions generated from the 11 accessions was 1.2884. H was 0.1773 and I was 0.2738, falling in between the self-pollinated species and the outcrossed species.

Comparing the phylogenetic tree reconstructed by the AG07 and BcMF15 genes with the dendrogram based on ISSR markers, the genetic relationships of ‘SZQ’, ‘ZQB’, ‘SHQ’, ‘SYM’, and ‘WYM’ were consistent. In the phylogenetic tree, ‘XJH’ and ‘XBY’ clustered with ‘XQCH’ and ‘XQCB’, whereas in the dendrogram, it was represented as a single cluster (‘XJH’) or clustered with ‘HXW’ (‘XBY’). ‘AJH’, which clustered with ‘HXW’ in the phylogenetic tree, was a single cluster in the dendrogram.

Establishment and validation of specific molecular markers for ‘Suzhouqing’. Among the ISSR primers, ISSR-880 was screened as the specific primer to identify accession ‘SZQ’. The amplified product of this primer was 400 bp (Fig. 3), exhibiting a specific band for ‘SZQ’. The ISSR-specific fragment in ‘SZQ’ was cloned and sequenced. The results revealed that the total length of the fragment was 424 bp, and the nucleotide sequence of the fragment is shown in Fig. 4. The sequence was searched against the B. rapa genome and was revealed to be an intergenic fragment.

A pair of SCAR primers was designed as SCAR-F and SCAR-R (underlined in Fig. 4), corresponding to the 133 to 153-bp and the 362 to 383-bp regions, respectively. The

Fig. 1. Maximum-likelihood phylogenetic tree of 11 nonheading Chinese cabbage accessions based on AG07 and BcMF15 genes. For definitions of acronyms, refer to Table 1. Images of each accession were placed in the tree accordingly.
designed SCAR primers were verified in 88 individuals of the 11 nonheading Chinese cabbage accessions. The results revealed that the primers amplified a clear target band of 251 bp in all eight individuals of ‘SZQ’, whereas the specific band was not produced in any of the other 10 accessions (Fig. 5).

Discussion

Genetic relationship among nonheading Chinese cabbage accessions. The 11 nonheading Chinese cabbage accessions in this study are cultivated as landraces under strong artificial selection for a long time. Thus, genetic differentiation is rather high, with virtually no gene exchange. Based on horticultural classification, the 11 accessions of nonheading Chinese cabbage are divided into the var. *communis* group (which includes ‘SZQ’, ‘SHQ’, ‘ZQB’, ‘SYM’, ‘WYM’, and ‘AJH’) and the var. *tai-tsai* group (which includes ‘HXW’ and ‘XBY’). The plants of the var. *communis* group are erect with smooth leaves, whereas those of the var. *tai-tsai* group are prostrate or semiprostrate with wrinkled leaves. Although ‘XQCH’, ‘XQCB’, and ‘XHJ’ belong to the var. *communis* group, the plants display a semiprostrate and wrinkled-leaf morphology.

In this study, the genetic relationship of accessions ‘SYM’, ‘WYM’, ‘SHQ’, ‘ZQB’, and ‘SZQ’ were consistent based on two single-copy nuclear genes and the ISSR markers. These five accessions were cultivated in Nanjing, Suzhou, and Shanghai with close geographic locations. Our results revealed a very close genetic relationship among these accessions, which could be also inferred from sequence-related amplified polymorphism (SRAP) and simple sequence repeat markers (Han et al., 2007; Ma et al., 2012). The other accessions showed little difference in the topology between the phylogenetic tree and the dendrogram, especially for those in the var. *tai-tsai* group. Two main factors might account for this inconsistency. One is that a hybridization event occurred in some accessions, such as ‘HXW’, during artificial selection, leading to lineage confusion and uncertainty. The other is that, compared with the nonspecific molecular ISSR markers, the single genes—especially those highly related to agronomic traits—should have experienced directional selection during artificial cultivation. So the evolutionary rate varied between these two.

Fragrant Bok choy, which has a rich aromatic flavor, is a unique and rare variety of nonheading Chinese cabbage with a long cultivation history of more than 100 years. In this study, ‘XQCH’, ‘XQCB’, and ‘XHJ’ represent the three major accessions of Fragrant Bok choy. Their morphologic differences reside only in color of the leaf and vein, richness of the flavor, and amount of fiber content. The results of this study revealed that these three accessions exhibited a similar genetic background. ‘XBY’ and ‘HXW’,
both belonging to the var. tai-tsai group, were more closely related to the three accessions of Fragrant Bok choy of the var. communis group, which is consistent with similarities in their morphologic characteristics: wrinkled leaves and prostrate plant. Accession ‘AJH’ is one exception, exhibiting a close genetic relationship with ‘HXW’ in the phylogenetic tree but a most distant genetic relationship with all the other accession in the dendrogram. ‘AJH’ is a landrace in Nanjing, Jiangsu Province. It is the shortest, almost prostrate, accession among the var. communis group. This accession exhibited a close genetic relationship with ‘HXW’, which is a semiprostrate accession in the var. tai-tsai group, indicating that the prostrate trait of the nonheading Chinese cabbage may be derived from the same ancestor. The analysis based on SRAP also exhibited consistent results (Han et al., 2007). Cao et al. (1997) proposed that semiprostrate var. tai-tsai, such as ‘HXW’, is the hybrid of prostrate var. tai-tsai and var. communis. Could be ‘AJH’ also the product of this hybridization event? This was further supported by Cao et al. (1997), who indicated that in the var. communis group, the green stem is an original trait whereas the white stem is an evolved one. And in our study, only ‘AJH’ exhibit white stems, indicating that this accession might indeed have undergone a special evolutionary process.

