MAIZE • PROGRAM • SPECIAL • REPORT

Development of New Stress-Resistant

Maize Genetic Resources

(UNDP Project GLO/90/003)

Final Report 1990-1996

Submitted to
The World Bank
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INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER
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Other Maize Program Special Reports:

International Testing: Evaluating and Distributing Maize Germplasm Products. R.N. Wedderburn, technical editor.

The CIMMYT Maize Germplasm Bank: Genetic Resource Preservation, Regeneration, Maintenance, and Use. S. Taba, technical editor.

Stress Tolerance Breeding: Maize that Resists Insects, Drought, Low Nitrogen, and Acid Soils. G.O. Edmeades and J.A. Deutsch, technical editors.

The Subtropical, Midaltitude, and Highland Maize Subprogram. M. Bjarnason, technical editor.

The Lowland Tropical Maize Subprogram. S.K. Vasal and S. McLean, technical editors.

Maize Genetic Resources. S. Taba, technical editor.

CIMMYT is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center works with agricultural research institutions worldwide to improve the productivity and sustainability of maize and wheat systems for poor farmers in developing countries. It is one of 16 similar centers supported by the Consultative Group on International Agricultural Research (CGIAR). The CGIAR comprises over 50 partner countries, international and regional organizations, and private foundations. It is co-sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), the United Nations Development Programme (UNDP), and the United Nations Environment Programme (UNEP).

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Abstract: A synthesis of results of research under the UNDP-supported Project "Development of New Stress-Resistant Maize Genetic Resources" (UNDP Project GLO/90/003), executed by the International Maize and Wheat Improvement Center (CIMMYT) from 1990 through 1996 to develop 1) efficient selection techniques for stress tolerance, and 2) maize source germplasm with tolerance to insects, drought, low nitrogen and acid soil conditions.

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Contents

Executive Summary

This report is a synthesis of results from the UNDP-supported Project **Development of New Stress-Resistant Maize Genetic Resources** (UNDP Project GLO/90/003), which has operated at CIMMYT, Mexico, from 1990 through 1996, with a total budget of US$ 6.6 m. Its objectives were to develop 1) efficient selection techniques for stress tolerance, and 2) germplasm sources with tolerance to insects, drought, low N and acid soil conditions.

Biotechnology and cytogenetics applications:

- A wide array of molecular techniques have been examined to determine if breeding for stress tolerance can be accelerated by using molecular markers.
- A survey of restriction fragment length polymorphisms (RFLPs) was carried out in 50 maize lines with variable levels of resistance to Southwestern Corn Borer (SWCB), plus 50 lines of importance to other breeding objectives of the Maize Program. The level of polymorphism was unexpectedly high.
- Software was developed which greatly aids in processing this type of data, and a database was formed and relationships among lines examined.
- Two crosses were made between resistant and susceptible lines (CML 139 x Ki3) and (CML 67 x CML 131). Insect resistance and several agronomic traits were mapped in the F_2 populations of these crosses, using RFLPs. These, plus a cross between short and long anthesis-silking interval (ASI) lines, have been used as a basis for molecular mapping of QTL associated with SWCB resistance and drought tolerance (i.e., short ASI). QTL were detected for each of these traits. For ASI, seven QTL were identified on chromosomes 1 (two QTL), 2, 5, 6, 8 and 10, and accounted for 50% of the phenotypic variability.
- In a comparison of marker-assisted selection (MAS) and conventional selection for resistance to Southwestern Corn Borer (SWCB), both methods produced lines that possess superior resistance to first generation leaf damage by SWCB over the original line, CML 204. The lines derived from the two methods were not statistically different for leaf damage ratings. For future MAS for SWCB resistance, the region on chromosome 9 could be transferred as a single region for ease and maximum cost effectiveness. MAS lines generally gave higher yielding hybrids than the conventionally-selected lines, when evaluated as hybrids in the environment where the recurrent parent was properly adapted, a difference which was significant when data were combined across three southern African location ($P < 0.10$). There was apparently selection during backcrossing in the conventional scheme for adaptation to local Mexican soil conditions that did not occur during MAS. MAS allows unbiased recovery of the recurrent parent in all but the QTL region(s), an advantage where selection for specific stress-tolerance traits takes place in environments which differ substantially from the target environment (See Appendix 3 for details).

- Mapping of recombinant inbred lines (RILs) for insect resistance and for drought tolerance is now well advanced. This will increase the precision with which QTL are identified, and reduce linkage drag when these QTL are transferred to a recipient line.

- Marker-assisted backcrossing is under way for insect resistance and for drought tolerance, using lines with a high degree of tolerance/resistance as sources and elite but susceptible lines as recipients. Polymerase chain reaction (PCR)-based methodologies have been adapted to increase the efficiency of marker-assisted selection and to reduce its costs. Results demonstrate that it is essential to make a new genetic map when the recurrent line is changed, although only one trial under drought conditions may be sufficient to identify the QTL of interest in the new F_2 population.

- There is considerable similarity between the genomic locations of QTL for ASI under drought and those associated with ASI under low soil N.

- Good progress has been made in the development of efficient *in situ* hybridization methods for detection of alien DNA in crosses with maize, and these are now being used to facilitate the introgression of apomixis genes into maize from *Tripsacum*.

- To date, 46 scientists from 21 countries have received short- or long-term training in our laboratories, and two formal courses on the "Molecular Marker Applications to Plant Breeding" have been held.

- RFLP probes have been distributed to a number of laboratories requesting these, and a set of laboratory protocols describing CIMMYT's non-radioactive, chemiluminescent technologies for RFLP detection has been distributed in English or Spanish to 314 scientists in 48 countries.

Host-plant resistance to maize insects:

- With our partners in national agricultural research programs in developing countries, we have developed and improved eight maize populations adapted to the tropics and subtropics for resistance, based on antibiosis, to major insect pests of the crop, including SWCB (*Diatraea grandiosella*) for midaltitude tropical environments; sugarcane borer (SCB), *D. saccharalis*, for lowland tropical environments; fall armyworm (FAW), *Spodoptera frugiperda*, for both tropical and mid-altitude environments; and corn earworm (CEW), *Heliothis zea*, for silk and ear resistance in floury material adapted to the Andean region. Improvement has been through recurrent selection, and has focused also on improving resistance to major diseases encountered in the target environments.

- With the aim of improving general agronomic performance, we have derived advanced inbred lines that combine good levels of pest resistance with elite agronomic performance, and have made these available to interested cooperators worldwide.

- Sources of resistance to the major storage pests of maize, the grain weevil, *Sitophilus zeamais*, and the larger grain borer, *Prostephanus truncatus*, have been identified, in collaboration with IITA, Nigeria.

- Collaboration between Project staff and INIFAP, Mexico, has led to the development of sources of resistance to corn rootworm, *Diabrotica* spp.

- More than 230 white and yellow grained insect resistant inbred lines (S_4-S_6) have been developed for lowland tropical and midaltitude zones, characterized for combining ability and heterotic response, and handed to the stress breeder or supplied to national program scientists. Advanced lines have been used to form various synthetic populations. The lines have also been extensively tested alone and in hybrid combinations under artificial infestation and under insect-free conditions. The best 6-7 white grain lines and 3-4 yellow grain lines will be formally released to cooperators in 1997 as CIMMYT maize lines (CMLs).

- A superior set of the S_6 white-grained insect-resistant lines is being used as a part of a post-doctoral project to examine dosage rate effects (i.e., do resistant hybrids need resistance genes from both parents, or only from one?) and to identify superior hybrids under artificially infested and protected conditions. Results show that resistance is largely additive, suggesting that to obtain a resistant hybrid it is probably necessary to have resistance in both parents.
- Collaborative research with staff of the National Autonomous University of Mexico (UNAM) has shown that soluble flavenoids are not involved in resistance to SWCB or FAW, though low leaf protein levels (below 2.1% nitrogen), high fiber, phenolic acid content in cell walls and leaf toughness play key roles.
- The project hosted an international symposium on host-plant resistance in 1994, and many national program collaborators attended and presented their findings.
- In on-station trials of hybrids which were formed among and between insect resistant lines (IR) and high-yielding elite inbred lines (AG), the AGxAG hybrids usually outyielded hybrids made from IRxIR crosses, even under quite severe natural or artificial insect attack. Crosses between AG and IR lines were generally lower yielding than AGxAG hybrids, but were more stable in yield under insect attack. In on-farm trials grain yield of agronomically elite hybrids was significantly higher under a moderate level of fall armyworm attack, where around 30% of the plants showed symptoms of leaf damage. Had the attack been rather more severe, the improved resistance of the IR sources would likely have resulted in higher yields from the insect resistant source hybrids. It seems entirely possible through conventional or marker-assisted backcrossing that the excellent yield potential of AG lines can be combined effectively with the high level of host plant resistance exhibited by IR lines so that high and stable yield is possible, even under a severe insect infestations.

Development of drought- and low N tolerant varieties:

- Six elite lowland tropical populations and one subtropical population (the latter in collaboration with CIMMYT, Harare) were improved for drought tolerance, and two lowland tropical populations improved for tolerance to low N. This work has emphasized the development of a robust selection methodology more than the production of germplasm.
- Detailed studies have been conducted to measure genetic gains after 2- 8 cycles of selection for drought tolerance. Results indicate that improvements in grain yield of about 5% per year (or 100 kg/ha/year) can be expected in diverse maize germplasm.*Selection has resulted in maize that can provide a 25-40% increase in yield under conditions where drought near flowering or during grain filling reduces yields from 6 to 2 t ha^{-1}.
- Gains were accompanied by a marked reduction in anthesis-silking interval (ASI), a trait which is easily observed in plants when they are under stress at flowering, is highly heritable, and appears to reflect plant growth rate in young ears.

- Selection for drought tolerance by exposing families of populations to a carefully-managed water deficit coinciding with flowering was effective at increasing grain yields under drought and under well-watered conditions. There is no yield penalty under good conditions associated with improved yields under drought.

- Evidence suggests that improvements in drought tolerance at the seedling stage will prove difficult.

- Evaluations of three cycles of selection for tolerance to low N in the lowland tropical population, Across 8328 BN, showed gains of 75 kg ha^{-1} $cycle^{-1}$ in grain yield at low N (2.8% $cycle^{-1}$; $P<0.10$) and 137 kg ha^{-1} $cycle^{-1}$ at high N (2.3% $cycle^{-1}$; $P<0.01$).

- Concerning low-N selection methodologies, intercropping maize with wheat in the winter and with sorghum in the summer has been effective for increasing N stress early in the life of the crop.

- Selection under drought has proven highly effective at increasing performance under low N, with ASI serving as a reliable indicator of partitioning to the ear under low N as well as under drought. Drought stress at flowering appears to be a highly effective selection environment for a wide range of traits -- including tolerance to shaded and high density conditions and low N, and improved performance in unstressed conditions.

- Two projects are under way to apply DNA marker-assisted selection, based on an index of the best quantitative trait loci (QTL) for ASI and grain yield, to improve drought tolerance in maize lines and populations.

- Six synthetic populations have been formed based on drought and low N tolerant S_7 lines or on S_7 semiprolific lines. Lines have also been characterized for their heterotic response using tester lines common to the Lowland Tropical Maize Subprogram.

- Testing of hybrids and synthetics has been significantly expanded in Mexico, in collaboration with the Mexican national program, INIFAP, and with private seed companies.

- Ten superior lines from La Posta Sequía and TS6, along with 7 elite lines from the Lowland Tropical Maize Subprogram, are being used in a diallel study; results indicate that drought tolerance is additive in gene action (both parents of a hybrid must possess tolerance for the resulting hybrid to exhibit the trait).

- Five La Posta Sequía lines, one TS6 line and two lines from Pool 26 Sequía will be released as CIMMYT Maize Lines (CMLs) lines in February, 1997.

- An inbred line database using Microsoft EXCEL software has been established.

- Two broad-based populations have been developed from germplasm components showing good levels of drought tolerance, for environments where drought stress is common. Their use is restricted by incomplete disease resistance. Two other populations with tolerance to low N have been formed from landraces, but show poor performance under high N conditions. Evidence suggests that selection for improved tolerance in elite populations gives better results than does assembling source materials from relatively unimproved or poorly adapted components allegedly possessing stress tolerance.

- Project findings were presented at the project-sponsored conference "Developing Drought and Low N-Tolerant Maize", in held in March, 1996. This was attended by 70 national program representative, most of whom presented their own research results.

Development of acid soil tolerant varieties:

- This activity is conducted at CIAT, Cali, Colombia, where large areas of naturally occurring acid soils exist. Germplasm is tested throughout South America and parts of Asia.
- The number of full-season maturity populations was reduced from six to four and populations were organized in heterotic pairs. This consolidation freed up resources to resume development of two early-maturity acid soil- tolerant maize populations.
- During 1995, 1,802 lines from CIMMYT-Cali and 305 lines from CIMMYT-Mexico were evaluated in acidic and nonacidic fields in Brazil, Colombia, and Thailand.
- Yield improvements under acid soils ranged from 0.5 to 8.4% per cycle and averaged 4.8% per cycle, and under nonacidic fertile environments yield improvements from -7.4 to 7.1% per cycle were obtained, averaging 1.9% per cycle. Selection has been effective in improving the performance of the populations across a range of environments.
- In an evaluation of experimental varieties, our best tolerant OPV (93SA-4) yielded 3.11 t/ha, compared to 2.97 t/ha for the best national program checks, over 15 acidic environments. Across two nonacidic fertile environments, our best OPV (93SA6) yielded 7.49 t/ha, compared to 5.18 t/ha for the best checks.
- Seven new open-pollinated varieties (OPVs) were developed, using elite S_1 lines to form synthetics, for evaluation in national program plots during 1996-1997.
- Sikuani V-110 was developed from the population SA-3 and has been released for use on the acidic *llanos* of Colombia as an essential component of a stable and sustainable cropping system for this very extensive acid soil area. At present this variety is thought to be grown on 10,000 to 15,000 ha in the *llanos*. Considerable farmer-to-farmer transfer of seed of this variety has occurred, making the extent of its usage difficult to estimate simply from seed sales. Two seed companies in Colombia have undertaken to increase and sell seed. There is little doubt that acid-tolerant varieties such as Sikuani have the potential to increase maize grain yields by 30% or more over local check entries under soil conditions of pH 4-5 and aluminum saturation levels of 60-70%. The original Sikuani cultivar is being released in Peru where it is also expected to have a significant impact. The use of Sikuani in pasture-based rotations in the *llanos* is expected to increase profitability and economic sustainability significantly. New hybrids are under development which may increase production under acid soil conditions by up to 70% over that achieved by Sikuani. These were evaluated in 1996.
- A total of 49 lines (29 highly tolerant and 20 highly susceptible), including 20 pairs of near isogenic lines for tolerance and susceptibility, were identified and are currently being studied using the amplified fragment length polymorphism (AFLP) technique to locate molecular markers for tolerance to soil acidity.

- Work in collaboration with physiologists at Cornell university has demonstrated that citric acid exudation by roots is involved in providing tolerance to Al toxicity in maize.

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Interaction with National Agricultural Research Programs:

- Many visiting scientists and trainees have interacted with all aspects of the Project over the past 6 years.

- All Project senior staff have traveled quite extensively in maize growing countries of the tropics and have presented Project findings to national program staff in many regional and national fora.

- In 1994 the Project hosted the international conference "Insect Resistant Maize: Recent Advances and Utilization," attended by some 40 developing country scientists.

- Three networks have been established, one for the development, testing and distribution of drought tolerant genotypes, the second with similar functions for low N-tolerant germplasm, and a third smaller and older network that focuses on development of insect-resistant germplasm. In addition a testing network for acid soil-tolerant germplasm has functioned in South America and Asia for a number of years.

- The **Drought Tolerance Network** consists of 32 collaborators in 26 countries. Three groups of international drought trials have been distributed to participants (1990, 1992, 1995), with a 50-60% successful return rate of data. Over the years the testing of varieties is being supplemented by the testing of topcross progenies.

- The **Low N Tolerant Network** was established in 1995 with around 20 collaborators in 15 countries. A set of low N-tolerant topcrosses, a late-maturing variety trial, and an early maturing variety trial were distributed in 1995. By mid-1996, a total of 179 sets of trials of drought- or low N-tolerant germplasm had been distributed for testing.

- The **Insect Resistance Development Network (IRDN)** was established in the late 80s and consists of 20 cooperators in 13 countries.

- A 4-week course on the development of host plant resistance to maize insect pests was attended by 14 national program staff in January 1993.

- In March 1993 Project staff conducted a course entitled "Breeding for Drought Tolerance in Maize".

The future:

- Several CIMMYT breeders routinely screen their germplasm for drought and low N tolerance, and some also test lines, topcrosses, etc., for insect resistance on a regular basis. There is also much interest of scientists in national programs in using screening techniques. We expect the routine use of abiotic stress environments during selection to continue to increase.

- Molecular techniques offer the promise of more rapid gains in stress tolerance and of being able to convert elite but susceptible inbred lines to resistance, without modifying combining ability. Several new projects are planned for the large-scale application of these methods.

- CIMMYT will endeavor to incorporate an appropriate degree of stress tolerance into all its elite maize germplasm products in the future, but resources may limit the scale on which this can be done. We do not anticipate releasing any new germplasm without its being accompanied by a well-validated rating for abiotic and biotic stress tolerance.

- Most methods developed by the Project and its predecessors are now well-proven and ready to be devolved to regional and national breeding programs. One project supporting these activities has already been funded for the SADC region of Africa, where declining soil fertility and drought are the major constraints to production. A second larger project, to be launched in 1997, will expand the development of stress-tolerant maize cultivars into East, West and Central Africa. It is our intention to launch a similar initiative in South Asia in the next 12 months.

II. PROJECT OBJECTIVE AND FUNCTION

1. State objectives of the project as stated in the original project document

To foster more efficient use of resources in maize production by providing a wide array of germplasm complexes, each possessing high levels of resistance or tolerance to one or more of the following stresses: stem borers and fall armyworm, drought, low soil nitrogen conditions and aluminum toxicity in acid soils. Products of this work will be environmentally safe and will contribute to sustainable production in developing countries. Within this broad objective:

1) To evaluate and identify sources of resistance to each of these stresses, including within the genome of the maize wild relative, *Tripsacum*.

2) To develop efficient methods for improving this germplasm, including systems of selection based on molecular markers, RFLPs, where appropriate, and the screening of germplasm products at sites in national programs where specific stresses are experienced.

3) To assist national programs to use this germplasm in an efficient manner by organizing stress-specific networks for testing and distribution, through research on genetic controls of stress tolerance, training of national scientists, conferences and publications.

2. What is the primary function of the project? Response: Experimental

3. List outputs included in latest project document

Task 1: Evaluate RFLP technology for identification and selection of QTL in maize and its utility in wide hybridization programs with maize. Evaluate the benefits of applying marker assisted selection (MAS) to improve quantitatively inherited agronomic traits, and assess the utility of the technology for increasing the efficacy of introgression of genetic segments from wild relatives of maize.

1.1. Characterize elite materials for their allele type for a selected set of RFLP probes.

1.2. Detect co-segregation of RFLP fragments and QTLs.

1.3. Identify suitable molecular probes/RFLPs for the identification of genetic segments in *Tripsacum*.

1.4 Transfer of, and training in, the use of appropriate technology.

Task 2: Identify and improve germplasm complexes with high levels of resistance to the major maize stem borer and armyworm pests. Deliberate attention will be given to developing germplasm with multiple pest resistances and good agronomic characteristics. Initial success will be based on evaluation of resistant sources in collaborating countries. Gains in improvement in resistance levels will be assessed towards the end of the project term by comparing initial versus later cycles of selection.

2.1 Develop sources of resistance to major insect pests.

2.2 Recombine sources of single specie/stage resistance; develop germplasm with multiple resistance.

2.3 Develop insect resistant experimental varieties, hybrids and inbred lines.

2.4 Investigate biochemical and biophysical bases and inheritance of insect resistance.

Task 3: Upgrade the strength of national programs in mass rearing of maize insect pests and host plant resistance (HPR) development.

3.1 Train national program scientists from target countries in entomology/insect mass rearing and HPR.

3.2 Build the experience in HPR of mid-level visiting scientists from target countries.

3.3 One in-country training workshop on insect rearing, managing and selection for HPR per year.

3.4 Strengthen national programs through visits and consultation by CIMMYT and other scientists.

Task 4: To develop effective field-based methods of selection for improved tolerance to drought and low soil N. Evaluation, under drought and low N, of synthetics and lines selected for contrasting levels of expression of single traits, or trait combinations.

4.1 Initiate recurrent selection in four elite populations for drought and two elite populations for low N.

Task 5: To develop new germplasm in broad genetic backgrounds as future sources of tolerance to drought or low N. This germplasm will serve to broaden the genetic base of stress tolerance.

5.1 Develop broad based populations with tolerance to drought and low soil N.

Task 6: To establish and coordinate a network of cooperators at selected national program sites. The network would foster the exchange of germplasm, techniques and information.

6.1 Establish two networks comprising source developers and source testers, for tolerance to low soil N and to drought.

Task 7: To develop and improve productive maize germplasm with tolerance to aluminum toxicity. Increase the level of tolerance to aluminum saturation levels in improved germplasm.

7.1 Develop better germplasm for acid soils with aluminum saturation.

Task 8: Develop and improve a laboratory methodology for selecting aluminum tolerant maize germplasm. More efficient selection of tolerant germplasm is expected through greater precision and testing uniformity.

8.1 Review current laboratory methodology for selecting Al-tolerant maize.

8.2 Comparison of laboratory and field evaluations for Al-tolerant maize.

8.3 Develop new laboratory screening methodology for Al-tolerance.

8.4 Written procedures for laboratory screening for Al-tolerance in maize.

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

NOTE: Detailed responses to each task are presented in Research Summaries later in the report. What appears here is simply a brief summary of major areas of progress.

Output number: 1

(See Research Summary: Chapter 2 for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 1: Evaluate RFLP technology for identification and selection of QTL in maize and its utility in wide hybridization programs with maize. Evaluate the benefits of applying marker assisted selection (MAS) to improve quantitatively inherited agronomic traits, and assess the utility of the technology for increasing the efficacy of introgression of genetic segments from wild relatives of maize.

1.1. Characterize elite materials for their allele type for a selected set of RFLP probes.

1.2. Detect co-segregation of RFLP fragments and QTLs.

1.3. Identify suitable molecular probes/RFLPs for the identification of genetic segments in *Tripsacum*.

1.4 Transfer of, and training in, the use of appropriate technology

Scheduled completion date as in original signed project document	Actual or expected completion date
December, 1996	December, 1996

2.a. Describe the present status of the output.

We have completed a survey of restriction fragment length polymorphisms (RFLPs) in 50 maize lines with variable levels of resistance to southwestern corn borer (SWCB), plus 50 lines of importance to other breeding objectives of the Maize Program. This database has been used to select the parents showing maximum differentiation at the RFLP level for the study the location of genomic regions controlling resistance to SWCB. Included here were all the initial improved CIMMYT maize inbred lines (CMLs) developed for insect resistance; most came from Antigua or Antigua x Rep. Dom. background. Software was developed which greatly aids in processing such data, and a database was formed. The level of polymorphism was unexpectedly high. Cluster and principal component analyses revealed groupings of germplasm that could be generally expected from the pedigrees of these lines, though no clear distinction between tropical and subtropical germplasm was observed. This database will provide useful information to breeders who wish to 1) identify parents which differ for a specific trait and 2) use markers to accelerate the rate at which this trait can be moved from a source line to a recipient, defective line.

On the basis of data provided by the above study and from field observations, two crosses were made between resistant and susceptible lines (CML 139 x Ki3) and (CML 67 x CML 131). Insect resistance and several agronomic traits were mapped, using RFLPs. Data have been presented at scientific meetings and arranged in a database and presented to the Coordinator of the *Eureka* network. Plans are to extend this study to a further 50 lines in 1997. The controlled crosses referred to above, plus a further cross between a short ASI line (P1) and a long ASI line (P2), have been used as a basis for molecular mapping of QTL associated with SWCB resistance and drought tolerance (i.e., short ASI).

For ASI, seven QTL were identified on chromosomes 1 (two QTL), 2, 5, 6, 8 and 10, and accounted for 50% of the phenotypic variability. The five QTL segments contributed by P_1, the short ASI line, were responsible for a 7-day reduction in ASI, and were stable over years and stress levels. Four QTL for grain yield and ASI were located at the same position, on chromosomes 1, 6, 8 and 10; one of these contributed short ASI but reduced yield.

Mapping of recombinant inbred lines (RILs) for insect resistance and for drought tolerance is now well advanced. RILs have the advantage of providing a more precise indication of the location of the QTL so that during marker-assisted selection there is less linkage drag from undesirable traits that are transferred within the QTL. Manuscripts relating to the original F_2 QTL map, or to the mapping of RILs have been published in peer review journals, or are in preparation.

Marker assisted backcrossing is under way for insect resistance and for drought tolerance, using lines with a high degree of tolerance/resistance as sources and elite but susceptible lines as recipients. A postdoctoral fellow funded under the project (1993-1994) has been comparing marker-assisted selection and conventional methodologies to transfer SWCB resistance into maize-streak-virus-resistant African germplasm and evidence on the relative efficiency (time needed to gain a specific phenotypic value for the trait concerned and to regain the overall phenotype of the African lines) of each approach is being analyzed. A similar project has been initiated for ASI for the line CML247. Polymerase chain reaction (PCR) based methodologies have been adapted to increase the efficiency of marker-assisted selection and reduce its costs. Although several QTL have been shown to be common to those identified in the original cross, the results demonstrate that it is essential to make a new genetic map when you change the recurrent line, although only one trial under drought conditions may be sufficient to identify the QTL of interest. There is considerable similarity between the genomic locations of QTL for ASI under drought and those associated with ASI under low soil N, though those associated with yield variation under these two stresses are expected to vary considerably in number and genomic location. Data from these and other experiments have been shared extensively with European researchers, especially the group at Hohenheim University.

Good progress has been made in developing efficient *in situ* hybridization methods for detection of alien DNA in crosses with maize. In this case the donor was *Tripsacum*, a near relative of maize that occurs naturally in Mexico. This methodology, and the substitution lines that were developed using it, have been taken up by a collaborative project between CIMMYT and The French National Research Institute for Development Cooperation (ORSTOM) to transfer apomixis from *Tripsacum* to maize, and has played an important part in improving the efficiency of that transfer.

To date, 46 scientists from 21 countries have received short or long-term training in our laboratories. Three students (Chile, Mexico and Peru) have done their MSc theses, and 10 students (Canada, Cuba, Ecuador, France, Germany and Mexico) have completed or are completing PhD dissertions in the Applied Molecular Genetics Laboratory. We have now held two formal courses entitled "Molecular Marker Applications to Plant Breeding" during 1995 and 1996, and a total of 32 young scientists from developing countries have attended these very popular courses.

RFLP probes have been distributed to a number of laboratories requesting them, and a manual of laboratory protocols describing CIMMYT's non-radioactive, chemiluminescent technologies for RFLP detection has been distributed in English or Spanish to 314 scientists in 48 countries. Three important computer programs that facilitate data entry, analysis and marker-assisted selection (HyperBlot, HyperMapData, HyperMAS) have been developed and distributed widely, free of charge. Each of these is expected to significantly increase the efficiency of mapping and MAS activities in labs that are equipped to do this research.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain: The goals of this task have been fully met and, with collaborative research funded from other projects, extended to include mapping of drought tolerance traits (ASI) as well as insect resistance. The identification of QTL associated with short ASI was an unexpected bonus and emphasizes the usefulness of concerted collaborative action among projects with similar aims but different funding sources.

Progress in MAS for SWCB has been in generally steady and based on careful field and lab research. Some concern remains that not all of the relevant portions of the genome have been identified with current markers and statistical techniques. This is a rapidly evolving field in which new techniques for analysis of data (such as cofactor analysis) are becoming available every year. Furthermore there appears to be a reasonable level of QTL x environment interaction; i.e., the location of QTLs is not always the same in each evaluation of resistance. This problem seems less of an issue with the anthesis-silking interval traits associated with drought tolerance. With insect resistance the problem relates to variation associated with field evaluation and the effects of the environment on both insect and plant behavior - all reasons pointing to the need to use MAS once a firm basis for it has been established. A final test of the progress made using MAS versus conventional approaches has been made during 1996 and awaits final analysis, and at that point a critical evaluation of MAS will be made. We are not however waiting for a final analysis before commencing MAS for insect resistance and drought tolerance. In both cases we are focusing on the conversion of elite adapted inbred lines in pilot conversion programs, and will move to a larger scale conversion of lines with the start-up in 1997 of the DGIS-funded projects with Zimbabwe and Kenya. We are also exploring the use of MAS to rapidly convert a population from a normal state to a drought-tolerant state, thereby saving years of recurrent selection under managed stress conditions (see Chapter 4). The use of MAS for reduced ASI under drought should sharply reduce both the cost and the time of identifying inbred lines and components of populations carrying drought tolerance. At the same time, it must be remembered that variation in ASI accounts for only 25-35% of the variation in grain yield under water stress, and the search for the traits which are associated with the remaining variation must go on. In other words, transfer of the short ASI trait will take us some of the way, but certainly not all of the way, towards drought-tolerant germplasm. When short ASI and high grain yield under drought are combined in MAS, then more progress will undoubtedly be made.

Research that focused on the transfer of traits from *Tripsacum* to maize is showing real returns: techniques developed by Project staff have been transferred to a French-funded apomixis project where they are being used to facilitate the transfer of the apomixis trait from *Tripsacum* to maize.

The transfer of new techniques, information and enhanced germplasm products is proceeding smoothly and CIMMYT's Applied Biotechnology Center and its Maize Program are now in an excellent position to assist national programs in the application of these techniques, as well as being able to apply them with much greater confidence in CIMMYT's own breeding programs.

3. If produced, to what extent, and by whom is the output being used?

In general we see the major beneficiaries of this group of outputs as the users of the new genetic products that are created. All national programs whose farmers are affected by SWCB or mid-season drought will benefit by having access to elite lines which have enhanced levels of SWCB or drought tolerance incorporated into them.

Advanced national programs, using MAS to transfer perhaps less complex traits which are normally difficult or impossible to screen for efficiently within their normal operations (e.g., drought tolerance in programs where there is no dry season nursery that is not affected by frost). The techniques are not yet established well enough to expect transfer to have taken place at this stage. The CIMMYT Maize Program will use MAS for insect resistance, drought tolerance and for several other traits outside of the scope of the UNDP Stress Project. Private seed companies are already using, or will use MAS.

Molecular techniques have greatly enhanced our understanding of the maize genome; this basic knowledge will benefit all breeders who seek to improve the efficiency of selection through marker-assisted or conventional means.

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

Output number: 2

(See Research Summary: Chapter 3 for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 2: Identify and improve germplasm complexes with high levels of resistance to the major maize stem borer and armyworm pests. Deliberate attention will be given to developing germplasm with multiple pest resistances and good agronomic characteristics. Initial success will be based on the evaluation of resistance sources in collaborating countries. Gains in improvement in resistance levels will be assessed towards the end of the project term by comparing initial versus later cycles of selection.

2.1 Develop sources of resistance to major insect pests.

2.2 Recombine sources of single specie/stage resistance; develop germplasm with multiple resistance.

2.3 Develop insect resistant experimental varieties, hybrids and inbred lines.

2.4 Investigate biochemical and biophysical bases and inheritance of insect resistance.

Scheduled completion date as in original signed project document	**Actual or expected completion date**
On going	December, 1996

2.a. Describe the present status of the output.

