Genetic diversity and population structure of *Seriphidium* Sub-genus of *Artemisia* from different terrains of Balochistan, Pakistan

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Abstract. Rehman A, Saeed S, Ahmed A. 2021. Genetic diversity and population structure of *Seriphidium* Sub-genus of *Artemisia* from different terrains of Balochistan, Pakistan. *Biodiversitas* 22: 2658-2664. *Seriphidium* is one of the largest sub-genus of *Artemisia* growing in different geographical populations and terrains of Balochistan Pakistan. This genus is economically important and used as folk medicine. The genus is highly variable, besides morphological characterization, molecular authentication is also needed for novel drug discovery. Three significant species of the genus viz., *S. quettense*, *S. oliverianum*, and *S. maritimum* were selected for morphological and molecular characterization and phytochemical investigation. Genetic variability assessment was carried out by using RAPD and ISSR amplified 231 loci, of which 111 were polymorphic (48% polymorphism). Phenolic contents and Flavonoids were estimated for phytochemical assessment. Results revealed that the highest phenolic compounds were in *S. quettense* when compared with other investigated taxa. In conclusion, molecular marker profiling together with phytochemical variation of all three species exhibited genetic diversity and chemical variation. The findings of the current investigation can further be used as a baseline study for the implementation of conservation strategies. In addition, phytochemical assessment can be utilized for modern drug discovery.

Keywords: Genetic variability, Hazarganji, phytochemicals, *Seriphidium quettense*, *S. oliverianum*, *S. maritimum*, Ziarat

INTRODUCTION

*Seriphidium* is the sub-genus of *Artemisia* belongs to the family Asteraceae. The habit of the genus is mostly herbs and shrubs. They are found in temperate climatic conditions frequently in dry or semi-dry habitats around the world. *Seriphidium* is the largest group within the genus Artemisia, and is considered as ecologically and economically important (Malik et al. 2017). Many species of this genus are known for their medicinal and other ethnobotanical uses. The known compounds from the genus are sesquiterpene lactones and terpenoids, making their taste bitter, producing strong aroma, and discouraging herbivores. Few species of this genus are also used as flavoring agents, food and some are used to repel insects (Shaﬁq et al. 2013; Ali et al. 2017). Phytochemical survey revealed that the genus *Seriphidium* produces essential oils and releases strong aroma, indicating volatile organic compounds (VOCs) (Shao et al. 2013; 2018). In recent world, these compounds are used as insecticides (Rizvi et al. 2020), bioherbicide (Shao et al. 2018), antioxidant and anticancer (Shaﬁq et al. 2013; Mokhtar et al. 2017; Liang et al. 2020), and many taxa are anthelmintic (Xie et al. 2019; Hussain et al. 2020). These compounds are natural and have no side effects. Pollination in this genus is through the wind-dispersed.

*Seriphidium quettense* (Podlech) is locally known as Zher or Terkha sperah. Synonym of *Artemisia quettensis* is a perennial, woody, aromatic shrublet endemic to Balochistan Province of Pakistan. The plant is well known for its medicinal and ornamental uses (Saeed et al. 2020).

*Seriphidium oliverianum* (J. Gay ex Besser) Bremer & Humphries ex Y.R. Ling. Bull. is a synonym of *Artemisia oliveriana*. A densely arachnoid-tomentose to glabrescent or ± glabrous, suffrutescent shrublet. The plant perennial and is abundantly found in Balochistan, Pakistan. Earlier various antioxidants were isolated from this plant and different biological activities investigated. Many important compounds like alkaloids, saponins, tannins, terpenoids, etc. are isolated from this species. The plant is also known for its use as anti-urease and anti-bacterial activities (Ali et al. 2017; Shaﬁq et al. 2017).

*Seriphidium maritimum* (L.) Poljakov is a synonym of *Artemisia maritimum* also found in Blochistan, Pakistan. It is also perennial and known as Sea wormwood. This species is not much used as a medicine. But some other ethnobotanical uses are well known about this species (Kumar et al. 2011).

