

FIELD EVALUATION AND UTILIZATION OF COLLECTIONS OF CEREAL GENETIC RESOURCES: THE CURRENT STATUS

Ardeshir B. Damania*

Genetic Resources Conservation Programme
University of California, Davis
California 95616-8602, (USA)

Large collections of cereals harbouring a sea of variations have been assembled in gene banks around the world. These collections need to be utilized in crop improvement to justify high amount of effort and time expended and the financial cost involved in maintaining them at sub-zero temperatures. Evaluation is the essential link between conservation and use. Various approaches to evaluation of germplasm have been described but the optimal is one that not only satisfies the criteria for current breeding goals but also takes into consideration the needs of the future since in-depth evaluation of large collections cannot be repeated at frequent intervals. The use of modern techniques, such as tissue culture, embryo rescue, and genetic engineering have resulted in greater use of wild and alien germplasm from the secondary and even tertiary gene pool for crop improvement. The rich genetic resources of such gene pools are increasingly tapped to overcome the constraints to food production in moisture limiting and other stress prone environments.

Key words : Breeding strategies, crop improvement, documentation, *Hordeum* spp., landraces, obsolete/primitive forms, *Triticum* spp., wild progenitors.

As a result of considerable increase in collection and conservation activity of crop plant genetic resources during the last two decades a very large quantity of germplasm accessions of cultivated and obsolete/primitive forms of crop plants have been assembled at various genetic resources conservation centers around the world. However, genetic resources merely kept safely in storage can be of little value to plant breeders for utilization in their programmes unless these are evaluated and the data is made available through communication and exchange. Evaluation is essentially the link between conservation and use. Keeping this in mind it would be conceptually much more fruitful to develop a working collection of germplasm for specific traits in order to

*Present Address : 18-20 Cawsji Patel Street, Fort, Mumbai 400 001, India

conserve germplasm in a more usable form. The information on a large number of stored genetic resources is often limited to an accession number, a seed source and sometimes, the country of origin.

Systematic evaluation of the biodiversity in large collections requires a multi-disciplinary team and an inter-disciplinary approach (Chang, 1985 a; Frankel, 1970). Interaction between members of the evaluation team should be at optimal level for more rewarding results and greater utilization of germplasm.

The aspects of evaluation and documentation of cereal genetic resources has been reviewed recently by Damania (1990). Problems arising during evaluation of large collection and suggestions to solve them have been given by Pecetti et al. (1991). The utilization of wheat genetic resources to meet diverse needs has been documented for cultivated types by Srivastava and Damania (1990) and for wild forms by Damania (1993). This paper will endeavor to stimulate discussion regarding correct handling of cereal germplasm in order to optimize its utilization for current breeding goals as well as for future contingencies.

GERMPLASM EVALUATION

The evaluation process in seed crops may involve some of the following steps (Chang, 1985a):

1. Seed multiplication and preliminary evaluation: During the initial cycle of seed multiplication the evaluator or germplasm curator should note some of the morpho-agronomic features and other observations of interest such as incidents of diseases and pests. Obvious duplicates are eliminated and promising selected accessions are re-planted for intensive further evaluation. Plant quarantine regulations, if applicable, must be observed.

2. Systematic characterization: For base collection maintained by conservation centers extensive observations are recorded. For instance, durum wheat (*Triticum durum* Desf.) germplasm evaluation at ICARDA records information on 29 different characters (Srivastava and Damania, 1989). However, handling such voluminous amount of data requires dedicated attention to detail and is manageable only if executed by a competent team of scientists.

3. Further evaluation of selected accessions: Mass screening of accessions from a large collection must be subjected to refined testing procedures at different locations as many economically desirable traits are controlled by polygenes which behave differently in diverse environments. After this exercise the number of accessions will have already shrunk to manageable size for the breeder to handle effectively.

Germplasm which survives detailed evaluation and testing is normally suitable as potential crossing material for one or two specific traits, hence it could be utilized as a donor of these traits rather than as lines for release as commercial cultivars.

At the present time several evaluation projects have been undertaken in response to current selection criteria of the breeders. This approach may not be optimal because the probability of a sample being examined for a given attribute is solely dependent on present needs. However, a more comprehensive approach can provide information suitable for a more wide use. It is not implied here that it may be possible to always ascertain breeding aims of the future. For example, at the turn of the century, short stature (dwarfness) was not considered a desirable trait as it was calculated to be linked to small grain size and hence low yields.

