CIMMYT's WIDE CROSS PROGRAM FOR WHEAT AND MAIZE IMPROVEMENT

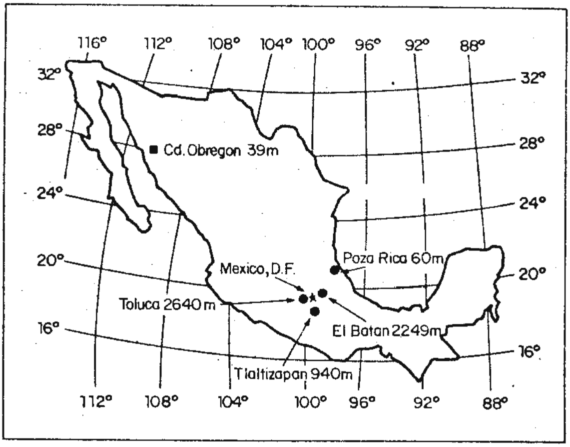
A. MUJEEB-KAZI and D. C. JEWELL

CIMMYT is in its fourteenth year of wide cross research in wheat and maize, and the program is an important part of the Center's over-all research effort. Traditional breeding techniques and wide cross research are seen as complementary. Progress in the former is predictable and dependable, while progress in the latter is less so, but has the potential to make quantum leaps. Recently developed techniques may allow wide cross research to proceed much more rapidly than it has to date, and permit the relatively quick development of germplasm with superior resistance to diseases and insects, as well as improved tolerance to salt, heat, drought, and aluminum. Specifically, CIMMYT is modifying prepollination and postpollination techniques, and experimenting with embryo-rescue techniques and novel cytogenetic systems (e.g., the 5B effect). The potentials of both tissue culturing and a recently developed transforming DNA technique are also being investigated. CIMMYT is optimistic that such research will, in the years to come, furnish superior germplasm for use in conventional breeding programs.

CIMMYT was established as an international agricultural research center in 1966, having evolved from a collaborative research program begun in 1943 between The Rockefeller Foundation and the Mexican Ministry of Agriculture.

Research is done at five experiment stations in Mexico (Fig. 1), four of which are operated by CIMMYT and one by the Mexican National Institute for Agricultural Research (INIA). The five stations range in altitude from near-sea level to 2,640 m and differ markedly in average temperature, moisture, and solar radiation. Within Mexico, then, experimental germplasm can be exposed to many of the environmental conditions of the developing world. The wide cross program in wheat and maize is a crucial part of CIMMYT's overall breeding work.

Centro Internacional de Mejoramiento de Maize y Trigo, A. C. (CIMMYT), Apdo. Postal 6-641, Col. Juarez, Delg. Cuahutemoc, 06600 Mexico, D. F., Mexico.



1. Locations and elevations of principal stations in Mexico at which CIMMYT conducts research (Cd. Obregon Station of the Instituto Nacional de Investigaciones Agricolas).

WIDE CROSS RESEARCH AT CIMMYT

Most of the work on wide crosses, started in 1972, was collaboration between CIMMYT and other centers. An early priority in CIMMYT's wheat wide cross research was to introduce high protein and high lysine into bread wheat (*Triticum aestivum* L.) from barley (*Hordeum vulgare* L.). To produce relevant hybrids and develop methods for transferring genes from related species to wheat for general crop improvement were also program objectives.

In maize, the priority was to develop hybrids with *Tripsacum*, a related species with genes for disease and insect resistance. Because available close relatives of maize are much fewer than those of wheat, however, the Maize Wide Cross Program is also investigating crosses between maize and more distant relatives such as sorghum.

The early efforts at CIMMYT did not result in many applied benefits; so when we joined CIMMYT, program priorities were changed. At first, in-house activities were intensified, and collaborative efforts with other institutions reduced.

Eventually, however, the wheat program focused on crosses, and the progeny of crosses, between wheat and *Elymus* spp. and between wheat and *Agropyron* spp. In the maize program, efforts were again directed to maize/*Tripsacum* spp. following intense but unsuccessful attempts to isolate sexually a maize-sorghum hybrid. By conservative estimates, this hybrid combination has a probability of less than one in three million of being isolated from sexual crosses (1).

The following criteria are now used to define wide cross research at CIMMYT:

1. A wide cross should, as in normal hybridization, result in an offspring (F_1) with chromosome complement of

both parents (the maternal complement may be haploid or diploid);

2. For CIMMYT to be interested in the resulting F_1, it should possess a phenotype intermediate between the parents;
3. The F_1 should be male sterile.

