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PGR NOTE

Collecting Vegetable Genetic Resources in India: Guidelines and Methods

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Collecting vegetable genetic resources (VGRs) is a cumbersome process hence requires specialised methods and knowledge on this group of crops. Successful collection of VGRs needs strategic approach in order to collect quality germplasm. To achieve the target, information on mode of propagation, plant part to be conserved and post-harvest processing of germplasm are pre-requisite to reduce mortality during transit. The international or national guidelines are available in public domain that do not much focus on collecting VGRs in general and crops of Indian origin in particular. Absence of which collecting bulky and perishable material is difficult for the explorers. It is therefore an attempt to put together the knowledge resources to guide the amateur collectors, PGR workers, and researchers. This manuscript provides maturity indices of major vegetable crops and specifications for some selected taxa having vegetative mode of propagation and future researchable aspects. Besides, methods of preparation of herbarium specimens of VGRs and their photographs are provided to support wild relatives and less-known vegetable taxa.

Introduction

Genetic diversity, a basic need for breeding to develop desirable genotypes can be assembled through exploration and germplasm collecting missions within the country in a cost-effective and time-efficient manner. A systematic approach is necessary to ensure the success of a collecting programme, which can be achieved only by devising a scientifically standardized collecting strategy prior to its execution. The strategy must take into account the eco-geographic considerations including present status of diversity, species prioritization, the planning and logistics followed by post-harvest handling of the germplasm (NBPGR, 2016).

In vegetable genetic resources (VGRs), germplasm collecting is generally followed by multiplication, characterization, evaluation and conservation for use. This essentially requires knowledge on characteristics of landraces/ primitive cultivars and wild relatives of various vegetable crops, prevalent in the area of collection. In order to minimize the resources, appropriate methods needs to be followed for sampling. Depending on the priority of crop to be collected, richness of diversity and areas to be explored, duration of survey, the number of visits and, sample type and sample size for collecting germplasm varies.

The VGRs constitute wide range of germplasm in the form of seeds, vegetative propagules, cuttings, suckers, etc. Majority of vegetable crops belong to different botanical families, and are propagated through seed and/ or vegetative means viz. potato, yams, taros, pointed gourd, cho-cho, etc. and a few are biennials (carrot, radish, onion, etc.); and others perennials (trees-drum stick). The methods of collecting the VGRs depends on bulkiness and fleshiness of fruit, mode of propagation and thus varies to a greater extent from other crop-groups such as cereals, millets, legumes, etc.

For collecting of germplasm, the national or international guidelines have already been published (Arora, 1991; Pareek *et al*., 2000; Dansi, 2011; NBPGR, 2014; Bhatt, 2018). However, these generally deal with collection methods and not the crop germplasm specifications and maturity at which the VGRs are to be collected (Dansi, 2011; ICAR-NBPGR, 2016). Hence based on data available and experience of the authors, this work focuses on collecting VGRs for the benefit of plant genetic resource workers, amateur explorers and others.

Among the total spectrum of genetic resources, landraces are probably the most important genetic resources adapted to an array of natural and cultural environments, making it very distinct (Harlan, 1975). Details on collecting of VGRs can be gathered from earlier field surveys reports, passport data and published literature especially in case of less-known and difficult

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to collect wild vegetables in general and wild relatives in particular before proceeding on an exploration of VGR (Pradheep *et a*l., 2015; Pandey *et al.*, 2018).

Knowledge on crop duration, local names, breeding system and population structure, regions of diversity and unique traits of the cultivars/landraces, maturity period of primitive types/ landraces/ local types in crops, wild relatives, and key morphological characteristics for identification of taxon are desired. While collecting landrace of any vegetables crop, local name(s), used in a prevailing area of occurrence are to be assured by repeated cross-checking from neighbouring areas/ regions to avoid the chances of duplicate collections under variable names.

Literature on horticultural crops/breeding vegetable crops throws light on propagation, and maturity of a species. During exploration, material is selected from farmers' field, handled for couple of weeks during transit till reaches the base camp or for conservation. Specific handling methods are to be followed during evaluation programme which are differently handled by breeders.

