Germplasm Exploration and Collecting: Principles and Practices

K.C. Bhatt
Division of Plant Exploration and Germplasm Collection, ICAR-NBPGR, New Delhi

There are over a quarter million plant species available on the earth. It is difficult to predict which of these species would be required to fulfill future needs. Hence, more the diversity is conserved and made available for future use, better the chances of fulfilling future demand. Generally, the exploration for germplasm collecting is conducted for desired genetic material (primitive and advanced) or capturing maximum variability to use in the crop improvement programme and also to study the genetic variability within the crops. Depending on the needs and objectives, crop specific or multi-crop exploration missions can be executed for capturing maximum variability from a wide agro-ecological/phyto-geographical region as well as for fulfilling the needs of breeders and conservation. In case of multi-crop/species collecting mission, a region is targeted and an attempt is made to sample as much as possible of the diversity of as many species as possible. Usually, they are planned when no systematic collecting in the area has been conducted before and/or when the area is difficult to reach and future visits are therefore unlikely.

For any collecting mission, the under-mentioned steps including prioritization of area/species, thorough gap analysis studies needs to be followed before execution.

- Planning
- Making contacts with local research organization
- Gathering equipment and preparation
- Meeting with local researchers in area to be surveyed
- Sorting out of collected samples
- Reporting to the Headquarters
- Preparation and publication of reports
- Distribution/ conservation of collected samples

Planning of exploration mission: principles and practices

Depending upon the priorities, gathering a prior knowledge of the crops/species before launching a mission is important. The areas to be explored and crops/ species to be collected should be prioritized after thorough gap analysis based on information from different sources including database/ National Genebank status. Priority should be given to the areas which have been inadequately covered/surveyed. Because, genetic variation in genepools is associated with variation in environmental factors, ecological conditions which are not represented are given high priority along with missing genotypes and taxa. The explorer should be well-versed with the nature and extent of diversity and breeding behaviour of the crop/species to be collected and plan well in advance to facilitate the preparations of the proposed missions except those to be carried out under special situations like rescue collecting. Visit to herbaria is essential to know the range of distribution, localities, diversity pattern and period of collection particularly for wild species. Collaborating institute/University should be communicated to depute a scientist well in advance with details of preparations/tour itinerary/tentative programme. Information on topography, climatic conditions, vegetation, crops in cultivation and their maturity, etc. needs to be gathered to finalize the itinerary of collecting mission. Besides, explorers should also establish local contacts especially at grass root level to seek the social, cultural, ethnic and other information of interest.

Tour itinerary: A tentative tour itinerary should be drawn up at an early stage of the planning, showing the main target areas (or even precise localities) to be visited within the overall target region, the roads/tracks to be followed and the proposed timings of each visit. The mode of transport should also be specified. Prior permission should be obtained from the concerned authorities for collecting in protected (biosphere reserves, sanctuaries, national parks) and restricted areas (border areas/some states in NEH region). Collecting/innerline permits may also

require specific areas to be mentioned. Letters of introduction to local government officials are often useful, and their preparation will require some rough idea of the itinerary to be followed. Maps will clearly be needed in planning the itinerary, but local contacts are essential for advice on the feasibility of following particular routes at different times of the year (Hawkes, 1980).

***Period of collecting*:** For seed producing cultivated crops/species, exploration should be undertaken when crops/species are physiologically mature and ready for harvest. In case of species with shattering nature because ripe seeds are generally quickly shed (crop wild relatives/wild species) are usually not available to the collector once this happens (though in some cases some collecting from the ground may be possible). For such species, missions are executed rather earlier (7-10 days depending on crop/ species) before their maturity. Further, longer duration (2-3 weeks) mission and repeat visits are suggested for collection of wild species. For vegetatively propagated crops/species, the targeted areas should be surveyed first for identification and marking of elite types at the time of flowering/fruiting and subsequently the collections are made at appropriate time. For conducting explorations within the country, the period should be of at least 10-15 working days and more than a month when conducted in foreign countries.

***Team composition*:** The collecting team should be familiar with basics of agriculture/plant genetic resources to meet the objective of the mission. Team consisting of 2-3 members including a collaborator and need-based local-aid be formed preferably a botanist/ breeder as leader. Area and route of explorations should be fine-tuned in consultation with the subject experts of local organizations as soon as the team reaches to the starting point keeping in view the targeted species and areas of the proposed mission.

***Items and equipments required*:** As per the nature of the germplasm to be collected (fruit/ seed/ vegetative propagule/ in vitro/ live plants) and the area(s) to be explored, the required items and equipments needs to be gathered before execution of exploration.