Output 2.1: In collaboration with national programs, private companies, and advanced research institutes, we have developed and improved several maize populations for the tropics and subtropics which possess good levels of resistance, based on antibiosis, to major insect pests of the crop, including southwestern corn borer (SWCB), *Diatraea grandiosella*, for midaltitude tropical environments; sugarcane borer (SCB), *D. saccharalis*, for lowland tropical environments; fall armyworm (FAW), *Spodoptera frugiperda*, for both tropical and mid-altitude environments; and corn earworm (CEW), *Heliothis zea*, for silk and ear resistance in floury material adapted for the Andean region. Materials include the:

- Multiple borer resistance population (MBR, Population 590)
- Multiple borer and disease resistance population (MBR-MDR, Population 590B)
- Multiple insect resistance tropical population (MIRT, Population 390)
- Multiple borer, *Exserohilum turcicum* resistance population (MBR-Et)
- CEW resistance populations (85, 86, 87 and 88)

To increase its usefulness to maize breeding programs in developing countries, we have recently focused on enhancing the agronomic performance and disease resistance of this source germplasm and on deriving subsets based on grain color. With the same aim, we have derived advanced inbred lines from the source germplasm that combine good levels of pest resistance with elite agronomic performance; these have been made available to interested cooperators worldwide.

At the behest of national programs, we have continued work to identify sources of resistance to the major storage pests of maize, the grain weevil, *Sitophilus zeamais*, and the larger grain borer, *Prostephanus truncatus* (LGB). CIMMYT is the only CG center developing source populations for LGB resistance; as part of this we have identified Caribbean landraces from germplasm bank collections which appear to possess good levels of resistance. We are collaborating with the International Institute of Tropical Agriculture (IITA) to model the relative importance of grain resistance in an IPM approach to *P. truncatus* control in western Africa. Finally, we are conducting joint research with the Mexican National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) to develop resistance sources for corn rootworm, *Diabrotica* spp. (CRW), and to identify lines and hybrids resistant to SWCB.

<u>Output 2.2:</u> Based on the results of recent trials (196 families from MIRT, 144 from MBR, and 121 from MBR-MDR) sent to collaborators in Brazil, China, Dekalb (Mexico), ICIPE (Kenya), India, Nigeria, the Philippines, the Republic of South Africa, the USA, and Zimbabwe, approximately 90 of the best families were selected for recombination, evaluation, separation into white and yellow grain fractions, and use by CIMMYT and national breeding programs.

Working in concert with collaborators in national programs and CIMMYT regional offices, during the project we have developed maize populations with the following combined resistance/tolerance traits. Seed of these populations has been made available to interested researchers in the above regions:

- Resistance to stem borer and maize streak virus (in collaboration with CIMMYT researchers and national programs in West and Southern Africa).
- Resistance to stem borer and downy mildew (in collaboration with CIMMYT researchers and national programs in Asia).
- Resistance to fall armyworm, stem borer, and maize stunt complex (in collaboration with CIMMYT researchers and national programs in Central America).

Output 2.3: As of 1995, CIMMYT entomologists had developed more than 230 white and yellow grain, insect resistant inbred lines (S_4-S_6) for lowland tropical and midaltitude zones. These lines are being used by the stress breeder, based on their combining ability, *per se* performance, and grain color, to form populations as sources of open pollinated varieties and new inbred lines, hundreds of which are being tested under a range of conditions. Regarding the materials of subtropical adaptation, the advanced lines used to form the synthetic populations are now in testing alone and in hybrid combinations under artificial infestation/inoculation with SWCB, FAW, SCB, and *E. turcicum* leaf blight, as well as under protected conditions.[1] Based on the promising results we have seen, we expect to make the best 6-7 white grain lines and 3-4 yellow grain lines available to cooperators in 1997. A superior set of the S_6 white-grained lines is being used as a part of a post-doctoral project to 1) examine dosage rate effects for borer resistance in hybrids as well as the role of vigor in their expression; 2) estimate the relationship between line *per se* and hybrid performance under both normal and insect-infested conditions; 3) identify single-crosses for developing second cycle lines that combine insect resistant traits with good agronomic performance; and 4) compare inbreds and hybrids selected for borer resistance with those selected for agronomic performance.

Selected lowland tropical materials derived from our entomology research are being grown under poor fertility conditions to verify the hypothesis that antibiosis resistance – rather than tolerance – is more effective under the low-input farming circumstance typical in many developing countries. The most promising tropical inbred lines are being tested alone and in hybrid combinations under both infested and protected conditions.

Lines developed for resistance to maize grain weevil are being evaluated in hybrid combinations and under infestation.

Output 2.4: Research in collaboration with the Universidad Nacional Autonoma de Mexico (UNAM) has revealed that soluble flavanoids are not involved in resistance to SWCB or FAW. Our other work suggests, though, that low leaf protein levels (below 2.1% nitrogen) and high fiber and phenolic acid content in cell walls play a key role in resistance. These results have been corroborated by our DNA mapping studies, which show that leaf toughness and reduced protein are associated with several of the same genome regions as field resistance. Regarding storage pests, the biochemical bases of resistance have been identified as phenolic acids in the pericarp and aleurone layers and kernel toughness; future research will further clarify factors which underlie the latter. Research at the University of Ottawa has shown the hydroxamic acid, DIMBOA, to be negatively correlated with rootworm damage; CIMMYT and the University of Ottawa are using this knowledge to facilitate screening for rootworm resistance in inbred lines, and a related genome mapping project is under way at CIMMYT. Some future work will focus on the mechanisms responsible for resistance to second generation borer attacks.

Finally, given the importance of dosage issues in developing resistant hybrids (e.g., for a single-cross hybrid, is it sufficient that only one of the inbred progenitors possess resistance, or must both?), several studies on the inheritance of resistance have been conducted under the project. It was found that the inheritance of leaf feeding resistance to FAW, SWCB, SCB, and ECB was due primarily to additive gene

[1] Testing is done simultaneously under stress and optimal conditions to identify germplasm that is resistant under pest attack but carries no associated "yield penalty" when cropping circumstances are favorable.

action, accompanied by a high general combining ability. Inheritance of resistance to storage pests and rootworm is also due to additive effects; with both general and specific combining ability being important in the case of rootworm. Related studies under way (such as that mentioned in 2.3) will expand our knowledge.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain:

Each output is being met according to the original set of goals laid out in the Project documents. Continuing challenges are the incorporation of insect resistance into elite germplasm sources (that is, how to use source germplasm); the improvement of the agronomic performance of insect-resistant germplasm, especially in the area of stalk strength and yield; the identification of adequate sources of insect resistance in elite inbred lines from the conventional breeding programs. Excellent progress has been made in identifying lines with adequate resistance and improved agronomic characteristics, and in consolidating the myriad subpopulations adapted to specific disease conditions.

3. If the status of the output is unsatisfactory.

A. What factors are causing it? (Check as appropriate and provide comments under questions 3.B & C on the next page.)

Not applicable

B. Explain item(s) checked in 3A, including how production of the output is affected.

Not applicable

C. What effect does this unsatisfactory status have on the achievement of the immediate objective?

Not applicable

4. If produced, to what extent, and by whom is the output being used?

Many shipments of insect-resistant seed have been sent to national program breeders, and several breeders made crosses onto their own adapted germplasm using pollen from MBR and MIRT progeny trials. Sources of insect resistance (and most other stress-resistant source germplasm) will only rarely be used as directly released varieties. Mostly we expect them to be crossed to locally-adapted elite germplasm to provide a good level of insect resistance in germplasm with a high yield potential and appropriate levels of disease resistance. It is difficult to track this usage of germplasm, especially in open-pollinated varieties. Our own Subtropical Maize Subprogram used several MBR lines in elite hybrid combinations with good results in 1995. The project stress breeder, Dr. David Beck, is evaluating many advanced lines that possess good levels of leaf feeding resistance; the best products have been exposed to a wider range of clients in the 1996 International Hybrid Trials. Steady progress has been made in identifying inbred lines with high levels of resistance and good general combining ability and in recycling lines. The second generation of inbred lines from these populations will be considerably superior to first in all aspects, but especially in yield, disease resistance, and reduced lodging. Finally, as noted above, breeders in several key maize production regions in the developing world are using recent products from the project that possess combined resistance to major insect pest species and to other locally important constraints (e.g., diseases such as stunt, maize streak virus, or downy mildew).

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

Output number: 3

(See Research Summary: Chapter 4, for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 3: Upgrade strength of national programs in mass rearing of maize insect pests and host plant resistance (HPR) development.

3.1 Train national program scientists from target countries in entomology/insect mass rearing and HPR.

3.2 Build the experience in HPR of mid-level visiting scientists from target countries.

3.3 One in-country training workshop on insect rearing, managing and selection for HPR per year.

3.4 Strengthen national programs through visits and consultation by CIMMYT and other scientists.

Scheduled completion date as in original signed project document	**Actual or expected completion date**
On going	December, 1996

2.a. Describe the present status of the output.

Output 3.1: No formal training course was offered in 1995, with the exception of two maize training sessions held in 1995 (one in Spanish and one in English). Informal training was provided to two students from the Agrotechnia, Saltillo, Mexico (Tino Almacende Sutaan and Ing. Rene Rodriguez de la Cruz) from June 15 to July 2. During this time both students learned about insect rearing and participated in lab. activities associated with mass-rearing of all four lepidopteran species handled by CIMMYT.

Output 3.2: In November 1994, CIMMYT hosted the international conference "Insect Resistant Maize: Recent Advances and Utilization," which some 40 developing country scientists attended. The CIMMYT entomologist participated in the 5th Asian Regional Maize Conference in 1995 at New Delhi and traveled throughout India, providing technical assistance and making arrangements for collaborative work on host plant resistance. Dr. Bergvinson also represented CIMMYT at an inter-center project review on the larger grain borer (LGB), *Prostephanus truncatus*. Two mid-level scientists from Africa worked as visiting scientists in the entomology unit during June - September, 1996.

Direct financial support to the China and Philippine national programs from this project will continue. Dr. Zhov Darong, Senior Research Entomologist, Institute of Plant Protection, Beijing, is responsible for the $10,000 provided by the entomology unit to support HPR breeding in Northern China. Efforts are being made to divert some of this money to Southern China (Nanning, Guangxi), where past CIMMYT collaboration has resulted in useful germplasm products. Dr. Eduardo Fernandez, Institute of Plant Breeding, Los Baños, Philippines, has been the administrator of the $3,600 received from the entomology unit for laboratory support in rearing *Ostrinia furnicalis*.

Output 3.3: No formal workshops were held in 1995, due largely to a change in entomology staff. The entomologist participated in a training workshop hosted by ICIPE, Kenya, in 1996.

Output 3.4: During 1995 the entomologist visited the national programs of Thailand, India and Honduras. He also visited colleagues at Mississippi to keep himself abreast of the latest techniques in insect rearing methods and breeding strategies that can in turn be made available to national programs. Direct consulting and technical assistance was given in Kenya, Zimbabwe, Ghana and Benin, in 1996.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain: As noted in the 1994 report, upgrading the capacity of national programs to conduct research on host plant resistance and to mass-rear and artificially infest with insects is not the same thing as actually making it happen. In general there is still a disappointing level of HPR research within national programs, and very few are capable of artificially rearing insects and infesting nurseries with them. The problem lies not with trained personnel, nor with methodology. Rather it is simply that the level of government support is not adequate to establish and maintain the needed facilities, and the turnover of national staff is often high. In the private sector of developing countries, methodologies associated with this research have not attracted a lot of attention because of the long-term commitment needed to achieve results. There is, however, an encouragingly high interest in the utilization of genetic sources of insect resistance developed by the Project, suggesting that in the future it may be necessary to invest in insect rearing facilities at regional research hubs where adapted maize germplasm can be properly evaluated for resistance on behalf of the national maize programs of the region. The one-on-one training at CIMMYT of mid-level entomologists committed to HPR is still regarded as a highly effective means of instituting such research in maize breeding programs of selected countries that have demonstrated a financial commitment to this type of work. Changes in personnel in the entomology unit in 1995 have made it difficult to sustain the level of interaction between this program and the national programs that was a hallmark of the previous entomologist.

3. If the status of the output is unsatisfactory.

A. What factors are causing it? (Check as appropriate and provide comments under questions 3.B & C on the next page.)

Not applicable; note comments under 2(b).

B. Explain item(s) checked in 3A, including how production of the output is affected.

Not applicable

C. What effect does this unsatisfactory status have on the achievement of the immediate objective?

Not applicable; see however comments under 2(b).

4. If produced, to what extent, and by whom is the output being used?

The techniques of mass rearing and artificial infestation were designed to be used by the national programs, both public and private sector. Some of the methods developed by Dr. Mihm before the commencement of the project, such as the "bazooka" insect dispenser for more uniform field infestation by larvae, have been widely used to breed for HPR in the USA and in several other industrialized countries. Other techniques, such as a uniform scoring system for insect damage, and efficient methods for utilizing insect resistant source germplasm to develop open-pollinated varieties and hybrids, are being used directly by national programs as they incorporate MBR, MIRT and other sources of resistance into adapted germplasm. The most widely used products from this research have undoubtedly been the germplasm sources (for example, the well-known borer-resistant inbred line, CML67) and uniform field infestation methods using larvae dispensed by the bazooka.

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

Output number: 4

(See Research Summary: Chapter 5 for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 4: To develop effective field-based methods of selection for improved tolerance to drought and low soil N. Evaluation, under drought and low N, of synthetics and lines selected for contrasting levels of expression of single traits, or trait combinations.

4.1 Initiate recurrent selection in four elite populations for drought and two elite populations for low N.

Scheduled completion date as in original signed project document	Actual or expected completion date
On going	December, 1996

2.a. Describe the present status of the output.

Six elite lowland tropical populations and one subtropical (in collaboration with CIMMYT, Harare) were improved for drought tolerance during the life of the project.

One population, a late yellow dent type, Across 8328 BN, has been improved for performance under low nitrogen, and a second population, Pool 16 BNSEQ, is being improved simultaneously for drought and low N tolerance. This work emphasizes the development of a methodology more than the production of germplasm.

Detailed studies have been conducted and analyzed to identify the genetic gains and mechanisms of improved stress tolerance after 8 cycles of selection for drought in Tuxpeño Sequía, and after 3 cycles of selection in La Posta Sequía, Pool 26 Sequía and Pool 18 Sequía. A recent review of the potential for improvement of drought tolerance in tropical grain was recently prepared by Project staff and presented at the Second International Crop Science Conference in New Delhi (Edmeades, et al., 1996), and the large proportion of the Project findings were presented at the project-sponsored conference "Developing Drought and Low N-Tolerant Maize", in held in March, 1996.

Selection for drought tolerance, by exposing families of populations to water deficit coinciding with flowering, was effective at increasing grain yields under drought and under well-watered conditions. Gains observed in populations that vary for genetic background and maturity average 100 kg/ha/year, or about 5% per year. Gains are around 100 kg/ha/cycle for full-sib selection and around 200-280 kg/ha/cycle for recurrent S_1 selection, and are positive and often large even under well-watered conditions. In other words, *there is no yield penalty under good conditions associated with improved yields under drought*. Selection has resulted in a 25-40% increase in yield for a farmer whose yields have been reduced from 6 to 2 t ha^{-1} by drought occurring near flowering and during grain filling. Gains were accompanied by a marked reduction in ASI, a trait which is easily observed in plants when they are under stress at flowering, is highly heritable, and appears to reflect plant growth rate in young ears. Total biomass production under drought or under well-watered conditions was unaffected by selection.

Screening procedures have been developed to identify families which are tolerant to drought stress at the seedling stage. Initial evidence suggests that improvements in drought tolerance at this stage will prove difficult, but differences in response by genotype were observed.

Studies to identify selection criteria for germplasm tolerant to low N consisted of a preliminary evaluation of diverse genotypes, of isopopulations developed by divergent selection for single traits or groups of traits in Across 8328 BN, and a detailed evaluation of changes which have occurred in this population over its first three cycles of selection. We expect to refine selection procedures to focus more on the roots, a long-overlooked component of the plant's adaptation to stress conditions.

Evaluations of three cycles of selection in Across 8328 BN in 1989, 1990, and 1991 revealed a linear increase of 75 kg ha^{-1} $cycle^{-1}$ in grain yield at low N (2.8% $cycle^{-1}$; $P<0.10$) and 137 kg ha^{-1} $cycle^{-1}$ at high N (2.3% $cycle^{-1}$; $P<0.01$). Six cycles of recurrent selection have been completed, the last two being among S_1 families rather than FS families. This has permitted the easy extraction of inbred lines from this population (see below).

A new technique for increasing the level of N stress early in the crop cycle continues to be refined. It consists of intercropping maize with wheat in the winter and with sorghum in the summer. Most maize grown under low N is now intercropped with wheat (in winter) or sorghum (summer). This provides a uniform N stress caused by the N which the secondary crop takes up. Providing the establishment is even, the secondary crop provides a uniform degree of competition for N which is available early in the crop's life, before maize canopy closure occurs.

Interactions between mechanisms of tolerance common to drought and low N have been examined. Our evaluations indicate clearly that selection under drought is highly effective at increasing performance under low N, with ASI serving as a good indicator of partitioning to the ear under low N as well as under drought. In our recent molecular studies, ASI mapped to several of the same genome regions under drought or low N conditions. Drought stress at flowering appears to provide a highly effective selection environment for a wide range of other target environments -- including shaded and high density conditions, low N, and unstressed conditions.

Two projects are under way to apply DNA marker assisted selection, based on an index of the best quantitative trait loci (QTL), for ASI and grain yield, to improve drought tolerance in maize lines and populations. (For details, see Task 1.)

Based on combining ability evaluations and the *per se* performance of the 100 lines from the Physiology Subprogram, we have selected 20 S_6 lines for further evaluation. We also are forming a synthetic population using 12 of the superior S_5 lines.

Semiprolific populations SPE, SPL and SPMAT were selected for general tolerance to stresses occurring around flowering and during grain filling. Hybrid combinations exceeding the yield of the hybrid check by 15-25% have been identified with lines from both SPE and SPL, and lines from SPMAT are also showing promising performance. We currently have 22 S_7 lines from SPE and 59 S_6 lines from SPL that are being studied and advanced further.

Twenty-nine S_5-S_6 lines from population La Posta Sequía C_3 (LPS) and 25 S_5-S_6 lines from TS6 C_1 have been selected based on their *per se* and top-cross performance in combination with elite tropical single-cross and inbred lines. During 1995-96, 24 hybrid trials with these materials were conducted in 20 environments including CIMMYT experiment stations and sites in collaboration with the Mexican national program and private seed companies, and through drought network trials. Ten superior lines from La Posta Sequía and TS6, along with 7 elite lines from the tropical subprogram, are being used in a diallel study as part of a post-doctoral project. Lines and hybrids used in this study have been evaluated in 10 environments including drought stressed, low N stressed and non-stressed conditions. Preliminary findings from this on-going project show that the mode of drought tolerance is largely additive and that dosage effects are important in developing drought tolerant hybrids. Two synthetic populations utilizing 18 lines from La Posta Sequía and 11 lines from TS6 have been developed. After extensive testing, we now plan to announce 5 La Posta Sequía lines, 1 TS6 line and 4-5 lines from Pool 26 Sequía in January, as CIMMYT maize inbred lines (CMLs), making the seed freely available to all interested cooperators. These drought-tolerant inbred lines from elite backgrounds are expected to generate a tremendous interest worldwide. Advanced early-maturing inbred lines from Pool 18 Sequía are under evaluation, prior to being made available to cooperators.

A significant accomplishment by the Stress Breeder during 1995 was the establishment of an inbred line database using Microsoft EXCEL software; data from 1996 on will be consolidated therein.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain: We believe that he output of this component of the project has greatly exceeded initial expectations, partly because the UNDP Project built on a solid research base established before the current project began. As a consequence, after 6 years of project support we can say with a high degree of confidence that we can improve the grain yield of tropical maize exposed to mid-season drought stress, and can do so at around 5% gain per year. The fact that grain yields can be improved under drought and low N conditions AS WELL AS under unstressed conditions, must be regarded as an important accomplishment. Stress tolerance does not have to accompany a loss in yield potential! The anthesis-silking interval remains the single most important secondary trait linked to barrenness and grain yield under drought, and our evidence links it also to performance under low N. The identification of QTL and alleles associated with this trait using RFLP markers opens up many new opportunities to speed the breeding for drought- and low N-tolerance in otherwise elite germplasm.

3. If produced, to what extent, and by whom is the output being used?

The outputs of this task are drought- or low N-tolerant populations, experimental varieties, lines and hybrids, proven methods of selection for tolerance to these stresses, and trained national staff. All of these have proved useful to national program collaborators, to private seed companies, to CIMMYT breeders, and to the scientific public. From 1990 through 1996, aside from the shipment of network trials (see below) we shipped 1140 individual genetic entities to approximately 50 collaborators from an average of 23 countries per year. In 1995-6 the demand for inbred lines with good GCA and with drought tolerance, outstanding performance under low N, or general stress tolerance increased greatly, and we anticipate it will increase even more in 1997. Publications are listed elsewhere in this report, and represent an important output as well, and our services as speakers are in reasonable demand. In 1996 one of our staff (G. Edmeades) was invited to address the Second International Crop Science Congress in New Delhi, and a another (D. Beck) was asked to present our findings to the prestigious Annual Corn and Sorghum Research Conference sponsored by the American Seed Trade Association in Chicago, IL.

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

Output number: 5

(See Research Summary: Chapter 6 for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 5: To develop new germplasm in broad genetic backgrounds as future sources of tolerance to drought or low N. This germplasm will serve to broaden the genetic base of stress tolerance.

5.1 Develop broad based populations with tolerance to drought and low soil N

Scheduled completion date as in original signed project document	Actual or expected completion date
On going	December, 1996

2.a. Describe the present status of the output.

Two broad-based populations have been developed for environments where drought stress is common. The process of forming these populations involved screening c. 300 landrace accessions from appropriate collection sites, and over 100 elite source materials. Both populations consist primarily of elite germplasm – 60% of lowland adaptation, 20% of subtropical adaptation and about 20% from temperate sources. Approximately 15% of the germplasm forming both populations, however, can be traced directly to superior landraces. One of these populations, DTP1, underwent two cycles of international testing using sites provided by the Drought Network collaborators. The other population, DTP2, was improved solely in Mexico. In 1996 we combined the best fractions of both populations to create DTPW C_8 and DTPY C_8, two populations that are distinguished solely on the basis of grain color. Both are intermediate in maturity but are rather susceptible to foliar and ear diseases. Both populations perform reasonably well in the tropics and subtropics, both carry a high level of tolerance to drought during flowering and grain filling, but their use is hampered by disease susceptibilities. We continue to evaluate additional materials under drought each year, in order to identify new sources of drought-adaptive traits which can be introgressed into these populations. Approximately 36 S_3 white-grained lines and 50 yellow-grained lines developed from the Drought Tolerant Population 1 (DTP1) were passed to the Stress Breeding Unit from the Physiology Subprogram in 1995. All selected lines have been advanced to S_4 and S_5, crossed to appropriate testers, and are currently under evaluation.

In addition, we have formed and improved a *latente* drought-tolerant subpopulation. This population is composed of germplasm reputedly carrying the *latente* complex of drought-tolerant characters (that is, an ability to defer developmental events under severe stress and an enhanced capacity to recover from severe drought stress at the vegetative stage). The *latente* population consists mainly of Corn Belt x selections from Michoacan 21 from DeKalb Genetics and from several other sources, and we are now attempting to tropicalize it. It is currently under evaluation to determine if it has any unique drought-adaptive traits, but the evidence is not convincing so far. Should this population show merit, it will be introgressed into DTPY.

For low N environments two broad-based populations, developed exclusively from germplasm bank accessions, have been developed. They are characterized by early and late maturity, and are of mixed grain color and texture. These populations are poor agronomically, but they are currently under improvement for yield and for reduced plant height under low N. Evaluation of these populations for yield during selection began in 1994, and at the same time we sought to maintain the traits for which they were originally selected. Inbred lines have been extracted from these populations, but at present they seem unlikely to contribute new and original genetic mechanisms that will enhance grain yield under low N conditions.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain: The source populations have been formed as envisaged from germplasm with unique tolerance to the stresses we are addressing. In doing so, a wide array of source germplasm was evaluated, and many landraces examined under drought or low N. The DTP populations are performing well under drought and under well-watered conditions, perhaps because they have 85% elite germplasm in background. Their broad genetic base has already been exploited by several national programs, and they have been well distributed to potential users through the S_1 progeny trials of DTP1. The population DTP1 has also been useful in the selection study of tolerance to drought at the seedling stage because of its broad genetic variability for a wide array of drought-related traits. We continue to observe useful genetic variation in new source germplasm for drought-related traits; this will be incorporated into DTP-Y or DTP-W over time. The division of the populations into color components and the extraction of inbred lines has been welcomed by collaborators, many of whom lack the facilities to undertake these tasks.

The development of broadly based populations comprising sources of superior performance under low N has proceeded more slowly, and the outcome has in general been less satisfactory. Thus far these populations have not included any elite germplasm showing good performance in infertile conditions, and as a consequence their performance under fertile conditions is not impressive. In 1996 we compared inbred lines and their topcrosses extracted from the BN populations under low N, and it seems unlikely that it will be worth continuing recurrent selection in these populations.

3. If produced, to what extent, and by whom is the output being used?

The broad-based drought-tolerant populations DTP-Y and DTP-W represent approximately 15% of the seed requests for stress-tolerant germplasm by national programs through the Physiology Subprogram. National program scientists are especially interested in receiving them as inbred families (S_1s or inbred lines) of a specific grain color, and demand for these is increasing rapidly. Population bulks remain popular as well. However, the frequency of elite inbred lines from these populations is lower than from elite source populations such as La Posta Sequía and TS6, and we will have to screen a rather larger number of lines to find ones which are fully competitive under drought *and* well-watered conditions.

There is much less demand for the broad-based low N populations, partly because they have never been tested internationally (and therefore are not widely known), but also because they have a number of agronomic defects that suggest they are not yet ready for wide distribution. As a result a decision was taken not to include these populations as progenies in trials distributed to participants of the low N Network, but instead to include them as population bulks in the variety trials (see below).

In general, broadly-based source populations have not delivered quality germplasm as quickly as we had hoped. this can be traced to the inclusion of components which, while they met our criterion of specific stress tolerance, did not carry the requisite resistance to the diseases of well-watered environments in the lowland tropics. We will continue reselecting among these populations, and send fractions of them to southern Africa and to Asia for regional adaptation. We remain optimistic that, at least for the DTPs, a useful broadly-adapted drought tolerant product will eventually be derived from them.

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

Output number: 6

(See Research Summary: Chapter 7 for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 6: To establish and coordinate a network of cooperators at selected national program sites. The network would foster the exchange of germplasm, techniques and information.

6.1 Establish two networks comprising source developers and source testers, for tolerance to low soil N and to drought.

Scheduled completion date as in original signed project document	Actual or expected completion date
On going	December, 1996

2.a. Describe the present status of the output.

Three networks have been established: one for the development, testing and distribution of drought tolerant genotypes; the second with similar functions for low N-tolerant germplasm; and a third smaller and older network that focuses on developing insect-resistant germplasm.

The first cycle of international drought-tolerance trials was conducted in 1990, and results were distributed to cooperators in 1991 (Edmeades et al., 1991). A second set of trials was announced in 1992, and results were distributed in 1995 (Edmeades et al., 1995). Efforts up till that stage had concentrated on the testing of DTP1 S_1 families and selected fractions of elite populations that were being improved for drought tolerance through recurrent selection. In 1995 a decision was taken to organize the trials through CIMMYT's International Testing Program, and to withdraw DTP1 from international testing and instead replace it with a series of topcross progenies of inbred lines that had demonstrated superior yields when tested under drought. Thus, at the end of 1995 a third set of drought-tolerant topcrosses, and late-maturing variety trial, early-maturing variety trial, and an extra-early maturing variety trial were distributed to those national program staff who requested them. The low N tolerance network was established in 1995, since the start of the development of selection methodologies was considerably later than that for drought or for insect resistance. In 1995, however, we were able to announce a set of low N-tolerant topcrosses, a late-maturing variety trial, and an early maturing variety trial, all with good tolerance to low N conditions for distribution to interested national program staff. By mid-1996, 179 sets of trials of drought- or low N-tolerant germplasm had been distributed, and results from around 20% of these have been returned to Maize International Testing staff for analysis. At present the international drought tolerance network comprises 32 scientists in 26 countries, while that for low N comprises 20 scientists in 15 countries. Cooperating countries are: Mexico, Nicaragua, Costa Rica, Guatemala, Panama, El Salvador, Haiti, Ecuador, Peru, Bolivia, Colombia, Brazil, Argentina, Thailand, India, Pakistan, China, Nepal, Indonesia, Philippines, Ivory Coast, Ghana, Nigeria, Egypt, Malawi, Zambia, Tanzania, Zimbabwe, Kenya, Mozambique, and Ethiopia.

The insect resistance development network (IRDN) was established in the late 1980s and consists of 20 cooperators who have the capacity to mass rear insects that resemble in life cycle and feeding habits those which challenge maize in the tropics and to uniformly infest experimental maize with the pests. Cooperators are located in the USA (6), Canada, India (2), China, Egypt, Kenya, Mexico (2), Zimbabwe, Ghana, Republic of South Africa, Iran, Philippines, and Taiwan. Three sets of S_1 progeny trials (Pop. 390; 590; 590B) have been shipped to network cooperators about once every three years (the time taken to complete a cycle of S_1 testing that includes international testing), and results are shared with all network participants. The next international trials for these populations will be ready for early 1997, and will include progenies, varieties and hybrids carrying insect resistance.

A 4-week course on insect resistance was provided to 14 national program staff in January 1993. Participants were arranged in pairs comprising a breeder and an entomologists -- the pattern they might be expected to follow in their home countries. The course strongly emphasized a "hands-on" approach to breeding for insect resistance. This was followed by a special course in March 1993, entitled "Breeding for Drought Tolerance in Maize". We felt that this short course would allow us to prepare a larger number of cooperators at a higher level of quality than a system of visiting scientists. As well, participants benefited greatly from the interaction among each other during the course. Nineteen scientists from fourteen countries participated in this course, and a description of the course is included in Chapter 10 of the Research Summaries. Participants commented that the information gained in both courses would influence the selection methodology and criteria they use in their breeding programs. A large number of seed samples have been shipped from the Entomology and Physiology Subprograms in response to requests generated by the course, indicating that germplasm distribution has been enhanced as well.

A visiting scientist from Somalia spent six months at CIMMYT in 1993 working on the development of drought tolerant germplasm. In March, 1996 a group of four visiting scientists from Colombia, Cape Verde, India and Ethiopia worked with us on drought tolerance research for 3-8 weeks each and now work in similar activities in their home countries. Later, a maize breeder from the Mexican national program, INIFAP, joined the Stress Breeding Subprogram for 6 months of training in hybrid production for stressed environments, and a second INIFAP scientist is presently on the same scheme for a further 3 months. In addition we have had four regular maize breeding trainees from Latin America and Africa who have spent 5 months as part of Physiology or Stress Breeding activities. Two students have completed their PhD theses in drought tolerance, and several others have completed their MS theses in aspects of acid soil tolerance.

In November, 1994, the Project Entomologist, Dr. John Mihm, organized an international symposium on entitled "Insect Resistant Maize, Recent Advances and Utilization," at CIMMYT, Mexico. This meeting was attended by 80 scientists, 40 from developing countries. Proceedings will be published in March, 1997. In March 1996, we organized the international symposium "Developing Drought and Low N-Tolerant Maize" at El Batan, Mexico. The event was attended by 110 scientists attended, 70 from developed maize-growing countries, and virtually all who attended made a presentation (a total of 36 oral presentations and 72 posters). Proceedings will be published in June, 1997. At each of these international symposia participants from the networks attended and were prominent in their presentations. Finally, in 1995, the proceedings were published from a 1992 meeting held in Brazil, to which the UNDP Project contributed ("International Symposium on Environmental Stress: Maize in Perspective". Editors: A. Toledo Machado; R. Magnavaca, S. Pandey and A. Ferreira da Silva. EMBRAPA/CNPMS and CIMMYT/UNDP. 449 pp.)

