Scientia Horticulturae 131 (2011) 58–66

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

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

# Isozyme diversity and genetic structure of barrenwort (Berberidaceae) populations

Maoyin Sheng[a,b,*], Lingjiao Wang[c], Kangning Xiong[a,b]

[a] Institute of South China Karst, Guizhou Normal University, 550001 Guiyang, Guizhou, China
[b] The State Key Laboratory Incubation Base for Karst Mountain Ecology Environment of Guizhou Province, 550001 Guiyang, Guizhou, China
[c] School of Vocation and Technology, Guizhou Normal University, 550001 Guiyang, Guizhou, China

ARTICLE INFO

*Article history:*
Received 6 July 2011
Received in revised form
14 September 2011
Accepted 17 September 2011

*Keywords:*
*Epimedium*
Isozyme variation
Genetic diversity
Phylogenesis

ABSTRACT

Genetic diversity in 36 populations of eighteen species of the genus *Epimedium*, including sixteen species of section *Diphyllon* collected from China and two species of section *Epimedium* collected from Germany was studied using isozyme variation at 11 loci for six enzyme systems (*EST*, *GLU*, *PGM*, *PRX*, *GPI*, and *SOD*). The mean number of alleles per locus ($A_p$) showed little variation, ranging from 2.09 to 2.73. The mean percentage of polymorphic loci (*P*) of the eighteen species was high, ranging from 72.73% to 100%. The mean expected heterozygosity ($H_e$) ranged from 0.229 to 0.479 of 36 populations studied. Intrapopulational genetic diversity ($H_T$) was 0.5981 while interpopulational genetic diversity ($H_S$) was only 0.3534. The dendrogram of 36 populations studied obtained, using genetic distances among taxa. Obvious grouping of populations consistent with taxonomic species was found in the cluster analysis, confirmed that the eighteen species studied were natural, with a clear boundary between each other. Based on the dendrogram, the phylogenic relationships of *Epimedium* species are in agreement with the flower size were found, and it was inferred that China should be the original centre of genus *Epimedium*.


## 1. Introduction

*Epimedium*, also known as Rowdy Lamb Herb, Barrenwort, Bishop's Hat, Fairy Wings, Horny Goat Weed, or Yin Yang Huo, is a genus of about 60 or more species of garden flowering plants in the family Berberidaceae (Wu et al., 2003; Xie and Sun, 2006). The large majority are endemic to Southwest China, with further outposts in Europe, and central, southern and eastern Asia (Ying, 2002). *Epimedium* are hugely popular as garden plants for the beautiful flowers in Japan, Europe and America (Xie and Sun, 2006; Zhang et al., 2002). *Epimedium* is green all the year round. While they can be successfully propagated in early spring, *Epimedium* are best divided in late August, with the aim of promoting rapid regrowth of roots and shoots before the onset of winter (Zhang et al., 2002). In China, some species have been cultivated widely, such as *Epimedium acuminatum*, *Epimedium pubescens* and *Epimedium wushanense*. Some varieties and hybrids have been in Western cultivation for the last 100–150 years (Wu et al., 2003). There are also many older Japanese hybrids and forms, extending the boundaries of the genus in cultivation (Guo et al., 2008).

Linnaeus recorded this genus and its type species, *Epimedium alpinum*, in 1753 [Linnaeus, 1753 (1957 reprint)]. After that, Morren and Decaisne (1834), Franchet (1886), Komarov (1908), and Stearn (1938, 2002) made monographic and systematic study of *Epimedium*, respectively. Up till now, Stearn (2002) established the most comprehensive classification system of this genus. In his monograph, he arranged the genus into two subgenera, *Epimedium* L. and *Rhizophyllum* (Fischer & Meyer) Stearn, mainly based on whether the flowering stem has leaves or not, and he divided the subgenus, *Epimedium*, into four sections mainly according to their geographical distribution and infrageneric relationship. Among the four sections, members of section *Diphyllon* (Kom.) Stearn were all endemic to China and subdivided into four series: *Campanulatae* Stearn, *Davidianae* Stearn, *Dolichocerae* Stearn, and *Brachycerae* Stearn based on corolla characteristics such as petals type, the form and relative size of the inner sepals and petals, and flowers dimension.

