



Genetic Diversity in *Podophyllum hexandrum* Royle from Himachal Pradesh, Northwestern Himalaya, India

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Abstract

Podophyllum hexandrum belonging to family Berberidaceae is a herbaceous rhizomatous species of great medicinal importance, now endangered in India. *Podophyllum hexandrum* is a valuable medicinal herb commonly known as the Himalayan May Apple, grows in the Himalayan alpine and sub alpine zones. It has long been used by the Himalayan natives and the American Indians. Medicinal use of *Podophyllum hexandrum* dates back to ancient times. Ten representative sites were selected and assessed for its population and genetic diversity. Further, representative plant samples were planted at two different locations for observing the diversity in lamina shapes. Plants of *Podophyllum hexandrum* were observed to have different number of leaves per plant viz. single leaf, two leaves, three leaves and four leaves per plant. The plant bearing single leaf were more abundant (48.1%) as compared to two leaved (35.4%), three leaved (16.9%) and four leaved plant (0.2%). A mature berry in the single leaved plant was observed for the first time. A considerable morphological variations in the lamina shape of leaf were observed and a total of ten lamina shapes were documented. The lamina shape and number of leaves per plant of most of the plants was found to vary in successive vegetational cycles when observed at two distinct geographical locations. Because the species is threatened at regional and global level, studies of its population biology and genetic diversity are imperative for successful development of its conservation strategies.

Keywords: Genetic diversity, lamina, morphological, population

1. Introduction

In the present scenario quality and quantity of medicinal plants are serious issues for the pharmaceutical and dietary supplements industries. Traditionally, the raw material of most of medicinal plants has being regularly harvested from the wild. Increasing public demand for these products is creating a serious environmental problem as demand is outpacing the supply and endangering the survival of many of these species in the world. Therefore, in the face of the emerging threats of destructive harvesting, habitat destruction and bio-piracy (Gadgil, 1996 and Sultan et al., 2008) it becomes imperative to document the valuable knowledge of these species (Gadgil, 1996). The Himalayan region is home of numerous highly valued medicinal plants including *Podophyllum hexandrum* (Vern. *Ban Kakari*) that grows in very restricted pockets in the Himalayan alpine and subalpine zones, now endangered in Himachal

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Pradesh (CAMP 2010). The term *Podophyllum* is derived from ancient Greek word 'podos' means a foot and 'phyllos' means a leaf. The name refers to the resemblance of leaves to duck's foot (Kumar et al., 2017). The genus *Podophyllum* is represented by five species including *P. hexandrum*, *P. peltatum*, and *P. sikkimensis*. Of these *Podophyllum* species, *P. hexandrum* is medicinally more valuable, containing comparatively higher concentration of podophyllotoxin (Muzafar Ahmad Rather and Shajrul Amin, 2016).

Underground parts i.e. rhizome and roots of the plant yield podophyllotoxin, an active ingredient that is effective in treatment of lung cancer, a variety of leukemias, and other solid tumors (Sharma et al., 2011). Podophyllotoxin is effective against many diseases, and is widely used as a starting compound for the semi-synthesis of anticancer drugs i.e. etoposide (VP-16-213), teniposide (VM-26) and etopophos (Biswas et al., 2018). The rhizomes of *P. hexandrum* contain three times more podophyllotoxin content in comparison to the other species, i.e. *P. peltatum* and *P. sikkimensis* (Venkatasubramanian et al., 2018). Kashmir it has been used in traditional system of medicine from time immemorial and is locally known as Banwangun, since its red colour fruit (berry) is of the size of a small brinjal (Malik et al., 2018). There has been massive extraction of its rootstock (official part) over the last several decades leading to destructive harvesting. This has led to severe reduction in its population density. A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites. Therefore, considering the importance, threat perception and need, the studies on its population, genetic diversity and the structure of population within a species may not only illustrate the evolutionary process and mechanism but also provide information useful for its biological conservation in the natural habitat.