These hybrids usually require specialized crossing and embryo rescue techniques. Hybrids that can be produced normally and are self-fertile, can be evaluated and selected as part of CIMMYT's ongoing breeding programs, and the wide cross programs need not be involved. This research should result in the accession of genetic information on species related to maize and wheat, not to form new species but to improve cultivated maize and wheat crops. Introducing genetic variability for traits with little or no known variability into the existing maize and wheat germplasm is emphasized.

THE WHEAT WIDE CROSS PROGRAM

The Wheat Wide Cross Program emphasizes bread wheat (T. aestivum L.) improvement, specifically the incorporation of

1. resistance to Helminthosporium sativum (known to cause seed decay, root rot, and leaf spots);
2. resistance to Fusarium graminearum (the pathogen of head blight and leaf spot); and
3. stress tolerance to salt, drought, heat, aluminum, and copper.

Several related genera are excellent sources of resistance or tolerance. They include several of the Aegilops, Agropyron, Elymus, Haynaldia, and Secale species. Until recently, however, the success rate for hybridizing wheat with related species has been alarmingly low, primarily because of problems associated with crossability barriers and poor embryo development.

We have now lowered the crossability barriers such that we can produce intergeneric hybrids. In general, prepollination or postpollination techniques, or both, together with manipulation of the embryo culture media may influence 1) pollen tube growth, 2) gynoecia longevity, 3) micropylar barriers, 4) delivery of male gametes, 5) initiation and assistance of seed set, and 6) embryo development.

Other more easily defined factors have contributed to overcoming crossability barriers. They include 1) cross direction, 2) varietal choice, 3) polyploid level of the related parent, 4) presence or absence of recombination in the F_1, 5) production of amphiploids from the F_1, 6) cytological differences in the backcross (BC) progeny, and 7) restoration of self-fertility by continued backcrossing.

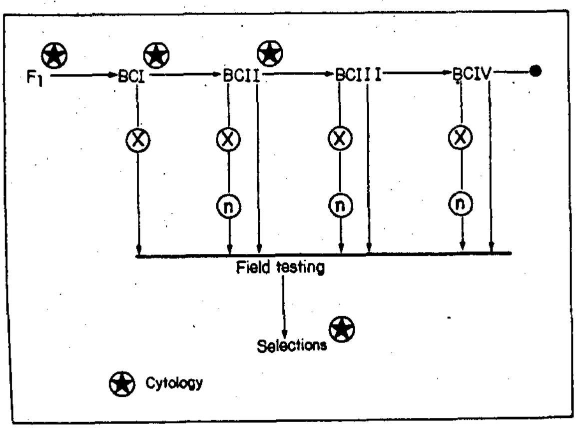
CIMMYT currently maintains 80 intergeneric hybrid combinations with wheat, the majority of which are new. Where possible, these hybrids have been made using commercially grown spring wheat varieties. In most of these F_1 hybrids, no recombination takes place between the wheat and alien chromosomes (chromosomes from the related species); in several cases, the BC1 progeny is cytologically abnormal (3, 4). Approximately 27,000 lines, derived from 5 of the 80 cytologically analyzed intergeneric combinations, have been field tested. Figure 2 illustrates the methodology used to advance an F_1 to field testing. Selection is based on plant type, on resistance to leaf rust, stem rust, *H. sativum*, and *F. graminearum*, and on cytological analysis.

About 60% of our effort in wheat wide cross program resources are devoted to advanced progeny from crosses with *Elymus giganteus* (2n=4x=28) (5, 7), a species moderately salt tolerant, free from leaf-spotting diseases, and resistant to *F. graminearum*. The chromosomes of this species are uniquely marked by chromosome banding and it is relatively easy to identify the chromosomal complement in the advanced progeny.

Lines have been tentatively identified for resistance to *H. sativum* or to *F. graminearum*. The advanced offspring have good plant type, but have not yet been tested for stress tolerance.

Fourteen different addition lines (normal wheat chromosome complement plus an additional chromosome from the related species) are possible with the tetraploid species *E. giganteus*. Five of them have been confirmed; seven others have been tentatively isolated; and one of the remaining two chromosomes has been substituted for a wheat chromosome. Only one chromosome has yet to be isolated in a wheat background. Specific resistances or tolerances have not yet been ascribed to specific *E. giganteus* chromosomes. Our objective is to introduce only the beneficial segments of these chromosomes.

