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RESEARCH ARTICLE

Evaluation of genetic diversity within asparagus germplasm based on morphological traits and ISSR markers

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Abstract *Asparagus officinalis* L. is a dioecious perennial plant globally known for its fine flavor and high nutritional value. An evaluation of genetic diversity in 46 asparagus accessions was carried out based on morphological and inter-simple sequence repeat (ISSR) markers. The result show that the coefficient of variation for 20 morphological characteristics is between 12.45 and 62.22%. Factor analysis revealed that nine factors explained 83.37% of the total variance. At Euclidean distance of 135.7, 46 accessions were divided into two clusters. Genetic similarity coefficient (GSC) based on ISSR data ranged from 0.60 to 0.97, suggesting a relatively abundant genetic base. Furthermore, the 46 asparagus accessions could also be grouped into three major clusters at a GSC of 0.74. And there is no significant relation between the two marker systems using the Mantel test. Clustering based on morphological traits compared with that based on ISSR data was not consistent, however, some common groupings were observed between two dendrograms. Therefore the results elucidated asparagus germplasm genetic background and determined hybrid parents, which will facilitate optimal application of asparagus germplasm resources and provide additional data for genetic improvement.

Keywords Asparagus · ISSR · Morphological traits · Genetic diversity · Genetic relationship

Introduction

The genus *Asparagus* of Asparagaceae family comprised about 200 species, which was divided into the subgenera *Asparagus, Myrsiphyllum* and *Protasparagus* (Clifford and Conran 1987). As asparagus is dioecious perennial plant, *Asparagus officinalis* L. is the economically most important species (Sarabi et al. 2010), which is cultivated widely in the world mainly in South and Central America, China and Europe (Prohens et al. 2008). Asparagus known as the "King of Vegetables" is one of the top 10 most popular vegetables because of unique texture and taste and high nutrition (Baxter et al. 2003). Asparagus is not only rich in proteins, selenium, and choline, but also in ascorbic acid, B vitamins, sapogenin and other phenolic compounds, which are responsible for various bioactive properties (del Árbol et al. 2016). Due to rapid development in recent years, China has become the leading producer of asparagus with a total cultivated area of 100,000 ha in 2010 (Chen et al. 2013).

Genetic diversity is usually evaluated by morphological and molecular markers (Muthusamy et al. 2008).

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Morphological characterization is fit to exploit the traditional approaches used for assessment of germplasm resource diversity with the advantages of economy, simple and intuition, although morphological characterizations are also usually susceptible to environmental influence (Vithanage et al. 1995; Linda et al. 2009; Singh et al. 2010). Moreover morphological variation is positively related to genetic diversity and can provide many valuable information for breeders (Moose and Mumm 2008).

Molecular markers are often used to characterize genetic diversity (Dalirsefat et al. 2009). Dominant markers are Amplified Fragment Length Polymorphisms (AFLP) and Random Amplified Length Polymorphisms (RAPD), however co-dominant markers are Simple Sequence Repeats (SSR), Restriction Fragment Length Polymorphisms (RFLP) and Inter Simple Sequence Repeats (ISSR) (De Vicente and Fulton 2003). ISSR markers are quick, highly reproducible, simple and polymorphic highly without prior information of genomic sequence, which only required small amounts of DNA (Bornet and Branchard 2001; Buhulikar et al. 2004; Coetes and Byrne 2005). Compared to other markers such as RFLP, SSR, RAPD, ISSR reveal more polymorphism (Marsh and Ayres 2002). They are more efficient than RAPD, simple than AFLP, SSR and RFLP, and also cheap than AFLP (Zietkiewicz et al. 1994; Domyati et al. 1996; Powell et al. 1996; Fang and Roose 1997; Kojima et al. 1998; Moreno et al. 1998; Mattioni et al. 2002; Liu et al. 2008; Wang et al. 2009, 2013; Denduangboripant et al. 2010).

Morphological/biochemical markers and molecular markers such as RAPD, SCAR and EST-SSR have been used to evaluate genetic diversity (Geoffriau et al. 1991; Jiang and Sink 1997; Sarabi et al. 2010; Marco et al. 2008; Castro et al. 2014). The genetic diversity of asparagus double haploids and wild relatives was also evaluated using AFLP markers (Casali et al. 2011). Additionally, genetic maps for asparagus have been constructed using RFLP, RAPD, AFLP, SNP and EST-SSR (Spada et al. 1998; Francesco et al. 2013). However, to date there are no systematic records of the genetic background, phylogenetic relationships, and sources of diversity for Chinese asparagus accessions, which has restricted the optimal application of asparagus germplasm resources for asparagus hybrid breeding due to insufficient asparagus genotype information (Zhou et al. 2012).