In recent years molecular marker techniques are used to assess the genetic diversity of plants. The technique is also used for correct plant identification and the conservation of medicinally important taxa (Ahmed et al. 2020). The PCR-based methods are preferable because of their easiness and utilizing less quantities of sample DNA. The polymerase Chain Reaction (PCR) can widely be modified to perform a wide range of genetic manipulations.
Studies on a number of RAPD and ISSRs markers provide a simple, potent, rapid, reproducible and low-cost means to assess genetic variability. RAPD and ISSR markers are the best, even if no prior information of the selected taxon is available. Further, such amplification does not require genome sequence information and leads to multilocus and highly polymorphic patterns (Sara et al. 2013).

This article deals with the genetic diversity in three Seriphidium species from different terrains of Balochistan, Pakistan. The species selected for molecular and phytochemical investigation are medicinally important, and the results may contribute to the conservation of threatened and endemic populations of the examined species.

**MATERIALS AND METHODS**

**Field surveys**

Field surveys were carried out over a two-year period (2018-2019) in different terrains of Balochistan, Pakistan, to investigate various Seriphidium species. Two mountainous ranges were selected for plant collection Ziarat (2,200-3,400 m asl.) and Hazarganji-Chiltan National Park mountain range from 1600-3300 masl (Figure 1). Field methods were followed as described by (Saeed et al. 2020).

**Sample collection**

Plant specimens were collected (in the replicates of three), identified and described morphologically. The voucher specimens were deposited in Botany Department, University of Balochistan, Quetta herbarium for future reference (Table 1).

**Morphology**

A total of 21 morphological characters were observed for morphological analysis listed in Table 3. Different magnifiers were used for observations of various parts.

**Phytochemical variation**

For phytochemical investigation, three samples were collected from each site. Total phenolic and flavonoid contents were estimated by using Double Beam Spectrophotometer (Hitachi u-2800).

**Flavonoids**

The method of Ordonez et al. (2006) was used for analysis of flavonoid contents. The amount of flavonoid measured at 420 nm absorbance. Total flavonoids contents were assessed by the quercetin (mg g⁻¹) equation Y=0.0255xR²=0.9812.

**Total phenolic contents**

The method by Slinkard and Singleton (1977) used to analyze total phenolic content was calculated at 765 nm absorbance. Total phenolic contents were expressed as mg g⁻¹ tannic acid equivalent by the equation Y=0.1216xR²=0.9365.

![Figure 1. Map of Balochistan, Pakistan illustrating two study sites of plant sampling. 1. Hazarganji-Chiltan National Park, 2. Ziarat](image)

| Plant name       | Vern. name  | Voucher no.       | Locality       | Elevation (m asl.) | Coordinates          | Flowering period     |
|------------------|-------------|-------------------|----------------|-------------------|----------------------|----------------------|
| S. quettense     | Zher / Tarkha | QUETTA000025     | Hazarganji     | 2800              | 30.1312°N; 66.4348°E | May-November         |
| S. oliverianum   | Tirkha      | QUETTA000358      | Ziarat         | 2500              | 30.3810°N; 67.7270°E | August-September     |
| S. maritimum     | Tirkha sperah | QUETTA000121     | Ziarat         | 2800              | 30.3810°N; 67.7270°E | August-September     |
Genetic diversity

The DNA extraction was carried out by using the method of Doyle (1991) with few modifications. The PCR reaction was carried out by using method of Saeed et al. (2017). A total of Twenty six primers (RAPD 17 and ISSR 09) were used (Table 2).

PCR reaction

The PCR reaction for amplification was carried out in total 20 µl volume. The PCR master mix was prepared to contain 3 mM MgCl2, 1X PCR buffer, 0.2 mM dNTPs mix, 20 pmol primers, 0.01% gelatin, 1U/rxn Dream Taq DNA polymerase and 25 ng DNA of template DNA. PCR amplification was performed using Applied Biosystems 96 well (USA), thermal cycler. For RAPD primers, PCR was programmed as mentioned below in table with initial denaturation at 94 °C for 3 min and then 36 cycles with denaturation at 94 °C for 1 min, annealing temperature at 36 °C for 1 min, extension step at 72 °C for 2 minutes and final extension for 5 minutes at 72 ºC. For ISSR primers, PCR was programmed as; initial denaturation at 94 °C for 3 min and then 40 cycles with denaturation at 94 °C for 1 min, annealing temperature 56 °C for 1 min, extension step at 72 °C for 2 minutes followed by an additional final extension for 5 minutes at 72 ºC. The amplified products were checked by electrophoresis in 1.8% agarose gels containing ethidium bromide (0.5 μg ml⁻¹) in 1X TAE buffer. The product was visualized by Gel documentation system under UV light and the size of markers was estimated by comparing it to the standard ladder (100 bp BIORON 0.2 mg ml⁻¹) in the gel.