2. Material and Methods

Extensive field surveys were conducted in different alpine and sub alpine zones ranging from 2050 m to 3636 m amsl (above mean sea level) of Himachal Pradesh (Figure 1) in northwest Himalaya for locating different populations of *Podophyllum*

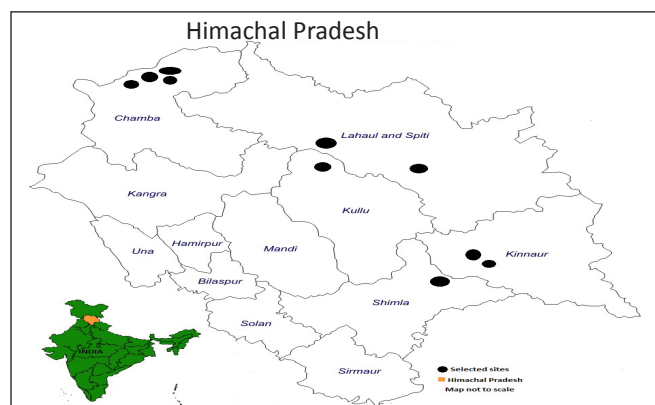


Figure 1: Study area

hexandrum Royle. Ten geographically distinct locations were selected to determine the distribution pattern on the basis of survey (Table 1).

The sites were visited and quadrats of size 100×100 m² were laid out at each site. All the plants of *Podophyllum hexandrum* in the quadrats were counted. All the plants were morphologically observed and on the basis of number of leaves per plant, the plants were divided into; 1) Single leaved, 2) Two leaved, 3) Three leaved and 4) Four leaved plants.

The morphological variations in leaves among and within natural population were also recorded. Also the representative plants (~30 numbers at each location) of different lamina shapes and number of leaves per plant were grown at two distinct geographical locations i.e. Village Rahla, District Kullu, Himachal Pradesh at 3048 m amsl and Village Shilly, District Solan, Himachal Pradesh at 1500 m amsl. This was done for observing the stability of lamina shape and number of leaves per plant in successive growing cycles.

The individuals of each plant type were assessed in the population and their percentage in the population was calculated as below:

Percentage of single leaved plants in population=(Total no. of individuals of single leaved plants at a site/Total no. of individuals of *Podophyllum hexandrum* at a site)×100

Percentage of two leaved plants in population=(Total no. of individuals of two leaved plants at a site/Total no. of individuals of *Podophyllum hexandrum* at a site)×100

Percentage of three leaved plants in population=(Total no. of individuals of three leaved plants at a site/Total no. of individuals of *Podophyllum hexandrum* at a site)×100

Percentage of four leaved plants in population=(Total no. of individuals of four leaved plants at a site/Total no. of individuals of *Podophyllum hexandrum* at a site)×100.

3. Results and Discussion

3.1. Distribution

The survey in alpine and sub-alpine regions revealed that the plants were located in dense forest in sub-alpine zones and as with increase in altitude (in alpine zone) the plants were found in open and rocky areas. Plants of *Podophyllum hexandrum* were found associated with some of the valuable medicinal plants such as *Valeriana jatamansi*, *Polygonatum cirrhifolium*, *Bergenia ciliata*, *Aconitum heterophyllum*, *Trilidium govanianum* in sites viz., Chanshal ghati, Nichar, Katgaun, Jagrauta, Topi, Kalatop, Paulanhi and Manali. In Koksar, plants of *Podophyllum hexandrum* were found in open rocky areas and in Chatru plants were found in pure forest of *Betula utilis*. During the present study, ten different populations of *Podophyllum hexandrum* at an altitude ranging from 2050m to 3636 m amsl were studied (Table 1) for status of plant on the basis of number of leaves per plant and leaf characteristics like shape, size etc. The Himalayan region is home to numerous highly valued medicinal plants