Less-known VGRs and crop wild relatives (CWR) are also important genetic resources and should be collected from kitchen gardens and wild, taxa like *Allium stracheyi* Baker, *A. roylei* Stearn, *Solanum nigrum* L., *Malva verticillata* L., *Rumex nepalensis* Spreng., etc. from their distributional range. Mode of propagation of most of the CWR is not known; hence explorer has to decide the type of material to be collected based on his knowledge and experience. Knowledge on method of propagation/seed multiplication and maturity indices of fruit/ seed/ propagule and the stage at which the crop is to be collected, especially in bulky vegetables/ cucurbits where seed extraction may reduce the bulk weight during transit but if the fruit is of less maturity and other samples are not available elsewhere, the collector has to decide based on maturity indices. Precautionary measures for post-harvest handling and transportation of germplasm to base camp/ Headquarters should be adopted.

Collecting vegetatively propagated material differs from that of collecting seeds material. In particular, it is more restrictive for the collecting timings of the mission. However, seed having good storability (except recalcitrant seeds), less bulky and easy to handle are preferred over the former (Fig. 1; Tyagi *et al*., 2016; Pandey *et al.*, 2019). To collect vegetative propagules in the form of roots and tubers (potato, cassava, yam, taro, sweet potato, etc.) and cuttings, suckers (pointed gourd, Malabar spinach), appropriate timing of the visit is the most important aspect of the planning. Vegetables such as onion, cho-cho, and drumstick are propagated by seed as well as vegetative means. Some of the crops may often produce very few seeds. A minimum of two separate visits can be made; in first visit the desirable genotype are marked as mother plant and second visit to the same site for collecting budwood. Hence, the decision is dependent on: a) the purpose of collecting; seeds of a new variety, vegetative samples for established varieties; b) site identification, and facilities available for multiplication; and c) breeding system of the species (monoecious/dioceous), seeds upon growing may turn up into male and female plants (pointed gourd).

Maturity Indices of Vegetable Crops

To enhance the efficiency of collecting mission, the aspects of seed/ fruit maturity indices for collecting are pre-requisite. An expert having physiology background can facilitate pinpointing the right stage of maturity prior to execution of collecting mission. A target should be set to extract maximum number of seed that qualify the gene bank standards (Tyagi *et al*., 2016). The collector should consider varietal differences, phyto-geographical areas, agronomic practices and temporal changes (season/ soil types/ irrigation/ stress). Information on seed maturity and right time of VGRs collecting especially for vegetatively propagated material are scarcely available in literature.

Due to asynchronized seed maturity or seed shattering (CWR/wild economic vegetables), care should be taken to collect seeds in case of *Allium*, brassicae, amaranths, *Luffa*, etc.. The inflorescence of amaranths and chenopods are harvested when 70 per cent of maturity (i.e. seed turns brown or black) or before the siliqua shatter in brassicae; gourds, cucumber, *Luffa* etc. are left to dry on the plants and harvested later when plant is completely dried. Others like melons, round gourd, ash gourd are harvested after drying of vine on fruit colour changes from green to yellow. In tuberous and bulbous vegetables, field-dry maturity is considered appropriate when bulb/ corm neck is completely dry/ papery or aerial part turns yellow.

In tuberous vegetable crops, like taros, propagules must be collected without causing damage to the material during harvesting or transportation. Most of the *Dioscorea*

Fig. 1. Collecting VGRs: a) pumpkin drying in sun for maturity; b) mature melon harvested for seed; c)farmer extracting seed from ash gourd fruit; d) wild onion packed for transportation; e) extracting pumpkin seeds in field; f) wild okra fully dried in field.

species are dioecious and bear male and female flowers on separate plants; flowering is erratic and seeds are seldom produced. *Dioscorea deltoidea*, a dioecious plant produces bulbils (small tubers from leaf axils) those can be used for propagation. For seed production male and female plants must be grown together. Vegetative propagation is done through the aerial and underground bulbils. Seed tuber weighing 125-100g optimum in lesser yam and its 200-250g pieces or whole tubers in white yam can be used for propagation. Giant *Alocasia* and the taros are propagated by rhizomes and needs to be cut between the upright stems to yield new plants. The information on maturity indices of VGR for seed/ vegetative propagule is given in Table 1 and 2.

