Full Length Research Paper

Genetic diversity and relationships among cabbage (Brassica oleracea var. capitata) landraces in China revealed by AFLP markers

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Genetic diversity and relationships among Chinese traditional cabbage landraces have not yet been well investigated. To explore the diversity, 83 landraces originating in Northern China, Southern China, Eastern Europe, Western Europe as well as other countries were evaluated by using AFLP markers. Results indicated that cabbage landraces exhibited a relatively low level of diversity. Among the 575 markers, 41.9% were polymorphic with an average PIC value of 0.354. Unweighted pair group method with arithmetic averages (UPGMA) cluster and population structure analysis consistently divided all landraces into two major groups reflecting geographic origins. Group 1 was a distinct group comprised of Northern China landraces and Eastern Europe landraces, whereas Group 2 was comprised of populations of Southern China landraces, Western European populations and other countries. Landraces with varied maturing times or head types could not be distinguished based on molecular data. The Northern China population was closely allied to the Eastern Europe population (D = 0.037). The integration of our data with historical documents confirmed that traditional cabbage landraces cultivated in North of China were first introduced from Russia.

Key words: Amplified fragment length polymorphism (AFLP), genetic diversity, cabbage (Brassica oleracea var. capitata), landraces, population structure.

INTRODUCTION

Cabbage (Brassica oleracea var. capitata) is one of the most widely grown and important vegetable crops consumed worldwide and are particularly widespread in many agricultural regions of China. More than 937 thousand hectares cabbage is planted in china every year (Fang, 2008). Although, local landraces have been widely cultivated in many Chinese provinces prior to the 1970s, during the last 40 years, these populations were rapidly replaced by many modern hybrid cultivars developed from genotypes with a restricted genetic base. The traditional local cabbage landraces distributed primarily throughout Northern China exhibit many high quality agronomic traits including disease resistance and ecological adaptation following a long history of natural and artificial selection in China. For instance, in the past four decades, most of the commercial cabbage hybrid cultivars in China were developed by crossing a traditional cabbage landraces with a newly introduced foreign cabbage breeding line (Fang et al., 2002). One of the most important black rot resistant cabbage accession PI436606 was developed from a Chinese traditional landrace Heiyedapingtou cultivated in North of China before 1970s (Dickson and Hunter, 1987).

To preserve the local germplasm, China launched a large scale nationwide germplasm collection for traditional head cabbage landraces. At the beginning of the 1980s, there were totally 221 head cabbage landraces

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collected and deposited into the National vegetables Gene Bank at the Institute for Vegetable and Flowers (IVF) of the Chinese Academy of Agricultural Sciences (CAAS). Some of them have been used as breeding materials of hybrid cultivars such as Heyexiaopingtou, Jingzhaoshen, Heyepingtou, Jixinganlan, Niuxinganlan, Dapingtou, Erwuye, Nanmuye and Erhutou. However, most of them have not been fully utilized up to till now.

Thus, to explore the promising breeding materials in the cabbage landraces conserved in China and improve cabbage varieties adapted to various biotic and abiotic stresses, genetic diversity, gene communication and cultivar exchange between different countries and planting regions must be explored to address these aims.

Several methodologies have shown efficacy in assessing genetic diversity within and among populations, including morphologic traits and isozymes. Among these molecular approaches, RAPDs have been the most commonly used PCR-based fingerprinting technique applied to analyze genetic diversity in *B. oleracea* to date (Hu and Quiros, 1991; Kresovich et al., 1992; Santos et al., 1994; Margalé et al., 1995; Lanner-Herrera et al., 1996; Phippen et al., 1997; Lazaro and Aguinalde, 1998; Koutita et al., 2005), largely due to its simplicity, efficiency in performance and no requirement for sequence generation. Recently, simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) have been advocated as the most common and effective marker systems for genetic analyses in different Brassica species cultivars (Sobotka et al., 2004; Tonguc and Griffiths, 2004; Zhao et al., 2005; Louarn et al., 2007; Van Hintum et al., 2007; Mei et al., 2010). Compared with codominant SSR, although, AFLPs might be associated with the inability to distinguish heterozygotes from homozygotes, many research suggested that the genetic estimates based on AFLPs and SSRs are highly correlated because the high numbers of polymorphic loci counterbalance the loss of information resulting from dominance (Powell et al., 1996; Gerber et al., 2000; Baraket et al., 2011; Mei et al., 2010). Recently, Van Hintum (2007) conducted research on the genetic diversity distribution in a selection of very similar groups of Dutch white cabbage accessions and confirmed that AFLP markers are extremely sensitive and especially possessed great utility in measuring minor genetic changes between some of the more similar cabbage accesses.