For the purpose of utilization, systematic analysis and description of samples is useful both in distinguishing between populations, identifying duplicates as well as providing information on the extent of variation within a given germplasm collection. It is axiomatic that the more documentation on a collection, the greater the chance of its rational utilization. Information from the site where a particular sample was collected may be extremely important.

Inadequate passport data very often inhibit effective utilization of collected germplasm. It must be emphasized to collectors and gene bank managers that passport data supply extremely valuable, in many cases the only available, information on the ecological adaptation of an accession and hence efforts should be taken to fill this important gap in documentation of germplasm (Frankel, 1987).

Information on the genetic variability of a sample is extremely useful and the eventual objective of every evaluator should be to describe the variation on the basis of a list of differences between and within samples in the sequence of nucleotides in the deoxyribonucleic acid (Erskine and Williams, 1980). At present, the study of storage protein (prolamins) variants by electrophoresis is the most convenient and rapid method available for detecting genetic differences close to the DNA in a cereal collection (Damania et al., 1983). However, it may be argued that the heterogeneity in storage proteins alone is of little value to the breeders or genetic conservationists because its correlation with any single agronomic character is obscure. Nevertheless, these markers can monitor the relative genetic diversity with a greater degree of accuracy than by other methods used in the past (Brown, 1978; Damania, 1983). A pre-screening procedure for identification of the ploidy levels and chromosomal aberrations such as deletions in wheat, with the use of electrophoresis has been described by Damania (1985). Electrophoretic technique can also be used

as a tool for elimination of duplicate stocks in gene banks (Damania and Somaroo, 1988).

In any survey of the distribution of genetic variability within a crop species of economic value or its wild relatives, the most obvious pattern which emerges is the variability associated with broad geographical regions (Allard, 1970; Damania et al., 1996). It is important to sample not only the broadest geographical variability but also to have samples from the extremity of distribution of the species, i.e. the marginal areas. According to the hypothesis of 'peripheral diversity' put forward by Yamashita (1980) it has been suggested that there is considerable accumulation of diversity where a species has reached its geographical limits due to physical or climatic barriers which it cannot traverse.

Evaluation of cross-pollinated and self-pollinated plants differs when it comes to describing variability within a population. Some have recommended considering 50 individual plants per cereal populations which are largely self-pollinated. Others have suggested taking at least 100 seeds from 50 individual plants of a cross-pollinating species. Unfortunately there can be no universal answer to this question because each species sampled from a particular geographic area may represent a unique problem in evaluation. A precise solution would require information on variates of each individual ecological system, observable 'marker' characteristics associated with and indicating variability and ways in which individual variates interact with climatic features of the area to produce the overall variation pattern.

Systematic description of samples for discrete traits has been limited to curtailing the phenotypic variation because of constraints in relating genotype with phenotype. Quantitative morpho-agronomic traits are also currently used in characterization. These traits are controlled by many genes which have a small effect which is quite often blurred by the environment. Consequently the correlation between genotype and phenotype is obscured.

Many evaluation studies have used ranking as a method of describing results. This ranking may change from one site to another for some quantitative characters such as plant height and days to heading (Damania, 1983). Such unstable characters cannot be adequately described when studied at a single location. Thus the concept of multi-location testing becomes imperative.

And lastly, there are those traits, such as resistance to diseases and tolerance to certain types of soils (such as saline) for which variability can only be observed at particular sites. Such traits are extremely important economically and every effort must be made to record them by carrying out the evaluation at particular sites where the incidence of that particular disease or pest is the greatest, the so called "hot spots". For example, for screening against resistance to *Septoria tritici* (leaf blotch) ICARDA use a humid and

high rainfall site located near Lattakia on the Mediterranean coast in Syria. For experiments on tolerance to salinity and drought affected site on the shores of salt lake Jabboul in northern Syria is used. Jana et al. (1983) first used this site to evaluate 3000 durum wheat accessions from various countries and ten lines were found to be highly tolerant to combined stresses of salinity and drought.

Wherever the purpose of evaluation is clearly known at the start the task is relatively simple. However, in the case of most wild and obsolete/primitive forms, evaluation aims to reveal potentially useful variability for direct use in the breeding programmes. This may necessitate initial characterization nurseries (Damania et al., 1995) and cataloguing of passport information followed by a more detailed field study in collaboration with the end-users of the germplasm.