In the present research, the aim was to assess the genetic relationships between asparagus germplasm resources in China based on morphological traits and ISSR markers and thus provided additional data on their possible practical value, which could be useful for further asparagus genetic improvement.

Materials and methods

Plant material

Asparagus germplasm resources were kindly provided by Dr. Chen Guangyu (Vegetable and Flower Institute, Jiangxi Academy of Agricultural Sciences, Nanchang, China) (Table 1), and were planted in July 2011 in Danzhou, Hainan province. Danzhou's climate is a tropical monsoon climate with average annual temperature 23.9 °C, annual rainfall 1763.2 mm and annual average humidity 83%. The soil is lateritic soil developed from granite. These asparagus plants were grown in greenhouses and watered twice a week. Thirteen accessions consisted of JK101–JK107, TC, 082, 097, 0914, NJ1189 and NJ1123 are population, other 33 accessions are commercial varieties. The Leaf samples were collected from forty-six asparagus accessions The leaf samples were collected by bulk sampling with thirty plants of each accession and then stored at − 80 °C until DNA extraction.

Twenty morphological characteristics were investigated and analyzed (Table 2) in the same asparagus field of leaf samples. The experiments were carried out in ten replicates using completely randomized design, one replicate was a plant, then ten plants from each accession were selected randomly, observations were taken on according to the criteria in Table 2 for each plant.

Isolation of genomic DNA

Total genomic DNA extraction from each sample (mixture of 10 plants of the accession) was done by a modified CTAB method (Yan et al. 2008; Wu et al. 2008). The concentration and purity of the DNA samples were assessed using a UV spectrophotometer. DNA was also quantified by electrophoresis, which was performed on a 1.5% agarose gel with 1 × TAE buffer.

PCR amplification

The optimal ISSR system in *Asparagus officinalis* L. was already established and 21 primers were screened from 100 candidate primers with gradient PCR being used to determine the optimal annealing temperature in previous experiments (Table 3). PCR amplifications were performed in 25 mL total volume containing 150 ng of each template DNA, 0.3 μmol/L primer, 15 μL 2 × Taq MasterMIX and DNA-free water. These amplifications were performed in the Biometra T Gradient thermal cycler (Biometra T1 Thermocycler, USA), which was programmed for an initial denaturation step of 5 min at 94 °C, followed by 30 cycles at 94 °C for 45 s, annealing temperatures (varied for each

Table 1 List of *Asparagus* germplasm resources

Serial number	Source	Accession name	remarks	Serial number	Source	Accession name	remarks
1	Walker seed	UC157	Dioecious	24	Jiangxi, China	Jing Kang Hong	Dioecious
2	Walker seed	Atlas	Dioecious	25	Jiangxi, China	JK101	Dioecious
3	Walker seed	Grande	Dioecious	26	Jiangxi, China	JK102	Male
4	Walker seed	Purple Passion	Dioecious	27	Jiangxi, China	JK103	Male
5	Walker seed	Jersey giant asparagus	Male	28	Jiangxi, China	JK104	Male
6	Walker seed	Jersey Supreme	Male	29	Jiangxi, China	JK105	Dioecious
7	Walker seed	Apollo	Dioecious	30	Jiangxi, China	JK106	Dioecious
8	Walker seed	Jersey Knight	Male	31	Jiangxi, China	JK107	Male
9	Limseeds	Avalim	Dioecious	32	Beijing, China	Jing green asparagus No.1	Dioecious
10	Limseeds	Backlim	Dioecious	33	Shandong, China	Shuo Feng	Dioecious
11	Limseeds	Gijnlim	Dioecious	34	Shandong, China	Champion	Dioecious
12	Limseeds	Grolim	Dioecious	35	Shandong, China	TC	Dioecious
13	Limseeds	Herkolim	Dioecious	36	Shandong, China	082	Dioecious
14	Limseeds	Thielim	Dioecious	37	Shandong, China	097	Dioecious
15	AsparaPacific	Pacific Purple	Dioecious	38	Shandong, China	0914	Dioecious
16	AsparaPacific	Pacific Challenger 1	Dioecious	39	Netherlands	Fortems	Dioecious
17	AsparaPacific	Pacific Challenger 2	Dioecious	40	Netherlands	Tallems	Dioecious
18	AsparaPacific	Pacific Endeavour	Dioecious	41	America	NJ1189	Male
19	AsparaPacific	Pacific Crusader	Dioecious	42	America	NJ1123	Male
20	AsparaPacific	Pacific Peak	Dioecious	43	Shandong, China	New century	Dioecious
21	AsparaPacific	Pacific Green	Dioecious	44	Italy	Precoce	Dioecious
22	Canada	Mill	Dioecious	45	Beijing, China	Jing purple asparagus No. 2	Dioecious
23	Jiangxi, China	Jing Kang 701	Dioecious	46	AsparaPacific	Pacific 2000	Dioecious

