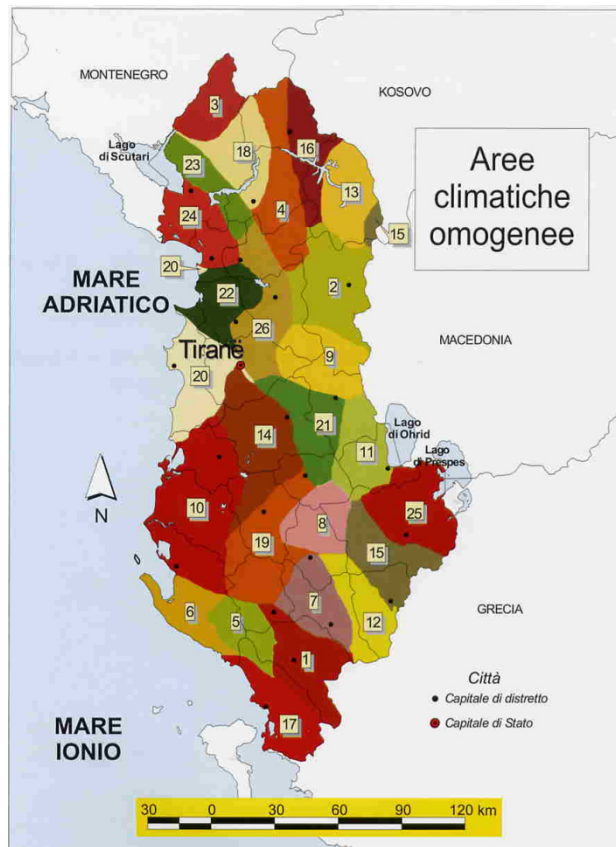


**DRAFT REPORT ON
GERMPLASM ASSESSMENT AND COLLECTION PROGRAM**

**REPORT OF
THE INTERNATIONAL SCIENTIFIC CONSULTANT IN ALBANIA**



Edited by Prof. Luigi Ricciardi

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REPORT ON GERMPLASM ASSESSMENT AND COLLECTION PROGRAM

This document is part of a report edited in March 2003 by Prof. Luigi Ricciardi (international scientific consultant, Di.S.S.P.A., University of Bari Aldo Moro) within an international co-operation on the safeguard and collecting of Albanian genetic resources with The Agricultural Services Project of Tirana (Albania) and the World Bank

SAMPLING STRATEGIES

Another important topic of discussion during the meetings regarded the optimal sampling strategies to adopt in the field and in the conservation of the genetic resources collected in a region.

This is an important task in particular because in the last years, the objectives in the collecting of crop genetic resources, at least with respect to the major world food crops, has changed radically. This to arise by the change in the strategy of safeguard and use of genetic resources.

Today, in fact, there is a greater emphasis on the collecting and conservation of germplasm of the wild and weedy relatives of the major crops; on the main nationally or locally important crops used for food, fibre, medicine or fuel, and particular importance is given to collecting genetic resources for direct use, in particular to replace lost or poorly representative samples or to meet current specific needs of breeders.

Information on the kinds and amounts of genetic variation in target species populations and its distribution in the target region is important in developing efficient sampling strategies. The knowledge of the genetic structure of plant populations has increased markedly (e.g. Doebley, 1989; Hamrick and Godt, 1989; etc.), providing efficient information on actions to develop robust and relevant sampling technologies and on the theory of species germplasm sampling (e.g. Oka,

1975; Marshall and Brown, 1981; 1983; Yonezawa, 1985; Namkoong, 1988; Chapman, 1989; Brown, 1992).

Sampling theory

According to Allard, it is useful to underline how most plant species contain high degree of genetic variation and consist of millions of different genotypes. Indeed, in many species each plant is genetically unique. There are several exceptions to this, even in naturally occurring populations. This is true especially for populations of self-pollinated or clonally propagated colonizers or crops, which can be depauperate in genetic variation and consist of only one or a few genotypes. Yet these species store considerable variation among populations. As a result, a plant collector can hope to sample only a fraction of the variation that occurs in nature. It is important that this fraction be as large as possible and contains the maximum amount of useful (now and in the future) variation. Therefore, when collectors perform their programs they must define procedures of sampling that will allow to collect the maximum amount of useful genetic variation, within a specified and limited number of samples (Marshall and Brown, 1983).