Table 2. Names and sequences of RAPD and ISSR used in analysis of genetic diversity

| Name of marker | Sequence       | Size of nucleotide |
|---------------|----------------|--------------------|
| RAPD          |                |                    |
| OPA-3         | AGTCAGCCAC     | 10                 |
| OPA-7         | GAACCGGGTG     | 10                 |
| OPA-9         | GGGTAACGCC     | 10                 |
| OPA-15        | TCCGAACCC      | 10                 |
| OPA-17        | GACCGCTTGT     | 10                 |
| OPA-18        | AGGTGGCCGT     | 10                 |
| OPB-1         | GTTTCGCTTC     | 10                 |
| OPB-4         | GACCTGGAGT     | 10                 |
| OPB-6         | TGCTCCTGCC     | 10                 |
| OPB-8         | GTCCACACGG     | 10                 |
| OPB-17        | AGGGAACGAG     | 10                 |
| OPC-06        | GAAACGACTC     | 10                 |
| OPC-07        | GTCCCGAGCA     | 10                 |
| OPC-11        | AAACTCGCCG     | 10                 |
| OPC-13        | AAGCCTCGTC     | 10                 |
| OPC-15        | GACGGATCAG     | 10                 |
| OPC-16        | CACACTCCAG     | 10                 |
| ISSR          | (CAC)7T        | 22                 |
| ISSR-2        | (GA)9C         | 19                 |
| ISSR-5        | (CAC)7GT       | 23                 |
| ISSR-15       | (GA)8C         | 17                 |
| ISSR-18       | (CT)8G         | 17                 |
| ISSR-21       | (CA)8G         | 17                 |
| ISSR-23       | (GT)8C         | 17                 |
| ISSR-29       | (AC)8C         | 17                 |
| ISSR-30       | (AC)8G         | 17                 |

Statistical analysis of data

For analysis of genetic diversity molecular markers, data were scored as 1 (present) and 0 (absent). RAPD and ISSR data were clustered and dendrograms based on similarity matrices were calculated by using Sequential Agglomerative Hierarchical and Non-overlapping (SAHN) algorithm by “Unweighted Pair Group Method with Arithmetic Mean” (UPGMA) through Numerical by using NTSYS 2.10 (Rohlf 1998). Plant secondary metabolites (PSMs) were analyzed statistically by using XLSTAT version (2007). In the graphs, values presented are mean ± SE. M. Morphological comparison was carried out by different qualitative and quantitative characters (Table 3). Three individuals per species were observed in the biometric analysis.

RESULTS AND DISCUSSION

The present study is the first comprehensive report on the morphological, chemical, and molecular diversity of three species of Seriphidium the one of the largest subgenus Artemisia groups. The data was collected from two high mountainous regions of Balochistan, Pakistan, i.e., Ziarat and Hazarganji-Chiltan National Park.

Morphological diversity

Life cycle and plant nature

Genus Seriphidium is a perennial shrub. S. quettense is perennial, woody and aromatic erect shrub. S. oliverianum is also woody and much-branched as compared to other two species. S. maritimum is perennial temperate shrub and aromatic similar to wormwood.

Plant height and diameter

Height of S. quettense is 38 to 40 cm and its diameter is 101 cm. S. oliverianum is 40 to 60 cm tall with large diameter 125 cm. The S. maritimum height range of 50 to 65 cm and diameter shorter than other two species 90 cm.

