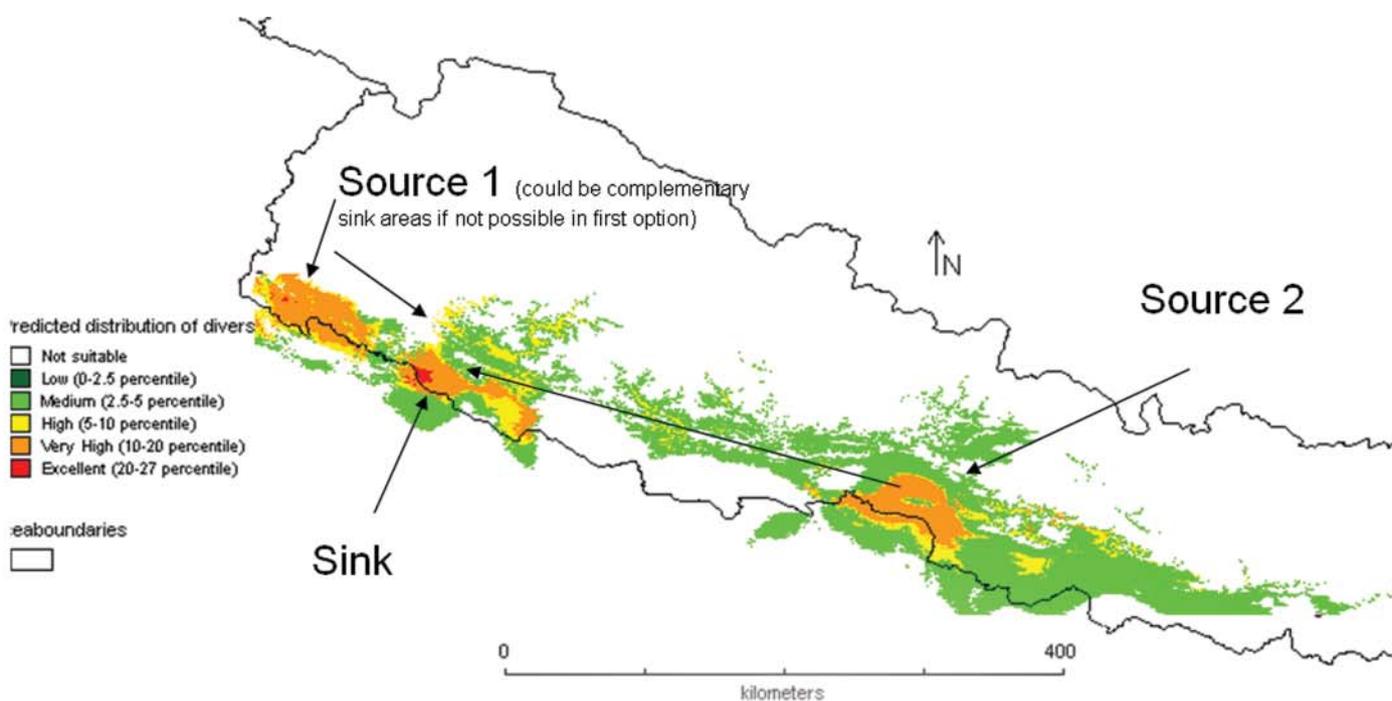


# In-Situ Conservation of Agro biodiversity and Forest Genetic Resources

B. Regmi , A. Subedi , A.R. Adhikari , K.N. Ganeshiah  
R Uma Shaanker and P. Shrestha

## Training Manual for Practitioners



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B. Regmi<sup>1</sup>, A. Subedi<sup>2</sup>, A.R. Adhikari<sup>1</sup>, K.N. Ganeshaiyah<sup>3</sup>, R Uma Shaanker<sup>4</sup>  
and P. Shrestha<sup>1</sup>

## Training Manual for Practitioners

1. Local Initiatives for Biodiversity Research and Development (LI-BIRD), P.O. BOX 324, Gairapatan, Pokhara, Kaski, Nepal
2. PhD Student, Netherlands
3. School of Ecology and Conservation and Department of Forestry and Environmental Sciences, UAS GKVK Bangalore, 560 065 India
4. School of Ecology and Conservation and Department of Crop Physiology, UAS GKVK Bangalore, 560 065 India

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# I. FOREST GENE-BANKS: THE CONCEPT AND ITS APPLICATION

Biodiversity losses around the world continue to mount despite considerable efforts to protect species and ecosystems. According to the latest FAO report (FAO, 2000), the annual rate of deforestation in the world during 1990-2000 was 9.4 million ha, and a recent report of IUCN (Walter and Gillett 1998) indicates that one out of every eight plant species is endangered or threatened with extinction. In particular, the tropical forests characterized by a great diversity of tree species, are under severe threats owing to the intense human pressures on them. Consequently, many of the tropical species, especially the economically important ones, are rendered either rare, endangered or threatened with incredibly low population sizes. Thus immediate measures are needed to arrest this decline in the forested areas and thence to save the endangered species therein.

Most of the conservation practices being followed have laid little emphasis on the conservation of genetic resources of the forest species. But the genetic resources of these species are important both for ensuring their survival and also for better prospecting and genetic improvement of the species. Thus it is being gradually realized that protection of genetic resources shall be an important element in the conservation practices we adopt. Unfortunately conservation of the genetic resources of the wild species can not be effectively achieved through the conventional approaches of ex situ and in situ methods (Uma Sanker et al., 2001). This is partly because the conventional methods of conserving genetic diversity are derived mainly for protecting the genetic resources of cultivated crops and the principles we adopt for these crops may not be easily translated for the conservation of the genetic resources of forest species. In other words forest genetic resources need to be treated differently from the conventional approaches.

## **Forest genetic resources**

The forest genetic resources have been under tremendous pressure from the high rates of deforestation, habitat alteration and indiscriminate extraction of forest products. This is particularly true in the tropical countries where a wide variety of non-timber forest product species are utilized by millions of people. The threats have endangered a large number of species and, in certain cases, have even driven them to extinction. Populations of many economically important forest species are highly fragmented with incredibly low numbers of individuals. It is feared that continued extraction of the species from the natural populations would irrevocably affect their genetic diversity with far-reaching consequences on their survival, adaptability and productivity.

Unfortunately, it may be not be productive to practice the conventional tools for conserving forest genetic resources for the following reasons:

1. In agriculture and or horticulture plants, we deal with accessions and provenances that are well defined. but in wild plants the accessions are not well known. The accessions of crop plants are most often stabilized populations such that maintaining a small population would suffice to conserve them. But in wild species, since the genetic diversity per se is

- not properly evaluated, it is difficult to define the required minimum population size to be conserved.
2. The germ-plasm of crop plants could be easily maintained on-farm, orchards or institutional farms in the form of very small and manageable populations. On the other hand, in forest species, this is not possible as most of these are out crossed, trees or large shrubs and hence require to be grown as large populations in unusually large areas (See table 1 below).
  3. In seed banks maintained for the crop plants, the genetic diversity is frozen and no opportunity exists for evolutionary dynamics. However, continuous generation of diversity through natural evolutionary processes is important especially for the wild species.

**Table 1. Dichotomy in conservation thinking**

Agriculture/Horticulture	Wild/Forest Species
<ul style="list-style-type: none"> <li>• Accessions/Provenances</li> <li>• Maintaining Germplasm               <ul style="list-style-type: none"> <li>– On Farm</li> <li>– On Institutional Farms</li> <li>– Orchards / Crops</li> </ul> </li> <li>• Temporal Snapshot</li> <li>• Less emphasis on Evolutionary dynamics</li> </ul>	<ul style="list-style-type: none"> <li>• Accessions not known</li> <li>• Large Populations               <ul style="list-style-type: none"> <li>– Difficult on Farms</li> <li>– <i>In situ</i> or Habitat sites</li> </ul> </li> <li>• Specific genotypes harvested</li> <li>• Natural Evolution</li> </ul>

Consequent to these differences, the conventional tools of conservation are not always effective for conserving the forest genetic resources. We review these difficulties below.

## Conventional methods in conserving forest genetic resource

### *In-situ Conservation*

*In Situ* Conservation emphasizes the conservation of genetic diversity of populations at their natural sites of origin (Shands, 1991). The method allows for the long-term conservation and, at the same time facilitates turnover of genetic variability of the populations concerned. Traditionally the in-situ conservation focused on conserving rare, endangered and threatened species mostly for animals such as Tigers, Panda, Elephants etc. The aim was to conserve the number of species. It did not pay attention at conserving genetic diversity of the species.

Perhaps the most successful strategy of *in situ* conservation is formulation of protected areas (PAs). PA are formulated worldwide but despite the establishment of the legislative PA network, more than 99% of the world protected areas are experiencing serious threats. The most severe threats to PAs are poaching, encroachment, agriculture, ranching, urban development, illegal and legal logging, and collection of non-timber forest products (World Bank 1999). Thus PAs may not be the best form of conserving forest genetic resources.

Since this approach aims at conserving the target species and its habitat, it is assumed that several of non-targeted species are also conserved (Adams and Burczyk, 2000). But It can not be tacitly

assumed that within the PAreas, genes and the gene assemblages of these non-targeted species are conserved because the protected areas may not contain the complete range of genetic diversity of these species. While PAs do promote dynamic evolution of species and contribute partially towards conserving non-targeted species, they may not be ensuring the conservation of the entire genetic variability.

In-situ conservation also focuses on conserving species through in-situ gardens, nature reserves, and on farm conservation sites etc. Unfortunately these approaches are not based on information on the distribution of genetic diversity. Even if spatially explicit information on the genetic variability is available, as being practiced, only a few sites with in-sufficient populations can be conserved through in situ approach; thus, at best these approaches conserve only a fraction of genetic variability. Consequently it is likely that in situ conservation may in fact lead to a loss of genetic variability due to genetic drift and inbreeding.

### *Ex-situ Conservation*

There seems little doubt that conservation of genetic resource is best achieved in natural habitats. However due to lack of information on where to conserve and logistics of conservation, off-site conservation (ex-situ) has been launched in a big way in practically all countries. They include botanic gardens, captive breeding in zoos, gene banks etc. Despite its widespread application in crop plants, ex situ approach can only be as effective as an arboretum in preserving the genetic variability of long lived forest tree species. The ex situ methods of conservation when applied to forest tree species suffer from (a) conserving only a limited number of individuals with a narrow genetic base and (b) permitting little or no habitat responsive evolution. The ex situ conservation will be best suited only in ensuring the survival of population or species in the face of their extinction in their natural habitats and do not serve as substitute for the conservation of the genetic variability of the natural populations (1994). The ex-situ conservation activities are only targeted to forest species and the approach is much costly. Furthermore in ex-situ conservation, complete variability is not represented in the conserved material.

## **New approach for conserving genetic resources**

While in the above we have argued how the existing methodologies can not be directly adopted for the conservation of the forest and agro biodiversity, we review here some good practices emerging in the recent past. It can be seen that while keeping the aim and the principle of conservation similar to conventional technique some radical alteration help a lot for meeting the missing elements of traditional conservation techniques. We also evaluate their suitability to our situation (Western Terai of Nepal).

Any approach for conserving genetic diversity of forest species shall attempt to

- a) conserve the most of the genetic diversity of the target species
- b) attain conservation in the most cost effective way *in situ*,
- c) retain the natural evolutionary processes and
- d) maintain a repertoire of the total genetic diversity as a bank for purpose of conservation and also utilization.

Accordingly we propose the following two approaches as the most useful methods for conserving the genetic diversity of the forest species (forest genetic resources), of agricultural crops that are in continuous interaction with the wild species (agro-biodiversity) and the species that are harvested both from the forest and farm-ecosystems.

#### **a. On farm Conservation of agrobiodiversity**

On farm conservation is an emerging new concept recently adapted by many government and non-government sectors in the developing countries. On-farm conservation of landraces refers to the process of maintaining the genetic races of crop plants in the very place where they are being maintained with all their present-day characteristics (Brush, 1995). The on-farm conservation is particularly important for a diversity-rich country like Nepal. Small and poor farmers in Nepal generally practice low-input farming and are dependent on, in addition to crop plants, wild and semi-wild species for food and other needs. For the conservation of diversity in these species, farmers and communities need to be targeted, particularly in diversity-rich areas.

#### **b. Forest gene-banks**

Forest gene bank (Uma Shaanker and Ganeshiah, 1997) is an *in situ* conservation approach which attempts to conserve total genetic diversity, among the populations as a unit and promotes evolution. Conceptually the forest gene bank serves as an *in situ* bank in which genes from as many diverse populations of the species are conserved and dynamically maintained. Operationally in these banks, an *in situ* site serves as the 'sink' population into which genes from distinct 'source' populations of the species are introduced. The forest gene banks facilitate a continuous turnover of the genetic material within and among populations. In this way, the forest gene bank is a modification of the existing protocols of *in situ* conservation with provision for substantial gene flow into it. The forest gene bank could also be viewed as facilitation of 'gene corridors' among populations to a central 'sink'. The forest gene banks facilitate the maintenance of the full allelic set of the species. Combining the gene pools would reduce inbreeding rate and enhance out crossing and mutation rates subsequently increasing the level of heterozygosity. The conservation stand should be established in areas where the threats by hybridization to endangered gene pools can be eliminated. Further, because of the continuous interaction between and among the different allelic sets maintained at the banks, genetic diversity would evolve in response to local selection pressures. Thus the "Forest gene" bank incorporates aspects of both *in situ* conservation and field gene banks. Forest gene banks are of particular relevance in the conservation of genetic resources of long-lived forest tree species. They could potentially be very useful in incorporating the genes or gene pools from threatened, endangered and fragmented populations. In fact, in such populations, forest gene banks could help avert the loss of diversity through drift, population extinction and other processes.

The salient steps involved in the establishment of gene banks are as follows (adapted from Uma Shaanker et.al., 2001):

- a. Mapping the geographical distribution of the species: It is necessary to identify the spatial distribution of the species and then to identify where populations of the species are reasonably large.
- b. Mapping the genetic diversity of populations: The sites identified during the preliminary assessment shall be used for estimating genetic diversity of the populations and hot-spots

- of genetic diversity. The assessment of the genetic differentiation of the populations needs to be done based on parameters of genetic diversity.
- c. Identification of *donor* and *recipient* populations: It involves in identifying the sites that can serve as donor (from where gene pool can be imported) and recipient (a genetic hot-spot and a sink to receive the gene pool from outside). The source and donor is identified based on the genetic variation in the populations, their presence of rare alleles, population size, local heterogeneity and threat to the population. The recipient or sink could be that which is allele-rich and has a broad genetic base with long-term demographic and genetic stability.
  - d. Introduction of gene from source to sink population: The actual process of genetic donation can be effected through the introduction of either seeds/saplings or pollen grains from the donors to the recipient population.
  - e. Monitoring of genetic diversity in the forest gene banks: Periodic injection of the genetic elements from previously identified populations could assist in effective forest gene bank with widest possible array of genetic variation.