Although, lots of works on genetic diversity analysis in *B. oleracea* have been reported, most of them pay attention to the diversity among different subspecies. To date, little work has been reported to evaluate genetic diversity and relationships between local cabbage landraces in China. In this paper, we used the fluorescence-based AFLP approach to investigate genetic diversity and relationships among head cabbage landraces collected from different planting regions of China. Such information will be of great interest for future genetic improvement of heading cabbage in China.

**MATERIALS AND METHODS**

**Plant materials**

A total of 83 heading cabbage landraces were obtained from the Institute for Vegetable and Flowers (IVF) at the Chinese Academy of Agricultural Sciences (CAAS), including 43 landraces collected from the provinces to the north of the Yangtze River (Northern China Population), 10 landraces from areas to the south of the Yangtze River (Southern China Population), 13 landraces from Russia and Ukraine (Eastern Europe Population), six landraces or open pollinated cultivars from Netherlands, Denmark and Germany (Western Europe Population) and 11 from Africa, India, Japan and Korea (here referred to as other countries population). All accesses, origins and main morphology are summarized in Table 1. All accesses were raised in the field at the IVF experimental center. Young accessions were collected from a pool of five plants of each accession and used for DNA preparation.

**DNA extraction and AFLP analysis**

Total genomic DNA was extracted from young leaves using the protocol of Doyle and Doyle (1987) with minor modifications. A total of 300 mg of fresh plant material was ground and initially extracted in tubes containing CTAB extraction buffer (100 mM Tris-pH 7.5, 700 mM NaCl, 50 mM EDTA pH 8.0) and iron bullets in a Retsch shaker. Two more extraction steps were preformed with chloroform/isooamlic alcohol (24:1). The DNA of each sample was retained in a final chloroform extraction and subsequently diluted in 100 µl dd water.

AFLP fingerprints were generated based on the protocol described by Vos et al. (1995) with minor modifications. Total genomic DNA (300 to 500 ng) was digested using two restriction enzymes (*EcoR*I and *Mse*I) and then ligated to adaptors. Pre-amplifications were carried out using E00 (5’-GACTCGCTAGGATCCATTC-3’) and M00 (5’-GATGAGTCCTGAGTAA-3’) primers. Selective amplification was performed with primers having three selective nucleotides each. Only E-NNN primers were labeled with IRD-700 or IRD-800 at the 5’ end for selective amplification. In order to identify primer combinations that yielded well scorable polymorphisms approximately, 80 primers were tested on six samples. Finally, 12 suitable combinations were selected for further analysis based on the number of unambiguously scorable polymorphic bands (Table 2). The AFLP amplification products were analyzed with a Li-COR model 4200 dual-dye automated DNA sequencing system. Electrophoresis conditions and data collection were as described by Myburg and Remington (2000)

**Data analysis**

All AFLP bands were treated as dominant markers and all weak and unresolved bands were discarded. Only clearly distinguishable polymorphic bands in the range of 50 to 500 bp were scored as present (1) or absent (0) to generate an binary data matrix for genetic analysis.

POPGENE version 1.32 (Yeh et al., 1999) was used to calculate Nei's genetic diversity (Nei, 1973) and Shannon-Weaver diversity index (I) (Shannon and Weaver, 1949) within populations. The probability of a polymorphism between two random genotypes (the polymorphism information content or PIC) was estimated using the following formula:
Table 1. List of accessions.

<table>
<thead>
<tr>
<th>Accession name</th>
<th>Label</th>
<th>Accession number</th>
<th>Type of line</th>
<th>Origin</th>
<th>Accession name</th>
<th>Label</th>
<th>Accession number</th>
<th>Type of line</th>
<th>Origin</th>
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<td>Damian dongganglan</td>
<td>Dam</td>
<td>V04A0213</td>
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<td>Liu</td>
<td>V04A0013</td>
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<td>V04A0125</td>
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<td>Gansu</td>
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<td>L</td>
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<td>L</td>
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</table>
Table 1. Data recorded for the 12 AFLP primer combinations employed to detect polymorphisms among 83 heading cabbage landraces.