The number of samples that can be considered during a program of safeguard and use of genetic resources is limited. Various factors are related to that limit and regard financial and personnel resources used in any stage of the program (exploration, classification, conservation, evaluation, utilization).

As seen, the development of efficient programs of germplasm safeguard and utilization requires decisions about the species that will be collected and information both on where to find wide variation of the species and where previous missions have been performed.

In this program, while the first point was already decided in relation to the species to collect indicated in the program (wheat relatives and officinal and medicinal species), the last two points are insufficiently documented.

Actually, for many reasons, limited actions of exploration and safeguard of genetic resources have been carried out in Albania (see for example: Hammer *et al.*, 1994; Gladis *et al.*, 1995; Ricciardi and Filippetti, 2000, 2001; Ricciardi *et al.*, 2002, 2003), and furthermore those actions particularly regarded cultivated species.

Poor information is given for officinal and medicinal plants and wheat relatives. However, as said, due to preliminary work started by the scientific staff, decisions have been taken on the species to collect and on the sites to visit. So, it remained to decide the strategies of sampling, in particular for: i) the number of plants to sample per site; ii) the total number of sites to sample; iii) the distribution of sampling sites within each area.

As said, optimal strategies of sampling are related to information on the kinds and amount of genetic variation in the species of interest. Furthermore, it is important to know how that genetic variation is apportioned among genotypes within populations, among populations within a region, and among regions within a selected area. Such pieces of information are not always available and, therefore, to formulate efficient strategies of sampling it is useful to use data of other species, which have been studied in basic work on the genetic of population, extrapolating these data to the species of interest.

However, before starting this action it is necessary to know how the genetic variation can be measured. This, at level of populations can be described by many parameters (e.g. allele and genotype frequencies, gene diversities, heterozygosity levels, disequilibrium coefficients (see Weir, 1990)), but for our purposes, the basic parameter for each population is the allelic richness or the number of distinct alleles at a single locus. In practice, when an estimate of this parameter is made, it is usually the average number of alleles for a large number of marker loci after the sample is taken. This parameter is the basic one for our purposes because later users of the genetic resources can adjust the frequencies of specific desired alleles at will. Thus, the allelic richness of a sample is a direct measure of its value.

In the formulation of appropriate strategies of sampling many studies have been carried out, but their description is not the main aim of this report. For their theoretical conclusions it is possible to consult the papers that the consultant has supplied to the scientific staff (Kimura and Crow, 1964; Marshall and Brown, 1975; Chapman, 1989; Brown and Marshall, 1995; Slatkin and Takahata, 1985; Schoen and Brown, 1991; etc.). Here, instead, it is more useful to give some practical points that must be highly considered during the missions.

By the studies above mentioned it derives that a good strategy of sampling is realized when at least one copy of 95% of the alleles that occurred in the target population at frequencies greater than 0.05 are included in the sample (Marshall and Brown, 1975). A sample of 59 random and unrelated gametes from the population is sufficient to reach this objective.

This can be obtained by collecting and bulking seeds or vegetative material from 30 randomly chosen individuals in a fully outbreeding sexual species, or from 30 random genotypes in an apomictic species, or from 59 random individuals in a self-fertilizing species. A sample of 50 individuals from each population will be considered as a benchmark.

Practical aspects of sampling

Consequently, a basic sampling strategy can be performed considering the:

Number and location of sampling sites: before starting the missions, the collectors must acquire environment data of the target region and information on the distribution of species of interest in the different areas. In relation to those data, and others based on ecological, botanical and agricultural differences, the region will be divided into a limited number of areas, in which collectors will operate in the search of populations. More sites should be sampled in areas where the target species is more common, or where it is evidently more variable for conspicuous polymorphisms.

