in Legume Genetic Resources for Semi-arid Temperate Environments: (eds. Alan Smith, and Larry Robertson): Proceedings of an International Workshop, Cairo, Egypt, 19-24 June 1987, : ICARDA

COLLECTION STRATEGIES FOR FOOD LEGUMES

Published in 1991

R.P.S. Pundir and M.H. Mengesha

Genetic Resources Unit, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru, P.O. 502 324, Andhra Pradesh, India

16

Summary

Food legumes are consumed as dry seeds, green seeds or the green pods with immature seeds inside. They are rich in protein and thus form a natural supplement to carbohydrate-rich foods. This paper addresses the principles of germplasm collection, with reference mainly to one important cool season food legume, chickpea (*Cicer arietinum* L.). This species is known to have originated in Turkey, and the crop is now cultivated under a much wider range of climatic conditions than occurs in that country, i.e., from temperate to subtropical. Chickpea is a self-pollinated crop. Germplasm accessions of chickpea are collected from dry seeds. Collection strategies and some guidelines are discussed in this paper. Other crops are referred to as examples of special cases or uses.

The purpose of collection

Man and earth's abundant flora and fauna have been together in evolution for thousands of years. Interactions between them and with the environment have resulted in innumerable patterns of variation and adaptation. Survival of the fittest by natural and human selection have enabled some plant species to become the crops which support the world's population today.

CP 365

ICRISAT Publication Series No. CP 365

The world population is increasing steadily, resulting in food scarcity and the need for improved agriculture and increased food production. Genetic improvement of crops is the main component of improved agriculture which can be achieved by utilising the diverse gene sources of a species. Hence, we have the need for germplasm collection and maintenance.

With the advent of new crop varieties, natural epidemics and urbanisation, local plant material is being eroded or becoming extinct. This hazard should be prevented. Some plant materials may not appeal now for our present requirements but may prove to be of paramount importance in the future. Plant material, therefore, has to be collected, while it exists, for utilisation in the present and future research programmes. This awareness for collection and preservation probably started at Vavilov's time. The International Biological Program (IBP), at its inception in 1964, set up a subcommittee to study ways and means to collect and conserve the vanishing plant genetic resources. Since then, many national and international research institutions have started this task. The International Board for Plant Genetic Resources (IBPGR) with its headquarters at Rome, was established in 1974 to catalyse a plant genetic resources network.

Geographical diversity

In as early as the 16th century, foresters recognised inherent differences within forest species. However, the practical importance of the variation associated with geography was realised mainly in the 19th century (Zohary, 1970). Vavilov is credited with establishing the phytogeographic basis for plant exploration. Awareness that variation in cultivated plants is unevenly distributed geographically and that the bulk of genetic diversity in our crops is confined to a relatively few areas or centers is mainly due to Vavilov. Centers of diversity are a fact, and intelligent exploration should be aimed at collecting the genetic variation existing in them. What is a classical example to illustrate this pattern? Over most of Europe as well as Siberia, wheat cultivation is based on a rather restricted number of varieties mostly hexaploid bread wheat, whereas in the Middle East many forms and types of wheat are grown (Zohary, 1970).

In chickpea, broadly two types are recognised (i) desi (meaning 'indigenous') which has purplish pigmented foliage, flowers and seeds of colours other than white, and with angular shaped seed (ii) kabuli types which have green foliage, white flowers and white seeds with an owl's head shape. The maximum variation in the genus Cicer can be found in Turkey (van der Maesen and Pundir, 1984) which is considered as the centre of greatest diversity or centre of origin (Ladizinsky, 1975). The wild progenitor of cultivated chickpea is very similar to the desi type. It is possible, therefore, that desi chickpea is more primitive than the kabuli type. In Turkey, west Asian and north African countries, people prefer kabuli types. Despite this human preference for kabuli for a long time period, one can still find desi types mixed in the kabuli population (Saxena, 1979), in contrast to other regions where one would find almost a pure stand of kabuli or desi. This indicates that in the area of origin more diverse populations co-exist than in other regions.

