Biochemical Systematics and Ecology 46 (2013) 120–126

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Genetic relationship between Chinese wild *Vitis* species and American and European Cultivars based on ISSR markers

Zhaobin Jing[a,b], Xiping Wang[a,b,*]

[a] College of Horticulture, Northwest A&F University, Key Laboratory of Horticultural Plant Biology and Germplasm Innovation in Northwest China, Ministry of Agriculture, Yangling, Shaanxi 712100, China
[b] State Key Laboratory of Crop Stress Biology in Arid Areas, Northwest A&F University, Yangling, Shaanxi 712100, China

ARTICLE INFO

Article history:
Received 29 January 2012
Accepted 30 August 2012
Available online 16 October 2012

Keywords:
Chinese wild *Vitis* species
Vitis
Genetic diversity
ISSR

ABSTRACT

Inter-simple sequence repeat (ISSR) markers were employed to detect the genetic diversity among 70 grape accessions including 52 clones of 17 Chinese wild grape species, seven interspecific hybrids, 10 *Vitis vinifera* L. cultivars, and one strain of *Vitis riparia* L. A total of 119 polymorphic bands with an average of 11.9 per primer were observed. The unweighted pair-group method (UPGMA) analysis indicated that the 70 clones or accessions had a similarity range from 0.08 to 0.93, indicating that abundant diversities exist among these accessions. Based on cluster analysis and principal coordinate analysis, all accessions could be divided into two major groups, the Chinese wild grape group, and the American and European cultivar group. The largest distance was found among *V. riparia* MichX, *Vitis piasezkii*, *V. vinifera* L. interspecific hybrid (*Vitis binifera* × *V. labrusca*) and the wild grapes native to China.

1. Introduction

Grape (*Vitis vinifera* L.) is one of the oldest and most important fruit crops in the world. The *Vitis* genus contains more than 70 species, with centers of origin in south Europe, Asia Minor, East Asia, and North and Central America (Wan et al., 2008). China is one of the major germplasm centers of *Vitis* species and more than 35 *Vitis* species originate in China (Wan et al., 2008). Chinese wild *Vitis* has many useful characteristics, including (i) high quality wine making attributes, such as high sugar content and moderate acidity (Li and He, 2000); (ii) high level of resistance to fungal diseases, including Powdery Mildew [*Uncinula necator* (Schw.) Burr.], Anthracnose [*Elsinoë ampelina* (de Barry) Shear], Ripe Rot [*Glomerella cingulata* (Ston.) Spauld et Schrenk], and Crown Gall [*Agrobacterium tumefaciens* Smith and Townsend] (He et al., 1991; Wang et al., 1995, 2007; Wang, 1979); (iii) highly efficient photosynthesis in *V. quinquangularis* (Zhu et al., 1994). In addition, Chinese wild *Vitis* can be easily crossed with American *Vitis* species, and the undesirable “foxy” flavor compounds in the berries of American *Vitis* species is nonexistent in Chinese wild *Vitis* species (He, 1999b; Wan et al., 2008). The selection for these desirable characteristics has enlarged the scope of applications for grape breeding, which results in many intermediate and transitional types of *Vitis* and causes difficulties in germplasm research of the wild Chinese *Vitis*.

* Corresponding author. College of Horticulture, Northwest A&F University, Key Laboratory of Horticultural Plant Biology and Germplasm Innovation in Northwest China, Ministry of Agriculture, Yangling, Shaanxi 712100, China. Tel.: +86 29 87082429; fax: +86 29 87082613.
E-mail addresses: wangxiping@nwsuaf.edu.cn, wangxiping89@gmail.com (X. Wang).

0305-1978/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2012.08.004

China has a long history in grape domestication. Wild Chinese grape species have enormous economic potential for grape breeding. The determination of the taxonomy of Chinese wild grape can be traced back to about the end of 18th century (Wan et al., 2008). In the past three decades, the taxonomy of Chinese wild grapes has been investigated through morphology, photochemistry, allozyme, and molecular marker analyses (He, 1999a,b; He et al., 1996; Niu and He, 1996; Wang et al., 2000a, b; Kong, 2004; Luo et al., 2001). However, the classification of a few Chinese *Vitis* species adopted by Chinese taxonomists has some controversy and confusion. For instance, Niu and He (1996) found that most types of *Vitis piasekii* are closely related to *Vitis amurensis*, while some types are closely related to *Vitis bashanica* according to the analysis of 110 traits and 22 Chinese grape species. Their analysis suggests that *V. piasekii* may be a polyphyletic species. Therefore, further studies are necessary to classify and elucidate the evolutionary relationships among the various *Vitis* species.