Majority of bulbous vegetables *viz.* onion, leeks are propagated by seeds as well as vegetative propagules (bulbs, rhizomatous bases, bulbils). *Allium* species being sensitive to photoperiod do not flower under all ecological conditions may be collected as bulb/rhizomatous part. To collect fresh bulbs of *Allium sativum* var. *proliferum* ('tree onion', 'pearl onion') use of moss grass or by any soft organic packing material mix with moist soil can be used to protect bulb from losing moisture and damage during transportation. *Allium ampeloprasum* produce aerial bulbils in flower head or underground bulbils in the basal part can be air dried, stored in refrigerator or air stored in moist free open area and sown in the month of November (about 13°C). In general aerial and underground bulbils have longer shelf life and are easy to transport.

There are more than 120 VGRs listed for the Indian region (Pandey *et al*., 2018). Some of the less-known/ localized vegetables- winged bean, teasel gourd, purselane, agathi, bread fruit, vegetable banana or other – mestha, roselle, are not included in this manuscript. However, one may decide on the basis of similarity in physiology, followed by expert opinion and literature consultation.

Target Sources: Diversity in VGRs

A preliminary check-list of VGR diversity available in selected area can be prepared by the routine visits, feedback from the residents/ locals, farmers' information, previous exploration reports and published literature. For example if landraces/ farmers' varieties/folk variety are the target species, then the main objective would be to collect them from farmers' fields, kitchen gardens, farm stores and local seed shops (if not available with farmer). Markets are reliable source of information on vegetable crops, varieties available and preferred by the locals in the area, location of cultivation sites and time of crop availability. Generally cultivation of local vegetable is confined to marginal land kitchen garden for self-consumption and for sale in local market. Assembling requisite number of propagules germplasm in appropriate quantity is therefore not always possible in such cases.

In VGRs, especially the ucurbits viz. *Benincasa*, *Cucumis*, *Cucurbita*, *Lagenaria*, *Luffa*, *Momordica* and *Trichosanthes*, the number of plants and mature fruits in a farmer's field is usually limited, and the seeds are usually obtained from one or a few fruits. A total of 15-20 cuttings are sufficient for species propagated through the vegetative methods. Material with dubious identity or unknown identity, vernacular name should be recorded along with herbarium specimen and photographs (closeup of flower, pods) for authentication.

The pointed gourd is usually propagated through vine cuttings and root suckers. To propagate from root suckers, tuberous roots of pointed gourd are dug in the early spring, subdivided, and replanted. Both pre-rooted and fresh vine cuttings are used for propagation. Vegetative cutting should have 8-10 nodes per cutting and should be partially or fully defoliated to check transpiration. Vine cuttings made in previous year and rooted during winter are planted in the spring after danger from frost is over. The concept of female: male ratio of 10:1 is optimum for ensuring maximum fruit set should be kept in mind. Cho-cho is propagated vegetatively either from the old rootstock or reproduced from mature fruits (viviparous germination). *Momordica subangulata* subsp. *renigera* is propagated by seed as well as tubers. Seeds have difficulty in germination (segregation is 1:1 male female ratio). Some of the less-known vegetable species like West Indian arrowroot (*Maranta arundinacea*) and Queensland arrowroot (*Canna indica*) are propagated by suckers/rhizomes and also from seed. A less-known introduced vegetable, the tree tomato (*Cyphomandra batacea*) is propagated by stem cuttings with leaves intact (especially during transit) to yield better survival. Other vegetables such as *Momordica cymbalaria*, *Solena amplexicaulis*, *Momordica dioica* are propagated through seeds as well as perennial rootstocks tubers while wild leafy types- amaranths and chenopods are propagated through seeds.