Certain characters occur much more frequently in areas other than the center of diversity. For example, in the USDA barley collections, 75% of the genotypes which are resistant to net-blotch disease come from Manchuria, an area which contributed only 12% of the total collection. Similarly, hessian fly resistant collections abound in north Africa (Allard, 1970). Resistance to pea root rot disease was identified only in germplasm from Ethiopia (Gentry, 1971 a,b). However, many examples can also be cited in which many strains carrying resistance to diseases occur with more or less equal frequency throughout the range of distribution of the species. Harlan (1975a) is of the opinion that each crop is a case of its own in respect to its geographic diversity, and a cropwise analysis reveals a certain consistency of evolutionary patterns.

The consideration of geographic diversity is more important for less researched crop species. For the well known crop plants like wheat and barley, ample information is available on distribution of types, characters and alleles, therefore, special collections can be organised as sources of desirable material. For crops like chickpea, faba bean and lentil where the available information is inadequate, germplasm collection based on geographic distribution would be more desirable.

Priority areas for collection

Germplasm collecting work is time consuming, requires expert personnel, finance, cooperation, travel feasibility, and awareness of and support from the germplasm users. It is also time specific and in the majority of the crop plants it can be done only for about one month in any one year. For collection in different regions, representation of geographic areas, countries and environmental contrasts need all to be considered. In a limited geographic area one can find several crop environments associated not only with altitude, soil type, hill slope, and aspect, but also with disease and insect pressure, and with rainfall.

It is to be expected that different material will arise from different environments. Plant material from diverse locations may look alike morphologically but might differ in reactions to biotic and abiotic stress factors. These factors should be taken into consideration while organising the collection mission.

The priorities of germplasm collection in different areas depend on:

(a) Germplasm already available in the genebank for the area,

(b) Antiquity of the crop. If the crop is newly introduced in the area, it would not be worth collecting samples there. It is believed that the longer a species has been present in an area, the more diverse will it be,

(c) Extent of germplasm erosion in the area as a result of the introduction of more remunerative crops and high yielding cultivars, which pose a threat to the native plant material. The latter should be collected and preserved before the new crops and newly bred cultivars can wipe out the native landraces,

(d) Urbanisation, which can also lead to the wiping-out of native plant species, and

(e) Drought, flood, fire, and civil war. These unfortunate tragedies, natural or man-made, can cause total loss of the native plant species to occur. If some of these disasters can be predicted in good time, all possible efforts should be made to salvage endangered material.

Species to be collected

While planning a collection mission, information should be obtained on which other plant material can be obtained in addition to the targetted species. However, though it may appear good to collect different plant species on a mission, this may not be feasible and appropriate if sufficient expertise and manpower are not available to accomplish the job. Often it is the case that the crop specific botanist, breeder or evolutionist would like to collect only one or two species which really interest him.

While this may be most effective, germplasm collectors often attempt the alternative of collecting several plant species in a single expedition. If this is the case, the collector is advised to obtain in advance, knowledge of the taxonomy, and herbarium specimens, of all species including wild relatives which occur in the target area. Study of herbaria can indicate where and when a mission should be organised in order to acquire ripe seeds.

Breeding system and collection strategy

Because food legumes are grain crops, mature seeds need to be collected and conserved for salvaging germplasm. Two types of sampling are generally made, namely 'random' (no selection) and 'biased' (selective). A germplasm collector usually takes random samples with the objective of capturing common alleles in the population.

Whyte (1958) recommended sampling more plants in a self-pollinated crop than in a cross-pollinated one. This is mainly because allelic distribution is broader in the latter. Working on wild oats, which are self-pollinated, Allard (1970) suggested collecting seeds from 200-300 plants to constitute a representative germplasm sample. Bennett (1970) advocated sampling from 200-500 plants from cereals as well as from other crops. Marshal and Brown (1975), though they did not elaborate on self- or cross-pollination, have the most convincing theory. They considered the aim of plant exploration to be the collection of at least one copy of each allele occurring with a frequency greater than 0.05 in the target population. The alleles which had a frequency less than 0.05 were considered of little interest to breeders, and in any event collecting these rare alleles from wild populations would not be of much use. Further, they calculated the minimum number of plants to be collected from a population, defining this minimum as the number of plants needed to obtain, with a probability of 0.95, all the alleles at a particular locus which occur with frequencies greater than 0.05. This minimum number varies according to the assumptions made about gene frequency distributions, but in general lies in the range of 25-50 plants. A larger sample may be appropriate, however, in populations which are highly variable for quantitative characters.

The above reports indicate that generally the minimum number of plants to be sampled is basically the same for inbreeding and outbreeding species. Indisputably, the larger the number of plants collected, the better the sample.

