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The core as a guide to the whole collection

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Abstract

The composition of a germplasm collection is dependent upon the way the collection has been accumulated and conserved. A curator's knowledge and good databases enable a collection to be systematically organised and patterns of variation revealed. However, deficiencies in the comprehensiveness and quality of information in the database need to be considered when selecting germplasm for a core collection. Users' knowledge of the germplasm collection varies; curators should therefore strive to make germplasm collections 'user friendly'. Evaluation has been conducted on a massive scale in some crops. Where a required genotype is not rare, evaluation can benefit from using a core collection. Examples from major crops in US agriculture with large germplasm collections reveal sustained yield gains over many decades despite the rather limited use of conserved germplasm. The rigorous selection during the breeding process suggests that the relatively few ancestral varieties contributing to released varieties should also form a portion of a core collection. Curators need to focus their attention on germplasm which will complement this in the composition of a core collection. The key to the development of core collections is reliable information on all the accessions in the whole collection. Old and new information can be used to sort germplasm and select accessions for a core collection. The use of core collections developed from the whole collection of cultivated and wild rice has enabled scientists to identify rapidly the germplasm they require.

Germplasm banks exist primarily to furnish germplasm and information on germplasm to current and future germplasm users. The conserved germplasm supplied to plant breeders for use in the development of improved varieties for farmers ultimately produces the economic return on investments made in germplasm collecting, preservation and evaluation.

The core collection, which is a part of the active collection, aims to reduce genetic duplication and redundancy while maintaining maximum genetic diversity (Frankel, 1984). There is no way at present or in the foreseeable future that genetically identical accessions in a gene bank can be identified with complete certainty. Thus, the responsibility for the long-term conservation of the whole collection, which lies with gene bank curators, will continue and will not be affected by the development of a core collection. The relationship between the whole (or base) collection and its core is the focus of this

chapter. In particular, we draw on our experiences with the collection of rice maintained at the International Rice Research Institute (IRRI) in the Philippines, where pilot studies have been initiated to determine the value of the core collection concept.

STRUCTURE OF A GERMPLASM COLLECTION

Germplasm collections are organised in different ways, but they all consist of some or all of the following: landraces and selected lines from landraces, elite breeding lines, released varieties, wild and weedy relatives of the cultigen, and genetic stocks (*see* Figure 1). These components show differences in genetic diversity within and between accessions; often, there are fewer accessions of the genetically most diverse germplasm such as wild relatives of crops.

The phases in the growth of the rice germplasm collection at IRRI, which are probably similar to those of some other collections, can be summarised thus:

1960-72: Donations of germplasm from existing collections

1972-85: Active collecting for rice landraces

1987-93: Active collecting for wild rices

Figure 1   **Components of crop gene pools**

Source: Adapted from Chang (1985)
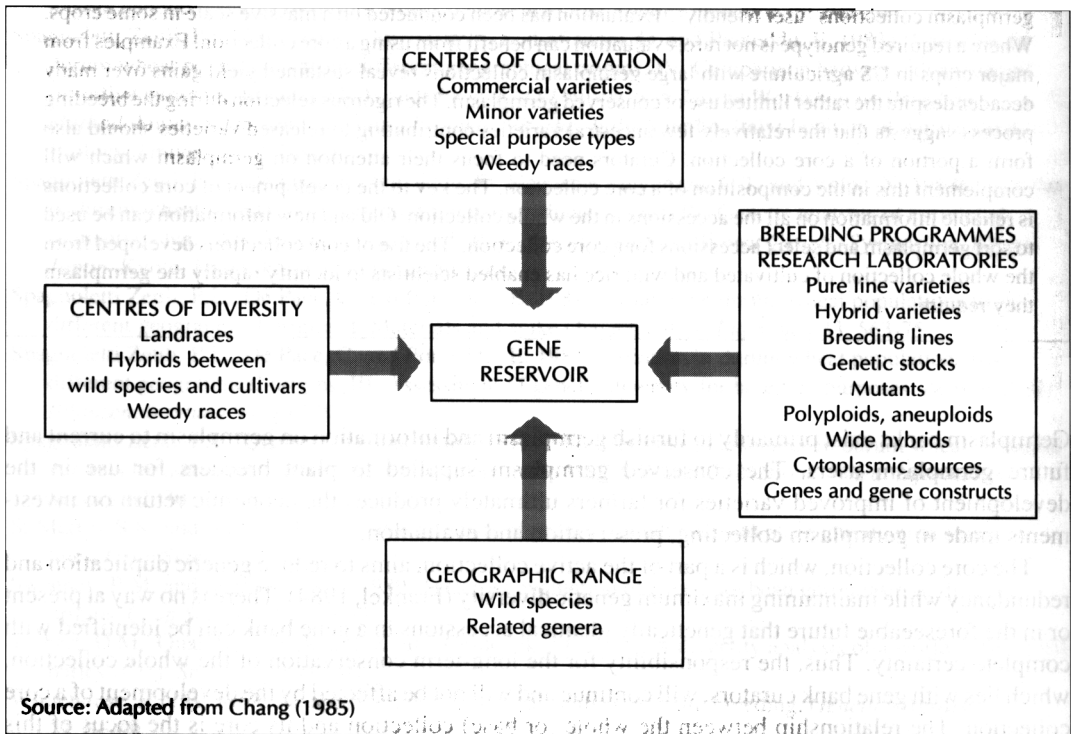
As a collection increases in size, so newly received germplasm requires careful checking for duplication of existing conserved germplasm before accepting it for long-term conservation. For seed-propagated species, this is a particularly difficult task.

It is often assumed that because a germplasm collection is very large, it is complete. The large size may hide the fact that germplasm from some areas may be over-represented, while that from other areas is unrepresented or under-represented. Although the germplasm collection at IRRI consists of about 80 000 accessions, it was only in 1992 that a comprehensive collection of rice cultivars was made in West Kalimantan, Indonesia. A four-volume work on the rice varieties of Mozambique (Gonçalves Valente, 1968) shows clearly that germplasm from Mozambique is under-represented in the collection (there are only eight Mozambique accessions in the collection). On the other hand, a collection of rice germplasm recently received from Cambodia had 59 variety names represented more than five times, suggesting considerable duplication during the collecting phase. The larger and more diverse the collection is, the more difficult it becomes to conserve germplasm in a routine way. Certain types of germplasm are more difficult to maintain than others, because of the environment in which they are grown or the inherent nature of the material, and the interaction between these factors (Chang, 1989). In selecting a core collection, these aspects should be taken into account to ensure that a representative sample of the whole collection is provided to germplasm users.

DATABASES

As germplasm collections increase in size, a good database is of fundamental importance in managing the collection and revealing patterns of variation. The database, coupled with a broad knowledge of the germplasm, becomes the basis for selecting accessions for the core collection.

Germplasm databases go through various developmental stages, usually starting with handwritten records before computerisation. The data obtained on an accession and incorporated into a database comprise three types: passport data (obtained at the collecting site), characterisation data (inherent characteristics of the germplasm) and evaluation data (germplasm screened in response to biotic and abiotic stresses). To this should be added management data which may or may not overlap with passport, characterisation and evaluation data.

New ideas, needs and concerns lead to improvements in germplasm databases from time to time. Thus, isozyme characterisation is now added to the characterisation database for rice. Isozyme classification increases our understanding of the genetic variation of varieties from different regions. In addition, varieties of certain isozyme groups have a shorter storage life; this information is helpful in managing the collection. Other biochemical and molecular characteristics of germplasm are likely to be incorporated into the existing germplasm databases in the future. Similarly, improved evaluation methods may stimulate re-evaluation of germplasm and evaluation for newly added descriptors (for example, tungro disease of rice is now known to be a complex of two viruses). New stresses arise requiring new evaluation methods (for example, evaluation is needed to identify germplasm resistant to parasitic plants of the genus *Striga*, which is increasing in importance in some African countries).

