

Establishing in situ gene bank of *Rauvolfia serpentina* (L.) Benth ex Kurtz in Western Nepal with a focus on conservation and sustainability

Abstract

Rauvolfia serpentina is a cosmopolitan in tropical habitats with more concentration in the tropics of Africa, Asia and America. Higher diversity has been recorded from the Indian Sub-continent region, especially around the gangetic plain areas. In Nepal, its distribution stretches from East to West up to an altitude of 900 m asl. Genetic resources are currently facing tremendous threats due to increased anthropogenic activities such as deforestation, habitat alteration and unsustainable harvesting for trade. A forest gene bank concept in which large range of gene sources is purposely put together is adapted to conserve genetic resources of the *Rauvolfia serpentina* plant. To enrich our knowledge regarding the natural habitat of the species, literature review, visit to the herbarium centre and field visits were preformed. All accumulated data was subjected to DIVA GIS to plot a predictive map of the plant habitat based on climate and altitude. The map depicts western *Terai* to be a safe zone for this species. Sinks and source area for the gene conservation concept revealed Bishnupur (Bardiya District) and Vajni (Kailali District) sites to be the sink areas, while other 34 sites as the source areas.

Keywords: conservation, germplasm, gene pool, policies, economic importance

Introduction

Rauvolfia serpentina or common name ‘Serpentine’ is a medicinal plant belonging to the Apocynaceae family and is an endangered plant. It is indigenous to the forest of South East Asia (Nepal, India, East Pakistan, Bhutan) including Burma, Bangladesh, Srilanka, Malaysia and Indonesia.¹⁻⁵ In Nepal, its distribution ranges from East to West up to an altitude of 900m above the sea level. This genus comprises of two species, among them *Rauvolfia serpentina* is one of them.² This *Rauvolfia serpentina* species is included in the endangered list by International Union of Conservation of Nature (IUCN), Critical by Conservation Assessment and Management Plant (CAMP), Nepal conservation List as Kha (Banned for export outside the country without processing), CITES included in Conservation List II. The Nepal Forest Act 1993, Forest Regulation 1995 and Amendment 2001 have banned the export of the Serpentine without processing. It is included in the national priority list for cultivation by the Ministry of Forest and Soil Conservation, Department of plant Resources, Kathmandu, Nepal (DPR 2012). Among several species, the genetic invasion has already been faced by *Rauvolfia serpentina* in Nepal. The large scale unsustainable over-harvesting of *Rauvolfia* has threatened its continuous survival in wild and genetic diversity has been threatened. This medicinal plant has been listed as protected plant in Nepal. Forest Act 1993 and CITES 1975 Appendix II has provided the legal protection from over-harvesting; however, extraction of this resource from the forest still continues. On the other hand, there is lack of enough scientific information on the accessions, genetic diversity and breeding system of ‘Serpentine’ in Nepal. Implementation of study to locate and assess diversity, explore the conservation ways and sustainable utilisation measures are apriority that require immediate actions in Nepal. The current research is one of the novel concepts in Nepal which integrate forest gene bank approach to secure the long-term conservation of globally significant biodiversity *i.e.*, *Rauvolfia serpentina* of the western *Terai* region of Nepal with the goal of its sustainable development.

Two broad approaches of conservation, viz., *in situ* and *ex situ* have already been shown as effective ways to conserve the global biodiversity. There are different types of conservation modalities at different levels, such as from gene to landscape levels that have been developed to further strengthen the conservation initiatives. The general conservation strategies for forest genetic resources are basically similar to those one that applies to crop species and consist of consecutive phases. The existing forest resources and their genetic variation is a starting point for conservation activities. After the priority species have been identified, their genetic diversity needs to be assessed so that the conservation activities and possible germplasm collection are focused on suitable areas. As an active conservation measure, locally isolated gene pools of critically endangered species could be combined into new *ex situ* and *in situ* conservation stands, or into the so-called ‘Forest gene banks’ in which large ranges of gene sources are purposely put together.⁶⁻¹⁵

The *Rauvolfia serpentina* species which possess a global significance and local importance for people’s livelihoods has been identified as one of the key species and present research approaches of conservation strategies are to establish the Serpentine gene bank in Nepal. The purpose of this study was, therefore, to identify the population and areas of the endangered species *Rauvolfia serpentina* and its geographical distribution map in the studied area which are rich and unique in genetic diversity (sink areas) and complementary habitats to further enrich it (source areas).

