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Research Article

Mapping Habitat to Establish the Gene Bank of *Aegle marmelos* (L.) Correa for Conservation in Nepal

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Abstract

Aegle marmelos (L.) Correa is an economically crucial plant mainly harvested and traded for medicinal and religious purposes. Most of the harvesting is made from forests. Due to rapid habitat change, deforestation and over-exploitation, it is feared that such a valuable plant and its precious genetic diversity may be lost from nature. Responding to these challenges, a major initiative has been launched in the Western Terai Landscape Complex to identify the threats and to identify hot spots (sinks) of genetic variability that can be targeted for enriching the forest gene bank concept. To improve our knowledge regarding the natural habitat of the species, literature review, herbarium center visit, and field visits were performed. All accumulated data were subjected to DIVA GIS to plot a predictive map of the plant habitat based on climate and altitude. The Sixteen measured populations distributed along East-West longitudinal gradient in Nepal were explored and tested using GIS maps. For conservation strategies, populations of the Khata site of Dhanaoura VDC, Bardia district is purposed as a hot spot and is a probable sink of the gene pool while remaining sites as a source. Therefore, the transformation of the gene in Khata of Dhanaoura VDC, Bardia district from other sites of the country, is required to conserve the genetic resources of *A. marmelos* in a constant and stable form. The provided information can be utilized to conserve genetic resources and to utilize the resources sustainability.

Keywords: Gene Bank; Diversity; Conservation Strategies; Western Terai

Introduction

Aegle marmelos (L.) Correa (Syns. *Feronia pellucid* Roth., *Cratevaeva marmelos* L.) is also called Bengal quince, Bael fruit, Indian quince, golden apple, holy fruit, stone apple, bel, bela (Nepali, Hindi); Sirphal, Malur, Amritfal, Mahafal, Sadafal, Willow (Sanskrit); Bel, Marmelos (Trade name) and other dialectal names are Ohshit, Opesheet in Burmese; Phneou or Pnoi in Cambodian; Oranger du Malabar in French; Maja, Maja batuh in Indonesian; Modjo in Java; Torum in Lao (Sino-Tibetan); Bilak or Maja pahit in Malayan; Marmelos in Portuguese; Matum, Mapin, and Tum in Thailand; Mbaunau, Trai mam in Vietnamese. The plant species belong to the Rutaceae family, and the distribution of the family Rutaceae Rue/Citrus (Latin for rue) is worldwide, especially in tropical regions, including 153 genera and 1800 species and it is characterized by the development of oil glands producing aromatic oil [1]. *Aegle* belongs to one of the monotypic genera of subfamily Rosidae, tribe clauseneae, and sub tribe Balsamocitrinae. It has a single species *Aegle marmelos* but other related which are purposed but were not written are *A. barteri, A. correa, A. decandra, A. glutinosa, A. sepiaria.*

A. Marmelos is indigenous to dry forests on hills and plains of central and southern India, southern Nepal, Sri Lanka, Myanmar, Pakistan, Bangladesh, also in mixed deciduous forests of former French Indochina: Vietnam, Laos, Cambodia, and Thailand 2]. It is cultivated throughout India as well as in Sri Lanka, northern Malay Peninsula, and Java, and to a limited extent on North Luzon in the Philippines Islands. It is grown in some Egyptian gardens and Surinam and Trinidad [3-5]. The tree is a subtropical species. It grows upto 1200 m, where the temperature rises to 48.89^{0}C in the shade in summer and descends to -6.67^{0}C in the winter, under prolonged drought. It will not fruit where there is no longer dry season, as in southern Malaya. Warm humid climate and sunny conditions favor higher yields. The *A. marmelos* fruit is said to be best on rich, well-drained soil, but it has grown well and fruit on the oolitic limestone of southern Florida. It grows luxuriantly in swampy alkaline or sandy loam soils with sufficient moisture and in soils with having pH range of 5 - 10. In Nepal, the plant is found from east to west upto an altitude of 1220m [2-6].