Project staff have traveled extensively throughout the life of the project, and have been well-represented at regional meetings of maize scientists. However, the major emphasis of this phase of the project has been on development of methodologies and sources of germplasm. As a consequence of the investment by the Project in conventional, managed stress and molecular breeding methodologies, we are now in an excellent position to move to more of an "extension" role in a subsequent phase of the Project.

Collaboration with Mexico has increased greatly through the life of the Project. Since 1987 there has been strong collaboration between the Physiology Subprogram and the INIFAP research station atCiudad Obregon, in the Sonoran Desert. This site was used to screen our germplasm for tolerance to heat and drought. In 1994 this expanded to include topcrosses from the Stress Breeding Subprogram, and more recently a number of inbred- or hybrid-oriented entries from conventional breeding programs as well. The Stress Breeding hybrid testing program has added five Mexican locations, including three with INIFAP, one with a Mexican University, and one with a private Mexican seed company.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain: The establishment of the three stress-specific networks represents satisfactory progress in this task. We have been successful in identifying national program scientists who are genuinely interested in cooperating with us in developing new germplasm sources and in testing products from selection. The limiting factors are usually the inability to rear insects artificially, lack of controlled irrigation, or lack of uniform test sites, which affect the timing, uniformity and intensity of the artificial insect infestations or drought stress required for proper selection. As well, the germplasm sources that served as vehicles for improved tolerance traits have serious limitations in adaptation – especially with regard to disease reaction, maturity and grain texture and color. Clearly the next phase is to establish networks around regional centers of excellence for selection conditions and infrastructure, working with the best adapted germplasm available in the region.

Thus far the networks have functioned well as a means of distributing germplasm, but have generally been less successful as a means of distributing information. Analysis of trial data has been slow, and each cycle of network testing has taken two plus years to complete. Interactions among network members have been somewhat ad hoc, mainly because there were no resources devoted specifically to network maintenance and communications. This suggests that the primary goals of networks have been to distribute germplasm and methods rather than to *develop* adapted sources of resistance. Quality of data generated by the networks, however, has increased noticeably over time (especially in the drought-tolerance network), and exchange of information has increased markedly in 1995 and will do so again in 1996-97.

The success of Project staff in attracting additional funding for regionally-based networks in southern Africa and more recently in East, Central and West Africa suggests that the future of these networks will rest on regionalization and on integration into the mainstream of maize breeding in each region and program. There are promising signs that this project has played a key role in focusing attention on these constraints in virtually every maize national program that CIMMYT has contact with.

3. If produced, to what extent, and by whom is the output being used?

The output of the networks is broadly adapted germplasm from CIMMYT carrying tolerance to either insect attack, to drought or to low N, that is available to all participants and to anyone outside the networks who requests it.. As well, locally-adapted germplasm supplied by network participants is beginning to be shared with other national programs. Information derived from CIMMYT's research and from joint network activities is also a product with considerable value to network participants, but also to the general maize research community.

A second output is better interpersonal relationships among network scientists who share common goals, germplasm and data. The movement of information through these networks is improving. The Symposia sponsored by the Project have been key points of contact among national maize researchers from all over the world, and their Proceedings will continue to provide benefit to all maize researchers in the tropics for the next 10-15 years.

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

Output number: 7

(See Research Summary: Chapter 8 for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 7: To develop and improve productive maize germplasm with tolerance to aluminum toxicity. Increase the level of tolerance to aluminum saturation levels in improved germplasm.

7.1 Develop better germplasm for acid soils with aluminum saturation.

Scheduled completion date as in original signed project document	Actual or expected completion date
On going	December, 1996

2.a. Describe the present status of the output.

This activity is conducted at CIAT, Cali, Colombia, by CIMMYT staff based at that site. The presence of acid soils is the principal reason why the program is based here rather than in Mexico.

Germplasm Development: During 1995 CIMMYT continued its efforts to develop and improve tolerance to soil acidity in tropical maize. Major germplasm development activities involved:

1. Reduction in the number of full-season maturity populations from six to four. Population SA-5 was merged with SA-3, and SA-8 with SA-7. For this 338 S_1 lines from SA-3, 368 from SA-4, 365 from SA-5, 352 from SA-6, 324 from SA-7, and 289 from SA-8 were evaluated for yield in acid soils in Colombia, Brazil, and Thailand, from which 96, 100, 47, 140, 73, 121 lines were selected,

respectively. The selected lines were crosses with appropriate heterotic populations and the topcrosses were evaluated in replicated trials in both acidic and nonacid environments in Bolivia, Brazil, Colombia, Ecuador, Guatemala, Ivory Coast, Mexico, and Thailand. The S_1 lines were advanced to S_2 in Colombia. Based on the heterotic pattern information, S_2's of selected S_1 lines will be intermated during 1996A to complete the process of consolidation of the populations. This consolidation released resources to resume development of two early-maturity acid soil- tolerant maize populations. For this, 571 lines were evaluated in acidic and nonacidic fields and 15 lines with white and 67 lines with yellow endosperm were selected and intermated to develop the two populations.

2. During the year 1,802 lines from our program and 305 lines from the program in Mexico were evaluated in acidic and nonacidic fields in Brazil, Colombia, and Thailand, and 36 lines with white and 142 lines with yellow endosperm were selected. Selected lines have been crosses with 5-6 testers and in a Design II mating system to develop approximately 880 hybrids for evaluation in national program plots during 1996.

3. We evaluated C_0, C_2, and C_4 of SA-3, C_0, C_1, and C_3 of SA-4, SA-5, SA-6, and SA-7, and C_0, C_1, and C_2 of SA-8 in five acidic environments, two nonacidic and low fertility environments, and one nonacidic fertile environment. Across the acidic environments, yield improvements ranged between 0.47 and 8.43% per cycle, and averaged 4.75% per cycle. Across the nonacidic low fertility environments, yield improvements ranged between -0.18 and 9.43% per cycle for an average of 2.99% per cycle. In the nonacidic fertile environment, yield improvement ranged between -7.35 and 7.10% per cycle and averaged 1.85% per cycle. The data suggest that we have been effective in improving the performance of the populations across a range of environments, and particularly in acidic soils.

4. Seven new open-pollinated varieties (OPVs) were developed, using elite S_1 lines, for evaluation in national program plots during 1996-1997.

5. An experimental variety trial was conducted with our newest OPVs where our best tolerant OPV (93SA-4) yielded 3.11 t/ha, compared to 2.97 tons/ ha of the best national program checks over 15 acidic environments. The superiority of our best OPV over the best checks is about 9% but in 11 of the 15 sites national programs had included our previous OPVs as their checks. Across two nonacidic fertile environments, our best OPV (93SA6) yielded 7.49 t/ha, compared to 5.18 t/ha for the best checks.

6. Bolivia, Ecuador, and Peru joined as our full and active collaborators in the project and grew topcross and variety trials.

Training, consultation and dissemination of results: During 16-20 January, 1995, a meeting was organized in Colombia to show to the participants recent developments in our program, discuss our 1994 results, and plan the collaborative research activities for 1995 and 1996. Two scientists from Bolivia, four from Brazil, three from Colombia, three from Ecuador, one from Peru, one from a regional institution (IICA-PROCIANDINO), four from CIMMYT-Mexico, and five from CIMMYT-Cali participated. The meeting included a side trip to Villavicencio where participants assisted in the harvest of acid soil-tolerant progenies and saw a number of agronomic trials.

The III Latin American and XVI Andean Regional Maize Meetings were held in Bolivia during 20-24 November, 1995. A total of 88 scientists participated: 12 from Argentina, 40 from Bolivia, eight from Brazil, seven from Colombia, three from Ecuador, one from Italy, one from Guatemala, one from Honduras, one from Nicaragua, three from Mexico, three from Peru, one from Uruguay, three from USA, and four from Venezuela. The participants presented their own research results and benefited from invited talks by distinguished scientists on the topics of biotechnology, regional integration, agricultural education, breeding for silage, and recent developments in CIMMYT's maize program.

Twelve scientists from the region participated in full-service training courses offered in Mexico and in Brazil and four scientists visited CIMMYT-Mexico to work with the colleagues at the head quarter's program.

We supported two Colombian students to pursue their M.S. degree at the University of Palmira (Colombia) and one Ecuadorian student to pursue his Ph.D. degree at the University of Piracicaba (Brazil).

Close collaboration is maintained with the acid soil tolerance program developed by EMBRAPA some years ago for maize at Siete Lagoas, and there is frequent exchange of information and views, collaborative testing of progenies and exchange of methodologies between the two programs.

The two scientists working in the program spent a combined total of 201 days visiting national program plots and working with national program scientists during the year.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain: In 1995, for the first time in the history of the UNDP Project funds were used to support this important component of our research, and the costs of the Post Doctoral Fellow (but not the senior scientist) were paid for by the UNDP Project in 1995. This will continue through 1996. Testing of yellow and white acid-soil tolerant genotypes in Asia (especially countries like Indonesia and the Philippines) is proceeding well, and the incorporation of downy mildew resistance into acid soil-tolerant germplasm will greatly extend its area of suitability. The regional network of breeders and agronomists addressing acid soil tolerance is now well-established.

3. If the status of the output is unsatisfactory.

A. What factors are causing it? (Check as appropriate and provide comments under questions 3.B & C on the next page.)

Not applicable

B. Explain item(s) checked in 3A, including how production of the output is affected.

Not applicable

C. **What effect does this unsatisfactory status have on the achievement of the immediate objective?**

Not applicable

4. **If produced, to what extent, and by whom is the output being used?**

As noted in the 1994 Progress report, acid soils cover 1,660 million ha of developing world agricultural areas, and about 43% of tropical soils are classified as acidic. Sixty-four percent of tropical south America, 38% of tropical Asia and 27% of tropical Africa, and 10% of mesoAmerica are considered acidic soils. Where pH falls below around 5.5 on weathered laterites, there is often a sharp increase in the proportion of aluminum which occupies the exchange complex of the soil. When this complex is more than 35% saturated by Al, there is a measurable reduction in maize growth. There are large areas (perhaps 5m ha of llanos in Colombia alone) with levels of Al saturation exceeding 60%. Liming these soils reduces Al saturation to acceptable levels, but this is often not feasible for logistical or economic reasons. Acid-soil tolerant maizes have the potential to be used on much of this land.

One example is the variety Sikuani V-110, released by the Cali-based program in 1994 (see last year's report), which will produce around 3 t/ha under hot lowland conditions and on soils with at least 60% Al saturation. This variety has the potential to displace rice from the widely-used rice-pasture rotation of the Amazon basin, and can stabilize maize production in the acid soil areas of Asia and Africa. As a consequence there is increasing interest in acid-tolerant maizes from the private sector and from national programs in Latin America, Asia and Africa. There is also much interest in determining mechanisms of acid soil tolerance and of the relationship between phosphorus use efficiency and acid soil tolerance, since acid soils are usually accompanied by acute P deficiency. There is a strong possibility that acid-soil tolerant lines will also be highly efficient in their ability to extract P from acid soils.

III. EVALUATION OF PROJECT PERFORMANCE - OUTPUTS

Output number: 8

(See Research Summary: Chapter 9 for details)

1. Repeat output (as stated in latest approved project documentation/revision):

Task 8: Develop and improve a laboratory methodology for selecting aluminum tolerant maize germplasm. More efficient selection of tolerant germplasm is expected through greater precision and testing uniformity.

8.1 Review current laboratory methodology for selecting Al-tolerant maize

8.2 Comparison of laboratory and field evaluations for Al-tolerant maize.

8.3 Develop new laboratory screening methodology for Al-tolerance

8.4 Written procedures for laboratory screening for Al-tolerance in maize.

Scheduled completion date as in original signed project document	Actual or expected completion date
On going	December, 1996

2.a. Describe the present status of the output.

<u>Output 1:</u> No change from the situation reported in 1994, which is repeated here. Literature searches of methodologies used to screen for aluminum tolerance have been carried out in previous years. They have revealed that several bioassays have been developed for fast screening of tolerance to Al-toxicity and P-deficiency. The most widely used is growing seedlings in nutrient solutions with varying levels of aluminum, and this has been adapted by EMBRAPA, Brazil, and their standard screen for Al-sensitivity in maize. The technique has proven to be efficient at differentiating tolerant and susceptible materials in

different crops. However, it has two major drawbacks: 1) it can only screen for the reaction to just one of the limiting factors for plant growth under acid soils (toxic levels of Al), and 2) the correlation between performance in nutrient solution and field results are not high (around 0.30). A new technique in sorghum and other cereals but not maize was published for rapid screening. It uses acid soils in pots instead of nutrient solution. The technique has the advantage of evaluating the response to several of the limiting factors for plant growth under acid soils and their interactions.

Output 2: No change from the situation reported in 1994.

Output 3: A total of 49 lines (29 highly tolerant and 20 highly susceptible), including 20 pairs of near isogenic lines for tolerance and susceptibility, were identified and are currently being studied using the amplified fragment length polymorphism (AFLP) technique to locate molecular markers for tolerance to soil acidity. We are also developing appropriate genetic stocks by crossing two highly tolerant and two highly susceptible lines to tag the genes responsible to acidity tolerance. This molecular information is expected to increase the efficiency of breeding programs, and shorten the time needed to identify and develop acid- tolerant maize cultivars.

Output 4: Written procedures for laboratory screening of germplasm: As the experiments are finished and are important, they are being published. A final document is not expected until near the end of the project in 1996.

b. The status is (x) Satisfactory ( ) Unsatisfactory

Please explain: As noted in the 1994 Progress Report, less progress on the development of laboratory screening techniques has been made than was originally envisaged. In general, this is because the correlation between such tests and performance in the field is low. Field resistance is a complex character involving the ability to accumulate biomass of roots and shoots, and the ability to partition that biomass at flowering in such a way as to establish a large ear. With the development of uniform acid soil screening sites at Villavicencio and Carimagua in the llanos of Colombia, and progress towards MAS (via the AFLP analysis described above) as a cost-effective way of discarding a large proportion of the non-tolerant backcrosses during the process of transferring tolerance, the need for laboratory screening has diminished. Molecular markers associated with acid soil-tolerance will sharply reduce the need for laboratory techniques. In essence, progress in molecular techniques, QTL identification and gene tagging have made the use of greenhouse screening methods redundant.

3. If the status of the output is unsatisfactory.

A. What factors are causing it? (Check as appropriate and provide comments under questions 3.B & C on the next page.)

Not applicable

B. Explain item(s) checked in 3A, including how production of the output is affected.

Not applicable

C. What effect does this unsatisfactory status have on the achievement of the immediate objective?

Not applicable

4. If produced, to what extent, and by whom is the output being used?

The output would be of use to those national programs and private seed companies that have access to greenhouse and lab. facilities, and who were committed to a breeding program for acid-soil tolerance in maize. Among countries with acid soil problems this may amount only to Brazil, Indonesia, Philippines, and perhaps Colombia. The primary use would be by Project staff themselves to reduce the proportion of progenies they would have to screen in the field.

IV EVALUATION OF PROJECT PERFORMANCE - OBJECTIVES

1. State how the achievement of the immediate objective(s) as stated in II.1 can be observed and/or measured.

- Through the identification of new sources of genetic variability for stress tolerance.
- Through the development of new methods of screening this variability.
- Through the transfer of resistance from sources with poor agronomic performance to elite germplasm products.
- Through the use in farmers' fields of improved germplasm showing resistance to one or more of: stem borers; fall armyworm; tolerance to acid soil; to drought; to low N.
- Through the increased capacity of national program scientists to undertake the above tasks themselves within their own programs.

2. Using the indicator/success criteria recorded in 1. above, provide your assessment of the extent to which the project has achieved or is likely to achieve its immediate objectives.

The first two criteria have been met quite satisfactorily. The third is taking place at present for insect resistance and drought tolerance through MAS, though not yet for tolerance to other stresses. This is not considered so essential for tolerances other than for insects because for other stresses selection for tolerance occurred within elite germplasm, through recurrent selection methods. Thus there was no real need to transfer these traits from sources which performed poorly (in an agronomic sense) to elite germplasm.

As noted in the 1994 Progress Report, it is difficult to gauge current use of germplasm in farmers' fields. There have been a large number of seed shipments of resistant/tolerant materials to national program scientists, and we have documented four direct releases of drought tolerant germplasm (all OPVs; El Salvador; Ecuador (2); China) over the past 2 years. One measure of the improved research capacity of national programs over time can be seen in the greatly improved quality of the data returned from the last round of drought network trials ('92-94) compared with that returned from the first round of trials ('90-91), and an improved quality of the scientific standards in presentations made by national programs on the topic of stress tolerance (see the Abstract Book for the upcoming conference on Development of Drought- and Low N-Tolerant Maize).

3. State the development objectives of the project as given in the original signed project document.

To improve the income and stability of income of resource-poor farmers in less well-endowed areas by undertaking a cooperative program of research to develop improved, stress resistant germplasm, to refine methodologies for improving this germplasm, to assist national programs in using this germplasm, and to provide advanced training to key national program scientists.

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4. Are there signs that the project is making or is likely to make a significant contribution towards the attainment of the development objectives? If so, please describe.

Steady and pleasing progress has been made on development of sources of stress tolerance/resistance in high-yielding backgrounds, and these have been shared with the national programs at their request. Such sources have shown increased stability of yield in the face of fluctuating levels of drought, fertility, insects and soil acidity. Release and adoption of stress tolerant germplasm is occurring slowly, but is expected to increase if resources can be directed towards promoting distribution and testing of stress-tolerant germplasm at the national program level.

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5 (a). Who are the beneficiaries of the project? (b) Explain how they are or will benefit from the project.

The immediate beneficiaries are national program scientists who receive improved sources of resistance, elite germplasm with high levels of resistance, and a suite of methods which will allow the scientist to extend this research to his/her adapted germplasm. The ultimate beneficiary will be the resource-poor farmer operating in marginal-stress-prone areas, who will experience a higher and more stable output from his maize farming endeavors, and the urban consumer who will enjoy lower and more stable food prices. The beneficiaries will be women as well as men, in the proportion with which they are found in the target areas.

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6. Has the project had any unforeseen effects either positive or negative? If so, briefly explain.

None that have been noted

7(a) On the basis of your analysis of parts III and IV above, give your overall assessment of the progress of this project in terms of achieving its immediate objective(s)

(x) More than planned in the original project documentation. In part this is because this project built on a solid research base that had already been established at CIMMYT in stress tolerance breeding, and in part because a number of other donors have contributed to the overall cost of attaining the goals of this project. The weakest area still lies in upgrading the capacity of national program scientists to undertake similar research, but as we have become more confident that we have selection methodologies and germplasm sources that work, we are increasingly ready to speed up that transfer of germplasm, information and scientific capacity.

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7 (b) What action do you recommend to be undertaken by any of the three parties involved (Government, Executing Agency, UNDP) to improve the effectiveness of this project?

By far the most serious problem this Project faces is what will happen to the research, the networks and the national program scientists involved with CIMMYT in this work, when the funding from UNDP ceases on December 31, 1996. If there is a substantial break in the funding, much of the momentum we have attained will be lost.

7(c). Description of the overall status of the project.

Above expectation; satisfactory progress towards developmental goals.

UNITED NATIONS DEVELOPMENT PROGRAMME

V: MONITORING RECORDS

Project Number and Title	Executing Agency	Expenditures	Date last report	Date this report
GLO 90/003/A/01/42	World Bank	Jan. 01, 1996 Sep. 30, 1996	15-Feb-96	18-Dic-96

1	2	3	4	5	6
Input item by budget line	Scheduled current calendar year Jan.-Dec./96	Actual date of report Jan. 01, 1996 Sep. 30, 1996	Actual end current calendar year 1996	Difference	Remarks

U.N.D.P. BUDGET MAIZE

SERVICES IN HOME OFFICE:					
Scientists	552,640	306,493	306,493	246,147	
Support Staff	95,745	53,100	53,100	42,645	
TRAVEL:					
Senior Scientists	17,762	9,851	9,851	7,911	
Subsistence Allowance	9,879	5,479	5,479	4,400	
OTHER REIMB. COSTS:					
Field Costs & Supplies	287,415	159,400	159,400	128,015	
Laboratory Costs & Supplies	44,690	24,785	24,785	19,905	
Field & Laboratory Office	31,803	17,638	17,638	14,165	
Training Courses Improv.	4,506	2,499	2,499	2,007	
Training Fellowships	0	0	0	0	
Collaborative Research	41,960	23,271	23,271	18,689	
Regional Workshops	0	0	0	0	
CONFERENCE:					
Conference	82,445	45,724	45,724	36,721	
Publications	1,922	1,066	1,066	856	
Central Services	175,610	97,393	97,393	78,217	
TOTAL	1,346,378	746,699	746,699	599,679	

Development Of New Stress-Resistance Maize Genetic Resources
Annex 2- Expenditures and Disbursements through December 31,1996

	Revised Project Budget	Expenditures through Sep 30,1996	Balance Available
Services in Home Office	3,341,546	2,948,396	393,150
Travel	232,249	165,997	66,252
Other Reimbursable Costs			
Field Costs	1,339,595	1,308,750	30,845
Laboratory and Supplies	344,402	386,296	(41,894)
Field and Fellowships	118,366	135,380	(17,014)
Training and Fellowships	125,715	133,863	(8,148)
Collaborative Research	120,076	46,218	73,858
Workshops and Conferences	101,421	92,949	8,472
Publications	23,583	33,419	(9,836)
Subtotal	2,173,158	2,136,875	36,283
Central Services	862,047	758,054	103.993
Total	**6,609,000**	**6,009,322**	**599,678**
Disbursements to Date		5,901,091.00	
% of disbursements to project budget		89%	
Balance		707,909.00	

Annex 1 - Budget and Expenditure by Task
January 1995 though September 1996

Project Task / Output	Estimated Budget	Previously Reported Jan-Dec-95	This Period Jan-Sep-1996
1. Evaluate RFLP technology for identification and selection of QTL in maize and its utility in wide hybridization. Evaluate benefits of MAS for introgressing genetic segments from wild relatives			
1.1 Characterize elite materials for their allele type for selected set of RFLP probes	0		-
1.2 Detection of cosegregation of RFLP fragments and QTL's	281	112	94
1.3 Identify suitable molecular probes/RFLP's for Tripsacum introg.	0	-	-
1.4 Transfer and training in appropriate technologies	24	9	8
Subtotal 1	**305**	**121**	**102**
2. Identify and improve germplasm complexes with high levels of resistance to major maize stem borers and insect pests;multiple pest resistance; good agronomics. Test in collaborating countries and assess rate of gain.			
2.1 Develop sources of resistance to major maize pests	222	112	65
2.2 Recombine multiple sources of resistance	158	79	45
2.3 Develop EVs, hybrid, and inbred lines	190	95	55
2.4 Investigate biochemical and biophysical bases and inheritance of resistance	63	32	18
		-	
Subtotal 2	**633**	**318**	**183**
3. Upgrade strength of NARS's in mass rearing of maize insect pests and host plant resistance			
3.1 Train NARS scientists from target countries	0		-
3.2 Train mid-level visiting scientists from target countries	40	27	18
3.3 One in-country training workshop per year	40	26	18
3.4 Visits of 3-10 days to national programs by CIMMYT	25	22	11
Subtotal 3	**105**	**76**	**47**
4. To develop effective field based methods of selection for tolerance to drought and low soil N. Evaluation under drought and low N of synthetics and line selected for contrasting levels of expression of single or groups of traits			
4.1 Recurrent selection in four elite populations for drought and in 2 elite pop.for low N	306	99	89
4.2 Develop lines with tolerance to drought and low N	305	98	87
Subtotal 4	**611**	**197**	**176**
5. Develop new germplasm in broad genetic backgrounds as future sources of tolerance to drought and low N			
5.1 Broad based populations development	123	69	60
5.2 Develop lines from these populations	53	30	27
Subtotal 5	**176**	**99**	**87**
6. Establish and coordinate network of cooperators at selected national program sites			
6.1 Two networks established, one for drought, one for low N.	127	53	46
6.2 Bring visiting scientists to Mexico for 1 month of training in methodology	48	20	17
6.3 Hold workshop on drought and low N in Mexico in 96	40	17	14
Subtotal 6	**215**	**89**	**77**
7. Develop and improve productive maize germplasm with tolerance to aluminum toxicity Increase the levels of tolerance to Al saturation in improvement germplasm.			
7.1 Better germplasm development	164	82	30
7.2 Develop inbred lines for tolerant populations	82	41	15
7.3 Map Al tolerance from crosses of susc and resist lines	27	13	5
Subtotal 7	**273**	**136**	**50**
8. Develop and improve a laboratory method. for selecting Al tolerant maize germplasm			
8.1 Review current literature on methodologies for screening	5	1	1
8.2 Compare laboratory and field evaluations under different levels of saturation	64	22	16
8.3 New laboratory screening methodology	19	7	5
8.4 Written laboratory procedures	10	4	2
Subtotal 8	**98**	**34**	**24**
Total	2,416	1,070	746

UNITED NATIONS DEVELOPMENT PROGRAMME

TECHNICAL COOPERATION PERSONNEL

Internationally-Recruited Project Professional Personnel Staff of Project 1990-96

Post Title	Name	Nationality	Gender	Entry	Departure
Senior Staff					
Cytogeneticist/Breeder	D. Jewell	Australian	Male	11/90	12/93
Stress Breeder	J. Deutsch	American	Male	11/90	12/93
Stress Breeder	F. Gonzalez	Mexican	Male	1/94	2/94
Stress Breeder	D. Beck	American	Male	6/94	12/96
Physiologist	G. Edmeades	New Zealand	Male	11/90	12/96
Entomologist	J. Mihm	American	Male	11/90	2/95
Entomologist	D. Bergvinson	Canadian	Male	1/95	12/96
Post-Doctoral Fellows (PDF) and Associate Scientists					
Assoc. Scientist Physiology	J. Bolaños	Guatemalan	Male	11/90	4/91
PDF, Entomology	S. Miller	American	Female	9/91	5/92
Assoc. Scientist Physiology	S. Chapman	Australian	Male	3/91	11/94
Assoc. Scientist Cytogenetics	N. Islam-Faridi	Bangladesh	Male	3/91	6/94
Assoc. Scientist Stress breeding	M. Willcox	American	Female	2/92	2/95
Assoc. Scientist Entomology	H. Kumar	Indian	Male	2/92	12/96
PDF, Physiology	A. Elings	Dutch	Male	4/95	12/96
PDF, Stress Breeding	J. Betran	Spanish	Male	1/95	12/96
Assoc. Scientist Breeder, Acid soils	L. Narro	Peruvian	Male	1/95	12/96
Assoc. Scientist (0.5) Biotech., MAS	M. Khairallah	Lebanese	Female	1/95	12/96

Notes on staffing. Staff whose operating costs were wholly or partially met by the UNDP Stress Project during 1990-96 were:

1. Dr. Renee Lafitte, Maize Physiologist/Agronomist, responsible for improving maize performance under low soil nitrogen. Dr. Lafitte was employed full-time in training, half-time by CIMMYT, and her salary and allowances were paid by core funds, while her operating costs were met by the UNDP Project. During her period of full-time employment on CIMMYT core funding, she maintained a research program in low N tolerance and these field activities were paid for by project funds.
2. Dr. Marianne Bänziger, Maize Physiologist/Breeder, July 1995 through July 1996. Dr. Bänziger's salary and allowances were paid from core, but her operating costs relating to selection for tolerance to low soil N were met by the UNDP Project. Responsible for establishing the Maize Low Fertility Tolerance Network. Departed the Project for an outreach stress breeding/physiology position, July 1996.
3. Dr. Shivaji Pandey, Maize Breeder, Leader of the Tolerance to Acid Soils Breeding Project based at CIAT, Colombia. Selected operating costs only, 1993-96.
4. Dr. Hernan Ceballos, Maize Breeder, part of the Tolerance to Acid Soils Project (resigned February, 1994. Selected operating costs only, 1993-94.

Other staff associated with stress tolerance research at CIMMYT, not funded by the UNDP Stress Project and whose research results are partially reported here are:

1. Dr. Marianne Bänziger, Post Doctoral Fellow, funded by the Swiss National Foundation and the Swiss Development Cooperation from July 1992 till July, 1995, to examine the relationship between N and drought stress tolerances, and to develop screening and selection methods for tolerance to drought stress during establishment. She took over the low N-tolerance research led by Dr. Lafitte. Her research results from the period when she was supported by the Swiss National Foundations (SNF) and Swiss Development Cooperation (SDC) are reported here.
2. Dr Jean-Marcel Ribaut, Post Doctoral Fellow, funded till mid-1995 by SDC and then by CIMMYT core funds, to develop a linkage map for QTL associated with anthesis-silking interval (a trait associated with drought tolerance) in tropical maize. This research is summarized in this report.
3. Dr. Martha Willcox completed her project on marker-assisted selection for Southwestern corn borer resistance in maize, participating in the mapping activities that this entails, and from Feb. 1995 was fully supported as a Senior Staff scientist under a companion UNDP Project (UNDP GLO/91/014).
4. Dr. Mireille Khairallah, Associate Scientist, 1992-94, who with Dr. Martha Willcox identified and mapped QTL associated with Southwestern corn borer resistance in maize. Partial financial support for Dr. Khairallah's position was provided by the project in 1995-96 (half-time position). Drs. Khairallah and Willcox were assisted by two German pre-doctoral students, Mr. Martin Bohn and Ms Susanna Groh. Mr. Bohn was directly involved in mapping QTL associated with insect resistance in two F_3 mapping populations, and Ms. Groh is characterizing recombinant inbred lines derived from one of those mapping populations.

   In addition, considerable time of Dr. Diego Gonzalez de Leon and Dr. David Hoisington of the Applied Molecular Genetics Section has been devoted to strategic issues and data analysis related to the Project.
5. Drs S. Pandey, H. Ceballos and R. Knapp, working in acid soil tolerance, provided research input to the Project variously from 1990 through 1996, but were actually supported by BID, CIDA and core funds during this time.

Research Summaries

Chapter 1: Introduction

I. Assessing the global importance of abiotic stresses

We have used several methods to determine the relative importance of production constraints. The first is through the survey of maize mega-environments conducted by CIMMYT, in which respondents were asked to list the biotic and abiotic challenges encountered by maize crops by adaptation zone, maturity class, grain color and grain texture classes (CIMMYT, 1988). Respondents were asked to consider only the frequency and severity of drought, and the questionnaire did not attempt to assess the importance of other abiotic constraints.

The alternative method to assessing relative importance of stresses relies on the accumulated experience of breeders and agronomists from CIMMYT and from the national programs, and from various estimates of stresses in the literature, resulting in estimates presented in Table 1.1.

This suggests that the largest causes of yield loss in farmers' fields are low fertility (principally N deficiency) followed by drought and varying plant competition caused by low planting densities, weeds and intercrops. A more quantitative approach to assessing the importance of abiotic stresses is through crop simulations combined with the use of global databases. In preparation for this we have conducted a series of experiments to better define our germplasm in terms of crop phenology and to assess the impact of both drought and nitrogen stress on leaf area development. These data will allow us to simulate the potential target area of our germplasm, to assess spatial and temporal variation within those areas, and to better set breeding priorities.

Respondents to CIMMYT's mega-environment the questionnaire were asked to place mega-environments into 4 classes, and we have associated an estimated annual yield loss with each class:

	Class	Estimated yield loss
Rarely stressed	1	5%
Sometimes stressed	2	10%
Frequently stressed	3	25%
Usually stressed	4	40%

Using these qualitative guidelines, yield losses can be estimated by ecology, maturity and region (Table 1.2). This led us to emphasize germplasm for the lowland tropics ahead of the subtropics. and to consider early and late materials of equal importance.

Table 1.1 The relative importance of abiotic stresses experienced by maize in farmers' fields, by ecology. Numbers are percentage annual yield losses compared with an unstressed condition. Total losses represent average decline in yield from the potential yield of improved germplasm grown in the specific ecology.