Biased samples are of more interest to plant breeders or the crop-specific germplasm botanists. These samples are primarily made to facilitate rapid utilisation of desirable gene sources in research programmes. The selectivity in biased sampling gives plants which are distinguishable morphological types, or have desirable biotic and abiotic traits, such as tolerance to drought, frost, disease or insect pests.

Sampling techniques

Field sampling of a species in plant exploration is usually aimed at obtaining the fullest possible geographic representation, and genetic variants, irrespective of the relative frequency or rarity of any gene or genetic complex. According to Bennett (1970), random sampling is usually employed, but may be modified to meet the demands of specific objectives. She suggested a procedure in which a starting point is selected at random at the collection site, and then (for example in cereals), taking a single spike at every second or third pace along a number of transects through the crop and collecting seed from 200-500 spikes. Allard (1970) suggested dividing the geographical area into east-west, and north-south transects and further subdividing into regions and sampling sites. Two hundred plants can be sampled from a site of size about 50x50 m. A similar procedure is described by Marshall and Brown (1975). However, when the information on the population structure of the target species is incomplete, they suggest sampling even fewer than 50 plants per site, but also using more sites.

Although the above sampling strategies have a good scientific basis, they have the disadvantage of being, expensive, time consuming and possible only when information on the population structure of the target species is available. This has been the case only for the very important and highly researched crops such as wheat, barley, and oats. Many geneticists, breeders, and evolutionists have assembled detailed information on these crops.

For many other less important crops, the required information is lacking. Yet they have to be collected, in many cases by a germplasm botanist from a far-off institution, who has been deputed to salvage local germplasm as early as possible before the existing types are replaced by new crops or newly bred uniform cultivars. This requires some compromise in sampling strategies.

The procedure usually followed by germplasm botanists of ICRISAT is to set priorities for different geographical regions of a species and collect samples along passable routes as well as in remote areas. The frequency of sampling can vary depending on population variability and intensity of cultivation of the crop. Usually the farmer's field is the "site" and the interval between two sites is about 5 km when the crop is cultivated in many fields and about 15 km when fields are not so frequent. We normally sample about 100 random plants, along a number of transects through the field. In some cases, farmers do not allow large scale sampling.

Sampling from the standing crop is most preferred. If the crop was harvested, samples can be taken from a threshing yard or from the farmer's home. "Market" samples can be taken of the produce from distant places or a mixture from various regions, but this can mislead research workers. However, sometimes there may be no alternative to using a market sample. Regarding sample size, the larger the quantity of seed the better the sample. Often it is feasible to collect 2000-4000 seeds.

Herbarium specimens of crop plants are not usually collected. This can be done, however, if a unique type is found or if some features of the plant material are not clear. Herbarium specimens are desirable for wild species and are necessary when taxonomic identification is doubtful. Herbarium specimens become more essential in a multi-species expedition. Plant species which are not producing seeds at the time of the expedition can be herbarium-sampled for botanical museum, or for identification purposes.

Special techniques of collection for dry areas

The cultivation of agricultural crops in dry areas depends on timing and amount of rain received. The sowing time may have to be earlier or later than usual depending on the onset of rain, and this will affect the time of crop maturity. Sometimes, it might happen that while sowing, very limited soil moisture was available and only a part of an area could be sown. The remaining part might be sown at a later date when the next rain occurs. Without sufficient moisture, some seeds can germinate early and others might germinate later, according to the rainfall pattern. This could result in a patchy crop stand, with a range of plant growth stages and maturity on different dates, particularly, if scattered rains occur towards ripening. If a germplasm collecting trip is to be organised under such conditions, information on the current season rain pattern and crop maturity would be needed for scheduling travel dates.

Collection of Rhizobia and mycorrhizae

Legumes have the potential to meet most of their N requirement through biological nitrogen fixation in symbiotic association with *Rhizobia*. The full potential of a genotype may not be expressed in all environments unless the right kind of *Rhizobium* is present (Date and Halliday, 1979). Such expectations have, perhaps, encouraged the collection from sites of *Rhizobia* in conjuction with the collection of plant germplasm.