*A. marmelos*is a deciduous medium-sized erect tree with few and irregular branches, approximately 10m tall, and grows in open places. Bark ash-colored with furrows, strong, very sharp, axillary thorns, and single or in pairs. Leaves are odd-pinnate, alternate, leaflets trifoliate rarely pentafoliate, ovate-lanceolate, gland-dotted, lateral sub-sessile or terminal long-petioled, crenate, obtuse. The flower is greenish-white, bisexual, in short panicles, sweet-scented; sepals 5, lobed, deciduous; petals 5, gland-dotted, numerous, oblong much longer than sepals; many stamens, filaments short and anther narrow, basifixed; ovary bright green with an inconspicuous disc. The fruit is about 6 cm in diameter (of about the size of a large orange), globular, ovoid or pyriform, greyish or yellowish-brown, has a faint aromatic odour and mucilaginous taste, with a hard smooth shell. Rind about three millimeters in diameter, and adherent to a pale-reddish juicy pulp in which is ten to fifteen cells, each containing several woolly seeds a large quantity of exceedingly tenacious mucilage, which when dried is hard and transparent. The flowering period is April to June; soon after the new leaves appear and the fruit ripens in 10 to 11 months from bloom- March to June of the following year.

Economic importance

Plant diversity has fascinated humankind throughout history. Medicinal plants have been used to cure a number of diseases. There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones. Apparently, *A. marmelos* trees are well known and respected all over the world for its healing properties, but mainly in the East by Natives as a valuable source of food and drink, to build or make tools and artifacts or things to use in daily life in the house and for rituals.

The Hindu, who expires under the *A. marmelos* tree expect to obtain immediate salvation. The fruits and leaves are used during the *Fulpati* of Dashain, the Hindus greatest festival. Wood is used in special religious ceremonies. Marrying Newar maidan, with Bael fruit, is still practiced in Nepal. It is official in both the *Pharmacopoeia of India and the British Pharmacopoeia.* Juice of root, about three teaspoonfuls twice a day, is given for fever; wood ash mixed with some water is spread over the swollen part of the body, especially when someone has a present [7]. The root bark and leaves are used in intermittent fever and is useful in hypochondriasis, melancholia, and palpitation of the heart. It is astringent, cooling, carminative, restorative, laxative, febrifuge, stomachic, uses for colitis, colic dysentery, diarrhoea, diabetes, ophthalmic, asthma complaints, flatulence, fever, and vomiting [4,7]. Stem and around seed yield gum and that gummy substances serve as an adhesive, also used as varnish for pictures and add brilliancy to water color paints [8]. The leaves cause abortion and sterility in women; the bark is used as a fish poison in the Celebes; tannin, ingested frequently and in quantity over a long period, is antinutrient and carcinogenic [3].

On the other hand, there is lack of scientific information on the accessions, genetic diversity, and breeding system of 'Bael' in Nepal. Implementation of study to locate and assess diversity, explore the conservation ways and sustainable utilization measures are the priority actions that are immediately required in Nepal. The current research is one of a novel concept in Nepal that integrates the forest gene bank approach to secure the long-term conservation of globally significant biodiversity i.e. *A. marmelos* of the Western Terai region of Nepal with the goal of sustainable development.

Two broad approaches of conservation, viz. *in-situ* and *ex-situ*, have already been shown to be effective ways to conserve global biodiversity. There are different types of conservation modalities at different levels, such as from gene to landscape levels, which have been developed to further strengthen the conservation initiatives. The general conservation strategies for forest genetic resources are basically similar to those applied to crop species and consist of consecutive phases. The existing forest resources and their genetic variation are a starting point for conservation activities. After the priority species have been identified, their genetic diversity needs to be assessed and located so that the conservation activities and possible germ plasm collection 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 so-called 'Forest gene banks' in which a large range of gene sources are purposely put together [9].

The *A. marmelos* species, which possess a global significance and locally importance for people livelihoods, has been identified as one of the key species, and the contemporary research approach of conservation strategies is to establish the *A. marmelos* gene bank in Nepal. The purpose of this study was, therefore, to identify the population and areas of the vital species *A. marmelos* 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).

Individuals within a species of plant are typically differing in morphology over a geographic range. Although some of this variation may be the random, ecological theory posits that a large proportion of this variation may represent the adaptive matching of morphotypes to a variable environment 10]. The variation in size, shape, coloration, behavior and physiology may be a product of current environmental differences between sites (phenotypic plasticity), a product of heritable (genotype differences) between the sub-populations at different locations, or a combination of both. Phenotypic variation is an essential pattern in variation to develop superior population stands. The significant difference is detected in the size, shape, number, organization, and color pattern of the essential plant organs (roots, leaves and stems) and of some complex structures (flowers, fruits and seeds) [11].