Up to now, lots of works have been adopted to deduce the system and evolution of this genus such as pollen morphology (Zhang and Wang, 1983; Liang and Yan, 1991), cytology (Sheng and Chen, 2008; Sheng et al., 2010), isozyme (Koga et al., 1991), molecular biology (Nakai et al., 1996; Wang et al., 2001; Sun, 2004; Sun et al., 2005), biogeography (Ying, 2002; Zhang et al., 2007), chemical classification (Guo and Xiao, 1999; Koga et al., 1991; Guo et al., 2008) and so on. Results showed that the infrageneric classification was generally consistent with its phytogeographic distribution, and

* Corresponding author at: Institute of South China Karst, Guizhou Normal University, 550001 Guiyang, Guizhou, China. Tel.: +86 851 6748683.
*E-mail address:* shmoy@163.com (M. Sheng).

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doi:10.1016/j.scienta.2011.09.017

the phylogenetic relationship among sections was basically clear in *Epimedium*. However, the systematics of Chinese *Epimedium* species still is not revealed well because lots of *Epimedium* species with multifarious corollas are nested together in China (Guo et al., 2008). As a result, it is essential to do more works for the genetics and evolution of genus *Epimedium*, especially for the Chinese *Epimedium* species.

Isozyme variability analysis has been become a very useful technique in genetic evaluation, widely adopted in many species. Up till now, some works of the isozyme variability analysis of genus *Epimedium* have been done (Koga et al., 1991; Xu et al., 2007). However, these works are not comprehensive and only based on a few *Epimedium* populations and enzyme systems. No more information is available of the conservation genetics, population genetics and systematic evolution by isozyme analysis in genus *Epimedium*, especially for the Chinese *Epimedium* species. In the present paper, to evaluate the variability in isozyme diversity sampled from 36 populations of barrenwort belonging to eighteen species, including sixteen taxa of section *Diphyllon* collected from China and two species of section *Epimedium* collected from Germany, were studied. The aims are: (1) to evaluate the isozyme diversity and genetic structure of the selected species and populations, (2) to clarify the natural taxonomy for *Epimedium* species, especially for the Chinese *Epimedium* species and (3) to identify the genetics and evolution for genus *Epimedium*, especially for the Chinese *Epimedium* species.

## 2. Materials and methods

### 2.1. Materials

On the basis of the specimen information and the on-the-spot investigations in the wild habitats of *Epimedium* species, a total of 1139 individuals from 36 populations of eighteen species, including sixteen species of section *Diphyllon* native to China and two species of section *Epimedium* native to Europe, were collected and grown in pots filled with potting soil in a greenhouse. All plant materials were cultivated in uniform cultivation conditions. Taxa, population localities, code names, ecology, and sample sizes were provided in Table 1.

### 2.2. Extraction of enzyme, electrophoresis, and staining

Of the 12 enzyme systems prescreened, 6 enzyme systems were used for the examination of variation, including esterase (*EST*, E.C.3.1.1.7), β-glucosidase (*GLU*, E.C.3.2.1.21), phosphoglucomutase (*PGM*, E.C. 5.4.2.2), peroxidase (*PRX*, E.C. 1.11.1.7), glucose-6-phosphate isomerase (*GPI*, E.C.5.3.1.9), and superoxide dismutase (*SOD*, E.C. 1.15.1.1). Without exhibited good resolution, another 6 enzyme systems, that is, acid phosphatase (*ACP*, E.C. 3.1.3.2), alcohol dehydrogenase (*ADH*, E.C. 1.1.1.1), malate dehydrogenase (*MDH*, E.C. 1.1.1.37), lsocitrate dehydrogenase (*IDH*, E.C. 1.1.1.42), lactate dehydrogenase (*LDH*, E.C. 1.1.1.27) and Glutamate dehydrogenase (*GDH*, E.C. 1.4.1.2), were not used.