Plant surface and stem nature

Plant surface presented in Figure 2. S. quettense is densely whitish hairy and color is pale green. Surface of S. oliverianum is densely arachnoid-tomentose to glabrescent and color is brownish yellow. The S. maritimum stem surface is also densely hairy and greenish in color.

Leaf length and width

Leaf length and width vary in all three species. Average leaf length and width of S. quettense is 2.7 x 1.4 cm respectively. The length and width of S. oliverianum is 3.9 x 2 cm and S. maritimum is 2.2 x 1.2 cm, respectively.

Leaf structure

Leaf structure varies as S. quettense leaves punctate-glandular covered with hair, greyish-green with petiole. Leaves of S. oliverianum are also long-petiole and oblong in shape. Leaves of S. maritimum are white and pinnate-sect with numerous segments.
Flower

Capitula of *S. quettense* is numerous and sessile, shape is ovate-oblong and arranged obliquely erect. *S. oliverianum* has sessile capitulum, oblong to narrowly ovate shape, arranged towards apices. *S. maritimum* inflorescence is generally in the form of a paniculate-raceme arrangement, flat and naked or covered by hair attached with small stalks. Shape of flower is ovoid and aromatic.

Flower size

*Seriphidium quettense* flowers are 3.5-4.5 x 2 mm long. Flowers of *S. oliverianum* are 3-4 x 1.5 mm long and the *S. maritimum* have short flowers of 2.5 x 1 mm length.

Flowering period

*Seriphidium quettense* flower period is between May to November, which is a comparatively long period compared with other two species. Flowering period of *S. oliverianum* is very short between August and September. The flowering period of *S. maritimum* is between October to November.

Genetic diversity

The present work is based on two different molecular markers RAPD and ISSR. These primers are used for the first time on three species of this significant genus *Seriphidium* from Balochistan. The sizes of amplified bands were 250 to 1000 bp from RAPD, and 200 to 1700 bp from ISSR. Combined molecular marker system of RAPD and ISSR were used to generate a dendrogram and cluster analysis. The present approach revealed better results and low chance of error as described earlier by (Saeed et al. 2020; Saeed et al. 2017). Out of total of 35 used makers, 26 were amplified, 17 RAPD and 09 ISSR exhibited polymorphism revealed by reproducible bands amongst various *Seriphidium* species. Table 4 explains the characteristics of banding patterns obtained by primers. The amplified primers showed 231 loci and 111 were polymorphic (48% polymorphism). The average bands scored per primers were 4.27.

![Figure 2. Morphological variation of three species. A. Seriphidium maritimum, B. S. quettense, C. S. oliverianum](image-url)
Based on the amplified bands, data were subjected to cluster analyses shown in Figure 3. Two clusters A and B were obtained. Cluster A represented the population of *S. quettense* collected from Hazarganj-Chiltan National Park. *S. quettense* is the endemic species of the region. While Cluster B comprised of two species collected from Ziarat, i.e., *S. oliverianum* and *S. maritimum*.

### Chemical diversity

Three species of *Seriphidium* were assessed for phytoconstituents. The total phenolic content (TPC) expressed as tannin equivalent and flavonoids content as quercetin equivalent within and among populations (Figure 4). The TPC ranged from 51 to 53 mg g⁻¹ within the population of *SQ*, 40 to 42 mg g⁻¹ within population of *SO* and 40 to 43 mg g⁻¹ within population of *SM* (Table 4). Mean concentration of TPC across the populations was 45.37±1.89 mg g⁻¹ whereas flavonoids showed diverse patterns within and among population. It ranged from 137 to 138 mg g⁻¹ within population of *SQ*, while in population of *SO* it ranged from 131-135 and population *SM* from 122-127 mg g⁻¹. Mean concentration of flavonoids across the populations was 132±3.15 mg g⁻¹.

