Biochemical Systematics and Ecology 70 (2017) 268–273

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Natural hybridization and introgression among sympatrically distributed *Rhododendron* species in Guizhou, China

Jing-li Zhang [a, 1], Yong-peng Ma [b, 1], Zhi-kun Wu [b], Kun Dong [a], Shuo-li Zheng [a], Yun-yue Wang [a, *]

[a] College of Horticulture and Landscape, Yunnan Agricultural University, Kunming 650201, Yunnan, China
[b] Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 10 December 2016
Accepted 17 December 2016

Keywords:
Natural hybridization
Rhododendron
AFLP

ABSTRACT

Natural hybridizations occur among *Rhododendron delavayi*, *R. decorum* and *R. irroratum*, however, there was little study that had addressed the interbreeding behaviors when the three species grew in the same geographic areas. In this study, 37 accessions, containing the three species and the putative hybrids, were obtained from Baili *Rhododendron* Nature Reserve, Guizhou, China. Examinations of hybridization patterns with AFLP markers have led to a total of 107 diagnostic DNA fragments, which were analyzed with Principal Co-ordinate, Structure and NewHybirds analyses. The data confirmed that the existence of hybrids originated from the interbreeding between *R. delavayi* and *R. irroratum* in Baili *Rhododendron* Nature Reserve. *R. decorum* did not appear to be involved in the hybridization. Furthermore, most hybrids detected were a result of backcrossing, indicating that this hybrid differed from F1 dominant hybrids between *R. delavayi* and *R. irroratum* found in previous studies.

1. Introduction

Interspecific hybridization plays a significant role in plant evolution, and many studies have shown the role of hybridization in generating adaptive variation, functional novelty and new species (Anderson, 1949; Arnold, 1997; Seehausen, 2004). Natural hybridization occurs fairly common within the genus *Rhododendron* L. (Ericaceae), probably due to relatively weak post-zygotic barriers, especially in subgen. *Hymenathes*. Breakdown of these external barriers can result in extensive natural hybridization (Chamberlain, 1982; Wu, 1986; Hu and Fang, 1994). Therefore previous studies strongly support the hypothesis that the formation of hybrids between sympatric species of *Rhododendron* is easy, and natural hybridization between closely related species in sympatric areas may be rather common (Milne et al., 1999; Zhang et al., 2007; Ma et al., 2010).

In the subgen. *Hymenathes*, *R. delavayi* Franch., *R. irroratum* Franch., *R. agastum* Balf. f. & W. W. Smith, and *R. decorum* Franch. are often found to be distributed sympatically in Yunnan and Guizhou provinces, China. Molecular evidence from previous studies has confirmed that *R. agastum* is a hybrid (Zhang et al., 2007; Zha et al., 2008, 2010). However, hybrids morphologically similar to *R. agastum* can be formed from the hybridization between *R. delavayi* and *R. decorum* (Zhang et al.,

* Corresponding author. Tel./fax: +86 871 5220389.
E-mail address: yunyuewang@126.com (Y.-y. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bse.2016.12.014
0305-1978/

2007; Zha et al., 2008), or the hybridization between *R. delavayi* and *R. irroratum* in different populations (Zha et al., 2010). Moreover, hybrids morphologically similar to *R. agastum* in two locations of Yunnan in China are mainly F1 hybrids between *R. delavayi* and *R. irroratum* (Zha et al., 2010).

During a field investigation at Baili *Rhododendron* Nature Reserve, we discovered a putative hybrid zone where *R. delavayi* and *R. irroratum* were present sympatrically. Unlike the similar flower traits among the hybrids in previous studies, Variations of many traits in the leaves and flowers were obvious among these putative hybrids, indicating a distinctive structure of the hybrid zone in Baili *Rhododendron* Nature Reserve. In addition, another species *R. decorum* is distributed at the altitude around 1750 m, which is very close to the putative hybrid zone at around 1550 m on the sites of Pudi and Jinpo. It is possible that *R. decorum* might be involved in the hybridization events. In this study, we aimed to answer the following questions: 1) Are these putative hybrids true hybrids? If yes, what are the parental species of these hybrids? 2) What is the status of these putative hybrids with varying morphological traits?