Constraint	Lowland tropical		Midaltitude tropical & subtropical		Highland tropical	
	Percentage loss					
Drought	15		15		19	
establishment		4		6		8
mid-season		7		7		3
terminal		4		2		8
N deficiency	16		13		13	
P deficiency	4		5		6	
Soil acidity/Al	4		4		2	
Variable density	4		3		3	
Competition (weeds, intercrops)	10		8		8	
Cold temperatures	2		4		4	
Heat	3		3		3	
Waterlogging	5		3		3	
Salinity	1		2		2	
Total abiotic	64		60		63	
[Biotic stresses	15		17		20]	
Total all stresses	79		77		83	
Area of maize sown (1988) ('000 ha)	32,720		16,632		5,574	

Table 1.2. Estimated annual loss of maize grain yield due to drought in the less developed world (excludes central and northern China), by ecology, maturity and region. Sources: CIMMYT, 1988; CIMMYT, 1992.

	Area ha ('000)	Yield loss due to drought %	Yield loss due to drought tons ('000)
Ecology			
Highland tropical	3,400	28	2,200
Transition[a]	2,300	7	400
Subtropical[b]	16,700	15	7,700
Lowland tropical	32,900	15	8,800
Temperate	5,300	23	4,900
Maturity class			
Early	15,600	22	6,500
Intermediate	22,000	13	7,000
Late	23,000	15	10,500

Region	Predominant grain color	Area ('000 ha)	Percent loss annually	Loss ('000 t/yr)
South America	Y	17,500	20	9,100
Central America	W	9,100	15	3,000
W Asia/N Africa	Y	2,100	17	1,700
W & Cent Africa	W	4,400	12	700
E & S Africa	W	10,900	13	2,100
Asian subcont.	Y	7,800	16	2,200
E & SE Asia	Y	8,800	14	5,200
Total		60,600	17	24,000

We estimate that nitrogen fertilizer is used, or should be used, to achieve better yields on some 90% of the maize area in the tropics. Poor utilization of applied fertilizer leads to both reduced economic returns and problems of environmental degradation. In many of the poorest regions, chemical fertilizer is unavailable or prohibitively expensive. There are some environments where the probability of drought or frost increase the economic risk associated with chemical fertilizer use to unacceptable levels, and those areas could also benefit from germplasm with improved tolerance to low soil fertility. The large area of lowland maize in the tropics led us to choose a lowland population to initiate the work on low N. Because of the prevalence of N stress, we based the selection of Population 28 on its excellent performance at both high and low N levels, rather than on mega-environment considerations. This late yellow dent germplasm is proving useful in many parts of South America and Asia, and has already been released in Haiti and Panama.

II. Assessing the importance of different types of insect pests of tropical maize

As with abiotic stresses, the relative importance of different biotic stresses which limit tropical maize production have been identified through mega-environment surveys (CIMMYT, 1988), the accumulated experience of CIMMYT staff, and consultation with researchers in national programs and country surveys. One such country survey was conducted for Mozambique (Fumo and Devries, 1994) in which farmers were asked the major problems faced in cropping maize. A total of 59% listed a lack of insecticides, 54% indicated drought, 54% indicated stem borers and 12% indicated grasshoppers.

In general, stem borers are considered the most important insect pest of maize around the world. Stem borers attack the plant at all stages of development but most damage comes through stalk tunneling, which restricts the movements of nutrients during grain fill. Yield reductions by stem borers commonly reach 25% with severe attacks resulting in yield reductions of over 50%. Stem borers also attack the developing ear, causing direct damage and providing portals for ear rot diseases to colonize ears, resulting in mycotoxin production. The most important stem borers are listed below:

Species	Common name	Continent(s)
Ostrinia nubilalis	European corn borer	N. America, Europe
O. furnicalis	Asian corn borer	Asia, Philippines
Chilo partellus	Spotted stem borer	Asia, Africa
C. suppresalis	Asiatic rice borer	Asia
C. zonellus	Maize stem borer	Asia
C. agamenon	Oriental corn borer	Mid-East, Africa
Sesamia cretica	Pink stem borer	Mid-East, N. Africa
S. calamistis	Pink stem borer	Africa
S. inferens	Pink stem borer	Asia
S. nonagroides	Pink stem borer	Mid-East, Africa
Busseola fusca	African maize stalk borer	Africa
Eldana saccharina	Sugarcane borer (African)	Africa
Diatraea saccharalis	Sugarcane borer (America)	Americas
D. lineolata	Neotropical borer	Cent. And S. America
D. grandiosella	Southwestern corn borer	North America
Elasmopalpus lignosellus	Lesser corn stalk borer	Americas

Armyworms, although not as important on a global basis, can cause reductions in excess of 40% in some tropical maize production systems. The most important species are the fall armyworm, *Spodoptera frugiperda*, (Americas); armyworm, *S. exigua* (Americas); and African armyworm, *S. exempta* (Africa). Armyworm typically attack young maize plants, causing seedling mortality but can also attack plants throughout development. Armyworm can cause ear damage in a similar fashion to that caused by the corn ear worm, *Helicoverpa zea*. Corn ear worm are most prevalent in the tropical highlands but are also found in subtropical and mid-altitude ecologies.

Insect pests not only restrict maize production but they also threaten food security by damaging maize during storage. Of the more than 20 economically important storage pests, the most important pests include: Maize weevil (*Sitophilus zeamais*); Larger grain borer (*Prostephanus truncatus*); Indian meal moth (*Plodia interpunctella*); and Angoumois grain moth (*Sitotroga cerealella*). Storage pests cause direct losses to stored grain, but can also increase the levels of mycotoxins by spreading molds within grain stores.

CIMMYT recognizes the potential to develop insect resistant germplasm for the above noted insect pests through artificial screening techniques. Host plant resistance is an environmentally safe means of controlling insect pests below an economic threshold while also being a technology which can reach resource poor farmers who have restricted access to high input technology such as insecticides. Maize populations which have elevated levels of resistance have now been developed through collaborative testing with researchers in National Research Centers in developed and developing countries. These populations have focused on developing high levels of antibiosis resistance, which restricts larval establishment and development.

References

CIMMYT. 1988. Maize production regions in developing countries. CIMMYT, Mexico D.F., Mexico.

Fumo, E., and J. DeVries. 1994. Maize varietal preferences and constraints to production in Central Mozambique. pp . 276-278. *In* D.Jewell, K.Pixley, S.Wadington and J. Ransom (Eds) Proc. Fourth Eastern and Southern Africa Regional Maize Conference. CIMMYT, Mexico.

Chapter 2: Task 1: Applied biotechnology and cytogenetics

Task 1: Evaluate restriction fragment length polymorphism (RFLP) technology for identification and selection of quantitative trait loci (QTL) in maize and its utility in wide hybridization programs with maize. Evaluate the benefits of applying marker facilitated selection to the improvement of quantitatively inherited agronomic traits and to the efficacy of introgression of genetic segments from wild relatives of maize.

Output 1:
Characterization of elite material for their allele type for a selected set of RFLP probes by means of the following activities:

Activity 1: *Describe elite maize inbred material for their RFLP allele type for each of a selected set of RFLP probes.*

I. Background
We have completed a survey of restriction fragment length polymorphisms (RFLPs) in 50 maize lines with variable levels of resistance to southwestern corn borer. This work provided the initial thrust for the development of a molecular genetic diversity database that was later enriched with a further 50 lines of importance to other breeding objectives of the Maize Program. This database has been used to select the parents showing maximum differentiation at the RFLP level on agronomic grounds within the set of lines selected by the breeders. For instance, the parental lines used to study the location of genomic regions controlling resistance to borers were selected in this way. In addition, the database has provided new insights into the genetic relationships between accessions and lines in current use at CIMMYT.

II. Germplasm
The borer-susceptible lines included in this study are from tropical and subtropical populations. The borer resistant lines are derived from Antigua accessions or from populations of Antigua by Dominican Republic accessions. This resistant source germplasm is known for its poor agronomic performance in subtropical and tropical environments. All the improved, resistant CIMMYT maize lines (CMLs) extracted from such germplasm were also included. Finally, B73 and Mo17 were included as representatives of US corn belt germplasm.

The DNA of each line was digested with either of two enzymes (*Eco*RI and *Hin*dIII) and RFLPs revealed with 120 probes representing loci uniformly distributed throughout the maize genome (genomic probes from the University of Missouri (UMC) and Brookhaven National Laboratory (BNL); see for example Gardiner *et al.*, 1993, *Genetics* 134:917-930). Given that in maize most polymorphisms that are revealed with one enzyme are well correlated with those revealed with a second one, only about half of the 240 probe-enzyme combinations have been used in the diversity analysis reported below.
A method for increasing the precision of RFLP morph determinations – i.e., for deciding which RFLP bands were similar and which dissimilar – was developed. In essence, a set of two DNA fragments derived from the bacteriophage l was included in each digested plant DNA before separation by electrophoresis. These fragments provided internal molecular weight standards that helped standardize migration distances of other sample bands or morphs and thus reduce some of the inherent limitations of band migration comparisons that characterize these studies. An extensive experiment was conducted to establish the reliability of band comparisons using a clustering algorithm applied to the corrected

migration distances. Thus we could establish the experimental threshold difference in migration distance between two bands to declare similarity or dissimilarity between them, a critical criterion for avoiding errors in band classification. A program, HyperBlot, was written that allows data capture using a digitizing tablet and computes the number of morphs for a given inbred x enzyme x probe combination and outputs binary matrices for further numerical analyses of the data.

III. Salient Results

HyperBlot significantly increased the quality of the data set generated for all the lines analyzed in the study. The binary data matrix (based on presence/absence of morphs for a particular inbred x enzyme x probe combination) was used to calculate dissimilarity matrices based on similarity coefficients or distance matrices using Nei's similarity measure (Nei and Li, 1979, PNAS 76: 5269-5273). These matrices were used for clustering and principal analyses as summarized below.

There is a strikingly high level of polymorphism in the germplasm in this study: The *average* dissimilarity measure used varied from 0.52 to 0.65, a range of relatively high values when set against that observed between the two Corn Belt types (0.55). Indeed, while these represent two very different groupings of Corn Belt maize, they also appear frequently as the most distant lines to any other of those surveyed here.

Cluster analysis yielded a dendogram that shows at least four groupings: Corn Belt types, Antigua and Antigua crosses, and two indistinct groupings within the tropical and subtropical germplasm. Lines with closely related pedigrees clustered tightly together. Principal component analysis of the same data, through which one can attempt to visualize the entire matrix of dissimilarities, tends to agree well with the pairwise comparison analysis resulting from the clustering algorithm. While there still is a large, ill-defined group of tropical and sub-tropical lines, the first three components of this analysis separate the Antigua, Mississippi and other selected lines under borer pressure.

Conclusions and Perspectives

This work indicates that RFLP analysis can be utilized to group lines by either cluster or principal component analysis. The resulting groupings correspond, in general, to known pedigree relationships and germplasm origins. The analysis of the chosen set of lines was not capable of distinguishing major groupings of lines extracted from broad-based populations (tropical and subtropical lines). The distance measures and band migration records however, do provide useful information on the potential parents for the development of populations for mapping quantitative trait loci (QTLs) for SWCB resistance as presented elsewhere in this document.

On a more general note, further studies of this kind will be needed to determine whether molecular marker data can be of immediate use to breeders at CIMMYT. However, the results are somewhat promising, and it has been proposed, on this basis, to conduct fingerprinting diversity analyses concurrently with one or more of the diallel studies that CIMMYT breeders have undertaken to define heterotic groups in tropical maize. This activity is now underway, and should provide definite answers to the question of whether molecular data have any predictive value in assigning an unknown germplasm source to a pre-defined grouping based on other data. Then, the question of whether these molecular marker data can be extended to predict single cross performance in tropical maize can be asked. Meanwhile, studies conducted on temperate maize germplasm have provided very variable and controversial predictions.

Activity 2: *Selection of appropriate germplasm for controlled crosses based on maximizing the differences in both RFLP allele type and agronomic trait performance.*

After RFLP determination, analysis showed that most CIMMYT lines characterized had very low similarity indices -- on the order of 0.35-0.40. This was particularly true when comparing the SWCB susceptible genotypes compared to the SWCB resistant genotypes. The choice of the parents therefore was determined primarily by the resistance level and its stability. The first set of materials chosen were CML139 (resistant), an Antigua-derived line from CIMMYT identified and partially developed by University of Mississippi, and Ki3 (susceptible), a Suwan 1-derived inbred line that was easily produced under Mexican conditions. The second set of materials chosen as verification lines contained CML67 (highly resistant), another Antigua-derived line developed at CIMMYT which had a higher level of resistance to borers and army worm than CML139, and CML131, a productive line selected for its extreme susceptibility to SWCB. In both crosses, several other morphological and agronomic traits were also different, giving us the opportunity to evaluate segregation in the F_2 population for a range of traits and therefore map the location of these trait loci as well.

Activity 3: *Analyze results and prepare manuscripts for publication in refereed journals.*
The germplasm characterization was presented at the American Society of Agronomy Meetings, 1989:

González-de-León D., D. Hoisington, D. Jewell, J. Deutsch (1989). Genetic evaluation of inbred tropical maize germplasm using RFLPs. *Agronomy Abstracts* 1989.

Hoisington, D.A., D. González-de-León (1989). The precision of RFLP morph determinations and comparisons in germplasm studies. *Agronomy Abstracts* 1989.

Activity 4: *Joint analysis of results from the EUREKA network participants and preparation of manuscripts for publication in refereed journals.*
The data have been provided to the coordinator of the Pan-European Funding Organization (EUREKA) network for analysis and preparation of acceptable manuscripts. An extended analysis of the data including a further fifty lines chosen from other germplasm sources was undertaken to make this wealth of information available both within and outside CIMMYT.

Output 2:
Detection of co-segregation of RFLP fragments and QTLs by means of the following activities

Activity 1: *Controlled crosses between germplasm that is suitable for subsequent field and laboratory analysis of the genetic control of resistance to southwestern corn borer (SWCB). RFLP analysis of QTL.*

Four F_2 populations were derived from the four parental lines chosen for reasons outlined in Activity 2 of Output 1, as follows: 1) CML139 x Ki3 (resistant x susceptible); 2) CML67 x CML131 (resistant x susceptible); 3) CML139 x CML67 (resistant x resistant); and 4) CML139 x CML131 (resistant x susceptible). The four parental lines of the crosses have been screened for probe x enzyme combinations not found in the germplasm RFLP survey. All probes revealing RFLPs between the parents and having good hybridization quality were used to genotype the 475 F_2s derived from the cross Ki3 x CML139, and the 170 F_2s derived from the cross CML131 x CML67. The other two F_2 populations were not used for QTL analyses.

I. QTL mapping of SWCB resistance in maize —Calibration cross Ki3 x CML139

A linkage map including 128 RFLP loci was constructed. It spanned a distance of 1975 centiMorgans (cM) and had an average density of 17 cM between two loci. The map was used along with the field measurement data in order to determine the location of the QTL controlling the resistance to SWCB. Leaf feeding damage from artificial infestation with neonate larvae of SWCB was measured in the $F_{2:3}$ families in three growing cycles at Tlaltizapan (1990B, 1991A, 1992A).

In addition to the two approaches for QTL analysis mentioned in previous reports -- namely, one-way ANOVA (using SAS) and interval mapping using a maximum likelihood approach (MapMaker/QTL — Paterson et al., 1988, Nature 335:721-726) -- we have used a newly developed approach, composite interval mapping (CIM) with the help of Dr. Chianjian Jiang, a biometrician who has joined our group in October 1994. This method integrates interval mapping with regression by using markers as cofactors (Jansen and Stam, 1994, Genetics 136:1447-1455; Zeng, 1994, Genetics 136:1457-1468). By using cofactors it minimizes the effects from other possible QTL while the testing is performed on putative QTL in one interval, thus increasing the power of QTL detection and reducing the possible bias in estimates of QTL effects and positions. QTL detecting power was further increased by joint analysis of multiple trials in different locations and years (Jiang and Zeng, 1995, Genetics 140:1111-1127) QTL by environment interactions (QTL x E) were also tested.

Based on these analyses, nine putative QTL for SWCB resistance were detected. These are on chromosomes 2 (2 QTL), 3, 5 (2), 6, 7, 8 and 9. Of those, only the QTL on 3, 6 and 9 were stable across environments (no significant QTL x E interactions). All QTL had the resistance contributed from the resistant parent and showed additive gene action, some had partial dominance and one of the QTL on chromosome 2 showed a dominance effect. The total phenotypic variance explained by those nine loci was 35%.

II. QTL mapping of SWCB and SCB resistance in maize — Verification cross CML131 x CML67

CML131 x CML67 was originally used as a verification cross to confirm the QTLs found in the initial cross reported above, and to indicate whether other alleles and/or loci may be involved in the genetic control of borer resistance. In this case, the linkage map was comprised of 100 RFLP loci and spanned a total length of 1535 cM and an average density of 17 cM. Both sugarcane borer (SCB) and SWCB feeding damage were rated in the $F_{2:3}$ families in Tlaltizapan during the winter seasons of 1992 and 1993. In addition, SCB feeding damage was assessed in Poza Rica during 1993. Some agronomic traits (e.g., grain weight, cob weight, moisture percentage) were measured in infested and protected observation trials in order to help determine the reduction in yield due to borer damage. All QTL analyses were carried out with the composite interval mapping procedure.

Leaf damage rating of SWCB was significantly affected by six putative QTL on chromosomes 1 (3), 5, 7 and 9, explaining 32.4% of the phenotypic variance. Significant QTL x E were found for only two of those QTL. Resistance to SCB was significantly affected by 10 putative QTL on chromosome 1,2 (2),5 (2), 7, 8,9 (2) and 10. These showed predominantly additive or partial dominance gene action and explained 65% of the phenotypic variance. Three QTL occupied the same locations for both insects. A joint CIM analysis was also performed on both traits to test for pleiotropy of the QTL regions for resistance to both insects. Seven out of ten regions were identified with significant pleiotropic effects. This result and the high genotypic correlation between SWCB and SCB leaf damage ratings in the $F_{2:3}$ families suggest that the resistance to SWCB and SCB found in CML 67 has largely the same genetic foundation and that breeding for resistance to one borer will provide resistance to the other. On the other hand, only two genomic regions on chromosome 5, 7 and 9 were common across both mapping populations, Ki3 x CML139 and CML131 x CML67. Some of the differences may be due to the different environments used to assay the resistance in the two populations (QTL x E), while others probably indicate that resistance in the two sources is partly based on different mechanisms.

III. Conclusions and Perspectives

It is important to keep several points in mind when doing QTL analyses for marker-assisted applications, especially of such complex traits as those studied here. Significant QTL x E interactions indicate the need to measure quantitative traits in several environments and with as much precision as possible. The low consistency of genomic regions detected in different populations for the same trait taught us the need to analyze for the QTL regions in the population where selection and transfer are to be achieved. This has been followed in the MAS work described below under Activity 6.

Activity 2: *Controlled crosses between germplasm that is suitable for subsequent field and laboratory analysis of the genetic control of increased tolerance to moisture stress at flowering time. Initial efforts will be oriented to the control of the anthesis-silking interval (ASI).*

From a cross between two tropical lines, P_1 (Ac7643) with a short ASI and drought tolerant, and P_2 (Ac7729/TZSRW) with a long ASI and drought susceptible, an F_2 population of 260 individuals was genotyped at 150 loci. In 1992 and 1994, F_3 families derived from the F_2 plants, were evaluated in the field under several water regimes for ASI, a number of morphological traits and yield components.

Activity 3: *RFLP analysis of the germplasm chosen for controlled crosses.*

ASI and drought tolerance: By comparing the field data and the allelic distribution at several loci along the genome, it was possible to identify QTL involved in the expression of the studied traits using two complementary mathematical approaches: Simple Interval Mapping (SIM, MAPMAKER/QTL) and Composite Interval Mapping (CIM). The identification and characterization of QTL involved in the expression of flowering parameters and yield components is presented in detail in two papers (Ribaut et al. 1996, Ribaut et al. 1997a), while in this report we will focus on ASI and grain yield (GY) QTL. For ASI, seven QTL (LOD scores greater than 2.5) were identified on chromosomes 1 (two QTL), 2, 5, 6, 8 and 10. These QTL accounted together for approximately 50% of the phenotypic variability, which represented a change of 10.5 days for ASI. Transgressive segregation was observed for ASI (Fig. 2.1). The five QTL segments contributed by P_1, the short ASI line, were responsible for a 7-day reduction in ASI, and were stable over years and stress levels. Unlike the rather stable ASI QTL, those QTL involved in the expression of yield components, such as grain yield (GY) (Fig. 2.2), ear number (ENO), and grain number (GRNO), were inconsistent across stress levels and explained only a low percentage of the phenotypic variance. The comparative study of QTL for ASI and GY identified through CIM showed that four QTL were located at the same positions on chromosomes 1, 6, 8 and 10 (Fig. 2.3). At common positions the distance between QTL peaks for the two traits never exceeded 20 cM. Both traits also presented a second QTL on chromosome 1, but the distance between the first and second peaks represented 57 cM (156 cM for GY and 213 cM for ASI), which was probably too big a genetic distance to consider them as common QTL.

Fig. 2.1 Distribution of adjusted means of ASI in segregating F_3 families from the cross Ac7643 x Ac7729/TZSRW under three water regimes: well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A). Parental mean values (P_1 and P_2), not measured in 92A, are reported under intermediate and severe stress.

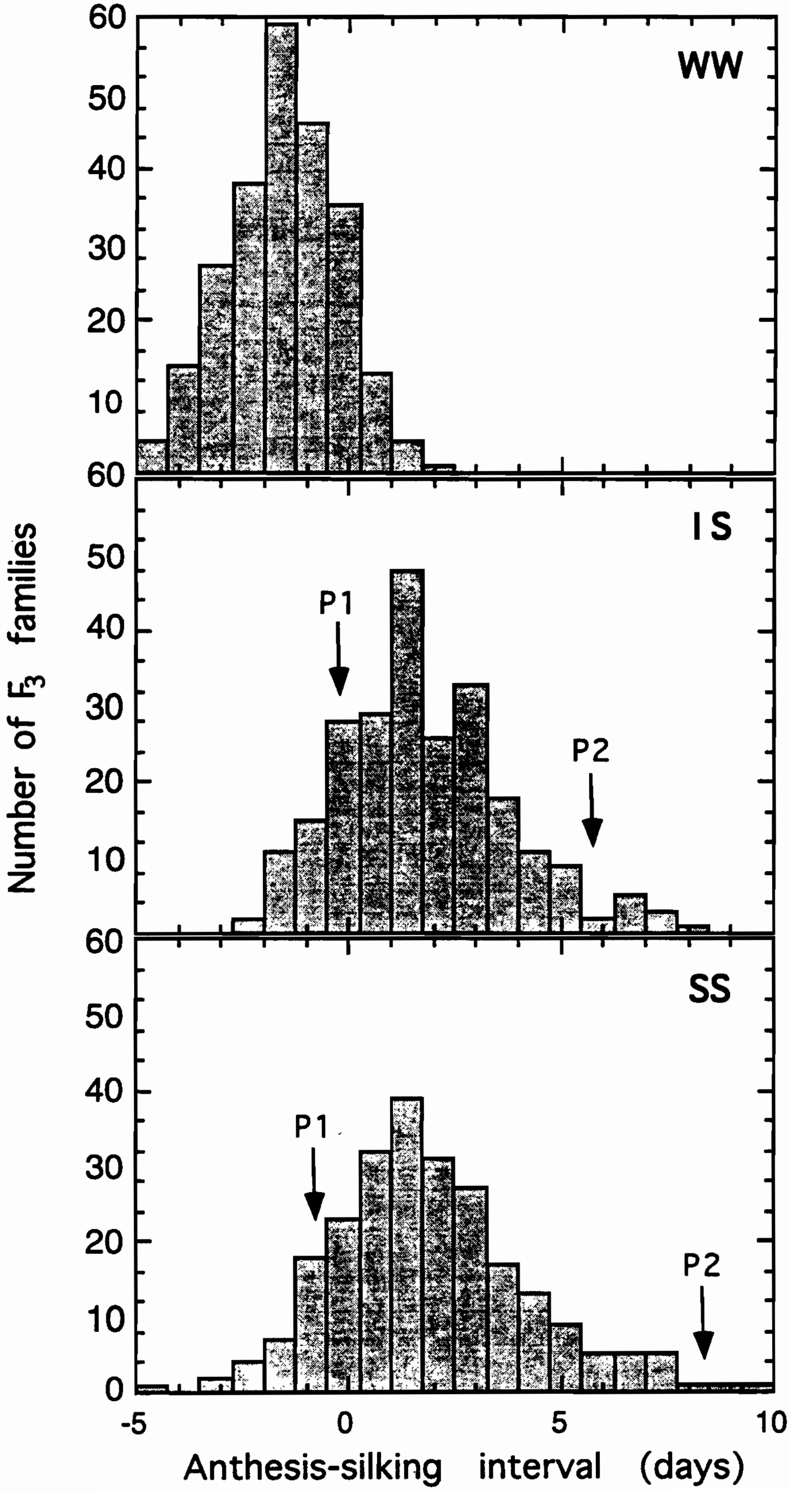


Fig. 2.2 Location of grain yield (GY) QTL detected using SIM (Mapmaker/QTL) under intermediate stress (IS) and severe stress (SS) in 94A. Genomic regions responsible for the expression of GY are represented by ellipses for LOD scores higher than 2.0. The width of the ellipses is proportional to the percentage of phenotypic variance explained by that QTL, and the magnitude of the allelic effects under SS conditions is represented in the bar graph for each QTL.

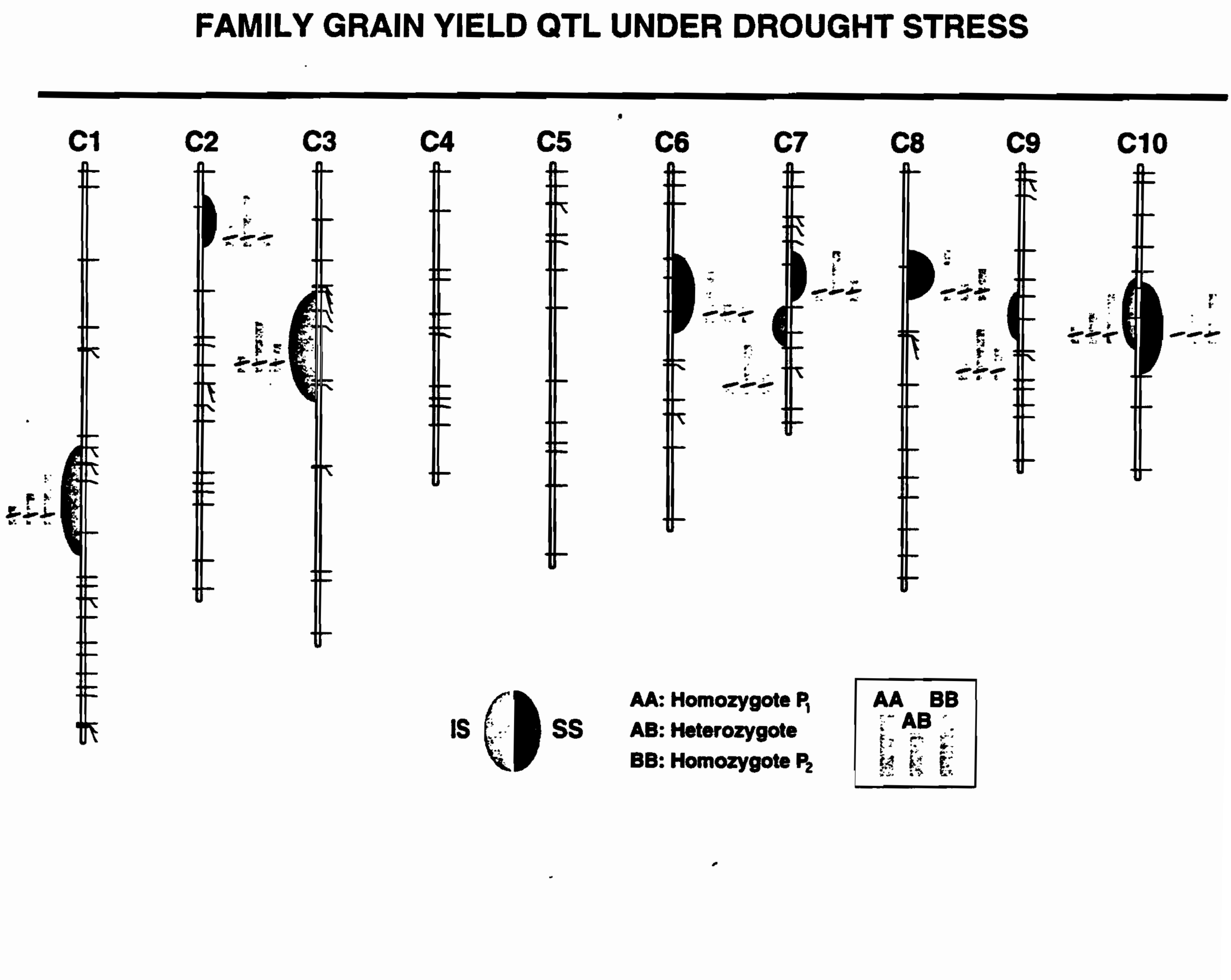
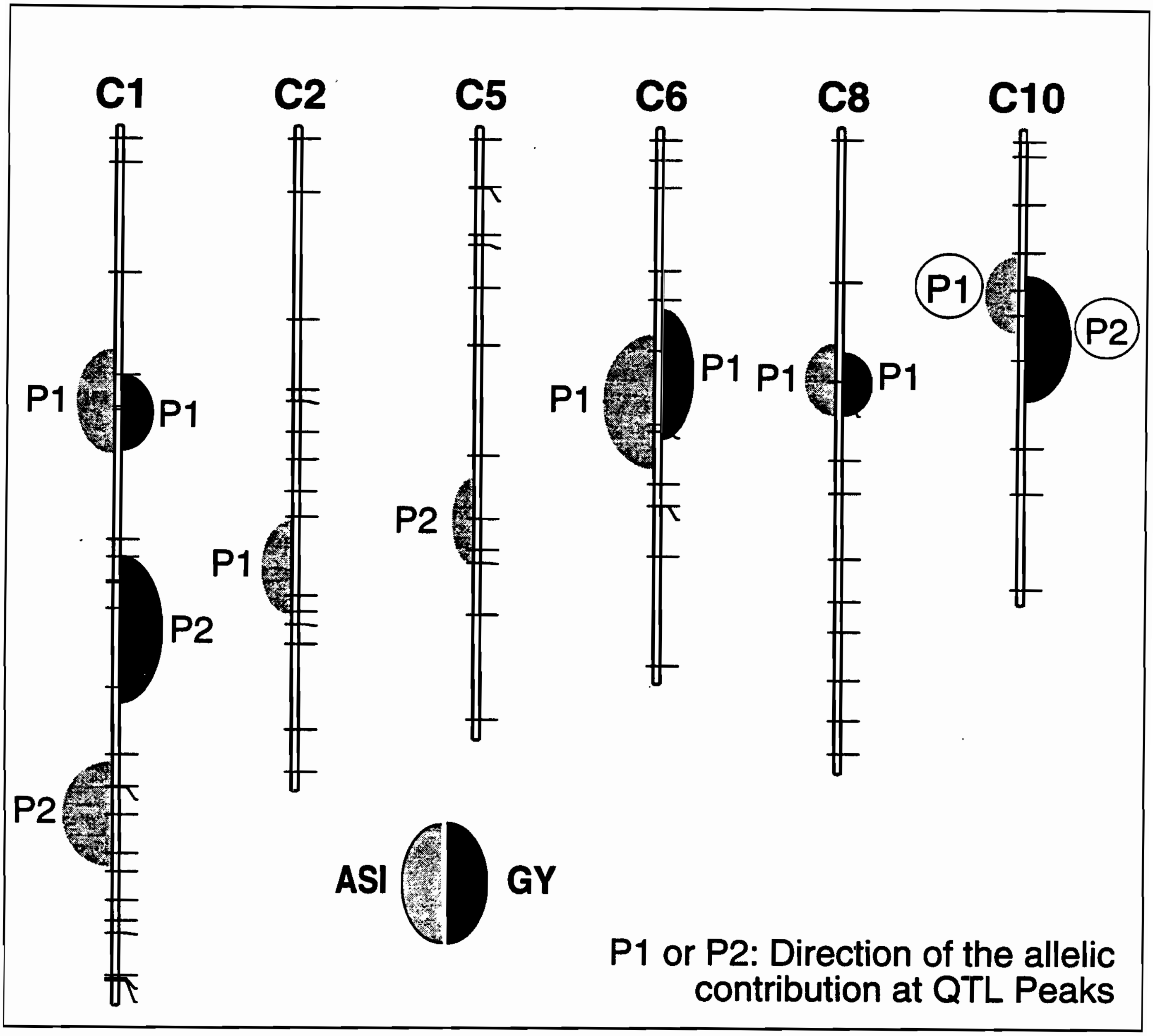


Fig. 2.3 Location on maize chromosomes of anthesis silking interval (ASI) and grain yield (GY) QTLs detected using CIM based on the combined data sets from both intermediate and severe stress field conditions. Genomic regions responsible for the expression of ASI (left) and GY (right) are represented by ellipses for LOD scores higher than 2.0. The width of the ellipse is proportional to the percentage of phenotypic variance explained by that QTL. The parental line contributing the allele for a short ASI or better yield is indicated for each QTL.