### Discussion

In the recent era, the indigenous folk medicines and traditional uses of herbs acquired importance in primary health care system. Most of the population of world depends upon medicinal plants and the phytochemicals isolated from these plants are being used for drug discoveries. Since the wild plants used in the drug discoveries, phytochemical screening and proper identification is required. Incorrect identification of plants due to similar morphological characters may cause loss in the production of herbal drugs.
Table 5. Phytochemical composition representing mean±SD values

| Plant code | Total phenolic (mg g⁻¹) | Flavonoids (mg g⁻¹) |
|------------|-------------------------|---------------------|
| SQ1        | 42.15±0.78              | 117.35±0.54         |
| SQ2        | 41.32±1.25              | 112.65±0.71         |
| SQ3        | 40.18±1.44              | 115.55±0.91         |
| SO1        | 43.74±1.31              | 102.25±1.24         |
| SO2        | 42.58±0.86              | 106.84±1.95         |
| SO3        | 45.54±0.21              | 101.51±3.12         |
| SM1        | 51.64±1.19              | 135.11±1.72         |
| SM2        | 45.86±0.82              | 130.82±1.94         |
| SM3        | 46.29±2.14              | 127.51±1.27         |
| Mean average | 45.37±1.89              | 132±3.15            |

Figure 3. Cluster analyses based on molecular markers

Figure 4. Patterns of phytochemical variations among different populations of Seriphidium

Molecular and morphological characterization resolves the misidentification of the taxa. Therefore, field surveys of Seriphidium plants have been undertaken in conserved region of Hazarganj-Chitnan National Park and Ziarat, Balochistan Pakistan. S. quettense, S. oliverianum, and S. maritimum were morphologically identified using taxonomic keys. The analysis of morphological criteria clearly reflects the differences among the populations of three Seriphidium species. Earlier S. quettense from various ecological zones of Balochistan was investigated (Saeed et al. 2020). Five species of Seriphidium from Balochistan Pakistan were characterized based on morphology (Peer et al. 2020). S. maritimum were characterized for its proper identification our finding is in agreement with its morphological characters (Kumar et al. 2011).

Genetic diversity plays an important role in the proper identification as well as planning of conservation of medicinally important taxa. The molecular marker system can be utilized to conservation medicinally important taxa (Saeed et al. 2016). Many researchers elucidated that change in the morphological characters may be due to harsh climate or weather. Plant adaptation property tends to alter their characteristics in response to any biotic factors. In the light of above-mentioned facts of morphological variation. The populations of S. quettense showed more variation within and among populations in comparison with other studied taxa that may be because the later is found in the same vicinity. This report contains genetic differences of three species of Seriphidium for the purpose of proper identification based on DNA fingerprinting by using RAPD and ISSR markers. A UPGMA tree exhibited the genetic distance amongst three populations of Seriphidium represented in Figure 2.

Results revealed a relatively high level of genetic distance of 0.80 of S. quettense when compared with the populations of S. oliverianum and S. maritimum that diverged at 0.61 and are closely related. Previously, Saeed et al. (2020) presented the highest degree of genetic similarity (0.53) that occurred between S. quettense from two different ecological zones of Balochistan. Phylogenetic studies provided the logical basis for classification of species. In current project, morphological characterization carried out along with the phytochemical and molecular characterization of various populations.

The selected plants are found to be used for medicinal purposes, total phenols and phenolic components were present. The similar level of total flavonoids found in all three species in our study is consistent with the results obtained by a photometric method exhibiting no significant differences. Total phenolics compounds varied from 40.18-54.41 mg g⁻¹. Although the average Phenolic contents and flavonoid content observed for our study species was similar to those reported (Saeed et al. 2020). Earlier different antioxidant and anticancer compounds were reported from genus Seriphidium (Deng et al. 2013; Mohamed et al. 2010). Our results suggested that other phytochemical investigations included GC-MS or HPLC may carry out for the studied taxa for isolation of medicinally important compounds from these three species.

The purpose of phytochemical investigation is to provide information about the best and suitable species of Seriphidium for further analysis. Further sequence analysis is strongly recommended for the authenticity of the species.

In conclusion, the present work on three species of Seriphidium in Balochistan, Pakistan reported here is an initial attempt that needs to be expanded towards a broad survey to validate the distribution of other species of
Seriphidium. The ecological variations may have played a role in the genetic diversity of Seriphidium species in study area. Results of the study may be utilized for future sustainable conservation plans.

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