

Chapter 2

Collecting the Wild Relatives of Crops in the Tropics

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2.1 Introduction

The wild relatives of crops, particularly those most closely related to crops, are very often plants of disturbed habitats. Such habitats are road sides and areas where there is much human disturbance. The adaptation to disturbance predisposes these wild species to the agricultural environment and hence this is one reason they were domesticated. These same habitats are ones that are vulnerable to urban expansion. Many populations of the wild relatives of rice that used to exist along the roads out of Bangkok, Thailand, have gone (Morishima et al. 1996). Whereas genetic erosion of crops is generally due to replacement by new varieties or new crops, the wild relatives of crops are most vulnerable to either habitat loss or habitat fragmentation (Hammer and Teklu 2008).

Wild relatives of crops have furnished important genes of crop improvement and help increase crop productivity. Two important genes from wild rice, resistance to grassy stunt virus not found in the cultivated rice genepool and a gene for male sterility that lead the way for hybrid rice development in China, are examples of the use of crop wild relative (Vaughan and Sitch 1991). Economic analysis of rice and other crops suggests genebank genetic resources including wild relatives of crops

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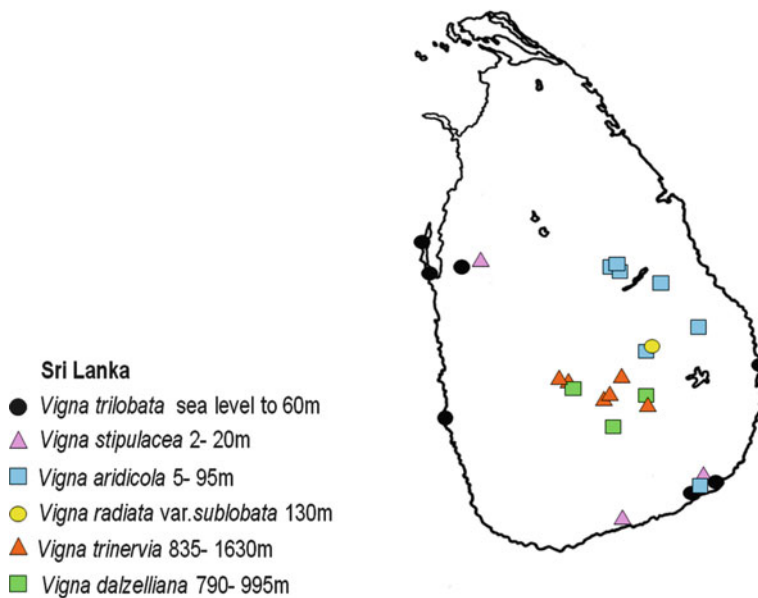


Fig. 2.1 *Vigna* species found in Sri Lanka during collecting missions (2000–2003). Prior to these missions' only two *Vigna* species were reported from Sri Lanka in the Revised Flora of Ceylon (From Tomooka et al. 2011 with permission)

directly and unambiguously have led to high economic return through increased crop production (Day Rubenstein et al. 2005; Evenson and Gollin 1997)

Scientists have been studying and collecting plant genetic resources for hundreds of years. Particularly with regards well known genera, therefore, surely we know all the species and these have now been collected. This is far from true, there remains much work to be done to collect and understand the wild species related to crops. *Vigna* is a genus that includes some of the world's most important legume crops such as cowpea and mungbean but much is still not known about the species in this genus. Prior to a comprehensive species specific collecting for wild *Vigna* in Sri Lanka only two *Vigna* species were recorded in the Revised Flora of Ceylon. After collecting six *Vigna* species are now known to grow in the country including a new *Vigna* species, *Vigna aridicola* (Fig. 2.1) (Tomooka et al. 2011). *Oryza*, the genus of rice, has not been surveyed in some areas of Latin America (Ecuador) and Asia (parts of Irian Jaya, Indonesia). Since there is still much to be learned about major crop genera for less well studied crops the gaps in our knowledge of their wild relatives remains even more incomplete.

Wild relatives of crops are still being described. A consequence is that inventory and survey is only possible for species that are known to science. Collecting is still necessary to uncover variation as yet unknown.

There are three main reasons for collecting the wild relatives of crops (von Bothmer and Seberg 1995):

- (a) To provide germplasm for taxonomic, phylogenetic and biosystematic research;
- (b) To conserve germplasm so that it may be characterized and evaluated;
- (c) To furnish germplasm for plant breeders.