In the previous studies, taxonomy and germplasm identification of some wild grape species native to China have been conducted based on morphological and isoenzyme approaches (He et al., 1996; Ma and He, 1998; Niu and He, 1996). However, morphological characteristics and isoenzymes of grapes are easily affected by developmental stages and environmental conditions (Luo et al., 2001). Molecular markers have been shown to be a powerful tool for the analysis of genetic diversity. A few studies have reported the use of DNA markers such as RAPD (Luo et al., 2001) to classify and analyze the evolution of wild grape species native to China. Inter-simple single repeat (ISSR) is recognized as one of the most useful molecular markers because it is simple, fast, reproducible, and cost effective (Zietkiewicz et al., 1994). However, there are no reports of evaluating genetic diversity of wild grape species native to China by ISSR markers to date.

In this study, we used ISSR markers to investigate genetic diversities and the relationship among Chinese wild grape species, and American and European cultivars. Our study provides certain theoretical basis for future germplasm conservation, evaluation, utilization, and grape breeding programs.

2. Materials and methods

2.1. Grape materials

A total of 70 grape materials including 52 clones of 17 wild grape species native to China, seven interspecific hybrid, 10 European cultivars, and one strain of *V. Riparia* L. were collected from the grape germplasm resources orchard, Northwest A&F University, Yangling, Shaanxi, China. Information for all of the 70 grape accessions is listed in Table 1.

2.2. DNA extraction

Genomic DNA was extracted from fresh young leaves using the CTAB method and purified according to the method reported by Qu et al. (1996) with minor modifications. DNA quality and concentration were checked with lambda DNA standards on agarose gels. The extracted DNA was stored at −20 °C after diluted to the required concentration.

2.3. Primer screening and PCR amplification

A total of 96 ISSR primer sequences were synthesized by Beijing Aoke Biological Technology and Service Co. Ltd. From these primers, ten (Table 2) were selected for the present study based on their reproducibility, clarity, and highly polymorphic nature of product bands. PCR amplifications were carried out in a 20 μl volume solution containing 10 × PCR buffer (100 Mm Tris-HC, pH 8.3; 500 mM KCl), 0.18 mM of each dNTPs, 0.75 mM of each primer, 1.87 mM of $MgCl_2$, 1 unit of *Taq* DNA polymerase and 20 ng of template DNA. PCR amplifications were performed as the following: an initial 5 min denaturation at 94 °C followed by 35 cycles of 45 s at 94 °C, 45 s at 52–58 °C, 90 s at 72 °C, and a final 5 min extension at 72 °C. PCR products were separated on 6% denatured polyacrylamide gels and detected by silver staining. Clearly and reproducibly distinguished bands were recorded and used in the following analysis.

2.4. Data analysis

Each ISSR fragment was scored as present (1) or absent (0) for each of the 70 DNA samples, excluding the weak and blurred bands, thus generating a binary data matrix. A locus was considered polymorphic if more than one band at the same position was detected for all the materials.

The binary data matrix was analyzed using NTSYS-pc version 2.1e software package (Rohlf, 2000). The pairwise genetic distances among all accessions according to Nei (1978), was calculated based on Jaccard's similarity coefficient. Cluster analysis was performed using the unweighted pair-group method with arithmetic average (UPGMA). The dendrogram was constructed using the NTSYS-pc version 2.1 software package (Rohlf, 2000) and MEGA version 4.0 (Tamura et al., 2007). POPGENE version 2.1e software (Yeh et al., 1999) was used to calculate Nei's gene diversity (D^a) and Shannon's information index (D^b). The average polymorphism information content (D^c) for each locus was calculated using the following formula

$$PIC = 1 - \sum P_i^2$$

where P_i is the frequency of the ith band (Powell et al., 1996).