The ideal way of collecting Rhizobia is to obtain fresh, firm and apparently functional (pink) nodules. These would mainly be available during the vegetative and flowering stages, and obviously would not coincide with the time of seed collection trips. When two trips are not possible, the collection of Rhizobia can be combined with that for seed. From the mature legumes the nodules, even if inactive, can be collected plus a sample of soil from around the plant for the subsequant isolation of rhizobia. However, if the nodules or soil samples are to be transferred across a country, plant quarantine measures have to be examined. Following a Rhizobial collection mission, specialized laboratory facilities are required for characterisation and authentication of the strains isolated from the samples. Date and Halliday (1979) have described these facilities along with the material and equipment required for such collection trips of short or long duration. They have also provided a list of laboratories in 12 countries which offer testing and characterisation services after nodules have been collected.

Compared to Rhizobia, an appreciation of the beneficial role of vascular-arbuscular (VA) mycorrhizae in crop growth is a relatively recent development. Collection of VA-mycorrhizae from the soil rhizosphere can be done at the flowering stage of a crop by removing fungal spores present in the plant roots and soil. The procedures for such collection are specialised and are yet to be published (Dr. K.R. Krishna, personal, communication). The collected spores can be purified and exported or imported with appropriate phytosanitation measures.

ICRISAT collects and maintains Rhizobia of its mandate legumes, chickpea, groundnut, and pigeonpea, and supplies Rhizobia and

VA-mycorrhizal fungi to all interested users. This work is not undertaken by the Genetic Resources Unit, as it requires a specialised team. ICRISAT scientists have undertaken the survey and collection of the fungi in India and west Africa.

Agreocological observations during collection

Experience from collecting many crops has shown that cultivars in regions where agriculture is not advanced are often closely adapted to the local environment and agricultural conditions and do not always perform well outside these environments. For example, the traditional chickpeas of northern India start flowering only after the cold period (December-January) is over, and so would not do well in other warmer areas. Quite often, germplasm collecting work is associated with describing plants which are particularly suited to specific environments. It is useful to breeders using the material if they know characteristics of the environment to which the material is specifically adapted. This information is equally relevant for studies of evolution. Therefore, it is desirable that the germplasm botanist/collector provides a clear account of the ecological and farming conditions of the place of collection of each accession.

Observations required:

a) Name of the place and its coordinates, or the exact location based on a permanent landmark, administrative unit, province and country,

b) Notes on topography, shading by mountains, sunshine hours, altitude, precipitation, rainfall-distribution, and drainage conditions

c) Soil type, depth and estimate of salt status, pH and electrical conductivity, whenever possible,

d) Irrigation facilities, other crops and associated vegetation and ground water table,

e) Crop season and specific local conditions which may modify the length of the season,

f) Local peculiarities, e.g., crop utilisation, consumer preference and storage practices,

g) Indications of hybridisation and introgression with wild and weedy forms,

i) Population variability status and taxonomic notes, and

j) Main anthropological, sociological and linguistic factors of the human population.

Organising an expedition

Germplasm collection will succeed best when it is sponsored or backed by well established institutions whose main objective is introduction, utilisation and conservation of the germplasm. To make a successful collecting mission, attention must be paid to the following points which are assembled by referring to several sources such as Bennett (1970) Hawkes (1979) and Harlan (1975b).

a) Planning: Germplasm collecting expeditions require advance planning. This is more so when collecting is be done in a foreign country or in regions for which specific permission is needed, for instance at a border area between countries or where there is political sensitivity. Letters of introduction, particularly to government authorities, should be carried. These may be particularly useful when the mission runs into some difficulty. Identifying collaborating agencies and scientists is also important, which requires contacting them in good time. The trip should be planned and implemented jointly in collaboration with national programs.

b) Objectives and collecting team: Objectives should be clearly determined and the collecting team must be technically competent to carry out the work. When the collection area has been determined, collecting one specific crop at a time is most effective, and the botanist must be an expert for the species. Two or more crops which are endemic to the same region and mature at almost the same time can be collected in one expedition, however, an increase in expert numbers would be required. Collecting teams should always be small: never more than three persons. Larger teams can be divided into groups, and each group allocated to a particular region. If there is a team leader, he should preferably be a botanist or a plant breeder.

c) Route: Route planning is essential and detailed road maps should be available. Regional, climatic, soil and vegetation maps should also be consulted. Local experts must be consulted during planning concerning feasability of the chosen routes. Travel on less important roads can hamper the mission during rains or while bridges or culverts are repaired. Therefore, one should discuss travel feasability with local bus or truck drivers every day during a trip.