For three out of the four common QTL, the parental line which contributed to reduced ASI, (i.e., P_1) at these three genetic positions contributed also to increased GY. However, on chromosome 10, one of the most important QTL for GY and GRNO, this correlation was not observed and the allele from P_1 contributed to reductions in both ASI and yield. This important result explains why with four QTL in common, the linear correlation between ASI and GY was "only" -0.4, and underlines the importance of checking not only the number of common QTL but also the allelic directions at these common QTL peaks.

Activity 4: *Different approaches will be taken to verify the location and effect of the identified QTLs for ASI and SWCB resistance.*
Verification involves more than one cross and several environments (sites/years) for SWCB. This has been described under "Activity 1" above. In addition, we have also used the recombinant inbred lines (RILs) developed from the same F_2 populations used for mapping as another means of verifying genomic regions associated with the trait(s) under study.

I. QTL mapping of RILs for resistance to SWCB and SCB in the two populations Ki3 x CML139 and CML131 x CML67.
This work was conducted by Ms. Susanne Groh, a student from the University of Hohenheim, as part of her PhD thesis. Recombinant inbred lines are formed by selfing within a cross of (susceptible x resistant) inbred lines. Because they have been selfed for a number of generations, they have much smaller recombination blocks, and therefore allow QTL mapping of much smaller regions. Thus the actual region on the chromosome associated with the trait of interest is identified more precisely. Accurate mapping of small segments is critical to the success of marker-assisted selection because this greatly reduces the amount of donor DNA not associated with the trait of interest which is transferred to the recurrent parent during backcrossing.

Leaf tissue was sampled from 10 plants per RIL for DNA extractions. Each line was RFLP genotyped using two enzymes and 140 probes showing polymorphism between the respective parental lines. A linkage map for each population was constructed using MAPMAKER. All field experiments were conducted at Tlaltizapan from 1994 to 1996. The CML131 x CML67 population was tested for leaf feeding resistance during three cycles under artificial infestation with SWCB larvae and under insecticide protection, using an alpha-lattice design with 2 reps. Resistance to SCB was evaluated during two cycles. The Ki3 x CML139 population was tested during two cycles under artificial infestation with SWCB. Standard agronomical traits were measured in both populations. Both populations were evaluated for leaf toughness as a possible resistance component in Ottawa, Canada, using a standard instron model for readings of the peak force needed to penetrate the lower epidermis of the leaves . Leaf protein content was measured in the CML131 x CML67 population. Composite interval mapping was used to combine data in a joint analysis across cycles for leaf feeding damage, using the adjusted entry means of each cycle.

In CML131 x CML67, nine QTL, located on chromosomes 1 (4 QTL), 5, 7, 8 (2) and 9 were detected in the joint analysis across three cycles for SWCB leaf feeding damage. The most important regions with stability across cycles, explaining the highest amount of the phenotypic variance, were detected on chromosomes 7, 8 and 9. The total phenotypic variance explained by all QTL was 52.4%. Only alleles from the resistant parent contributed to lower leaf damage ratings. Eight QTL were detected on chromosomes 1 (3), 5, 7, 8 (2) and 9 for SCB leaf feeding damage. The QTL on chromosome 9 represented the most important region, explaining 25.8% of the phenotypic variation. Only the second QTL on chromosome 8 showed significant QTL x E interaction; all other QTL showed non-significant environmental effects and were detected in both cycles. All alleles contributing to lower leaf damage came from the resistant parent and explained 52.8% of the phenotypic variation. Eight QTL were in common between both insects. Across both insects, there were six regions in common between the F_2/F_3 mapping and the RILs.

In Ki3 x CML139, five QTL were detected on chromosomes 1, 6, 8 and 9 (2). Two QTL showed significant QTL x E interaction and were detected in only one cycle each, while the QTL on chromosomes 8 and 9 were stable across both cycles. The contribution towards resistance of the QTL on chromosome 6 came from the susceptible parent. The total phenotypic variance explained was 35.5%. Two QTL for SWCB resistance on chromosomes 1 and 8 existed in common between both populations, while only the QTL on chromosome 9 was in common between the F_2/F_3 mapping and the RILs in this population. Although only additive effects (and not dominant ones) can be detected in RILs, this is not enough to explain the low number of QTL in common in the two generations. Again, QTL x E interactions can be another explanation.

Mapping components of resistance:
In CML131 x CML67, five QTL were found for leaf protein content on chromosomes 1 (2), 5, 8 and 9, explaining 38.5% of the phenotypic variation. All QTL for protein content were detected in genomic regions that carried a QTL for SWCB and SCB resistance. Five QTL were detected for leaf toughness in CML131 x CML67, with two regions in common with insect resistance. In Ki3 x CML139, only two QTL were found for leaf toughness, with one region in common with SWCB resistance.

II. QTL mapping of RILs for tolerance to drought.
This work was partly conducted by Damien Liberherr, visiting scientist, during a six month stay at CIMMYT. The data produced is now being analyzed.

Activity 5: *Analyze results and prepare manuscripts for publication in refereed journals.*
The results produced from mapping maize resistance to stem borers and tolerance to drought are being or have been published in refereed journals as follows, in addition to write ups in non-refereed publications and presentations at various meetings:

- Ki3 x CML139 QTL mapping, in preparation
- CML131 x CML67 QTL mapping, 2 manuscripts for Crop Science, one published, the other submitted.
- Mapping in RILs, 1-2 manuscripts in preparation.
- ASI QTL mapping, 2 manuscripts for Theoretical and Applied Genetics, one published, the other accepted.

Activity 6: *Integrate appropriate RFLP technology into the germplasm program.*

Marker Assisted Backcrossing for SWCB Resistance (see also Appendix 3):
Work on the application of marker-assisted selection for Southwestern corn borer resistance was begun in 1993. A postdoctoral fellow funded under the project (1993-1994) compared marker-assisted selection and conventional methodologies to transfer SWCB resistance into maize-streak-virus-resistant African germplasm. We hope to provide evidence of the relative efficiency (time needed to gain a specific

phenotypic value for the trait concerned and to regain the overall phenotype of the African lines) of each approach, and thus be in a position to analyze the possible impact of molecular marker technologies for improving complex traits such as insect resistance in CIMMYT germplasm.

Parental screening of an insect susceptible line, CML 204 and an insect resistant line, CML 67, showed 82% of the 223 clones used detected a RFLP between CML 204 and CML 67 with at least one of the two restriction enzymes used. F_1 crosses between CML 204 and CML 67 and the BC_1F_1 progenies were genotyped using 109 maize genomic and cDNA clones. A linkage map was constructed 1458 cM in length with a density of 15 cM, and showed a large number of common loci (around 40) with other linkage maps. QTL analysis was carried out, based on field observations made on the BC_1F_2 population, using the maximum likelihood approach, and threshold LOD score of 2.5. Three QTL were identified, on 7L, 9S/L and 10L. MAS was initiated using these three regions.

The marker assisted selection proceeded as follows: BC_1F_1 individuals were selected that were heterozygous for the resistant parent genotype at the QTL regions and that possessed the maximum amount of fixed recurrent parent genotype for those parts of the genome outside the QTL regions. BC_2F_1 individuals derived from three selected BC_1F_1 individuals were analyzed genotypically in a second round of MAS. The same criteria was applied: QTL regions were selected that had the donor parent QTL present in heterozygous form, and all other regions were selected to have the maximum amount of fixed recurrent parent. The selfed ears of selected individuals from these BC_2F_1 plants (BC_2F_2 ears) were grown for a third round of selection. In this round the criteria changed to select for fixation of the donor genotype in the QTL regions. These selected individuals were again selfed and the resultant BC_2F_3 lines were evaluated under infestation and compared with lines selected by conventional means (See Annex 3).

The conventionally selected lines underwent two cycles of selection under artificial infestation. This selection began with the selection of 10 BC_1F_2 lines based on their resistance to foliar feeding by SWCB. The BC_2F_2 lines derived from these most resistant BC_1F_2 lines were again artificially infested. The most resistant lines were selfed and these resulting BC_2F_3 lines are those that were evaluated in the combined trial.

The evaluation of this work was directed towards comparing lines selected using molecular markers with lines selected by conventional selection. These lines, selected from the same original population by the two methods, were evaluated during two cycles in replicated trials under artificial infestation in the CIMMYT station at Tlaltizapan, Morelos. These lines have also been sent to Zimbabwe and South Africa for evaluation under conditions of natural infestation with African insects.

In addition to being evaluated as lines, these marker-assisted and conventionally-selected lines were tested as hybrids at four locations to evaluate the recovery of the recurrent genotype. We know the amount of unwanted donor genome which was still retained outside the QTL regions in the selections made using markers, and we are doing RFLP analysis on the conventionally-selected lines to gain the same information. Yield of the topcross hybrids from these lines, however, will give an idea of the biological significance associated with varying amounts of retained donor DNA. The four locations of these trials were Tlaltizapan, Mexico; Harare, Zimbabwe; Rattray Arnold Farm, Zimbabwe; and Petit, South Africa. The emphasis on testing in southern Africa was because the recurrent parent, CML 204, and the lines used as testers in the hybrid trials, CML 202 and CML 206, were developed in Harare, Zimbabwe, and are well-adapted to that environment.

Data from these trials are still being analyzed. Results of preliminary analyses of the infested line trials are shown in Table 1.1. The leaf damage ratings from the line trials show an advantage to conventional selection for producing insect resistant lines. However topcross data (Table 1.2) show a superiority of marker assisted selection for producing higher yielding hybrids in the targeted African locations. The only location where the topcrosses of the conventionally selected lines out-yielded those bred from lines selected with marker assistance was in Tlatizapan, Morelos -- the site where the conventional selection was conducted over four growing cycles. A possible factor here is that selection may have favored adaptation to the Tlaltizapan station soils, which are alkaline and frequently cause iron chlorosis in maize. Marker assisted selection seems to have been more successful in recovering the yield potential of the recurrent line in its targeted environment. In a comparison of marker-assisted selection (MAS) and conventional selection for resistance to Southwestern Corn Borer (SWCB) (see Appendix 3), both methods produced lines improved for resistance to first generation leaf damage of SWCB compared with the original line, CML 204. The lines derived from the two methods were not statistically different for leaf damage ratings. For future MAS for SWCB resistance, the region on chromosome 9 could be transferred as a single region for ease and maximum cost effectiveness. MAS lines generally gave higher yielding hybrids than the conventionally-selected lines, when evaluated as hybrids in the environment where the recurrent parent was properly adapted, a difference which was significant when data were combined across three southern African location ($P < 0.10$). There was apparently selection during backcrossing in the conventional scheme for adaptation to local Mexican soil conditions that did not occur during MAS. MAS allows unbiased recovery of the recurrent parent in all but the QTL region(s), an advantage where we find it necessary to conduct selection for specific stress-tolerance traits in environments which differ substantially from the targeted environment. Two manuscripts describing the selection process and the results will be submitted to scientific journals in 1997.

Table 2.1. Leaf damage ratings of lines selected by either conventional or marker assisted selection methods after artificial infestation with first generation larvae of SWCB (*Diatraea grandiosella*).

Line	Selection Method/ Description	Leaf damage rating	Significance LSD
CML67	Donor parent	4.82	c
BC2F3 82(1)-4	Conv. Sel.	6.59	abc
BC2F3 38-4-4	Conv. Sel.	6.76	abc
BC2F3 36-3-6	Conv. Sel.	6.80	abc
BC2F3 82(1)-5	Conv. Sel.	7.03	ab
BC2F3 218-40-175	MAS	7.03	ab
BC2F3 95-11-3	Conv. Sel.	7.25	ab
BC2F3 218-39-245	MAS	7.38	ab
BC2F3 218-40-57	MAS	7.42	ab
BC2F3 218-40-82	MAS	7.45	ab
BC2F3 218-39-31	MAS	7.67	ab
CML204	Recurrent Parent	7.83	a

Table 2.2. Contrasts of topcross yields (kg/ha) between conventionally-selected and marker-assisted selected lines evaluated as hybrids.

Conventional vs. Marker Assisted Selection	Difference (kg/ha)	Std Error (kg/ha)	Pr > |t|
Harare, Zimbabwe	-192	89	0.044 *
Petit, South Africa	-308	193	0.121
Rattray Arnold, Zimbabwe	-136	107	0.217
Combined, Africa	-212	197	0.285
Tlatizapan, Mexico	400	111	0.002 **

Marker Assisted Backcrossing for ASI and Drought Tolerance:

Is there a need? Drought is unpredictable, varying in timing and intensity. Selected plants have to perform well under both well-watered (WW) and drought conditions, complicating breeding for yield and generally leading researchers to evaluate under drought only material identified as performing well under well-watered conditions.

Selection under drought involves several considerations. First, the efficiency of the selection generally decreases due to the decrease of the grain yield heritability, as already well described by several authors (e.g., Bolaños and Edmeades 1996). The decrease is related to a decrease in genetic variance and an increase in the error variance (Edmeades et al. 1989). Second, selection under drought is very time consuming since in the tropics we have only one dry cycle per year, and if the years with undesirable rainfalls are taken into account, then 15 years are probably needed to conduct seven cycles of selections under drought. Marker assisted selection (MAS) can help reduce this time and improve the effectiveness of conventional breeding.

A MAS evaluation project for ASI, similar to that described above for SWCB resistance, has been initiated for ASI. Polymerase chain reaction (PCR) based methodologies have been adapted to increase the efficiency of marker-assisted selection and reduce its costs. Based on a backcrossing scheme, using P_1 as the donor line and CML247 as the recurrent line, a MAS experiment was initiated two years ago. CML247 is an elite tropical line from CIMMYT, with outstanding combining ability, good yield per se under well-watered conditions, but has a relatively long ASI that significantly reduces its yield potential under mid-season drought. By combining genetic data from an F_2 population derived from this new cross and an F_3 family evaluation in the field under severe stress conditions, QTL identification for traits of interest was achieved. The results indicated that the QTL for ASI were quite consistent across the two crosses. Of the five QTL from the original cross carrying the allele for short ASI from P_1, only the QTL on chromosome 6 was not detected in the second cross. The QTL on the short arm of chromosome 1 was shifted by 40 cM in the new cross, and the three other QTL on chromosomes 2, 8 and 10 overlapped across the two crosses. A new ASI QTL was detected on the short arm of the chromosome 3. These results demonstrate that it is essential to make a new genetic map when you change the recurrent line, although only one trial under drought conditions may be sufficient to identify the QTL of interest.

Based on these findings, eight individuals out of 400 backcrossed plants (BC_1F_1) were selected using RFLP markers. The selection was restricted to markers flanking the five QTL of interest. In the next BC cycle 300 plants were selected out of 2300 BC_2F_1 plants in a first preselection step conducted with three suitable PCR-based markers mapping at selected QTL positions (Ribaut et al., 1997b). The use of these markers allows a substantial increase in the pressure of selection at each backcross step, thus making the selection process more efficient by reducing the number of backcross cycles. This preselection step allowed us to quickly reduce the initial population in size to one that can then be handled more efficiently using RFLPs for genotyping at further loci of interest, and to evaluate the remaining percentage of the donor parent genome in the different preselected individuals. Finally eight plant will be selected again, and in the next plant cycle (97A), selfed progeny (BC_2F_2) of the selected plants will be screened to identify those having fixed P_1 alleles at QTL position of interest.

Marker Assisted Selection in a Population for ASI and Drought Tolerance:
The second MAS project concerns the improvement of an open-pollinated population. CIMMYT physiologists conducted eight cycles of full-sib recurrent selection in the population "Tuxpeño Sequía" during the dry season in Mexico. Selection was based mainly on an index comprising increased grain yield, the maintenance of the planting-anthesis interval and reduced ASI. In this study, changes in allelic frequencies over cycles of selection at loci of known map position were quantified (Ribaut et al., 1995). It was found that the most significant allelic shifts occurred at or near the QTL previously detected for ASI. It can be postulated that these changes result from the selection pressure applied by breeders at each cycle, in the direction of reduced ASI while increasing various yield components under drought. It is intriguing to speculate whether plant selection based on the presence of alleles that increased their frequency at specific genomic positions across a few cycles of selection, could provide a significant improvement of the drought tolerance in the population. MAS on 400 plants from cycles C_0 and C_4 is now under way.

Preliminary results under low N conditions.
From several comparative field evaluations conducted by CIMMYT physiologists, it seems that yield improvement under drought conditions also increases yields under conditions of low soil N (Bänziger et al., 1997). To complete these results from a genetical viewpoint, two trials of the segregating F_3 families derived from P_1X P_2 were conducted under low N. Comparing ASI values evaluated on the same material, but under different stress conditions and locations -- i.e., under drought at Tlaltizapan (cycle 94A) and under low N at Poza Rica (cycle 96A) -- the linear correlation between the two measurements (0.55) was highly significant. However, the linear correlation between ASI and GY was larger under drought (-0.45) than under low N (-0.2). Preliminary results on ASI QTL seem to demonstrate that most of the genomic regions identified under low N were already identified under drought. For yield component QTL, large variability in their location is expected because of the variation among the trials and differences the nature of the two stresses.

Activity 7: *Compare the results of the research on QTLs done under this project with the results of research on QTLs generated by other participants in the EUREKA network and prepare manuscripts for publication in refereed journals.*
From the EUREKA meeting held in Einbeck, Germany, November 1993, it was clear that the different contributors to the network had measured different traits and had used sometimes different approaches even if measuring the same trait. Therefore, it was concluded that it would be difficult to combine the various datasets and test for the consistency of QTL across populations. However, the Hohenheim group is still looking at possibilities of combining certain data sets for this purpose. We have collaborated closely with the group at Hohenheim led by Dr. A. Melchinger in analyzing the data sets produced here, and sometimes we have compared our results to those of others as a means of identifying reliable analytical techniques.

Output 3:
Identify suitable molecular probes/RFLPs for the identification of genetic segments in Tripsacum *(a wild relative of maize).*
CIMMYT has been involved in research on wide hybridization of maize since the mid 70's. This research has been viewed as long term and complementary to both CIMMYT breeding objectives and to activities in genetic conservation of the wild relatives of maize. In 1990-91 the CIMMYT Wide Cross Program was sufficiently advanced so that an expansion of these activities was warranted. This expansion was implemented through support from UNDP; a Post Doctoral Fellow/Associate Scientist, Dr. N. Islam-Faridi, was added to the project from May 1991 to July 1994 to work full time on this research.

Research focused on the maize-*Tripsacum* system. *Tripsacum* is the only known relative of the genus Zea in the new world. Its adaptability to a large variety of ecological conditions, from the semiarid zones of central Mexico to the humid lowland tropics in Southern Mexico and Guatemala, makes it an interesting source of genetic variability for maize improvement. A important collection of *Tripsacum* accessions is available at CIMMYT, and this can be used to search for interesting alleles.

Hybrids between maize and *Tripsacum* have been obtained by many researchers during the last 50 years, and evidence for spontaneous gene exchanges between them have accumulated. A major barrier to the utilization of this genetic resource for maize improvement, though, has been the absence of suitable methods for identifying 1) the chromosome segments carrying the alleles of interest, and 2) maize into which *Tripsacum* chromosome segments could be introgressed. With the recent advances in molecular genetics, the identification of genomic segments involved in a given phenotypic response has become a routine activity in many crops, including maize. In the context of the UNDP project, our main objective was to develop efficient techniques to analyze and enhance the potential for gene exchanges between maize and *Tripsacum*. This was done using both traditional cytogenetics and improved molecular techniques, i.e., RFLPs and *in-situ* hybridization.

Activity 1: *Select appropriate "wide cross" germplasm derived from hybridization between maize and* Tripsacum *for RFLP analysis.*
Interesting alleles have been identified in *Tripsacum*. The more promising ones are those responsible for apomixis, an asexual mode of reproduction through seeds. Apomixis bypasses both meiosis and fertilization, producing offspring that are exact genetic replicas of the mother plant. Apomixis, which could allow fixation of heterosis, has been perceived as one of the more promising traits for maize improvement in developing countries. Apomixis does not exist in maize, but is a common mode of reproduction in polyploid *Tripsacum*. Other interesting characteristics have been identified in the CIMMYT *Tripsacum* collection, including resistance to *Striga*, a parasitic weed which causes major crop losses in Africa, and for which no source of resistance has been identified in maize to date.

Activity 2: *Analyze the selected germplasm for RFLP alleles and determine the relationship between selected RFLP linkage blocks in* Tripsacum *and maize.*
In a collaborative project with French-funded scientists working at CIMMYT, many *Tripsacum* species have been characterized using maize RFLP clones. This work has provided valuable information regarding the use of the RFLP technique in *Tripsacum*. The analyses confirmed that maize and *Tripsacum* have essentially the same affinity to the publicly available maize clones that are commonly used for genome mapping. Hence, most of the maize mapping information can potentially be used for molecular

genetic studies in *Tripsacum*, opening new avenues for the manipulation of segments of interest. A good example has been provided with the identification of molecular markers linked with the apomictic mode of reproduction by Dr. Yves Savidan's group, using maize RFLP markers. After the departure of Dr. Nurul Islam-Faridi in July 1994, the plant materials and a detailed listing of all seeds that remained from the Wide Cross Program were handed over to Dr. Savidan. These materials are now being used mainly within the apomixis project, aiming at the transfer of the apomictic mode of reproduction onto maize cultivars. The staff of the apomixis project worked closely with UNDP project staff (Dr. Islam-Faridi). Many technical developments from the UNDP work have been transferred to the apomixis project, and wide cross research under UNDP Stress Project was closed. The techniques of *in situ* hybridization for the detection of alien chromosome segments were taught by Dr. Faridi to six scientific staff/PhD students and one person from the Postgraduate College, Montecillo, Mexico, before his departure from CIMMYT. Those techniques have had a major impact on the efficiency of the on-going apomixis project.

Activity 3: *Devise and apply suitable genetic strategies for marker facilitated selection of the introgression of genetic segments of* Tripsacum *into maize.*
To introduce new variability into maize, the envisioned strategy was, through conventional back-crossing, to isolate in a tropical maize background individual chromosomes of *Tripsacum* accessions possessing phenotypic trait of interest, to test these maize-*Tripsacum* addition lines in the field to confirm the phenotypic expression of the trait, and to transfer the corresponding alleles in the maize genome.

Genomic *in situ* hybridization techniques have been adapted to the maize-*Tripsacum* system. They have been utilized to analyze plants regenerated from the long term tissue culture of a maize-*Tripsacum* hybrid and various generations of maize-*Tripsacum* hybrids and backcross derivatives. This was done to determine the utility of both approaches for obtaining recombination. In both cases, clear recombinants could be identified. Moreover, a plant containing a triploid complement of maize chromosomes and a few additional *Tripsacum* chromosomes was identified during routine cytological analysis of the maize-*Tripsacum* backcross progeny. This plant was of particular interest, because it putatively contained unpaired maize chromosomes that would be available to pair with potentially homeologous segments of the *Tripsacum* chromosomes. The progeny of this plant were used to identify and isolate a maize chromosome carrying a *Tripsacum* translocated segment. This observation led us to propose a general cytological system for promoting the interchange of genetic material between maize and *Tripsacum*.

Activity 4: *Prepare manuscripts for publication in refereed journals.*
Three manuscripts on the wide cross research are in the final stages of preparation for submission to refereed journals. Two further manuscripts are in the early stages of development (see Appendix 1).

Output 4:
Transfer of and training in the use of appropriate technology through the following activities:

Activity 1: *Train selected scientists in the use of marker facilitated selection where appropriate.*
To date, 46 scientists from 21 countries have received short or long-term training in our laboratories. Three students (from Chile, Mexico and Peru) have done their MSc theses, and 10 students (from Canada, Cuba, Ecuador, France, Germany and Mexico) have completed or are completing their PhD dissertions in the Applied Molecular Genetics Laboratory. In addition, we have hosted and trained 12 visiting scientists (from Brazil, France, Germany, Mexico, South Africa, Spain, Switzerland, Syria and the UK) for less than

a month and 21 visiting scientists (from Argentina, Australia, Austria, Brazil, Chile, China, Egypt, France, India, Mexico, Peru, Switzerland and the USA) for a month to a year.

In addition, we have now held two formal courses on molecular marker applications to plant breeding during 1995 and 1996. The first course was sponsored by the International Triticeae Mapping Initiative (ITMI), Pioneer Hi-Bred International, Inc. and CIMMYT, Int. and lasted two weeks. Eighteen young scientists from 13 countries received training in theoretical and practical aspects of plant genome analysis and its application to plant breeding problems in both wheat and maize. For the practical aspects, in addition to laboratory experiments, all participants were also trained in data capture and analyses. The high success of the first course and the availability of funds from ITMI, Pioneer, CIMMYT and Ciba-Geigy allowed us to offer a similar but slightly extended course (three weeks) in October 1996, attended by 14 participants from 12 countries. Additional marker techniques such as AFLPs and new statistical methods for QTL analysis were added to the curriculum. In addition, we invited two instructors from Agriculture and Agri-Food Canada, Winnipeg, Canada and from Cornell University, Ithaca, NY to contribute to the course. Details of the contents and a list of participants in each course are included in Chapter 10.

Activity 2: *Make available to selected national agricultural research systems (national programs), the RFLP probes required for marker facilitated selection together with related information.*
A number of preliminary products from UNDP-funded research have been successfully used in several other laboratories:

a) RFLP probes available for distribution:
CIMMYT has permission to distribute three sets of clones developed at the University of Missouri, Columbia (UMC), at the Brookhaven National Laboratory (BNL), and at California State University (CSU). As requested, those have been provided to 16 different laboratories in 11 countries (Brazil, Colombia, Cuba, France, Mexico, Netherlands, Philippines, Thailand, UK and USA).

b) Laboratory protocols:
In view of the large amount of data that would be generated in research to apply marker technologies to breeding, low-cost, large-scale RFLP protocols were developed and published (Hoisington, 1992). The related detection techniques are based on non-radioactive, chemiluminescent technologies that we have adapted, making them especially useful for developing country laboratories. A second edition of these protocols has been released in both English and Spanish (Hoisington et al., 1994 and 1995) and includes large-scale applications of RAPD (random amplified polymorphic DNA) techniques. The protocol manuals have now been distributed to 314 scientists in 48 countries.

c) Software for molecular data capture and MAS:
Much effort has been dedicated to developing a software package for capturing, storing, verifying and analyzing molecular marker data. First, new tools for the analysis of molecular diversity data were developed as part of the germplasm diversity study. To increase the precision with which any two bands are compared and thus reduce the risk of considering that they are different when in fact they are not, a system of internal DNA controls was included with each plant DNA sample. These controls were a high (24.8 Kb) and a low (1.5 Kb) molecular weight band isolated from the DNA of the l bacteriophage. A Hypercard (Macintosh computer) program (HyperBlot) was written that allows the digitized entry of band migration and the analysis of band similarities/differences using internal DNA controls and

clustering algorithms (Hoisington and González de León, Agronomy Abstracts, 1989). This software package has been distributed to other laboratories free of charge.

Second, a program (HyperMapData) was developed for capturing RFLP mapping data, verifying them in several ways, and analyzing and outputting them in a range of formats for further analysis using current genetic mapping software packages (e.g., Mapmaker, JoinMap) as well as commonly used statistical software packages (e.g., SAS or SYSTAT).

Finally, a program (HyperMAS) was written that takes the output of the program above as well as the linkage map derived thereof. It calculates the proportions of markers derived from the parental plants in each of the individuals of segregating populations derived from those parents. This has proved to be an essential tool for the efficient selection of individuals of interest in marker assisted selection programs. Given a target genotype that includes certain desired genomic segments of a donor parent in a background of the recurrent parent, the program classifies all individuals in a segregating population according to how well they match this target while retaining a minimum of unwanted donor parent genome. Graphical genotypes of all the chromosomes of each individual can be generated and used for visual comparisons and to facilitate selection.

d) Methods

The methods we have developed for molecular marker analyses and recently for marker-assisted selection are being taught both in our formal courses and to visiting scientists (described under Activity 1 above). Additional formal training will be conducted in relation to the DGIS-funded projects for applying marker assisted selection in Kenya and Zimbabwe to develop locally adapted insect resistant and drought tolerant germplasm.

*Chapter 3: Task 2: Host plant resistance and development of insect-resistant germplasm sources**

Task 2: Identify and improve germplasm complexes with high levels of resistance to the major maize stem borer and army worm pests. Deliberate attention will be given to developing germplasm with multiple pest resistance and good agronomic characteristics to the extent possible. Initial success will be based on evaluation of resistant sources in collaborating countries. Gains in improvement in resistance levels will be assessed toward the end of the project term by comparing initial vs. later cycles of selection.

Output 1:

Develop sources of resistance to major maize insect pests through the following activity:

Activity 1: *Screen and identify potential sources of resistant germplasm.*

Background

In the past, many maize breeding programs used relatively restricted groups of genetic materials to form the basis of the improved varieties and hybrids. The major emphasis was on improving grain yield and grain quality for food and feed. Extensive use has been made of CIMMYT populations to develop experimental varieties and hybrids for international testing and distribution to national research programs. However, in most maize growing areas in the developing world, insect pests cause yield reductions and grain damage, as well as facilitating the entry of fungi which produce mycotoxins. The varieties and hybrids which are derived from high yielding populations do not have high levels of antibiosis resistance to stem borers and armyworms. For this reason, new populations were established in the mid-1980s at CIMMYT which brought together a diverse range of germplasm with antibiosis resistance to insect pests of maize. The stress breeding subprogram has utilized early inbred lines with insect resistance to develop advanced lines with both good resistance and good agronomic traits for developing improved varieties and hybrids.

3.1 *Screening and identifying sources of resistant germplasm*

We have continued screening bank accessions and elite germplasm to find new or better sources of resistance to stem borers, armyworms, maize weevils, spider mites, rootworms and corn earworms. One of the most effective methods of deploying new germplasm quickly and widely in a breeding program is to develop source populations that recombine insect resistance alleles and then to extract lines from these populations for variety and hybrid products. We have developed such populations for the lowland tropics, for the subtropics and midaltitude regions, and for the tropical highlands.

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* For additional detail, see Appendix 5.

3.1.1 Resistance to stem borers and armyworms

Activities have focused on resistance, rather than tolerance, assuming that source populations will be destined for areas with heavy infestation (as in West Africa during the minor cropping season) and relatively low yield potential (tolerance is related to plant vigor), and, moreover, aiming to reduce insect populations (which resistance can achieve by slowing insect development and affecting reproduction). The target species are southwestern corn borer (SWCB) *Diatraea grandiosella;* sugarcane borer, *D. saccharalis;* and fall armyworm (FAW), *Spodoptera frugiperda.*

Achievements: A study has supported the notion that first generation stem borers cause the greatest yield losses (Table 3.1). Estimated relative yield losses associated with first generation feeding damage is 10% with an additional 12% loss due to tunneling by first generation larvae. Approximately 3% of the yield loss is attributed to second generation attack during anthesis.