On the basis of previous experiences, and as weak guide, a sample of 50 sites per species and region is recommended to consider a pertinent sample. This number could increase or decrease when clear reasons for this variation exist;

Number of individual plants sampled at a site: the sample size at a site should be about 50 individuals. This number should be increased when duplications of the sample are expected, in the case that seeds show low viability and in relation to possible loss of some individuals during transport and quarantine. Generally, if it is not possible to collect from 50 individuals at each site because populations are small or shattering has already started, more sites should be sampled;

Choice of individuals: many studies have been performed on the better techniques to sample genetic resources (Marshall and Brown, 1975,1981; Porceddu and Damania, 1992). Random sampling is generally the most reliable and desirable method for crop populations, while for populations of wild species a stratified random sampling could be more efficient because of the presence of local subpopulation.

Biased sampling of rare phenotypic variants in a population is to be avoided, except when such plants clearly merit separate and distinct recognition (e.g. a rare disease-free individual in a heavily diseased field). In this case the sample must be separately collected, receiving separate collecting number.

It will often be advantageous to keep a minimum distance between sampling points to avoid excessive sampling of closely related individuals;

Number and type of propagules per plant: the last decision for the plant collector concerns the kind(s) and amount(s) of material to be collected from each plant chosen for sampling. The material can be different (pollen, seeds and vegetative cuttings or tubers, bulbs, etc.) (Brown and Briggs, 1991).

The seeds are the best material in relation to their easy handling and storage, while vegetative material appear to be less appropriate particularly for the timing of the mission. Cuttings are often the most convenient vegetative samples to collect, but they may require the use of “*in vitro*” techniques.

An important matter to consider regards the variation that is expressed by the genetic resources collected. This will depend on the mating system of the considered species. In the case of autogamous species, seeds will closely resemble the parent plant, as well as for vegetative material, while for allogamous genotypes the seeds will differ from the parent showing within-family diversity (Yonezawa and Ichihashi, 1989). In these species, to increase sample variation, it will be convenient, from each individual, to collecting seeds from several fruits, bulking them.

It will also be better to collect equal number of seeds from each genotype considered. As previously said, the more appropriate number of seeds per genotype to harvest will also depend both on the division that will be necessary to operate on samples for their distribution to collectors and on the capacity of storage of gene banks involved in the missions.

On the basis of what previously said, we can summarize that the strategy for efficient sampling must take into consideration four fundamental criteria:

- 1) sample about 50 populations in an ecogeographic area or mission;
- 2) sample about 50 individual plants within each population;
- 3) generally, at each site of sampling, choose the genotypes at random. When habitats show particular climatic and environmental variations, it will be recommendable to sample separately in each microenvironment;
- 4) sample sufficient seeds or vegetative material per each genotype to assure its best representativeness in eventual samples which will be duplicated.

Clearly, during the programs, in relation to many factors, adjustments to those criteria could be necessary. Some cases are reported below.

For example, a population found only in a narrow geographic range would merit sampling from fewer sites, but with an increased number of individuals at each site, and an increased number of seeds per individual.

In the case of populations that can be scarcely present in a site (i.e. wild relatives) it may be remote the probability to find 50 individuals per population. In this case, it will be useful to increase the number of sites and that of seeds collected on each plant.

When for a species to collect are available seeds and vegetative materials it is advisable, if it is possible, to collect both the kinds of reproductive organs.

Finally, in relation to the mating system of the species, it is useful to confirm that for allogamous species the number of populations in an ecogeographic region can be reduced and the number of individuals per site increased without great loss of variation. When the interested species are autogamous, the populations will be different due to their alleles and on the basis of their variation in genetic polymorphism. Therefore, it will be necessary to collect a large number of populations, even limiting the number of individuals collected at a site.

At this stage of the program and in relation to the sampling strategies to adopt in the missions regarding the areas in which to sample, the consultant and the Albanian researchers are waiting to visit the sites above indicated to confirm the choice of the species to collect, to establish optimal dates to start missions and rational routes to collect Albanian germplasm.

In these days, the period to develop these actions (it depended on meteorological situations) has been decided; it will be in the first or second week of April.

TECHNICAL ADVICE TO COLLECT GENETIC RESOURCES

For all the discussion above presented, in this draft report also some technical advice about the missions of genetic resources collection that will start in a few months are reported.

All the draft report will soon be discussed (April 2003) with the Albanian staff and, where it is necessary, scientific points will be explained, giving others information, news and advice to perform all the work of exploration and collection in a rational and efficient way.