Although improving yield and yield stability are the ultimate goals in most plant breeding programmes the most sought after characters in exotic landrace germplasm are disease and pest resistance, tolerance to stresses such as temperature extremes, drought, salinity, etc. and nutritive values. For example, in the case of wheats, these characters are: stable resistance to various rusts, and *Septoria tritici*; new sources of genes for semi-dwarfness; greater tolerance to stresses such as droughts, salinity and temperature extremes; earliness; cytoplasmic male sterility; as well as other factors related to wide adaptability. Some of these traits are highly heritable, others are tightly linked to undesirable traits.

METHODOLOGY FOR EVALUATION

Evaluation process begins when a sample is collected because it is at the site itself that the collector takes certain decisions regarding the sampling procedures he applies to the population which determines the efficiency of subsequent evaluation. Therefore, when collected germplasm samples arrive at a genetic resources centre the process of their evaluation and documentation has already commenced. For a review of sampling variation in genetic resources of seed crops see Porceddu and Damania (1992).

In order to save time and effort, multiplication of the seed material is normally carried out at the same time it is evaluated. But sometimes, especially in the case of wild germplasm, this is not possible due to paucity of seed in the original collection. In this case the multiplication has to be carried out in the first season (or in the glass house for precious wild material) and later evaluated in the second season.

A population structure of a species is defined as the totality of ecological and genetical relationships among individual members which may co-evolve as a result of gene exchange but may also diverge under localized forces of

evolutionary change (Jain, 1975). Landraces and obsolete/primitive cultivars are as a rule products of several years of crop evolution and it is vital to preserve their genetic composition during and after evaluation. Instances have been reported where polymorphic cereal populations have undergone radical changes in their genetic composition in one growing cycle (Shevchuk, 1973). However, in the case of samples collected from village markets or those which are subjected to biased sampling methods, it is sufficient to safeguard and maintain their genes and not necessarily their gene frequencies within populations.

It is ideal to evaluate accessions of germplasm at suitable sites in countries of origin but this is not always practical for several reasons. Alternatively, the ecological environment of growth for the purposes of preliminary evaluation could be made as identical as possible to that of the collection area. However, this is not always possible for in a world collection samples originate from all corners of the globe where cereals are grown and from different ecological zones. Therefore, no single location can be entirely suitable for all samples. ICARDA is fortunate in being located within the centre of diversity for wheat (*Triticum* spp.) and barley (*Hordeum* spp.) and, as such, is as near to an ideal site for evaluation of these two genera (Srivastava and Damania, 1989). Evaluation carried out at such almost ideal site not only minimize the effect of natural selection on the samples' genetic make-up but also provide an adequate harvest of seed quantity sufficient enough to make further multiplication redundant.

Several International Agricultural Research Centers (IARCs) have more than one location for the evaluation of their germplasm. For instance, the International Centre for Maize and Wheat Improvement (CIMMYT), evaluates samples of maize that originate from an altitude below 1500m asl at Tlaltizapan, Morelos (940m asl) whereas those samples from above 1500 m are grown at El Batán (2249 m asl).

The relationship between active collections of national programmes and base, long-term collection located at an IARC is an important link in the chain of evaluation work and the flow of data and other information. For example, in the preliminary evaluation and subsequent regeneration of germplasm ill-adapted to the environment of the centre cooperative arrangements with the national programmes play a vital role. These arrangements also help in the eventual flow of selected germplasm and data (through international nurseries) into the national breeding programmes.

All IARCs possess their own base collections of the mandate crops and these usually operate through a genetic resources unit. A base collection may be duplicated at a suitable national gene bank to avoid total loss due to natural or man-made calamities. The centres with common mandate crops

such as CIMMYT and ICARDA could exchange and meticulously compare accessions to minimize the maintenance of obvious duplicates and to ensure that no distinct ecostrain is over-looked in the inventorial process. Original names and accession numbers should be maintained as far as possible when germplasm is received at the genetic resources units in order to cross reference them later to avoid duplication. Therefore, major germplasm collections like the ones maintained at the IARCs should be encouraged to standardize their data systems and achieve sufficient compatibility for easy accessibility and exchange of germplasm data.

Systematic evaluation is an expensive and time consuming process as any worker in this discipline will agree. Therefore, it is imperative to carefully choose the traits which one wishes to evaluate. Priority-wise, the inclusion of traits in an evaluation programme will result in optimal use of physical facilities, manpower and financial resources.