5’-TGGAGAGGAGAGGAGACTAATTTGATCTCTCAATCTCAATATCGAAAA CGCTGCCGGAGAAAACATCGTTTAGCGGCAGGACTAATCTTTGCTGTCGAC GGAGCTGGAAGCAGCTCTTTTTCTCTCCAGATCTCTAACCAGTTCTCCTCA CCTCTCTCTGTTTGACCCTTGGTTTTGTCTCGCTACTTTTGGATCTCTTTTC CTCAGCTATTGCGATATTCCGATTGCCTGATCGTCATTTTTCTCGAA TCCATTGTCATGGACAAATCTGTGATTAGATGCAGCCACGATAGA TCGAAACGTCCGGTTTTCCAGATTTAATAACCTCGTTGACAGATCAGTA AACATGCTTTACGATTGATGAAAAACGGCTATAACGTTACATTTACCGATT GGATCTTCTCTCTCTCTCCA-3’

Fig. 4. The nucleotide sequence of a Suzhouqing-specific fragment amplified using primer intersimple sequence repeat-880. The underlined sequences were designed sequence-characterized amplified region primers.

Fig. 5. Verification of the Suzhouqing-specific intersimple sequence repeat–sequence-characterized amplified region primers by polymerase chain reaction (PCR) in eight individuals from each of the 11 nonheading Chinese cabbage accessions. (A–K) PCR results from the eight individuals of ‘SZQ’, ‘ZQB’, ‘WYM’, ‘XQCH’, ‘AJH’, ‘SYM’, ‘HXW’, ‘SHQ’, ‘XBY’, ‘XQCB’, and ‘XHJ’, respectively. M, DL2000 DNA marker. Lanes 1 through 8 are PCR products from eight individuals.
Molecular markers for the identification of ‘Suzhouqing’. In this study, the universal sequences of matK, rbcL, psbA-trnH, and internal transcribed spacer (ITS) were first used to differentiate the 11 accessions, and no variations were discovered (data not shown). Then, the leaf formation-related gene AG07, and the male sterile-related gene BcMF15, were used in the study. The results showed that ‘Suzhouqing’ could be identified specifically from the other 10 accessions based on 21 specific nucleotide variations of the AG07 gene. As one of the major targets of cultiva- 
tion, the leaf shape might have undergone strong artificial selection (Li et al., 2016). During the long-term cultivation of nonhead- 
ing Chinese cabbage, the AG07 gene has undergone relaxed purification selection stress (Ka/Ks = 0.17 in our study; Ka/Ks ratio between the number of nonsynonymous substitutions per nonsynonymous site (Ka) and the number of synonymous substitution per synonymous site (Ks)). And rich variations have accumulated in the 11 accessions of nonheading Chinese cabbage [τ = 0.01040, comparable with 0.00472 in 150 accessions in Liang et al. (2016)]. Those nucleotide variations serve as reservoir and play an important role in the formation of leaf types in nonheading Chinese cabbage. The gene sequences of the two groups of nonheading Chinese cabbage with wrinkled leaves or smooth leaves were divided into two distinct groups (Fa = 0.594*), indicating a significant correlation between the leaf morphology and the AG07 genotype. As one of the regulators in leaf adaxial/abaxial patterning, AG07 might serve as one of the target genes of selection during the diversification of leaf morphology shape in nonheading Chinese cabbage, which is worth paying more attention to for agronomists.

The SCAR marker developed based on ISSR markers can uniquely identify accession ‘SZQ’ in 11 popular accessions in the Yangtze River Delta, with an identification efficiency of 100%. Compared with the traditional morphology-based or nonspecific molecular markers, this PCR-based identification technique has the advantages of high accuracy, good reproducibility, stability, and reliability. Definitely, further studies should be carried out for the application of this specific marker in more nonheading Chinese cabbage accessions. ‘Suzhouqing’ is a unique landrace with a long history of cultivation in Suzhou, Jiangsu Province. The development of molecular markers for its specific identification also provides a theoretical basis for the protective identification of other agricultural crops.

Conclusions

‘Suzhouqing’ could be specifically identified from the other 10 popular accessions in the Yangtze River Delta based on 21 specific nucleotide variations of the AG07 gene. Sequence variations show a strong correlation with leaf morphology, suggestive of partial causal links between the two. ISSR-880 was screened as the specific primer to identify accession ‘Suzhouqing’, and a specific discrimination ISSR-SCAR marker was explored, which amplified no target band in any other accessions. The development of molecular markers for the specific identification of ‘Suzhouqing’ could provide a theoretical basis for the protective identification of other important agricultural crops.

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