There may be considerable differences between populations, between plants of the same population growth in different sites or even between plants of a single community growing together. Species are taxa with different genome compositions capable of interbreeding e.g. Under conditions of sympatry and are reproductively isolated from other such groups (at least when not growing under extremely distributed conditions). If the differences are primarily genetic rather than environmental, the variation is usually specified as racial, ecotypical or cline. Two significant processes promote the differentiation of geographical races into new species: random and genetic drift and natural selection 12]. When genetic drift is the primarily mechanism, such a relationship between phenotypic similarities and geographical characteristics has been investigated along various gradients; such as latitude/longitude [13,14]; climate [15]; rainfall [16]; and soil and nutrients [17,18]. Genetic variability of species is necessary for productivity and to adapt to shifting ecological conditions. It is, therefore, itself a natural resource and is essential to give attention to the management of renewable genetic resources for their continuous supply of goods and services. Knowledge of genetic diversity should help to identify what to emphasize in conservation and management efforts. It is always desirable to know that species are the most appropriate or commercially valuable for a particular site, and there are several trials established for comparing species. However, the global awakening of interest in the genetic management of forest tree populations has not yet been witnessed in Nepal.

Materials and Methods

The research was carried out at the Western Terai Landscape Complex. For the conservation and sustainable use of globally significant biodiversity in Nepal's Western Terai Landscape Complex and establish 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 Bardia districts that extends from $28^0$59'13'', $80^0$06'05'' and $28^0$04'14''- $81^0$30'07'' (Figure 1C). The area lies in Terai Duar Savana Grassland Ecoregion, one of WWF's global 200 ecoregions, covering a total area of 3466 sq. km. of Terai Arc Landscape [19].

Topographically, the area has two distinct zones: the lowland Terai in the south and Churia hills in the North. The research utilizes local knowledge with scientific studies, such as taxonomic surveys and ecological studies, to formulate a sound conservation strategy for the establishment of the *Aegle marmelos* Plant Gene Bank and its management in WTLC areas of Nepal. The area oc-

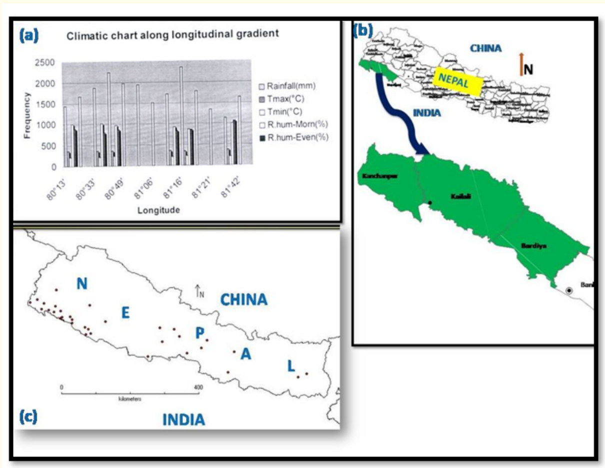

Figure 1: a) Climatic data of the study area, b) Nepal for mapping of the species, b) Study area.

cupied by forest is 2069 km^2 (59.7%), cultivated land 1102 km^2 (31.8%) and others 295 km^2 (8.5%). *Shorea robusta* forest, tropical deciduous riverine forest, tropical evergreen forest, *Terminalia* forest, *Dalbergia sissoo-Acacia catechu* forest, *Pinus roxburghii* forest, and subtropical deciduous hill forest are the types of forest decorate and enrich the area. More than 80% of the people in this area are involved in agriculture. Bardia National Park (968 km^2) with Buffer Zone (237 km^2) and Shuklaphanta Wildlife Reserve (305 km^2) with Buffer zone (153 km^2) is the protected areas of this territory. Dry and hot climates are found in the region. Thirteen climatic stations were found within the area. The maximum and minimum temperatures, rainfall, and relative humidity in the evening and morning are shown in figure 1a. Altogether, 46 places of Nepal that were extracted from literature (9), Interview (4), and herbaria (17); 16 places of the Western Terai have been surveyed during the course of the study.

