

# Introduction to Procedures for Preparing Herbarium Specimens of Cultivated Plants

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## Introduction

The herbarium is collection of dried plant specimens identified and grouped on the basis of similarities and using a standard system of classification. In the development of herbarium specimen, the plant material is collected, processed and preserved that all parts are represented and full information on locality, habitat and plant characters are recorded. Herbarium is traditionally associated with gardens, teaching and research institutions and their holdings are valuable source for study of natural build up, distribution and loss of diversity. Herbarium, in relation to plant genetic resources activities can be used for identification of specimens, locating source areas for survey and collection and study of diversity in natural conditions. The present paper briefs about introduction to procedures for preparing herbarium specimens of cultivated plants with special reference to procedures grossly followed at NHCP, ICAR-NBPGR, New Delhi.

## Role of herbarium

The primary purpose of herbarium is to present a picture by means of representative herbarium specimens of the composition of flora; to record the distribution of all plants and to classify them in a system which will depict their phylogenetic history becomes the ultimate goal. Herbarium studies going towards achieving this goal are:

- a) Herbarium material and taxonomic studies associated with it form the basis for grouping species and classifying them and classification system of plants are primarily based on herbarium material.
- b) Herbarium studies provide a fairly close approximation to random sampling for the total species area. In biosystematic studies, the task of sampling widely scattered populations great; the herbarium provide a convenient means by which one can justify or otherwise the extrapolations being made from sample populations.
- c) Study of relationship based on new evidences, a considerable amount of details of characters is preserved after drying. Besides, macro morphological traits, the micro morphological traits and depositions are comparable with those from fresh specimens. In addition, epidermal features (hairs, trichomes) pollen grains remains practically the same, chemical constituents such as phenolics, alkaloids, also remains same as in living plants.
- d) Additional use of herbarium in various studies is a repository for voucher specimens of studies on cytology, chemistry, breeding etc., which are the only means where by a subsequent worker can check the material from previous research.
- e) The status of plant species about their area of availability, variability pattern flowering/fruiting time, status regarding rare/endangered/endemic types, etc. gathered from herbaria may help the explorers and researchers in setting their targets.

## Management of herbarium

The broad outlines of procedure adopted in the management of a herbarium comprise collection and processing of plants, identification and incorporation

### *Collection and processing of plants*

The herbarium adds to its collections by way of following methods:

- collections made by staff/explorers
- material included as gifts or received in exchange

- retention of part of the material received for identification
- voucher samples deposited at the end of specific researches.

Plants are collected at random along a zigzag/diagonal transect, using a strong knife, a pair of pruning shears or secateurs. A collecting pick is used to dig out underground parts. Polythene bags or vascula (metal collecting cans) are also used to collect the material. Delicate plants that tend to wilt fast may be collected and pressed right in the field between blotters. A tag with collector number and date is attached to individual plants while collecting.

The specimen should consist of one or more whole plants complete with roots, stem, leaves, flowers and if possible fruits. Normally the minimum number of specimens in a collection should be two but if the plants are small an attempt is made to collect several (at least 5-6) specimens of the same plant from the same locality. Where the plants are shrubs or trees, it is necessary to select a portion as a representative specimen the size of which is normally determined by the size of the mounting sheet. When flowers and fruits are too large to be pressed with the leaves, they are kept separately in polythene bags or boxes. Extra specimens of the same collection can be made depending on the requirement and duplicates when collected may be used for exchange/studies. All additional specimens notes as collector's name and collection number, place and date of collection, and features of the plant not shown by the dried specimen as soil types, associated flora, distribution, etc. should be recorded in the field note book.

Using blotters or newspapers each specimen is neatly arranged with all plant parts well spread. Care should be taken to avoid overlapping of plant parts. A corrugated sheet is placed between the blotters and the specimen if specimen is to be dried using artificial drying method. The whole bundle is placed in a plant press and tied using a strong belt or strap. Some points to be noted while pressing include the following:

- Large specimens should be pruned and folded to the required size giving V or N shape.
- A few leaves should be pressed with lower side up; and flowers split open if gamopetalous.
- A large specimen may be split into two or three parts and pressed separately but with the same number.
- Plants with very large leaves may be represented by a leaf (split or folded) along with some important parts like ligules in bamboos, leaf sheaths in the Araceae, etc. Special notes or outline features may also be enclosed.
- Underground parts that lose their identity on drying may be depicted by photographs/outlines.