Table 1. Maturity indices of vegetable crops [largely adopted from AVRDC- Asian Vegetable Research and Development Centre (1990), the feedback through explorers and validated from published sources]

Crop(s) $	Mode of propagation; maturity indices
Amaranths, chenopods	Seed; leaves and inflorescence turning brown, followed by seed shattering on touching
Ash gourd	Seed; fruit wall hard to pinch, skin turning greyish white; seed turning brown and hard
Asparagus	Vegetative; plant starts yellowing followed by leaf fall
Bitter gourd, spine gourd, sweet gourd, teasel gourd	Seed; fruit softening followed by turning to pale red to pale orange-brown, aril/ pulp turning red and slimy
Brassica (leafy types)	Seed; pods turning brown, cracking of the lower pods initiated
Brinjal	Seed; fruit beyond edible stage, fruit pericarp turn dull yellow and seed hard, tough walled
Cabbage, cauliflower, broccoli, other cole crops	Seed; head cracking followed by siliquae formation; seed turning dark brown
Carrot, radish, turnip	Seed; inflorescence turns brown and brittle; fruit formation, stem defoliate, wooden brown colour base-seed shatter on touching
Cassava	Stem cuttings# from 8-14 months old plants; seed
Chekkurmanis (*Souropus androgynus*)	Seed/stem cuttings; woody stem defoliated, leaves start drying
Chilli and other peppers	Seed; fruits turn red, pericarp lose moisture (start wrinkling)
Cho-Cho	Vegetative cuttings/ fruits; viviparous fruits detach from vine of mature plant (sprouting preferred)
Coriander and other members of Apiaceae	Seed; umbel turning brittle, flaccid/dry; seed dark brown and shedding
Cowpea	Seed; two third of pods turn brown; well-filled pods that snap readily, loose green colour, turgidity
Cucumber, long melons	Seed; vine drying, fruits with hard pericarp, pale yellow/golden-brown, seeds with hard skin; rattling in pepo
Curry leaf	Seed; fruit start ripening (hard/ shrivelled fruits are not viable); plant the fruit/remove the pulp; soft, loose pulp purple-violet colour
Drumstick	Seed-pod turning brown, hard and brittle; cuttings with brown hard wood (pencil thickness, 20-30cm)
Fenugreek	Seed; leaf/ pod turning brown followed by skin turning hard
Garden pea, French bean, dolichos bean, cluster bean, velvet bean, other beans	Seed; plant showing senescence, drying, pod turning darker-brown, dry and brittle; seed fully developed and hard
Onion, garlic, leek and other *Allium* species	Cloves/ bulbs- leaf turning complete brown; tops beginning to dry out and topple down (approximately 10 to 20 per cent fallen); bulbs scales drying, leaves drying and collapse of neck. For seed producing onions: seed#turning to brown-black
Indian round melon	Seeds; fruit wall soft, brown, seeds black
Malabar spinach	Seed/stem cuttings#; seed turning black, leaves turning brown on stem
Meetha karela (*Cyclanthera pedata*)	Seed; pod turning pale seed colour change to black, splitting of fruit
Okra	Seed; capsule turning hard, brown, drying, splitting; seeds mature to black colour
Onion	
Pointed gourd	Vegetative cuttings; fruit wall turning to pale orange, over mature if thumbnail cannot penetrate flesh readily; stem cutting from plant of mature bark
Potato	Mature tubers (seed tubers); aerial parts turning yellow, dried; seed extracted from fully mature fruits when fruit colour turning violet-brown
Pumpkin, bottle gourd, water melon, squashes	Seed; fruits wall turning hard to pinch, brownish yellow (beyond edible stage); colour of lower part turning darker, vines drying
Ridged gourd and sponge gourd	Seed; fruit hard, fibrous wall, opening on the tip clear and seeds black and dry (over mature if thumbnail cannot penetrate flesh readily)
Snake gourd	Seed; fruit wall turning to pale red (over mature if thumbnail cannot penetrate flesh readily)
Snapmelon	Seed; fruit wall cracking/degenerating, aroma on full ripening followed by seed maturity; colour change to pale brown
Spinach	Seed; spines of the seeds can be broken easily
Tomato	Seed; fruit fully ripe (90 per cent skin turn red, soft); seeds slipping when fruit is cut
West Indian arrowroot	Sucker/rhizome#, occasionally by seeds; leaves turn yellow, stem senescent
Yam and taro, giant taro, elephant foot yam and other edible aroids	Tuber; leaf turning complete brown; just rightly mature with initiation of sprouts; large enough (over-mature if tough and fibrous); senescence of the aerial parts
Yam bean	Seed# harvested from dry, yellow-brown pods; sprouted root tubers of previous year left in unirrigated soil, with hard periderm