To predict species distribution, DIVA GIS software version 4 was used, where applied the BIOCLIM model. This tool helps to predict the areas of distribution of species in a defined geographical range. It maps area of high, medium and low occurrence for species suggest the areas where the chance of species occurrence is maximum and lowest. This map will help in four ways: a) Preparing the distribution map of species under study, b) The areas where we need to focus our study or the areas where we can avoid, c) If the species is threatened then it suggests the areas where it likely to be conserved and d) So it reduces the cost and time of conservation activities. Climatic and altitudinal parameters were used to predict the distribution of the *Aegle* species. Based on the available area on the map, further survey points were determined and performed ecological studies. Information regarding the distribution of the *Aegle marmelos* in Nepal was obtained after visiting herbarium center namely National Herbarium and Plant Laboratory (KATH) and Tribhuvan University Central Herbarium (TUCH) for herbaria, available literature, direct field visits, and other academic research and public sources. The GPS coordinate value was obtained and digitized using the GIS software DIVA version 4. The economic importance and status of the species at which *Aegle marmelos* were also extracted through participatory inventory methods like group discussion, key informal survey, informal talk, field observation, transect walk, cooperation with primary consumers, and traders. By using DIVA GIS, a map was prepared based on the coordinate values that were recorded. The 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 the adjoining areas were performed. Random quadrates of size 20x20 m^2 were laid on their habitat where *A. marmelos* were more in number; otherwise, a visual survey was 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.

Local knowledge acquisition for local use-values and community understanding of diversity was carried out on field tenure. Herbarium specimens were collected, prepared herbarium, and deposited safely as voucher specimens for further detailed studies. For taxonomic identification, of the plant morphological studies were conducted by using the standard book of Flora, the Annotated checklist of flora by [20]; Flowers of the Himalaya by [21] and consulting with local healers, internet surfing, a tally by the photograph and checking through the herbarium center. By direct field visits on each and every population, unique genetic diversity and its distribution areas (sink and source regions) were identified. Gene banks (Sinks) and donors (Source) were identified through an ecological niche model.

Results

Mapping habitat and field observations

Based on the secondary information (i.e. Literature, interview with experts and visit of herbaria) and eco-geographic surveys that was recorded in target sites the predicted distribution diversity map of the *A. marmelos* in Nepal has been developed by using DIVA GIS. Development of the GIS map has facilitated easy access of plant's natural habitat. It makes easy to do further research as well as conservation activities. The preliminary findings suggested the tropical region of Terai is comparatively rich in diversity than other parts (Figure 1c). Furthermore, the landscape of this region is varies in eco-geography; socio-cultural use-values of forest resources has further enriched the genetic diversity of the *A. marmelos*. These regions falls the higher temperature (Reached upto 46^0C in summer) and relatively dryer (Precipitation less than 25 mm) part than eastern Terai of Nepal. In almost all cases, the *A. marmelos* were found to occur along the river, stream, lakes or wetlands. Field survey and consultation with different expert/stakeholders/sectors of the country it is cleared that although the tree is distributed in most parts of southern Nepal, it was primarily concentrated in the western Terai. The highest density was seen in Khata (Dhanaoura VDC) Bardia district (45.9 ± 31.21 in 20 x 20m^2) and the least at Ghadariya of Kailali district (1.5 ± 0.71 in 20 x 20m^2). Overall, the observed density in different populations of Western Nepal is tabulated in table 1. There were 16 populations six in Kanchanpur, five in Kailali, and five in Bardia districts. Three communities (Khata, Shuklaphanta, and Krishnapur) were sub-divided into two groups each due to barriers present between them. The total number of individuals' does not appear to exceed 35 (personal observation), except for the Khata population. The sapling of *A. marmelos* in different populations was almost absent, except in Khata.

Accordingly, it is predicted that the population in Western Nepal might be more genetically diverse than the populations in the remaining parts of the country. The altitudinal diversity patterns based on the primary and secondary surveys of *A. marmelos* in Nepal are constructed using the DIVA GIS computer tool (Figure 2A). At present, *A. marmelos* fruit is locally threatened (proposed by observing its demographic structure and decreasing habitat) because it is not an economical crop. Now days, some attention has been given towards its use by the people of the study area because in Khata and Radhakrishna sites, the community forest user groups had opened a juice extraction cottage factory in their area. The factory was based on the *A. marmelos* product i.e. fruit, but at field observation time it was not running. In the national market, different branded *A. marmelos* juice can get quickly.