Collecting germplasm is only of value when there is a repository that can handle effectively the germplasm that is collected such as a genebank or botanic garden. Even a genebank or botanic garden may not be able to handle all germplasm if there is no specialist that understands the requirements for growing and conserving specific germplasm. In planning a collecting mission it is necessary to know where the collected materials will be maintained effectively. This chapter provides a guide to collecting wild plant genetic resources. For a much more comprehensive coverage of the collecting plant genetic resources subject readers are directed to Guarino et al. 1995 and Smith et al. 2003.

2.2 International and Legal Considerations

The “Global Plan of Action for the conservation and sustainable use of Plant Genetic Resources for Food and Agriculture (PGRFA)” (GPA) was adopted by the global community in 1996. The GPA has become a road map for monitoring PGRFA conservation and use. The first of the 20 priority areas in the Global Plan of Action (and its updated version FAO 2011) is “Survey and inventorying PGRFA” and the seventh priority area is “Supporting planned and targeted collecting of PGRFA”.

The Convention on Biological Diversity (CBD), an international treaty, came into force in 1993 and now has more than 185 contracting parties. The CBD is a watershed agreement because it recognizes the sovereign rights of States over their natural resources, while having provisions for access and benefit sharing. The CBD and more recent treaty the International Treaty of Plant Genetic Resources for Food and Agriculture (ITPGRFA 2009) require that those involved with seed conservation and collecting are fully aware of the implications of these Treaties on their work.

The CBD requires that collectors of germplasm follow the correct, up-to-date, legal procedures. A set of rules for collecting germplasm has been produced to help guide collectors (FAO 1993). Among the guiding principles is the need for collectors to obtain “prior informed consent”. A second principal is clear understanding that after collecting, the germplasm is properly documented to ensure that any benefits from the use of the collected germplasm are fairly and equitably shared with the donor of that germplasm. Both of these principals reflect the CBD that countries have sovereign rights over the genetic resources within their borders.

With respect to international partnership on collecting and conserving germplasm, agreements between the parties involved are needed. These usually need to

be prepared well in advance, based on discussions among the parties involved. A helpful guide to developing access and benefit sharing agreements can be found in Cheyne (2003). Collecting conducted within a country often requires a collecting permit(s) and they need to be obtained in advance from the appropriate authorities

2.3 Preparation Phase

The main activity in the preparatory phase is making sure “all the homework is done”. This includes having very clear collecting objectives. Legal aspects of collecting are one of the preparatory activities of collecting PGRFA (see above). Another major preparatory activity is obtaining as much information as possible about the target germplasm and area where the collecting is to take place. It may be necessary to undertake a comprehensive eco-geographic survey (Maxted and Guarino 2003). Much information may be available via the internet but it is often also very worthwhile visiting herbaria with material of the target species to accumulate data that might not be available on the internet. While there may well be much information on target taxa it is important to realize that, particularly in tropical regions, there is still much that is unknown and undocumented.

Contact with local officials in the target area in advance may facilitate the collecting mission. If given advanced warning local people knowledgeable of the target area can assist during the collecting mission and provide contacts and seek local permission for collecting.

However much preparation is done, it is necessary to expect the unexpected and be as prepared as possible for potential “good” and “bad” situations. For example, finding a new species will require that herbarium specimens are prepared to enable the new species to be described; the herbarium specimens are essential for naming a new species and must be deposited in reputable herbaria. If the vehicle you are using breaks down and you need to stay out at night have the necessary preparations been taken including taking the medicine needed to prevent malaria infection? A comprehensive check list of various types of equipment that may be needed during a collecting trip is provided (Appendix 1)

2.3.1 *In the Field*

The objective of collecting for ex situ conservation is to efficiently gather and document the genetic diversity of the target species in a particular area. Hence the job of collecting in the field has two distinct components (a) sampling the plant populations and (b) documenting as thoroughly as possible the sampled population and its environment.

2.3.1.1 Sampling

Sampling in the field needs to be as comprehensive as possible and this may involve, not just collecting the seeds or vegetative parts, but also herbarium specimens, DNA samples and associated symbiotic micro-organism (ENSCONET 2009).

