# Genetic structure and variation in *Perovskia abrotanoides* Karel and *P. atriplicifolia* as revealed by molecular and morphological markers

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## ABSTRACT

In this study, 63 accessions of *Perovskia abrotanoides* from 16 populations were collected from different parts of Iran. Four accessions from *P. atriplicifolia* were also included specifically from southeastern parts of the state. Evaluations of the genetic variation by morphological characteristics and ISSR markers revealed a high morphological variation among and within the studied populations. The lowest coefficient of variation (CV) was obtained for seed length (10.93%) while the highest belonged to leaf width (33.9%). For molecular analysis, thirteen primer combinations were used to produce 110 polymorphic bands. Cluster analysis with STRUCTURE software classified the accessions into three major groups. The results were in most cases confirmed by principal coordinate analysis (PCoA) and morphological classifications. According to AMOVA, 77.33% of the total genetic variation occurred within the populations, while only 22.67% was observed among them. The populations exhibited a relatively high genetic differentiation (Gst = 0.85) and a low gene flow (Nm = 0.36). Among the populations, SESiKh from southeastern Iran showed the highest percentage of polymorphic loci PPL (51.82%), with a Shanon index (*I*) of 0.29 and a heterozygosity of 0.21. Furthermore, the central and southeastern populations exhibited the lowest admixture of accessions, while the northern and northeastern ones showed the highest admixture. The results of this study may provide a better understanding of the genetic variation and evolutionary dynamics of the genus *Perovskia* for the beneficial improvement of its breeding programs.

## 1. Introduction

The genus *Perovskia* belongs to the Lamiaceae family. Three species of this genus have been identified, among which *P. abrotanoides* and *P. atriplicifolia* have been used in ornamental and landscape design applications in many European and Asian countries. Wild populations of these species are mostly found in Iran, Afghanistan, Pakistan, and Turkmenistan (Arabi et al., 2011; Beikmohammadi, 2011). *Perovskia* has wide applications in the pharmaceutical industry. It is used as an antipyretic drug in Pakistan (Aoyagi et al., 2006) and for healing such disorders as atherosclerosis, cardiovascular disease, liver fibrosis, and cardiac fibrosis (Beikmohammadi, 2011; Fang et al., 2010). Furthermore, the roots of *P. abrotanoides* have considerable amounts of tanshinons, which is a valuable diterpenoid alkaloid (Zaker et al., 2015). The roots of *P. abrotanoides* are used in different regions of Iran to treat leishmaniasis and skin diseases (Duke et al., 2002). Its anti-inflammatory (Hosseinzadeh and Amel, 2001; Nassiri Asl et al., 2002) and anti-malaria (Esmaeili et al., 2008) effects have been well established. Its applications for the treatment of urinary tract infection (Ballabh et al., 2008), rheumatoid arthritis, and osteoarthritis as well as

its anti-spasmolytic activity (Cetin et al., 2007) have been reported.

Wild and native germplasms of indigenous plants are considered in each country as their valuable genetic resources whose conservation and sustainable use are crucial to breeding programs (Polignano and Alba, 1995). Moreover, breeding programs greatly rely on genetic diversity to resolve the major problems of susceptibility to diseases and environmental stresses, as well as low harvesting index and productivity observed in most wild medicinal plants (Rahimmalek et al., 2009). Nowadays, high grazing, droughts, and growing urbanization and industrialization have led to serious declines in most of the genetic resources. To face these challenges, plant breeders have been concentrating their efforts on maintaining variation in natural germplasms in an attempt to improve upon their breeding programs. Furthermore, assessment of inter- and intra-population genetic variations plays a crucial role in developing breeding strategies for a plant species. For this purpose, application of molecular markers along with their morphological traits has been identified as robust tools for the evaluation of genetic diversity in wild medicinal plant species (Wagner et al., 2005; Gharibi et al., 2011; Rahimmalek, 2012). Among the molecular markers, ISSR has been widely employed for the assessment of genetic

relationships in medicinal plants (Baruah et al., 2017). ISSR offers such advantages as high reproducibility, high polymorphism, low DNA requirements, easy handling, and high genomic distribution (Fadaei Heidari et al., 2016).