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>Number of band</th>
<th>Polymorphic band</th>
<th>Polymorphism rate (%)</th>
<th>PIC</th>
</tr>
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<tbody>
<tr>
<td>E-AGG/M-CCT</td>
<td>39</td>
<td>10</td>
<td>25.6</td>
<td>0.339</td>
</tr>
<tr>
<td>E-AQC/M-CGC</td>
<td>19</td>
<td>6</td>
<td>31.6</td>
<td>0.412</td>
</tr>
<tr>
<td>E-AGT/M-CCG</td>
<td>52</td>
<td>19</td>
<td>36.5</td>
<td>0.303</td>
</tr>
<tr>
<td>E-AGG/M-CCG</td>
<td>81</td>
<td>49</td>
<td>60.5</td>
<td>0.333</td>
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<tr>
<td>E-ACC/M-CCT</td>
<td>48</td>
<td>30</td>
<td>62.5</td>
<td>0.318</td>
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<tr>
<td>E-AAG/M-CCC</td>
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<td>28</td>
<td>42.4</td>
<td>0.345</td>
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<tr>
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<td>37</td>
<td>74.0</td>
<td>0.348</td>
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<tr>
<td>E-AQC/M-GAG</td>
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<td>7</td>
<td>38.9</td>
<td>0.412</td>
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<tr>
<td>E-AGA/M-CCG</td>
<td>43</td>
<td>18</td>
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<tr>
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<td>E-AGA/M-CCT</td>
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<tr>
<td>E-ACC/M-CTG</td>
<td>65</td>
<td>18</td>
<td>27.7</td>
<td>0.301</td>
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<tr>
<td>Total</td>
<td>575</td>
<td>251</td>
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<tr>
<td>Average</td>
<td>47.9</td>
<td>20.9</td>
<td>41.9</td>
<td>0.354</td>
</tr>
</tbody>
</table>

\[
\text{PIC} = 1 - \sum_{i=1}^{k} P_i^2
\]

Where, \( k \) is the total number of alleles detected for a given marker locus and \( P_i \) is the frequency of the \( i \)th allele in the set of genotypes investigated (Anderson et al., 1993).

Similarity was calculated as the proportion of AFLP markers where the comparison of two accessions exhibited the same score (SM_{xy} = (n_{11}+n_{00})/n) and where \( n \) was the number of markers scored. Cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA). Dendrograms were constructed using the UPGMA algorithms in the MEGA 4.0 software (Tamura et al., 2007).

A Bayesian approach with the program structure 2.2 was used to identify population groups (Falush et al., 2007; Pritchard et al., 2000) The number of population clusters (\( K \)) was set to vary between 1 and 10 with 600,000 Markov Chain Monte Carlo (MCMC) iterations and a burning period of 60,000. The value of \( K \) with the highest likelihood was selected as the optimal number of clusters in the sample at which, Pr(X/K) no longer increased with increasing values of K.

All cabbage landraces were subdivided into five geographical populations (Northern China, Southern China, Eastern Europe, West Europe and other countries) according to the accession country of origin. A classical analysis of molecular variance (AMOVA) within and among population components was analyzed using WINAMOVA 1.55 (Excoffier, 1992).

RESULTS

Levels of polymorphism

In this study, 12 pairs of EcoRI/MseI primers, which were
selected from 128 pairs of primer combinations, showed
clear banding patterns with notable polymorphisms. These
primer pairs were used to fingerprint 83 *B. oleracea*
accessions of different geographical origins. A total of 575
scorable amplification products ranging from 50 to 600 bp were
obtained, of which 251 were polymorphic with an average of 20.9 polymorphic
bands per primer combination. The levels of polymorphism were
calculated based on the percentage of polymorphic bands,
which varied from 24.4% for the E-AGA/M-CCT primer combination to 74.0% for the E-ACA/M-CCT primer combination (Table 2). The polymorphism content
estimation information for the 12 primer combinations ranged from 0.301 to 0.412, with an average of 0.354.

Table 2 shows the selected primers, number of polymorphic bands, rate of polymorphism and PIC among
accessions.

### Genetic relationship and population structure among all landraces

In this study, all possible pairwise comparisons were
used to assess genetic similarities of the 83 head
cabbage based on the 251 polymorphic AFLP markers; a
high range of similarity among landraces was observed.
The genetic similarities ranged from 0.413 in a pairs of
genotypes ('Da pingtoerhao' versus 'Kaifengnixiu') to
0.933 in a pair of genotypes ('Sumuqinerhutou' versus
'Erheiganlan'), with an average value of 0.736.

