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# AFLP analysis of genetic variation in wild populations of five *Rhododendron* species in Qinling Mountain in China

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ABSTRACT

The genetic diversity in 22 populations of 5 wild *Rhododendron* species in Qinling Mountain in China was evaluated using amplified fragment length polymorphism (AFLP). Four AFLP selective primer combinations generated a total of 135 bands with their sizes ranging from 67 kb to 501 kb, among which 123 bands were found to be polymorphic, accounting for 92.04% variations. POPGENE analysis indicated that all the five species had high levels of genetic diversity, with percentage of polymorphic loci (PPL) ranging from 87.2 to 99.8% and effective number of alleles (ne) varying from 1.4205 to 1.9957. Shannon's Information Index (*I*) differed from 0.4286 to 0.6921, while Nei's gene diversity (*H*) varied from 0.2711 to 0.4989. The 5 species showed similar levels of genetic variations, with Hs and Ht ranging from 0.2253 to 0.4041 and from 0.2400 to 0.4994, respectively. The coefficient of gene differentiation among 22 populations (Gst) was 0.0923, accounting for 90.8% genetic diversity and only 9.2% genetic variations among the populations tested. The differences among populations were low in comparison with the previous studies on other species using the same technique. Low level of the genetic differences among *Rhododendron* populations investigated in the present study might be due to their outcrossing reproductive system. The analysis of molecular variance (AMOVA) also showed that variations among the species and populations were low, mainly due to a high variation within the populations. Based on unbiased genetic distances determined by an unweighted pair group method using arithmetic mean (UPGMA) phenogram, all populations could be grouped into *Rhododendron* species.



## 1. Introduction

Qinling Mountain is located in a transition region between the northern temperate zone and the northern subtropical zone in China and is recognized to be a natural boundary between northern and southern climate in China. There exists a huge reservoir of biodiversity in Qinling Mountain.

The genus *Rhododendron* is a typical genus in Qinling Mountain and is beneficial to maintaining the stability of the ecosystems. The wild *Rhododendron* is one of the most valuable plants because of its cold-resistance and ornamental value (Gen, 2008). Recently, *Rhododendron* germplasm is endangered mainly because of human being's activities and excessive excavation. In order to prevent *Rhododendron* from the extinction and maintain its diversity, it is necessary to investigate the genetic variations of the genus *Rhododendron* in Qinling Mountain.

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Molecular markers are commonly used to access the genetic variation of natural plant populations and estimate genetic diversity (Bouzat, 2001; Manel et al., 2007). Most of the previous studies on assessing *Rhododendron* genetic diversity employed the techniques such as horizontal starch gel electrophoresis (Gao et al., 2000; Ng and Corlett, 2000), random amplified polymorphic DNA (RAPD) (Iqbal et al., 1995 Javed et al., 1995), ITS technologies (Gao et al., 2002) and inter-simple sequence repeat (ISSR) (Liu et al., 2010). Owing to poor repeatability by RAPD, low ability to measure genetic diversity by the isozyme technique and inability to analyze allelic molecules by ISSR, all the techniques mentioned above can not be used to evaluate frequency of allelic molecules. When genetic information of a given species is unknown, amplified fragment length polymorphism (AFLP) molecular marker technique is preferred to probe its population genetic and genetic relationship, because of its high multiplex ratio, reproducibility and time-efficient (Zawko et al., 2001; Zhang et al., 2010). AFLP technique has been successfully used to characterize plant populations and estimate genetic diversity in several species such as *Isoetes malinverniana* (Gentili et al., 2010), *Jatropha curcas* (Pamidimarri et al., 2010) and *Typha taxa* (Na et al., 2010). There have been studies on analyzing population genetic variations of different species and cultivars in the genus *Rhododendron* by AFLP technique (De Riek et al., 1999; Dendauw et al., 2002; Chappell et al., 2007, 2008).

A better understanding of the genetic diversity and differentiation is not only necessary for conservation of a given species, but also helps realize population dynamics, adaptation and evolution process of the species (Fritsch and Rieseberg, 1996). However, information on the genetic variation of *Rhododendron* germplasm in China has been quite limited (Ng and Corlett, 2000).

The objective of the present study was, therefore, to evaluate genetic variation level and to understand the distribution of population genetic diversity in five *Rhododendron* species in Qinling Mountain. Such information is expected to provide basic knowledge of the genetic variations among the populations and help develop strategies suitable for conservation of *Rhododendron* species.

## 2. Materials and methods

### 2.1. Sample collection

Five *Rhododendron* species (*R. calophytum*, *R. purdomii*, *R. concinnum*, *R. clementinae* and *R. capitatum*) were collected from 10 counties located in Qinling Mountain in Shaanxi province (Fig. 1). Twenty two populations in total were collected: 4 from *R. calophytum*, 7 from each of *R. purdomii* and *R. concinnum* and 2 from each of *R. clementinae* and *R. capitatum*. The 5 *Rhododendron* species including 22 populations represent a majority of the wild genus *Rhododendron* genetic background in Qinling Mountain. The detailed information on the 22 populations is supplied in Table 1. The distance between individual samples collected was at least 50 m. Ten samples were randomly selected from each population. Fresh young leaves were removed from sample shoots, wrapped by wet paper, brought back to laboratory and stored at −70 °C until usage.

### 2.2. DNA extraction

Genomic DNA from 0.1 g fresh leaf tissues was extracted using plant genomic DNA Kit (Tiangen, Beijing, China), according to the manufactory's instructions. Purified total DNA was detected by 1.5% agarose gel electrophoresis. DNA concentration was

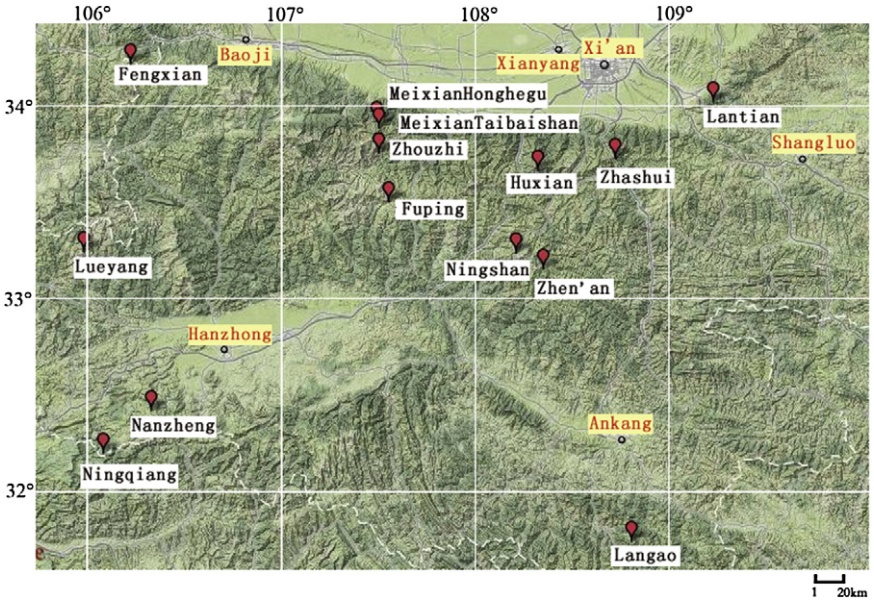


**Fig. 1.** Locations of the five *Rhododendron* populations collected for the present study in Qinling Mountain in China.