

Paper 3

GENETIC DIVERSITY AND PLANT IMPROVEMENT

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ABSTRACT

This paper examines the genetic diversity available to plant breeders and the narrow genetic base agriculture is founded upon. Problems of genetic erosion are discussed. Methods for increasing available germplasm are covered, e.g. probing of gene pools, chemical hybridising agents, facilitated recurrent selection, wide crosses, biotechnology and genetic engineering. The importance of worldwide sharing of germplasm and information is emphasised. This paper promotes the conservation and evaluation of germplasm as a natural resource.

KEYWORDS

Plant domestication, crop improvement, germplasm, genetic erosion, male sterility, hybrid wheat, genetic engineering.

INTRODUCTION

Of the quarter of a million species of higher plants estimated to exist, 3000 have been domesticated, and 150 are extensively cultivated. Modern agriculture is therefore based on an extremely limited array of the plant kingdom which is responsible for 98% of world food production. Cereal grains make the largest contribution, about 41%, and wheat (*Triticum aestivum* L.) is most important, supplying more than 20% or about 509 million metric tons (1985-86).

Plant domestication and improvement, the genetic manipulation of plants for the benefit of humanity, is as old as agriculture itself. It began when women, gathering food for their families, noted variations (genetic diversity) among and within plant species. Plants with larger seed which did not shatter but were easily threshed, were saved and planted the following year. These early plant breeders did not have Mendelian genetics or the scientific methods of plant improvement available to them; however, they were keen observers of the plants around them because survival often depended upon their selections.

Duvick (1984) provided examples of how yields have been increased through plant breeding and improved management practices over three time periods in this century. He compared the relative grain yields for wheat, maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.)

Table 1. Average grain yields of four major US crops in three time periods.¹

Crop	1900-1930 (kg/ha)	1930-1955 (kg/ha)	1955-1982 (kg/ha)
Wheat	887	987	1770
Maize	1666	1958	4841
Sorghum	1037 ²	1004	2979
Soybean	³	1138	1636

¹Data obtained from various volumes of US Department of Agriculture's Agricultural Statistics, US Government Printing Office, Washington, DC.

²For years: 1919-1930.

³No data.

Moench.), and soybeans (*Glycine max* L. Merrill) in the United States during these periods (Table 1). Increases in grain yield were not observed until 1930 in wheat, maize, and sorghum. A substantial increase in yield was noted between 1930 and 1955, with an even greater jump in yield between 1955 and 1982. The rate of increase for soybean between the same periods was much less. Factors contributing to the increase in grain yields included the release of improved varieties after 1930, the development of hybrid corn and sorghum, the semi-dwarf stature, mechanisation, increased use of fertiliser (especially N), herbicides, and related pesticides. Of the total yield increase it has been estimated that genetic improvements are responsible for 50% or more of the total gains depending on the specific crop.

Such improvements have only been possible because plant breeders had the necessary genetic diversity to manipulate and select for the desired attributes. N.I. Vavilov (1926) is generally credited with recognising the need for genetic diversity. He noted, 'plant breeding is evolution at the will of man and like all evolution is dependent upon variation'. Today we accept that genetic diversity is the basis of all plant improvement programmes.

Concerns are now being expressed that this genetic diversity is being exhausted in major food crops. As a consequence, breeders may be spending a higher percentage of their effort breeding for yield stability rather than improvement. Soybean in the US may be an example of this.

The problem of exhaustion of diversity raises several issues: the status of existing genetic diversity, means of increasing existing genetic diversity, and the continued exchange of this genetic diversity.

GENETIC EROSION

Genetic vulnerability is not a major threat to production of US field crops (Duvick, 1984) however, it must be regarded as an important and potentially dangerous problem.

The development and dissemination of agronomically superior cultivars have resulted in the replacement of many heterogeneous land race cultivars. Hawkes (1981) noted that, ironically, this process causes genetic diversity to be reduced or lost. Increased yield levels and specific demands from industry have meant that breeders are often reluctant to use older land race cultivars in programmes since the probability of obtaining superior progeny is low. Thus, the development and dissemination of new higher yielding cultivars narrows the genetic base. Breeders also discard potentially valuable genetic stocks because they do not fit immediate objectives.

Genetic diversity is also reduced by cultural practices such as multiple cropping, intercropping, or reduced use of tillage systems. Older cultivars may decline because their heterogeneity means they do not conform to a particular management practice. Herbicides and cultivation for weed control may reduce the opportunity for introgression of genes from weeds to crops (e.g. *Aegilops* to wheat).

Widespread disruption of wild habits through farming, grazing, or logging may increase soil erosion and change native ecosystems.