UTILIZATION OF GERMPLASM COLLECTIONS

The criterion for assessing the success of a crop-oriented germplasm evaluation project is the use to which the conserved genetic resources are put. Varietal improvement and the incorporation of yield stability in the improved cultivars through the use of landraces has also been impressive in the cereals. For example, Duwayri *et al.* (1987) crossed "Stork", a semi-dwarf high yielding cultivar under optimum conditions, with "Haurani" the local well adapted landrace in Jordan and Syria which produces reasonable yields under stress conditions. A number of lines which resulted from these crosses appeared promising in low as well as moderate rainfall zones.

There are three ways in which obsolete/primitive forms and wild relatives of our cultivated cereal crops can be utilized (Frankel, 1970): 1. Introductions for direct use as crops. 2. Introductions which can confer particular traits to the adapted cultivars such as, disease resistance, protein content, etc. This type of utilization is the most prominent way in which obsolete/primitive forms can be utilized and the only way for the wild relatives. 3. Introductions to increase yield *per se*, irrespective of resistance to physical or biotic stresses present in the environment.

However, the wild species remain the least collected, conserved and exploited category of germplasm, especially from the secondary gene pool. The extent of evaluation and initial usage (some times called "enhancement") among the three categories of germplasms are almost proportional to the degree of utilization.

Plant breeders in wheat producing regions of the world mostly use breeding material with known agronomic data and background. They are reluctant to cross their commercial varieties, even after these have begun to

deteriorate in performance, with an obsolete/primitive landrace or wild species simply because they do not want to introduce undesirable genes into their breeding programme (Srivastava and Damania, 1989). Lack of information and the unavailability of substantial seed quantities are additional impediments in the use of these germplasms.

Spagnoletti and Qualset (1992) make a strong case for the maintenance of a "core collection" in order that genetic resources in gene banks are utilized in crop improvement programmes by breeders. The concept essentially advocates separating a few hundred samples from a much larger collection consisting of thousands of samples. This core collection may contain a wide range of variability for traits which are considered desirable by current breeders of the crop. The composition of the core collection may change from time to time as newer needs for particular traits become evident or as material which was not previously available joins the main collection as new acquisition.

It becomes obvious that if greater use of obsolete/primitive and wild material has to be made it is essential to remove (or at least suppress) the close linkage between desirable traits and unfavourable alleles. This may be done through transporting the germplasm to areas similar to the native habitats where selection can be carried out under favourable conditions of soil, photoperiods and temperatures. For wild species, particularly the putative progenitors, either a naturally introgressed population or an artificially directed back-crossing programme would improve their chances of inclusion in a breeding programme (Chang, 1985b). This preparatory activity is often referred to as germplasm enhancement or pre-breeding.

Sears (1956) gives a good example of pre-breeding efforts involving a wild relative of wheat. *Aegilops umbellulata* was initially crossed with *T. aestivum* cultivar but the F1 was male sterile and had to be back-crossed to *aestivum* twice. The progenies of this back-cross were tested for leaf-rust resistance which was present in the wild species. A resistant plant was isolated carrying 21 bivalents. This plant was then crossed with the then high yielding cultivar "Chinese Spring" to produce "Transfer" which was widely used in North America as a rust resistant cultivar. Since then other wild species of wheat have been utilized by Canadian and U.S. breeders as a source for improving winter-hardiness, short stature and cytoplasmic male sterility (Stalker, 1980).

The current situation in cereal breeding demands continual evolutionary studies and mobilization of new and different sources of germplasm. Most of these potential sources incorporate the true landraces, obsolete/primitive cultivars and the wild relatives from the primary gene pool.

At the International Rice Research Institute (IRRI) the utilization of these forms has already yielded promising results in producing lines with disease resistance as well as tolerance to drought and salinity. A strain with an

apparently desirable trait, e.g. disease resistance, may not be truly resistant; not being infected by chance or a result of genotype \times environment interaction under the prevailing test conditions. Therefore, any resistant accession found in the preliminary evaluations of large collections must generally be retested, often with several replicates and in more than one season by refined techniques to ascertain the true nature of the resistance. The same applies to abiotic traits such as tolerance to temperature extremities, drought and salinity.