primer according to Table 3) for 60 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min; the program was held at 4 °C until the tubes were removed. The amplified products were separated by electrophoresis in 1.0% (w/v) agarose gels and at a constant voltage of 110 V in 1 × TAE buffer for approximately 50–90 min. These products were stained with GlodenView (SBS Genetech, China) and then visualized under UV light. Fragments sizes were determined by comparision to size markers (Real-Jimes 100 bp plus DNA ladder).

Statistical analysis

Summary statistics on morphological traits were computed by SPSS 17.0 after data standardizing to estimate germplasms and cluster analysis. Shannon's information index (I) (Lewontin 1972) was obtained by POPGENE 3.2 version 1.32 (Yeh et al. 1999).

Amplification products were transformed into a binary character matrix (1 = presence, 0 = absence). The matrix was then used for the following analyses: the percentage of polymorphic loci (PPB), Nei's gene diversity (h) and Shannon's information index (I) (Lewontin 1972; Nei 1973) using POPGENE 3.2 (version 1.32) (Yeh et al. 1999). The unweighted pair-group method with arithmetic means (UPGMA) was performed using NTSYS2.0 software (Rohlf 2000) and bootstrap analysis was conducted by 1000 replicates. Moreover, Mantel correspondence tests were performed based on the distance matrices in order to estimate the correlation between morphological and ISSR data (Legendre and Fortin2010).

Table 2 Morphological characters analyzed in *Asparagus* accessions

Number	Character	Unit
1	Plant height	cm
2	Plant height to first panicle branch	cm
3	Number of primary branches	–
4	Number of secondary branches	–
5	Internode length of primary branches	cm
6	Internode length of secondary branches	cm
7	Diameter of main stem	mm
8	Diameter of primary branches	mm
9	Diameter of secondary branches	mm
10	Length of primary branches	cm
11	Length of secondary branches	cm
12	Number of scales under the lowest panicle branch	–
13	Spear length	cm
14	Spear diameter	mm
15	Fresh weight of spears	g
16	Number of spear scales	–
17	Length of spear scales	mm
18	Width of spear scales	mm
19	Number of cladodes	
20	Foliage length	mm

Results

Analysis of morphological characteristics

The mean value and ranges of variability of each character are presented in Table 4. There were different degrees of variation in the 20 characteristics, the range of the coefficient of variance (CV) was between 12.45% and 62.22% and the mean coefficient of variation was 28.81%. The range for individual characters varied widely, from 2.84 for "Diameter of secondary branches" to 275.7 for "Plant Height". Internode length of secondary branches (62.22%), fresh weight of spears (62.02%), internode length of primary branches (43.28%), length of secondary branches (39.64%) and diameter of secondary branches (35.87%) were characteristics with a high coefficient of variance. The trait with the smallest coefficient of variance was plant height. The mean Shannon diversity index (I) of morphological characters was 1.99. Number of secondary branches, foliage length, plant height to first panicle branch and length of spear scales were characteristics with a high Shannon diversity index, while the lowest Shannon index was obtained for "Fresh weight of spears".

The existence of significant positive and negative correlations between traits was shown using simple correlation coefficient analyses. For example, plant height to first panicle branch and number of scales under the lowest panicle branch had the highest positive correlation. The characteristics of plant height to first panicle branch, number of primary branches, diameter of secondary branches, length of primary branches, number of scales under the lowest panicle branch, spear length, number of spear scales, length of spear scales, width of spear scales, number of cladodes and foliage length contributed to nine factors and explained 83.37% of the total variance using principal factor analysis (Table 5).