2. Material and methods

2.1. Plant materials

In April 2011, 37 *Rhododendron* trees were sampled from the Baili *Rhododendron* Nature Reserve, Guizhou, southwestern China. Three species, *R. delavayi*, *R. irroratum*, and *R. decorum,* grew sympatrically. The petal color is red in *R. delavayi*, yellow in *R. irroratum*, and white in *R. decorum*, but the putative hybrids have pink petals or pink petals with light yellow throat. We collected 5, 5, 12 and 15 individuals of *R. delavayi*, *R. decorum*, *R. irroratum* and the putative hybrids, respectively, based on their morphological traits (Table 1).

2.2. DNA extraction and AFLP procedures

Genomic DNA was extracted from dried leaves using a modified CTAB method (Doyle and Doyle, 1987). DNA concentrations were determined by comparison with a serial dilution of standard lambda DNA, and the quality of DNA was checked by a Nucleic Acid-Protein instrument (Bio-Rad).

AFLP experiments were performed according to the protocol described by Reisch (2007). Double digestion of genomic DNA was performed for 2 h at 37 °C in a mix of 20 μL using 2 units (U) of MseI and 10 U of EcoRI. Adapters were then ligated to DNA in a volume of 20 μL for 2 h at 22 °C using 2 U of T4 DNA Ligase (Shanghai Sangon Biological Engineering Technology, Shanghai, China). Preselective polymerase chain reactions were performed in a reaction volume of 20 μL. PCR parameters were chosen as follows: 2 min at 94 °C; 25 cycles of denaturing at 94 °C for 20 s, annealing at 56 °C for 30 s, and extension at 72 °C for 2 min; followed by 2 min at 72 °C and ending with 30 min at 60 °C. The preselective products were diluted 20-fold and were used for selective PCR with the following three sets of primers: E-AAC/M-CTA, E-ACC/M-CTT, E-ACA/M-CAG. Selective amplifications were carried out in a volume of 21 μL, and PCR reactions were performed with the following touchdown profile: 2 min at 94 °C; 10 cycles of denaturing at 94 °C for 20 s, annealing for 30 s at 66 °C and then reduced by 1 °C for the next 10 cycles, elongation at 72 °C for 2 min, followed by 25 cycles for denaturing at 94 °C for 20 s, annealing at 56 °C for 30 s, and 2 min elongation at 72 °C; with a final extension of 30 min at 60 °C. Finally, the PCR products were added to a mixture of Sample Loading Solution (Beckman CoulteR, Fullerton, California, USA) and CEQ Size Standard 400 (Beckman Coulter). The fluorescence labeled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter).

2.3. AFLP data analysis

Raw data were collected and analyzed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Individuals were scored for the presence or absence of each fragment in binary mode (1/0) in crv-files. Bins were built using the AutoBin option with a peak height of 800 and a bin width of 2. Fragments were then assigned to bins with a selective height and checked manually. When ambiguous electropherograms were detected, the AFLP procedures were repeated to test for reproducibility. In the AFLP data matrix, the presence of a band was scored as 1, whereas the absence of the band was

Table 1
Sample sources of the *Rhododendron* species used in this study.

Species	Sample number	Subsection	Location	Altitude	Longitude	Latitude
R. delavayi	5	*R.* subsect. *Arborea*	Jinpuo spot	1550 m	N27°10.884′	E105°55.572′
R. decorum	5	*R.* subsect. *Fortunea*	Liulong town	1730-1750 m	N27°15.439′	E105°58.642′
Putative hybrids	15	*R.* subsect. *Irrorata*	Pudi spot	1550 m	N27°10.884′	E105°55.572′
R. irroratum	12	*R.* subsect. *Irrorata*	Pudi spot	1550 m	N27°10.884′	E105°55.572′