Subtle gene transfers are essential for the germplasm to be useful to breeders in conventional breeding programs. They are facilitated by 1) suppression of the 5B effect, 2) irradiation, 3) tissue culture, and 4) pentaploid-induced translocation (all



2. This scheme represents the movement of germplasm from the intergeneric F1s to field testing.

recently incorporated into the wheat wide cross program). To produce new germplasm for breeders, CIMMYT and collaborators have evolved the following steps:

- identify CIMMYT wheat with highly crossable genes,
- transfer the 5B chromosome stocks to these cultivars,
- develop a monosomic series in one of these cultivars,
- ascertain their suitability for tissue culturing (callusing and regeneration), and
- remake some of the hybrid combinations using lines adapted to tissue culturing and having the desired crossability genes.

Ideally small pieces of the alien chromosomes should be inserted into closely related chromosomes; however, insertions of alien genetic material into less closely related chromosomes can be equally valuable. The benefits breeders have derived through introgression of alien genetic material are best exemplified by CIMMYT's IB/IR wheat varieties. These have been released globally because of their wide adaptation, yield stability, aluminum tolerance, and resistance to *Septoria tritici* (8). Significant improvements in wheat through translocations made by institutions other than CIMMYT include

- stem rust resistance: *A. elongatum* and the wheat chromosome 6A;
- leaf rust resistance: *A. intermedium* and the wheat chromosome 7A;
- leaf rust resistance: *Ae. umbellulatum* and the wheat chromosome 6B;
- leaf rust and powdery mildew resistance: *S. cereale* and the wheat chromosome 4A; and
- green bug resistance: *S. cereale* and the wheat chromosome 1A.

Significant practical success from the wheat wide cross program is expected within the next decade, particularly since hybrid production has been simplified and the methods for successful hybrid exploitation, well documented. Once the desired variation has been recognized in the wild species, and its expression in a hybrid established, the choice of method to introduce the alien variation follows logically from the relative affinity of the chromosomes involved. The introduction of alien variation can only increase the range of variation for specific traits from which plant breeders can select (6).

The genes introduced into wheat cultivars are not entirely different from the genes already present; and their expression will be affected by the same factors affecting the genes normally found in cultivated wheat. Two situations exist: mutable and nonmutable. An example of a mutable situation is the introduction of genes conferring resistance to disease. The pathogen is free to mutate, and the resistance is expected eventually to fail. The plant breeder must then identify a different source of resistance. Examples of nonmutable situations include tolerance for salt, drought, low copper, and high aluminum. An alien gene or genes conferring tolerance for these conditions should be very long lasting and could substantially contribute to food production in areas where the lack of such tolerance is a limiting factor.

The practical potential of wide hybridization is probably greater with wheat than with most other crops because 1) wheat hybridization is easy; 2) the species cytogenetics are well understood; and 3) bread wheat, being a hexaploid (2n=6x=42), has a large amount of genetic material so it is well buffered against introgression of alien material. The introduction of genetic material from species with relatively close evolutionary ties to wheat has great potential and will continue to be exploited by CIMMYT.

THE MAIZE WIDE CROSS PROGRAM

Current emphasis in the maize wide cross program is on maize/*Tripsacum* and the resulting progeny. *Tripsacum* possesses wide adaptability, tolerance for waterlogging and drought, and resistance to several foliar diseases and insect pests. Several approaches are being used to incorporate genes from this relative into maize.

We currently maintain more than 130 F_1 hybrids between maize and *Tripsacum*. These involve 5 different maize pools in combination with 17 different collections representing 6 taxa of *Tripsacum*. They include both diploid (2n=2x=36) and tetraploid (2n=4x=72) species.

One of the techniques by which we obtain a maize population with *Tripsacum* genes was developed by Harlan and de Wet (2, 9). It requires sequential backcrossing of the F_1 with maize, together with maintenance of the F_1 chromosome number. After several generations of maintaining this F_1 chromosome number the backcross plants with the normally expected chromosome number are selected and backcrossed again. Plants are then selected for near-normal maize chromosome number and for phenotypic differences. The selected plants are recombined to form a population that can be tested for desirable traits. This method is applicable only to tetraploid *Tripsacum* spp. because maintaining the F_1 chromosome complement for several generations is not possible with the diploid *Tripsacum* spp.

The main difficulties in using this procedure are

1. time (the F_1 and recovered F_1s take about 18 mo to flower);
2. difficulty in obtaining backcross progeny, because a dominant gene in some *Tripsacum* spp. prevents backcrosses to maize without a similar gene; and
3. applicability to only the tetraploid *Tripsacum* spp.