The database at IRRI has about 5 million items of information on the 80 000 conserved accessions of rice. However, the information available on each accession and on the germplasm collection as a whole depends upon many factors, including the relative importance of the information, the cost and the time taken to obtain information on the trait. Some traits may have priority for evaluation, but no reliable screening techniques have been developed to obtain reliable data on them. One example is the identification of germplasm adapted to different edaphic stresses. However, some stresses can be

quickly, accurately and simply scored, such as the clipping method for evaluating the reaction of rice to bacterial blight (*Xanthomonas campestris* pv. *oryzae* ([Ishiyama] Dye) (Kauffman et al., 1973).

The accumulation of data in a well-organised database permits analysis which can provide new insights into the germplasm. For example, analysis of green leafhopper resistance revealed that a high proportion of resistant accessions are distributed in West Asia (Vaughan, 1991a). Similarly, a search for rice germplasm which has both good elongation ability and flood tolerance revealed that no single accession with both traits was available out of the 903 accessions screened. These two useful traits are probably mutually exclusive.

Good information on a germplasm collection is also essential to enable curators to respond appropriately to seed requests. Between January 1991 and June 1992, all 122 foreign seed requests for germplasm received at IRRI's germplasm bank were specific in nature (*see* Table 1). Germplasm users are often unaware of the core collection concept and the existence of core collections. When non-specific or broad seed requests are received, germplasm curators should promote accessions in a core collection as these would be a relevant starting point for experimental purposes.

Table 1 Foreign seed requests for conserved germplasm received by the germplasm bank at the International Rice Research Institute, 1991 to mid-1992

Total number of requests	122
Total number of countries	18
Type of request:	
Specific varieties/accessions	95
Specific trait/environment	19
Specific group/origin	3
Combination of variety, trait and site	5

USE OF THE WHOLE COLLECTION

Germplasm collections are used by scientists for basic, strategic, applied or adaptive research. Scientists vary in their knowledge of collections, and curators could help by making collections 'user friendly' for those least familiar with the collection. In general, the major use to date has been for strategic research, particularly evaluation of germplasm for specific traits for use in plant breeding. The use of exotic germplasm in basic research, such as biodiversity studies and adaptive research involving multilocational testing of germplasm, is likely to increase in the future. Results from the use of germplasm in basic and adaptive research should help in targeting new germplasm as potential parents for plant breeders.

Evaluation

Evaluation of some germplasm collections has been done on a large scale. In particular, the international agricultural research centres based in the tropics have taken a lead in the conservation, evaluation and use of their mandated crops. At the International Crops Research Institute for the Semi-

Arid Tropics (ICRISAT) in India, screening *Arachis* germplasm for various abiotic and biotic stresses has revealed many desirable accessions. One desirable accession was found, on average, in every 144 accessions evaluated for the 16 stresses described by Moss et al. (1989).

The multitude of evolving rice pests and diseases in the tropics has led IRRI to undertake large-scale evaluation of rice genetic resources. Over 20 000 accessions of rice have been evaluated for their reaction to 10 biotic and abiotic stresses (*see* Table 2). For all these stresses, many accessions showed resistance or moderate resistance. For some stresses, the resistant varieties were concentrated in a particular geographic region or regions; a good example is resistance to the brown planthopper in southern Indian and Sri Lankan germplasm (Khush, 1979). Clearly, a core collection approach could have helped in reducing the effort expended on evaluation. Finding many resistant accessions enables genetic analysis of these materials to determine whether or not the genetic basis for resistance is the same.