The genetic resource survey by Frankel and Bennett (1975) raised questions about the status of genetic diversity of various crop species within existing germplasm collections. Some accessions do not represent the populations from which they come because collections are often based on only a few plants. The genetic integrity of the material might also be challenged by possible outcrossing even in mostly self-pollinating species, unsuitable growing conditions and resultant natural selection, human error during propagation, and inadequate facilities and unreliable records.

A greater effort must be made to evaluate accessions for their desired attributes. To simply collect more material will not serve the intended purpose of such collections.

The disastrous impact of genetic erosion on the diversity of plant populations has been clearly identified by Harlan (1975) and by the report by the US National Academy of Science (1978). Extensive germplasm collections exist for some crop species; however, future exploration, evaluation, computerisation, and adequate facilities for the preservation of this international treasure must receive much higher priority. Reitz (1975) states, 'many will say germplasm will be looked after tomorrow. Well, tomorrow is now'. There will not be a tomorrow for much of today's germplasm unless action is taken immediately.

ENHANCING EXISTING GENETIC DIVERSITY

Induced mutations and polyploidy create genetic variability. In self-pollinated species such as wheat, there appear to be other opportunities.

Probing winter and spring wheat gene pools

The late Dr Joseph Rupert, a Rockefeller Foundation scientist working with the International Maize and Wheat Improvement Center (CIMMYT) in Chile in the 1960s, initiated a programme to systematically cross winter and spring wheats. Such crosses have been used by breeders in many countries to incorporate additional genetic sources of disease resistance, dwarfism, or agronomic attributes. In the US, some famous cultivars resulted from such crosses. Redit and Reliance released in 1924, were products of crosses between Turkey (winter) x Florence (spring), and Kanred (winter) x Marquis (spring), respectively. Even Thatcher, a widely used cultivar after its release in 1934, was produced from Marquis-Iumillo (spring) x Marquis-Kanred (winter). Cultivars which gave rise to the Green Revolution in wheat, obtained dwarfing genes from Norin 10-Brevor 14, a winter parent, and the daylength insensitivity response from several spring parents like Gabo.

Today, wheat breeders at CIMMYT and Oregon State University (OSU) are systematically probing and recombining spring and winter gene pools.

F₁ seed resulting from winter x spring crosses, is integrated with the CIMMYT International Spring Wheat Programme by three and four-way crosses using spring parents. Thus, the spring habit is retained while desired attributes are transferred from the winter donor parent. After evaluation and selection, advanced lines are disseminated through various international nurseries coordinated by CIMMYT to spring wheat cooperators.

In Oregon similar F₁ winter x spring populations are crossed to winter or facultative parents using three and four-way crosses. The segregating populations are grown under a range of biotic and abiotic stresses. After appropriate evaluation in different environments superior F₆ lines are included in a screening nursery which is sent annually to cooperators in winter and facultative wheat-growing regions.

Breeders at both CIMMYT and OSU have successfully employed a material flow system or shuttle breeding approach, by growing segregating populations at different sites and applying various selection pressures alternately and/or simultaneously. This approach has identified progeny with superior and stable yields. Materials from the winter x spring programme were compared with locally adapted check cultivars in 39 countries in 1983. The percentage yield increase of winter x spring lines varied from 7 to 282%, depending on the particular site. Several winter x spring-derived lines were superior at both low and high-yielding sites.

Increased genetic diversity has provided an

opportunity to modify the growth pattern of the wheat plant. Differences in vernalisation requirements and responses to photoperiod can be more suitably matched with periods of more favourable environmental conditions. Of particular interest have been changes in physiological maturity, rate and duration of grain fill, and the rate of dry down of the grain. Other attributes enhanced by the increased diversity are additional sources and more durable-type disease resistance, improved drought tolerance, improved tolerance to acid soils, additional dwarfing genes, increased protein quantity and quality, greater spike fertility, and improved quality of several end products.

Chemical hybridising agents

The discovery of genetic fertility restorers in the 1960s to complement male sterile cytoplasm appeared to offer opportunities for the development of hybrid wheat. After much research in the private and public sectors, many have abandoned this approach. Scientists at the Cargill Company, who currently have several wheat hybrids on the market are the exception. In regional yield trials in the Midwest US, these hybrids have performed well even when grown in stress environments. Whether the extra yield advantage will offset the additional seed costs remains to be seen.