Clearly, further and more precise information about the program will be given in the final report (which will be presented on May 31st, 2003) and, however, after the consultant, together with the national coordinator and other components of the staff, will has performed a mission in various sites of Albania where genetic resources of the species of interest will be collected in the future.

Organization of missions

The efficiency of a scientific program aimed to safeguard, collect, and exploit plant genetic resources depends by the organization of missions that will be performed to collect germplasm, on the information of various kind regarding the environment (ecological, geographical climatic, etc.) in which the exploration will be developed and on the germplasm that will be considered. Before starting the missions of exploration it is necessary to draw efficient logistic plans.

Collecting team

An important point regards the size and composition of the collecting team. As said in the first part of this report, the components of the scientific staff appear to be culturally well matched and this, in relation to cars availability, could enable to have

two teams that will perform the collecting missions at the same time, sharing the different sites of harvest (the suitability for the adoption this strategy will be soon discussed with the Albanian researchers). However, in the team, where the recommendable number of persons will be of 3-5, it will always be useful to benefit from the presence of a botanical expert.

Of great importance will be the participation of an expert driver that should have knowledge of the area that will be explored. It will be important to equip furnish the vehicles with all the useful accessories (Table 13).

Sampling actions

Before starting the missions an accurate itinerary should be drawn, showing the regions, the districts and the sites that will be visited.

The intensity of sampling will vary in relation to the environment and to the consistence of the material to collect. If there is uniformity of conditions for both the factors mentioned, then infrequent harvests performed by coarse-grid procedures could be adopted (e.g. every 10 or 20 Km). On the contrary, where environment and populations are more variable the sampling must be more frequent. In these cases it could be optimal to have temporary bases where to come back and where to take care of the harvested samples; for example, to clean and dry them or to put in order information and passport data.

Sampling of the officinal and medicinal species

The procedures of sampling for officinal species and wheat relatives have been discussed in detail in the paragraph specifically concerning that theme. However, specific information will here be given as to collection of the species belonging to the family of the *Lamiaceae* (thyme, sage, winter savory, rosemary, oregano, peppermint and basil) and the *Asteraceae* (chamomile), which are all characterised by a mostly allogamous system of reproduction, showing entomophilous pollination.

Anyway, under particular conditions, such as the absence of pollinators or population isolation, some self-fertilisations or less extreme forms of autogamy may take place, which can reduce the variability of the accession in a specific site. It is, therefore, good rule that the operator, in case of sites in which there are particular conditions, such as a low number of individuals, geographical isolation, etc., must gather many seed per sample in order to have a good representativeness of the variation in the sampling of that accession.

In the seed collection it is necessary to collect only the driest inflorescences, shortly before the natural seed dispersion and, if possible, in order to reduce the amount of the material to carry, to just simply provide on-site seeds recovery, through inflorescence shaking. For chamomile, instead, since the dissemination often happens shortly before complete dehiscence of the flower, it is preferable to gather the flower when not completely dry.

If in the sampling phase in a determined site the seed of a species is no more available, because already disseminated, it is possible to have recourse to agamic propagation. As a matter of fact, many of these species, excepted basil and chamomile (as already discussed in the special section), may be also propagated agamically or through tuft or by cutting. Under such circumstances, the operator has to keep to the directions about propagation techniques reported, for each species, within the special section., Furthermore, special attention must be given to the sampling modality which must be like the one carried out for autogamous species, because the population variability will be distributed, in this case, among clones (tuft or cutting), and then it is necessary to harvest many clones for each population in order to have a significant representativeness for that population.

As concerns bilberry, it is necessary to harvest particularly ripe fruits and provide, if possible, immediately to the extraction (drawing) of seeds from fruits, to dry them speedily and place them into plastic bags, taking care, as soon as possible, to practise an accurate cleaning and drying of the seeds. Should it be impossible to immediately clean the seeds, it is however necessary to place the bilberry berries

inside plastic and well-closed bags, in order to avoid, in case of fruits' squeezing, the accidental emission of juice which might consequently damage other materials.

Finally, for juniper, as describe in the special section, it is necessary to have a particular care in the choice of the fruits of the 2nd year, situated in the most basal parts of the fruit-bearing branch, because only from theme it is possible to obtain viable seeds.