Specific recommendations to four questions for a basic sampling strategy have been proposed:

- (a) How many sites should be collected? About 50 populations in an eco-geographic area or during a mission;
- (b) How many plants per population should be collected? Sample about 50 plants per population;
- (c) How should samples be selected in a population? Sample individuals randomly within and among plants at each site but with attention to microenvironments if the habitat is heterogeneous;
- (d) What quantity of material should be collected per plant? Sample sufficient good quality seeds or vegetative material per plant to assure that each plant is represented in duplicates of original collections (modified from Brown and Marshall 1995).

These recommendations are guidelines and highlight some important issues. For example, when collecting wild species ensure that attention is paid to varying habitats at a site. During a collecting mission for wild *Vigna* in northern Thailand at one site four species were found in different parts of the site that varied in disturbance and shade cover (Fig. 2.2). At the end of a collecting mission samples collected will be divided for safe duplication hence it is necessary to have a sufficiently large sample of good quality material to permit duplication.

While a basic strategy about sampling is a useful guide perhaps as important is the good advice that follows:

There is no substitute for common sense, based on biological knowledge, to guide the collection of seeds of wildland plants. (Young and Young 1986)

The collector's biological knowledge of germplasm being collected depends on how well the plants being collected have previously been studied. However, generally it is known in advance if the material is inbreeding, outcrossing or vegetatively reproduces (including parthenocarpy). This information is core to determining sampling strategy because the genetic system determines the apportionment of genetic variation among and within populations. The approach to sampling will be affected by what plant parts are being collected – seeds, vegetative parts, or whole plants. In addition, there is the “common sense” elements that includes not causing irreparable damage to the population being sampled and using time wisely.

Collected germplasm needs to be as healthy and in the case of seeds fully mature on collecting. It is one thing to sample according to an appropriate design for the species of interest, however, it is also necessary to ensure that what is collected is of “good quality” and that the quality is retained until planted for rejuvenation. Therefore, timing of collecting is crucial to ensure that it is the best time for collecting

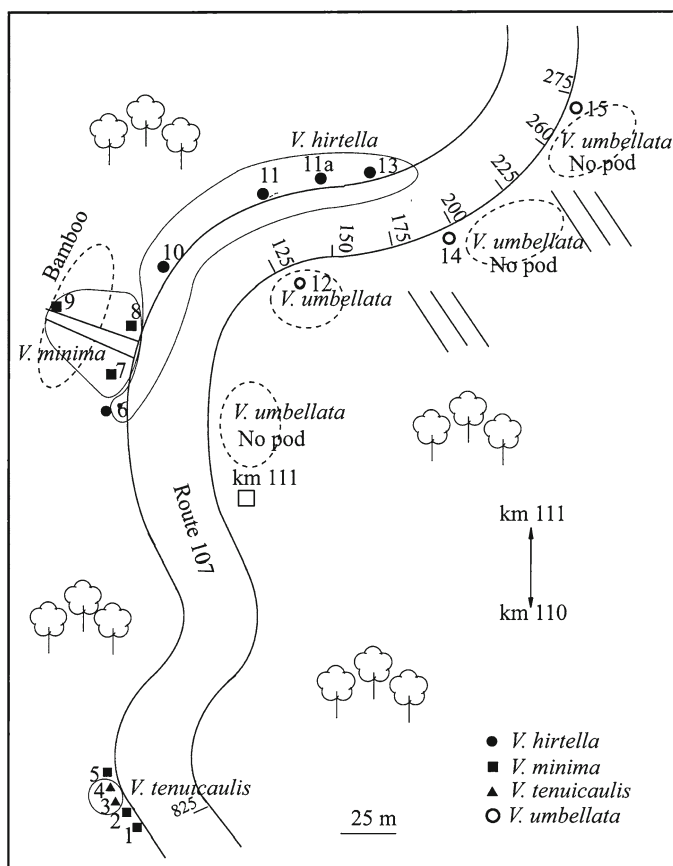


Fig. 2.2 Diversity of *Vigna* species found at one site in northern Thailand on the road between Chiang Mai and Fang. The different species were isolated by ecological conditions such as in forest (*V. minima*) or out of the forest (*V. hirtella*, *V. tenuicaulis* and *V. umbellata*) and time of flowering (*V. umbellata* flowers later than the other species at this site)

germplasm of excellent quality. The long term value of the collection will be impacted by the quality of the germplasm that is collected and the speed with which it is processed for temporary storage or planting. Issues associated with collecting seeds at the appropriate time can be found in Hay and Smith (2003).