Euclidean distance coefficients were calculated based on 20 quantitative characteristics with Ward's method. Forty-six accessions could be classified into eight major clusters at a Euclidean distance of around 45.2 (Fig. 1). And cluster A and cluster B were observed at Euclidean distance of 135.7, whereas cluster A mainly included tall accessions with green and purple, cluster B contained only short accessions with green. Cluster A had morphological characteristics as follow: the plant was tall, the first panicle branch was high, there were few secondary branches, the stem was thick, the primary branches were long, there were a large quantity of scales below the first branch, and the

Table 3 ISSR primers and optimal annealing temperatures

Number	Primer	Annealing temperatures (°C)	Number	Primer	Annealing temperatures (°C)
807	$(AG)_8T$	52.8	840	$(GA)_8YT$	49.8
808	$(AG)_8C$	56.8	841	$(GA)_8YC$	52.8
810	$(GA)_8T$	48.4	842	$(GA)_8YG$	51.2
811	$(GA)_8C$	48.4	855	$(AC)_8YT$	55.6
812	$(GA)_8A$	52.8	856	$(AC)_8YA$	51.2
816	$(CA)_8T$	54.2	861	$(ACC)_6$	55.6
818	$(CA)_8G$	49.8	864	$(ATG)_6$	51.2
825	$(AC)_8T$	54.2	889	$DBD(AC)_7$	49.8
826	$(AC)_8C$	55.6	890	$VHV(GT)_7$	54.2
827	$(AC)_8G$	49.8	891	$HVH(TG)_7$	55.6
834	$(AG)_8YT$	46.3			

Table 4 Basic statistical data for morphological characteristics of asparagus

Character	Min	Max	Range	Mean	SD	CV (%)	I
Plant height	22.80	298.50	275.70	225.31	28.05	12.45	2.01
Plant height to first panicle branch	4.50	79.50	75.00	39.87	11.79	29.57	2.08
Number of primary branches	16.00	77.00	61.00	41.37	9.25	22.35	2.02
Number of secondary branches	10.00	45.00	35.00	27.03	5.91	21.88	2.11
Internode length of primary branches	0.30	16.10	15.80	4.66	2.02	43.28	1.92
Internode length of secondary branches	0.10	13.10	13.00	3.85	2.39	62.22	1.91
Diameter of main stem	6.99	22.34	15.35	12.55	2.63	20.91	1.98
Diameter of primary branches	0.90	5.95	5.05	3.12	0.82	26.38	2.05
Diameter of secondary branches	0.17	3.01	2.84	1.15	0.41	35.87	1.93
Length of primary branches	10.78	210.30	199.52	90.84	21.33	23.48	1.92
Length of secondary branches	3.10	92.30	89.20	34.57	13.70	39.64	2.04
Number of scales under the lowest panicle branch	4.00	26.00	22.00	13.50	3.52	26.06	2.01
Spear length	2.97	50.70	47.73	36.59	5.44	14.86	2.02
Spear diameter	4.03	21.21	17.18	10.38	2.75	26.53	2.00
Fresh weight of spears	3.07	97.90	94.83	17.76	11.02	62.02	1.72
Number of spear scales	12.00	43.00	31.00	27.44	5.10	18.57	1.93
Length of spear scales	6.73	14.94	8.21	10.43	1.68	16.14	2.08
Width of spear scales	4.24	14.78	10.54	9.12	1.90	20.79	2.06
Number of cladodes	3.00	41.00	38.00	17.45	4.62	26.48	1.91
Foliage length	8.30	39.20	30.90	20.89	5.57	26.68	2.09

SD standard deviation, *CV* coefficient of variance, *I* Shannon diversity index

Table 5 Eigen value and cumulative variance for nine factors based on principal factor analysis