Table 1: Sites characteristics of *Podophyllum hexandrum* in Himachal Pradesh

No.	Name of site	Altitude (m)	Latitude	Longitude	Dominant species
1.	Chanshal ghatti (Shimla)	3636	31°11'48.54" N	77°59'18.33" E	<i>Quercus leucotrichophora</i> , <i>Cedrus deodara</i>
2.	Nichar (Kinnaur)	2890	31°30'00.13" N	78°00'11.94" E	<i>Cedrus deodara</i>
3.	Katgaun (Kinnaur)	2855	31°37'00.19" N	78°00'01.92" E	<i>Cedrus deodara</i> , <i>Abies pindrow</i>
4.	Koksar (Lauhal & Spiti)	3160	32°22'20.00" N	77°17'26.40" E	<i>Bergenia ciliata</i> , <i>Caragana versicolor</i>
5.	Chatru (Lauhal & Spiti)	3389	32°29'20.06" N	77°17'16.38" E	<i>Betula utilis</i>
6.	Jagrauta (Chamba)	2270	32°32'42.79" N	76°01'10.98" E	<i>Cedrus deodara</i>
7.	Topi (Chamba)	2200	32°33'14.01" N	76°01'36.96" E	<i>Cedrus deodara</i> , <i>Pinus wal-lichiana</i>
8.	Kalatop (Chamba)	2423	32°54'14.07" N	76°01'36.17" E	<i>Cedrus deodara</i>
9.	Paulanhi (Chamba)	2510	32°33'19.36" N	76°01'42.35" E	<i>Abies pindrow</i> , <i>Cedrus deodara</i> , <i>Quercus floribunda</i>
10.	Manali (Kullu)	2050	32°20'05.20" N	77°12'26.50" E	<i>Cedrus deodara</i> , <i>Abies pind-row</i> , <i>Picea smithiana</i>

including *Podophyllum hexandrum* Royle (Berberidaceae) which is a herbaceous rhizomatous species of great medicinal importance. It has long been used by the Himalayan natives and the American Indians. Medicinal use of *Podophyllum hexandrum* dates back to ancient time. The plant has been described as Aindri- a divine drug in Indian traditional system of medicines, the Ayurveda and has been also used by the Chinese system of medicines for the treatment of various ailments. Of the natural components with anti-cancerous activities, podophyllotoxin occupies a very important position. Podophyllotoxin is a natural occurring lignans, which is extracted from *Podophyllum hexandrum* and *Podophyllum peltatum*. *Podophyllum hexandrum* of Indian origin contains more than twice podophyllotoxin than its American counterpart, *Podophyllum peltatum*. The plant extract has also been found to possess radioprotection by modulating free radical flux involving the roles of lignans present (Sultan *et al.*, 2010). The rhizome of *Podophyllum hexandrum* is being indiscriminately harvested in large quantities from the wild to meet the ever increasing demand for the crude drug. As a result of this and lack of organized cultivation, *Podophyllum hexandrum* has been reported as a endangered species from the Himachal Himalayas. The population size of *Podophyllum hexandrum* is very low (40-700 plants per location) which is declining every year, and some of the population have virtually disappeared owing to anthropogenic and overexploitation (Bhadula *et al.*, 1996 and Airi *et al.*, 1997). It is distributed in restricted pockets throughout the temperate and alpine Himalayan region. Since the species is already endangered and exploitation of its underground parts continues to exceed the rate of natural regeneration, it needs immediate attention for conservation. Population biology and genetic diversity studies are important for the development of conservation strategies. In order to develop *Podophyllum hexandrum* as an alternative crop so as to meet the growing demand of

pharmaceutical industry, it would be essential to evaluate its natural population for podophyllotoxin content and growth and yield parameters so as to isolate a better strain/variety with higher growth and yield potential. The aim of present study was to study the genetic diversity in the diminishing wild population of *Podophyllum hexandrum* in the North-Western Himalayas.

3.2. Status of the *Podophyllum hexandrum* on the basis of number of leaves per plant

Relative percentage of different types of *Podophyllum hexandrum* plants in different populations of Himachal Pradesh revealed that out of total plants of *Podophyllum hexandrum*, overall single leaved plants (48.1%) were more abundant as compared to two leaved plants (35.4%), three leaved plants (16.9%) and four leaved plants (0.2%). Four leaved plants were observed only in single population at Manali. Single leaved plants with mature berry were observed only at Jagrauta (9.1%) and Manali (1.6%) populations (Figure 2).



Figure 2: Single leaved plants with mature berry

Among different populations, out of total *Podophyllum hexandrum* plants observed, maximum percentage of single leaved plants was recorded at Manali (63.9%), followed by Nichar (60.0%), Katgaun (60.0%), Chatru (55.6%), Chanshal