The model of forest gene bank proposed here has provided a paradigm shift in the process of gene conservation of forest trees. By allowing for infusion of genetic material from source to sink population, the model encourages gene flow among small fragmented populations of tropical trees and thereby leads to genetic enrichment of the populations. Such enriched populations could form the storehouses or repositories of the genetic variability of the species. However there are certain criticisms on the concept:

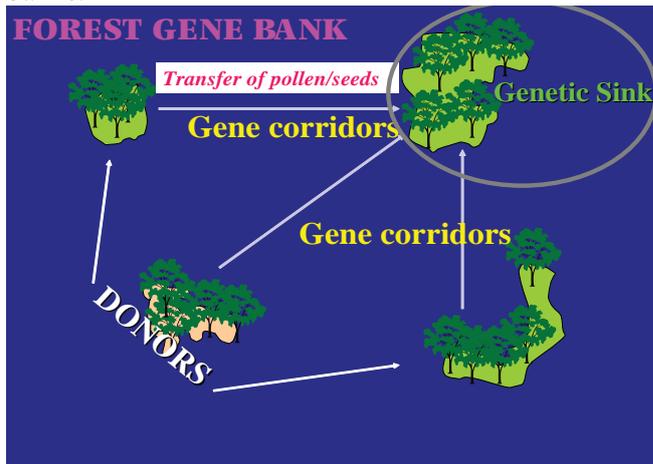
- a. Gene mixing could lead to out breeding depressions and loss of alleles due to competition (Campbell and Sorenson, 1984; Campbell and Waser, 1987).
- b. Gene flow across populations may prevent or disrupt local adaptive combinations (Antonovics and Bradshaw. 1970; Adam and Burczyk, 2000) and,
- c. Mixing populations may lead to loss of genetic differentiation and hence reduce but not increase genetic variability (Dole and Sun, 1992).

Despite these debates the forest gene bank model proposed here could form an important step forward in the effort to resurrect the genetic diversity of forest trees, especially of those which are on the brink of genetic bankruptcy. In a way the forest gene bank model can serve as a 'genetic management' approach towards tree species where the conventional models of conservation may not be applicable.

BOX

### Forest Gene Bank: The concept

Conceptual frame work of Forest genebank require identification of a) Genetic Sink, which hold highest genetic diversity perhaps representative of all the populations of the species, and b) Genetic Donors, which complement the genetic diversity of the Sink. The genetic material from the sources is introduced in to sinks and the sinks are conserved for ever as the in situ forest gene banks.



### Forest Gene Bank: Execution:

Establishment of forest gene bank requires the identification of 'sink' populations in to which genes from the identified 'source' populations can be infused periodically. Sink populations could be among those whose mean genetic distance with the rest of the populations would be the most representative of the 'global' population, while the source populations could be those which represent distinct genetic clusters (or provenances). Uma Shaanker and Ganeshaiyah (1997) estimated the genetic diversity of a set of large viable population in south India and identified such sinks and sources. They found that the populations at BRT Hills, which are the most representative of the studied populations could serve as the sink into which genes (either through pollen grains or seeds) can be infused periodically from other sites at Pechiparai, Kollihills and Thenmalai which form distinct genetic clusters. They proposed that long-term monitoring and management of such forest gene banks can ensure the conservation of the genetic resources of *Phyllanthus emblica* in a cost-effective manner.

The forest gene bank as proposed here combines the virtues of both in situ conservation sites and field gene banks. In these banks, the 'global' genetic diversity of the species is allowed to 'evolve' as it would in any other natural habitat. Thus the process of conservation of the genetic resources is not only all encompassing but also dynamic. This approach is useful in the conservation of genetic resources of trees whose populations are highly fragmented and sparse, economically important and rare and threatened plants. Further in small populations, the forest gene banks can restrain the loss of genetic diversity through drift and extinction.

(Adopted from Uma Shaanker, R, KN Ganeshaiyah, M Nageswara Rao, G Ravikanth, 1997, A new approach to conservation of genetic resources of forest trees: promise and processes. in Forest Genetic Resources: Status, Threats and Conservation Strategies (ed Uma Shaanker, R, K N Ganeshaiyah and K S Bawa) Oxford IBH Publishing Co.)

## II CONTEXT OF THE MANUAL

Landscape level conservation is a new paradigm in conservation arena crafted to address the issue of expanding human needs and their pressure on the ecosystem. In this program, conservation is extended beyond the boundary of protected areas to cover larger landscape of different land-use patterns.

With an aim to develop replicable landscape-level management model(s) to safeguard the biological wealth and vital ecological functions in Nepal, WTLCP was launched in August 2005 after signing an agreement between the Government of Nepal and the UNDP. The eight-year long project is a joint initiative of Government of Nepal and seven national and international organizations.

The project's landscape approach envisions integrated ecosystem management to achieve the multiple objectives of conservation, sustainable natural resource management and poverty alleviation by reorienting biodiversity management approaches and its institutional arrangements. The project was designed to address major biodiversity threats of agriculture encroachment and squatting in forestlands; high grazing pressure in the forests; overexploitation of forest resources; and the replacement of traditional agricultural crop varieties and landraces with modern cultivars.

The focus of the project need to target the conservation efforts in Forest core i.e. protected areas, forest buffer i.e. buffer zone and agriculture landscape i.e. productive sites. The conservation should target wild species in the forest core, wild relatives in the forest buffer and agrobiodiversity in the agriculture landscape (see figure 2).

There is relative gap of technical knowledge on establishing forest gene bank concept in Nepal. In this context, this manual will be useful in guiding the field level technician, researchers and professionals to establish forest gene bank. Specifically it will

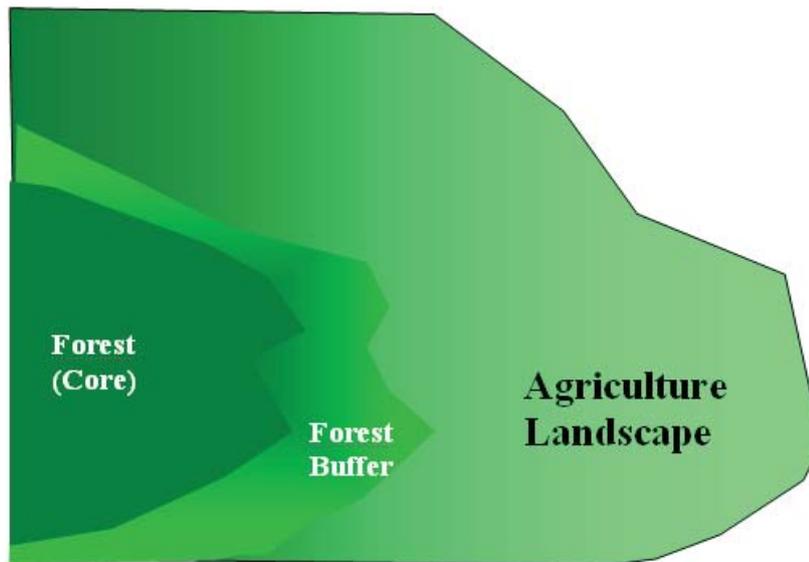
- a. Provide the basic knowledge on the importance, process and methodology of forest gene bank establishment
- b. Guide field based technicians to collect information, document, analyze and interpret findings
- c. Facilitate in the establishment of forest gene bank (sink and source)

### **Elements of conservation**

- a. *Agrobiodiversity*: Agricultural biodiversity is considered as contributive component to sustainable livelihoods of rural and marginal farming communities. It is regarded as one of the novel and innovative strategy to conserve locally threatened as well as globally important crop and animal genetic resources. The major category of this conservation approach is livelihood of communities and on Farm Conservation strategy. It needs different approach to conservation.
- b. *Agro-Forest Biodiversity*: Agro forest biodiversity offers great opportunities for the people living in the vicinity of the corridor and buffer zone areas. Large number of communities

is dependent on the forest resource and it is one of their major sources of livelihood. It is therefore to have focus on the agro forest biodiversity. The In situ Conservation approach is most appropriate in this area.

- c. *Forest Genetic resources* are globally significant and are important to conserve. The forest genetic resources have medicinal and commercially values. It also contributes directly to the livelihood of communities. The concept of forest gene bank is appropriate strategy.



*Fig 2. Stratification of Biodiversity Elements to be conserved*

## **Criteria used for identifying globally significant and locally relevant species**

### *a. Globally significant elements of Biodiversity*

- Rare and Threatened
- Endemic
- Focal species
- High diversity (Richness in intra-specific and inter-specific)
- Presence of wild relatives or wild biodiversity
- Already listed in Conservation criteria (IUCN, CITES, Forest Act and others)

### *b. Locally relevant elements of Biodiversity*

- Linked with daily livelihoods
- Socio-cultural values
- Highly potential for value addition or marketing (potential to enhance livelihoods)
- At the risk of extinction (verge of extinction)

Matrix table 2. Checker board for globally significant and locally relevant (e.g)

Category	Globally Significant	Locally Relevant
Agricultural/Food	Cell G F Rice, Finger millet, Mango, Wild pig (Pigmy hug), Gaddi buffalo, Jackfruit, Khaila cattle, Home garden agrobiodiversity, Small animal agrobiodiversity	Cell-LF Rice, Mango, Local terai goat, Wild, Jackfruit, Gaddi buffalo, Khari goat, Fingermillet, Guava, Terai cattle, home garden agrobiodiversity, small animal agrobiodiversity
Medicinal/ Industrial/ Commercial	Cell GC Sarpaganda, Amala, Asparagus, Piper longum, Bahunia species, Chattiwaan, Casia fistula, Mushroom, Neem	Cell LC Mushroom, Asparagus, Harro Barro, Amala, Bel, Jamón, Amara, Tejpatta (Cinamomum), Kusum, Asuro, Bayeer, Sakini chicken, Wild boar, Local buffalo

### III PROTOCOLS AND METHODOLOGY

<b>Title</b>	<b>Establishing <i>In situ</i> Gene Bank of Rauvolfia serpentina (L.) Benth. ex Kurtz in WTLCP areas for Conservation and Sustainable Use</b>
<b>Purpose/ Objectives</b>	<ul style="list-style-type: none"> <li>• To assess and map the distribution of genetic diversity of Rauvolfia in Western Terai Landscape Complex (WTLC) and adjoining areas</li> <li>• To develop <i>In situ</i> conservation strategies for Rauvolfia genetic diversity in WTLCP.</li> <li>• To formulate sustainable utilisation mechanisms of Rauvolfia genetic resources to enhance the local livelihoods</li> </ul>
<b>Rationale</b>	<p>‘Rauvolfia’ is a globally significant wild medicinal plant genetic resources’ and its primary center of origin lies in Indian sub-continent region (Arora and Nayar 1984). The large scale unsustainable over-harvesting of Rauvolfia has threatened its continuous survival in wild and genetic diversity has been threatened yet. 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 is still continue. At another hand, there is lack of scientific information on the accessions, genetic diversity and breeding system of Rauvolfia in Nepal. Implementation of study to locate and assess diversity, explore the conservation ways and sustainable utilisation measures are the priority actions that immediately require in Nepal. Therefore, the WTLCP-Agrobiodiversity component has identified Rauvolfia as one of the key species. Under the project, an economical and comprehensive way of conserving Rauvolfia diversity was explored through the implementation of forest gene bank approach (Shankar and Ganeshaiyah, 1997). This approach included locating <i>in situ</i> habitats of the species that retained high levels of inter and intra species diversity into which the complementary diversity from other areas can be introduced. Such gene banks are conserved <i>in situ</i> with least efforts. This proposal aims to follow the conservation of Rauvolfia. It will provide basis for assessing the genetic diversity using morphological, biochemical and molecular characterization purposes. In addition, this information will be useful in designing interventions and designing project monitoring and evaluation framework.</p>

<p><b>Research Questions</b></p>	<p>What is the amount and distribution of genetic diversity (inter and intra-specific) of Rauvolfia in WTLCP and adjoining areas?</p> <p>Where is the rich genetic diversity of Rauvolfia located in WTLCP?</p> <p>What is the extent of genetic erosion in Rauvolfia in WTLCP?</p> <p>Does forest gene bank approach is useful for <i>In situ</i> conservation of genetic diversity of Rauvolfia in the context of WTLCP?</p>
<p><b>Hypothesis</b></p>	<p>Tropical areas of western <i>terai</i> landscape represent the primary center of origin of Rauvolfia. The landscape is varies in ecogeography; socio-cultural use-values of forest resources and presence of protected areas has enriched the genetic diversity (Inter and intra-specific) of Rauvolfia.</p> <p>The increasing unsustainable over harvesting has threatened the Rauvolfia diversity in WTLCP and adjoining areas.</p> <p>Forest gene bank concept has been already useful to conserve several forest genetic resources including non-timber forest products such as <i>Phyllanthus</i> sp., therefore, this strategy will be useful to conserve the Rauvolfia genetic diversity conservation in the context of WTLCP</p>
<p><b>Location</b></p>	<p>Distribution areas in Nepal and within the vicinity of WTLCP project areas</p>

<p><b>Process/Steps or Methods for field study</b></p>	<p><b>1. Develop the ‘Predicted distribution Map’ (niche map) for Rauvolfia diversity in Nepal</b></p> <p><b>1.1 Literatures review</b></p> <ul style="list-style-type: none"> <li>• Review literatures on the species availability, status, habitat and use values</li> <li>• Review national and international policies governing its conservation and use</li> <li>• Review project documents to identify areas where species is recovered by others.</li> <li>• Sources of secondary information are: TU Central library, IOF library, NARC, IUCN, NAST, ICIMOD library, WTLCP project documents, Publication from department of plant resource, Department of Forest Research, Department of National Park and Wildlife Conservation, Ayurvedic literatures, policy documents (PRSP, 10<sup>th</sup> Five year plan, Tri-Yearly Interim Plan, Biodiversity strategy, Forest Act and Regulation, Plant protection Act), Other relevant publications in books, journal, proceeding, articles etc.</li> <li>• Collect data on general distribution, other relative species available in the area, x y coordinates and variants if any</li> <li>• Internet searching</li> </ul> <p><b>1.1 Consultation with relevant experts and organization</b></p> <ul style="list-style-type: none"> <li>• Consult with relevant scientists, academicians, policy makers and researchers</li> <li>• Collect information on a) distribution sites of Rauvolfia in Nepal b) listing sites that are rich in Rauvolfia d) priority sites for future surveys in WTLCP and adjoining areas</li> <li>• Potential organizations for consultation are: CDB-TU, IUCN-Nepalgunj, Green Energy Mission, Protected Areas Officials and others</li> </ul> <p><b>1.1 Herbarium study</b></p> <ul style="list-style-type: none"> <li>• Visit the TUCH, KATH and record the information on diversity, distribution, locality and coordinate values</li> </ul> <p><b>1.4 Develop the predicted distribution map of Rauvolfia in Nepal and WTLCP area</b></p> <ul style="list-style-type: none"> <li>• Synthesize the available information from above steps (literature reviews, herbarium, consultation meeting and Interviews)</li> <li>• Identify the coordinate values using available tools (map, GPS record)</li> </ul>
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- Prepare the predicted distribution map of Rauvolfia in Nepal using GIS tools
- Locate the potential areas for detail surveys in WTLCP and adjoining districts

## **2. Assess the distribution and diversity of Rauvolfia in WTLCP and adjoining districts**

### 2.1 Organise a field consultation meeting at WTLCP to finalise the specific sites for detail study

- Invite Traders/collectors, park authorities, district forest office, FECOFUN, IUCN-Nepalgunj and other relevant organization/ personnel in consultation meeting
- The sites must be heterogeneous which include protected area, buffer zone, national/community forest and private land
- Finalize the specific sites for surveys

### 2.2. Getting prior inform consent or approval to conduct surveys in Protected areas or community forest

### 2.3 Ecogeographic surveys in specific sites

- Preparation of multidisciplinary survey team members from WTLCP project stakeholders. The survey team members should have following expertise: a botanist (with knowledge on ethnobotanical survey methodology), forester with knowledge on sampling methodology, Key informant of Rauvolfia from local area who can also translate the ethnic language.
- Organize orientation programme on survey methodology and ecological sampling procedures a) transect walk b) Random or stratified sampling methods. A guideline for these methods has been given in Appendix III.
- Prepare Resource Map of each survey sites
- Prepare a format for sample and information collection as per the methodology. A guideline for data collection is given in Appendix IV.
- Collect the information along the transect or in sampling quadrants • Collection of voucher specimens for herbariums (at least three sets) and collection of root/leaf materials for biochemical and molecular studies
- Organize FGD with local community to validate the documented information, local name and use-values.