Factors	1	2	3	4	5	6	7	8	9
Eigen value	3.97	3.08	2.38	1.60	1.49	1.41	1.11	0.94	0.69
Variance %	19.87	15.38	11.92	7.98	7.43	7.07	5.53	4.72	3.47
Accumulative variance %	19.87	35.24	47.17	55.15	62.58	69.65	75.18	79.90	83.37

quantity of spear scales was large. However morphological characteristics of Cluster B was as below: the plant was short, the first panicle branch was low, there were a lot of secondary branches, the stem was thin, the primary branches were short, there were a few of scales below the first branch, and the quantity of spear scales was small. Subcluster A1a consisted of six accessions which came from Walker Seed (1 accession), New Zealand (1 accession), Canada (1 accession), Jiangxi (1 accession), Shandong (1 accession) and America (1 accessions). Subcluster A1b comprised nine accessions which came from Walker Seed (1 accession), Limseed (2 accessions), New Zealand (1 accession), Jiangxi (2 accessions), BeiJing (1 accession), Shandong (1 accession) and America (1 accession). Subcluster A1c also consisted of Walker Seed (1 accession), Jiangxi (2 accessions), Shandong (2 accessions). Subcluster A1d comprised seven accessions which came from Walker Seed (1 accession), New Zealand (2 accessions), Jiangxi (1 accession), Shandong (2 accessions) and Netherlands (1 accession). Subcluster A1e also consisted of three walker Seed accessions and 1 Limseed accession. Subcluster A2 was comprised of Jersey supreme and JK105, cultivars which came from Walker Seed and Jiangxi respectively. Subcluster B1 consisted of 2 Limseed accessions, 3 New Zealand accessions, 1 Jiangxi accession and 1 Shandong accession. Subcluster B2 comprised six accessions which came from Limseed (1 accessions), Netherlands (1 accessions), New Zealand (1 accession), Italy (1 accession), Jiangxi (1 accession), BeiJing (1 accession). Morphological dendrogram result showed that the genetic relationship between Jing kang701 and Mill was the closest, whereas UC157 and Precoce's was the farthest. Accessions from different regions had both difference and crossing, which may had something to do with the similar geographical habitat of origin areas and introduction each other.