SN	Locality	Density per 20x20 m^2	Min^m den	Max^m den
1	Shuklaphanta1	9.5 ± 3.41 (From 4 quadrat no.)	6	14
2	Shuklaphanta2	9.5 ± 3.41 (4 quadrat no.)	6	14
3	Shankarpur	2.5 ± 0.71 (2)	2	3
4	Daiji	3.33 ± 1.53 (3)	2	3
5	Krishnapur1	5 ± 1.63 (4)	3	7
6	Krishnapur2	5 ± 1.63 (4)	3	7
7	Malakheti	2 ± 1.0 (3)	1	3
8	Devariya	4.3 ± 0.57 (3)	4	5
9	Ghadariya	1.5 ± 0.71 (2)	1	2
10	Ghodaghodi	2.25 ± 0.96 (4)	1	3
11	Vajni	5.25 ± 1.25 (4)	4	7
12	Motipur	2 ± 1 (3)	1	3
13	Thakurdwara	2 ± 0 (1)	2	2
14	Khata1	45.9 ± 31.21 (10)	2	87
15	Khata2	45.9 ± 31.21 (10)	2	87
16	Belwa	3.2 ± 1.64 (5)	1	5

Table 1: Density of the species.

Similarly, the production of the general distribution of the species was analyzed and a map was formed (Figure 2, B). Different colors determine the size of the species. The map showed an irregular distribution of the species throughout the country, whereas Terai of the country is rich in *A. marmelos* diversity.

The predicted distribution diversity map of the species (Figure 2B) and altitudinal wise diversity pattern (Figure 2A) showed that the Khata population of Bardia district is the best for the species diversity that is circled in figure 2C and rich in *A. marmelos* diversity that resulted from the density extracted from field visits (Table 1). Using geographic information system tools (GIS), it is possible to display the potential areas suitable for the species according to climate and soil requirements. Through probability analysis, the potential distribution of the species was identified according to climatic conditions (Figure 2D). This indicates that attention should be given to the highly probable areas for the collection and development of new production areas.

Causes for threats of the species

Pressure on species and population are often a direct result of harvests for both commercial and subsistent needs. Other factors include habitat loss, forest fragmentation, and exotic species. All these factors probably act synergistically. As being of this plant gregarious when the fruits get ripen and fall, some are washed away by water and deposited in unsuitable habitat reducing of seed germination. At the same time few seeds had the opportunity to fall at land and remain dormant due to the dry season, i.e. March/April. During the rainy season, over flooding occurs and covers the whole land for nearly two months, which destroys fruit before seeds are ready to germinate.

Conservation strategies

As from uni- and multi-variate analysis of morphometric characters as well as ZOI of antibacterial activities, it was clear that *A. marmelos* of Western Terai had high genetic diversity (researcher observation, manuscript prepared and on review).Variation within and between the natural population offers possibilities for improved adaptation sites and production requirements. But many of these natural populations are in danger of depletion or extinction, mostly due to human activities and encroachments. It is, therefore, further local and international efforts are required to preserve and improve the genetic resources of all populations. To conserve them in the forest gene concept, the Khata of Dhanaoura VDC, Bardia district (Figure 2D) was declared as the sink, and the remaining sites were sources, which is discussed in the discussion unit.

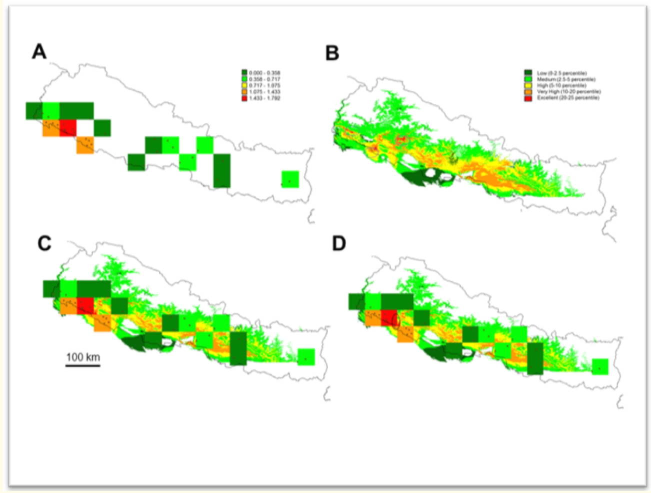

Figure 2: A) Altitude wise diversity patterns of the species, B) General Distribution of the species, C) Matching diversity and prediction, D) Suggested potential sink area of *Aegle marmelos* gene bank establishment i.e. Khata of Dhanaoura.