Table 2   Number of rice accessions in the germplasm collection at the International Rice Research Institute tested for 10 stressses and found to be resistant or moderately resistant

Stress	Number of germplasm accessions evaluated	Accessions with resistance or moderate resistance to the stress (score 1 to 3)
Brown planthopper	44 335	682
Green leafhopper	50 137	1 403
Rice whorl maggot	22 949	697
White-backed planthopper	52 042	871
Bacterial blight	49 752	5 512
Blast	36 634	9 616
Sheath blight	23 088	2 153
Drought resistance at early vegetative stage	28 319	4 288
Drought resistance at late vegetative stage	22 873	1 826
Recovery from drought stress	24 432	15 115

Lehmann (1984) reported that 11 accessions of barley in the Gatersleben collection out of 6000 tested were identified as being resistant to six isolates of leaf rust (*Puccinia hordei* Otth.). Since the variation among these accessions was limited, it was thought that the resistance was based on a single gene, *Pa7*. On the other hand, 71 strains of barley resistant to a new race of stripe rust (*P. striiformis* West.) suggested that several different genes contributed to the resistance. For biotic stresses caused by organisms that may rapidly evolve, seeking different resistance genes is an essential part of an evaluation programme.

To improve the quality of soybean, evaluation was undertaken in the USA to find germplasm lacking various chemical constituents of the seed. About 3300 accessions of wild and cultivated soybean were evaluated; of these, only two were found that lacked the kunitz trypsin inhibitor (Hymowitz, 1980). After testing 6499 soybean accessions, two were found that lacked the enzyme lipoxygenase-1 (Hildebrand and Hymowitz, 1981). A core collection approach is unlikely to have

helped reveal these rare genotypes. When seeking rare genes, the relative costs of systematic screening of the entire collection compared with using a mutation approach to generate the desired trait, or even with introducing alien genes through genetic transformation, may need to be considered.

Use

Surveys of US crops indicated that the increase in yield as a result of genetic work was 0.5-0.8% per year during the 1960s and 1970s for wheat, cotton and soybean (Boerma, 1979; Meredith and Bridge, 1984; Schmidt, 1984; Specht and Williams, 1984). Hybrid maize in the USA showed yield gains of 1.4-1.78% per year between 1930 and 1980 (Duvick, 1984). However, gains in sorghum hybrids were about 2% per year in the 1970s (Miller and Kebede, 1984).

Despite these considerable and sustained gains, the number of plant introductions contributing to the genetic improvement of US crops has been rather limited. The total number of accessions in the US soybean collection is about 14 000. Delannay et al. (1983) reported that eight cultivars contributed about 65% of the genes of US soybean cultivars. Some 62 plant introductions, out of 30 000 in the total US wheat collection, contributed to breeding hexaploid wheat (*T. aestivum* L.) in the USA; of these, seven were landraces, 11 were other species and 44 were improved wheats (Cox, 1991). Frey (1991) stated that 'most of the oat germplasm utilised and developed in the USA until 1970 can be traced to seven landraces.' The US world oat collection consists of about 20 000 accessions, of which about 25% are wild and weedy species. The US rice collection consists of about 20 000 accessions but rice cultivars can be traced to only 45 plant introductions (Dilday, 1990).

The average annual growth rate of rice yield in Asia since 1965 has been 2.0-2.5% (David, 1991). Germplasm from the rice breeding programme at IRRI, which has been available to national programmes for direct use or use in their breeding programmes since the early 1960s, has been derived from 66 112 crosses involving 3985 parents not derived from IRRI lines. Of the parents used in crosses at IRRI, 124 varieties have each been used in over 100 crosses (*see* Table 3), and constitute the IRRI

Table 3   Number of times non-IR varieties or their derivatives have been used in crosses at the International Rice Research Institute, 1962-92

Number of times used in crosses	Number of varieties
>100	124
90-99	17
80-89	22
70-79	24
60-69	39
50-59	55
40-49	77
30-39	130
20-29	221
10-19	544
2-9	1919
1	813
Total	3985

breeders' collection (the breeders' active collection would include many of their own lines). From the thousands of crosses made at IRRI, less than 100 lines derived from these crosses have been released as varieties by national programmes.