List of items and equipments for collecting

Survey / collecting items	Global Positioning System (GPS), digital camera with additional memory card, binocular, magnifying glasses, handheld microscope, digital Vernier calliper and portable balance, Haversack/ kitbag, seed envelopes, cloth bags, polythene bags, aluminium & tag labels, drying sheets, old newspapers, plant press, moss, rubber bands, packing tape, *sutli* (thick and thin), secateurs, scissors, knife, digger, torch light, measuring tape, passport data book, field note book, pencil, ballpoint pen and permanent marker.
Reference material	Regional/ national flora, digital herbarium, lap-top and accessories, list of local names of plants, road-map, vegetation/climate map, list of rest-houses/ lodges, hotels, resting/ stay places and list of local contacts (phone, fax, e-mail).
First Aid-Box	Anti-malaria pills, anti-allergen tablets, pain killers, anti- amoebic and anti-diarrhoeal tablets, mosquito repellent, antifungal/ antibacterial/ antiseptic creams or lotions, cotton-packs, band-aid, dettol, dressing gauze, water-purifying tablets, etc.

Collecting strategies and sampling method

At the actual collecting sites, there is a need to apply sampling method which will ensure that the genetic diversity of crop/species represented in the sample collected. For sampling in any region/site, inaccessible areas of valleys, isolated hills, villages at the edge of deserts, forests, mountains and isolated coastal belts which may hold rich genetic diversity, potential/ trait-specific germplasm and wild relatives should be approached for collecting. While for cultivated species, farmers' field, backyard/ kitchen garden, threshing yard, farm store, local village market, etc. are ideal sites to collect the germplasm. Drier tracts (vs. humid), un-irrigated areas (vs. irrigated),

valleys (vs. hills) should be approached to capture maximum diversity and sites having stress situations viz. saline habitat, un-irrigated/ drought conditions, desert (cold and hot), flood prone areas should be identified as target areas for collection of trait-specific material. For biotic stress tolerant material, hot-spot areas should be visited to collect healthy plants in fields where severe pest damage is evident. The frequency of sampling (number of samples per site) should be decided based on on-the-spot observations on the variability available. In general, more sites per target area are preferred to sample the targeted species rather than sampling from a few sites.

***Sampling method*:** While collecting seeds, the explorer should keep in mind the required quantity of material to be sampled for long-term conservation (2,000 and 4,000 seeds for self- and cross-pollinated crops, respectively) besides meeting the requirement of characterization, evaluation and related studies. The optimum sample size per site would be the number of plants required to obtain, with 95% certainty, all the alleles at a random locus occurring in the target population with frequency greater than 0.05 (Hawkes, 1976; Marshall and Brown, 1975). In case of species with extremely small-sized seeds, low seed-set, asynchronous maturity and low seed viability, care should be taken to collect adequate sample size. Such species have been increasingly focused on from 1998 onwards. Of nearly 320 species of CWR considered a priority for collection (Arora and Nayar, 1984). In case of extremely variable populations, one can either make larger samples (bulking), or take as sub-samples if observed interesting variants, and be given separate collecting numbers. In general, random sampling should be followed by collecting single spike/panicle or fruit/berry/pod from at least 50 plants along a number of transects throughout the field (Hawkes, 1976, 1980) to obtain a representative and adequate sample. In a situation when wild population with few individuals occur, one should collect from all the plants so as to make the representative sample from that site. In case of certain wild and semi-domesticated species occurring in small pocket with scattered populations (treated as sampling site) having specific use/traits, the seed should be bulked. However one should not deplete the populations of farmers' planting stocks or wild species, or remove significant genetic variation. In case of large tubers, only a portion, e.g. head or proximal ends in yams, crown or tuber in taro and other aroids should be collected. In case of scion collection, the sample size will depend upon the number of rootstocks available but not less than ten per sample. In case of cuttings and rooted suckers (e.g. grapes, ornamentals, passion fruits, black pepper, beetle vine, banana, cardamom, etc.) 15-20 cuttings may be sufficient. Material with dubious identity or unidentified material, vernacular name should be collected along with herbarium specimen and photographs for authentication. The detailed guidelines for preparation and processing herbarium specimen should be followed as per Jain and Rao (1977).

***Recording of information*:** Information on both the essential and optional fields needs to be recorded in the passport data sheet at the site of the collection. However, in any circumstances, the explorer should not leave the information blank on essential fields namely sample labelling; sample identification; sampling information and collecting sites. Information on genetic erosion should also be gathered particularly on the depletion of landraces cultivated over the time and the reasons for their loss in general using a unstructured format. Observations on the distribution pattern and frequency status of crop wild relatives, rare, endangered and threatened species of PGR importance should be recorded for their sustainable management. In addition, on-site observations on some important quantitative and qualitative characters/traits should also be recorded using descriptors developed. Ethnobotanical observations and new uses of plants, especially those collected from tribal dominated tracts, are currently recorded as a database which would be available for reference in future collections

***Post collection handling*:** The extraction and cleaning of seed should be done preferably on the same day or immediately after completing the expedition and process for their drying under shade/ sun/ controlled conditions. The seeds with short longevity (recalcitrant type) should be processed at the earliest and care should be taken during threshing/ cleaning to avoid damage. In a situation, when delay in processing is anticipated, all precautions should be taken to maintain its viability. The observations on variability parameters on fruit/ pod/ seed should be recorded along with photographs for report writing, documentation and publication. The clean and dried material

should be kept in the envelopes with proper label specifying its botanical name and collector number. One set of the material along with passport data should be sent for accessioning, conservation (LTS/MTS) and another set be sent to the collaborating institute for initial seed increase (if required), maintenance, characterization and evaluation. The vegetatively propagated material should be sent for establishment/ maintenance in field genebank or at suitable site. The material for *in vitro* and cryo-genebank should be handed over to the concerned curators. The elite material, if any, should be studied in detail to generate supporting information as well as for validation of the known trait(s) for its registration with NBPGR.