Duration of missions and equipments

As concerns the duration of the collecting missions, it will depend on various factors (maturity of seeds to collect, different periods of species harvest, etc.), but it is very important to know that the duration is in relation to the period of viability of the material that will be harvested and stored. When seeds or vegetative materials collected are characterized by a low viability they must be early stored at gene bank, a fact which will be highly influencing the number of days of missions.

It is important that the mission staff have rational equipment for any circumstance. In tables 14 and 15 advisable camping equipment and medical supplies are reported.

Data descriptors and passport

All the genetic materials collected must be well documented to give to the users (particularly breeders) optimal chances to use them in subsequent programs. Furthermore, there are minimum data that must be recorded to provide an efficient flux of information among collectors, breeders and gene banks.

To promote standardization of data, the International Plant Genetic Resources Institute (IBPGR) produced for many species descriptor lists in which passport, characterization, and evaluation data are reported. These are not definitive, but they give an idea of what is important to consider during the phases of harvest and storage of genetic resources. An example of a list of essential data descriptors to record during missions is reported in table 16.

In relation to the species that will be collected in our program, particularly for officinal species, there are not related lists; therefore the consultant with Albanian researchers has prepared a passport (Table 17) containing essential descriptors that will be used during the collection of accessions.

As can be seen, passport is a list of data recorded at the time of collecting, regarding information on the germplasm samples and the collecting sites. To speedily acquire that information, passport data have been pre-printed and reported on a single sheet of paper, with clear captions and enough space for text.

These sheets will be distributed to the collectors that will report descriptors relative to each sample on a single sheet. Then, all the sheets relative to one single species, population and visited site will be collected in a book. However, it will also be useful to keep a field notebook in which to report particular data on genotype tolerance or resistance to biotic and abiotic stresses and daily to record other general information related to the travel, visited sites, number of samples collected and opinions about the harvests. It is very important to remember that information must be reported writing in pencil or indelible pen.

In table 18 a list of the basic equipment for data gathering and recording has been reported. Clearly, some of the listed materials are essential and other desirable.

A first group of information reported in sample passport (Tab. 17) regards data useful to univocally identify the Organization involved in the mission, the name of collector or collectors and the number of the collected sample.

Different kinds of registration can be used for those data. It is convenient for each collector to use progressive numbers of identification of the samples. In addition, when it has been decided to collect both seeds and vegetative material of a species, it will be useful to give the same collecting number if the seeds are taken from the same plant from which a vegetative sample has also been taken. If an adjunct of other material (specific for some trait) is carried out to the collected sample of germplasm it will be better to give the same number as the germplasm.

The date of collection should always be indicated. It can be useful to understand reasons of material damage or, in the future, to decide when to repeat a mission. It is important to decide the data format to be used (i.e.: day/month/year or month/day/year).

It could be very useful in the field or at base to take photos of the populations considered and/or of single samples or genotypes. These photos should also be numbered, reporting the correspondent numbers on passport of the sample.

Information on genus, species, subspecies, etc., is indispensable to identify and classify botanically the acquired samples. When doubts persist on species classification, this has to be underlined in the passport and right identification has to be confirmed coming back to the base. It will also be useful to report possible vernacular name of specific genotypes collected and the common utilization of the species products.

Data must be reported on the type of material collected. Generally, the most used can be: seeds, inflorescences, vegetative materials (herbaceous cuttings, bud-wood cuttings, tufts, tubers, etc.), *in vitro* materials, pollen.

The status of sample refers to whether wild, weedy or cultivated populations have been sampled. Other genetic resources that could be considered are: landraces, obsolete and improved varieties, advanced improved varieties, breeding and/or research materials, interspecific derivatives, etc..

The meaning of weedy species must be considered taking into account those species related to crops, but not actually cultivated themselves, which have been subject to disturbance often caused by human activity for establishment and reproduction and which are therefore found on the edges of cultivations, often in close proximity to their cultivated relatives.

A landrace may be defined as a set of populations or clones of a crop species, originally developed by farmers, maintained by them over a long period and recognized by them as belonging to a single entity. However, the term is often used as synonymous with traditional variety. Improved varieties are the product of

scientific plant breeding. Also other kinds of genetic resources could be considered (pure lines, bulks, etc.).