2.4 Characterization through plant morphology, biochemical and molecular markers studies.

- Prepare 'Rauvolfia Descriptor' for characterization purposes based on the results of Step 1 & 2 (Taxonomic and Local knowledge)
- Carry out plant morphology characterization based on descriptor (herbarium and field data)
- Biochemical and molecular characterization/DNA markers studies (separate protocol will be prepared for this activity)
- Development of suitable statistical method since the data will be on morphometric and molecular studies.

### **3. Mapping the diversity of Rauvolfia**

3.1 Mapping the intra and inter-specific diversity

- Perform GIS tools and statistical analysis
- Mapping species (other related species) and genetic diversity using GIS tools

3.2 Locating the source and sink Areas

- Identify the areas with viable populations
- Locate the areas with high genetic diversity (inter and intra-specific) or Genetic Hotspots and Species Rich Hotspots
- Identify the areas that overlap between Inter and Intra-specific diversity
- Listing potential Gene Banks (sinks) and donors (Source)

### **4. Organize stakeholders' workshop to finalize the sink area**

- Sharing the study outputs: With Forest user group members, DFO, FECOFUN, Protected areas authority and project team members
- Agree and finalize the source and sink areas (if the sink areas selected within PA then get permission from PAs authority).

### **5. Establish the Raulvofia gene banks**

- Identify the population and sample size
- Consider Ecological factors prior transferring the genetic material at Sink
- Gather information on soil status, climate, topography, and other ecological consequences prior transplanting the species from different sources to sink and vice versa.
- Collect seeds or propagules from source and transfer the subsequent individuals that represent the diversity of each source to sink areas

	<ul style="list-style-type: none"> <li>• Enrich Gene Banks with genetic materials from Donors</li> <li>• Formulate strategies for protection of the gene pool at sink from possible threats</li> <li>• Implement regular monitoring programmes (viability, disease/ pest, growth etc.) in sink area</li> <li>• Conserve it as Rauvolfia <i>in situ</i> Gene Bank</li> </ul> <p><b>6. Conservation and sustainable use</b></p> <ul style="list-style-type: none"> <li>• Develop institutional working modality for Rauvolfia gene bank management</li> <li>• Collaborate with protected areas and forestry authorities for management of gene banks</li> <li>• Involvement of local institution and building their capacity for sustaining the conservation activities</li> <li>• Implement sustainable harvesting mechanisms in source areas i.e. within the community/national forest areas through training and orientation to local communities</li> <li>• Develop community based nurseries and initiate Rauvolfia cultivation in farmers fields</li> <li>• Value addition of Rauvolfia products (processing and packaging) and link with market chain.</li> <li>• Scaling up the good practices of gene bank for the management of other forest species</li> </ul>
<b>Activity Leader</b>	
<b>Team Members</b>	
<b>Consultants</b>	
<b>Reporting Outputs</b>	
<b>Budget (NRS)</b>	

<b>Title</b>	<b>Establishing <i>In situ</i> Gene Banks of Mango (<i>Mangifera indica</i> L.) in WTLCP areas for Conservation and Sustainable Use</b>
<b>Objective</b>	<ul style="list-style-type: none"> <li>• To assess the distribution and diversity of mango genetic diversity in Western <i>Terai</i> Landscape Complex (WTLC) and adjoining areas</li> <li>• To develop <i>In situ</i> conservation strategies for mango genetic diversity in WTLC.</li> <li>• To develop sustainable utilisation mechanisms for potential mango genetic resources to enhance the local livelihoods</li> </ul>
<b>Rationale</b>	<p>Mango is one of the important tropical fruits of Nepal and covering the 9% of total fruit cultivated area which ranked second in all fruits cultivated in Nepal (Gautam and Dhakal 1994). The total area under mango cultivation in the country is 13, 904 ha with annual production reached up to 1,03,784 metric tons (ASD 2005). The <i>terai</i>, lower hills and mid hills are the regions from where several different genotypes of mango have been reported. Home gardens, commercial orchards, religious or cultural places, places around river gorge and forest are the areas of mango cultivation or existed as wild entities (Subedi <i>et al.</i> 2005). This fruit species is closely linked with local peoples' livelihoods, socio-culture and economic benefits. Despite several desirable traits reported in local mango such as ability to grow in dry or marginal areas, the ability to grow at high altitude, the absence of fiber (in some cases), high fruit setting rate, off-season fruiting. However, in recent years the original habitats of local mango have been severely changed in view of the biotic, economic and other pressures such as extensive demand of local mango tree trunks in brick factories. This has resulted genetic erosion and loss of potential local genotypes. At another hand, being trees it is difficult to maintain the genetic diversity in on farms or <i>ex situ</i> gardens at long-term. Therefore this study aims to conserve mango diversity of WTLC and adjoining areas. The project will implement the complementary strategy to conserve the genetic diversity which is cost effective, ensure long term conservation and promote sustainable use by adopting the principles of forest gene bank in the context of WTLC.</p>

<p><b>Research Questions</b></p>	<p>Where is the rich genetic diversity of Mango located in WTLC and adjoining areas?</p> <p>What is the total amount and distribution of genetic diversity (inter and intra-specific) of Mango in WTLC?</p> <p>What is the extent of genetic erosion of genetic erosion of Mango diversity in WTLC?</p> <p>What are the potential local mango genotypes to be promoted in WTLC to enhance the local livelihoods?</p> <p>Does forest gene bank approach is useful for <i>In situ</i> conservation of genetic diversity of Mango in the context of WTLC?</p>
<p><b>Hypothesis</b></p>	<p>Tropical areas of WTLC is diverse in ecogeography, rich in socio-cultural diversity and presence of protected areas are rich in Mango genetic diversity (Inter and intra-specific) due to.</p> <p>The original habitats of Mango have been rapidly changing owing to the biotic, economic and other pressures. Therefore, genetic diversity is eroding and in the verge of extinction from WTLC.</p> <p>The rich diversity of mango genotypes contained several potentialities which can be promoted in WTLC on community based approach to enhance the local livelihoods.</p> <p>Forest gene bank approach has been already demonstrated to conserve several forest related species such as Amala in India, therefore, it will be equally useful to conserve the Mango genetic diversity conservation in the context of WTLC.</p>
<p><b>Location</b></p>	<p>WTCLP sites (Bardiya, Kailali and Kanchanpur districts) and adjoining districts</p>

<p><b>Process/ Steps/ Methodology</b></p>	<p><b>1. Develop the predicted ‘map of distribution’ (niche map) for Mango in Nepal</b></p> <p>1.1 Literatures review</p> <ul style="list-style-type: none"> <li>• Project documents review - <i>In situ</i> Agrobiodiversity Conservation project, TFT projects and other documents published by DADO, NARC, DFO, National Park and others</li> <li>• Internet searching</li> </ul> <p>1.2 Consultation with relevant crop experts</p> <ul style="list-style-type: none"> <li>• Prepare a checklist for consultation meeting and data collection (Please refer Appendix I)</li> <li>• Organize meetings with DADO, NARC, Protected Areas Officials and NGOs personnel to identify a) list down the sites<sup>4</sup>for mango growing or areas where mango still exist as wild b) record the different mango genotypes in different areas) list down the sites for unique mango genotypes and genotypes name d) priorities the potential sites<sup>5</sup> based on consultations</li> </ul> <p>1.3 Develop the predicted distribution map</p> <ul style="list-style-type: none"> <li>• Synthesize the available information from literature reviews, project baseline surveys and consultation meeting outputs</li> <li>• Identify the coordinate values using available tools</li> <li>• Prepare the predicted distribution map using GIS tools</li> <li>• Interpret the map to locate potential sites for details studies</li> </ul> <p><b>2. Locate and assess the distribution and genetic diversity of Mango</b></p> <p>2.1 Organize diversity fairs at village level (potential villages in each districts) to document the farmers unit of diversity (FUD) (See the Appendix II for detail field methodology)</p> <ul style="list-style-type: none"> <li>• Organize orientation programme to community members</li> <li>• Develop a format to document the local information (Address of collection source, local name of genotypes, unique use/values, age of tree, fruit bearing nature, Seedlings/Grafted, Availability status)</li> <li>• Exhibition of fruit samples in fair</li> <li>• On site validation through focus group discussion to get consistency in farmers naming and locating the custodians</li> <li>• Collect fruit samples and herbariums for morphological characterization (5 fruits per genotypes of single tree-IPGRI 1989) and leaf samples for molecular studies</li> <li>• Visit the field to get coordinate values and marking on tree trunks for scion or bud collection purposes</li> </ul>
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5 Sites may represent specific village, wards, commercial orchards, forest, protected areas, religious or scared places, river areas or premises of public places such as school or horticultural farm etc.

6 Potential sites: Potential sites must represent the heterogeneous environments such as cultivated areas, forest areas and interface of between these two etc.

## 2.2 Ecogeographic surveys in protected and forest areas

- Prepare a format for sample and information collection (Address of forest, Local name of genotypes, Coordinate value, Population status (approx area and number), Age of tree)
- Organize surveys (include at least one horticulturist, if possible Key Informants from village and Forestry people in group), if necessary make sub-groups
- Prepare field map to locate the tree trunks, record the coordinates and altitude and mark by enamel over tree trunks for future scion collection
- Collection of fruit samples and herbariums for morphological characterization and leaf materials for molecular studies

## 2.3 Characterization of fruit morphology (See the Appendix III for detail field methodology) and molecular markers studies.

- Carry out fruit morphology characterization based on IPGRI Mango Descriptor 1989 (Please refer Appendix III)
- Molecular characterization/DNA markers studies (Dr. Jwala could help to develop protocol on this)
- Development of suitable statistical method since the data will be on morphometric, variety based and molecular studies.

## **3. Mapping the diversity of mango**

### 3.1 Mapping the intra and inter-specific diversity

- Perform multivariate analysis, PCA, ANNOVA test etc
- Mapping species (other related species) and genetic diversity using GIS tools

### 3.2 Locating the source and sink Areas

- Identify areas with viable populations
- Locate the areas with high genetic diversity (inter and intra-specific)
- Identify the areas that overlap between Inter and Intra-specific diversity
- Listing potential source and sink areas

## **4. Organize stakeholders' workshop to finalize the sink area**

- Sharing the study outputs with farmers from potential sink and source areas, DADO, DFO, NARC, Protected areas authority and project team members

	<ul style="list-style-type: none"> <li>• Agree and finalize the source and sink areas</li> </ul> <p><b>5. Develop protocol for transferring the source population to sink (Developing Mango Gene Banks)</b></p> <ul style="list-style-type: none"> <li>• Identify the genotypes and sample size</li> <li>• Grow seedlings for root stocks, collect scion and grafting (establish nurseries)</li> <li>• Transfer seeds and saplings (grafted plants) of subsequent individuals that represent the diversity of each source to sink areas</li> </ul> <p><b>6. Establish and Maintain the Mango gene banks (Maintaining the Mango Gene Banks)</b></p> <ul style="list-style-type: none"> <li>• Implement regular monitoring programmes (viability, disease/ pest, growth etc.) in sink area</li> <li>• Implement sustainable use mechanisms in source areas such as community based mango nursery establishment</li> <li>• Training and orientation on local mango cultivation</li> <li>• Explore value addition (or commercial importance) and establish or strengthened the linkages with market chain in source areas</li> </ul>
<b>Thematic leader</b>	
<b>Activity leader</b>	
<b>Team members</b>	
<b>Professional Input</b>	
<b>Data analysis/ Synthesis</b>	
<b>Technical Outputs</b>	
<b>Activity plan</b>	
<b>Estimated Budget</b>	

<b>Title</b>	<b>On farm conservation of rice genetic diversity in the western <i>terai</i> landscape complex of Nepal through species diversity mapping and seed bank approach</b>
<b>Purpose/ Objectives</b>	<ul style="list-style-type: none"> <li>• To develop species diversity maps to locate, assess and analyse the extent and distribution of rice genetic diversity in WTLCP and its adjoining areas based on geographic distribution, farmers descriptor, morphological and molecular characterisation measures</li> <li>• To integrate the good practices of community seed bank and forest gene bank approaches as an institutional support system for on farm conservation of rice genetic diversity in WTLCP</li> </ul>
<b>Rationale</b>	<p>Rice (<i>Oryza sativa</i> L.) is a globally important crop. In Nepal it is a major crop for food security at national and household levels. Nepal is considered as a centre of origin of Rice. Altogether four different wild species of rice and its several wild relatives have been already documented from Nepal. From the ancient time, a wide range of rice diversity under cultivation as landraces and they have been long adapted to local environment and specific cultural regimes, being better suited to diverse farming systems, agro-ecology, diverse socio cultural settings and the needs of farmers.</p> <p>At other hand, there is an increasing trend of rapid loss and genetic erosion in rice landraces particularly from the <i>terai</i> region of Nepal due to replacement by a few modern varieties (MVs). Large numbers of rice landraces are grown in small plots which increasingly threatened by MVs due to their higher yield potential, better market demand and price with comparison to local landraces (Chaudhary <i>et al.</i> 2004). Since rice landraces are the affordable option to poor farmers for their livelihoods, it is urgent need to develop strategies to conserve local rice diversity and promote its' use particularly in <i>terai</i> region of Nepal.</p> <p>Therefore, the WTLCP-Agrobiodiversity component has identified Rice as one of the key species. Under the project, an economical and comprehensive way of managing Rice diversity will be explored which will be least cost and effective in the context of WTLCP. This protocol will employ the species diversity mapping as entry point to locate, measure and analyse the extent and distribution of rice diversity in WTLCP. The map will strategically provide the basis for identifying the communities or villages to establish the community rice seed banks as an institutional support system to conserve and use the rice diversity.</p>

	<p>The good practices of community seed bank developed during the implementation of project <i>In situ</i> agrobiodiversity in Nepal will be scaled up in WTLCP. Various community based participatory approaches such as participatory plant breeding, participatory variety selection and landrace enhancement programmes can be integrated with this protocol to continue maintain the rice landraces on farm and developing ‘choice of diversity’ to farmers which secure their livelihoods.</p>
<p><b>Research Questions</b></p>	<p>What is the amount and distribution of genetic diversity of rice (wild species, wild relatives, landraces and modern varieties) in WTLCP and adjoining areas?</p> <p>Where is the rich genetic diversity of rice located in WTLCP?</p> <p>What is the extent of genetic erosion in rice landraces in WTLCP?</p> <p>Does species diversity mapping methodology is useful to locate, measure and analyse the rice diversity?</p> <p>Does community seed bank approach is useful as institutional support system to maintain the rice diversity on farm in the context of WTLCP?</p>
<p><b>Hypothesis</b></p>	<p>Tropical areas of western <i>terai</i> landscape represent the primary center of origin of rice, therefore, a rich genetic diversity (wild species, wild relatives, landraces and modern varieties) of Rice exist in WTLCP and its adjoining areas.</p> <p>The increasing threat to local landraces due to varetial replacement by few modern varieties is common in <i>terai</i> region of Nepal which has resulted varietal loss and genetic erosion in landraces in WTLCP and adjoining areas as well.</p> <p>Species diversity mapping has been used to locate, measure the extent and distribution of genetic diversity in several forest based genetic resources, therefore, the good practices of this methodology can be reviewed and applied in the context of agrobiodiversity such as Rice.</p>

	<p>Community seed bank strategy has been already useful to on farm manage the rice diversity in central <i>terai</i> region of Nepal, therefore, this strategy will be scaled up in WTLCP and good practices of forest gene bank will be integrated with it to develop as an institutional support system to conserve the Rice genetic diversity on farm in the WTLCP areas.</p>
<b>Location</b>	WTLCP and adjoining areas