Isozyme assays were conducted on young leaf tissue. The youngest fully expanded leaves (about 230 mg) were homogenized with 90 μl extraction buffer [sucrose 16.7% (w/v) and sodium ascorbate 8.3% (w/v) in 50 mM Tris–HCl, pH 7.4] at −20 °C. Crude extracts were centrifuged for 5 min at 8160 × *g*. Supernatant (5.5 μl/well) was loaded onto precast, agarose isoelectric focusing (IEF) gels (Isolab, Akron, OH). Gels with pH gradients 3–5 (50%), 3–7 (25%), and 3–10 (25%) were used for esterase, β-glucosidase, phosphoglucomutase and peroxidase; gel with pH gradients 3–7 (50%) and 4–5 (50%) for phosphoglucoisomerase; and gel with pH gradients 3–10 (75%) and 3–7 (25%) for superoxide dismutase. Staining procedures were those of Wendel and Weeden (1989) with minor concentration, pH, and ingredient modifications; namely, *EST*, 0.6 M Na phosphate buffer pH 6.1, 50 mg α-naphthyl acetate and omitted β-naphthyl acetate; *PRX*, 0.1 M Na-acetate buffer, 0.4 mL hydrogen peroxide, 40 mg 3-amino-9-ethylcarbazole. The IEF gels were run at constant power and voltage limited to 1500. The first run was 60 min at 40 W and the second was 20 min at 60 W.

### 2.3. Electrophoretic patterns and data analysis

Electrophoretic bands corresponding to multiple alleles at each locus were named 1, 2, 3, and so on, for the order of mobility from slow to fast, and alleles were indicated by *a*, *b*, *c*, and so on. The known active subunit composition and the minimal conserved number in diploid plants for each of the enzyme were used to infer the number of loci and alleles (Wang, 1998; Weeden and Wendel, 1989; Huang et al., 1994a,b).

Chi-square ($\chi^2$) tests were applied to assess the heterogeneity of allele frequencies for each locus among populations. Mean number of alleles per polymorphic locus ($A_p$), percentage of polymorphic loci [*P* (99%; frequency of most common allele $\leq 0.99$)] and expected heterozygosity per population ($H_e$) were estimated. Genetic diversity parameters ($H_T$, $H_S$, $D_{ST}$ and $G_{ST}$) were also obtained. Genetic distances among populations and taxa were analyzed as described by Nei (1978).

GeneStat-PC 3.3 (Lewis, 1993) was used to compute Nei's gene diversity statistics (Nei, 1978), SAS (1988) for $\chi^2$ tests and NTSYS-pc (Rohlf, 1998) to obtain the dendrogram using the genetic distances of Nei (1978) and the unweighted pair group method (UPGMA) (Sneath and Sokal, 1973).

## 3. Results

### 3.1. Isozyme variability

Six enzyme systems, that is, *EST*, *GLU*, *PGM*, *PRX*, *GPI*, and *SOD*, exhibited good resolution were used in this study. A total of 11 loci and 42 alleles were scored from 1139 individuals of the 36 populations. The frequencies of each allele are given in Table 2 . All loci except *EST-1* were polymorphic, and the number of alleles per locus varied among species (Table 2).

The quaternary structure of *EST* enzyme system might be monomeric or dimeric, and three loci were detected in each population (Table 2). Only one allele was found at *EST-1*; *aa* was the genotype in all populations studied. Three alleles were identified at *EST-2*. In *E. acuminatum*, *Epimedium ecalcaratum*, *Epimedium simplicifoilum*, *Epimedium franchetii*, *Epimedium baojingense*, *Epimedium parvifolium*, *Epimedium davidii* and *Epimedium qingchengshanense* populations, only two alleles, *EST-2*$^a$ and *EST-2*$^b$ were found, whereas in *Epimedium yinjiangense*, *E. alpinum* and *Epimedium pubigerum* populations, *EST-2*$^a$ and *EST-2*$^c$ were found, and in *E. pubescens*, *Epimedium myrianthum* and *Epimedium sagittatum* populations, *EST-2*$^b$ and *EST-2*$^c$ were found. Four alleles were detected in *EST-3*. *ab* was the dominant genotype in *Epimedium letorrhizum*, *Epimedium shuichengense*, *E. yinjiangense*, *E. davidii* and *E. qingchengshanense*, whereas *aa* was the dominant genotype and *EST-3*$^c$ was a rare allele in *E. wushanense* and *E. franchetii*.

*GLU* enzyme system was dimeric. One locus detected with four alleles in all populations. The allele frequencies varied remarkably among species. The genotype of *E. acuminatum* and *E. ecalcaratum* was *aa*, whereas *bb* was the dominant genotype of *E. wushanense* and *Epimedium luodianense*, *cc* was the genotype of *E. pubescens*, *ab* was the dominant genotype of *E. letorrhizum*, *E. shuichengense*, *E. simplicifoilum*, *E. yinjiangense*, *E. franchetii*, *E. baojingense* and *E.*