The strong selection pressure that results in millions of lines from thousands of crosses being reduced to a few released varieties suggests that the ancestral parents of released varieties should also be a component of a core collection because of their unique genes, useful gene combinations and good combining ability. The germplasm curator has to determine which germplasm to include when developing a core collection. Particular attention may need to be given to newly received germplasm in the collection which has not undergone multiple testing over many years.

In a number of crops, such as oats and rice, one of the principal approaches is to infuse exotic germplasm to overcome stagnant or declining yields (Frey, 1991; Khush, 1991). Developing new plant types is an important approach to make a significant increase in yield potential if yields have reached a plateau.

The active role of germplasm curators in stimulating or participating in enhancing germplasm to develop agronomically acceptable, genetically desirable and diverse populations would overcome the primary constraint in the use of exotic germplasm for cultivar development. In addition, well-classified germplasm can immediately highlight segments of the whole collection or core collection suitable for use by the breeder.

DEVELOPMENT AND USE OF CORE COLLECTIONS WITH PARTICULAR REFERENCE TO RICE

Information on the diversity of accessions in the whole collection is crucial to the development of core collections. Wild species collections tend to be relatively small, with good passport data, and if well classified taxonomically they can be readily incorporated into a core collection. Small germplasm collections permit comprehensive biodiversity studies, leading to better classification on which selection for a core collection can be based (Hamon and van Sloten, 1989).

With large collections, basic information such as passport data is often absent. For example, only about 25% of the cultivated rice germplasm at IRRI has some passport data, compared with 72% of the wild rice accessions. Efforts to develop core collections for major crops pose problems beyond the mere size of the collection. The advice given by Brown (1989b) to use a stratified sampling strategy to choose germplasm for a core collection has helped in the development of core collections for some major crops (Brown et al., 1989; Erskine and Muehlbauer, 1991; Vaughan, 1991a).

Problems encountered in developing and using core collections reflect those encountered with using the whole collection, such as inadequate coverage of certain geographic regions, lack of information on accessions and inadequate seed stocks of some accessions. The use of accessions in a core collection may be limited by its size and the cost of a particular experiment. For example, several thousand accessions may be analysed for isozyme diversity for the same cost as studying only scores or a few hundred using restriction fragment length polymorphisms (RFLPs). At IRRI, two small core collections have been developed to test the usefulness of the core collection approach. However, our principal interest in establishing a core collection is the safe duplication of accessions representing the broad diversity of the genus *Oryza* in several locations around the world; a duplication of the whole collection for this purpose would not be feasible.

The scientific basis for the two major rice varietal groups, *indica* and *japonica*, was established by Kato et al. (1928). Oka (1958) subdivided *japonica* varieties into temperate and tropical types. Using

electrophoresis, Glaszmann (1987) surveyed about 2000 carefully chosen accessions and summarised the diversity by recognising six isozyme groups which largely corresponded to specific types of varieties from different regions. Thus, groups I and VI correspond to *indica* and *japonica* rices (both tropical and temperate). Group IV, however, is represented by the so-called Rayada varieties, found only in a few villages on the edge of the Madhumati river in Bangladesh (*see* Table 4).

Table 4   Varietal types and isozyme groups in rice

Origin	Isozyme group					
	I	II	III	IV	V	VI
Oka's testers (7)	indica	—	—	—	—	japonica
Iran, Pakistan	—	—	—	—	sadri	—
North-western India	—	—	—	—	basmati	—
Bangladesh	Aman	aus	early deepwater	rayada	—	—
South-eastern Asia	lowland	—	—	—	—	upland
Java, Bali	Tjereh	—	—	—	—	bulu
China	Hsien	—	—	—	—	keng

Source: Glaszmann (1986)

Our current knowledge of rice diversity based on geographic, morphological, agronomic, biochemical and molecular characteristics has resulted in the development of a small core collection of about 270 accessions of *O. sativa* which represents the known genetic diversity of rice (Glaszmann, 1987; Bonman et al., 1990). Using a similar approach, Vaughan (1991a, b) designated a core collection of wild rices to enable researchers to evaluate this germplasm efficiently.