One of the greatest constraints for sampling in the field is time. Initially it is necessary to canvass an area to find target plants and then determine their distribution in a locality and any obvious ecological or phenotypic variation in the sampling area. There is the need to balance the need for surveying a location, taking samples, including herbarium specimens, and documentation of these samples within and between populations. The approach taken will depend on multiple factors but the nature of the germplasm being collected will be the main determinate. For example, sampling large vegetative propagating plants may be more time consuming than

collecting seeds of herbaceous species. For examples of sampling different types of species see Guarino et al. (1995) and for palms Dransfield (1986) and Martin (2004).

Techniques for collecting herbarium specimens have been described by various authors (e.g. Martin 2004; Zippel et al. 2010). Herbarium specimens are particularly important for the less well known or indeed unknown wild species that are being collected.

There are now simple methods available for collecting DNA in the field such as using Whatman FTA® paper (Vitha and Yoder 2005). If a collector on return to base has access to molecular biology laboratory that can analyze DNA this may enable a collection to be rapidly characterized. Such molecular characterization can be helpful in guiding future collecting (Fay 2003).

When collecting some species, it is necessary also to consider collecting associated organisms. For example, it should be standard procedure to collect root and/or stem nodules from leguminous species (Date 1995). Sampling or photographing the organisms associated with species may be helpful to provide information on pests, diseases or pollinators

2.3.1.2 Documentation

Unfortunately early and very important germplasm collections in genebanks were undertaken before the “computer age”. At that time passport data was generally limited to a few notes in a field note book. The most important passport data is exact location, that today should if possible be recorded with a Global Positioning System (GPS), as well as the “unique” number – passport number that identifies the collected germplasm. *The only unique identifier of a collected population is the passport number* and it is essential that this remains associated with the sample at all times. Much germplasm is currently duplicated in the global genebank system; however, because germplasm is exchanged using genebank number that is not associated with original passport number the extent of duplication is unknown.

There are many examples of plant collecting data sheets in the literature (e.g. Zippel et al. 2010) a comprehensive review of data to record in the field is provided by Moss and Guarino (1995). The data to collect in the field as follows:

- (a) A preliminary description of the locality this can be elaborated later after consulting maps;
- (b) GPS location;
- (c) Habitat (site) data including landform, slope, dominant plant species, degree of disturbance etc.
- (d) Information on species/populations collected such as color, height, relative abundance, diseases observed etc.;
- (e) Ethnobotanical information if travelling with knowledgeable residents of the area.

Collectors can combine information gathered by visual observation onto data sheets with GPS readings and (digital) photographs of locality, site, plants and plant

parts. Additionally simple sketch maps of a collecting site and locality that includes, if possible, a permanent marker such as road mile marker. These simple sketch maps are particularly helpful in the situation where recollecting/resurveying is conducted, for example, to monitor genetic erosion. An example of a collecting site map redrawn from a sketch maps is shown (Fig. 2.2). An example of the type of passport data that should be collected when sampling wild species is provided (Appendix 2).

Combining passport data with other available databases such as climatic data and soil type data can lead to learning much about germplasm. When samples have been characterized and evaluated, characterization and evaluation databases can also be used to analyze collections. A simple example for Japan is shown that illustrates combining location of collecting site with seed colour (Fig. 2.3) (Takeya et al. 2009). Such information can be displayed on Google Earth® (Takeya et al. 2009). DIVA-GIS, a freely available software, is available that allows analysis of genebank and herbarium databases to elucidate genetic, ecological and geographic patterns in the distribution of crops and wild species (Hijmans et al. 2001). Analysis of passport data can provide insights into which collected samples should be targeted for specific evaluation. While cold tolerance would not be expected to be associated with germplasm growing close to the equator that is where the cold tolerant rice variety called Silewah from Indonesia was found. The clue to why this rice variety was cold tolerant was the altitude it was growing, 1,300 m.

Of particular help in ensuring that collected germplasm is used after collecting is to obtain ethnobotanical information from local informants. In rural areas people tend to have knowledge about a wide variety of both cultivated and wild plant species. This information can provide valuable insights into the potential wider use of the germplasm. Information related to gathering ethnobotanical data can be found in Cotton (1996) and Martin (2004).