ghati (53.9%), Jagrauta (45.5%), Paulanhi (42.0%), Topi (40.0%) and Kalatop (40.0%). Minimum percentage of single leaved plants was observed at Koksar (20.0%). The percentage of two leaved plants out of total plants observed ranged from 20.0% to 46.7% in different population with maximum (46.7%) at Topi and minimum (20.0%) at Katgaun population. Three leaved plants percentage ranged from 10.0 % to 41.9% in different populations with maximum (41.9%) recorded in Koksar and minimum (10.0%) recorded at Nichar. Out of the total ten populations, three leaved plants occurred at eight populations only. At Chanshalghati and Chatru only single leaved plants and two leaved plants were recorded. Most of the two leaved plants and three leaved plants were with berry. The four leaved plants observed only at Manali were with berry. The representative plant of different lamina shape and number of leaves per plant were planted at two locations i.e. Rahla and Shilly. Plants grown in successive seasons have considerable variation in lamina shape and number of leaves per plant. The lamina shape of plants was observed to change to another lamina shape in successive growing cycle. The number of leaves per plant were also found to vary in successive growth cycle as reported by Bhadula et al., 1996 who also observed morphological differences in plants of *Podophyllum hexandrum* when planted out of their natural

3.3. Inter and Intra population leaf polymorphism in *Podophyllum hexandrum*

Considerable variation in number of leaves per plant, leaf/lamina shape, leaf size and leaf incision was observed in plants of different populations. The number of leaves per plant varied from 1 to 4 in different populations (Figure 3). On the basis of

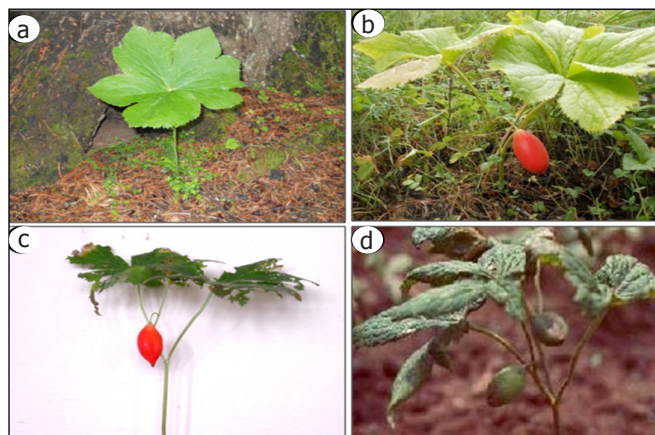


Figure 3: Leaf variants of *Podophyllum hexandrum* Royle, a: Single leaved; b: Two leaved; c: Three leaved; d: Four leaved number of leaves per plant, plants of *Podophyllum hexandrum* has been grouped into four type i.e. Single, Two, Three and Four leaved plant. The plants of different populations were observed to have different type of lamina shapes. Lamina shapes observed in plants of *Podophyllum hexandrum* has been grouped into ten different types of Lamina shapes. The number of lobe per leaf varied from 1 to 9 in different lamina shapes. During the present study, *Podophyllum hexandrum*

genotypes collected from ten different locations ranging from 2050 m to 3636 m amsl altitude from Himachal Pradesh exhibited variation in number of leaves per plant. The numbers of leaves observed were 1, 2, 3 and 4 per plant (Table 2). These morphological variants has also been reported on the basis of presence of 1, 2, 3 and 4 leaves per plant from Garhwal Himalayas (Purohit et al., 1998 and Bhadula et al., 1996), Kashmir Himalayas (Sultan et al., 2008) and North-Western Himalayas (Alam et al., 2009). In general, 48.08 percent plants had single leaves, 35.35% had two leaves, 16.94% had three leaves and 0.20% had four leaves. These findings are in line with findings of Alam et al., 2009 who also observed 39.5% plants with single leaf, 30 percent with two leaves, 20% with three leaves and 10.5% with four leaves. One of the interesting and novel features observed in the present study was that single leaved plant bear mature berry which was not observed by Alam et al., 2009 and Purohit et al., 1998 who observed single leaved plant bearing abortive berry.

Considerable morphological variation in the lamina shape of the leaf was observed during the present study. The plant of *Podophyllum hexandrum* has been observed to have ten different types of lamina shapes (Figure 4). The number of