Do's and Don'ts

Do's

- Get acquainted with the International Code of Conduct for Plant Germplasm Collecting and Transfer of FAO (1993).
- Always keep a route map of the target area with list of important places and the distance covered during travel to facilitate report writing.
- Before entering into a forest take the help of forest guards to have forehand knowledge of possible dangers in the target area. If needed, help of a gunman is taken during survey in dense forest.
- Explain the purpose and get consent from the farmers for collecting germplasm**.**
- Keep important telephone numbers of concerned officers including district authorities, hospitals, dispensaries and police station.
- Keep your identity card and a certificate from Head of Organization for proposed mission.
- Honour social customs of local inhabitants of the target area.
- While talking and discussing with ladies, be polite and respectful to them
- After day's collection and before retiring to bed, have a glance at your equipments, passport data and collected material for need-based updating.

Don'ts

- Do not provide lift to strangers in your vehicle under any pretext.
- Do not indulge in unnecessary discussion related to politics, religion and local beliefs with the local people.
- Do not make false promises with donors.
- Do not plan the expedition during important festivals and peak election campaign in the target area.
- Do not enter any house for seed collection in absence of male members of the family.
- Do not eat unknown wild fruits since some of them may be toxic or internally infected.
- Do not collect the seed in large quantities from any household if the farmers wish so.
- Over-collecting of the genetic diversity with similar attributes should be avoided to save time and energy in collection and evaluation and to save space in the genebank.

References

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NATIONAL BUREAU OF PLANT GENETIC RESOURCES, NEW DELHI -110012

PASSPORT DATA SHEET

Date.....Collector's No.....Accession No..... Botanical Name..... Common Name (English)..... Crop/Vern. Name..... Cultivar name.....Region Explored..... Village/Block..... District.....State.....Latitude….……^{0}N Longitude….……^{0}E Altitude……..m Temp..... Rainfall	
COLLECTION SITE	1. Natural wild 2.Disturbed wild 3.Farmer's field 4.Threshing yard 5.Fallow 6.Farm store 7.Market 8.Garden 9.Institute 10.....
)GICAL STATUS	1.Wild 2.Weed 3.Landrace 4.Primitive cultivar 5.Breeder's line
FREQUENCY	1.Abundant 2.Frequent 3.Occassional 4.Rare
MATERIAL	1. Seeds 2.Fruits 3.Inflorescence 4.Roots 5.Tubers 6.Rhizomes 7. Suckers 8.Live plants 9.Herbarium 10……..
BREEDING SYSTEM	1.Self-pollinated 2.Cross-pollinated 3.Vegetatively propagated
SAMPLE TYPE	1.Population 2.Pure line 3.Individual plant
SAMPLE METHOD	1.Bulk 2.Random 3.Selective (non-random)
HABITAT	1. Cultivated 2.Disturbed 3.Partly disturbed 4.Rangeland 5…….
DISEASE SYMPTOMS	1.Susceptible 2.Mildly susceptible 3.Tolerant 4.Resistant 5.Immune
INSECT/ PEST/ NEMATODE INFECTION	1. Mild 2.Moderate 3. High
CULTURAL PRACTICE SEASON	1. Irrigated 2.Rainfed 3.Arid 4.Wet 5..... 1.Kharif 2.Rabi 3.Spring-summer 4.Perennial type
ASSOCIATED FLORA	1. Sole 2.Mixed with……
SOIL COLOUR	1. Black 2.Yellow 3.Red 4.Brown 5……..
SOIL TEXTURE	1.Sandy 2.Sandy loam 3.Loam 4.Silt loam 5.Clay 6.Silt
TOPOGRAPHY	1.Swamp 2.Flood plain 3.Level 4.Undulating 5.Hilly dissected 6.Steeply dissected 7.Mountainous 8.Valley
AGRONOMIC SCORE	1.V poor 2.Poor 3.Average 4.Good 5.V good
ETHNOBOTANICAL USES	
PART(S)	1.Stem 2. Leaf 3.Root 4. Fruit 5.Flower 6.Whole plant 7.Seed 8.Others
KIND	1.Food 2.Medicine 3.Fibre 4.Timber 5.Fodder 6.Fuel 7.Insecticide/ Pesticide 8.Others
HOW USED	.....
INFORMANT(S)	1.Local Vaidya 2.Housewife 3.Old folk 4.Graziers /Shepherds 5.Others
PHOTOGRAPH	1.Colour/Video
FARMER'S/ DONOR'S NAME.....ETHNIC GROUP.....	
ADDRESS	
PLANT CHARACTERISTICS/ USES ADDL. NOTES	