Table 3.1. Subtropical hybrid yield trial infested with southwestern corn borer, *Diatraea grandiosella* at different phenological stages, Tlaltizapan 1996.

			Percent yield loss			
Hybrid	Description	Protected yield (t/ha)	First gen.	First gen. borer	Second gen.	First + second gen. borer
TL8645/P47S3/ Mp78:518	IR yellow	9.36	-1.13	12.96	3.77	**12.25**
S89500F2/89[P500SIWD]F246	IR white	10.42	15.00	20.00	6.49	**10.77**
P33C2(STE)-11-1-B*6/P45C6F83-3-13	IS yellow	10.18	38.86	46.58	22.76	**43.25**
CML78/CML321	IS white	11.94	30.00	20.15	-6.63	**33.85**
Average		10.47	20.68	24.92	6.60	**25.03**
Corrected percent yield loss			9.91	11.95	3.16	

Source populations: By advancing lines from resistant populations under artificial insect infestations, we have obtained lines which possess both moderate antibiosis resistance and good agronomic traits. To increase the usefulness of these materials, lines derived from source populations were evaluated in 1991 and 1994 by international collaborators in national programs for adaptation and for resistance to *Ostrinia nubilalis, O. furnicalis, Diatraea grandiosella, D. saccharalis, Eldana saccharina, Chilo partellus, Busseola fusca,* and *Spodoptera frugiperda,* as well as for resistance to streak virus, corn stunt complex, *Puccinia sorghi, Exserohilum turcicum,* and downy mildew. Insect resistant populations developed at CIMMYT include the original multiple borer resistant population (MBR, Population 590), a multiple borer and disease resistant population (MBR-MDR, Population 590B), and a multiple insect resistant tropical population (MIRT, Population 390). Populations 590 and 590B are adapted to subtropical and midaltitude environments; 390 is adapted to the lowland tropics. C_0 of Population 590 was formed in 1985 and was recombined in 1988, 1991, 1993, 1995. C_0 of Population 590 was formed by combining C_1 of Population 590 with lines resistant to *E. turcicum* in 1988 with subsequent recombinations in 1993 and 1995. MIRT was formed in 1987 with subsequent cycles of selection in 1991, 1993 and 1995. The populations were tested internationally through a network established by Dr. J.A. Mihm. All populations were recombined within either a white or yellow grain color group in 1995, based on requests from national program collaborators.

The improvements in host plant resistance in subtropical maize populations are summarized in Table 3.2. The most dramatic was observed in C_1 of Population 590, which was the first stem borer population formed at CIMMYT. Given that germplasm from the United States and Caribbean were incorporated, the first cycle improved the adaptation as well as the level of resistance in this population. Population 590 has improved in yield potential under insect pressure to provide greater yield stability, although yield under protected conditions has not improved. Following the first Insect Resistant Progeny Trial (IRPT) in 1986, Population 590 was found to be very susceptible to *E. turcicum* and *P. sorghi*. Efforts were made in 1987 to incorporate disease resistance into the population by recombination with disease resistant lines to form MBR-MDR, Population 590 B. C_0 of this population was found to be susceptible to both SWCB and FAW, with an improvement in resistance and yield for C_1 but no clear gains in C_2. During each cycle of selection this population has been screened for *E. turcicum* and *P. sorghi* under natural and artificial inoculation to maintain disease resistance.

Lines from Populations 590 and 590B have been used to form both white and yellow insect resistant populations. The yellow population is more resistant than the white; however, both populations provide good yield stability under artificial infestation with both SWCB and FAW. Population 47 has also been selected for stem borer resistance and shows good yield stability, while Population 45 has not been screened for insect resistance and shows significant reductions in yield under stem borer and FAW attack.

Table 3.2. **Improvements in host plant resistance in subtropical maize against southwestern corn borer (SWCB), *Diatraea grandiosella*, and fall armyworm (FAW), *Spodoptera frugiperda*, Tlaltizapan, 1996.**

Population	Insect damage ratings			Yield (t/ha)		
	SWCB (1-9 scale)	Tunnels per plant	FAW (1-9 scale)	Protected	SWCB	FAW
Pop. 590						
$MBRC_0$	8.40	2.26	5.27	6.09	3.73	3.94
$MBRC_1$	5.42	1.11	3.72	6.48	5.82	6.11
$MBRC_2$	7.12	1.21	3.95	6.07	5.66	6.21
$MBRC_3$	5.07	1.01	3.50	6.53	5.72	5.90
$MBRC_4$	4.61	0.88	3.41	6.01	5.98	6.30
$MBRC_4$Am*	5.26	0.91	3.21	6.45	5.57	6.34
$MBRC_4$Bc	4.22	0.45	3.07	6.44	6.26	6.55
Pop. 590B						
$MBR/MDRC_0$	7.26	1.71	5.17	5.18	4.95	5.12
$MBR/MDRC_1$	6.63	1.41	3.84	6.50	4.93	5.49
$MBR/MDRC_2$	6.98	1.83	4.24	6.61	5.52	6.29
$MBR/MDRC_2$Am	4.85	0.76	4.18	5.64	5.61	5.41
$MBR/MDRC_2$Bc	7.10	1.91	4.75	6.01	4.74	6.03
Resistant synthetics						
SIRsynAm	5.07	0.72	3.37	7.28	7.47	8.03
SIRsynBc	6.26	1.18	4.47	6.65	6.30	6.25
Checks						
Pop.45	8.31	2.20	5.71	6.52	4.87	5.36
Pop.47	6.56	1.29	4.36	7.23	6.53	7.27

* "Am" = yellow grain; "Bc" = white grain.

The insect resistance of MIRT – which was developed under artificial infestations with FAW and SCB, as well as being evaluated through international testing prior to recombination – has also benefited from subsequent selection under insect pressure. The cycles of selection trial conducted in 1996 did not receive severe FAW attack and was not infested by SCB (Table 3.3). This trial will be repeated in 1997 to characterize changes in resistance and yield stability under severe insect attack.

Table 3.3. Improvements in host plant resistance in tropical maize against fall armyworm (FAW), *Spodoptera frugiperda*, Poza Rica, 1996.

Population	FAW rating (scale 1 - 9)	Yield (t/ha) Protected	Infested
$MIRTC_0$	7.46	6.93	6.91
$MIRTC_1$	7.25	7.20	7.81
$MIRTC_2$	6.67	7.48	6.94
$MIRTC_3$	6.67	7.49	6.70
$MIRTC_3$ Bc	6.43	6.67	7.31
$MIRTC_3$ Am	5.93	7.04	7.48
Checks			
Pop.43	7.90	10.65	9.62
Pop.2[illegible]	7.92	9.85	9.52

Am = yellow grain; Bc = white grain.

Early lines available from the entomology unit: One of the major activities of the entomology unit is to deliver new sources of resistance to the breeders at CIMMYT and interested researchers in national programs. Lines which have been delivered include:

- 120 S_3 lines derived from Population 390 MIRT (multiple insect resistant tropical) and over 200 sub-tropical lines (S_3-S_5), in 1993.
- Advanced lines (S_{4-6}) from the entomology unit were transferred to the stress breeding subprogram in 1995, including 46 white and 61 yellow lowland tropical lines and 92 white and 37 yellow subtropical lines. Most of the germplasm was derived from Populations 590 B (MBR-MDR) and 390 (MIRT).
- 110 S_3 lines derived from MIRT and 140 sub-tropical lines (S_3-S_5), in 1996.

An additional population which has become popular with four subprograms at CIMMYT is Population MBR - E.t. This population was developed by recombining elite MBR lines with *E. turcicum* resistant lines during the early 1990s; it now provides elite lines which have good combining ability as well as insect and disease resistance. Advanced lines from MBR-E.t. were also passed back to the subtropical subprogram for recombination with elite subtropical lines in 1995 (22 whites and 9 yellows). More than 2,300 samples of seed were shipped to international collaborators in national programs, universities and seed companies during 1995 and 1996.

Advanced line screening: As of 1994, the entomology unit began to characterize existing CIMMYT inbred lines (CMLs) and promising new lines for resistance to stem borers and fall armyworm. Most lines have been found susceptible, but several developed in collaboration with the entomology unit have shown moderate resistance. Lines adapted to subtropical and midaltitude ecologies include:

Pedigree	Origin	Yield under SWCB (t/ha)	Rating SWCB	Yield, no infestation (t/ha)	Number of maize plants	Yield under FAW (t/ha)	Rating FAW
MBR-ET(W)#-56-1-1-1-B-B-6-1	TL95B 6225 52-1	1.603	6.5	3.399	19	1.940	4.75
89[SUWAN8422]/[P47s3/Mp7 8:518]#-183-1-2-1-2-2-B-#-B	TL96A-1964-14	1.592	6.75	3.288	17.5	1.993	5.5
TR res EC/MBR IPTT-ECBMo.88-90-1-1-7-(1-6)#b1 14#b 14#b-1-3-B-#-B	TL96A-1964-21	1.400	5.25	2.507	18.5	2.042	4.25
89[TL8645]/[P47s3/Mp78:518] B-24-1-1-4-1-3-B-#-B	TL96A-1965-9	1.339	6.5	3.381	15	2.940	5
P590B C2 S5/P390 C1 S1 F16-2-4-b-b-B#-B	TL96A-1966A-18	1.174	7.25	2.489	17.5	2.010	4
P 590 C4 F153-b-2-1-1-B#-B	TL96A-1966A-36	1.147	7	2.519	19.5	1.330	6.5
89[SUWAN8422]/[P47s3/Mp7 8:518]#-57-3-6-1-1-3-B-#-B	TL96A-1964-13	1.124	7.25	1.836	15.5	1.176	4.75
MBR-ET(W) F2-10-2-2-1-b-b-B#-B	TL96A-1966A-2	1.034	5.75	1.267	14	1.958	4.75
MBRHC96-1#-1-1-1-6-3-#-b-3-b-b-B-#-B	TL96A-1966B-2	0.938	6.25	2.362	17	1.743	4
P590B C4 F220 -1-1-b-b-B-#-B	TL96A-1966B-22	0.962	7	1.906	17	1.464	5
MBR-ET(W)#-10-2-2-2-B-B	TL95B 6165 -12	0.909	6.5	1.389	13	1.961	3
MBR/MDR F84-3-3-5-3-1-1-B-#-B	TL96A-1965-18	0.866	7.5	2.394	18	1.403	4
MBR-ET(W)#-14-3-1-3-B-B	TL94B 6225 -4	0.863	4.25	1.287	15	0.816	5.25
MBRHC96-1#-1-1-1-6-3-#-b-2-b-b-B-#-B	TL96A-1966B-1	0.831	6.5	2.760	15	2.670	4.75
MBR-ET(W) F2-278-1-2-1-b-b-B#-B	TL96A-1966A-14	0.718	7.75	2.080	19.5	1.714	5.5
TR res EC/MBR IPTT-ECBMo.88-91-2-1-2-(1-6)#b1 16#b 16#b-3-3-B-#-B	TL96A-1964-28	0.718	6.5	4.584	17	4.871	5.25
89[TL8645]/[P47s3/Mp78:518] B-188-2-4-3-1-1-B-#-B	TL96A-1965-13	0.648	7.5	2.887	18	2.065	5.5
CML67 (resistant check)	TL96A	1.124	4.25	1.330	15.5	1.223	4
CML131 (susceptible check)	TL96A	0.000	9.75	1.528	18	0.674	7.75

3.1.2 Corn earworm resistance in highland tropical maize

Introduction: The corn earworm (CEW), *Helicoverpa zea* (Boddie), is an important pest of highland maize. Larval feeding on maize husk, silk and cobs during grain fill cause direct losses as well as providing portals of entry for various *Fusarium* spp., which reduce grain quality by producing mycotoxins in the ear. Damage associated with the CEW is most severe on floury maize, the preferred type in many highland areas. A major difficulty in working with highland germplasm is its lack of tolerance to inbreeding and its relatively poor yield potential compared to that of tropical, subtropical, or midaltitude maize. In the past five years, the entomology unit has attempted to combine CEW resistance sources with high yielding germplasm and pools of floury maize not used by other subprograms.

The substance maysin in the silks of 'Zapalote Chico' has been reported as imparting resistance to CEW (Wiseman *et al.*, 1992). Considerable effort has been made to elucidate the biochemical composition of maysin in Z. chico and other maize inbreds, but there are few reports on the successful use of this characteristic in improved maize varieties and hybrids, perhaps owing in part to the landrace's poor yield potential and disease resistance. In CIMMYT, efforts were initiated to incorporate CEW resistance genes from Z. chico into maize populations. The germplasm under improvement for resistance to CEW includes: 1) Population 85, an early white, semident population developed with 60% germplasm from the tropical highlands, 20% from temperate highlands and 20% from the tropical and subtropical areas; 2) Population 86, an early yellow semident developed from 55% tropical highland, 25% temperate highland, and 20% tropical /subtropical germplasm; 3) Populations 87 and 88, late white and yellow semidents developed from 55% tropical highland, 25% temperate highland, and 20% tropical/subtropical germplasm. These populations are being improved for CEW resistance *per se* by S_{2-3} full sib recurrent selection, and two cycles have been completed under infestation with CEW. Lines have also been derived from populations 85, 86, 87 and 88 under CEW pressure which have also been improved for agronomic characteristics.

In addition, in 1990 nineteen landraces of 'Zapalote Grande' from the germplasm bank were identified as having resistance to CEW. These landraces have larger ears and are adapted to midaltitude environments, while those of Z. chico have smaller ears and are adapted to the lowlands. Each landrace was selfed to generate S_3 lines. These were recombined to form the first cycle of selection, which has now been advanced to S_3 and recombined in 1996. The S_3 lines from C_1 were also crossed with S_5 (early white semident) lines derived from Population 85.

In 1994, S_5 lines from CEW resistant populations were crossed to CML242 (early white) and CML245 (early yellow) from the highland program. These hybrids were evaluated in 1995 for insect resistance and yield performance with 40 hybrids being used for line recycling. In 1995, S_4 lines from C_1 of the Z. grande population were crossed to CML 242 and CML245. These hybrids were not high yielding, but did possess good levels of CEW resistance. Lines derived from these hybrids will be returned to the highland breeding program and cooperators in national programs in 1997.

Achievements: After one cycle of recurrent selection, Population 85 possessed a large number of S_3 lines with damage levels below 4. Population 85 or Population 86 x Zapalote chico produced a greater number of S_3 lines resistant to CEW than Population 85 or Population 86 alone, and the same was true in the second cycle of selection. When lines derived from Population 85 were crossed with S_3 lines of Z. grande and F_1 hybrids infested with CEW, 8 highly resistant hybrids were identified.

One MS thesis has been completed (1996) in collaboration with the entomology unit to determine inheritance of CEW resistance. This study concluded that resistance is conferred by four loci that show quantitative inheritance for resistance from both Z. chico and Z. grande. Based on reciprocal crossing studies in 1995, equal levels of resistance are conferred, whether the resistant parent is used as the female or male. Based on these results the entomology unit recommends that the agronomically-elite line be used as the female when producing CEW resistant hybrids.

3.1.3 Sources of resistance to storage pests

Post-harvest losses due to storage pests are a major threat to food security in many parts of the developing world, where proper storage facilities are generally not available. Two insect pests of maize which have been researched considerably in the past two decades are the maize weevil, *Sitophilus zeamais*, and the larger grain borer (LGB), *Prostephanus truncatus*. The maize weevil has worldwide distribution and is considered to be an early colonizer of maize ears in the field. The LGB is a wood borer which attacks grain stores within a month of storage. This pest, which was introduced from Central America into Tanzania and Togo and has since expanded into neighboring countries, can completely destroy grain stores within five months, making it the most aggressive storage pest of maize. An integrated approach involving biological control agents and better cleanliness in storage systems is being tested, but host plant resistance is not well developed in traditional maize varieties. Furthermore, researchers have not looked hard enough for resistance sources, despite the economic viability and ecological soundness of host plant resistance and its compatibility with other control measures. For this reasons, CIMMYT has attempted to identify resistant sources in the past five years.

Achievements: In collaboration with Canadian colleagues, CIMMYT has identified three collections resistant to the grain weevil. They are Sinaloa 35, race Chapala; Yucatan 7, race Naltel; Oaxaca 130, and Sinaloa 2, race Chapala. The biochemical mechanism for this resistance has been elucidated and is reported in section 3.4. In order to demonstrate the effectiveness of these resistant sources and to improve the agronomics of resistant material, these sources have been crossed with La Posta soft *opaque*$_2$, which is very susceptible to weevil attack. Approximately 100 S_3 lines have been extracted from these crosses which show improved agronomics and moderate levels of resistance. Lines extracted form these landraces have also been crossed with Zapalote grande to augment their level of resistance to CEW. Finally, since 1993 research has intensified to identify sources of resistance to the LGB (Table 3.4).

Table 3.4. Frequency distribution of larger grain borer damage to S_2 lines derived from germplasm bank accessions.

	Percentage of kernels damaged										
Entry	0	10	20	30	40	50	60	70	80	90	100
TE69A-1209-5:BRVI147	2	0	0	0	0	1	1	0	0	0	0
FL84-4022-61:CUBA 63	0	2	1	2	1	2	1	0	0	0	0
FL85-5006-81:CUBA140	0	0	0	0	0	0	0	0	0	1	0
FL85-5001-101:GUAD-5	46	23	12	13	25	16	3	2	1	3	0
FL85-5007-41: CUBA60	1	0	0	1	2	2	0	0	0	0	0
FL86-6010-030:SCX-GP1	0	0	0	0	0	1	0	0	0	0	0
TE69A-1197-1:CUBA44	6	3	3	1	4	1	2	0	1	1	0
TE69A-1204-5:BRVI 117	0	1	1	0	2	4	2	1	0	1	0
TL70B-2989-0:GUAD -11	9	4	2	6	9	11	2	7	10	4	2
TL71B-1505-0:CUBA89	1	0	0	2	0	1	0	0	0	0	0
TL74A-1817:CUBA113-2377	1	0	3	0	4	5	0	0	0	1	0
Susceptible check	0	0	0	0	0	0	0	0	0	0	30

CIMMYT is collaborating with IITA to model the relative importance of resistance in an integrated approach to *P. truncatus* control in western Africa. The model will evaluate the importance of resistance in delaying LGB population growth and thus facilitating biological control.

3.1.4 Sources of resistance to corn rootworm

Introduction: Although corn rootworms (CRW), *Diabrotica* spp., are not considered a major insect pest in tropical maize cropping systems, they are of considerable importance to Mexico. Host plant resistance work for subterranean insect pests is difficult due to destructive sampling techniques, and for the rootworms this is compounded by the difficulty in rearing these insect pests for artificial infestation. CIMMYT has worked since 1990 to identify CIMMYT lines and germplasm bank collections which are resistant to CRW. Sources of resistance were found in eight landraces of Mexico, and lines were selfed for crossing onto agronomically elite germplasm. This work has been conducted in collaboration with the Mexican National Institute of Forestry, Agriculture, and Livestock Research (INIFAP; Jose Blas Maya, Jalisco) and Agriculture Canada (Dr. R.I. Hamilton, Ottawa).

Achievements: Two methods for screening germplasm were developed during the project. Field evaluations involve two row-plots and the application of granular insecticide at the time of planting to one row. Plants are classified based on relative height reduction and lodging between the protected and unprotected rows. This protocol requires fields with uniform CRW infestations, and such fields are best found in Jalisco. When CRW eggs are available for artificial infestation, plants are destructively sampled 10 weeks after planting for damage ratings. The second screening method is based on rapid quantification of phytochemicals responsible for resistance. Plants are grown in a hydroponic system, and after 10 days the roots are harvested. From these absorbency readings were obtained and provided estimates of levels of the resistance factor DIMBOA. Using this phytochemical screening technique, S_3 lines have been selected on the basis of large roots (tolerance mechanism) and elevated levels of DIMBOA (antibiosis mechanism). Selections have also been made for small roots and low levels of DIMBOA for genome mapping studies. Using these techniques, 12 resistant lines and three susceptible checks have been identified. In 1996, a DNA mapping project was established using one resistant line and one susceptible line obtained from this program. A map of resistance factors should be completed by early 1997.

3.2 Developing multiple species/stage resistance

Background: Tropical maize crops are often attacked by several pests or face a daunting combination of biotic and abiotic constraints, during a single cycle. Examples of such environments and combined stresses include:

- Eastern Africa (stem borer and maize streak virus).
- Asia (stem borer and downy mildew).
- Latin America (fall armyworm, stem borer, and maize stunt complex).
- Subtropical and highland areas (corn earworm and ear rot).
- Dry areas (drought and spider mites).

Developing multiple resistance/tolerance in single varieties or hybrids requires the use of several sites and a network approach involving many collaborators to evaluate germplasm under different stress conditions. The entomology unit has organized an international network of breeders and entomologists with access to mass rearing facilities to provide uniform screening of populations and experimental varieties.

3.2.1 Networking to evaluate progenies under artificial infestations

Introduction: Insect Resistant Progeny Trials (IRPTs) were sent to cooperators worldwide – including ICIPE, IITA, national programs in selected developing countries, and collaborators in North America – in 1986, 1988, 1991, and 1994. Germplasm improvement for specific traits has generally been achieved through research with one or a few collaborators. Examples of this are the shuttle breeding programs for :

- Spider mite resistance and drought tolerance: Dr. Tom Archer, Texas A&M University
- Resistance to maize stunt complex and FAW: Research scientists in Nicaragua and El Salvador.
- Downy mildew and stem borer resistance: University of the Philippines and CIMMYT-Bangkok
- Maize streak virus and stem borer resistance: IITA and CIMMYT-Harare
- Corn rootworm resistance: INIFAP-Centro de Jalisco, Agriculture Canada-Ottawa, USDA-Missouri.

Achievements: Through the IRPTs more than 12 experimental varieties have been developed and elite lines from each cycle have been identified and recombined for each cycle of selection. Help from our network of collaborators was useful in separating source populations into grain color groups. These populations have now reached C_4 for MBR (Population 590 subtropical), C_2 for MBR-MDR (Population 590 B subtropical), and C_3 for MIRT (Population 390 tropical). More specific achievements include:

- Release of two mite-resistant lines with Texas A&M.
- Generation of 250 tropical and 120 subtropical lines with combined maize stunt and fall armyworm resistance.
- Identification of 20 lines with combined downy mildew and stem borer resistance.
- Identification of 120 lines with combined maize streak virus and stem borer resistance, now in used by the Zimbabwean breeding program.
- Identification of 8 lines with resistance to the western corn rootworm which are now being used in a genome mapping project in collaboration with the University of Ottawa (PhD Thesis).

3.2.2 Recombination of materials showing resistance to two or more species

Introduction: Developing sources of resistance to several insect species is important because many of the leaf feeding and stalk boring insects overlap in their distribution. Population improvement and the development of experimental varieties has been based on the results obtained from IRPTs or through more focused shuttle breeding activities described in Section 3.2.1. In order to combine traits, key test environments must be identified, as well as the selection pressure needed to identify genetic variation in the population. For most of the multiple stress populations, insect resistance is considered to be more difficult to achieve than disease resistance (downy mildew, maize stunt complex, maize streak virus and common foliar diseases), so more weight is generally given to insect selections than disease selections.

Achievements: During 1995, the three main source populations were recombined based on data obtained from eight international cooperators and from evaluations conducted in Mexico.

- Multiple Borer Resistant Population (Population 590): In 1994, 144 S_1 families were generated and sent for international testing to 12 locations. The results received from 8 locations have been used in a selection index to make recombinations during cycle 1995B. The weighting of the selection index was based on the location (adaptation), quality of the data, and the relative importance of the insect species used in the trial. To facilitate the adoption of insect resistant sources we have made recombinations within each color group, as well as the traditional method of mixed-color recombinations. Twenty-three white and yellow lines were identified for full-sib recombination.
- Multiple Borer Resistant and Multiple Disease Resistant (MBR - MDR) Population (Population 590 B): Families of this population were crossed onto elite sources which had resistance to leaf blights and rusts. The population was further selected under disease pressure at El Batan and stem borers at Tlaltizapan. After one cycle of selection, 121 S_2 lines were sent to collaborators. For recombinations, a slightly modified version of the selection index for Population 590 was used. Based on this index, 28 white yellow lines were identified for recombination. During 1996, S_1 lines from both subtropical populations have been screened for disease resistance (*E. turcicum* and *P. sorghi*).

- Multiple Insect Resistant Tropical (MIRT) Population: In 1994, the second cycle of selection for the MIRT population was completed and 196 S_2 families were sent to 11 international collaborators. The results from 7 collaborators were used to identify 39 white and yellow lines for recombination. Prior to flowering additional selections were made to reduce the number of families to 34 yellow and 31 white lines.

It is apparent that very considerable gains in resistance have been made; nonetheless, the gains may be diminishing. The entomology unit may therefore discontinue the improvement of these source populations to focus attention on line recycling and generation of new populations with multiple resistance and second generation stem borer resistance. During 1996 the first trial for second generation stem borer resistance was sent out prior to the formation of C_2. The first IRPT for second generation stem borer resistance will be sent in 1998.

3.3 Development of experimental varieties, hybrids, and inbred lines under infestation in good and marginal environments

Introduction: With the increased emphasis on line development for hybrid products, a variety of synthetics and hybrid products has been developed in the last four years. The challenge facing the maize program is how to adequately test these materials, given the narrow adaptation that lines often have in comparison to synthetics and populations. An additional complication in evaluating insect resistant germplasm is the scarcity of research on optimal soil management practices to evaluate germplasm for insect resistance. The stress breeding subprogram has attempted to address these issues to deliver resistant materials that are broadly adapted and provide good yield stability under severe insect pressure.

The two areas which have received the most attention have been tillage practices and soil fertility. Trials were conducted on three experiment stations during 1993-1995 under zero tillage without mulch, zero tillage with mulch, minimum tillage, and conventional tillage to elucidate the importance of these practices on insect damage to maize. All hybrids planted under zero tillage + mulch suffered significantly higher damage by SCB and FAW. Retention of a higher level of soil moisture by mulch could have provided more favorable conditions for larval feeding. Leaf feeding damage by SWCB was not affected by the tillage systems. Finally, during 1995 and 1996 the importance of soil fertility was studied, with special emphasis on low nitrogen and its impact on host plant resistance.

Achievements: In the past four years considerable understanding has been gained on the importance of soil management practices in evaluating insect resistant germplasm. Grain yield on zero tillage plots is generally higher than under conventional or minimum tillage, except in trials under SWCB. These observations indicate that zero tillage with mulch can be used effectively to characterize insect resistant germplasm, as mulch appears to create a favorable microenvironment for insect establishment. Moreover, this management strategy is the preferred one for resource-poor farmers, as it requires fewest inputs and helps conserve fragile tropical soils.

Vigor has been considered as an important component in resistance/tolerance to insect pests in maize. Under this construct, low nitrogen environments reduces plant vigor, which in turn increases plant susceptibility. However, in our studies under severe low nitrogen stress (1-2 t/ha yield potential), stem borer and armyworm establishment was extremely poor, with virtually no differences for yield between infested and protected plots. Based on our understanding of the biochemical basis for multiple borer resistance in maize, we now consider low nitrogen content in the leaf to be an important component in maize resistance to insects in poor fertility soils. Leaf nitrogen drops sufficiently within 35 d after sowing to be unsuitable for larval establishment, especially for stem borers, which appear to have higher nitrogen requirements than fall armyworm. Results from experiments planned for 1997 promise to provide insights on how farmers can optimize fertilizer use while reducing insect establishment on maize.

Testing of experimental hybrids, synthetics and lines: In 1990, trials of resistant and susceptible materials were conducted under infestation at eight locations in the US, Canada, and Mexico to evaluate the stability of resistance to ECB, SCB, SWCB, and FAW. The hybrid formed from our most resistant lines was the most resistant material at all locations and against all insects tested. Across locations, the experimental varieties (EVs) developed from MBR and MIRT against single species had similar leaf feeding damage ratings and were classified as moderately resistant (i.e., yields under infestation of 5-6% less than those on protected plots, whereas susceptible varieties showed 25-30% yield reductions).

In 1992 a new trial was begun using the latest EVs from the MIRT and MBR populations. The trials were planted under moderate and high nitrogen levels. Leaf damage ratings were similar for the moderate and high nitrogen plots. On average, percentage yield reduction due to the damage (mean leaf damage rating 6.4) was almost doubled at the moderate nitrogen level (20% at 100 kg N/ha vs 12% at 200 kg N/ha). Mean yields under protection were 8.1 t/ha at 100 kg N/ha and 8.4 t/ha at 200 kg N/ha. Resistant EVs and hybrids showed a smaller reduction than susceptible cultivars, but the results were not entirely consistent.

During 1994, 196 advanced insect resistant lines (S_{4-5}) handled by the entomology unit were planted at Tlaltizapan and Poza Rica for further improvement of insect resistance. At the same time, a series of lines were passed to the stress breeding subprogram for determination of general and specific combining ability (GCA and SCA) on at least two testers (Tuxpeño and ETO EVs). These lines comprised 390 S_{4-5} lines of Population SCB and FAW, and 234 S_3 second generation lines from the same populations. From MBR and MIRT, 594 topcross progenies under evaluation with and without insect infestation were used to identify elite hybrids which will be tested in CIMMYT's conventional hybrid trials. At the same time, location-specific experimental open-pollinated varieties were generated from the international testing of Populations 590, 590B and 390, and these have been made available to national program collaborators as adapted sources of these populations.

During 1995, the entomology unit handed over to the Stress Unit 92 white and 37 yellow midaltitude insect-resistant lines (S_4-S_6) and 46 white and 61 yellow insect-resistant lowland tropical lines (S_4-S_6) (see below). Based on international testing of MBR-MDR and MIRT, experimental open-pollinated varieties will be generated for specific insects within regions of interest. Lines identified with disease resistance (streak for Africa, downy mildew for Asia) will be made available to national programs who need specific disease resistance as well as high levels of insect resistance. Also in 1995, two white dent-flint hybrids were developed (MBR-E.t x MBR Blanco Elite) and included in the International Subtropical Hybrid Trial that was widely evaluated. Resistant and susceptible hybrid checks were also provided by the entomology unit.

Information describing the performance of insect resistant hybrids versus conventionally selected hybrids under moderate infestation of FAW in the field is described in Appendix 5.

Advanced insect-resistant germplasm handled by the stress breeding subprogram

Significant progress has been made in the development of insect resistant inbred lines, hybrids and synthetics. This work has been done in close collaboration with the entomology, subtropical, and tropical lowland breeding programs.

Subtropical insect resistant germplasm: Three subtropically-adapted synthetic populations were formed from elite insect resistant lines based on combining ability, *per se* performance, and grain color. These synthetics have been distributed to numerous collaborators for use as open-pollinated varieties and as a source of new inbred lines. We have commenced selfing in these materials to develop second-cycle lines, and now have 129 S_4 lines from Subtropical Insect Resistant (SIR) Tuxpeño white, 132 S_4 lines from SIR non-Tuxpeño white, and 65 S_4 lines from SIR yellow being evaluated under insect infestation. We are also topcrossing them to elite tester lines to evaluate their performance in hybrids under both optimal conditions and insect pressure.

Advanced lines used in the development of the three SIR synthetics have gone through a period of extensive testing both as lines *per se* and in hybrid combinations. Elite lines were evaluated *per se* in 15 trials during 1995-96, where they were subjected to SWCB, SCB, FAW, *E. turcicum* and *Fusarium* ear rot pressure. During this period the lines were evaluated in hybrid combinations with 4-8 elite tester lines from the subtropical program. The hybrid trials were conducted under both protected and infested conditions at 5-8 locations each, with assistance from Mexican researchers in public and private organizations. In 1995-96, we shipped 32 SIR hybrid trials. Based on this extensive *per se* and combining ability evaluations we have identified five white-grained and four yellow-grained lines which we plan to announce as insect-resistant CMLs in February, 1997 (Table 3.5). We have already distributed significant quantities of these lines to interested national program collaborators, and expect to be sending much more after the official announcement. These may be the first set of lines ever released that combine both excellent agronomic characteristics and insect resistance.