<p><b>Process/Steps or Methods for field study</b></p>	<p><b>1. Develop the ‘Predicted distribution Map of Rice diversity’ in WTLCP and adjoining areas</b></p> <p>1.1 Secondary data collection and analysis</p> <ul style="list-style-type: none"> <li>• Review the project documents–PRA studies, project site selection and baseline reports</li> <li>• Review of relevant documents published by DADO, NARC, workshops/conference papers and others</li> <li>• Review of diversity fairs data (one fair has been already organized)</li> <li>• Listing rice landraces, improved variety, wild species and its’ wild relatives along with agro-ecological information such as production domain, custodians, documenting local use values and conservation status<sup>34</sup> at village level</li> <li>• Listing the unique and rare diversity of rice and its distribution areas at village level</li> <li>• Identify the X Y coordinates and altitude range of above villages using the different sources such as information collected from Global Position System (GPS), DADO, DDC, Village profile or interpretation from the District Map.</li> </ul> <p>1.2 Organize rapid biodiversity assessment in project villages</p> <ul style="list-style-type: none"> <li>• Organize a meeting with DADO and NARC to list down the potential villages which are rich in rice diversity (landraces, wild species and its wild relatives). The criteria for selection of villages will be diversity on existing agro-ecosystems, replacement of local landraces by modern variety and socio-cultural factors.</li> <li>• List down the villages based on above categories and select randomly ... % from each category</li> <li>• Organize FGD with key informants to rapidly assess the rice diversity. The assessment should prepare an inventory of rice, diversity (local landraces, modern variety, wild species and its wild relatives), its production domain, unique diversity, local use-values and local status (decreasing, increasing and same).</li> <li>• Record the X Y coordinates and altitude range of villages</li> </ul> <p>1.3 Development of prediction distribution map of rice</p> <ul style="list-style-type: none"> <li>• Data entry from the results of steps 1.1. and 1.2</li> <li>• Run GIS software to analyze and interpret the results</li> <li>• Develop the prediction distribution map of rice in WTLCP and its adjoining areas</li> </ul>
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- Identify the villages which are rich in rice diversity for further detail assessment and studies

## **2. Characterization and assessment of rice genetic diversity in WTLCP and adjoining areas**

### 2. Organize rice diversity fair at District Level

- Organise rice diversity fairs at district level. Include the villages which has been identified as rich in rice diversity from the step 1.3
- Organize orientation programme on methodology of diversity fair at district level to project stakeholders
- Follow the steps and procedures of diversity fair preparation as developed in *In situ* project (Sthapit *et al.* 2006)
- Collect passport data and other information in registers during the fair: Local name, landraces/improved/wild/wild relatives, address of custodians or location of habitat, agro ecosystems, local use-values, local status (increasing, decreasing and same)
- On site validation on farmers naming and information recorded in registers through focus group discussion
- Organise four cell analysis exercise to identify the common, rare and unique rice diversity
- Collect the sufficient seed samples to establish diversity blocks for further detail morphological and molecular studies
- Visit the villages to get coordinate values and altitude data

### 2.2 Ecogeographic surveys in protected, forest and wetland areas

- The specific purpose of this activity is to collect samples and document the information of wild species or wild relatives of rice
- Before organizing collection mission in protected areas get approval with PA authority
- The information should be collected during the survey include: Taxonomic name, local name, source of collection, detail address, approx. area coverage, local use-values, conservation notes such as any threats observed etc, coordinate values and altitude.

### 2.3 On farm characterisation and assessment of rice diversity

2.3.1 Establish rice diversity blocks for consistency test on farmers naming , morphological and molecular characterization purposes

- Collect the seeds materials of different landraces as per farmers naming during the diversity fair and from the survey sites
- Collection of herbarium specimens (three sets)
- Maintain them in diversity blocks as per the farmers practice in different agro-ecological domains
- Organise traveling seminar at diversity blocks during the crop maturity stage to custodians farmers, resource farmers and cop experts
- Validate the farmers naming through discussion and field observation
- Collect the data and samples for morphological studies
- Characterize the rice diversity as per the Rice Descriptor (as an example of rice descriptor will be provided later on)
- Collect the sufficient seed samples for molecular studies (a separate protocol for this study should be prepared before the implementation of this activity)

### **3. Mapping the diversity of Rice**

#### 3.1 Mapping the intra and inter-specific diversity

- Data analysis of steps 1 and 2
- Perform GIS tools and other statistical analysis
- Mapping species (other related species) and genetic diversity through GIS tools
- Develop species diversity maps of rice

#### 3.2 Locating the source and sink Areas

- Here sink represent the ‘Seed bank’ and source represent the areas from where rice diversity could be collected and added to seed banks
- Locate the areas with high genetic diversity (inter and intra-specific) or Genetic Hotspots and Species Rich Hotspots
- Identify the areas that overlap between Inter and Intra-specific diversity. These villages will be the candidate for establishing rice seed bank.
- Listing potential villages Seed Banks (sinks) and donors (Source)

#### **4. Organize stakeholders’ workshop to finalize the sink area (Seed banks sites)**

- Sharing the study outputs: Farmers, CBOs, DADO, NARC, and WTLCP project stakeholders

	<ul style="list-style-type: none"> <li>• Agree and finalize the sink areas (Seed bank areas) and source areas</li> <li>• During selection of sites for seed banks a network of sites should be envisioned which represent the diverse agro-ecology, socio-cultural values and conservation needs of WTLCP. These sites ultimately join with each other to form a networks of seed banks functional at landscape level</li> </ul> <p><b>5. Establish the Community based on farm rice seed banks</b></p> <ul style="list-style-type: none"> <li>• Identify the diversity, population and sample size</li> <li>• Collect sufficient genetic materials (seeds) from source and transfer the subsequent population that represent the diversity of each source to sink areas</li> <li>• Enrich Seed Banks with genetic materials from Donors</li> <li>• Formulate strategies for protection of the gene pool at sink from possible threats</li> <li>• Implement regular monitoring programmes (viability, disease/ pest, seed multiplication etc.) in sink area</li> <li>• Manage it on farm seed bank of rice</li> </ul> <p><b>6. On-farm management of rice seed banks and its sustainable use</b></p> <ul style="list-style-type: none"> <li>• Identify and develop community based institutional system for the long-term management rice gene banks through village level workshop</li> <li>• Review and scale up the good practice of seed bank of Bara ecosite (<i>In situ</i> project) for management of seed bank management</li> <li>• Establish ‘Community based code of conduct’ for seed bank operation such as seed exchange and collection system within and outside the community or village</li> <li>• Build the technical (seed viability, seed quality maintenance) and managerial capacity of community based institution managing the gene bank and farmers groups at source areas through exposure visit, training and orientation</li> <li>• Maintenance and increase the seed source in diversity blocks and farmers fields</li> <li>• Document the good practices and scaled up to other areas of Nepal</li> </ul>
<p><b>Activity Leader</b></p>	

<b>Team Members</b>	
<b>Consultants</b>	
<b>Reporting Outputs</b>	
<b>Budget (NRS)</b>	

## IV TOOLS AND TECHNIQUES

**Background:** There are several computer based tools and techniques available nowadays to assess, analyze and predict the biodiversity and its various components such as GARP, MAXENT. In this manual we are particularly introducing the DIVA GIS software. DIVA GIS is developed by Robert S. Hijmans, Luigi Guarino, and Andy Jarvis, Rachel O'Brien, Prem Mathur (latest version 5.4 and for further detail please check [www.diva-gis.org](http://www.diva-gis.org)) and it has following advantages:

1. Simple to use
2. Specifically suited for agricultural, and forest plants
3. In built data on several climatic parameters is available
4. It helps developing predictions for the future distribution of the species as well in the context of climate change
5. Freely available

We have given a step by step process to locate, assess, analyse and predict the biodiversity in given space and time with the context of *In situ* and *Ex situ* biodiversity conservation at the landscape level. To make it illustrative we have referred the datasets from the mango research conducted by Global On Farm Agrobiodiversity Conservation Project in Nepal. In this chapter there are two modules. The first module gives some basic information on preparing the suitable datasets that are required for the analysis. Second module offers a detailed and illustrative step for selected tools and techniques relevant for biodiversity conservation and forest gene bank establishment.

## MODULE A : DATABASE PREPARATION

**Step 1: Recording the coordinates:** To use DIVA GIS, it is necessary to have coordinates (X and Y) of species or variety under the study area. So when you go to survey it is better to take a Global Positioning System (GPS) to record these values. If you do not have access to this equipment then we can identify from secondary sources such as map interpretation or data received from R & D organizations.

**Step 2: Data entry and Screening :** After completing the survey or data from secondary sources, the next step is to prepare a suitable format in Microsoft Excel sheet. The format could be varied as per the study objectives. However, basic elements could be species name, source of collection, address of collecting source, local use-values, **coordinate values**, altitude etc. See the figure as one example.

1	Local name	Farmers name	Address	District	Source of collection	Longitude (X)	Latitude (Y)	Altitude (m)
2	Chaksa	Yadubir Sahi	Navadurga-4, Dadaban	Dadeldhura	Home garden	80°42' 29	29°17' 39	350
3	Langda	Yadubir Sahi	Navadurga-4, Dadaban	Dadeldhura	Home garden	80°42' 29	29°17' 39	350
4	Rithya	Krishna Dev Bhatta	Samajji- 1, Pali	Dadeldhura	Village garden	80°34' 31	29°20' 24	1400
5	Mithe	Som Raj Pandey	Baglung Municipality-11, Niraye	Baglung	Sacred place	83°36' 27	28°16' 43	750
6	Gilhe	Som Raj Pandey	Baglung Municipality-11, Niraye	Baglung	Sacred place	83°36' 27	28°16' 43	750
7	Bahure	Tara Nath Paudei	Pang-1, Saharshadhara	Parbat	Home garden	83°37' 91	28°14' 89	700
8	Lamche	Dayaram Rijhal	Pang-2	Parbat	Home garden	83°38' 21	28°15' 11	900
9	Nalte	Dayaram Rijhal	Pang-2	Parbat	Home garden	83°38' 21	28°15' 11	900
10	Kalkatiya	Manilal Ram	Kanchanpur-6	Saptari	Mango orchard	86°38' 12	26°38' 46	150
11	Siplya	Manilal Ram	Kanchanpur-6	Saptari	Mango orchard	86°38' 12	26°38' 46	150
12	Jalmare	Abul Hasan	Kalyanpur-6	Saptari	Road side	86°38' 23	26°38' 50	150
13	Bhaunth	Rajendra Chaudhary	Kalyanpur-5	Saptari	Sacred place	86°47' 46	26°28' 10	100
14	Green bombay	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85°11' 34	28°02' 16	650
15	kalkatte kale	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85°11' 34	28°02' 16	650
16	Farse	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85°11' 34	28°02' 16	650
17								
18								
19								
20								
21								
22								

Fig 1. A example dataset format required for DIVA GIS prepared in Microsoft Xcel sheet

**Step 3. Fine tuning the data :** It is essential to fine tune the data and based on the requirement of the software. For instance it is important to have the geographic coordinates in an uniform analysis mode. A decimal system is universally used in geographic computing. In the decimal system, latitude and longitude are described by a single number each, and no letters with the sign indicating the hemisphere (+ = N or E, - = S or W) (e.g., - 12.57083). We need to convert longitude and latitude in degrees, minutes and seconds to decimal degrees, the following formula is used:

$DC = h. ( d + m/60+s/3600)$  where DC is the decimal coordinate; d is the degrees, m the minutes and s the seconds of the sexagesimal system. Decimal degrees should normally be recorded with 4-5 decimals. See Table below for references.

The simplest way : When you are working on Xcel sheet the existing coordinate value such as 80°30'40" should be changed by entering the following formula in Xcel sheet <+80+30.40/60>. The value will soon change to decimal and appears as 80.5066. By this way repeat for remaining coordinates values. See the figure below

	A	B	C	D	E	F	G	H	I
1	Local name	Farmers name	Address	District	Source of collection	X	Y	Altitude (m)	
2	Bahure	Tara Nath Paudel	Pang-1, Saharshadhara	Parbat	Home garden	83.632	28.248	700	
3	Bhaunth	Rajendra Chaudhary	Kalyanpur-5	Saptari	Sacred place	86.791	26.468	100	
4	Chaksa	Yadubir Sahi	Navadurga-4, Dadaban	Dadeldhura	Home garden	80.705	29.290	350	
5	Farse	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85.189	28.036	650	
6	Githe	Som Raj Pandey	Baglung Municipality-11, Niraye	Baglung	Sacred place	83.605	28.274	750	
7	Green bombay	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85.189	28.036	650	
8	Jalmare	Abul Hasan	Kanlyanpur-6	Saptari	Road side	86.637	26.642	150	
9	Kalkatiya	Manilal Ram	Kanchanpur-6	Saptari	Mango orchard	86.635	26.641	150	
10	kalkatte kale	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85.189	28.036	650	
11	Lamche	Dayaram Rijhal	Pang-2	Parbat	Home garden	83.637	28.252	900	
12	Langda	Yadubir Sahi	Navadurga-4, Dadaban	Dadeldhura	Home garden	80.705	29.290	350	
13	Mithe	Som Raj Pandey	Baglung Municipality-11, Niraye	Baglung	Sacred place	83.605	28.274	750	
14	Naithe	Dayaram Rijhal	Pang-2	Parbat	Home garden	83.637	28.252	900	
15	Rithya	Krishna Dev Bhatta	Samalji-1, Pali	Dadeldhura	Village garden	+80+34.31/60	29.337	1400	
16	Sipiya	Manilal Ram	Kanchanpur-6	Saptari	Mango orchard	86.635	26.641	150	

Fig 2. Changing the coordinates value into decimal values

Note: Note the circled area showing the formula to convert the coordinate value in decimal value

#### Step 4. Data sorting

It is necessary to sort out the information so that it will be easier for the analysis. The sorting will keep the dataset alphabetically. See the figure below for references.

	A	B	C	D	E	F	G	H	I
1	Local name	Farmers name	Address	District	Source of collection	Longitude (X)	Latitude (Y)	Altitude (m)	
2	Bahure	Tara Nath Paudel	Pang-1, Saharshadhara	Parbat	Home garden	83.632	28.248	700	
3	Bhaunth	Rajendra Chaudhary	Kalyanpur-5	Saptari	Sacred place	86.791	26.468	100	
4	Chaksa	Yadubir Sahi	Navadurga-4, Dadaban	Dadeldhura	Home garden	80.705	29.290	350	
5	Farse	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85.189	28.036	650	
6	Githe	Som Raj Pandey	Baglung Municipality-11, Niraye	Baglung	Sacred place	83.605	28.274	750	
7	Green bombay	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85.189	28.036	650	
8	Jalmare	Abul Hasan	Kanlyanpur-6	Saptari	Road side	86.637	26.642	150	
9	Kalkatiya	Manilal Ram	Kanchanpur-6	Saptari	Mango orchard	86.635	26.641	150	
10	kalkatte kale	Jee Nath Aryal	Malang-7, Baireni	Dhading	Mango orchard	85.189	28.036	650	
11	Lamche	Dayaram Rijhal	Pang-2	Parbat	Home garden	83.637	28.252	900	
12	Langda	Yadubir Sahi	Navadurga-4, Dadaban	Dadeldhura	Home garden	80.705	29.290	350	
13	Mithe	Som Raj Pandey	Baglung Municipality-11, Niraye	Baglung	Sacred place	83.605	28.274	750	
14	Naithe	Dayaram Rijhal	Pang-2	Parbat	Home garden	83.637	28.252	900	
15	Rithya	Krishna Dev Bhatta	Samalji-1, Pali	Dadeldhura	Village garden	80.572	29.337	1400	
16	Sipiya	Manilal Ram	Kanchanpur-6	Saptari	Mango orchard	86.635	26.641	150	

Fig 3. Sorting the datasets alphabetically

**Step 4. Save as Text file :** Now, the data sheet should be saved to Formatted text. So click ‘Save as’ icon and select the <Text (Tab Delimited) (\*.xls)> or shape files<sup>1</sup> and save it. See the figure below for reference. After completing these four steps your dataset is ready for DIV GIS analysis.