Discussion

The world is endowed with a rich wealth of medicinal plants. Man cannot survive on this earth for long life without the plant kingdom because the plant products and their active constituents play an important role. Herbs have always been the principal form of medicine and presently, they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in the count with the body's own defence. There is a widespread belief that green drugs are healthier and more harmless or safer than synthetic ones. Medicinal plants have been used to cure several diseases. Although the recovery is slow, the therapeutic use of medicinal plants is becoming popular because of its inability to cause side effects and antibiotics resistant microorganisms.

Forest products contribute immensely to the livelihood of forest-dwelling communities in terms of household self-sufficiency, food security, income generation, accumulation of savings, and most importantly, risk minimization. However, very little is known about the resource availability and quantity of extraction and its impact on the conservation of biodiversity of the forests. Hence, mapping these resources would offer a perspective on the economic value of forest resources. In this direction, maps have been developed for *A. marmelos* in Nepal and especially in WTLC.

In statistics, the concept of a population is an abstraction signifying theoretically large assemblage of individuals from which a particular group under consideration is a sample. Most biologist uses the term, however, imply the total of organisms belonging to a specific taxonomic group (or taxon), which are found in a particular place at a specific time. The population unit of outstanding significance in the study of variation is quite different from the local interbreeding group of individuals shared a common gene pool [22].

Environmental/climatic factors, genes, and population structure were responsible for population genetics. The difference among individuals is of three main types: developmental, environmentally induced and intrinsic [23]. Phenotypic variation is largely due to phenotypic plasticity [24], although genetic differentiation can also play an important role [25]. Differences in population genetic diversity arise due to features related to the extent of distribution of the species and or the population as well as the successional stage of population. The breeding system, seed dispersal, and most importantly, the geographical range had predictive roles in shaping the genetic structure of communities. More spread species as well as species that are out crossed, tend to have greater diversity within the population. The *A. marmelos* occurs in gregarious groups along with river systems in water-logged conditions (field visits). Populations along the same water sources (rivers, lakes, ponds, etc.) have higher chances to be related and result in low genetic variation between populations because during floods, the fruits (seeds) are often carried to long distances and deposited along the banks of rivers giving rise to gregarious stands. Thus, population sampling across longitudes may not give the desired diversity in the *A. marmelos*.

Genetic drift and the founder effect have been implicated as determining factors of the population genetic structures of several species [26]. Thus, even if the breeding unit within the *A. marmelos* populations is the actual size of the population, genetic drift has probably been responsible in some part for both the low variation within populations and the extensive variation between populations. Low levels of gene flow combined with the effects of random genetic drift and local populations to the selection, cause small, geographically distant populations on diverse genetically. Morphometric diversity data are essential for the conservation and management of the *A. marmelos* species. Because a large proportion of morphometric variation (manuscript prepared and on review/publication) in the *A. marmelos* was found to be among individuals within populations, a considerable amount of genetic variation of the species could be obtained when sampling a large number of plants from one or two populations rather than smaller collections from many different sites, and the same would be true for any conservation plan.

Threat and conservation strategies

Human beings are peril for the species through the loss, degradation, and fragmentation of natural habitats; the over exploitation of resources; the catastrophic effects of induced plants and animals, including pests and diseases; and the impacts of environmental pollution, resulting not only in the localized loss of biodiversity but also on the broader endangerment of plants through, acid rain and the global climate change. A similar result of [27] the natural home of *A. marmelos* in Basanta and Laljhadi corridor of WTLC is encroached and destroyed by landless, *Mukti Kamaiya* (Free bonded laborers), and flood victims. It is important to note that threats may be proximate in the local usage of resources or from commercial interests outside the target area. Thus, an epistemology of various factors is necessary for an adequate assessment of their impacts and devises possible mitigation.

The increasing exploitation of the tree species by habitat loss, forest fragmentation, and exotic species has focussing attention on the optimal strategies for the conservation of their genetic resources. In this species because native populations are highly fragmented and rapidly shrinking, conventional methods of preservation of genetic resources are besieged with problems. The optimal strategy for *in-situ* conservation of the genetic resources of the *A. Marmelos* will be prescribed by the effective population sizes and distribution of the genetic diversity within and between populations. Quantification of extraction levels, particularly for species that are being harvested by a large number of people for their subsistence needs, together with the relative importance of species to the livelihoods, is critical to the design of appropriate conservation measures. Theoretically, small populations have to risk losing genetic variation due to the effects of random genetic drift [28]. In the context of these, plants from different populations of Western Nepal are needed to be conserved.