Table 3.5. Subtropically-adapted insect resistant CIMMYT maize lines (CMLs) released by the Stress Breeding Unit in 1997.

CML No	Pedigree	Yield (1-5)*	Plant Ht. (cm)	Stand-ability (1-5)*	GCA	Special Resistance/ Tolerance
	White-Grained					
330	89[SUWAN8422]/[P47s3/Mp78:518]#-7-1-1-1-1-1-B-#-B-B	1.5	155	2	Avg.	SWCB,FAW
331	89[SUWAN8422]/[P47s3/Mp78:518]#-183-1-2-1-2-2-B-#-B-B	1.5	181	2.5	Exc.	SWCB,FAW
332	89[SUWAN8422]/[P47s3/Mp78:518]#-183-1-7-3-1-2-B-#-B-B	2	122	2	Good	SWCB,FAW
333	P590C3F373-1-1-7-B-#-5-2-B-#-B	2	182	3	Good-Exc	SWCB,FAW
334	P590C3F374-2-1-2-B-#-1-1-B-#-B	1.5	219	3.5	Exc.	SWCB,FAW
	Yellow-Grained					
335	89[SEYF]/[P47s3/Mp78:518]B-23-1-5-1-1-2-B-#-B	2.5	137	3	Good	FAW
336	89[TL8645]/[P47s3/Mp78:518]B-24-1-1-4-1-3-B-#-B	2	149	2	Good	SWCB,FAW
337	89[TL8645]/[P47s3/Mp78:518]B-24-1-3-2-1-2-B-#-B	2	144	2	Good	SWCB,FAW
338	P.590B F84-3-3-5-3-1-1-B-#-B	2	169	2.5	Exc.	SWCB,FAW

A superior set of the S_6 SIR white-grained lines is being used as a part of a post-doctoral project designed to address several important practical issues regarding the development of insect resistant hybrids. These lines have been crossed with elite lines from the subtropical program in a study to: 1) examine dosage rate effects for borer resistance in hybrids, as well as the role of vigor in their expression; 2) estimate the relationship between line *per se* and hybrid performance under both normal and insect-infested conditions; 3) identify single-crosses for developing second cycle lines that combine insect resistance with good agronomic performance; and 4) compare inbreds and hybrids selected for borer resistance with those selected for agronomic performance. Preliminary results (Table 3.5) show that having one insect resistant line in your hybrid should provide acceptable yield performance under SWCB pressure. However, for insect foliar feeding, two resistant lines may be necessary to minimize damage levels. Excellent single-cross combinations that combine insect resistance and good agronomic traits have been identified for use in developing second-cycle lines. Hybrids demonstrating top-end yield potential under non-stressed conditions and superior performance under SWCB and FAW pressure have been identified.

Early generation recycled lines developed from a set of full-sib families, formed by intercrossing 16 elite lines derived from Population 590 (MBR), have been advanced to the S_5 level under SWCB pressure. Currently, we have 123 S_5 lines under evaluation both for SWCB and *E. turcicum* resistance. These lines have been topcrossed to two elite subtropical tester lines and evaluated in the 1996B cycle at three locations in Mexico. Superior lines identified should be ready for release in 1998.

In 1995, 92 white-grained and 37 yellow-grained subtropical S_5 lines were supplied by the entomology unit. They have been evaluated as lines under protected and infested conditions for several cycles, and the superior fraction further selfed and crossed to elite tester lines from the subtropical program. The hybrids formed were tested in the 1996B cycle at four locations. The superior lines and hybrids will be further tested in 1997. Superior lines identified will be released in 1998.

Tropical insect resistant germplasm: Early generation lines derived from a series of tropical insect resistant synthetics were advanced under SCB and FAW pressure in 1995 and 1996. The tropical insect resistant synthetics were previously formed based on *per se* performance, combining ability, and grain color of a set of advance tropical lines. Numbers of S_4 lines harvested at the end of the 1996B cycle and the source population from which they were derived are listed below:

Number of lines	**Source Population**
70	Population SCB/FAW GCA Yellow
46	Population SCB/FAW Tuxpeño Yellow
35	Population SCB/FAW Non-Tuxpeño Yellow
47	Population SCB/FAW Tuxpeño White
62	Population FAW Non-Tuxpeño White
69	Population FAW GCA White

These lines will be top-crossed to elite tester lines from the tropical lowland program in the 1997A cycle.

Approximately 125 white-grained and 90 yellow-grained S_3-S_4 insect resistant lines were evaluated in combination with elite tester lines from the tropical program in 1995 under both protected and SCB infested conditions. Superior lines identified were further inbred and crossed in additional hybrid combinations, and a 70 entry trial was conducted. This trial included single-cross and three-way cross white-grained hybrids that had at least one insect resistant line as parent. The trial was evaluated in five environments (one infested with SCB, one infested with SWCB, and three protected locations, including a Mexican national program site). Similarly a 72 entry trial of yellow grained single- and three-way-cross hybrids were evaluated in the same environments. Based on the results of these trials, we have identified 30 white-grained and 40 yellow-grained hybrids which have been extensively tested both as lines and in hybrid combinations (24 trials in 28 environments) during 1995 and 1996. We plan to release two white-grained lines as CMLs in February, 1997 (Table 3.6).

Table 3.6 Tropical CIMMYT maize lines (CMLs) released by the Stress Breeding Unit in 1997.

CML No.	Pedigree	Yield (1-5)*	Plant height (cm)	Stand-ability (1-5)*	Grain Text.	GCA	Special Resist/ Tolerance
	White grained						
339	LA POSTA SEQC3-H297-2-1-1-1-3-#-#-B-B	3	169	3.5	F	Exc.	Drought
340	LA POSTA SEQC3-H20-4-1-1-2-3-#-#-B-B	2.5	179	3	SD	Good-Exc.	Drought
341	LA POSTA SEQC3-H1-2-2-2-1-1-#-#-B-B	1.5	160	3.5	SD	Exc.	Drt+, Low N
342	LA POSTA SEQC3-H1-2-2-3-2-1-#-#-B-B	2	145	3	SD	Exc.	Drt+, Low N
343	LA POSTA SEQC3-H17-1-2-3-2-1-#-#-B-B	2.5	163	2	SF	Exc.	Drt+
344	TS6C1-F118-1-2-3-1-2-#-#-B-B	3.5	140	2	SF	Good	Drt+, Low N
345	Suwan-1/Lin.IITA*MpHib.C1SCB-F72-1-1-1-1-#-5-B	3	156	2	F	Good	SCB, FAW
346	AC90390SR(SCB)-F430-1-1-2-3-2-2-#-#-B	3	152	3.5	F	Good	SCB, FAW
	Yellow grained						
347	G26SEQC3-H71-1-1-2-1-B	3	150	3	SD	Good	Drt+
348	G26SEQC3-H83-1-1-2-1-B	3	175	2.5	SF	Good	Drt+

(1-5): 1=Excellent; 5=Poor. GCA = General combining ability + Moderate drought only

Advanced lines from Population 390 (MIRT) and several insect resistant tropical synthetics have been advanced under SCB pressure. Approximately 52 white-grained and 127 yellow grained S_6 lines have been increased, evaluated for *per se* performance, and crossed to elite tropical testers. The hybrids formed will be evaluated in the 1996B cycle.

The 46 white-grained and 61 yellow-grained tropical S_5 lines received from the entomology unit in 1995 have been evaluated for agronomic performance, and the superior fraction further selfed and crossed to elite tropical lines. Hybrids formed have been tested during the 1996B cycle; superior lines and hybrids will be further evaluated in 1997.

A set of S_4 lines developed for resistance to the maize grain weevil (*Sitotroga* spp.) were advanced and evaluated in hybrid combinations. Additionally, ears from these lines were evaluated in the laboratory under controlled conditions, where they were infested with grain weevils. Seven white-grained and three yellow-grained lines were selected, based on laboratory and field evaluations. Additional hybrid combinations have been formed with these 10 lines, and we also have made a synthetic population with the seven white-grained materials.

Although difficult to measure, we believe the impact of CIMMYT's insect resistant maize germplasm has been minimal to date. This is probably largely due to the agronomic problems associated with this material (see Appendix 5). However, we now have material that combines excellent insect resistant with good agronomic characters, and we believe our material will soon begin to have major impacts in insect-plagued areas.

3.4 Biochemical and biophysical bases and inheritance of resistance in maize

Introduction: The availability of insect resistant lines provides researchers with the exciting opportunity to identify the biochemical basis and the inheritance of insect resistance. Knowing this, management practices can be developed which complement resistance mechanisms and breeding efforts can better target desirable traits.

Achievements: <u>Biochemical and biophysical composition of resistant germplasm:</u>

1. Stem borers: As part of a PhD thesis, D. Bergvinson (University of Ottawa) quantified several biochemical factors possibly involved in resistance and correlated these factors to field resistance data, as well as with biophysical data (leaf toughness). Reduced leaf protein, elevated fiber content and elevated phenolic dimers in the hemicellulose (dehydrodiphenolic acids) were found to account for 85% of the observed variability in field ratings for 13 genotypes which showed a range in insect resistance. Leaf protein below 12% (2.1% nitrogen) is not sufficient for stem borers to become properly established. Likewise, tough epidermal cell walls which are high in fiber and cell-wall-bound phenolic acids restrict the penetration of neonate larvae into the mesophyll. This work has been followed up with mapping studies (PhD thesis, Susanne Groh, Germany) using the cross CML67 x CML131. This research showed that leaf toughness and reduced protein have many of the same QTLs as field insect resistance, thereby confirming the importance of these biochemical factors in stem borer resistance. Collaborative research with the Mexican Autonomous National University (UNAM), Mexico City, has revealed that soluble flavanoids are not important resistance factors against *D. grandiosella* and *S. frugiperda*.

2. Storage pests: In collaboration with Canadian researchers, the biochemical basis of resistance to *Sitophilus zeamais* has been identified as phenolic acids in the pericarp and aleurone layers. Phenolic acids conjugated to amines are found in high concentration in the aleurone. Feeding studies using synthetic phenolic amines have shown these compounds to be toxic to both *S. zeamais* as well as *P. truncatus*. Kernel toughness is also negatively correlated with insect performance factors (delayed development, reduced survival and fecundity). Cell-wall-bound ferulic acid is considered important in the expression of kernel toughness, but it is still uncertain in which tissue (e.g., pericarp, endosperm, or germ) this is most important.

3. Corn rootworms: Research at the University of Ottawa has shown the hydroxamic acid, DIMBOA, to be negatively correlated with rootworm damage. CIMMYT and the University of Ottawa have used this information to conduct biochemical screening to advance rootworm resistant lines showing good field resistance. Progenies from a cross between two of these lines are now being mapped at CIMMYT's Applied Biotechnology Center, as part of a PhD thesis project.

Inheritance of resistance

1. Stem borers: In PhD thesis research (Catherine Thome, Cornell) involving eight CIMMYT lines, it was found that the inheritance of leaf feeding resistance to FAW, SWCB, SCB, and ECB in the MBR population was primarily due to additive gene action accompanied by a high general combining ability. Dominant genetic response was also important. Genetic background of the susceptible was important in maximizing the dominance response. Crosses with susceptible US germplasm tended to show an additive response, whereas crosses to susceptible tropical germplasm showed a dominant resistant response.
2. Storage pests: Inheritance of storage pest resistance has been the subject of a PhD thesis project by Antonio Serratos (University of Ottawa/CIMMYT). Resistance to *S. zeamais* was primarily an additive genetic response, which suggests that the triploid endosperm is an important factor in resistance.
3. Rootworms: A study in collaboration with the University of Ottawa and Agriculture Canada using CIMMYT and northern subtropical germplasm showed an additive genetic response, with both general and specific combining ability being important.

Chapter 4: Task 3: Strengthening national programs' capacity for host-plant resistance research

Task 3: *Upgrade strength of national programs in mass rearing of maize insect pests and host plant resistance development.*

Background: Equipping national program scientists in developing countries in insect mass rearing and host plant resistance improvement is an important step in delivering insect resistant germplasm to national programs. If proper training is not made available then the likelihood of resistance being maintained when germplasm improved for insect resistance is being used is reduced.

The challenge facing national program scientists interested in host plant resistance is the high recurring cost of insect production. Funding is often sporadic, making it extremely difficult to maintain a stable program. Collaborators in stronger national programs (e.g., India, Philippines and China) have managed to maintain a good level of activity in host plant resistance, but many countries, especially African national research programs, do not have the financial support to sustain this type of research activity.

CIMMYT has provided support to those national programs with the physical infrastructure and political support to ensure some level of success in sustaining research activity. In order to provide some level of political support, public awareness of insect associated losses in maize will be required.

4.1 Train national program scientists from target countries in entomology/insect mass rearing and host plant resistance improvement through the following activity:
4.1.1 Training courses at CIMMYT Mexico for entomology and breeding/ crop management. Each trainee will make a two-week visit to a US university maize insect host plant resistance program, if possible and timing appropriate.

The first Entomology Training Course was held in El Batan in January 1993. Twelve participants, a breeder and an entomologist from each program came. The Course outline and participant list is included in Chapter 10.

In addition, 2-4 lectures on HPR and breeding for improved insect resistance are presented to the trainees who are here for the maize improvement course held twice a year (one in Spanish, one in English). Advanced Maize Breeding Courses were also provided at CIMMYT: Sep.1 - Oct. 15, 1993; Jan 10 - July 10, 1994; and Sept - Oct., 1996. In these courses, ideas were exchanged on the constraints encountered in the use of host plant resistance for pest management in less-developed countries, and how some of these constraints can be overcome.

4.2 Build the experience of mid level visiting scientists from target countries through the following activity:
4.2.1 *Visiting scientists will come to CIMMYT Mexico or to CIMMYT outreach centers for collaborative research on utilizing resistant materials.*

During 1994 there was one mid-career entomologist who spent time with the Entomology program. Mr. Pedro Injante from the national program of Peru visited the Entomology laboratory for one month to learn rearing, infestation and evaluation methodologies for corn earworm resistance in maize. There was however a strong national program representation of professional entomologists at the international conference "Insect Resistant Maize: Recent Advances and Utilization", with special reference to CIMMYT's insect-resistant germplasm. This conference, which had about 80 participants and 60 scientific papers, took place at CIMMYT in November, 1994, and many developing country scientists contributed papers and participated in discussions. Contributions will be incorporated into a bound proceedings (see Appendix 2). Editorial comments by Dr. J. Mihm should be completed in early 1997.

Dr. Mihm also participated in one CIMMYT regional maize conference in 1994 (in Harare), taught classes in the Advanced Breeding course at CIMMYT, and consulted with numerous national program visitors to CIMMYT. In 1995, Dr. Bergvinson participated in the 6th Asian Regional Maize Workshop in Ludhiana, India. During the workshop, researchers in host plant resistance planned future collaboration to develop maize populations for Asian. Mass rearing supported through this project is being used to artificially infest this population in India (New Delhi, Ludhiana), Philippines, China and Thailand. During the Asian Regional Workshop and a workshop on maize stem borers in Kenya, mid-career entomogists were approached to work with CIMMYT entomologists during advanced training. However, no suitable candidates could be found which work closely with breeders and who would therefore have the opportunity to apply the techniques learned at CIMMYT, HQ, to their national breeding program.

3.3 One in-country training workshop per year.
4.3.1 *Planning and conducting screening/evaluation trials with artificial infestation and protection, with the aim of strengthening interregional collaboration.*

The in-country training workshops have been largely subsumed by the activities described under Outputs 4.1 and 4.2. In-country workshops in a formal sense have proven difficult to manage, largely because there are few national program entomologists in any single country who are integrated in national maize breeding programs. As well, the logistics of such workshops are more difficult to manage within the national program than they are at CIMMYT's Headquarters. Visitors to CIMMYT HQ see and learn a great variety of additional useful facts, especially about suitable germplasm for their home programs and other new breeding techniques. All in all, we feel that formal training courses held at CIMMYT (rather than in-country) are more effective, and make far more efficient use of the project Entomologist's scarce time. Future developments in host plant resistance research may well revolve around a centralized mass rearing laboratory that supplies a number of nearby countries with insect larve.

4.4.Strengthening national programs through visits and consultation by CIMMYT and other scientists.
3.4.1 *Visits of 3-10 days by CIMMYT scientists to interact with national program scientists in planning and inspecting experiments, visiting farmer's fields, and consulting with decision makers.*
As noted before, upgrading the capacity of national programs to conduct HPR research and to mass-rear and artificially infest with insects is not the same thing as actually making it happen. In general there is still a disappointing level of HPR research within national programs, and very few are capable of artificially rearing insects and infesting nurseries with them. The problem lies not with trained personnel, nor with methodology. Rather it is simply that the level of Government support is not adequate to establish and maintain such facilities, and the turnover of national staff is often high. In the private sector of developing countries, methodologies associated with this research have not attracted a lot of attention because of the long-term nature of the research commitment needed to achieve results. There is, however, an encouragingly high interest in the utilization of genetic sources of insect resistance developed by the Project, suggesting that in the future it may be necessary to invest in insect rearing facilities at regional

research hubs where adapted maize germplasm can be properly evaluated for resistance on behalf of the national maize programs of the region. The one-on-one training at CIMMYT of mid-career entomologists committed to HPR is still regarded as a highly effective means of instituting HPR research in maize breeding programs of selected countries that have demonstrated a financial commitment to this type of work.

Dr. John A. Mihm traveled to several regions (India, China, Zimbabwe, Egypt, Brazil, Nicaragua, El Salvador) at the request of the national programs and of our regional staff. Regional staff, who travel regularly to the countries in the region and participate in the planning of national programs, have most of the responsibility for planning and inspecting host-plant resistance experiments. As we begin to develop more insect resistant material, protocols on testing the materials and evaluating the results will be provided to regional staff and cooperating national programs. This will maximize the efficiency of the travel.

David Bergvinson has travelled to Thailand to establish collaborations with entomologists working in host plant resistance. After meeting with maize researchers in the region, entomologists at Tak Fa, working with *Heliothis armigera,* showed a keen interest in extending their host plant resistance program to include *Ostrinia furnicalis*. CIMMYT outreach is working closely with the Tak Fa research station to establish stem borer rearing facilities there, in collaboration with CIMMYT entomologists.

CIMMYT was also invited to participate in a *Prostephanus truncatus* workshop hosted by Zamarano (Tegucigalpa, Honduras) in 1995. During the meeting contacts with IITA, IIBC and the Escuela Agricola Panamericana were made. The workshop provided opportunity for farm tours to talk with farmers on the successes and difficulties associated with storage pests and the various control options available to them. David Bergvinson and David Jewell were invited to attend a workshop held in Nairobi, Kenya in 1996. The papers presented focused on the control of stem borers pests in Africa. Prior to the workshop, we met with other IARCs (IITA, ICIPE, ICRISAT) and with key national program leaders (SADC and CORAF) to identify areas of research where collaborative activities could take place to address issues of stem borer control in Africa. After the workshop, a list of proposed activities and collaborators was presented to national program entomologists in attendance. Comments and ideas obtained from this discussion were incorporated into the collaborative activities proposed for the coming five years. One issue that arose from the discussion was the lack of support for agricultural research and the lack of public awareness in maize losses to insect pests. As with the Thailand program at Tak Fa, the Entomology program at the University of Zimbabwe will be equipped through this project to increase their capacity to mass rear *Chilo partellus and Busseola fusca.*

Chapter 5: Task 4: To develop effective field based methods of selection for improved tolerance to drought and low soil nitrogen. Evaluation, under drought and low N, of synthetics and lines selected for contrasting levels of expression of single traits, or trait combinations.

Output 1:

Recurrent selection in four elite populations for drought and two elite populations for low N, involving the following activities:

Activity 1: *Based on mega-environment data, select appropriate populations (three for lowlands and one for subtropics for drought; two lowland populations for low N).*

Identification of populations for improved drought tolerance

At the onset of the UNDP Project the Physiology Subprogram was selecting for drought tolerance in 4 elite populations.

- La Posta Sequía, a late lowland white dent population (carries streak resistance, suited to lowland Africa; also for southern China);
- Pool 16 Sequía, an early white dent well suited to shorter season areas of West Africa, and carries some streak resistance. Improvement was shared with CIMMYT's West Africa breeder and the Early Germplasm Development Unit, to whom the population was passed in 1991.
- Pool 26 Sequía, a late lowland tropical yellow dent suited to Brazil, Ecuador, parts of Asian subcontinent free of downy mildew. Improvement in this population was halted in 1992, subject to an assessment of progress.
- Pool 18 Sequía, an early lowland tropical yellow flint/dent, suited to E & SE Asia, W Asia & N Africa and the Indian subcontinent in areas free of downy mildew).

Three additional populations were added:

- Tuxpeño Sequía C6 (TS6), a lowland white population. This population had a previous history of improvement for drought tolerance (Bolaños and Edmeades, 1993a; 1993b; Bolaños et al., 1993), and is suited to lowland Mesoamerica, parts of Africa relatively free of streak, and to southern China. It will be handled in the future by CIMMYT in collaboration with the Central America Regional Maize Program.
- ZM601, a late white flint/dent subtropical population, which was improved for two cycles in collaboration with CIMMYT's Harare Maize Breeding Program. Testing is carried out under drought in Mexico and under rainfed stressed and unstressed conditions in Zimbabwe. Selection is for performance in both sites.

- Pool 16 BNSEQ: This population is derived from a combination of Pool 16 Sequía and the conventionally selected Pool 16 from CIMMYT's main program, and carries some streak tolerance as well as improved earliness. Because drought and low N stress often occur together in short-season areas, progenies are being exposed to both stresses.

Two additional drought tolerant populations, DTP1 and DTP2, have been formed, and are being improved under the auspices of the Project (see Task 5). In addition, the Early Germplasm Development Unit, from 1990 through 1993, improved for drought tolerance a group of early and intermediate maturity lowland yellow and white flints, which nicely complemented the activities of this Project. Thus, the populations that were improved for drought tolerance had the potential to serve as sources in 80% of the areas where drought is a significant problem. On a cautionary note, however, in selecting populations on the basis of mega-environment data, we have focused principally on drought tolerance, especially on the development of effective selection methodologies, and have exerted only minor selection pressure for disease resistance. Lack of resistance to downy mildew and streak virus in the lowlands and to *Puccinia sorghi* and *E. turcicum* in the subtropics is limiting the immediate use of some of these source populations at present. Nonetheless, versions of La Posta Sequía and/or Tuxpeño Sequía C6 have been released in China, El Salvador, Guatemala and Ecuador, and Pool 26 Sequia has been released also in Ecuador.

Identification of populations for low nitrogen environments

We estimate that nitrogen fertilizer is used, or should be used, to achieve better yields on some 90% of the maize area in the tropics. Poor utilization of applied fertilizer leads to both reduced economic returns and problems of environmental degradation. In many of the poorest regions, chemical fertilizer is unavailable or prohibitively expensive. There are some environments where the probability of drought or frost increase the economic risk associated with chemical fertilizer use to unacceptable levels, and those areas could also benefit from germplasm with improved tolerance to low soil fertility. The large area of lowland maize in the tropics led us to choose a lowland population to initiate the work on low N. Because of the prevalence of N stress, we based the selection of Population 28 on its excellent performance at both high and low N levels, rather than on mega-environment considerations. This late yellow dent germplasm is proving useful in many parts of South America and Asia, and has already been released in Haiti and Panama.

Although there is a strong need to include a population with white grain for improvement under low N because of the extremely low rate of fertilizer use in Central and Southern Africa (average of 11 kg of nutrients per hectare of arable land are applied), where white grain is preferred), we have found that selecting for drought tolerance greatly improves performance under low N as well. Accordingly, we have added only an early-maturing white dent (Pool 16 BNSEQ) to our low N-tolerant populations. Its earliness and drought tolerance will add tolerance to low N and escape from drought.

Activity 2: *Review research literature and examine existing data; determine promising selection methods.*

Drought tolerance

a. A brief summary of key findings in the literature: maize grain yields and drought stress

Grain yield under stress is normally more highly correlated with kernel number per plant than with individual grain weight. Maize is unusually susceptible to drought stress at flowering. In part this is because male and female flowers are separated by 30-100 cm, because silks must extend by up to 30 cm to escape the husk, and because pollination demands that delicate stigmatic tissue and pollen grains are exposed to a desiccating environment. A characteristic of maize under environmental stress is an increase in the anthesis-silking interval (ASI).

Recent evidence from temperate hybrids suggests that direct effects of pollen supply on grain number per plant can be expected only when pollen production is reduced by 80%, or when ASI exceeds 8 d (Westgate and Bassetti, 1990). In open-pollinated varieties with greater variation in flowering date, an ASI of 10-12 d could probably be tolerated before pollen supply limited grain set. Pollen viability is normally little affected by plant water status, but is significantly reduced by temperatures greater than 38-40^{o}C. Tassel blasting (failure to extrude anthers) is also observed in the field at these temperatures when the atmosphere is dry.

Severe water stress prior to flowering can result in the failure of the developing ovule. Silk growth is very sensitive to turgor and follows leaf water status quite closely. In some circumstances slow-emerging silks may enter a senescent phase and abscise from the ovule prior to fertilization, though this is not thought to be a major source of yield loss under drought (Westgate and Bassetti, 1990). When late-emerging silks on drought-stressed plants are pollinated, fertilization can be shown to have taken place, but grain development is arrested shortly afterwards, giving rise to patchy grain formation, bare ear tips or complete barrenness. Boyle et al. (1991) were able to offset most of the effects of drought stress at flowering by infusing the stems of stressed plants with a sucrose-rich solution. We conclude that the continuing development of fertilized ovules is related to the *flux* of assimilates from current photosynthesis (Schussler and Westgate, 1991; Edmeades et al., 1993) rather than to the concentration of carbohydrates in the stem or even in the developing kernel (Westgate and Boyer, 1985). Silk delay may rather be a symptom of reduced assimilate flux than the direct cause of barrenness.

b. Examination of existing data: recurrent selection for drought tolerance in 'Tuxpeño Sequía' and in other advanced drought-tolerant populations

We have relied heavily on our own experience in methodology accumulated over the past 21 years of research on drought tolerance. Much it was accumulated during the selection of the population 'Tuxpeño Sequía', a population broadly adapted to the tropical lowlands. For a more complete description of selection methods used in this population, see Bolaños and Edmeades, 1993a; 1993b; Bolaños et al., 1993.

In summary, eight cycles of recurrent full-sib selection for improved drought tolerance in the tropical maize population Tuxpeño Sequía resulted in a yield gain of 500-800 kg ha^{-1} at all yield levels. Gains in drought tolerance were at no cost to yield in well-watered environments. Selection did not change osmotic adjustment, predawn and noon leaf water potentials or staygreen, but reduced tassel biomass, plant height and the biomass of superficial roots (Bolaños et al., 1993). Yield gains were associated with increased ear growth rate prior to anthesis, rapid silk growth, reduced tassel growth, reduced growth of surface roots, and higher harvest index, all manifestations of increased partitioning of current photosynthate to the developing ear. The study established an important role for managed stress environments, which were used to expose genetic variation for specific traits, especially those which control partitioning of assimilate at flowering and thereby affect harvest index.

Secondary traits emerging from this study as being important were reduced barrenness (or increased ears per plant) under drought and a reduced anthesis-silking interval, traits which are indicative of partitioning to the ear during the all-important flowering period. A shortened ASI under stress is a simple external indicator of changes in ear growth rate at flowering. The process of favoring ear growth can be accelerated by actively selecting for small tassel size, reduced ASI, and to some degree, for shorter plants as adjuncts to selection for increased grain yield. Improved radiation interception from leaves which do not roll should also favor increased production under drought, and erect leaves are though to be more water-use efficient than horizontal leaves. These considerations, then, formed the basis of selection criteria used during the first three years of the UNDP Project.

c: Methodological studies using a broader range of maize populations; evaluation of progenies under drought and low N.

These two activities have gone hand-in-hand. We have opted to develop methods for improving drought and low N tolerance by using normal progeny evaluation trials that form part of a regular breeding program.

<u>Drought: flowering stress</u>

Breeding methodology: Almost all of these populations have been improved in a similar manner. A recurrent S_1 selection scheme was used, in which a large number (600-1500) of S_1 families were prescreened under summer drought and heat stress in the Sonoran Desert near Ciudad. Obregón. The superior 200-250 families were grown in Tlaltizapán during winter from remnant seed under the three water regimes described, and the best 50 families were recombined to form the subsequent cycle of selection. Canopy temperature and relative leaf and stem extension rate were replaced as secondary selection traits by upright unrolled leaves, small tassels, and lodging resistance, while grain yield, ASI and "stay green" under stress were retained as the principal traits on which selection was based.

Selection environments: Each of the managed selection environments used has a specific purpose. The dry heat of Obregón (temperatures often exceed 37^{o}C) exposes lines susceptible to upper leaf firing and tassel blasting. At Tlaltizapán the well-watered environment allows expression of yield potential, while the IS regime exposes genetic variability for lower leaf senescence and for grain yield. Under the SS regime, observed variability for grain yield is small, but this stress exposes variability for ears per plant and ASI. There is little variation from 1.0 in ears per plant in these predominantly single-eared populations until stress applied at flowering becomes severe. Plots managed in this way are not attractive in appearance, but the method provides an unique opportunity to expose genetic variation for partitioning of current assimilate to the ear at flowering.

The evaluation of S_1 families in replicated yield trials has allowed us to examine broad-sense heritabilities for various traits at different yield levels. We found that heritability for grain yield remained in the range of 0.5-0.6 until yields were reduced by drought to below 20% of the well-watered control. At these low yield levels the genetic correlation between grain yield and ASI approached -0.80, and that for grain yield and ears per plant increased to 0.90, indicating that ASI and ears per plant were good surrogates for grain yield in these environments. In fact, the heritability of ears per plant, unlike that for grain yield or ASI, increased with declining yield levels. We believe that it was the deliberate exposure of progenies to severe drought stress at flowering that allowed us to increase the harvest index of Tuxpeño Sequía across the whole spectrum of grain yields.

Refinement of selection criteria for progeny screening: Concomitant with the screening of progeny under drought stress, we have attempted to evaluate the importance of individual secondary traits in two ways. The first is by linear phenotypic correlations between grain yield in single row plots and an array of traits which were thought indicative of improved plant water status, or of improved water use efficiency. Correlations conducted among S_1 families of five tropical populations show that grain yield under drought stress is highly associated with ears per plant and kernels per plant (Table 5.1. The negative correlation between yield and anthesis date reflects the pressure for earliness (escape) that occurs when stress increases in intensity with time. If flowering date is not held constant during selection, populations will become about 2 d earlier per selection cycle. Among other traits, ASI (untransformed) accounted for the most variation in grain yield. High grain yield under stress was weakly associated with a low canopy-air temperature differential, unrolled leaves, a high leaf chlorophyll concentration and small tassels. Leaf death score, relative leaf and stem extension rate, leaf erectness score and plant height were essentially unrelated to grain yield under stress (Table 5.1). The lack of association between yield and traits related to plant water status confirms the notion that ASI and barrenness are affected more by changes in assimilate partitioning to the ear than by plant turgor. As with Tuxpeño Sequía, it is likely that correlations related to plant water status were affected by competition between neighboring families in the small single-rowed plots used in this study, though studies conducted under low N suggest that this is not a major factor under that stress (Bänziger and Edmeades, 1995). With this in mind, we continue to apply mild selection pressure for erect leaves, since theory suggests that erect leaves may increase water use efficiency in pure stands.

Table 5.1. Phenotypic and genetic correlations between grain yield and selected traits under severe drought stress for S_1 progenies drawn from several maize populations. All phenotypic correlations were significant at $P < 0.01$. Scores are on a 1 (unrolled, upright or green) to 5 (rolled, lax or dead) scale. Phenotypic correlations are pooled data from up to 11 trials with the specified total number of observations; genotypic correlations are unweighted averages for the number of trials specified (from Bolaños and Edmeades, 1996).