A UPGMA dendrogram was generated to evaluate
similarity values among landraces (Figure 1). The
resulting dendrogram resolved two major clusters of
genotypes with low bootstrap values. However, general
interpretations can be made from the results. The
Chinese landraces from the Northern provinces of China
(36 of 43 or 84%) and Eastern European landraces (5 of
8 or 63%) were allied in Group 1. Group 2 was comprised of
the Chinese landraces in the Southern provinces of China
(7 of 10 or 70%) and Western European landraces
(4 of 6 or 67%). The landraces from other countries
(Africa, India, Japan and Korea) were distributed in both
groups. No obvious clustering based on morphotypes
was evident, because landraces with various maturing
times or head types were not clearly distinguished based
on molecular data. These results suggested that most
polymorphisms do not contribute to the phenotypic variation in head cabbage.

Population structure generated similar results using
structure 2.2 software (Figure 1). The results indicated
that a K=2 value was the best average assignment rate.
The software provided the coefficients of estimated
ancestry per individual in each group. In the plot of
ancestry estimates shown in Figure 1 (parallel to the
UPGMA dendrogram), each individual is represented by
a single horizontal bar broken into two segments, with
lengths proportional to the individual’s estimated ancestry
fraction from each of the two groups. Model-based
groups were congruent with dendrogram classifications.

The ‘red bar’ group corresponds to the first cluster in
the UPGMA dendrogram (Group 1), while the ‘green bar’
corresponds to the second cluster (Group 2). Population
composition of Groups 1 and 2 generated by structure 2.2
showed a more defined delimitation supporting different
geographical origins. Group 1 included 51 landraces,
representing the Northern China population (represented by 37 landraces) and the Eastern European population
represented by 10 landraces). 32 landraces comprised
Group 2 and circumscribed the Southern China popu-
lation (represented by 7 landraces), the Western
European population (represented by 5 landraces) and
the other countries population (represented by 11
landraces).

### Genetic diversity among geographical populations

Genetic diversity among cabbage landraces was
congruent with geographical origins based on AFLP data. To further explore the genetic diversity among the five
geographical populations, POPGENE 1.32 was used to
calculate Nei’s genetic diversity (h) and the Shannon-
Weaver index (I). Total genetic diversity (h) and the
Shannon-Weaver index (I) for all cabbage landraces was
0.317 and 0.483, respectively. Genetic diversity was
highest in the Northern China population, with a mean h-
value of 0.319 and I-value of 0.477. These measures
were lower in the Southern China population, which
exhibited an h-value of 0.261 and I-value of 0.398.
AMOVA partitioned population genetic diversity and
indicated that the major portion of genetic diversity was
within geographical populations, even when all the land-
races were analyzed together (Figure 2a) or only Chinese
landraces (Figure 2b) were analyzed independently.
Genetic differentiation between different geographical
origins was extremely low. This result suggested that
local differentiation in Chinese head cabbage landraces
did not arise following introduction into China. Low
genetic differentiation among landrace populations was
confirmed by high gene flow (Nm = 3.509).

### Genetic relationships among geographical populations

Gene differentiation between the five geographical
populations was further explored by generating estimates
of genetic distance based on AFLP allele frequencies.
Pairwise comparisons of the five populations revealed
high genetic identity values ranging from 0.906 to 0.964,
which also indicated high similarity and closer genetic
distance between different geographical populations.
Relationships between populations were further
illustrated by a UPGMA dendrogram, based on Nei’s
Figure 1. UPGMA dendrogram and population structure analysis of the 83 head cabbage landraces based on AFLP data. Numbers on the branches correspond to bootstrap values (values smaller than 30 were not included). Estimated population structure of each landrace is represented by a horizontal bar, which is partitioned into two colored segments that represent the individual estimated levels of the two groups.

The dendrogram showed that the Northern China population was closely related to the Eastern Europe population genetic distance (0.037), but distant from the Southern China population (0.059). Conversely, the Southern China population showed a close genetic relationship to cabbage landraces of other countries (0.047), but appeared to be more distant from the Eastern Europe population (0.087) and Western...
Figure 2. Summary of the genetic differentiation ($\Phi_{st} = F_{st}$) among and within groups as determined by AMOVA. (A) All landraces together; (B) China landraces (1,000 permutations); $P < 0.001$.

Figure 3. UPGMA cluster analysis dendrogram based on Nei’s genetic distances among the five populations of head cabbage landraces.
Europe population (0.086).