The collecting source will indicate the place where the sample has been collected (field, farm store, market, seed companies, etc.).

In the passport also are reported descriptors that are related to the knowledge on genetic erosion degree of a species in a site. In this regard, it is useful to estimate the frequency of populations in the area around the collecting site and their consistence. We decided to estimate this descriptor by means of the attributes: abundant, frequent, occasional and rare.

Data and descriptions on the prevalent habitat around the area of sampling will be important information. In addition, other descriptive notes could be added. These descriptors may give better information on the localization of collecting sites. Notes on them must be precisely recorded. They could regard the geographic origin of the sample, therefore information on country, district, precise locality where the sample has been collected must be reported. Moreover, a brief description of the main road near to the site and distances from its to the main villages should be described.

The exact locality of sampling must be reported. It will be useful to mark on adapt map the position of the sites visited, also indicating their latitude, longitude and altitude. These data can be read consulting a map (scale of 1: 250,000 or larger) or, for altitude, using an altimeter.

Other information will regard the topography of region and will be aimed to refer to the variation in the elevation of the land surface. Topography traits (flat, undulating, rolling, hilly, steeply dissected, mountainous) will be recorded taking into account FAO guidelines for soil description (1990).

A characteristic that will be assessed will be the soil texture. This gives information on the relative amount of primary particles of different size classes in the fine earth fraction of the soil; in addition its determination is useful to have indication on the edaphic preference of a species. For the determination of this attribute the

extent to which the moist soil may be shaped by hand is used. The descriptors of texture inserted in our passport are: sand, silt, loam, clay and highly organic.

Indications on the degree of stoniness of the soil will be reported in the passport. The attributes that will describe the degree of stoniness will be: none, low, medium and high.

Among the soil characteristics, also the soil pH will be recorded. Generally, soil pH values range from 3.5 to 12, with minimum values for acid soils, 7 for neutral soils and 12 for strongly alkaline ones. Samples for this analysis will be taken into the 20 cm of soil and analyzed with a specific test.

Information about soil drainage will be acquired and reported in the passport sheet, discriminating for: poor, moderate, good and excessive soil drainage.

Other descriptors that will be recorded in the passport will regard the cultivation or the growth of species near the site of collecting and the presence of recurrent pathogens and pests, which can induce damages to the species.

Finally, for samples showing problems in taxonomic identification and classification, data will be reported on the name of the researcher that will classify the sample, on his Institution, on the date of analysis.

Sample treatments

An important task in collecting missions regards sample conservation in the course of missions. This is in relation to the types of seeds or vegetative materials that will be collected in the field. Some advice on these topics must be given.

For seeds, it can be advised to collect an equal number of seeds (at the same maturity stage) from each plant sampled, avoiding the collecting of damaged seeds.

They must be stored in permeable containers such as cotton or paper bags that enable aeration of samples, reducing the possibility of mould formations (a sample of those will be showed to Albanian researchers that will provide for their supply). During the transport, occurring high degree of humidity, it will always be necessary to minimize environment and seed moisture.

In the case of vegetative material samples, these rapidly can deteriorate after harvest. In addition, they are also easily damaged in transport. For these reasons, they must be properly prepared and housed in suitable containers. Materials that do not sprout soon after harvests generally require only to be placed individually in strong paper bags or newspapers and loosely accommodated in boxes with soft packing material. Also for these samples excessive temperatures and humidity must be avoided, promoting samples ventilation.

Growing materials generally require greater care. Stem cuttings must be operated at middle portion of the stem and they consist of several nodes. Leaf surface must be reduced to a minimum, avoiding rotting. Fleshy stems must be stored in wet papers or newspapers that must be put in insulated cool boxes. Samples should be examined regularly to keep wrapping papers moist.

On arrival at gene bank, the material will need to be planted out fairly quickly. On this purpose, during the meetings with Albanian researchers, it has been pointed out how sites where it will be possible to grow the acquired genetic resources are the experimental fields of the: Agriculture University of Tirana, National Seed Institute of Tirana (Germplasm Unit), Cereal Institute of Lujnia, the Botanical Garden of Tirana.