#: more commonly used method for propagation; abstracted from Pandey *et al*. (2018); $: refer Pandey *et al.* (2018) for botanical names

Table 2. Specifications# for vegetative propagules of some selected VGR

Crop (s)	Details of material
Aerial yam	Aerial tubers (potato sized) borne in axil of leaf preferred over root tubers
Asparagus	Tuberous, mature roots (texture dry) of over one cm or more in diameter
Cassava	Basal midleaf/ stem cuttings# from 8-10 month old defoliated plant (seed germination only 50 per cent)
Chekkurmanis	Stem cuttings (woody stem 20-40cm)
Chinese chives	Rhizomatous underground rooted stem with apical bud
Chinese onion; chives	Bulbs with shining dry/ papery skin
Chinese potato	Tubers produced during storage period; sprouting tubers give better results
Chinese yam; white guinea yam	Big sized mature tubers (cut into pieces) or whole tuber if small
Cho-cho	Vegetative cuttings (15-20cm long and 0.5-0.8cm diameter); fruits (with sprouting preferred over non-sprouting) for good survival and rejuvenation
Coleus	Vine cuttings from 3-4 week old plants
Drumstick	Stem cuttings of 1.5-2.0cm sized diameter with mature brownish bark, preferably from old branches; seeds from fully mature pods (wild plants shatter seeds)
Elephant foot yam	Ripe fruit; cormlets (mortality is high); tuber pieces of 125-100g pieces or whole tuber if small, whole rhizome without injury
Garlic and leek	Cloves/ bulbs# from mature plants with skin shiny and dry; aerial bulbils from mature plants with shiny, dry skin (often seen under adverse conditions)
Giant Alocasia	Offsets [(in spring/ rainy season)/ stem cuttings (root readily)]; seeds (from mature dried cob along the spadix)
Greater yam	Tuber top with intact skin (125-100g optimum) or whole if small tubers/ aerial bulbils
Ivy gourd	Vegetative cuttings with 3-4 nodes of 10-20cm long x 1-0.5cm diameter from mature vine
Japanese bunching onion	Bulbs with thick, dry, papery skin
Malabar spinach	Leafy stem cutting from mature plant with 2-3 nodes, 30-40cm long x 2 cm diameter; fruit with juicy red pulp, dark black skin, and dried on plant (can be used for planting)
Onion and shallot	Bulb completely dry, shining papery tunic easily detachable; ideally bulbs to be collected when top leaves die down after flowering
Pointed gourd	Stem vine cuttings of female plants (with 8-10 nodes) from mature branch (cutting size: 10-15cm long and 0.5cm diameter wide), dark brown and hard stem preferably with roots
Potato	Mature tubers with skin peeling well set and healthy tuber# with at least two to three well developed eyes; (seeds formed in temperate areas used generally for research purpose)
Spine gourd	Terminal cuttings from 2-3 month old plants, taproot tubers of senescent plants of 2-3 year
Sweet potato	Fresh vine cuttings from senescent tubers
Tannia	Tops/suckers#, corms/cormels
Taro	Cuttings (stem of about 30-45x5-7cm) suckers/cormels/corm
Top onion	Bulb with outer skin completely dry, papery; mature aerial bulbils borne in flower head (shatter on touching)
Tree tomato	Stem cuttings from 1-2 year old tree (preferably of size 30-45x 1.5-2.5cm) with brownish-mature skin
West Indian arrowroot, Queensland arrow root	Suckers or rhizomes with two or more nodes each from 10-12 month old plants at senescence; occasionally propagated by seed