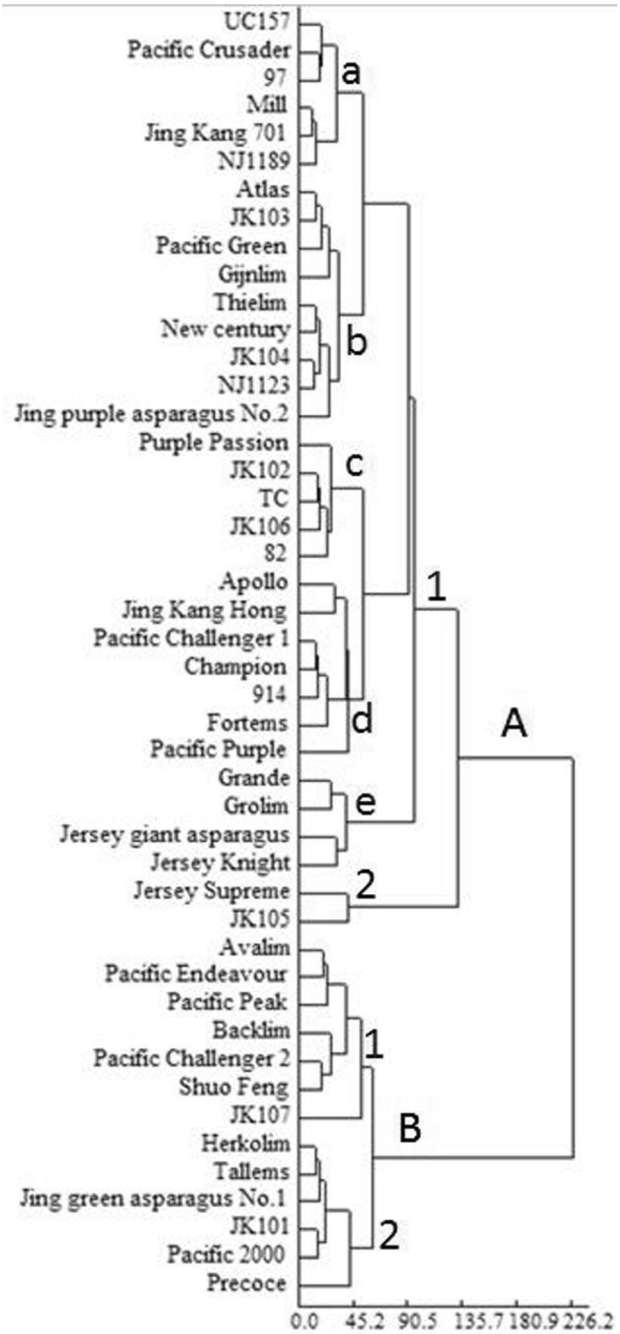

Fig. 1 Cluster of asparagus germplasm based on morphological characteristics

ISSR analysis

Twenty one ISSR primers produced 275 bands across the 46 accessions, of which 227 were polymorphic. The number of amplified fragments varied from 7 (825) to 19 (891) across the accessions for each primer pair. The average number of polymorphic bands per primer was 10.81. Complete polymorphism (100.0%) was observed with primers 812 and 825, while the lowest level of polymorphism (28.57%) was observed with primer 861 (Table 6). The values of PPB, h and I were 82.01%, 0.243 and 0.377 respectively.

The genetic diversity index can be expressed via the value of genetic similarity coefficient (GSC). That GSC among germplasms was small, and the genetic relationship was far indicating high genetic diversity; on the contrary, a large GSC indicated low genetic diversity. Our research results showed that GSC among 46 accessions was large, ranging from 0.60 to 0.97, and the closeness of the genetic relationships differs, which indicated high genetic diversity; GSC between the Champion and Jersey Supreme was 0.60, indicating a least close genetic relationship; GSC between Herkolim and Grolim was 0.97, indicating a closest genetic relationship.

Genetic difference and genetic relationship are inversely proportional to GSC. If GSC is larger, the genetic difference is smaller and the genetic relationship is closer; conversely, the genetic difference is larger and genetic relationship is the less close. In this research, there are 100 pairs of germplasm which GSC is lower than 0.7, which are of high hybridize preponderance and can provide a reference for parental selection for cross breeding of asparagus.

A dendrogram was assembled with the unweighted pair-group method with arithmetic averages (UPGMA) base on GSC. With the exception of Pacific Challenger 2, all accessions were placed in 2 major clusters, A and B (Fig. 2). Within group A, Purple passion and Pacific purple were genetically distant from the other members of the cluster, which form a compact group (A1). This was also the case for Jingpurple No. 2, NJ1189 and Pacific 2000 which occupied discrete branches within group B and separated clearly from subgroup B1. Within group A1 accessions cluster due to origin from WalkerSeed, Limseed, New Zealand, Canada and Jiangxi. Whereas group B1 cluster because of origin from Jiangxi, Shandong, Beijing, Netherlands, America and Italy. Moreover, we observed germplasms of the same origin mostly form a cluster together. This was probably because they were originated from a common ancestor. Moreover, Mantel test was carried out with the distance matrix based on morphological marker and ISSR marker to evaluate their cooperativity, and the result showed that there is no significant relation there between.

Discussion

The morphological characteristics of the same species were similar, but the outward manifestation differed from individual to individual due to environmental factors (Vithanage et al. 1995). Morphological characteristics were so susceptible to external environmental factors that the real

Table 6 The amplification results of ISSR primers in Asparagus accessions

Primer pairs	Total number of amplified bands	Number of polymorphic bands	Percentage of polymorphic bands (PPB) (%)
807	16	15	93.75
808	15	14	93.33
810	13	12	92.31
811	13	11	84.62
812	14	14	100.00
816	10	9	90.00
818	13	11	84.62
825	7	7	100.00
826	9	6	66.67
827	15	14	93.33
834	14	13	92.86
840	11	8	72.73
841	12	9	75.00
842	12	10	83.33
855	16	14	87.50
856	11	7	63.64
861	14	4	28.57
864	12	7	58.33
889	12	10	83.33
890	17	16	94.12
891	19	16	84.21
Total	275	227	–
Average	13.09	10.81	82.01