Kailali, which issued to be known as the district with the largest area covered with forests, has lost its identity with nearly half of the forest area denuded during the five decades, as mentioned by [29]. With regard to these now and on time, protection and conservation of the plant resources are essential. Now days, the application of genetic principles to the forest tree improvement program is gaining popularity in many forest regions of the world. Genetic improvement of a species is a form of regulating its variation toward a set of goals [30]. It is directed to raise forest productivity in quality and quantity. Moreover, justice for future generations of people requires evolutionary protection potential, and [31] believes that such protection is feasible. It can be accomplished in a program of genetic conservation that includes directional selective breeding in at least one and probably in multiple populations [32]. Such a program is also adapted here for the *A. marmelos* trees to conserve genetic resources that occurred in different populations.

Identifying the unique areas of *A. marmelos* diversity for conservation

Irrespective of the pollination and dispersal modes and the breeding system of the species, small populations on average suffer more from mating constraints than large populations. The demographic status of a species could in fact be used as an approximate indicator of the state of the genetic diversity of the populations [33]. Accordingly, it noticed that the population in the Khata site might be more genetically diverse than any other population (Table 1). Therefore, it is the purpose of a sink area for *in-situ* conservation and other remaining sites are as the sources.

The conservation efforts lack clarity on issues of 'what' and 'where' to conserve. We do not yet have a consensus about the list of all that needs to be conserved and how best to conserve them. Accordingly, it is predicted that the population of the *A. marmelos* in the Western Nepal might be more genetically diverse than the populations in the remaining parts of the country (Figure 2B). Not only has this, the Shannon index showed (Figure 2C) the Khata population of the Western Terai is the best site for the productive area to propose sinks area. The data obtained from the field visits, the Khata site has the largest population and density, which supports the site, is correct for the sink.

Predicting species distribution is an indispensable element of conservation biology and ecosystem management. Multivariate models are commonly used to define habitat suitability and combined with the GIS; allow the creation of potential distribution maps [34] from the model, predicted suitable existence regions for the *A. marmelos* in Nepal. There are several appropriate areas in WTLC, with the most significant area around Khata sites of Dhanaoura VDC, Bardia district, and others scattered throughout the entire area.

The Khata of Bardia district has been identified as unique in diversity in terms of diversity in local names that are recognized by local communities as different diversity as well as various ecological and climatic data. Based on secondary information and ecogeographic surveys at target sites, the predicted distribution of diversity of *A. marmelos* in Nepal has been developed. The preliminary findings suggested that the tropical region of Western Terai is comparatively rich in diversity than other parts of Nepal (Figure 2B). These WTLC regions fall under the higher temperature and relatively dryer parts than the Eastern Terai of Nepal [35]. The altitudinal diversity pattern also suggested that Western Terai is rich in diversity (Figure 2A). Similar results of [36], that is, high temperature and dry weather, have supported the density of diversity of the *A. marmelos*. Besides, the plant will not bear fruit when there is no long, dry season and that is available in Western Terai. Western Terai, because of the great variety of its climatic regimes and land forms has more known biological diversity in its flowering plants in terms of species richness, than any other parts of Nepal [37]. Many widely spread species are not listed as threatened or endangered, nonetheless; they lose the integrity of their gene pools, foreclosing evolutionary potential and resilience. Thoughtful planning could result in the conservation of genetic diversity in these widespread species, preserving their evolutionary fitness by maintaining populations that have adaptations to local conditions. Knowledge of the factors that initiate and support patterns of variation is instrumental in establishing effective and efficient conservation practices [38].

It is imperative that viable strategies to conserve the surviving populations and their genetic resources of at least critically important species/population are formulated to arrest further loss. Without knowing the normal habitat of the species/population, formulating of any conservation rules is unsuccessful. An extensive geographic distribution map of *A. marmelos* has been developed, while there have been attempts to map the geographic distribution of the important species, such works are needed but lacking in Nepal.