Interest in hybrid wheat has been renewed by the development of chemical hybridising agents by a number of private companies. Male sterility depends on applying the treatment at the correct stage of growth of the female lines, and on chemical concentrations. The latter factor may vary depending on winter or spring-type wheat and location. There is also a strong cultivar x genotype interaction which may be a function of the morphological development of floral structure. A male shedding abundant pollen is needed. Although chemical hybridising agents avoid much of the breeding work required in the cytoplasmic male sterility system, they are not free of concerns about combining ability, amount of outcrossing, the extent of hybrid vigour, and seed production. Also the economic question of seed cost for the farmer must be answered. Currently there is at least one company marketing chemically-induced hybrid wheat.

Hybrid wheats are attractive since they allow genetic variability to be used fully. Currently breeders working with diploid self-pollinating species are restricted to those genes acting in an additive manner. In wheat and other polyploids, however, there may also be interactions between genomes which would contribute to hybrid vigour without the need for cross-pollination.

Facilitated recurrent selection

Various types of recurrent selection have long been used to develop favourable gene combinations in cross-pollinated species. Chemical hybridising agents plus recurrent selection may enhance genetic diversity in self-pollinating species. This is particularly true for traits where minor genes are involved. In wheat, this approach would be

useful to accumulate minor sources of resistance to diseases like barley yellow dwarf virus, septoria (*Septoria tritici*), foot rot, etc. It is also possible to achieve more durable resistance using this method. In the final analysis, chemical hybridising agents may play a more important role in population improvement than in the production of hybrid crops. However, the lower profit incentive of this approach may not attract many private chemical companies.

Facilitated recurrent selection employing dominant and recessive male sterile genes may be used to increase genetic diversity in normally self-pollinating species. Different sources of resistance to several diseases of barley have been combined at Montana State University. Recurrent selection populations using genetic male sterility can accumulate genetic factors for resistance or tolerance to several biotic and abiotic stresses.

Wide crosses

Interspecific and intergeneric hybridisation in wheat have been used to acquire basic cytological, evolutionary, or phylogenetic information. Increased interest among wheat breeders may have been prompted by the development of triticale. A. Myeeb-Kazi (1984) at CIMMYT has incorporated disease resistance (*Helminthosporium sativum* and *Fusarium* spp.) and stress tolerance (salt, drought, aluminium, and copper) into wheat from species of such alien genera as *Aegilops*, *Agropyron*, *Elymus*, *Haynaldia*, and *Secale*. Several researchers are combining wheat and barley (*Hordeum vulgare* L.) in an attempt to transfer the Yd2 gene for barley yellow dwarf resistance into wheat. *In vitro* interspecific hybridisation makes such wide crosses a reality (Collins *et al.*, 1984).

Biotechnology and genetic engineering

Recent findings in the fields of molecular biology and genetic engineering may allow the gene to be domesticated. New sources of genetic diversity may result from recombining genes in combinations not possible through hybridisation.

Plants have been regenerated from cultures of callus tissue, cell-suspensions, and protoplast fusion. Today the most promising of these appears to be tissue culture. It is now possible to regenerate plants of some agricultural species from callus. The organ or tissue of the plant most suitable for explanting, varies from immature embryos, meristem tips, leaf sheath tissue, mesocotyl region, to roots. The appropriate media must also be identified and a range of growth hormones tested at varying concentrations to ensure callus is induced, and shoots and roots are initiated. Thousands of potential plants can then be evaluated for various attributes in a petri dish: herbicide tolerance, resistance to disease toxins, improved nutritional properties etc. Some investigators report an increase in genetic diversity for a number of traits resulting from tissue culture, variability which is difficult to explain when genetically uniform material is used. In some species a number of chromosomal rearrangements have been noted

in regenerated material. It appears that tissue culture provides an excellent screening technique at the cellular level and specific traits can be identified.

Genetic engineering of plants where specific genes can be isolated using restriction enzymes, cloned, and transferred into unrelated species, potentially allows access to genetic diversity across the entire plant kingdom. Reports frequently mention the transfer of genes for herbicide resistance from one genus to another, however, there are many constraints to be overcome before this technique becomes a viable tool for the plant breeder. First, one must locate the desired gene which is a huge task in plants such as polyploids. The desired genes may reside in the nucleus, chloroplasts, or even the mitochondria. Once a gene is isolated, a vector or some other transfer method must be found to relocate the gene in the host cell. *Agrobacterium tumefaciens* has been a successful vector in some dicotyledons; however, the lack of a suitable vector remains a problem for monocotyledons such as wheat, although direct injection of DNA may be possible. Once a gene has been introduced it must be expressed at the correct time in the correct tissue. In addition, many agronomic traits are quantitatively inherited and a single gene or block of genes may not have the desired effect.