genetic inclination was concealed (Linda et al. 2009). However, morphological characteristics were the outcome of interaction between environmental factors and genetic factors, so morphological characteristics can reveal the manifestation of genetic diversity of germplasms under the same cultivation and management conditions to a certain extent (Moose and Mumm 2008; Singh et al. 2010). Besides, morphological characteristics played an important role in discovering new plant resources, about which there are many reports on successful cases, such as rice (Chakanda et al. 2013), maize (Couto et al. 2013), wheat (Li et al. 2012) and pea (JHA et al. 2013). These researches indicated that a great variety of modalities are helpful for breeding. Our results showed that the average variation coefficient of the 20 morphological characteristics is 28.81%, the largest variation coefficient was internode length of secondary branches (62.2%), the CV for fresh weight of spears, internode length of primary branches, length of secondary branches, diameter of secondary branches and plant height to first panicle branch were above 29.57%. In other words, the morphological variation was significant. This indicated that the phenotypic plasticity is high. Some characteristics of the same variety also may have significant variation and form a phenomenon of complicated intraspecific polymorphism. Therefore, there was a large range of choice for phenotypic character of significant variation in practice, namely more importance should be attached to the leading indexes of morphological differentiation when morphological indexes was selected to screen elite asparagus germplasm resources and breeding, which was in line with our focus in production (Chen et al. 2013). Specifically, for cultivation of high-yield variety of asparagus, plant height to first panicle branch, diameter of main stem, spear diameter, fresh weight of spears and diameter of primary branches should be taken as the primary indexes. These abundant genetic resources provided rich resources for breeding of asparagus. It was expected to improve plant type of asparagus by means of systematic breeding, so as to obtain superior clones better for photoassimilates accumulation. Besides, the morphological diversity index of asparagus took on an opposite tendency against the change of its variation coefficient, which agreed with the research result of Zhang et al. (2009).

The 46 asparagus accessions could be classified into eight major clusters based on the morphological traits analysed in the study. Close morphological relationship, most notably in height were observed between the accessions of group B in the dendrogram in Fig. 1. This was

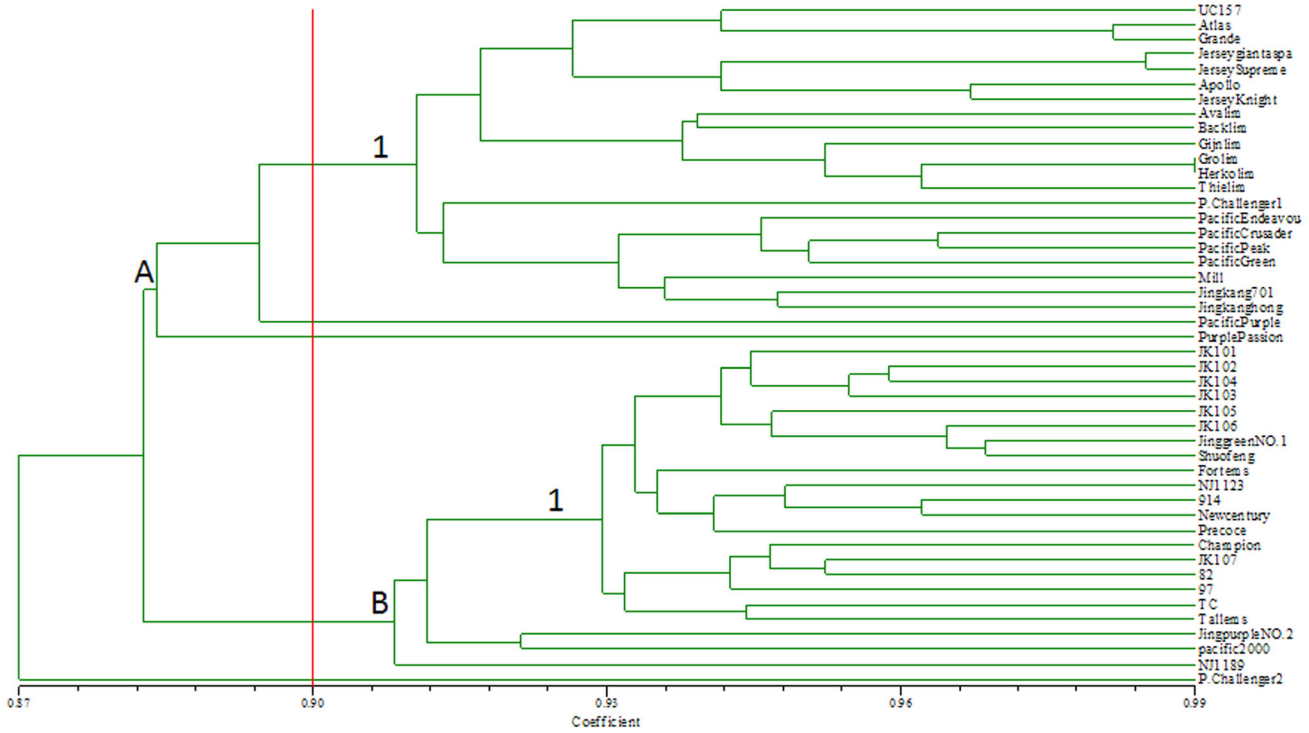

Fig. 2 Dendrogram showing genetic relationships between asparagus germplasm based on ISSR markers

congruent because these accessions came from a similar environment with similar habitat and they may be derived from common ancestors.