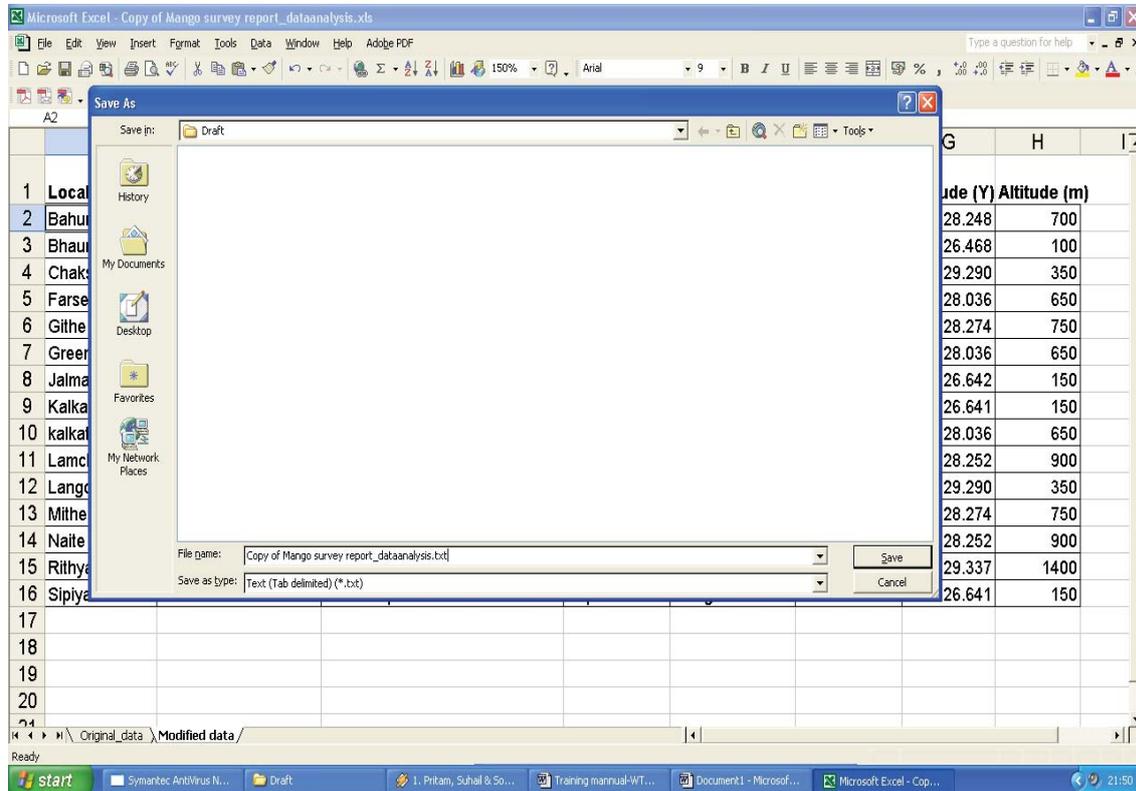


Fig 4. Saving the datasets into text format

<sup>1</sup> Shapefiles are so called vector databases, describing the location of points (e.g., collecting locations), areas (e.g. soil types, countries)

## MODULE 2 : DIFFERENT TOOLS USED

As per the study purpose, we could analyze and prepare map on species diversity, its distribution, locating hot spots, prediction of diversity, selecting the reserves for conservation etc. Each analysis requires different steps so it is described in respective chapters.

### TOOL 1: Mapping the predicted distribution

**Purpose of this tool:** 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 (preference) for species suggest the areas where the chance of species occurrence is maximum and lowest. This map will help in three ways:

- b) Preparing the distribution map of species under study
- c) The areas where we need to focus our study or the areas where we can avoid
- d) If the species is threatened then it suggests the areas where it likely to be conserved (helpful to species recovery and translocation project)
- e) So it reduces the cost and time of project

However, it is suggested here that to conduct some sample surveys to validate the predicted areas before any conservation decision made.

**Step 1. Open the DIVA GIS :** First of all make sure that, you have closed the Xcel sheet under which you are working. Open the DIVA GIS software's then a main menu will appear. Click on <Data> Select <Import Points to Shapefile> then select <From text file.(Txt)>

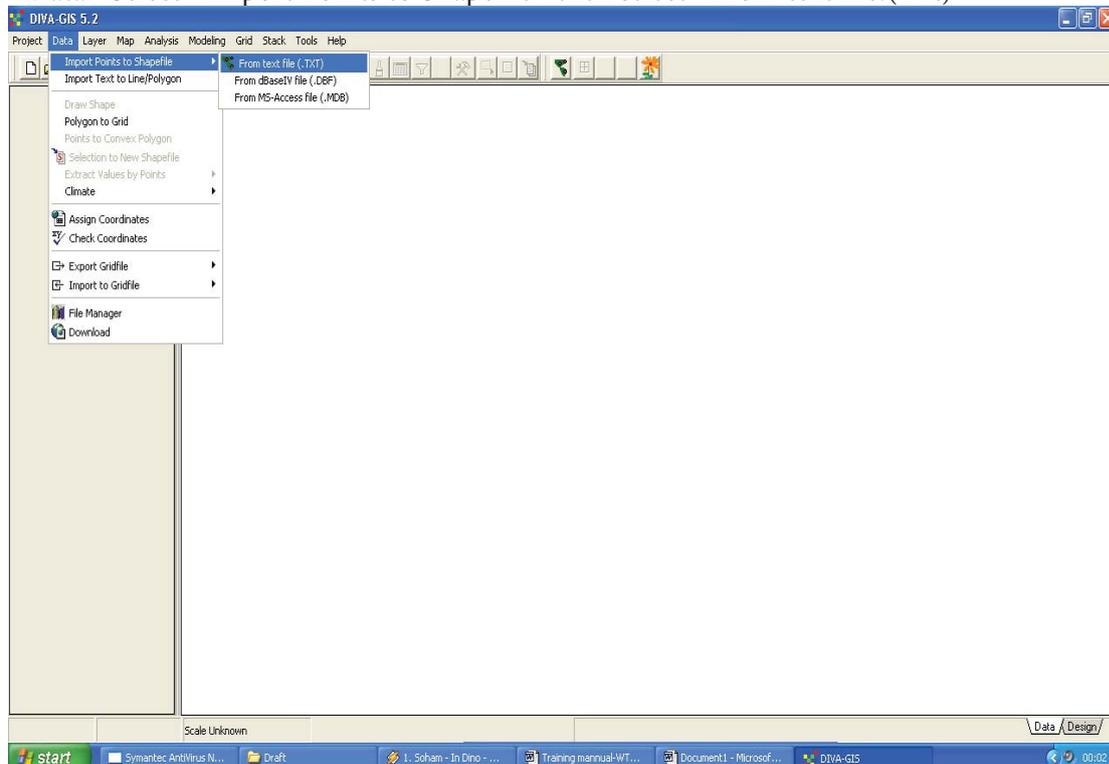


Fig 5. View of main menu of DIVA GIS showing to import the data from text saved file

**Step 2. Selecting Input File and getting points of distribution :** By clicking on <From Text File.(Txt)> you will get another small window as shown below where you need to select and click on <Input File>. By clicking on this you will be asked the location to of the text file (i.e.Xcell sheet saved as Text file). You then need to choose the text file. When you selected it automatically gives the Output file and other information. The final output of this looks like below. Then press on <Apply> icon. You will get points distributed in the menu. See the next figure

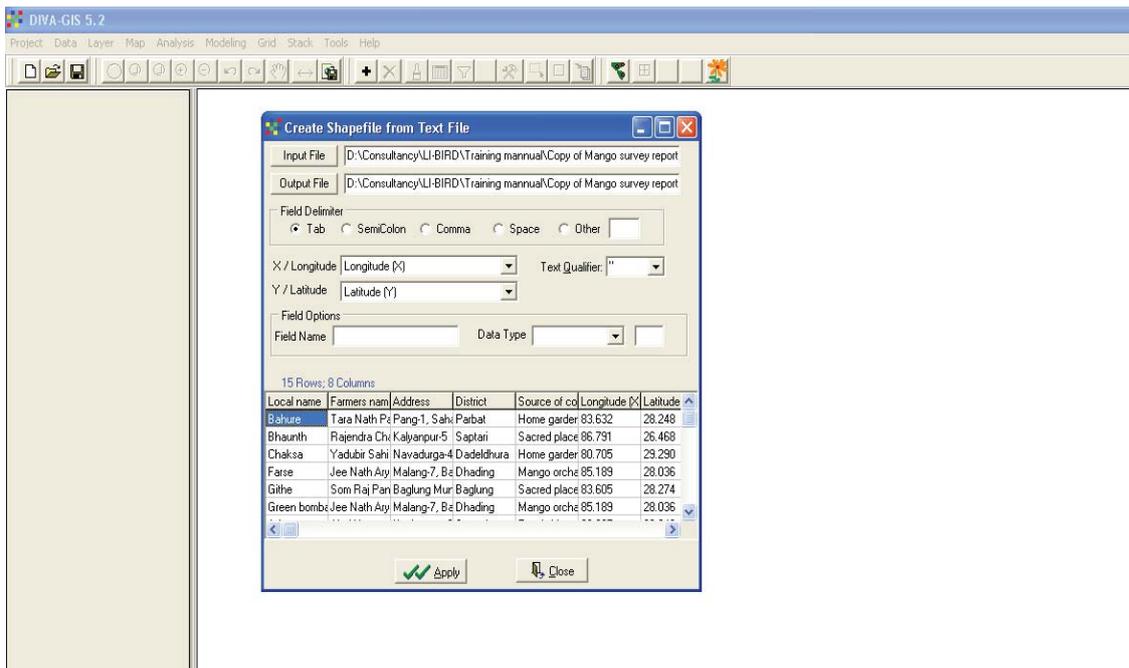


Fig 6. Applying the text file for analysis in DIVA.

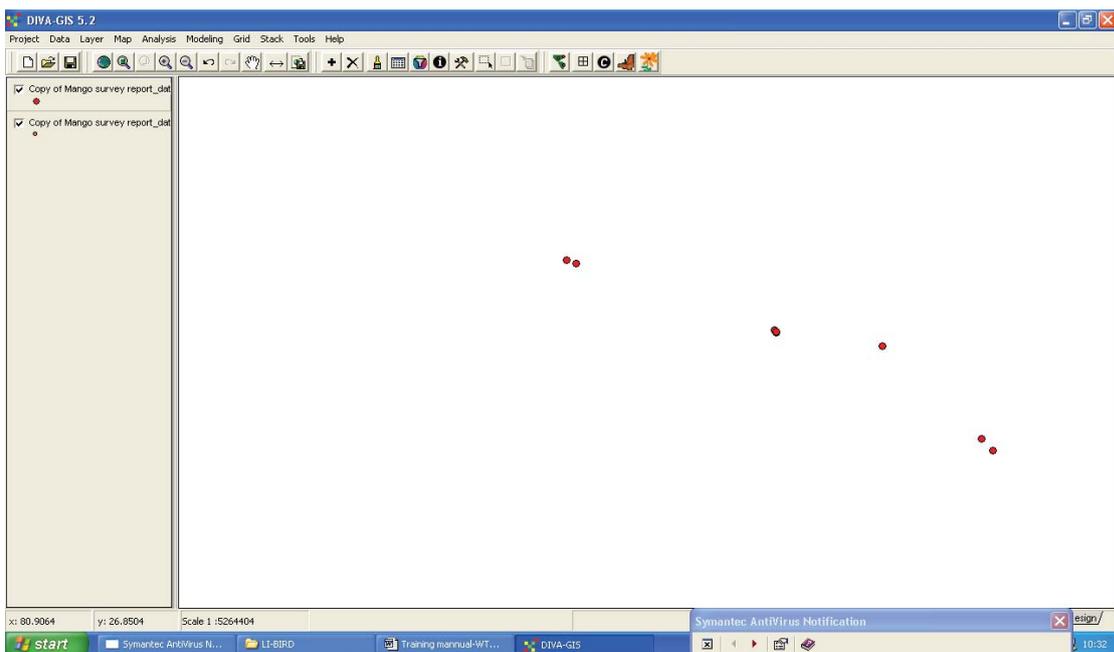


Fig 7. Results obtained after the analysis (note: red circled showing the distribution of diversity within the study range)

**Step 3. Mapping the predicted distribution :** After receiving above picture, now we select icon <Modeling> in DIVA GIS window. Select <Bioclim/Domain> another small window will be appeared as shown in figure below. Select <predict> icon. Give <Output> file name and click on <Apply> the software runs for sometime and a prediction distribution map will appear on the screen.