2.3.2 Post Field Collection

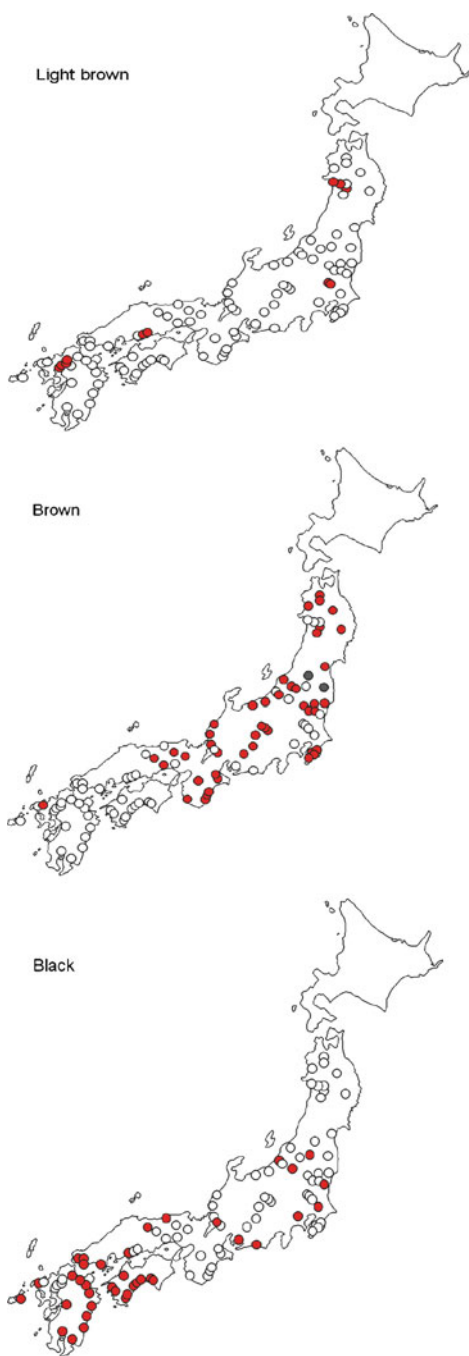
On returning to base there are a series of activities that need to be undertaken by the collector(s). These include securing the collection, writing a report on the collecting mission and ensuring all data that was collected is entered into the appropriate database.

How the collection is secured will depend on the material collected. Quarantine clearances maybe required for material that crosses borders. The collection should be duplicated for safety at separate locations. The collection should also be put into appropriate storage conditions or planted to ensure the collection maintains its genetic integrity. Herbarium materials, DNA samples, soil and/or rhizobia samples should be deposited appropriately.

A trip report is needed to summarize all aspects of the trip and should include:

- The objectives of the mission;
- The environment of the target region;

Fig. 2.3 Variation in seed color of *Glycine soja* across Japan shows clinal variation for *black* and *brown* seed color but not for light brown seed color (Modified from Takeya et al. 2009)



- Logistic and scientific planning;
- Details of the execution of the mission (timing, itinerary, sampling strategy and collecting technique)
- General observations during the mission (such as appropriateness of timing, ethnobotanical information);
- Summary of mission results (table of collected material and where it was found);
- Details of where collected materials will be deposited;
- Recommendations for follow-up;
- Acknowledgments;
- Relevant literature (after Toll and Moss [1995](#)).

The mission report should be completed as soon as possible on return to base and distributed to all concerned and also deposited in a library where it will be catalogued for future reference.

The collector is responsible for ensuring passport data is entered into databases accurately and that the passport database is accessible to others and also backed up safely.

2.4 Concluding Comments

There are many types of collecting mission such as species specific or multi-species, locally organized or centrally organized, single visit or multiple visits. The approach taken by the collectors may vary but central to a collecting mission is to conduct it safely with respect to the collecting team and plants/populations being collected. A single chapter on this topic can only present some general guidelines for more specifics consult the references below and search the internet with respect to the specific wild germplasm of interest.

Acknowledgements The authors acknowledge Dr. Worapa Seehalak, Kasesart University at Kampaeng Sean, Thailand, for Fig. [2.2](#).