Pandey *et al*. (2018)

Herbarium Voucher Specimens and Photography

Herbarium specimens can serve as a useful resource for taxonomic identification of materials, especially those where identity or nomenclature is not accurately determined during the collecting, and for the less-known/ wild species of VGRs. The basic standard methods (Jain and Rao, 1977; Fuller and Barber, 1981; Rao and Sharma, 1990) along with modified methods developed by the National Herbarium of Cultivated Plants (NHCP), New Delhi (Pandey *et al*., 2017) for cultivated plants can be followed.

As a rule, voucher specimens should be prepared during collection, especially for the wild vegetables, and variants of vegetables (local cultivars) where taxonomic identity needs authentication and confirmation.

Density of spines in brinjal and non-spiny types can be depicted in the voucher with stem and leaf, besides the fruit/ calyx. If possible, fruit at young stage may be included to show the shape variation. In cucurbits, the processing of herbarium specimen needs initial drying in the field to avoid limping/overlapping of parts. In melons character differences are evident even at the initial

stage of fruit development and can be used to delineate intra-specific separation of taxa. A holistic understanding of the plant morphology can be re-ascertained if wet collection is supplemented to the germplasm.

Close-up of the plant stand and wide angle population, leaves and branching pattern, vegetative parts (in case of vegetatively propagated taxa- tubers, corm, suckers in whole and cross/longitudinal section), fruits/ pods/seeds, flower and flowering habit, the pistil, anther, open and closed/ bud as well as variability collected and may be photographed.

Guidelines and Research Thrust for VGRs

None of the national and international guidelines (Guirino *et al*., 1995, rev 2011; NBPGR, 2016) exclusively deal with vegetable group in general and also crops of Indian origin. Internationally, guidelines on collecting for vegetatively propagated crops focus more on roots and tubers donot sufficiently explain the methodology and specifications required while selecting material (Dansi, 2011). Following the general guidelines that are available in public domain are difficult for amateur collectors, PGR workers, researchers and information on specific group is insufficient. They may not have knowledge on specific VGRs. Present work is an exclusively attempted on specifications on the VGRs and their maturity index that an explorer needs while deciding to collect a bulky fruit that is available in a given time.

Information presented here is synthesized primarily from the field experience of the authors, and further validated from literature to facilitate explorers. Unavailability of such information in one place prompted the authors to compile the information for the use by young explorers who are dealing with crop specific explorations such as vegetables which are a difficult group mainly due to: 1) small number of seeds available during collection from kitchen garden, and small scale cultivation; 2) fleshy fruit that are bulky, tubers/ roots perishable and prone to fast damage. Table (3) below gives the comparison on guidelines for the two:

Information arising from this work is provided in this manuscript which emerges as de-novo approach through the experiences of the collectors and base knowledge by breeders for collecting quality germplasm material in VGRs. This would facilitate collecting of quality germplasm besides reducing loss of material during transit. Some of the research thrust areas are given below:

- Evaluation of per cent mortality using different methods during transit
- Methods on enhancing survival using different modes
- Selection of ambient conditions for storage
- Fruit maturity of crop *vis-a-vis* seed health

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Table 3. Comparison of general and VGRs collecting methods in PGR

Item	General collecting	Collecting VGRs
Guidelines	Cover general points; hardly cover VGR	Specialised for VGR
Source of availability of germplasm	Wider sources	Specific confinement in kitchen garden, small scale cultivation
Quantity of material gathered	Sufficient	Seed/propagule not sufficient; need multiplication before conservation
Mode of multiplication	Seed	Vegetative and seed both
Post-harvest methods	Not specific	Deal with perishable, bulky material
Maturity indices	Not focused	Species specific and specialized
Specifications for vegetative propagule	General and can applied widely	Very specific for different species

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