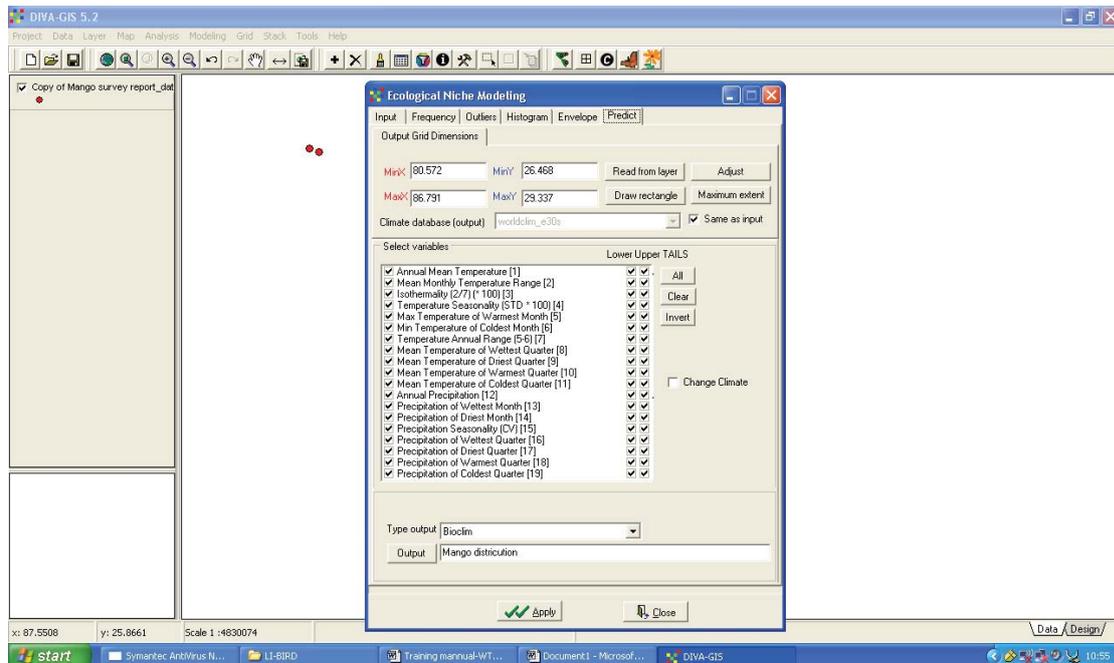


Fig 8. Preparation for developing prediction map using Bioclim menu

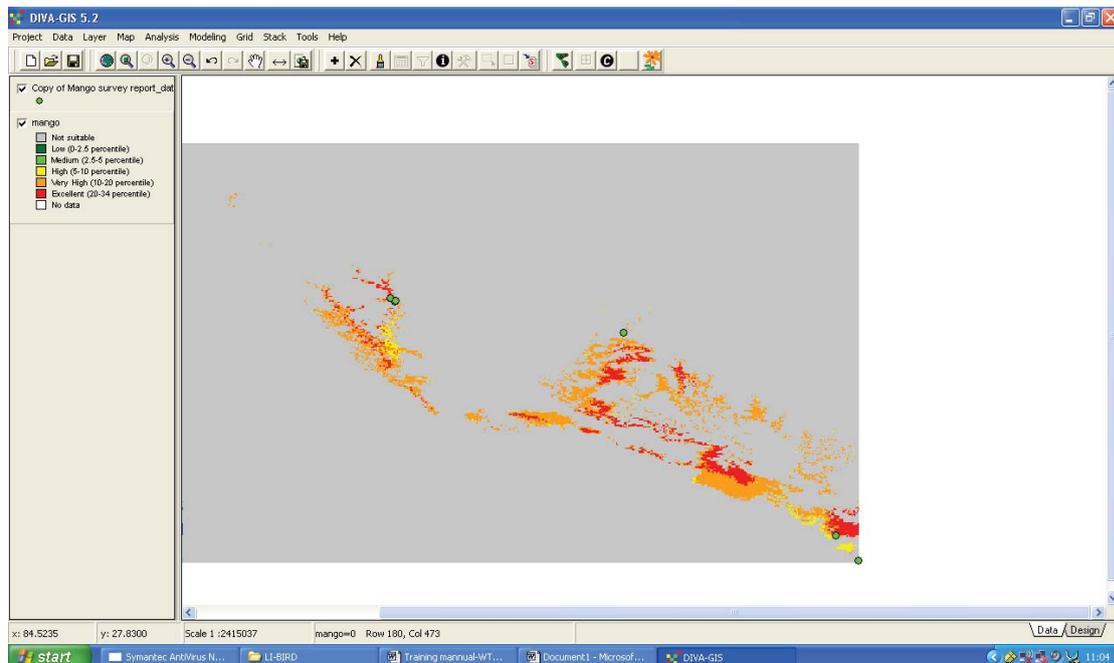


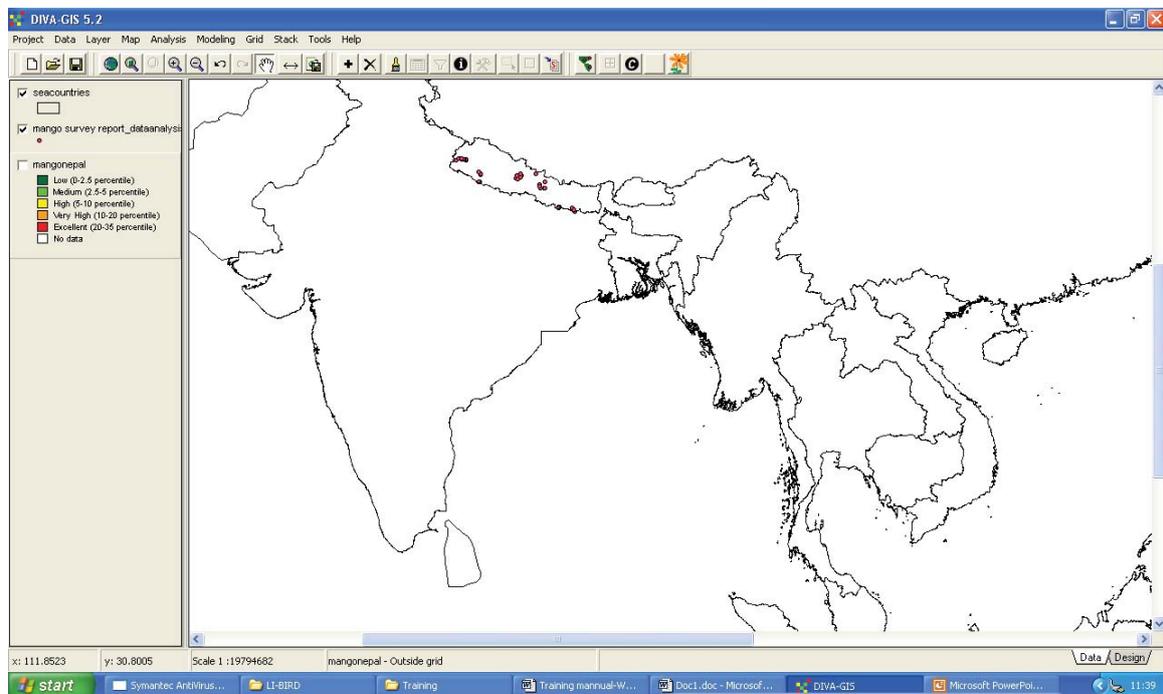
Fig 9. A predicted distribution map of mango. Note red colour represents the high (probability of occurrence) percentile.

The above map gives a distribution of Mango. At left hand side there is legend showing the percentile of prediction on the basis of colour. The red coloured zones are the predicted highest probability of

to find the species while yellow color shows the lowest probability. Now researchers can locate the survey sites for further collection of samples and information. This map also to identify potential areas for developing the conservation sites for mango. By double clicking on legend we can change the value and colour of map.

**Step 4. Preparing prediction map in political boundary :** If we desire to see the map overlaid on political boundaries where we are conducting the study then we can add layer on the existing data. For this step, we need an additional shape file of the political maps of country or geographical regions. Gridfiles and shape files for all countries of the world are available from the DIVA-GIS website.

Select the <Layer> and <Add Layer> a separate window appeared to choose the geographical areas. You have to select one shape files as per your objectives and click on <Open> you will get the your study areas as points in the political map. See below figure



*Fig 10. Species distribution points showing in political map by adding layer over it*

Now, you highlight the legend of prediction that you already analysed. See the left hand side of below figure. Now you got the final result of prediction of Mango in Nepal (as an example with few data).

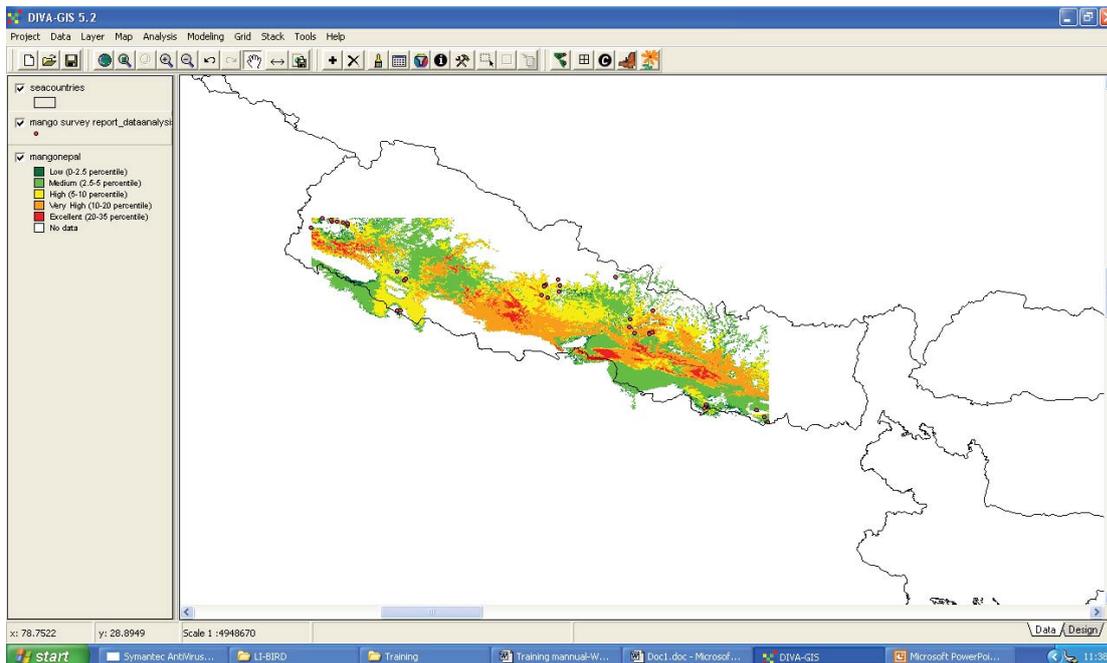


Fig 11. Predicted map of Mango

## TOOL 2: MAPPING THE DIVERSITY

By using this tool we can analyze and map the species under our objective in time and space. For this go to main menu of DIVA GIS and repeat the similar process from step 1 & 2 of mapping the predicted distribution. Here we again get the distribution of points. Now select <Analysis> then <Point to Grid> now we will get a variety options for further analysis such as <Richness>, <Estimators of Richness>, <Turnover>, <Diversity>, <Reserve selection> <Statistics> etc. Based on research objectives and given options we could do further analysis. Here we have provided some examples which are more relevant in the context of WTLCP.

### 1. RICHNESS ANALYSIS

**Concept:** The number of species (or whatever other units) observed in an area depends to some extent on the effort invested in recording there. Because a complete census is rarely feasible, in most cases only a sample of an area is surveyed. The estimate can give both a measure of the completeness of the inventory and also allow for better comparison with the species richness of other localities.

**Step 1.** For analysis the species richness go to the <Analysis> menu, choose the <Point to Grid<sup>2</sup>> and select <Richness> menu. Small window will appear and select <Options>. Again small window appear of Grid options. Select <Cell size> and make a suitable size suitable for study. Select <Draw Rectangle> and draw it over the points to cover within it the click on <ok>.

---

2      Grids are central to the analytical capabilities of DIVA-GIS. A grid divides the world into equal sized cells. It helps us to proceed with more objective comparison.

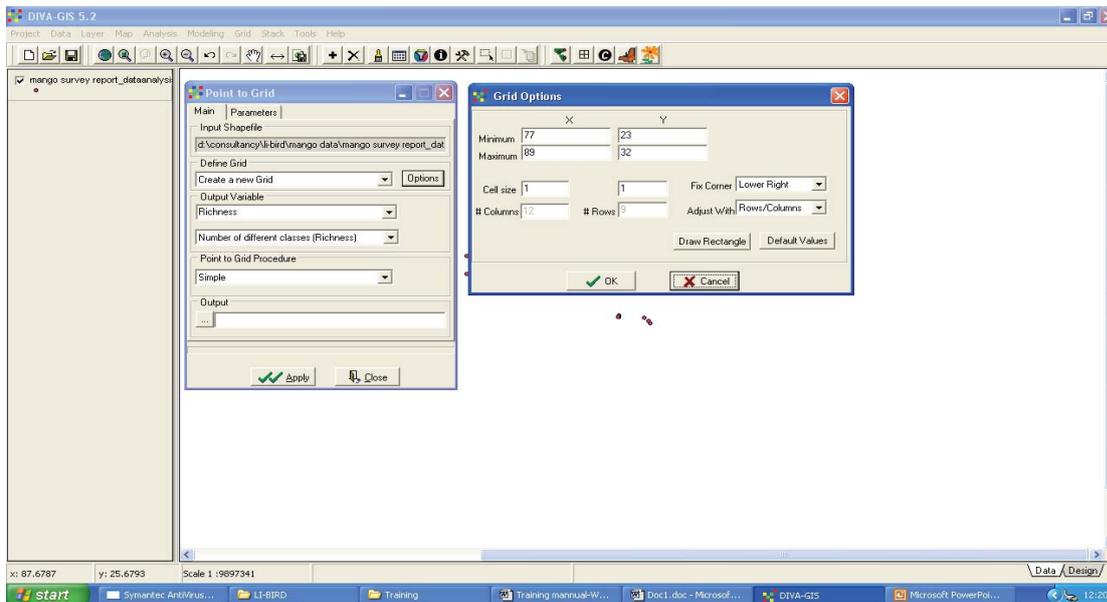


Fig 12. Showing the point grid file and grid options file menu

Return to first window and select the icon <Parameters> and give the field. This field is based on the activity purpose on which we want to measure the diversity such as Species name, Altitude, Collection source. Select one of them and click on <Apply>. You will get another saving window as Gridfile as shown in figure.

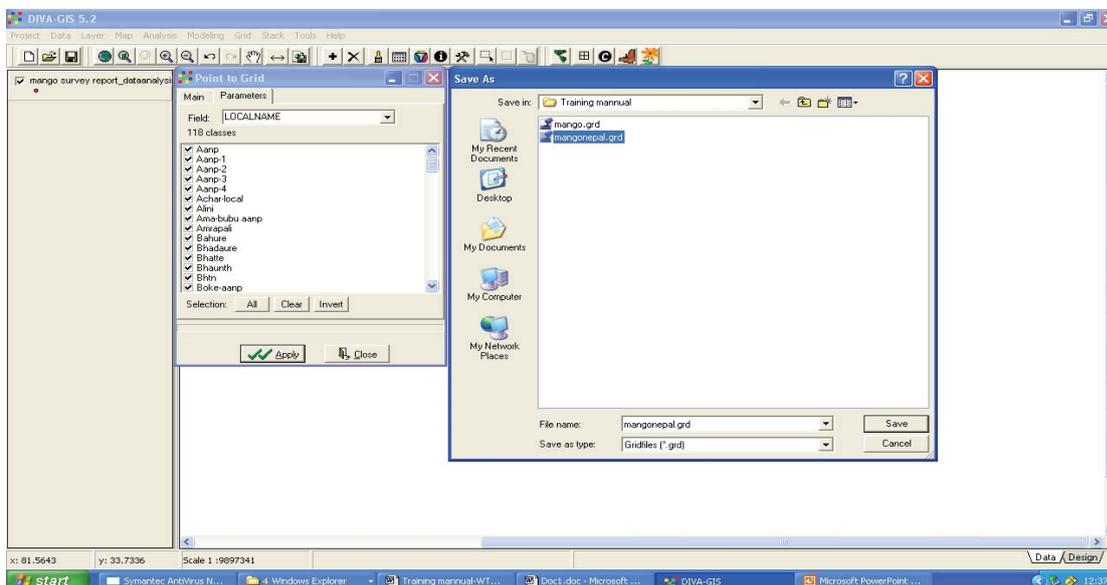
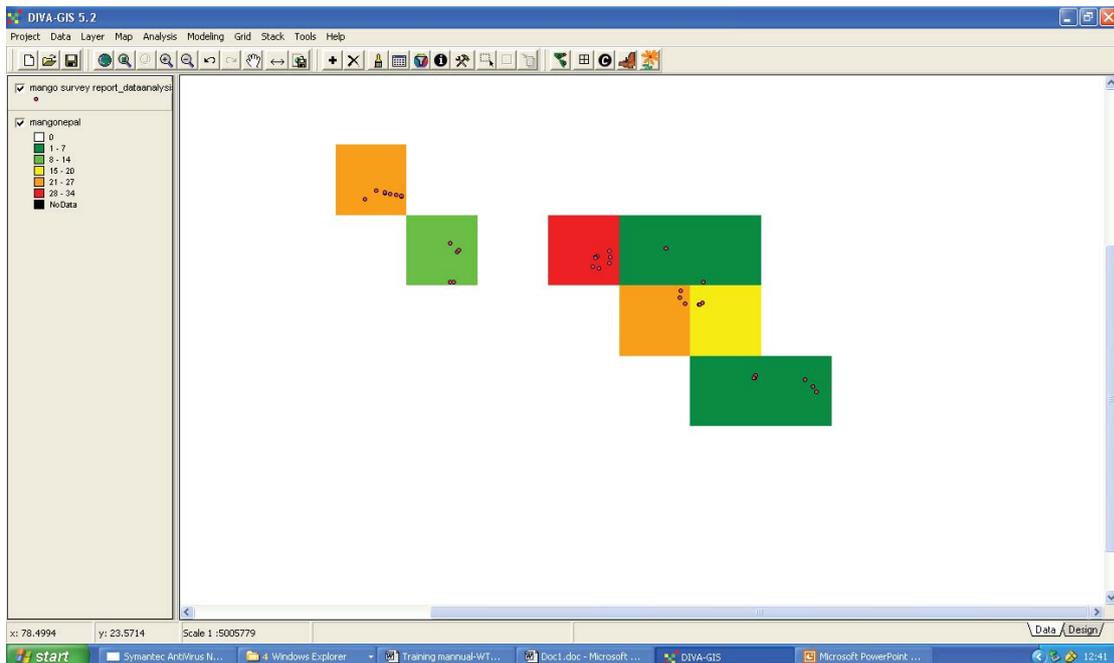