Most of the medicinal plants in the Lamiaceae family found to grow in arid and semi-arid regions have been identified (Sonmezdag et al., 2017). Unfortunately, many of these species have been reported to be facing increasing extinction risks, which has encouraged scientists to study the genetic conservation potentials of these valuable species. The genetic diversity of certain medicinal plants in the Lamiaceae family has been extensively investigated; *Thymus daenensis* (Rahimmalek et al., 2009), *Salvia* (Aghaei Jeshvaghani et al., 2015) and *Stachys* (Kharazian et al., 2015). However, no molecular or morphological reports are available on the genetic variability of *P. abrotanoides* despite its high medicinal value.

The present study was conducted to achieve the following objectives: I) to evaluate for the first time the genetic variation within and among *Perovskia* populations using morphological and ISSR markers, II) to determine the level of population differentiation and relationships between molecular and geographical distribution patterns in this species, and III) to evaluate the genetic structure of the *P. abrotanoides* populations studied.

## 2. Material and methods

### 2.1. Plant materials

*Perovskia abrotanoides* has a wide distribution in Iran. In this study, 63 accessions of *P. abrotanoides* and four accessions of *P. articifolia* were used. *P. abrotanoides* were collected from different geographical locations in central, northern, northeastern, and southeastern parts of Iran. This species has been distributed from the Alborz Mountains (South of the Caspian Sea to Khorasan) to the central parts of the Zagros Mountains in Isfahan province as well as the Taftan Mountain Range in the south-east of Iran. *Perovskia* mostly grows in high altitudes with available water storage. The samples were collected in 16 separate populations at a distance of at least 10 km and they were identified by Dr. Mehdi Rahimmalek using the Flora Iranica (Rechinger, 1982). In each population, five accessions were collected and transferred to laboratory on freezing ice and they were frozen at −20 °C. Morphological measurements were also carried out in nature in triplicates. Detailed information on collected sites are presented in Table 1.

### 2.2. Extraction of genomic DNA

The genomic DNA was extracted from young leaf samples using a

modified CTAB method as described by Murry and Thompson (1980). DNA quality and quantity were examined using the Nanodrop Array (Spectrophotometer Nano Ar, 2015) before the DNA samples were diluted to a concentration of 10 ng/μl.

### 2.3. ISSR analysis

Out of the 15 ISSR primers screened, 13 primers that produced higher numbers of reproducible bands were chosen for the ISSR analysis of *Perovskia* populations (Table 2). The total volume of PCR reaction mixture was 15 μl containing 10 ng of DNA, 7 μl of the Master Mix Red (Ampliqon, Finland) including 10 pM from each primer, 0.25 mM dNTPs, 4 mM MgCl$_2$, 1 U *Taq* DNA polymerase, and 10 × PCR buffer. The annealing temperature was optimized for each primer using the gradient PCR program (Table 2). A thermocycler (Bio-Rad thermal cycler) was used at the selected annealing temperature to start the PCR which was carried out in three steps. The first step of initial denaturation was carried out at 95 °C for 5 min followed by 40 cycles of 1 min at 95 °C, 1 min at the optimized annealing temperature (47–62 °C), and 2 min at 72 °C, terminating the process by a final extension step of 10 min at 72 °C.