d) Date of collection: For seed crop plants, information on crop maturity is essential and should be gathered from previous reports and by consultation with local experts. Allowance must be made for climatic and seasonal fluctuations which might influence the date of crop maturity. There is always a need to allow more time for an expedition than would normally have been thought necessary.

e) Transport: Mode of transport will vary depending upon the country and resources available. However, in normal circumstances the best is a diesel vehicle, with four-wheel drive, high/low gear ratio, long wheel base, heavy duty springs, covered and lockable. The vehicle should have fittings such as a roof rack and spares such as a complete tool box, spare wheels and tyres, fuel cans, engine driven winch and a chain or nylon rope.

f) Equipment:

i) Camping equipment: The team should plan their travel in such a way that they reach some city, town or institution by the end of each day to obtain accommodation and food. However, if necessary, they should carry items for camping and food preparation. Light weight tents, sealed ground sheets and mosquito nets, water-proof sleeping bags, air mattresses, cooking equipment, food, utensils, water containers, lamp and torch will be required. A transistor radio/tape recorder is also very useful to remain aware of news and for recreation,

ii) Scientific equipment: Altimeter, field compass, two cameras, pocket lenses, pH meter and binoculars are required,

iii) Sampling equipment: Thin cloth bags, paper packets (porous), labels, secateurs, leather gloves, field books, rubber bands, plant presses, drying stove and cardboard boxes are needed, and

iv) Medical supplies: Team members must have all necessary vaccinations in advance. They should be careful with unfamiliar food and drinks. They should carry medical items such as insect repellents, antimalarial and antibiotic tablets, mexaform, anthistamine cream, pain killing tablets, bandages, water filter and water purifying tablets.

References

Allard, R.W. 1970. Population structure and sampling methods. Pages 97-107 in Genetic Resources in Plants Their Exploration and Conservation (Frankel, O.H. and Bennett, E., eds.), IBP Handbook, No. 11, Blackwell, Oxford and Edinburgh.

Bennett, E. 1970. Tactics of plant exploration. Pages 157-179 in Genetic Resources in Plants Their Exploration and Conservation, (Frankel, O.H. and Bennett, E., eds.), IBP Handbook, No. 11, Blackwell, Oxford and Edinburgh.

Date, R.A. and Halliday, J. 1979. Collection of strains of Rhizobium. Pages 21-26 in Handbook for the Preservation and Characterization of Tropical Forage Germplasm Resources (Mott, G.O., ed.). CIAT, Colombia.

Gentry, H.S. 1971a. Pisum resources - a preliminary survey. Plant Genetic Resources Newsletter 25: 3-13.

Gentry, H.S. 1971b. Pea picking in Ethiopia. Plant Genetic Resources Newsletter 26: 20-24.

Harlan, J.R. 1975a. Geographic patterns of variation in some cultivated plants. The Journal of Heredity 66: 182-191.

Harlan, J.R. 1975b. Practical problems in exploration: seed crops. Pages 111-115 in Crop Resources for Today and Tomorrow, (Frankel, O.H. and Hawkes, J.G., eds.). IBP2, Cambridge University Press, Cambridge.

Hawkes, J.G. 1976. Manual for field collectors (seed crops.) AGPE: Misc. 7, FAO, Rome, 34 pp.

Ladizinsky, G. 1975. A new _Cicer_ from Turkey. Notes Roy. Bot. Gard. Edin. 34:201-202.

Marshall, D.R. and Brown, A.H.D. 1975. Optimum sampling strategies in genetic conservation. Pages 53-80 _in_ Crop Genetic Resources for Today and Tomorrow (Frankel, O.H. and Hawkes, J.G., eds.). IBP 2, Cambridge University Press, Cambridge.

Saxena, M.C. 1979. Survival of desi chickpeas in the kabuli landraces. International Chickpea Newletter 1:4-6.

van der Maesen, L.J.G. and R.P.S. Pundir, 1984. Availability and use of wild _Cicer_ germplasm. Plant Genetic Resources Newsletter 57:19-24.

Whyte, R.O. 1958. Plant exploration, collection and introduction. FAO Agric. Stud. 41, Rome.

Zohary, D. 1970. Centers of delivery and centers of origin. Pages 33-42 _in_ Genetic Resources in Plants: Their Exploration and Conservation, (Frankel, O.H. and Bennett, E., eds.). IBP Handbook no., 11, Blackwell, Oxford and Edinburgh.