**Drying methods:** The press containing specimens is left tied for 24 hours (sweating period). When first opened, all plant parts are neatly rearranged on fresh blotters. The bundle with specimens is again put in the press for 24-36 hours. Same process is repeated for about a week or until complete drying is effected. Thick or succulent material require more time. Used blotters may be recycled after drying. Plant specimens are usually processed right in the field or at least the same day. Left overnight they tend to deteriorate.

In an alternative method, specimens after processing for 24 hours in field press are rearranged and placed over a heat source (drying chambers, ovens, stoves, etc.). The temperature is adjusted to 46-50°C. The heated air passing through the corrugated sheets placed between the blotters dries the specimens. Time taken for drying varies and this procedure may be modified based on type of material. Special techniques are adopted using micro-wave succulents or fleshy material for effective drying.

**Preservation:** The collected specimens are poisoned immediately after collection or at the time of mounting usually by:

- Chemical treatment using alcohol is particularly done to avoid the microbial damage under high humidity conditions in the field.

- Dipping the whole plant in a saturated solution of mercuric chloride in ethyl alcohol and processing the same for drying. For mounted specimens a brush may be used.
- Pouring 10% formalin over specimens contained in the press and placed in an airtight polythene bag.

Specimens are fumigated when infestation of pests is high inside the cupboards. Volatile poisonous liquids like carbon disulphide, methyl bromide, carbon tetrachloride are also used sometimes. Specimens should remain in airtight condition for 3-4 days. These chemicals pose a health hazards and should be handled with great care. Deep freezing technique has been found most effective and user friendly and used to control of pests/pathogens. Bundles of specimens (15-20) packed in double layered polythene bags are tightly sealed and exposed to -200C for 48-72 hours at 6 to 8 months interval.

**Mounting of specimen:** Completely dried, poisoned specimens are mounted on good quality, standard-sized mounting sheets/boards (11.5 x 16.5 in). Different materials such as glue, paste, narrow strips of glued linen, a needle and thread, etc. are used for mounting. The glue or paste is usually applied using a brush. An alternative procedure is to spread the glue over a sheet of glass over which the lower side of the plant is placed before mounting on sheet. Stiff/bulky plant parts are usually tied using needle and thread. The herbarium label (4.5 x 2.75 in) containing information on plant name, family, local name, date of collection, place of collection, collector number, status (flowering/vegetative) should be pasted on the bottom right hand corner with information typed or filled with permanent ink. A paper pouch for extra plant parts may be pasted on the bottom left hand corner.

In the label, the latest accepted name should be filled. Annotation or determinavit (det.) slips are small slips attached to the herbarium sheet to indicate name changes/correct identity of a plant. The person annotating should put his name and signature with date along with institutional affiliation.

## 2. Identification of the plant specimen

Identification methods involve study of the plant characters; careful examination and comparison of the characters vis-avis the description in the regional floras using family, genus and species keys, and cross matching with already available identified specimens. When no clues are available plants from adjacent regions are looked at and reference is made to larger herbaria.

## 3. Incorporation of specimens

After mounting, labeling and identification, specimens are given a Herbarium Accession Number. This is a unique number and may be specimen in herbarium is arranged in a hierarchical system. : Species - genera - family - order and so on; a standard system of classification such as that of Bentham and Hooker, or Engler & Prantl (Lawrence, 1964) is used to organize collection. The group/species of interest for study can be located in herbarium of cultivated plants (NBPGR), using the following guidelines.

- A family is generally separated from the one above and one below by marker flap.
- Collections from different localities/regions are demarcated using different colour folders, corresponding to different phyto geographic zones.
- Genera and species under them are usually arranged alphabetically.