**DISCUSSION**

This study makes the first report of the investigations into genetic diversity in a collection of 53 Chinese head cabbage landraces compared with 30 representatives from Europe, Africa, India, Japan and Korea. The rates of polymorphisms were relatively lower (41.6%) compared with polymorphisms (70%) between *B. oleracea* subspecies (Farnham et al., 1996), which demonstrated that head cabbage landraces are very similar in genotype and had relatively low diversity among all genotypes analyzed. PIC has been used in marker comparison studies analyzing levels of polymorphism in various *B. oleracea* subspecies (Tonguç and Griffiths, 2004; Louarn et al., 2007). The PIC value of dominant markers ranged from zero for monomorphic markers to 0.5 for markers that are present in 50% of the plants and absent in the other 50% (Anderson et al., 1993). In our work, relatively lower PIC values (average 0.317) suggested that *B. oleracea* var. *capitata* cultivars represent populations of low genetic diversity. This result confirmed the narrow genetic diversity observed in main cabbage breeding materials reported in a previous investigation (Fang et al., 2002).

The UPGMA dendrogram and population structure analysis divided all 83 head cabbage landraces into two groups. Group 1 was a distinct group of Northern China and Eastern European landraces (mainly from Russia and Ukraine), whereas Group 2 is primarily comprised of landraces from the Southern China population, Western European population and other countries. Northern China landraces and Eastern Europe landraces are intermingled in Group 1 and do not form a clear sub-cluster between the eco-geographical populations. Similar results were observed in Group 2. These observations suggested that landraces from the North of China possess a relatively close relationship to that from their Eastern European neighboring countries. The differentiation between Northern China and Southern China landraces was suggestive of different ancestry and cultural history of head cabbage in the Northern and Southern areas of China.

Another important observation was that landraces with varied maturing times or head types could not be definitively distinguished based on molecular data. The incongruity between morphological and molecular data can be explained by the absence of significant genetic distance between these main economical traits in cabbage cultivars relative to diversity in geographical origin. Most polymorphisms observed did not contribute to phenotypic variation, which indicated that only a few genes are involved in the favored economical traits.

To further explore the genetic diversity within and among geographical populations, POPGENE 1.32 was used to calculate Nei’s gene diversity (*h*) and the Shannon-Weaver diversity index (*I*). The results indicated that the old China landraces had a relatively higher genetic diversity, which revealed a more valuable gene pool in the landraces reserved at IVF. Partitioning of population genetic diversity among geographical populations showed that the major portion of genetic diversity was within geographical populations and the genetic differentiation between different geographical origins was extremely low.

Many factors, including breeding-system, seed exchange and agricultural practices influence genetic diversity, including the proportion of variation distributed within and between populations (Hamrick and Godt, 1996). The high genetic similarity among different geographical populations can be explained by the short cultural history of head cabbage and the active exchange of seeds among different countries.

Late-maturing cabbages are the oldest group of head cabbages (http://www.history.org/history/CWLand/resrch3.cfm) and have been popularly planted at large scales in the north of China in the 19th century, according to historical documents in China (Wu, 1848). To date most of the head cabbage landraces reserved in IVF were originated in Northern China (78.9%). One of the most valuable observations of this study was that the Northern China population had closer relationships with the Russia and Ukraine landraces (representing the Eastern Europe populations) and relatively lower relationships with other European countries and the Africa, India, Japan and Korea populations.

There are different opinions about the cultivation history of cabbage in China. Some Chinese researchers believed that Chinese traditional head cabbage was first introduced from Russian (Jiang, 1981). Alternatively, some others suggested that head cabbage was first introduced from the Netherlands or some other Western European country through the south of China (Ye, 1986). Based on molecular data, the close genetic relationships between head cabbage landraces originating in Northern China and Eastern European countries confirmed the Chinese historical documents relative to the cultivation history of head cabbage by the end of 17-century (Fang, 1690). Due to the significantly lower levels of diversity within the Southern China population, we can presume a fairly shorter history of head cabbage cultivation in the south compared with the north. Combining the historical documents to date, we support the hypothesis that head cabbage was first introduced from Europe through the Mediterranean Sea, the Middle Asian Region, Russia and then to different parts of North China by the end of the seventeenth century.

In conclusion, we presented a profile of the genetic diversity among cabbage landraces of different geographical origins. Our findings also provided a systematic reference for future cabbage breeding programs, contri-
buting to the traditional China head cabbage germplasm bank. In addition, we also contributed information regarding the genetic diversity and allocation of possible heterotic groups. For the last 50 years, the majority of commercial hybrid head cabbage cultivars in China has been and is presently crossed with cultivars introduced from outside China. The literature did not provide much information regarding the diversity among traditional China landraces and landraces from outside China. Our study analyzed 83 China landraces from varied geographical sources and disclosed another important genetic pool in the limited head cabbage germplasm. The divergence exhibited in this group provided us valuable information regarding the relationship among these accessions and will assist breeders in generating new F1 hybrids.

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