### 2.4. Molecular data analysis

The polymorphic bands in each gel were scored as present (1) or absent (0) (Fig. S1). The cluster analysis and principal coordinate analysis (PCoA) were conducted using NTSYSpc Version 2.02 (Rohlf, 1998). The polymorphic information content (PIC) was calculated using the simplified formula (due to Anderson et al., 1993): $PIC_i = 2fi(1-fi)$, in which $fi$ represents the percentage of the $i^{th}$ present band. Genetic similarity among all the populations was calculated according to the Simple Matching (SM) similarity index, using the similarity of qualitative data (Simqual) routine. The tree dendrogram was constructed using the unweighted pair group method average (UPGMA) clustering procedure. The Mantel test (Mantel, 1967) was used to detect the correlation between each two dendrograms. The cophenetic correlation coefficient was generated by means of the COPH routine (in NTSYSpc Version 2.02 software) in order to check the goodness of fit between the clusters in the dendrogram and the similarity coefficient matrix. Gene diversity and analysis of molecular variance (AMOVA) were calculated using Arlequin version 3 software. STRUCTURE software was employed to evaluate the degree of accession admixture and better classification of the studied populations (Pritchard et al., 2000)

**Table 1**
Geographical location of 68 Perovskia accessions.

| No | Population code | species | Location | Geographical region | No. accession per population | Altitude | longitude | latitude |
|---|---|---|---|---|---|---|---|---|
| 1 | CEsNa | *P. abrotanoides* | Natnz, Esfahan, Iran | Center | 5 | 33° 35′N | 51° 76′E | 2102 |
| 2 | CEsKa | *P. abrotanoides* | AbyanehB, Esfahan, Iran | Center | 5 | 35° 49′N | 51° 34′E | 1190 |
| 3 | CEsAb | *P. abrotanoides* | AbyanehA, Esfahan, Iran | Center | 5 | 33° 59′N | 51° 59′E | 2222 |
| 4 | CEsKk | *P. abrotanoides* | Varguran, Esfahan, Iran | Center | 5 | 32° 63′N | 51° 65′E | 2550 |
| 5 | CEsKs | *P. abrotanoides* | Kesheh, Esfahan, Iran | Center | 5 | 33° 39′N | 51° 77′E | 2450 |
| 6 | CEsMe | *P. abrotanoides* | Mezdeh, Esfahan, Iran | Center | 5 | 33° 27′N | 51° 84E | 1977 |
| 7 | CEsGh | *P. abrotanoides* | Ghohrud, Esfahan, Iran | Center | 5 | 33° 67′N | 51° 42′E | 2350 |
| 8 | SESiKh | *P. abrotanoides* | KhashA, sistan va Baluchestan | South-East | 5 | 28° 15′N | 61° 15′E | 1410 |
| 9 | NGoSa | *P. abrotanoides* | Sari, Gorgan. Iran | North | 3 | 36° 56′N | 53° 06′E | 132 |
| 10 | NSeDa | *P. abrotanoides* | Damghan, Semnan, Iran | North | 2 | 36° 16′N | 54° 36′E | 1170 |
| 11 | NSeSM | *P. abrotanoides* | Mehmandust, Semnan, Iran | North | 4 | 36° 27′N | 54° 75′E | 1127 |
| 12 | NSeSh | *P. abrotanoides* | Shahrud, Semnan, Iran | North | 3 | 36° 41′N | 55° 02′E | 1380 |
| 13 | NEKRGS | *P. abrotanoides* | Gardaneh Sandogh Shekan, North Khorasan, Iran | North-East | 4 | 37° N | 59° E | 2000 |
| 14 | NEKRKL | *P. abrotanoides* | Kalat,orth Khorasan, Iran | North-East | 3 | 37° 8′N | 59° 30′E | 2100 |
| 15 | NEKRAd | *P. abrotanoides* | Abardeh, North Khorasan, Iran | North-East | 3 | 36° 39′N | 59° 27′E | 1468 |
| 16 | SESiKhA | *P. atriplicifolia* | KhashB,sistan and baluchestan, Iran | South-East | 4 | 28° 15′N | 61° 15′E | 1410 |
| 17 | CEsYA | *P. abrotanoides* | Yahya abad, Esfahan, Iran | Center | 1 | 33° 35′N | 51° 76′E | 2102 |