Material and methods

The research was carried out at Western *Terai* Landscape Complex. For the conservation and sustainable use of globally significant biodiversity in Nepal’s Western *Terai* Landscape Complex and establishment of effective management systems and building capacity of community, local organizations and line agencies, the Ministry of Forests and Soil Conservation (MFSC) implemented the Western *Terai* Landscape Complex Project (WTLCP) in three districts

namely, Kanchanpur, Kailali and Bardiya districts that extends from 28°59'13", 80°06'05" and 28°04'14"- 81°30'07". The research utilizes local knowledge with scientific studies such as taxonomic surveys, ecological studies to formulate a sound conservation strategy for the establishment of *Rauvolfia serpentina* plant Gene Bank and its management in WTLCP areas of Nepal. Altogether, 21 localities of Nepal that were extracted from literature and herbaria 14 places of the western *Terai* have been surveyed during the course of this study.¹⁶⁻¹⁹

To predict species distribution DIVA GIS software version 4 was used where applied the BIOCLIM model. Climatic and altitudinal parameters were used to predict the distribution of the *Rauvolfia* species. Based on the availability area on the map, further survey points were determined and performed ecological studies. Information regarding the distribution of the *Rauvolfia serpentina* in Nepal was obtained after visiting herbarium centres namely National Herbarium and Plant Laboratory (KATH) and Tribhuvan University Central Herbarium (TUCH) for herbaria, available literature, direct field visit and other academic research and public sources. GPS coordinate value was obtained and digitized using GIS software DIVA version 4. The economic importance and status of the species where *Rauvolfia serpentina* exist were also extracted through participatory inventory methods like group discussion, key informal surveys, informal talks, field observations; transect walks, co-operation with primary consumers (Dhami, Guruwa) and the traders. By using DIVA GIS a map was prepared based on the coordinate values that were recorded.²⁰⁻²⁶ An ecological niche model was used in GIS and explored the potential map for the distribution of the plant. To know about the ecology and status of the plant eco-geographic surveys in predicted diversity rich areas in WTLCP and adjoining areas were performed. Random quadrates of size 5x5m² were laid on habitats where Serpentine were more prevalent, otherwise just visual surveys were done for ecological studies to quantify population status. The species population along with other related species were surveyed and located using GPS and development of the distribution map of this species was performed.²⁷⁻³²

Local knowledge acquisition for local use-values and community understanding on diversity was carried out on field tenure. Due to being the endangered species as well as shrub plant less number of herbarium specimens were collected, prepared herbarium and deposited safely as vouchers specimens for further detail studies.

For taxonomic identification, of the plant morphological studies were conducted by using standard book of Flora namely Annotated checklist of flora² Flowers of the Himalaya⁷ and consulting with local healers, internet surfing, tallied by photograph and checked through the herbarium centre. By direct field visit on each and every population, unique genetic diversity and its distribution areas (sink and source areas) were identified. Gene Banks (Sinks) and Donors (Source) were identified through ecological niche model.

Results and discussion

The indigenous communities 'Tharu' of the survey districts known *Rauvolfia serpentina* plant by vernacular name *Jharbaruwa* or *Chandmaruwa*. In different parts of Nepal it is known by *Sarpagandha*, *Pagalbuti*, *Dhabalbaruwa*, *Dhaulebir*, *Baruwa*, *Dhalaliya*, *Chandramar*, *Sarpagandh* (Sanskrit), *Dharmaruwa* (*Derai*), *Chyangro* (*Chepang*), *Chotachand*, *Chandravama*, etc. *Rauvolfia serpentina* plant population was found as scattered, with 2/3 mature individuals' plants. From Key informant interviews with

Traditional healer groups or locally known as Guruwa and Dhami it is known that Root is the major part of Serpentine used in traditional medicines as well as commercial purposes. Nepal and its adjoining country India has witnessed its legacy from the time of Charaka and Susruta for traditional knowledge systems of medicinal plants and this plant was mentioned. That plant is especially used by the Dhami Guruwa to get relief from various central nervous system disorders both Psychic and motor, including anxiety states, excitement, and maniacal behaviour associated with psychosis, schizophrenia, insomnia and epilepsy. However juice of leaves has been also reported for the remedy of opacity of the cornea. Consumption of small pieces of leaf lowers blood pressure. Root of that plant is bitter, sharp pungent and has been used as an antidote for scorpion stings as well as snake bites. The extracts of root have been used during intestinal disorders, particularly diarrhoea and dysentery. Roots mixed with other plants for treatments of cholera, colic and fever. Its roots are believed to stimulate uterine contraction and recommended for use in difficult childbirth cases.³³⁻³⁶