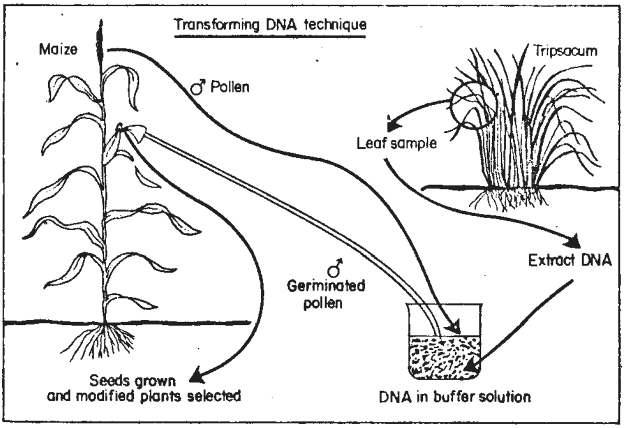
We are therefore continuing efforts to isolate new F_1 hybrid and backcross progeny for single addition lines that could, perhaps by irradiation, be used to incorporate the beneficial genes into the maize chromosome complement. The Harlan and de Wet method (2, 9) is being used, where possible, for the F_1 hybrids involving tetraploid spp.

We are also initiating collaboration in tissue culture of maize/*Tripsacum* F_1s in an attempt to use somaclonal variation to recover annual tripsacoid maize plants from the perennial F_1s more rapidly than by the classical method.

A subset of the existing tripsacoid maize was obtained from the University of Illinois and has been crossed to CIMMYT's breeding material. The resultant progeny is being selected for resistance to Southwestern Corn Borer (Diatraea grandiosella); it shows promise and may be screened for other beneficial traits.

We have also been evaluating other techniques. In collaboration with The Plant Breeding Institute, Cambridge, we are attempting to determine whether or not maize and sorghum nuclei can coexist following microinjection of the pollen nuclei into the micropyle. This should help in determining future strategies. For example, if maize and sorghum chromosomes can coexist in the same cell, even for a short time, protoplast fusion, followed by irradiation, might be used to transfer sorghum genes to maize.

We are just completing 18 mo of collaboration with the University of Illinois to evaluate a "transforming DNA technique" for incorporating small segments of alien DNA into maize. This involves extracting DNA from a donor species and soaking maize pollen in a solution of this DNA before pollination, to incorporate small donor DNA segments into the developing embryo (see Fig. 3). This technique has been used successfully to transfer genes for cob color and rust resistance from one maize inbred to another (de Wet, pers. comm.). Tripsacoid plants have been recovered, both at the University of Illinois and at CIMMYT, using maize as the male and female and Tripsacum as the DNA donor. The technique has tremendous potential, as it may allow us to quickly introduce genetic material from both diploid and tetraploid Tripsacum spp., and it may permit introducing genes from other, more distantly related genera. The program intends to expand its efforts with this new technique, not only to synthesize tripsacoid maize populations, but alsot to test the technique's potential to incorporate sorghum genes into maize.



3. Diagram of the method used for incorporating alien material by the transforming DNA technique.

EXPECTATIONS

Nearly all maize and wheat breeding in the world today is of the conventional type, where progress is, of course, still possible. And success in wide crosses is expected to be slow, proceeding from plants that are near relatives to plants that are more distantly related.

However, in the next decade, wide cross research is expected to provide new sources of genetic variation for conventional breeding. Thus wide cross research should, in the near term, significantly contribute toward feeding the world's growing population. In the longer term, the quantum leaps referred to at the beginning of this paper are well within the realm of possibility.

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UK/Mexican Link in Genetic Engineering

Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT)

by Dr. Mujeeb-Kazi

The project on introduction of genes for disease resistance and stress tolerance from alien sources into wheat funded by the British Overseas Development Administration is being carried out in the Centre of Arid Zone Studies in Bangor, Wales; P.B.I., Cambridge, England and CIMMYT, Mexico. Disease resistance transfers are targeted for ***Fusarium graminareum*** (head scab) and ***Helminthosporium sativum***. Attempts are presently directed towards identifying alien sources that possess resistance to these two diseases with disease screening in progress in Toluca (Mexico) for ***Fursarium*** and in Poza Rica (Mexico) for ***Helminthosporium sativum***. Once resistant alien species are identified the breeding via genetic engineering will be conducted in P.B.I., England with field testing of the material produced being undertaken in Mexico.

The salt tolerance stress objective has been advanced to a meaningful stage. Alien species were identified as salt tolerant by the Centre in Bangor, hybrids of these species with wheat were produced in CIMMYT, Mexico, and P.B.I. England, and studied in greater depth at P.B.I., England using a systematic methodology. The CIMMYT group is approaching the task using an applied breeding methodology and it is hoped that these efforts will complement each other.