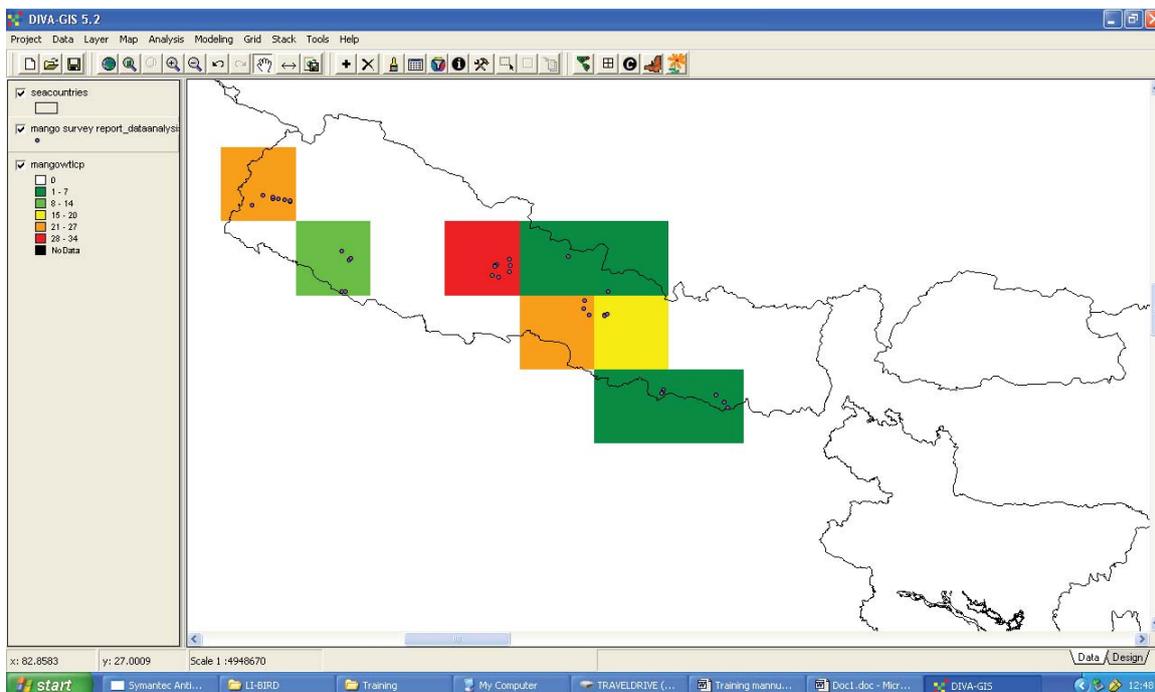
Fig 13. Saving the grid file

Select the already existing Gridfile or give another new name. Now you will get analysed map of diversity as shown in figure.



*Fig 14. Diversity analysis of Mango, red colour indicating the high richness for local mango as per the farmers name*

See the legend at left side which gives you the richness measurement values. You can follow the same procedure for the other indicators of your datasets. This map can be layered by adding layer of political map by following the Step 4 of Preparation of prediction map showing the analysis of local mango using the Shannon-Weaver diversity index. .



*Fig 15. Species richness showing in political map of Nepal*

Once you will have the crop or species diversity you have choice to go to the properties and add or remove the row. You can follow the same procedure for the crop or species diversity mapping.

## 2. TURNOVER

**Concept:** Turnover (or beta-diversity) is a measure of the rate at which species assemblages change in space. It indicates how different a number of nearby areas are. Imagine two large areas with similar numbers of species overall, but one with different species in all its grid cells and another with the same species in all its grid cells. The first area would have a high turnover, the second area a low turnover. Use the same procedure as diversity analysis the only thing you need to choose is the option of turnover when you enter the analysis menu.

**Steps:** The steps for Turnover analysis are similar with Species richness method. Instead of <Diversity> selection you will choose the <turnover> in <Analysis> main menu and remaining process is same until the final map you get it.

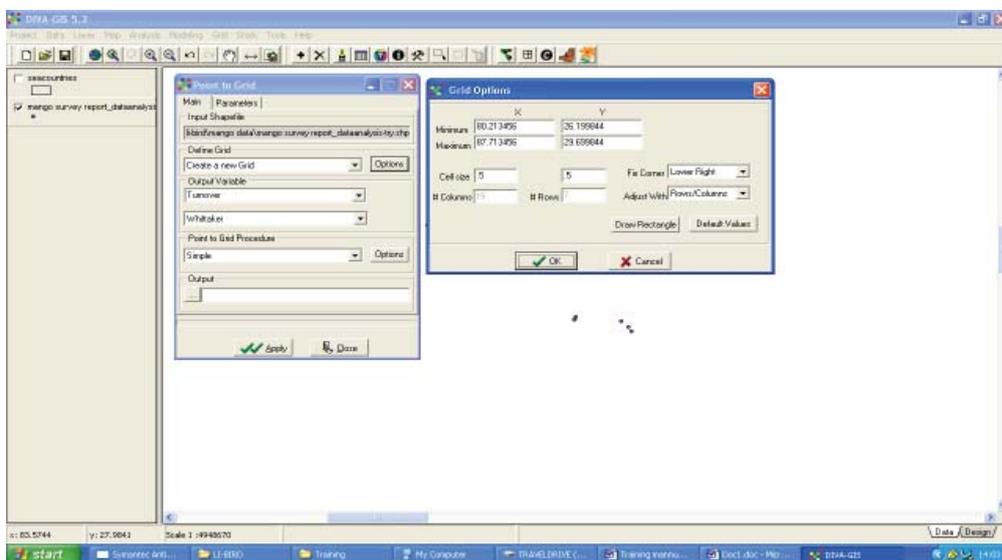


Fig 16. DIVA GIS window showing the process to initiate the turnover analysis

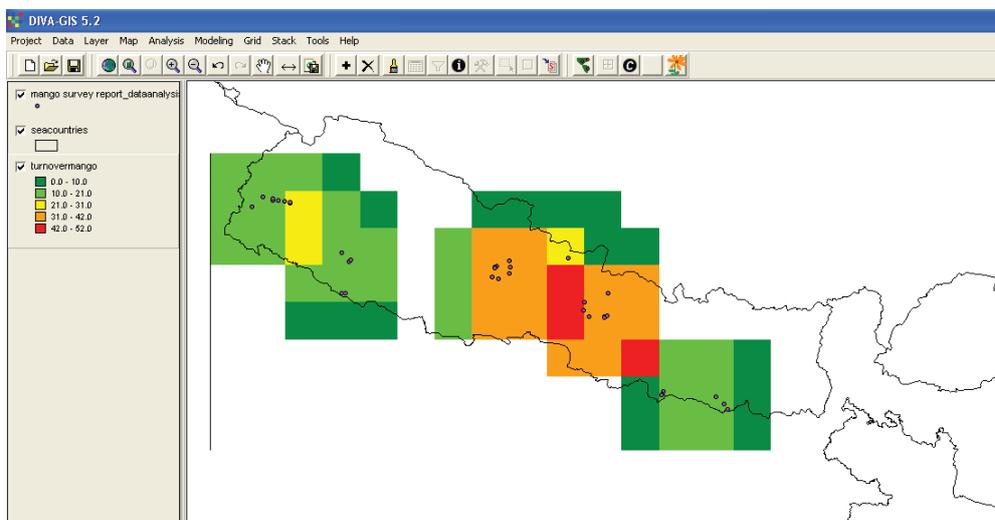


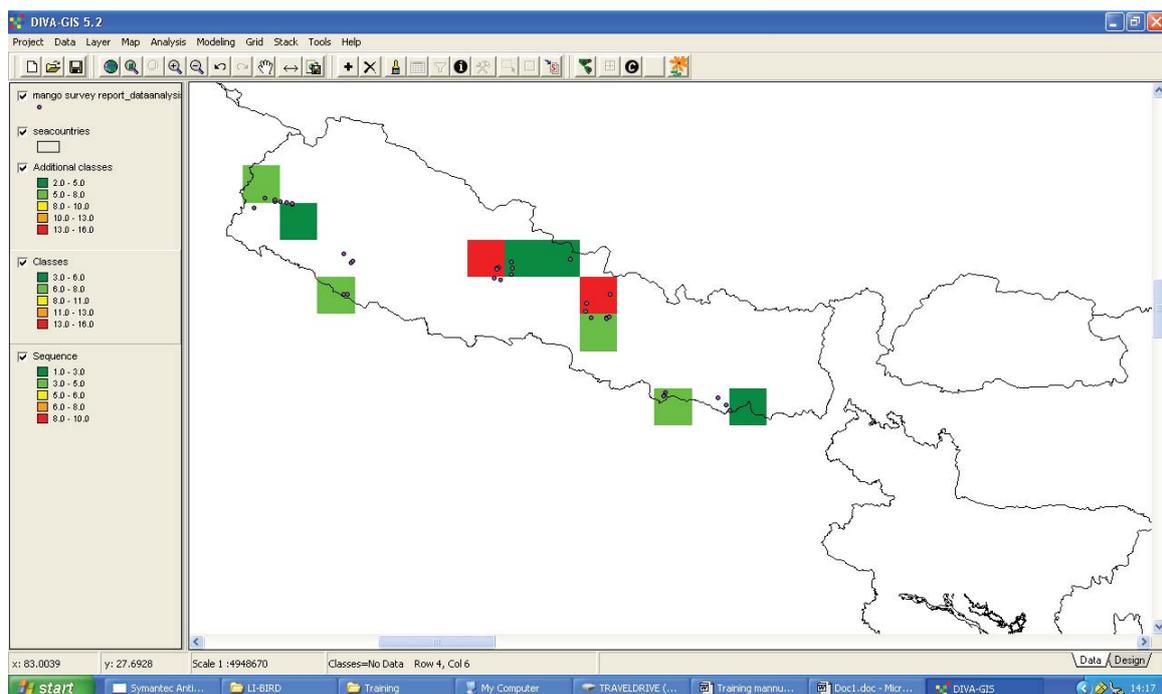
Fig 17. Results after the turnover analysis

## TOOL 3. IDENTIFYING SINK AND SOURCES

### 1. RESERVE SELECTION

**Concept:** The reserve selection procedure aims to identify sets of grid cells that are complementary to each other, i.e. to locate the grids that capture a maximum amount of diversity in as few cells as possible. Instead of using simple richness, an adjustment can be made in which rare observations get a higher weight. This reserve selection is important to identify sink and source populations.

**Steps:** The method employed is similar with that used for Richness analysis and turnover methods. Go to <Analysis> menu, choose the <point to Grid> and select <Reserve selection> menu. After entering into the reserve selection category you will find a menu name as point to grid. The main menu consists of input shapefile, define grid, output variable, point to grid and output variable. You need to go to parameter menu and select the field (could be coordinates, species, crops etc). Go to grid option and draw a rectangle. Then go to apply menu and click. After this you need to save the project, give a file name and save it. Then return to apply menu click it and you will have the outputs in your screen.



*Fig18. Results after reserve selection analysis showing the different classes and sequences*

You may want to classify grid cells based on the values they have in different grids (for example presence and absence of different species etc.) Use the cluster of points for the addition of sink.

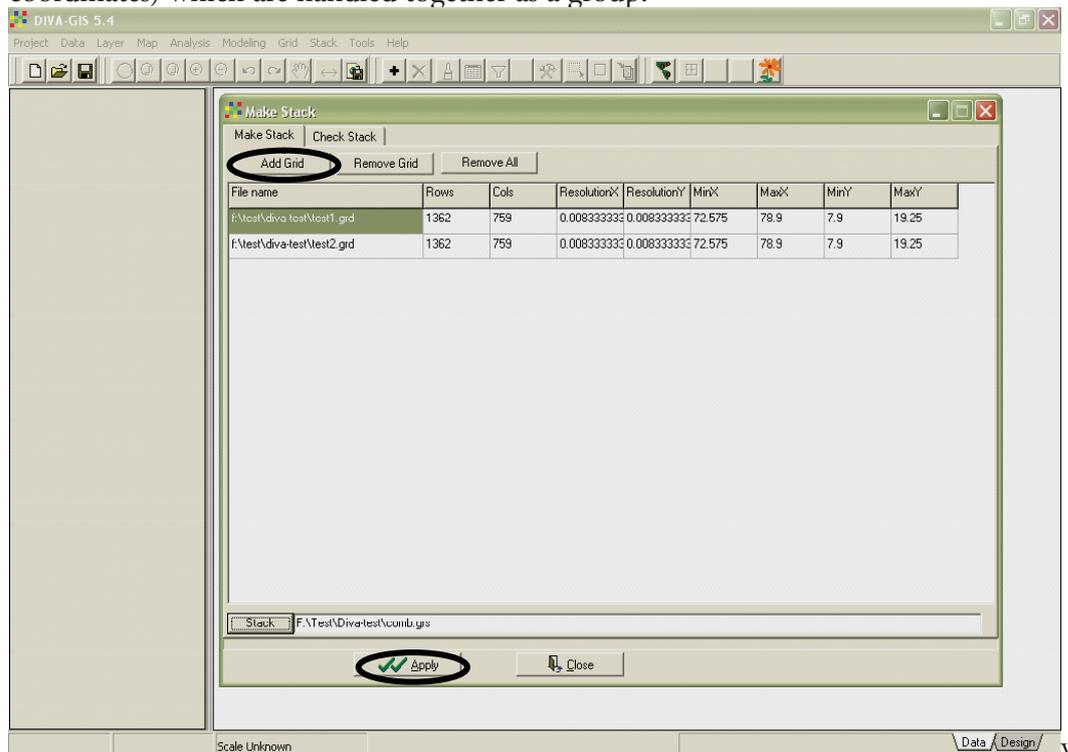
The output will show the grid based on the reserve selection. The grids show the species or crop richness and diversity captured in few colored grids. The grid with maximum number of crop/species can be treated as sink and other grid with relative less but with rich species/crop as the sources. Based on the number of records of occurrence of species in grids of different category, explicit species diversity map can be prepared. The contours of species richness of any species can be prepared which will provide a powerful input for managing the genetic resources as well as conservation planning. The pollen or seed from source could be brought and introduced in the

sink.

## 2. Clustering

You may want to classify grid cells based on the values they have in different grids, for example if the different grids represent the presence and absence of different species. You can do this in the *Cluster* menu.

Stack files has to be generated for clustering. A **stack** is a set of grids with the same dimensions (number of columns and rows, and resolution) and location (minimum and maximum x and y coordinates) which are handled together as a group.