Research has found that the herbal market demands of the *Rauvolfia serpentina* all over the world is above 600 tonnes as root, whereas it is exported from about 0.2-0.3 tonnes from Nepal. As prescribed by the Forest Regulation 1995 and its third amendment 2005, the government royalty for dried root *Rauvolfia serpentina* is US\$ 0.44 per Kg. Herb production and processing company paid US\$ 0.88 for per kg of Serpentine. Similarly Gorkha Ayurvedic Company paid US\$ 1.33 for per kg. In European countries like in Germany its root is selling in US\$ 3.26 per kg (Acharya, 2007).⁸ It was observed that the plant trader could not sell the product because premature collection was done by the rural healer and caused massive depletion on distribution of the species. It is used even in minor health issues though it is valuable and an endangered plant species.

The plants are more frequent under the shade of *Shorea*, *Ficus*, *Terminalia*, *Holarrhena*, *Cassia*, *Dalbergia*, *Mangifera* and *Adina* species. Relatively it prefers to grow in exposed forest floors where colonization and seedlings were seen more frequently. This species is distributed throughout the tropical and sub-tropical parts of Nepal. The present study has added several additional localities of *Rauvolfia serpentina* in Nepal particularly around WTLCP of Nepal (Figure 1), however a higher density has been found in western *Terai* region of Nepal (Figure 2). Field visits revealed that the density and status of the species was seen dense in two sites i.e. Vajni and Bishnupur where density found 5 but other sites had covered just 2/3 number from whole habitat. Serpentine plants were concentrated 2-5 numbers in large extended area and even some plants were seen uprooted or removed by Local herbal healer to perform traditional medicinal treatment. Due to its spatial distribution and aforementioned circumstances confirmed that the species serpentine is under massive pressure. This fragmented spatial distribution of the *Rauvolfia serpentina* is characteristic of a population under edaphic-climatic and demographic pressure. Genetic and demographic processes related to small population size play a major role in putting the species into endangered status. The species of *Rauvolfia serpentina* used in this study were of typical populations that have been under great force of genetic stochasticity. The genetic stochasticity is loss of genetic diversity related to the combined effects of genetic drift and inbreeding in small populations.^{9,37,38}

Based on secondary information (i.e. Literature, interview with experts and visit of herbaria) and eco-geographic surveys that were recorded in target sites, a predicted distribution diversity map of the *Rauvolfia serpentina* in Nepal has been developed by using DIVA GIS

(Figure 2A). In Nepal using GIS for spatial distribution happens is a new field of research. Development of the GIS map has facilitated easy access of the plant's natural habitat. It makes it easier to do further research as well as conservation activities. Field survey and consultation with different expert/ stakeholders/sectors of the country it is cleared that density of the *Rauvolfia serpentina* is more at western *Terai* (Figure 2A). More over tropical and sub-tropical areas of Nepal including western *Terai* landscape represent the primary centre of origin of 'Serpentine'. Furthermore, the landscape of this region is varied in eco-geography; socio-cultural use-values of forest resources have further enriched the genetic diversity of Serpentine. The *Terai* region exhibits higher temperature (Reached upto 46°C in summer) and relatively dryer (Precipitation less than 25mm) properties than eastern *Terai* of Nepal. Discoverlife.org on its electronic website has also shows the natural map of the *Rauvolfia serpentina* in the world, where in the case of Nepal that point was given towards western *Terai*. In field observations most of the population were found nearer to the wet land or marshy land. The altitude diversity patterns formulated by GIS also suggest that western *Terai* is rich in diversity (Figure 2B).



Figure 1 Distribution of the *Rauvolfia serpentina* in Nepal.

The tropical and sub-tropical countries *i.e.* India, Indonesia, Malaysia, Brazil, Bangladesh, Nepal, etc of the world are extremely rich in non-timber forest genetic resources which are used by millions of people to meet their livelihoods and are becoming an important source of income. On other hand, these forest based genetic resources are currently facing tremendous threats due to anthropogenic activities such as deforestation, habitat alteration and unsustainable harvesting for trade. It is because of those aforementioned reasons, a large number of species have already been critically endangered in their centre of origin and driving toward the verge of extinction. Therefore, conservation of forest based genetic resources has become a global concern which urges development of effective conservation strategies at local, national and international levels. Different models were proposed by the workers for the conservation of endangered species. Among these one is "a novel approach of quasi *in situ* conservation of endangered plants"⁹ and in their approach, *ex-situ* collections are maintained in natural or semi-natural environment, wherein preserving both neutral and adaptive genetic diversity is complimentary to the *in situ* conservation strategy. Another one is the gene bank approach that was propose.⁶ Based on this reference of endangered species and immense resources, the nature of this study focused on the conservation of the *Rauvolfia serpentina*. An ecological niche model

was used in GIS and explored the potential map (Figure 2A). Based on the availability area on the map, further survey point were determined and ecological studies performed. The model indicated that western *Terai* of the Nepal has more availability of the species. The increasing unsustainable harvesting for legal/illegal trade has already threatened the 'Serpentine' diversity in WTLC and adjoining areas.

