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Characterization of genetic relationship of dragon fruit accessions (*Hylocereus* spp.) by morphological traits and ISSR markers

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ABSTRACT

Dragon fruit, a kind of fruit belonging to *Hylocereus* spp., has brought about interest in China not only due to its important economic value with high contents of nutrients, but also due to the striking tolerance to drought stress. Fifteen morphological traits and 111 Inter-Simple Sequence Repeat (ISSR) markers generated from 16 primers were firstly employed to discriminate 50 accessions recently selected in China, as well as to evaluate their genetic relatedness. According to univariate statistical analysis of morphological traits, high variations were observed between or/and within the wild and cultivated lines. The dendrogram of 50 accessions was constructed based on morphological traits. Taking 7.50 as a threshold, three major clusters could be observed, i.e. the first included nine genotypes, which mainly belonged to wild accessions; the second clusters standing for the red pulp genotypes; the third consisting of the white pulp accessions. Each ISSR primer generated 4–10 obvious DNA bands ranging from 100 bp to 1500 bp, with the average of 6.9 per primer. The percentage of polymorphic bands (PPB) varied from 25.00% to 100% with an average of 66.12%, and DNA markers generated from primers UBC824, UBC891and UBC900 could efficiently fingerprint 50 genotypes. Polymorphism information content (PIC) among the tested genotypes varied from 0.49 to 0.93 with an average of 0.85, suggesting a high genetic diversity among the tested genotypes. Based on UPGMA method, 50 genotypes could be grouped into two major clusters at a genetic distance of 0.23. Although there existed discrepancy in genetic relatedness between morphological and ISSR loci, a significant correlation between the data of two evaluation methods was observed using Mantel correspondence test.

1. Introduction

Dragon fruit, a type of fruit belonging to *Hylocereus* spp. of Family Cactaceae originating from Latin America, has been intensively developed as an economic fruit crop in northern, central and southern American (Wichienchot et al., 2010). It had widely brought about high interest due to its attractive color (Adnan et al., 2011), pleasant taste (Castellar et al., 2006), high content of nutrients (Tze et al., 2012), senescence-retarding (Lim et al., 2012; Zhuang et al., 2012) and cancer-preventing effects (Yusof et al., 2012). Moreover, dragon fruit can withstand prolonged drought. Therefore, it has demonstrated a high potential for horticultural development, especially in areas where drought is a limiting factor for other fruit production (Yusof et al., 2012; Choo and Yong, 2011). It has been widely cultivated in South China, especially in the karstic regions where frequently suffer from severe drought stress. However, much of the interest in this crop has just focused on analysis of nutrients, cultivar selection, as well as cultivation techniques, etc. (Tel-Zur et al., 2012; Tze et al., 2012). To date, few studies have been conducted for the assessment of genetic diversity and DNA fingerprint construction in dragon fruit, which are requisites for full use of dragon fruit germplasm and for its proprietary right protection (Ahlawat et al., 2010).

Conventionally, morphological traits had been used to differentiate plant germplasms as well as to elucidate their genetic relationship (Bianco et al., 2011). Earlier, morphological traits had been employed to unravel the genetic relationship of dragon fruit crops (Esquivel et al., 2007; Grimaldo-Juárez et al., 2007). However, morphological traits were susceptible to the influence of environmental factors (Ferreira et al., 2010). Molecular markers, which

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reveal directly polymorphism at the DNA level, had showed to be a very powerful tool for fingerprinting germplasm, elucidating genetic diversity, and evaluating genetic relationship among genotypes, etc. (Innis et al., 2011; Almajali et al., 2012). Junqueira et al. (2010) applied RAPD markers to assess the genetic variation among and within 16 dragon fruit genotypes maintained at Embrapa Cerrados Germplasm Collection from Brazil. However, RAPD markers are not so reliable as compared to other markers. Inter-Simple Sequence Repeat (ISSR) markers developed by Zietkiewicz et al. (1994) demonstrated several advantages over other markers, thus was widely used for assessment of genetic diversity and construction of the DNA fingerprints in many plants (Ferreira et al., 2010; Liu et al., 2011). Nevertheless, there have been no reports on the application of ISSR markers for unraveling genetic diversity as well as for fingerprinting germplasms of dragon fruit so far.

To provide valuable information for the genetic improvement of dragon fruit in South China, the objectives of the present study were: (i) to unravel the genetic relatedness of the dragon fruit germplasms by both morphological traits and ISSR markers; (ii) to fingerprint the genotypes using ISSR markers; and (iii) to investigate the coincidences and divergences of the distances between two marker systems.

2. Materials and methods

2.1. Plant materials

To better elucidate the genetic diversity of dragon fruit germplasm, the 50 accessions (Table 1) were selected to carry out the downstream research based on the morphological characters including the stem and fruit traits. Tender stems were harvested from the Dragon Fruit Germplasm Collection of Guizhou Institute of Fruit Tree Science (P.R. China) for DNA extraction, and stored in a −20 °C freezer for no longer than two weeks before DNA extraction.