Despite current constraints in biotechnology and genetic engineering, new opportunities will result making the genetic diversity found throughout the plant kingdom more accessible.

Without question, the major contribution of molecular biology initially will be to further our understanding of the more fundamental aspects of plant growth and development.

DISSEMINATION OF GERMPLASM

All plant improvement programmes rely on worldwide sharing of germplasm and information. When the pedigrees of most cultivars are compared, it becomes clear that many breeders from different countries have contributed in a stepwise fashion to plant improvement. Wheats of the Green Revolution provide such an example. Norin 10 contributed dwarfing genes from Japan and was crossed with Brevor at Washington State University. Brevor originates from Turkey and Australian parentage. Day length-insensitive genes were also contributed by Australian cultivars. Finally these characteristics were combined by Rockefeller Foundation scientists (CIMMYT) in Mexico. The semi-dwarf, day length-insensitive cultivars were distributed throughout the spring wheat regions of the world. This germplasm now provides parental material for cultivars of the future.

Today, CIMMYT scientists distribute germplasm of seven F2 bread wheat nurseries selected for various abiotic stresses (e.g. moisture, temperature, aluminium toxicity), and distribute five screening, yield, and crossing block trials. When the international triticale and barley nurseries are included, CIMMYT sends about 1770 trials to 272 cooperators in 103 countries where spring cereal grains are

grown. As a complementary effort, the winter x spring programme at OSU is sending selected F2 populations, screening nurseries, yield trials, and crossing blocks to 51 different countries and to 111 breeding programmes where winter or facultative germplasm is required.

The University of Nebraska initiated the International Winter Wheat Performance Nursery in 1968. This nursery consists of 30 entries of established cultivars obtained from various countries and is sent to 68 sites in 38 countries.

A significant feature of international nurseries is the agronomic data collected by the different programmes which is compiled, analysed, and returned to each cooperator before the next crossing season. Thus, in addition to providing improved germplasm, these nurseries also serve as surveillance nurseries where new biotic and abiotic stresses can be identified and information disseminated about new sources of genetic resistance or tolerance.

There are numerous other regional and international nurseries, many of which are designed for specific objectives such as the US Department of Agriculture International Wheat Rust Nurseries. Also, the sharing of germplasm between breeding programmes both within and between countries has been of great benefit.

With more countries adopting some form of cultivar protection laws, the future sharing of information and germplasm may be slowed. The continuing exchange of material is vital and any policy or law which restricts the free exchange of material, ideas, or concepts is of concern.

SUMMARY

Genetic diversity has been, and will continue to be, the lifeline of all plant improvement programmes. The demand for greater genetic diversity to feed a hungry world comes at a time when existing germplasm is being genetically eroded at an ever increasing rate. Many of the primary and secondary centres of origin are being drastically and permanently altered. Plant scientists today rely on the previous contributions of others; therefore, they must share a responsibility and obligation to ensure that future scientists will also have the necessary genetic variability.

Old and new technology are available to scientists to create additional, useable genetic diversity to improve agricultural crops. The systematic probing of winter and spring gene pools in wheat, the development of hybrids in normally self-pollinated species, the use of facilitated recurrent selection, wide crosses, and emerging biotechnology and genetic engineering are tools which can enhance existing genetic diversity and create new diversity.

These opportunities will never lessen the need to collect, evaluate, and disseminate genetic germplasm — an irreplaceable natural resource.

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SYMPOSIUM DISCUSSION

Prof. D. von Wettstein, Carlsberg Laboratory

Why propagate hybrid wheat? You have said there are no yield plateaus in wheat. It is an allo hexaploid species so you should be able to have all the heterozygosity you wish. It would be much easier not to have to deal with gametocides, buying new hybrid seed, etc.

Kronstad

I agree that with 3 genomes there is sufficient opportunity to increase yield. My concern is that private companies may not be interested in developing chemicals and they may not be available to us although we certainly have the male sterility genes.

Sir Otto Frankel, CSIRO

I want to emphasise the importance of breeding lines as genetic resources. In Simmonds book he says breeding lines may replace landraces in future genetic collections because many of them have accumulated desirable genes and gene complexes which would be useful to plant breeders, more so than the naturally selected land races of the past. This should be borne in mind and perhaps put into practise in the next decade.

Dr B. Imrie, CSIRO, Brisbane

You suggested soybean may be approaching a yield plateau somewhat lower than the cereals. I think the history of the development of that crop is much shorter than cereals and they still have a long way to go. We have achieved, under experimental conditions in our laboratories in Brisbane, yields of 8.5 t/ha. It is just a matter of getting the cultural characteristics together with varieties and we will find yield levels that approach those of cereals.