The herbarium sheet on which specimen is mounted records all information about the plant sampled. Herbarium label gives basic information on the specimen when collected from its natural habitat, the location, date of collection, collector's name, identity etc. There are small slips (Determinate slips) on which the identification details are given. Any name changes done during subsequent studies are added on these slips along with the person making change. Demarcating flaps are used to separate one family with the other.

Types specimens are housed separately with special care and are not handled routinely. A system of different coloured genus folders for different geographic regions is used to facilitate quick review of general distribution of a species. Duplicate collections are properly numbered and

placed separately. Loan material being precious is therefore always handled with special care and returned to the lending institute at the earliest. Normally cultivated plants of a region are placed at the end of family in a separate folder.

### **National Herbarium of Cultivated Plants (NHCP)**

The National Herbarium of Cultivated Plants located at the NBPGR differs in its mandate from the existing herbaria in that emphasis is laid on the collection of the wide range of variability available in crop plants in different agro ecological situations and also generated through breeding and selection. Equal emphasis is also laid on the collection of wild relatives and related types. The herbarium thus serves as a referral source in the study of crop plant taxonomy, related uses in taxa, crop evaluation and monographic works on cultivated plant taxa. The NHCP serves a complementary role to the existing herbarium such as BSI, FRI and NBRI.

The current holdings in the NHCP is nearly 23,000 herbarium specimens, representing 266 families, 1509 genera and 4201 species. In addition, medicinal plants are well represented here as herbariums well as the vouchers/crude plant products of different medicinal plants and economic products/seed samples collected from wide agro-climatic habitats in the countries. Some of the examples of rare/endangered medicinal plants in India are represented by species such as *Podophyllum hexandrum*, *Nardostachys grandiflora*, *Chlorophytum spp.*, *Picrorhiza kurroa*, *Orchis latifolia*, *Aconitum heterophyllum*, *Commiphora wightii*, *Inula racemosa*, *Onosma bracteatum*, *Taxus baccata*, *Swertia chirata* and *Aristolochia bracteata*.

**New Initiatives-NHCP:** To represent the diversity in the form of landraces, cultivars of crop groups- cereals, millets and other taxa where only variation is available as spike/ inflorescence, maintaining a set of ideal specimens with variability in panicle/spike collection has been found useful; initiatives have been taken for this group in general and will be followed for fruits (dried pods, sliqua etc.).

Representation of bulky underground material like *Amorphophallus*, *Dioscorea* etc., may be depicted by photographs/outline diagrams followed by wet collections. Succulent tuber/root can be made as herbarium specimens by small sections of the peels and transverse sections. Bulbous plants like *Allium* and rhizomatous taxa such as *Curcuma*, *Zingiber*, *Canna* and *Xanthosoma*, leaf with petiole, rhizome/bulb cut in section and inflorescence can be represented. Root material - carrot, radish, sugar beet, turnip etc. can be represented as sections giving outline of the root shape and peel mount of the surface; the specimen can be added with photos of variability within the group.

**Digitisation of holdings-** Herbarium specimens were scanned after authentication of identity using flat-bed scanner (high resolution HP Scanjet 3500C) at a resolution of 300 dpi (600 to 1200 dpi for close up of flowers, trichomes, seeds and other micro details of plant parts; and for selected specimens). A total of nearly 7,000 digitized scans (jpeg images) are saved in family-wise and genus-wise folders and are linked to database by unique identity numbers assigned to herbarium specimens (HS numbers). The herbarium digital resource was configured in web-based software in collaboration of AKMU (<http://192.168.1.59/herb/Hindex.aspx>).

**Use of GIS in herbarium database:** applications of geoinformatics technology in NHCP was found to ensure integrated approach to use geospatial technologies (remote sensing, geographic information system, global positioning system and information system) in precise way to map, quantify and predict agro-biodiversity rich areas besides other applications in agricultural sector. Locality information (village /block / district/state) and geo-coordinates (latitude and longitude) recorded by global positioning system used in geo-referencing and diversity distribution mapping. The initiatives have been taken to start use of this to link location data for CWR.

## Suggested Readings

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