Continued collecting and biodiversity studies require that these core collections are updated. Recently, varieties silent for the allele for the isozyme aminopeptidase-2 have been found in rice varieties from eastern Indonesia (Vaughan and Juliano, 1992). Recent exploration for wild rices has resulted in a new species being added to the collection, and new ecotypes of other species from isolated areas have been found (Vaughan and Sitch, 1991).

The use of these core collections has permitted rapid identification of germplasm for some traits. Thus, all accessions of the small Rayada group of varieties, highlighted in the isozyme study, were found to be resistant to leaf scald, caused by *Microdochium oryzae* (Hashioka and Yokogi) Samuels and Hallett (Bonman et al., 1990). Since there are only 19 Rayada accessions among the 80 000 in the germplasm collection at IRRI, neither random nor stratified sampling would have been likely to include one of these accessions in the designated 270 core accessions.

Broadcasting rice is replacing transplanting rice in many areas of Asia. Consequently, the *O. sativa* core collection was used by Yamauchi et al. (1993) to find varieties suitable for directly sowing under the surface of flooded rice soil. The results showed that deepwater rices and summer (aus) rices from north-eastern India and Bangladesh were most suited to these conditions. This information has led to further in-depth studies comparing these varieties with currently used commercial varieties.

The wild species core collection has been used to find sources of resistance to the tungro virus complex, one of the most serious diseases in Asia. Out of the 208 accessions of 19 species tested, 15 accessions of four species were not infected with one of the two forms of this virus, the rice tungro

bacilliform virus (RTBV), and had no or very low infection by rice tungro spherical virus (RTSV). Of these, three *O. rufipogon* accessions were susceptible or moderately susceptible to the green leafhopper vector. Although more than 40 000 rice accessions have so far been screened (Kobayashi et al., 1993a, b), no good sources of resistance to both RTSV and RTBV are available. In addition, two African species, *O. glaberrima* Steud. and *O. barthii* A. Chev., exhibited a previously unreported symptom of tungro infection (Kobayashi et al., 1992). However, use of the wild species core collection has shown that insufficient information is available on intraspecific diversity, and its composition will need revision as more information on this critical issue becomes available.

CONCLUSION

From this review of the relationship between a core collection and the larger collection which it represents, several important points emerge which give an indication of the issues which need to be addressed in the development and use of core collections:

Germplasm collections are still growing and the studies of biodiversity in germplasm collections are increasing. Both these factors imply that significant new collections and information on the whole collection will require periodic revision of core collections.

More 'user friendly' databases to guide germplasm users to the germplasm most suited to their needs are now available in many gene banks. Too few users know what is meant by a core collection. The role of the germplasm curator in the development, maintenance and promotion of this part of the whole collection will be necessary if more useful results are to be achieved in a cost-effective way.

The role of genetic resources centres in conserving products of biotechnology will become increasingly important for some crops. How these will be maintained and whether they become a part of a core collection will need to be addressed. For example, would an alien addition line series of a wild rice in the background of a cultigen be more useful in a core collection than several accessions of the wild species, which is difficult for plant breeders to use directly?

An economic analysis of the return on investment in rice genetic resources conservation suggests that this return 'far exceeds the cost of managing and collecting' germplasm (Evenson, 1989). However, the same analysis shows that 'the estimated impact of special search landrace materials turns out to be quite large. This has considerable relevance to genetic resource management because these special search materials are found on the fringes of the collection.' Such an analysis is encouraging for those conserving germplasm and underlines the importance of rare genes and appropriate means of finding them.

The core collection approach to evaluating and understanding conserved genetic resources has been shown to be useful. Sustaining the efforts to evaluate conserved genetic resources for a wide range of traits will increasingly require the use of such an approach as financial and personnel resources for evaluation activities become more scarce.

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