Based on the primary and secondary data, a map was drawn at GIS and got denser in the western region of the country, although with high density at Bishnupur and Vajni areas and sparse distribution at other sites (Figure 2C & 2D). Angular described an endangered species is usually signified by small and isolated populations that already experienced strong effects of genetic drift. Therefore, according to¹⁰ relocation or reintroduction of the endangered species should take into account the potential risks of inbreeding depression, in addition to local adaptation and spatial structure of adaptive variation. So to conserve this precious resource *i.e.* *Rauvolfia serpentina* the Shankarpur village of Kanchanpur and Dadehri village of Bardiya districts have been identified as unique in diversity, and recognized by local communities as different diversity as well as various ecological and climatic data. From field visit and sampling of the species, it is concluded that Vajni site (Figure 2D) from Kailali and Bishnupur sites (Figure 2C) from Bardiya are potential sites for the sink whereas Parasin, Shankarpur sites of Kanchanpur, Motipur sites of Kailali and Danoura of Bardiya as well as other 28 sites are the sources of the gene for the conservation in gene banks. The present research gives clear ideas about the identification of exact genetic hot spot of the *Rauvolfia serpentina* and decides where to conserve the plant species. All the visited sites are endangered for its habitat. So the introduction of the gene from the source to the sink will cause better conservation of the *Rauvolfia serpentina* in WTLC areas of Nepal. Uma Shankar⁶ proposed the Forest gene bank approach and has been already useful to conserve several forest genetic resources including non-timber forest products such as *Phyllanthus* sp., therefore, this strategy will be also useful to conserve the 'Serpentine' genetic diversity conservation in the context of WTLC. Following national park policy is needed to use the site as a sink area because the Bishnupur Area is included inside of Bardiya National Park.

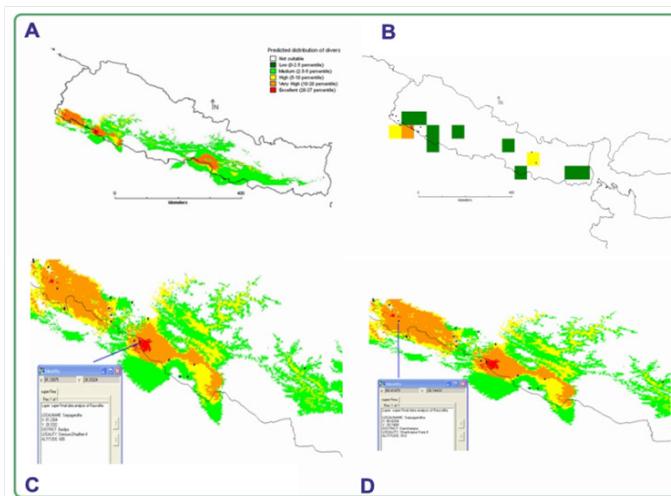


Figure 2 A) Prediction of diversity of Serpentine in Nepal; B) The altitude wise diversity assessments of Serpentine in Nepal; C) Suggested potential sink areas of Serpentine gene bank establishment *i.e.* Bishnupur site; D) Suggested potential sink areas of Serpentine gene bank establishment *i.e.* Vajni site.

Conclusion and recommendation

The research depicts western *Terai* to be a safe zone for this species *serpentine*. For the conservation of the *Rauvolfia serpentina* species by the gene conservation concept, it is concluded that Vajni site from Kailali and Bishnupur sites from Bardiya are potential sites for the sink while other 34 sites are as the source areas. Constant anthropogenic encroachment by victims of flood even in the sink areas (Vajni) is putting intensive pressure in the loss of Serpentine population plus its habitat fragmentation, which indicates prompt application of conservation of these natural resources. Usage of molecular tools will definitely provide much more needed information rather than simply morphological studies. In addition, raising awareness programs need to be implemented so as to safeguard this declining species. Nevertheless, prompt revisit and review of the policies and strategies at the government level plus the inception of the gene bank will definitely help sustain this species.

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Conflicts of interest

The author declares there are no conflicts of interest.

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