The genetic diversity information of germplasm resources was an indispensability to breeders (Innark et al. 2014), and molecular markers with high polymorphism including ISSR were widely applied in genetic diversity analysis and genetic mapping (baliyan et al. 2014). The existing researches showed that molecular markers were highly effective for genetic diversity analysis of asparagus, which was also proved in our research (Khandka et al. 1996; Aceto et al. 2001; Sica et al. 2005; Marco et al. 2008; Sarabi et al. 2010; Casali et al. 2011; Castro et al. 2014).

Since ISSR marker works well in detecting very slight genetic variation, it is usually applied in research of plant genetic diversity (Zietkiewicz et al. 1994; Moreno et al. 1998 Wang et al. 2009, 2013). For genetic diversity analysis, ISSR marker has proved to be a method of low cost and high efficiency, and has been successfully applied to determine genetic relationship (Moreno et al. 1998; Gilbert et al. 1999; Shao et al. 2010). Our research has further proved this. PPB showed no correlation with the total bands. For example, primer 891 produced 19 bands, but PPB reaches 84.21% only. However primer 825 produces 7 bands, PPB is up to 100%. Similar results also were reported in researches of *Rheum tanguticum* (Hu et al. 2011), *Asparagus cochinchinensis* (Ou et al. 2011) and *Haplocladium microphyllum* (Mao and Fang 2014). Our research results showed that the genetic diversity of the 46 asparagus germplasm resources was high, which disagrees with that of researches of Geoffriau et al. (1991), Lallemand et al. (1994), Marco et al. (2008), and Castro et al. (2014, but agrees with that of researches of Sica et al. (2005), Sarabi et al. (2010) and Casali et al. (2011). This may be because they adopted wild asparagus resources as research materials, which showed that the utilization of wild asparagus resources was very necessary to expand the genetic diversity of asparagus (khandka et al. 1996). Therefore, our research results also showed the importance of indigenous varieties and wild resources of asparagus, which can promote the optimal application of asparagus germplasm resources in crossbreeding, and provided a reference for genetic improvement of asparagus germplasm resources.

There was no significant relation between morphological characters and the molecular marker, which agreed with the reports of Shao et al. (2010) and El-Bakatoushi et al. (2013). Probably because different sites between them were taken, many morphological characteristics were affected by the physiological status of individual plants and surrounding environment, and there was no link between marker and the gene controlling the morphological character (Shao et al. 2010; Solouki et al. 2008). In contrast, farajpour et al. (2012) showed a correlation of some morphological character with a series of related genes. However, there were some common groups in both morphology

and ISSR marker clustering results. The genetic relationships between Jersey knight and Jersey giant or between JK102 and JK106, as shown in the morphological dendrogram result, were close, and as shown in the molecular marker dendrogram result, also were near. It was the same with that between Avalim and Backlim. On the contrary, the genetic relationship between Herkolim and Tallems, as shown in the morphological dendrogram result, was close, but as shown in the molecular marker dendrogram result, was not near. It was the same with that between Pacific Crusader and 97. The results of this research also have proved the argument reported before that morphology doesn't conform to DNA molecular marker in dendrogram result (Papadopoulou et al. 2002; Almajali et al. 2012.). Besides, for parental selection for crossbreeding, at the time of attaching importance to genetic difference among parents and the closeness of genetic relationships, stress also should be laid on the desirable characters of parents according to the breeding objectives.

Conclusion

Morphological traits and ISSR markers provided a comprehensive insight into the genetic diversity of asparagus accessions. Our results indicated that asparagus germplasm in China was relatively abundant for breeding purposes. And the results will facilitate optimal application of asparagus germplasm resources for varietal development through sexual hybridization, and provide additional data of practical value for further exploitation of asparagus germplasm resources by genetic improvement. The genetic background present in the Chinese asparagus accessions showed the importance of asparagus landraces and genetic diversity analysis of germplasm resources at both the molecular and morphological level was necessary in order to facilitate their effective use in the breeding programs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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