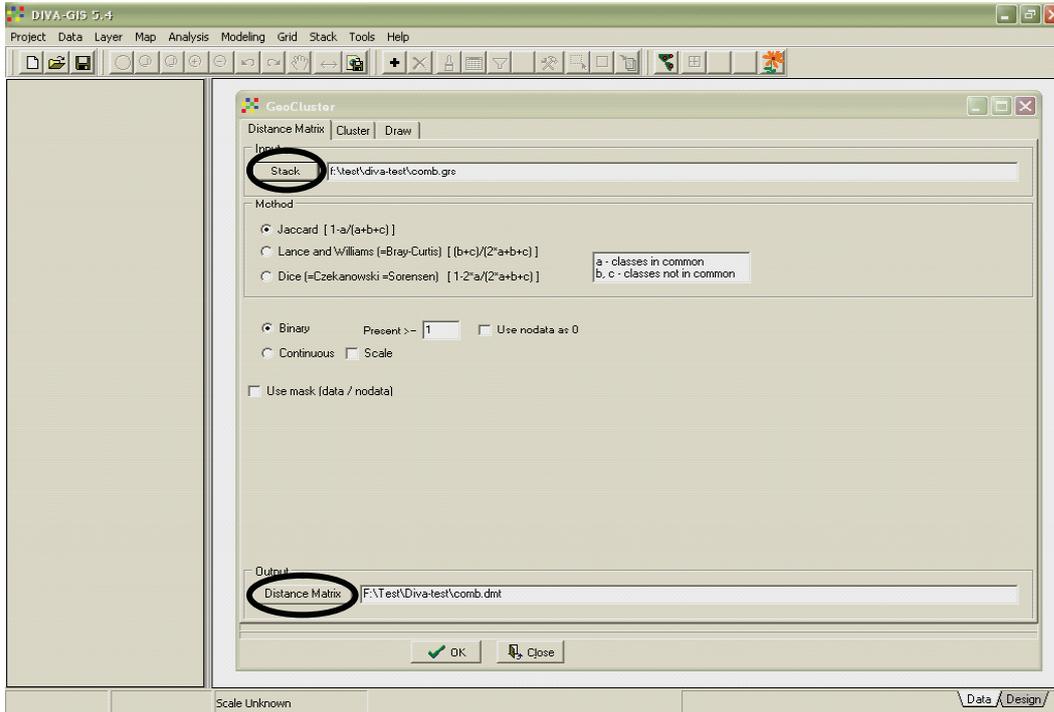


Take this stack file for clustering

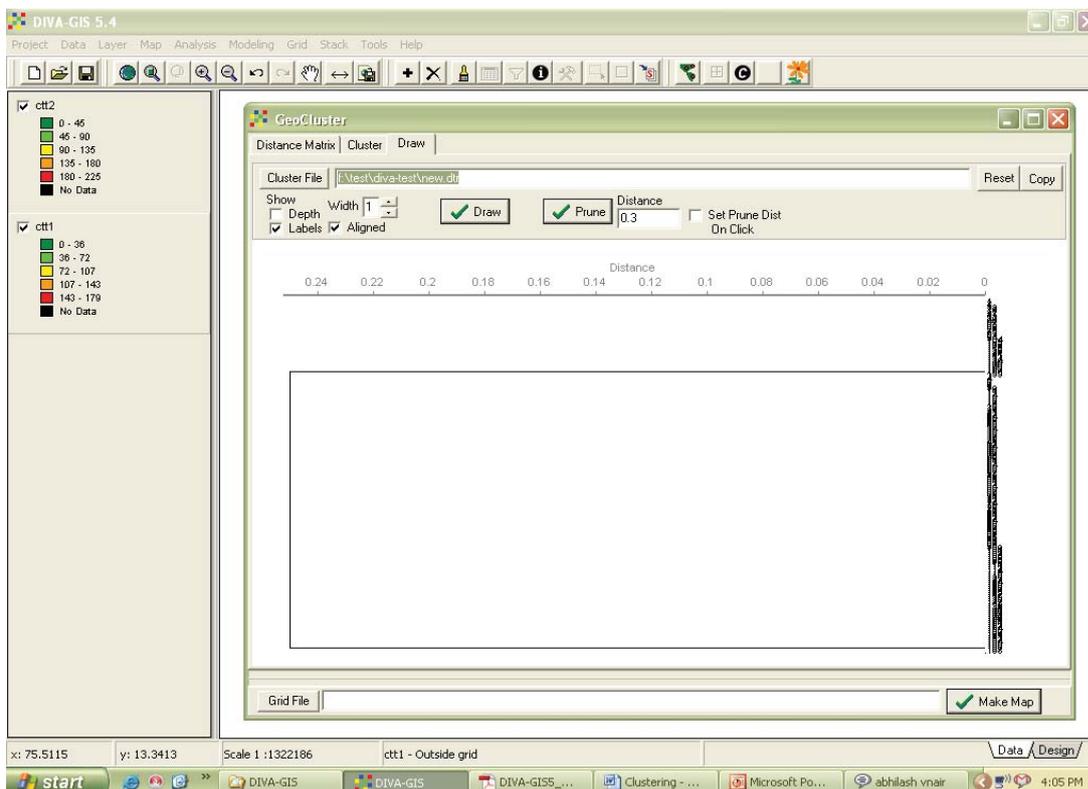
In the “Distance Matrix” tab, calculate a matrix of distances (file extension DMT) between grid cells by specifying a distance measure.

In the “Cluster” tab, use the DMT file produced above to calculate a cluster file (file extension DTR) using either the weighted or unweighted UPGMA algorithm.

Note: If the two grids do not overlap then the distance matrix generated will be empty.



In the “Draw” tab, use the DTR file you just produced to draw a dendrogram of the distance relationships among grid cells, decide at what level of distance to prune the dendrogram, and finally produce a new grid in which the values of the grid cells will correspond to which cluster in the dendrogram the cells belongs to.



## Diversity Mapping with Molecular Market Data.

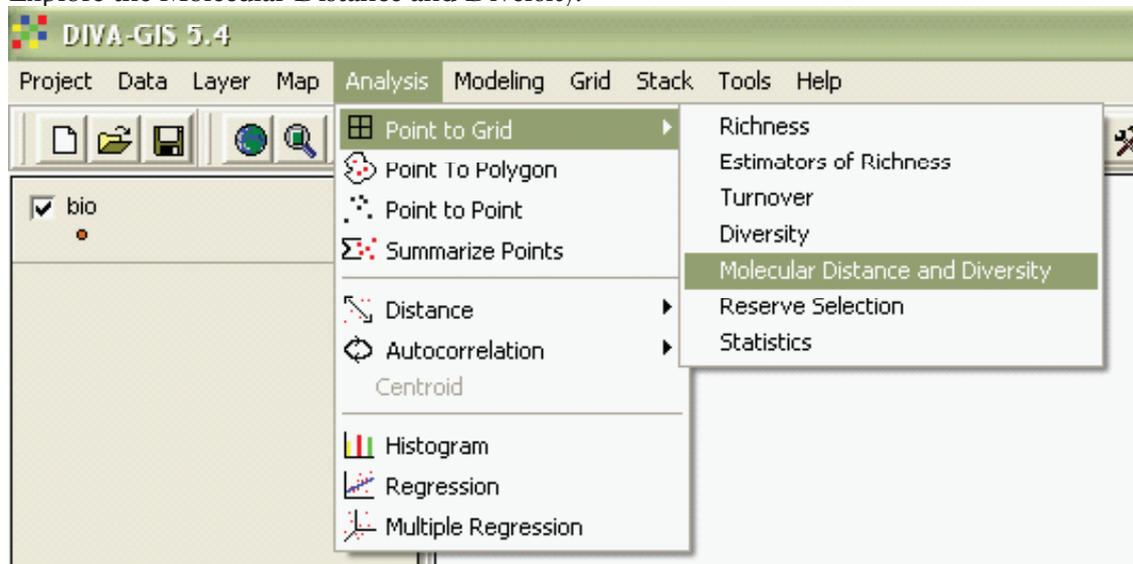
Molecular markers data associated with point localities can also be analyzed in DIVAGIS. At this moment, only absence/presence data (e.g., of bands on an electrophoretic gel) can be analyzed. Molecular distance, molecular diversity and number of haplotypes can be calculated.

The Input data should be as follows:

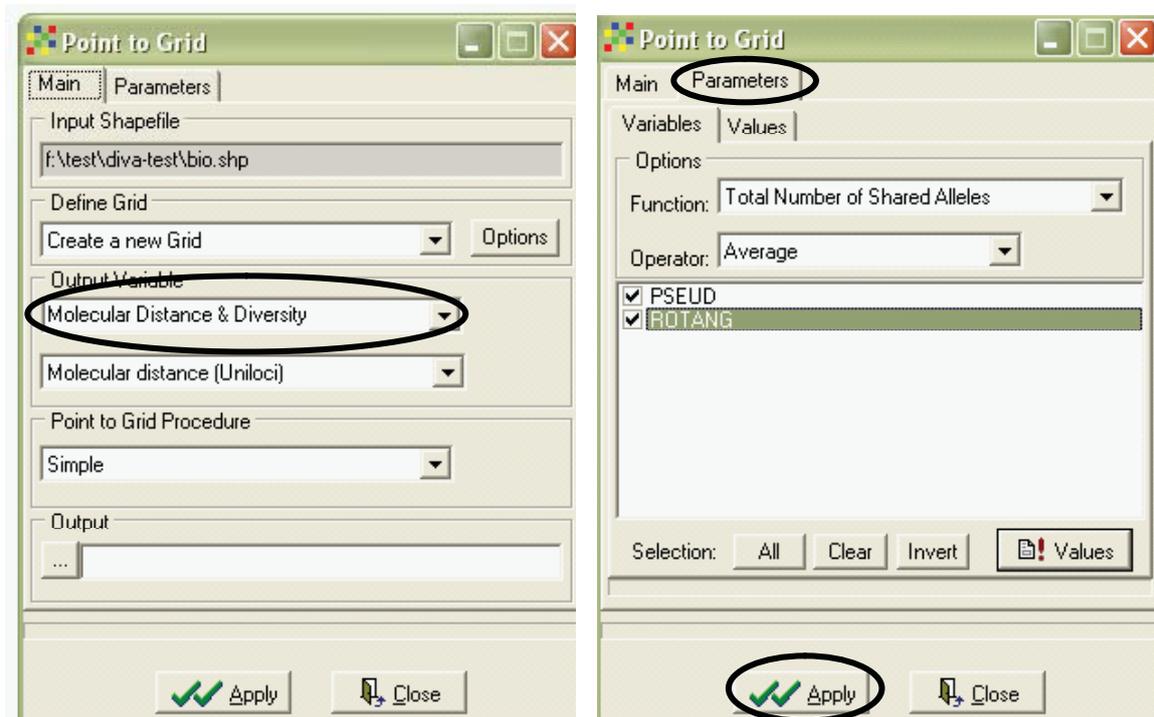
Species	X	Y	variable	Pseud	Rotang
xy	75.9792	13.0208	B1	0	0
xy	75.9708	13.0125	B2	1	1
xy	76.0042	12.9958	B3	1	1
xy	76.0542	12.9958	B4	1	1
xy	76.0708	12.9792	B5	1	1
xy	76.0625	12.9708	B6	0	1
xy	75.9958	12.9542	B7	1	0
xy	76.0458	12.9458	B8	1	0
xy	76.1125	12.9458	B9	0	0
xy	76.0458	12.9375	B10	0	0

Convert the text file to Shapefile (refer Import points to shape file).

Explore the Molecular Distance and Diversity.

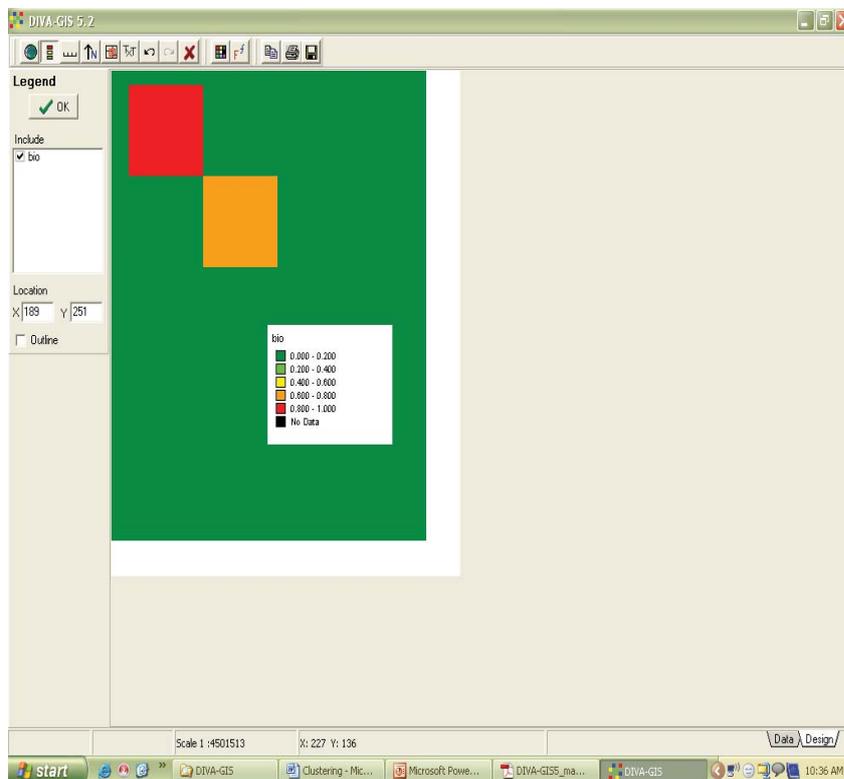


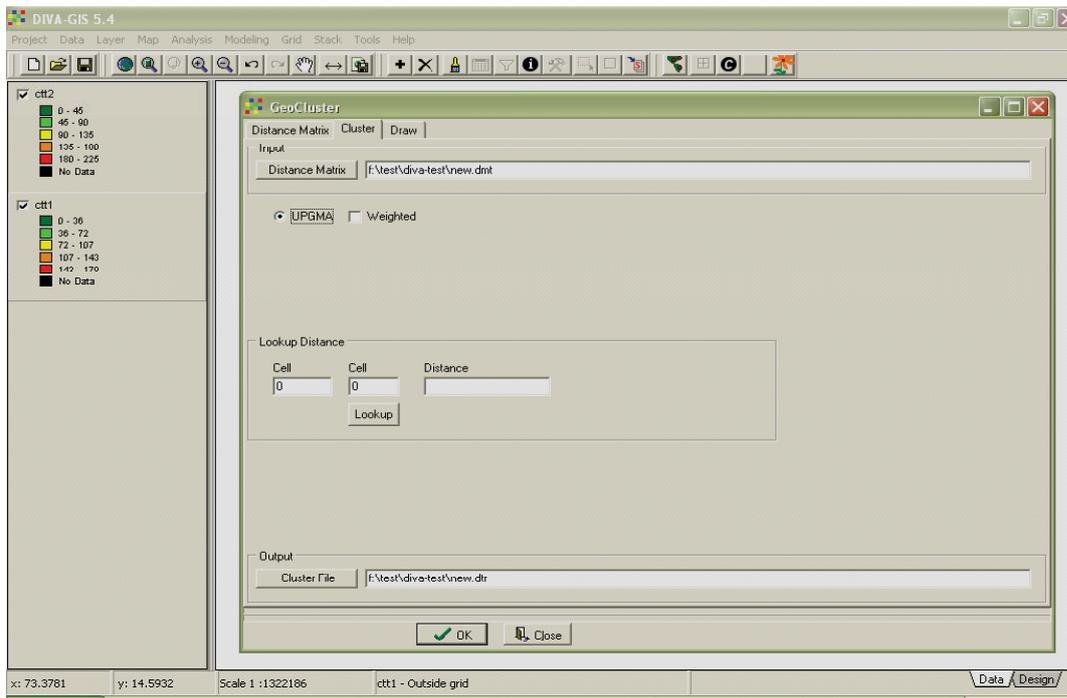
The fields that have the data must be selected using the *Parameters* window.



After selection click on the apply window.

Diva will generate the grid file that shows the presence and absence.





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