Modeling habitat requirements of the species is an increasingly important tool both for investigating the requirements of species and for planning conservation reserves [39] and for the understanding of the pattern of biodiversity. In particular, using existing data and modeling species' distribution to orient field effort reduces the cost of field surveys. Distribution modeling is essential to ensuring consistency, while reducing the time and costs of large-scale studies of biodiversity involving large numbers of species. Remembering to this, the mapping of the *A. marmelos* in three natural habitats was using ecogeographical data and GIS too (Figure 1c).

Moreover, the current conservation strategies depend excessively on several independent programs without any coordination among them. The biological elements (specific genes, species *perse* in an ecosystem, a set of taxonomically related species, etc.) and the spatial and other habitat features (unique micro- and macro-habitats, ephemeral zones, sensitive patches of the ecosystems, unique forest types and the whole ecosystem as such) are the essential elements that demand conservation attention. These elements and their interactions together provide a mosaic of conservation priorities.

The *A. marmelos* plant is a potential renewable natural resource. Therefore, the conservation and sustainable utilization of this plant must necessarily involve a long-term program. A holistic and systematic approach envisaging interaction between social, economic, and ecological systems will be a more desirable one. In most widely accepted scientific technologies of biodiversity conservation are the *in-situ* conservation and *ex-situ* conservation. These approaches suffer from several limitations, as they represent only a proportion of global variability (*in-situ*) or relative isolation of the populations with a narrow genetic base (*ex-situ* and field banks).

It is only in their habitats (*in-situ*) that breeding populations of plants can survive to evolve. Even if we put a particular variety of a specific species under millions of hectares of cultivation, the species can still go extinct in the wild, leading to loss of specific strains or genes absent in the cultivated stock [40,41]. Therefore, no cultivation strategy can ever ensure the survival of the species. In fact, in order to sustain a viable cultivation program, it is essential to maintain the intra-specific diversity or the germplasm of species because it is the germplasm, which provides opportunities for selecting the desired variety or for breeding a new one. The ideal way to maintain the intra-specific variation within the species is the way nature does it i.e*.,* through an *in-situ* conservation strategy. Wild populations need to being preferably conserved as gene pools rather than be used as raw material for consumption.

These approaches suffer from several limitations, as they represent only a proportion of global genetic variability (*in-situ*) or relative isolation of the populations with a narrow genetic base (*ex-situ*). Plants located outside their natural environment automatically deprive their ability of become involved in the natural evolutionary process. A combination of *in-situ* and *ex-situ* methods can do a large used to overcome this limitation.

Authors [42,43] proposed the forest gene bank concept, which is especially important in the conservation of genetic resources of trees. The model combines the virtues of the *ex-situ* gardens (of collating diverse gemplasm) and *in-situ* gardens (of allowing the conservation to be dynamic). It is proposed that in *A. marmelos*, a forest gene bank could be created for the conservation to be dynamic. It is proposed that in the *A. marmelos*, forest gene bank could be established for the preservation of genetic resources in the Western Terai Landscape Complex, and it may be for the whole country. Based on both the demographic and genetic diversity parameters, populations of Khata site of Dhanaoura VDC, Bardia district could serve as a sink for the gene pool, while those at the remaining populations can serve as a source. The understanding of biodiversity and its manifestation in a given area clearly recognizes the regional or bioregional nature of biodiversity. To manage biodiversity within such regions has become the most practical approach.

Conclusion

Geographical distribution mapping of the species was done. The genetic diversity of *A. marmelos* in natural stands from the study area i.e. Western Terai Landscape Complex, has been estimated. *A. marmelos* is the most important medicinal as well as religious plant of human use; we must give attention for the conservation of this plant in its natural habitat to generate the genetic resources to future prospective in the national level.

In Hindu religion, the conservation of *A. marmelos* is given more emphasis. Due to little knowledge of its economic importance, it has not been widely cultivated and, at present, this plant is a very rare crop. Even the cultivation of the plant cannot preserve/conserve wild genes of the plant; it may be mutated or lost. Therefore, plant genetic conservation is a significant issue. Concerning this, the *A. marmelos* in this study are trying to develop conservation strategies.

Based on the predicted distribution map, Shannon index mapping, and demographic size (density and number) here, it is proposed that the forest gene bank be established at Khata of Dhanaoura VDC. The gene pool from other sources can be incorporated into such forest gene banks. Genetic resources of the *A. marmelos* are cultivated *in-situ* at a Khata site of Dhanaoura VDC, Bardia district, into which the gene pool from remaining other sites is infused periodically (*ex-situ*) and facilitates the continuous turnover of the genetic material.

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