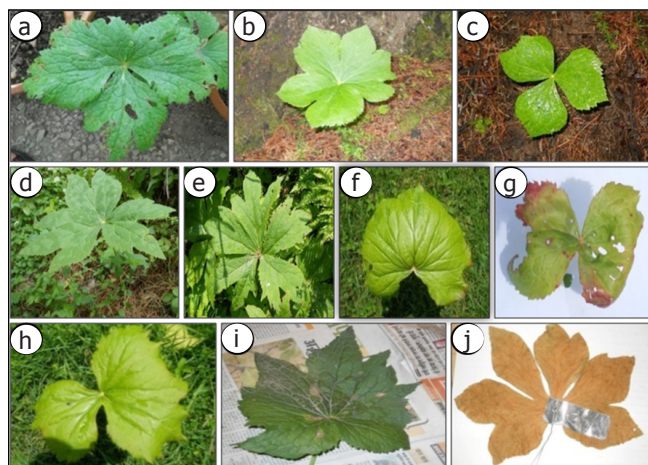


Figure 4: Different lamina shapes in *Podophyllum hexandrum*

lobes per leaf varied from 1 to 9 in different lamina shapes. Bhadula et al., 1996 has also observed morphological variation in leaf characteristics of *Podophyllum hexandrum* from Garhwal Himalayas.

The different type of lamina shapes/morphotypes observed is described as below (Figure 4):

- Lamina type A: Two lobed with smaller lobe almost entire. Larger lobe further divided into four partially separate sub lobes with the incision not reaching upto center margin.
- Lamina type B: Lamina prominently two lobed those are unequal in shape and size. The smaller lobe is further partially bilobed at the incision between the two prominent veins. The larger lobe is further partially sublobed into unequal four lobes with incision upto middle between margins to center.
- Lamina type C: Lamina trilobed, incision going deep down

Table 2: Percentage distribution of different type of plants in different populations of *Podophyllum hexandrum*

Sites	One leaved (%)			Two leaved (%)			Three leaved (%)			Four leaved (%)	
	WB	WOB	Total	WB	WOB	Total	WB	WOB	Total	WB	Total
Chanshalghatti (S1)	-	53.84	53.84	23.08	23.08	46.16	-	-	-	-	-
Nichar (S2)	-	60.00	60.00	20.00	10.00	30.00	10.00	-	10.00	-	-
Katgaun (S3)	-	60.00	60.00	20.00	-	20.00	20.00	-	20.00	-	-
Koksar (S4)	-	20.00	20.00	35.35	6.66	42.01	35.33	6.66	41.99	-	-
Chatru (S5)	-	55.55	55.55	33.33	11.11	44.44	-	-	-	-	-
Jagrauta (S6)	9.09	36.36	45.45	18.18	9.09	27.27	27.27	-	27.27	-	-
Topi (S7)	-	40.00	40.00	6.66	40.00	46.66	13.33	-	13.33	-	-
Kalatop (S8)	-	40.00	40.00	26.66	6.66	33.32	26.66	-	26.66	-	-
Paulanhi (S9)	-	42.00	42.00	42.00	-	42.00	18.00	-	18.00	-	-
Manali (S10)	1.61	62.36	63.97	20.09	1.61	21.7	5.78	6.45	12.32	2.08	2.08
Overall Distribution (%)		48.1 %			35.4%			16.9%		0.2%	

Abbreviations used: WB: With berry; WOB: Without berry; S: Site

to the center, lobe are more or less equal in size and shape.

- Lamina type D: Lamina prominently divided into three almost equal lobes with incision deep upto the center. Each lobe further subdivided into 2-3 sublobes with the incision upto the middle from margin to center.

- Lamina type E: Lamina is prominently divided into five lobes with incision deep upto the center. Each lobe is further subdivided into 2-3 equal or unequal sublobe with incision reaching upto 1/3rd from margin toward center.

- Lamina type F: Simple lamina, entire lamina is without any incision or lobe.

- Lamina type G: Lamina completely lobed with each lobe almost equal in shape and size.

- Lamina type H: Lamina completely bilobed with smaller and larger lobe. Each lobe is entire without any incision or lobe.

- Lamina type I: Broad lamina with partially lobing into 7-9 lobes. Lamina incision almost upto 1/3rd of the lamina length from margin to center.

- Lamina type J: Lamina lobular with incision reaching upto 2/3rd length from margin to center. Two large lobes are further subdivided into two sublobes, one lobe is entire and another with a large cleft.

4. Conclusion

In order to develop this endangered species, *Podophyllum* as an alternative crop to meet the demand of pharmaceutical industry, it would be essential to evaluate its natural population for podophyllotoxin content and growth and yield parameters so as to isolate a better strain/variety.

5. Acknowledgement

Authors are highly thankful to the local inhabitants of the areas surveyed in Himachal Pradesh for their willingness to share the valuable knowledge and wholehearted co-operation. Dr. Y. S.

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