In a program of germplasm safeguard, important work phases also regard operations that must be carried out returning to base. Attention must be taken to samples because seeds and vegetative materials may be at risk. Those operations will be applied both in the case that samples must be split for conservation and distribution to other Institutions and when samples must be stored.

Very soon seeds may reduce their viability and this phenomenon is highly correlated to various factors, most especially temperature and seed moisture content. It is well known, particularly for orthodox seeds, that decreasing temperature and moisture content will result in an increase of seeds viability. In contrast, there are seeds, named recalcitrant seeds, which have not benefits when reduction of temperature and moisture is operated.

A first action to be carried out early consists in drying the seeds. The hotter and more humid the climate, the more unfavorable the conditions are for drying seeds. As an example, it can be said that at a RH of more than 75%, the equilibrium moisture content of non-oily seeds will be >13% and that of oily seeds >9%. Since viability loss in orthodox seeds is greatest at intermediate moisture content of 15-30%, it is dangerous that seeds stay at these levels of moisture for long time. Therefore, the seeds must immediately be put under an artificial system of drying to quickly reach moisture content of 15% or less. Drying is most efficient if samples are stored in bags, with good ventilation and in the shade.

Different procedures will be applied in the case that natural drying is not possible. This happens when the environment of first storage of the samples presents high humidity or when samples must be definitively stored at humidity and temperature preferential for medium or long conservation.

In this case hot air can be used, but this method is not recommended, particularly when the seeds are moist. An alternative is to dry seeds by dehumidification at low temperature.

However, an efficient method to dry seeds both during the collecting in the field and in seed treatment performed at base (logistically complicated in the former case) can be developed by using silica gel. It is possible to use a ratio of seeds to silica gel of 3:2 and place the seeds in a thin layer just above the dehydrated gel, inside a desiccator or any container with a moisture-proof lid and large area:volume ratio. Zhang and Tao (1989) report periods of two days to two weeks (depending on seed size) to dry seeds from >15% moisture content to <7%.

When seed moisture content has been reduced it is possible to start the phase of seed cleaning.

To minimize the risk of pests and diseases, seed samples must be cleaned to eliminate soil, weed seeds, parasites. Chemical treatments should be avoided because they can damage seed viability. However, if necessary samples can be treated, describing on bags the chemical used.

Another important action that will be performed returning to the base regards the processing of all the collecting data, which will be distributed with germplasm samples. Then, all data must be checked and sorted, adding information from reference sources. In this phase, the computerization of data is desirable.

Sample distribution and germplasm conservation

Completed the operations described above, it will be necessary to split samples. A part will be used for storage, while other seeds could be used both to be distributed to gene banks and/or in field tests of seed multiplication and/or evaluation of the accessions.

In relation to seed distribution, it is important to note that when a species collection is established there will be parts of that aimed at different objectives. Base collections are established for long periods of storage and are not actively grown. Generally, it is advised that indicatively the number of seeds of those collections should be very high and it is related to the kind of population. According to the Manual for Field Collectors, edited by the Food and Agriculture Organization of the United Nations (Hawkes, 1976), which will be distributed to collecting staff (see annexe 1), base collections of highly variable populations should be established with about 12,000 seeds; this number could be lower: about 4,000 seeds, for fairly uniform populations. For base collections, as safety criterion, also duplicates should be established, containing 3,000 and 1,000 seeds, respectively.

Another type of collections are named active collections. They are materials stored for medium to short-term period (5-20 years) and are used for multiplication, distribution to breeders, evaluation and other purposes. These active collections should be established with 5,000 seeds for allogamous species and 3,000 for autogamous ones.

By means of these data it can be said that for the collections of allogamous species the suggested optimum total number of seeds per population sample is of about 20,000 seeds while, in the case of autogamous species, it is of about 8,000

seeds. These indicative data also should be considered during our phases of exploration, with the aim to have germplasm samples composed of a number of seeds numerically consistent.

However, as said, various factors could limit the optimum number of seeds/sample collected. It is useful to clarify that when, for various reasons, the number of seeds collected doesn't reach the indicated amounts, nevertheless the accessions will be stored in the best way and, before starting sample distribution, a multiplication of the original seed samples collected in the field will be necessary. Then, normally also samples of 1,000-1,500 viable seeds are acceptable for base collections.