Appendix 1

A comprehensive list of equipment that may be needed during a collecting mission (Modified from Engels et al. 1995; Way 2003)

Collecting equipment	Two spare tyres, pump and pressure gauge
Mobile phone with relevant telephone numbers in target area	Puncture repair kit and plentiful supply of repair patches
Maps	Heavy duty jack and power levers
Compass	Chain and nylon rope
Altimeter	Shovel and pick
Global Positioning system	
Plant identification guides	Camping equipment
Micro-scale magnifier	Tents and accessories
Binoculars	Tarpaulin and ropes
Secateurs/hand pruners/tree pruners	Sleeping bags
Pocket knife	Mosquito netting
Leather gloves, plastic gloves (for aquatics)	Camp beds and air mattresses
Collecting bags (large, medium and small)	Small folding table and chairs
Paper bags (large, medium and small)	Battery operated hand torches and spare batteries
Plastic bags (large and small)	Cooking stove and spare fuel
Tags for numbering specimens	Matches
Herbarium press and straps	Cooking pots and utensils
Newspapers	Candles and/or lamp
Blotters or absorbent paper	Water containers (small and large)
Portable stove and stand	
Passport data sheets	Medical supplies
Camera with sufficient memory, spare camera batteries	Water-purifying tablets
Clip board, field notebook and, pencils	Insect-repellent cream
Indelible pens	Antihistamine cream
Cardboard boxes	Antiseptic cream and wipes
Adhesive tape	Antibiotic tablets and cream
String	Fungal infection remedies
Sealable container for seed drying	Antacid tablets
Silica gel	Sachets of oral hydration solution
Whatman FTA® paper for collecting DNA	Eyewash
Mortar and Parafilm® M for extracting DNA onto Whatman FTA® paper	Lip salve
Insect proof clothing	Aspirin, paracetamol or other pain-killer
	Anti malarial tablets for both prophylaxis and treatment
	Snakebite sera
Vehicle accessories	Disposable hypodermic syringes
Basic set of spare parts	Cotton wool
Toolkit	Splints
Spare petrol can, large funnel and plastic tubing	Bandages and plasters
	Scissors
	Cigarettes (heat from burning cigarette is an effective way to remove leeches)

Appendix 2

Appendix 2.

Names and Addresses of collectors in Local language

Names and Addresses of collectors in English

Basic data

Scientific name		Date	dd	mm	yy
Local name		Collecting no.			
Plant no. codes		Site no.			

Location

Latitude					Longitude		
Geocode method	GPS	DGPS	Estimate	Map	Google Earth		
Land holder	(In local language)				(In English)		
Village	(In local language)				(In English)		
Nearest town	(In local language)				(In English)		
State / Country	(In local language)				(In English)		

Site

Topography	Hill	Plain	Mountains			
Altitude (m)						
Altitude method	Altimeter	DEM	GPS	Estimate	Map	
Land use						
Soil characteristics*				Geology*		
Climate*				Aspect / Slope		

Habitat

Associated vegetation type		Forest	Bushes	Cultivated	Grassland	Other specify ()	
Associated plants specific		Dominant sp.					
		Other spp.					
Shading (%)	Heaby	Medium	Light	Open (none)			
Degree of disturbance	High	Medium	Low	None			
State	Vegetative	Flowering	Mature	Past maturity			
Status	Wild	Weedy	Cultivated	Mixed			
Introgression	Yes (extent)			No			
Disease assessment	Leaf				Pod / seed		
Pest assessment	Leaf				Pod / seed		

* Information may be obtained from maps

Names and Addresses of collectors in Local language

Names and Addresses of collectors in English

Plant characteristics

Leaf pub.	High	Medium	Low	None	
Viable seeds/pod (10)					
Ovules/pod(10)					
Flower color					
Phenology status	More flowers than fruits		More fruits than flowers		
	Only fruits		Fruits already dispersed		
Frequency	Rare	Few	Frequent	Very frequent	Highly frequent
Species characters					

Population characteristics

Population variation			
Population size (m ²)		Sampling area (m ²)	
Number of plants sampled		Seed collected from	Plant Ground Both

Check list

Photo numbers	Site	Habitat	Plants
Herbarium voucher	Yes / No, Number:		
Other samples taken (e.g. rhizobium / soil)	Yes / No, What was sample?		
Sampling methods	Random	Regular	Transect (linear)
	Core of population	Edge of population	Other
Plant no. codes		Collecting no.	
Ethnobotanical information			

Sketch Map

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<http://www.springer.com/978-1-4614-3775-8>

Conservation of Tropical Plant Species

Normah, M.N.; Chin, H.F.; Reed, B.M. (Eds.)

2013, XIV, 538 p., Hardcover

ISBN: 978-1-4614-3775-8