2.2. Morphological characterization

According to the documented characterization (Grimaldo-Juárez et al., 2007), 15 morphological traits, which generally characterize the genotypes of dragon fruit, were described and quantified (Table 2). Characters for each accession included qualitative characteristics and quantitative traits, i.e. rib thickness, rib width, areole spacing, number of fruit scale, spine length and number of spine (Esquivel et al., 2007). To avoid errors, data for each trait were measured by the same person within a single day.

2.3. Molecular characterization

Genomic DNA was extracted from approximately 0.1 g of tender stem tissue using a modified CTAB method (Wen et al., 2004). The quality and concentration of the DNA were confirmed by electrophoresis on 1% agarose gels with λ DNA/*Hin*dIII markers.

Following an initial screen of 100 ISSR primers (Biotechnology Laboratory, University of British Columbia), 16 that yielded maximum numbers of reliable and reproducible polymorphisms were then used to performed PCR amplification (Table 3). The amplification reactions were carried out in a 10 μl volume containing 1 μl DNA (20 ng/μl), 5.0 μl PCR mix (Tiangen, China), 0.8 μl primer and 3.2 μl nuclease-free water using Thermal Cycler S1000TM (Bio-Rad, Hercules, CA, USA) programmed for an initial denaturation step of 5 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 45 s at the specific annealing temperature and 1 min at 72 °C, and ended with a final extension step of 5 min at 72 °C. Each reaction was repeated at least twice, and only reproducible bands were scored. The PCR products were electrophoresed using 1.6% (w/v) agarose gel at a constant voltage of 5 V/cm, then viewed under UV light using a Gel Doc (Bio-Rad) apparatus. ISSR markers were scored as presence (1) or absence (0) of bands. The sizes of bands were estimated by comparison with D2000 DNA ladder.

2.4. Data analysis

The variables of morphological traits were standardized before calculating distance. The Euclidean distance (D_{ij}) between accession i and j was defined as: $D_{ij} = \sum(Y_{im} - Y_{jm})^{1/2}$, where Y_{im} and Y_{jm} stand for the mth morphological or ISSR data of accession i and accession j, respectively. Efficiency of each marker in giving polymorphic DNA bands was shown by polymorphism information contents (PICs) as $\mathrm{PIC} = 1 - \sum P_{ij}^2$, where P_{ij} is the frequency of the jth allele for the ith marker.

Cluster analyses were performed using the un-weighted pair group method analysis (UPGMA). A cophenetic matrix was derived from the distance matrix using the COPH (cophenetic values) program and the goodness of fit of the clusters was tested by comparing the original distance matrix with the cophenetic value matrices. To investigate the correlation between morphological and ISSR data, Mantel correspondence tests were further carried out based on their distance matrices (Legendre and Fortin, 2010). All calculations were conducted with NTSYS version 2.10e software.

3. Results

3.1. Morphological traits

The morphological traits highly diversified among the genotypes. According to univariate statistical analysis, the mean, SD, range and CV of phenotypic genetic variation for each trait were computed. Based on pairwise comparisons among the genotypes, a matrix of Euclidean distance was obtained, which displayed considerable diversity. The minimum (1.79) of Euclidean distance was detected between line No. 15 and line No. 16, while the maximum (8.35) was gained between line No. 4 and line No. 11. For the wild accessions, the distance value ranged from 2.94 to 6.24, and those of the cultivated lines were 1.79–8.06. The UPGMA dendrogram was obtained based on morphological traits (Fig. 1A). A cophenetic correlation of $r = 0.73$ was obtained, which indicates a good fit between the original distance matrix and the clustering analysis. Taking 7.50 as a threshold, four clusters could be observed: the first included nine genotypes, i.e. lines No. 1, 2, 3, 5, 6, 8, 12, 14, and 47 which mainly belonged to the wild accessions; the second standing for the red pulp genotypes; the three consisted of the white pulp accessions.

3.2. ISSR analysis

The 16 selected primers generated 111 discriminatory and reproducible amplified fragments, giving altogether 74 polymorphic bands. Each primer generated 4 (UBC810) to 10 (UBC891) obvious bands, with the average of 6.9 per primer (Table 3). The scored bands ranged from 100 bp to 1500 bp, and three primers, i.e. UBC835, UBC895 and UBC900, were shown to be the most polymorphic (Table 3). The electrophoresis pattern obtained with primer UBC835 was illustrated in Fig. 2. The percentage of polymorphic bands (PPB) varied from 25.00% (UBC810) to 100% (UBC895) with an average of 66.12%. PIC among the tested genotypes varied from 0.49 (UBC810) to 0.93 (UBC891) (Table 3).

Based on these markers, a Euclidean distance matrix was computed and a dendrogram was obtained using a UPGMA cluster analysis. A cophenetic correlation of $r = 0.62$ ($n = 1225$, $P < 0.001$) was detected, which indicated a goodness of fit between the