At ICARDA selected germplasm for low rainfall areas is tested at Breda and Bouider for wheat and barley accessions, respectively. This is done over several seasons with the weakest accession being eliminated after each selection process and only the truly drought tolerant lines are retained for crosses to incorporate this attribute in the cultivars.

Invariably in all situations the early generations derived from wheat \times alien species hybridizations tended to be poor agronomic plant types. However, by employing appropriate breeding methodologies, plant types can be significantly improved phenotypically up to the state that alien disomic chromosome additions or derivatives with subtle alien introgression are produced.

The utilization of species even from the primary gene pool or wild progenitors presents some problems as several undesirable traits from the wild show up in the progenies of crosses and these need to be eliminated by repeated selection process. For example, in a simple *Triticum durum* \times *T. dicoccoides* cross characters from the latter species, such as brittle rachis, glume hairiness, profuse unsynchronized tillering, hybrid necrosis, grass clumping and loose crown, persist in subsequent generations but rapid progress can be made by making a top cross of this material with durum wheat. The *Triticum durum* \times *T. dicoccoides* cross also transfers some disease resistance qualities from the wild species to the cultivated. These crosses have also shown that selection for high protein content as well as yield can be transferred to the cultivated form. Whereas the durum wheat have a relatively low percentage of protein and the *dicoccoides* a higher figure, in the resulting progenies high percentage protein is maintained. Similarly, the 1000-kernel weight of the durum varieties is much higher than that of the *dicoccoides* and in most of the progenies high 1000-kernel weight is retained (Srivastava and Damania, 1989).

The wild progenitor of barley, *Hordeum spontaneum*, was probably domesticated within the 'fertile crescent' region. This region is, therefore, in a unique position to assess its potential for improvement of barley yields in the dry areas. In recent years the barley project has commenced evaluation of *H. spontaneum* collected from the region where it can be found growing under

diverse ecological conditions. Because it also occurs in very harsh environments it is considered to be a probable source of resistance to drought.

The utilization strategies of *Horedum spontaneum* are as follows : a) screen for tolerance to drought; b) evaluate the extent of diversity within the species for agronomic traits; c) select a number of accessions to initiate a crossing project with cultivated barley and d) evaluate a number of early generation families between the wild progenitor and cultivated barley plants with non-brittle rachis. Evaluations carried out so far have revealed considerable variability in the collections for growth habit, cold resistance and days to heading. Accessions with a relatively good potential for crosses with improved cultivated barleys were identified (Ceccarelli *et al.*, 1987). Crosses will be made with selected cultivated lines (used as maternal parents) adapted to arid environments. Early generations of the hybrid material will be grown at several sites representing the actual barley growing environments.

CONCLUSIONS

The assessment of use of germplasm collections in crop improvement for the major cereals has revealed the following:

1. The use of exotic germplasm: The successful use of landraces and wild species in cereals has been more extensive in the developed countries, which lack original indigenous germplasm, than in the developing countries. This has been partially due to the fact that exploitation of these germplasms was undertaken earlier by the former. After the creation of the IARCs the benefits of such germplasms have improved crop production on a global basis. Both Sudan and Syria have become self-sufficient in wheat production for the first time this century largely due to the adoption of improved germplasm developed by the IARCs.

2. Constraints to the use of exotic germplasm: Many plant breeders have been reluctant to devote a greater part of their resources for the exploitation of landraces and wild species in the past. This was because the potential value of these germplasms was not fully known. Insufficient germplasm pre-breeding, lack of communication between gene banks and users and little or no feedback from users are some of the other reasons often cited for poor utilization of the exotic genetic resources.

3. Support for plant genetic resources programmes: Extensive use of landraces, obsolete/primitive forms and wild species appears more tenable when the process of conservation, evaluation and exchange of germplasm is strengthened and adequately funded. Improved communication channels and maintenance of core collections can also augment breeders' use of exotic genetic resources.

4. Use of computers and software packages: Software packages designed for analyzing large quantity of evaluation data have greatly reduced time and effort needed for arriving at tangible conclusions and the selection of genotypes with superior performance. This in turn has led to the publication of germplasm catalogues which have greatly facilitated dissemination of information on germplasm collections to actual users, thus encouraging greater utilization of services rendered by gene banks.

5. Applications of bio-technology: Modern techniques such as chromosome engineering, anther culture and haploid breeding in cereals, could serve as a powerful tool towards reducing research time and can expand considerably the horizon of plant improvement.

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