Specifically ***Agropyron junceum*** (2n = 2x = 14) is a grass species with a high level of salt tolerance. Its hybrid with wheat has 28 chromosomes (21 of wheat and 7 of ***A. junceum***). The hybrid upon treatment with a chemical (colchicine) produces doubled chromosome progeny that is fertile, possessing 56 chromosomes. The efforts are to develop advance lines that each have 42 chromosomes of wheat and each of the ***A. junceum*** chromosomes (7 such lines are possible since this is the basic number in ***A. junceum***). The lines when obtained will be screened for salt tolerance and eventually field tested in saline sites. It is anticiped that the genetics of salt tolerance will be complex, consequently additional procedures will be required to make the product useful for applied breeding programs.

So far the salt-tolerance expression has been identified in the doubled plant for Nacl levels up to 250 mol m-3. Procedures that permit identification of the ***A. junceum*** chromosomes have been completed, employing differential staining (chromosome banding) and biochemical markers (grain protein and isozyme markers). The schematic of the procedure is represented in Figure 1 as is a C-banded karyotype of the basic. 7 ***A. junceum*** chromosomes in Figure 2.

This ODA funded collaborative program is headed by Dr. Gareth W. Jones in Bangor, Dr. Colin N. Law in P.B.I. and Dr. A. Mujeeb-Kazi in CIMMYT.

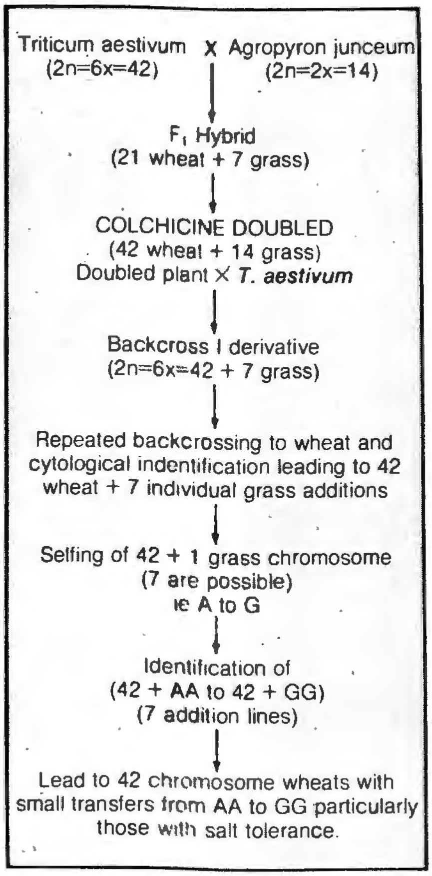

Fig. 1: Schematic showing research development trends for salt tolerance transfers from *Agropyron junceum* (2n=A2x=14) to wheat (*Triticum aestivum*)

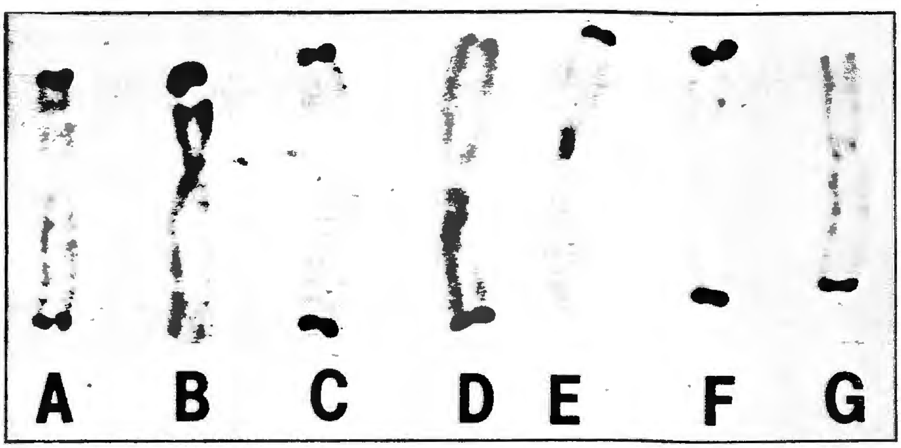

Fig. 2: A C-banded karyotype of *Agropyron junceum* (2n=2x=14) be used to transfer its salt tolerance potential to wheat (*Triticum aestivum*)

The Bioquimex Laboratories have been awarded the 1986 Medal for Merit in Foreign Trade for their work in processing the cempasuchil flower for colouring, both for the internal and external markets.