	No. of observ.	Phenotypic correlation	No of trials	Genotypic correlation
Ears plant^{-1}	2449	0.77	9	0.90 ± 0.14
Kernels ear^{-1}	2227	0.50	7	0.71 ± 0.22
Kernels plant^{-1}	2227	0.90	8	0.86 ± 0.15
Kernel weight	2227	0.46	9	0.14 ± 0.17
Days to 50% anthesis	2449	-0.40	9	-0.58 ± 0.12
ASI	2449	-0.53	8	-0.65 ± 0.24
Leaf and stem extension rate	1321	0.10	4	-0.10 ± 0.33
Canopy-air temperature diff.	1089	-0.27	2	-0.20 ± 0.15
Leaf rolling score	2033	-0.18	9	-0.03 ± 0.18
Leaf erectness score	2033	-0.18	1	0.00
Leaf death score	2449	-0.11	9	0.11 ± 0.24
Tassel branch number	1793	-0.16	1	0.15†
Plant height	2449	NS	-	-
Lodging percent	-	-	9	-0.03 ± 0.15

† Calculated from a single evaluation of S_3 lines

A second approach used to determining the worth of secondary traits is by divergent selection within each group of 220-250 S_1 families constituting a single selection cycle. Thus after three selection cycles it is possible to have up to three sets of divergent synthetics for any trait of interest. When these and the cycle bulk are grown under a range of drought stresses, we can assess realized heritabilities and the adaptive value of the trait in synthetics that differ principally for the trait in question. Using this method Bolaños and Edmeades (1991) showed that leaf osmotic potential had little adaptive value in tropical maize, but that its realized heritability was 0.46. Other preliminary unpublished data suggest that realized heritabilities of leaf extension rate, canopy temperature, and leaf chlorophyll concentration are low, and that those for leaf erectness, tassel size, senescence scores and ASI are relatively high. Of these, only reduced ASI and occasionally small tassel size appear to have consistent significant adaptive value under drought. Delayed senescence during grain filling, cool canopy temperatures and upright leaves we also consider worth including in an ideotype of a stress-tolerant water-efficient tropical maize plant growing in a competitive situation, based both on theoretical grounds and on results which show some advantage to those traits. Although the heritability of grain yield declines under severe stress, heritabilities of ASI and ears per plant, both highly correlated with yield, have proven to be more stable across stress levels, and serve as suitable surrogates for grain yield in severely stressed environments (Edmeades and Bolaños, 1996).

In summarizing our information on the value of individual secondary traits, correlation analyses and divergent selection studies suggest selection should be firstly for improved grain yield under all water regimes, increased ears per plant under stress, reduced ASI under stress, and reduced tassel size. Less important traits under stress are cool canopy temperature and unrolled green leaves with a long duration. Erect leaves may increase water use efficiency.

Recurrent selection methods and realized gains under drought: summary:

We selected five diverse tropical lowland maize populations for improved tolerance to water deficits which occurred at flowering and grain filling, using either a recurrent full-sib or S_1 selection scheme and a selection intensity of 8-30%. Drought stress in the dry Mexican winter and on heavy clay soils was imposed by withdrawing irrigation 10 to 21 d before flowering until maturity in an intermediate stress (IS) treatment, and from 21 to 35 d prior to flowering until maturity in a severe stress (SS) treatment. All progenies were also evaluated under normally irrigated (WW) conditions to determine yield potential. Yields of IS and SS regimes averaged 40-60% and 10-25% of the WW yield. Selection of superior progenies initially was based on an ideotype with high grain yield under SS and IS, delayed foliar senescence under SS and IS, and reduced canopy temperatures and ASI under IS and SS. The selected fraction had the same mean male flowering date as the bulk of the population to avoid selecting 'escapes'. Subsequently selection for reduced canopy temperatures was replaced by selection for an increased number of ears per plant (EPP) under drought, upright leaves and small tassels. All traits were combined in a selection index used to identify superior progenies. These were then intercrossed and a new set of progenies formed.

Evaluation of progress occurred after 2, 3 or 8 cycles of selection, depending on the population, and was conducted in large replicated plots (> 16 m^2) grown under the same water regimes as those used during selection, or under naturally rainfed conditions. Initial and final selection cycles of the populations were also grown under low and high N over two seasons. Yields under low N averaged 47% of those under high N.

From the most recent cycle of selection in each population 100 S_2 lines were extracted at random and topcrossed to two elite inbred line testers. The same procedure was followed with matching versions of the populations which had been improved through multilocation progeny testing. The performance of hybrids formed from these pairs of source populations was then compared under SS, IS and WW conditions in Tlaltizapan, under IS and WW conditions under very hot conditions in Obregon, and under low N and normal N conditions in Poza Rica.

Results: Phenotypic and genetic correlations among progenies for traits included during selection are presented in Table 5.1. Grain yield showed a strong dependence on ASI and ears per plant (Fig. 5.1). Realized gains from selection averaged 102, 67 and 100 kg ha^{-1} yr^{-1} under SS, WW and low N, and represented 5% gain per year of selection under stress (Table 5.2). Gains were accompanied by a significant reduction in ASI and barrenness under water deficit, and an increase in harvest index under all water regimes. Gains under low N were almost identical to those observed under water deficit. Selection for staygreen under drought increased staygreen under low N.

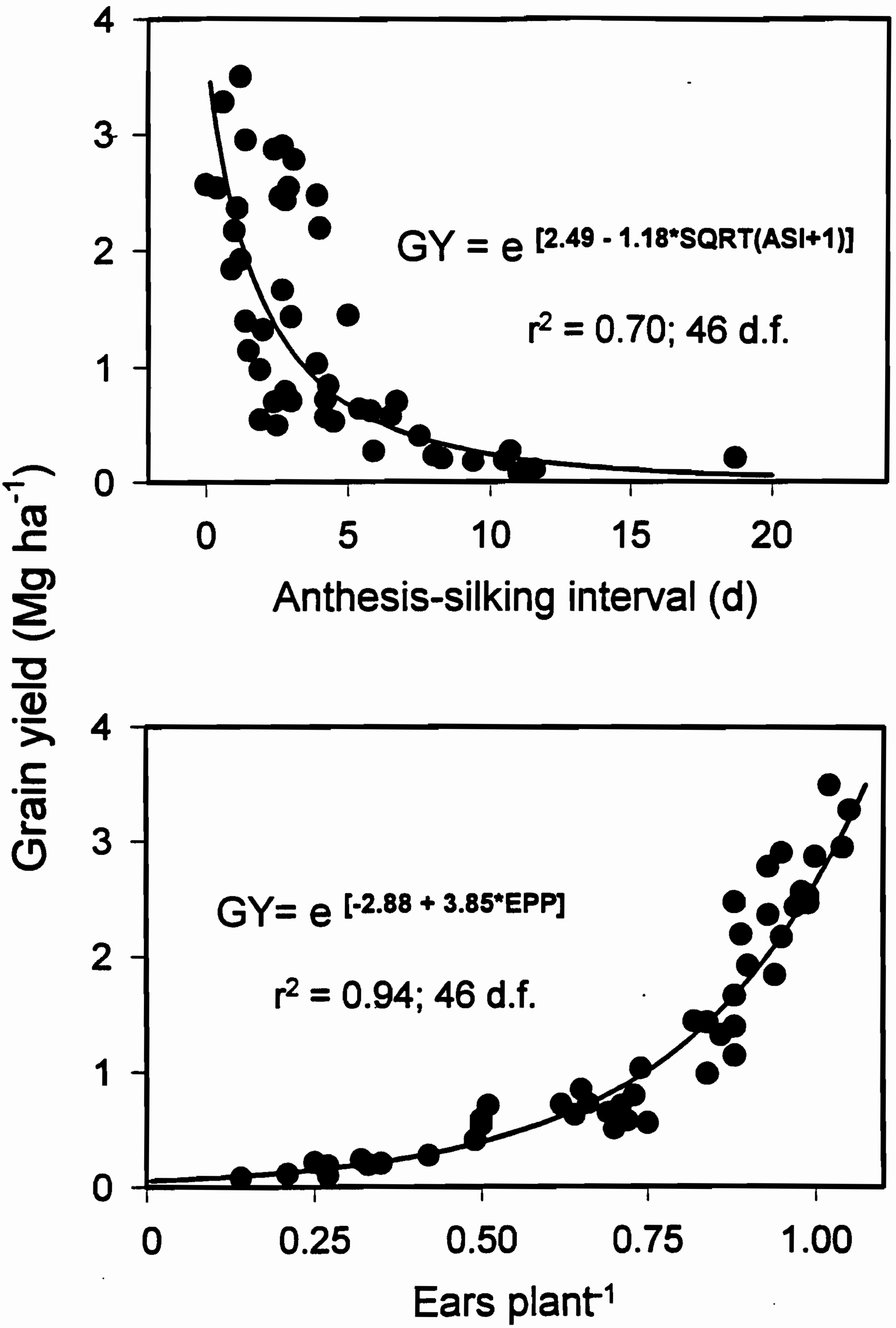
The subtropical drought population – No formal evaluations took place in the subtropical population ZM601, since only two selection cycles were completed. During the severe regional drought of 1992, however, ZM601 drought selection was the highest yielder in a variety trials conducted at the severely stressed site of Makaholi (K. Short, pers. comm.).

Table 5.2: Effects of selection for drought tolerance on gains per selection cycle in four maize populations when evaluated at 3-6 water stressed (SS), at 5-8 well-watered (WW), or at 2 low N sites, in Mexico (Mex.) or internationally (Int.). *, **, ns: sig. slope at *P*<0.01, *P*<0.05, *P* >0.05 (adapted from Bolaños and Edmeades, 1993a; Byrne et al., 1995; Edmeades et al., 1997).

Population	Change in yield (kg ha^{-1} $cycle^{-1}$)			Anthesis WW (d)	ASI SS (d)	Ears $plant^{-1}$ SS
	SS	WW	Low N			
Evaluation 1988/91						
Tuxpeño Seq. (Mex.)	100**	125**		-0.40**	-3.0**	0.03**
Tuxpeño Seq. (Int.)	52ns	101**		-0.24**	†	†
Evaluation 1992/4						
La Posta Seq. (Mex.)	229**	53ns	233	-0.52**	-1.18**	0.07**
Pool 26 Seq. (Mex.)	288**	177**	207	-0.93**	-1.50**	0.08**
Tuxpeño Seq. (Mex.)	80**	38**	86	-0.32**	-0.44**	0.02**
Pool 18 Seq. (Mex.)	146**	126**	190		-2.13**	0.05**

† Gains in ASI and ears $plant^{-1}$ from international trials were averaged over all water regimes, and were -0.35 d $cycle^{-1}$ ($P < 0.01$) and 0.011 $cycle^{-1}$ ($P < 0.01$)

Fig. 5.1 Relationship of grain yield to anthesis-silking interval (ASI) and to ears per plant (EPP) in 3509 inbred maize progenies grown under a range of available water. Data are means of 50 trials containing subsets of the progenies (after Bolaños and Edmeades, 1996).



The frequency of hybrids tolerant to drought increased when lines were derived from drought tolerant populations, compared with lines extracted from related populations improved through multilocation testing. The probabilities of extracting drought tolerant hybrids (with yields 50% greater than the mean of all topcrosses under drought) from these source populations were 2-6 times greater when drought-tolerant sources populations were used as the source of inbred lines Table 5.3).

Table 5.3 Chances of selecting a topcross that yields 30% or 50% better than the mean of all topcrosses under stress if lines come from a stress-tolerant source population instead of a conventionally-selected equivalent.

	Mean yield t ha^{-1}	Chances of topcross yields	
		30% > mean	50% > mean
Tuxpeño Sequía	1.14	3.4	5.9
La Posta Sequía	1.76	3.0	3.2
Pool 26 Sequía	1.81	3.3	5.0
Population 28 BN	1.90	1.9	2.3

A similar evaluation of progress in the early maturing population, Pool 18 Sequía, after 3 cycles of recurrent selection was carried out at 4 droughted sites and at 6 well-watered sites. Gains from selection averaged 146 kg/ha/cycle (10.3%/ cycle) under drought, and 126 kg/ha/cycle (2.4%/cycle) under well-watered conditions, for a gain of 134 kg/ha/cycle (or 3.8%/cycle) across all sites. The highest yielding entry, Pool 16 C20, also has a history of selection under drought in the Physiology and Germplasm Development Units.

Summary: Selection for drought tolerance, by exposing families of populations to water deficit coinciding with flowering, was effective at increasing grain yields under drought, well-watered conditions and under low N. Selection has resulted in a 25-40% increase in yield for a farmer whose yields have been reduced from 6 to 2 t ha^{-1} by drought occurring near flowering and during grain filling. Gains were accompanied by an increased partitioning to the ear and by fewer, larger ear spikelets that had a greater success rate in forming grain under water deficit (Edmeades at al., 1993); results indicate that ASI is a reflection of partitioning to the ear under drought *and* under low N. Success in selection was partly attributed to use of an index of traits that collectively described a drought-tolerant ideotype. Gains under water deficits were at no cost to yield in unstressed environments because partitioning to the ear was increased in all environments. This constitutive trait required a carefully managed drought stress to expose symptoms (ASI; barrenness) indicative of its genetic variation in these diverse maize populations.

Mapping the traits: QTL identification for ASI and yield components in maize has been completed using an F_2 population derived from a cross of lines contrasting for ASI (see Chapter 2 for details). A successful marker-assisted selection strategy should be based on an index of best QTL for both ASI and grain yield. Two marker-assisted projects, based mainly on ASI and yield QTL to improve drought tolerance in maize lines and populations, are presently underway (Ribaut et al., 1996). Recent data on ASI under low N conditions indicates that this also maps to many of the same positions as ASI observed under drought, suggesting that ASI is indeed a trait related to general stress tolerance in maize.

c. Seedling drought stress

The main drought tolerance program focuses on developing genotypes which tolerate drought around flowering or during grain-filling. In 1993 we started a project to develop a method to screen for improved seedling establishment under drought. Large areas in the tropics are planted at the beginning of the rainy season. The crop often receives enough water to germinate, but is severely affected if the next rain is delayed. Yield losses due to post-emergence drought stress are in some areas as high as those due to drought around flowering (Table 1.1).

A total of 196 S_1 families was screened in 1993A under a water gradient (line source). The 20 best and the 20 worst families, according to survival, biomass production, leaf rolling, and regeneration after irrigation, were recombined the same cycle. S_1s were produced during the subsequent summer cycle. With this selection scheme, an evaluation of progress and of correlated traits should be possible after two cycles of selection (1995A).

Selection environments: Screening was conducted at Tlaltizapán during the dry season. Soil water content was first reduced by planting a green manure crop which is removed when the crop shows severe wilting symptoms. Progeny are than planted, and irrigation is applied to create a water gradient along the row (6 to 40 mm) to obtain different moisture zones within each family. Survival, biomass production, unrolled leaves under drought, and regeneration after irrigation were chosen as the main selection criteria, since we believe that these traits could be measured by scientists in national programs. We have also examined some of more sophisticated measurements mentioned in literature (e.g. ABA, osmotic adjustment) for the screening process. The concentrations of ABA in leaf tissue were determined by staff in the laboratory of Dr. Steve Quarrie, John Innes Institute, Norwich, UK. Correlations among these traits are shown in Table 5.4, and heritabilities in Table 5.5.

Table 5.4. Phenotypic (above diagonal) and genotypic correlations (below diagonal) measured with 225 S_1 families of DTP1 SIBA C_2 under post-emergence drought stress at Tlaltizapan, Mexico in 1994/95.

	— Plant count — initial	final	Biomass	Leaf rolling	Seed weight	Leaf ABA concentration
Initial plant count		0.19*	0.29***	0.15*	-0.07	0.09
Final plant count	0.49***		0.24**	-0.16*	0.06	0.02
Biomass	-0.26***	0.52***		-0.15*	0.04	0.15*
Leaf rolling	0.34***	0.02	0.12		-0.05	-0.02
Seed weight	-0.11	0.12	0.22**	0.16*		-0.02
Leaf ABA concentration	-0.08	0.01	-0.10	0.18*	0.15*	

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$.

Table 5.5. Broad-sense heritabilities measured with S_1 families of DTP1 SI, DTP1 SIBA and DTP1 SIWA under post-emergence drought stress at Tlaltizapan, Mexico between 1992 and 1995.

	– Plant count – initial	final	Biomass	Leaf rolling	Seed weight	Recovered plants	Leaf ABA conc.
DTP1 SI C_0 S_1	0.28	0.42	0.27	0.54	0.94	0.32	na
DTP1 SIBA C_1 S_1	0.20	0.48	na	na	na	na	na
DTP1 SIWA C_1 S_1	0.59	0.35	na	na	na	na	na
DTP1 SIBA C_2 S_1	0.14	0.41	0.28	0.64	0.99	na	0.56
Mean =	0.30	0.42	0.28	0.59	0.96	0.32	0.56

na = not available.

Since the approach to selecting for seedling drought tolerance is quite different from the one used for flowering stress, we expect to select for different drought adaptive traits as well, such as water uptake and osmotic adjustment. These characteristics might be valuable not only during early establishment, but may constitute an additional type of drought resistance which would be advantageous during the whole life of the crop.

After three cycles of divergent selection we are now in the process of evaluating progress in this area. It is concluded that selection for improved survival under post emergence drought stress is rendered difficult under field conditions, as environmental variation is high. Secondary traits, as leaf rolling and leaf ABA concentration, have higher heritabilities but do not show an obvious relationship with survival. Unless genetic variation has already been exploited through natural selection, more precisely controlled selection conditions and other plant characteristics may be needed to identify superior genotypes. However, in looking for effective solutions, it is clear that agronomic solutions, such as improved planting date recommendations based on risk assessment or modified planting methods, may lead to more short-term impact than genetic solutions in reducing yield losses due to post-emergence drought stress.

II. Low nitrogen

a. A brief summary of key findings in the literature: maize performance under low nitrogen

Most modern high-yielding cultivars have been developed under conditions of high soil N, and while they often outyield older ones at all N levels, it is not clear whether that selection strategy is optimal for materials targeted specifically to N-limited environments. It has been suggested, for example, that traits related to high nutrient uptake are particularly important when N supply is adequate, but that the ability to produce a large amount of grain per unit of N in the plant is critical when N is limiting. There may also be a cost associated with low N-adaptive traits in terms of yield potential.

It has been suggested that nitrogen use efficiency in maize (grain yield per unit of N applied) can be increased by increasing the sink strength through prolificacy or altered endosperm composition. Genotypic differences in maize performance under low N have also been related to differences in N and biomass partitioning within the plant, especially in terms of the amount of N remobilized from vegetative tissues. Patterns of N uptake and partitioning before flowering have also been shown to be critical for the maintenance of grain numbers in N-limited environments. Low nitrogen has been shown to influence both the number of florets ear^{-1} and the fraction of those florets which form kernels, while drought or shading near flowering have their primary effect on the number of aborting kernels. A greater biomass floret^{-1} at flowering is associated with reduced abortion of fertilized florets, and low N supply tends to reduce the biomass floret^{-1}, resulting in the low number of grains ear^{-1} which is typical of N stress.

It has been suggested that greater synchrony of grain growth within an ear may result in reduced tip grain abortion and a greater number of grains row^{-1}, but experimental tests of this hypothesis have been inconclusive. The abortion of tip kernels after pollination has been found to be associated with a slower growth rate of those kernels, and the lower sink strength and growth rate of kernels in apical positions have been related to kernel volume at the onset of the linear grain-filling phase. It is possible that a reduction in the number of florets ear^{-1} allows more uniform development of the kernels within a row, and therefore less kernel abortion.

The morphological and physiological responses of maize to continuous N stress include reduced plant size, reduced radiation use efficiency, accelerated senescence, increased mobilization of vegetative N to the grain, and reduced plant N concentration. A number of these effects on crop growth can be easily evaluated in the field among progenies, allowing the identification of families on the basis of minimal direct effects of N stress as well as on the basis of grain yield.

Selection methodology: Because we still have some doubts about the importance of vigor in the expression of genetic variability for tolerance to low N, we have continued to use full-sib recurrent selection in the population Across 8328 BN. About 225 full-sib families are sown in two replicates under both high and low N in Poza Rica during the summer season. Fifty superior families are recombined during the winter in Poza Rica, and the resulting full-sib families are evaluated during the following summer.

Selection environments: Two low nitrogen blocks have been developed at the Poza Rica experiment station, one in 1985 and one in 1988. These areas have received only about 40 kg of N ha^{-1} as chemical fertilizer since they were initiated as low N areas. For the first several seasons, all residues were removed, but once a low N level was achieved residues were incorporated. Each block is rested for one season approximately every 3 years, and a green manure crop (preferably one which does not fix N) is sown. Other practices reflect standard station management. Yields of maize grown on these blocks are generally 30-40% of those on high N blocks.

Considerable soil variability was revealed under low N which was not apparent in the high N treatment. This variability was visually related to differences in soil texture which result from the alluvial history of the site. The use of a lattice design with a small block size (10 to 16 rows) greatly improves the experimental efficiency under low N, the improvement in efficiency of the lattice design relative to the RCBD for grain yield varying from 70 to 110%. Large increases in efficiency due to incomplete blocking are also observed for chlorophyll, plant height, ear leaf area, and senescence measurements under low N. In contrast, the relative efficiency for grain yield of incomplete block designs under high N does not exceed 20%.

In 1991, the large continuing soil variability and the observed low heritability of leaf chlorophyll concentration suggested the use of chlorophyll concentration in adjacent plots as a useful covariate. In that season, the efficiency of the lattice design (with 15 rows per incomplete block) was 60% better relative to an RCBD, while the improvement with the covariate analysis was 80%.

b. Examination of existing data: recurrent selection for tolerance to low N in 'Across 8328 BN' and evaluation of gains.

Selection criteria used to select the first three cycles of the population 'Across 8328 BN', which is under improvement for low N environments, were based on the results of a preliminary trial conducted in 1986. Certain traits were found to be closely correlated with grain yield under low N for a group of eighteen cultivars and also for progeny within the population (Lafitte and Edmeades, 1987). These characteristics were combined in a selection index which comprised grain yield and plant height with high and low N, maturity at high N, and leaf chlorophyll, ear leaf area, and leaf senescence at low N.

Experimental varieties (EVs) formed from both C_0 and C_2 of Across 8328 BN were evaluated in four seasons, and the adaptive value of all traits included in the selection index (except leaf chlorophyll) was confirmed (Lafitte and Edmeades, 1994a). Yield differences in the EVs selected for yield alone under low N were comparable to those in the EVs selected for a combination of yield across N levels and secondary traits. Those EVs selected using a combination of yield and secondary traits did, however, tend to show more consistent differences in those traits which are expected to be associated with performance, such as delayed senescence, and were the only EVs which showed an differences in total biomass production.

Performance at each of the two N levels showed no negative association, and selection for yield alone under low N did not result in a decrease in yield potential under high N. In fact, selection for yield under low N alone was at least as successful at identifying families which performed well at high N as was selection for yield across N levels.

Based on the performance of divergently selected experimental varieties, realized heritabilities were slightly greater for yield under low N than for yield under high N, indicating that, at these yield levels, similar progress can be made for yield under low N as for yield at high N. Heritabilities of yield across N levels, chlorophyll concentration, and senescence rate tended to be smaller than those of yield at either N level.

Subsequent analyses of a large number of progeny trials at Poza Rica growing under high and low N has shown that:

a) the broad-sense heritabilities for grain yield are around 20% higher under high N than low N, simply because error variance declines with yield at a slower rate than genetic variance. Note that this contrasts with realized heritability which reflects progress for yield resulting from both primary and secondary trait selection.

b) the efficiency of a lattice design for progeny evaluations is around 20% higher than that of the RCBD under low N

c) If the target environment is a low N environment, then selection in a low N environment is more efficient than selecting in a high N environment and relying on "spillover" of gains into the low N environment. In fact, when yield reductions in the target environment are more than 40% of the high N yield, gains are significantly greater ($P<0.05$) when selection is conducted under low N. This finding has important implications for the rest of the maize breeding program at CIMMYT, given the amount of N-deficient maize that is commonly observed in the lowland tropics.

Evaluation of progress under low N: An evaluation of progress was conducted for the first three cycles of selection in Across 8328 BN. For eight trials, gains of 75 kg ha^{-1} $cycle^{-1}$ and 135 kg ha^{-1} $cycle^{-1}$ were observed under low N and high N. Cycles 0 and 3 of the population Across 8328 BN were evaluated for root growth and for ear growth characteristics to better understand the physiological basis of observed yield differences in earlier evaluations. The root growth of cycle 3 is greater than that of cycle 0 when N is available in the soil. Cycle 3 produces fewer florets per ear, has a longer lag phase after pollination before linear grain growth begins, and a higher rate of grain filling during the linear phase than does cycle 0. These changes in ear growth parameters may be responsible for the increased number of grains per ear in cycle 3 under conditions of N stress.

Additional evaluations have been conducted to assess the relationship between tolerance to drought and tolerance to low N. In two of the drought populations, significant gains were also seen under low N. The low N population showed no improvement under drought.

Results- Evaluations of three cycles of selection in Across 8328 BN were conducted in 1989, 1990, and 1991 at Poza Rica. Cycles 0, 1, 2, and 3 were evaluated under two N levels in four seasons. Detailed reports on these results are contained in Lafitte and Edmeades, 1994a; 1994b; 1994c; 1995. Analysis across seasons revealed a linear increase of 75 kg ha^{-1} $cycle^{-1}$ in grain yield at low N (2.8% $cycle^{-1}$; $P<0.10$) and 137 kg ha^{-1} $cycle^{-1}$ at high N (2.3% $cycle^{-1}$; $P<0.01$). The yield component responsible for this increase at high N was grain number per ear, which increased by 7.8 grains $cycle^{-1}$. Ears per plant also increased by 0.02 ears $cycle^{-1}$ across N levels, but that change was not statistically significant.

Despite attempts to maintain a constant plant height at high N, height increased by 8.4 cm $cycle^{-1}$. Ear height, which was not controlled in the selection process, increased even more than did plant height. Anthesis date also changed significantly, increasing by about 0.3 d $cycle^{-1}$ when averaged across N levels. Silking date averaged across N levels remained unchanged, so the interval between male and female flowering was also reduced by 0.3 d $cycle^{-1}$. The number of florets per ear declined significantly with selection at a rate of 8.2 $cycle^{-1}$ across N levels. That change, along with the increase in grains per ear, resulted in a highly significant linear increase with selection in the percentage of florets which formed grains. Selection for tolerance to drought in a tropical maize population also resulted in a decrease in the number of florets formed per ear (Edmeades et al., 1993), and it was suggested that a reduction in the number of florets allows scarce resources to be divided among fewer grains, resulting in a lower rate of grain abortion.

Selection increased total biomass at maturity by 205 kg ha^{-1} $cycle^{-1}$ across N levels and seasons ($P<0.05$). Selection resulted in an increase in weights of leaves and stems at flowering, and weights of stems and grain at maturity, but in a decline in N concentrations of leaves, stems and grain. Harvest index was unchanged by selection. Total N uptake at maturity differed among entries ($P<0.10$), but the change was not linear with selection. Selection had a highly significant effect on the timing of both N and biomass accumulation. Selection cycles tended to differ in N uptake prior to flowering ($P<0.10$), with later cycles accumulating more N. There was a significant linear increase of 0.14 g m^{-2} $cycle^{-1}$ in the amount of N mobilized from vegetative tissues during grain filling. These results suggest that selection pressure for performance under low N in elite germplasm results in a significant adjustment in the crop's C and N economy, with a resulting improvement in the efficiency with which N is utilized to produce biomass. It appears that selection under low N may be affecting different plant parameters than do traditional selection programs.

When C_0 and C_3 were sown in large pots from which roots could be recovered, root biomass of C_3 was significantly greater than that of C_0 when N was applied at a rate equivalent to 250 kg ha^{-1}. These differences were observed at depths of 0.25-1.0 m when plants were harvested 2 wk after silking. In this study, the low N treatment produced only 30% of the above-ground biomass and 23% of the grain yield per plant compared to the high N treatment, a rather severe level of N stress, and no significant differences were observed in root growth between the cycles of selection at low N.

Ear growth after silking was evaluated in cycles 0 and 3 of Across 8328 BN and cycles 0 and 8 of Tuxpeño Sequía. The number of florets per ear has decreased with selection. The length of the exponential phase of grain growth immediately following pollination was greater in the advanced cycles of selection. This difference more than offset the smaller weight $floret^{-1}$ at silking (though not at anthesis), so the weight $kernel^{-1}$ was considerably greater in the advanced cycles of selection at the end of the lag phase, which is the critical period when kernel biomass strongly influences kernel abortion. Improvement under conditions of abiotic stress has resulted in a greater number of grains ear^{-1}, not only through a reduction in the number of florets ear^{-1}, but also in the development of a longer lag phase following pollination. The rate of change in the number of florets ear^{-1} with selection has been greater in Tuxpeño Sequía than in Across 8328 BN (Bolaños and Edmeades, 1993; Lafitte and Edmeades, 1995), and the increase in the length of the lag phase has been more rapid in Across 8328 BN. Neither of these mechanisms could be exploited indefinitely, since they would both eventually restrict yield potential. It is possible that the delay in senescence and increased mobilization of N from vegetative tissues to the grain which has occurred with selection (Lafitte and Edmeades, 1995) allowed grain filling to continue in C_3. These traits result in more kernels ear^{-1} and a higher success rate among fertilized florets in both high and low N environments. In the case of the population selected for tolerance to drought, greater weight per floret in the period after silking resulted in more ears per plant in the high N experiment, and an improvement in all yield components in the low N environment.

Based on these results, modified selection criteria were developed, and selection in Across 8328 BN was resumed in 1991. Chlorophyll content has been removed from the selection index because of its low heritability, and increased pressure is being applied to reduce plant and especially ear height. Scores of husk color have been included to extend grain-filling duration, while increased emphasis is placed on the maintenance of flowering date. A total of 8 cycles of recurrent selection have been completed, the last two being among S_1 families rather than FS families. This has permitted the easy extraction of inbred lines from this population (see below).

A new technique for increasing the level of N stress early in the crop cycle continues to be refined. It consists of intercropping maize with wheat in the winter and with sorghum in the summer. This year almost all maize grown under low N was intercropped with wheat (in winter) or sorghum (summer). This provides a uniform N stress caused by the N which the secondary crop takes up. Providing the establishment is even, the secondary crop provides a uniform degree of competition for N which is available early in the crop's life, before maize canopy closure occurs. If the season is unusually cold, the wheat provides too great a level of competition; if the plant density of sorghum is too high, the same thing occurs. At present the intercropping technique provided a level of N stress not attainable by normal methods in our nurseries.

c. Drought and low N interactions

Results of the trial to evaluate ear growth showed that improvement for drought tolerance in Tuxpeño Sequía resulted in excellent performance under low N. In 1992B, we started to examine a broader spectrum of drought tolerant materials under low N. In 1992B, N stress was incomplete, resulting in high grain yields. Results (Table 5.6) indicate that we made considerable progress under low N while selecting for drought tolerance in at least two populations. In that evaluation, the advanced cycles of Tuxpeño Sequía and Pool 26 Sequía outyielded C_0 by 15% and 13%, respectively (Table 5.6). Gains in yield under low N due to selection for drought tolerance averaged 86 kg ha^{-1} yr^{-1}. Populations selected for performance under low N was also planted under drought, but did not demonstrate any improvement under that stress.

Table 5.6. Grain yields (oven-dry basis) and gains from selection in four tropical maize populations improved for 2-8 selection cycles under mid-season drought stress. Selection cycles were evaluated in five experiments differing in N supply at Poza Rica, México, between 1992 and 1994. C_0 represents the original selection cycle, and C_n represents a population improved for n cycles under mid-season drought stress.

Entry	Grain yield	Gain cycle^{-1}	Gain year^{-1}
	---------- t ha^{-1} ----------		
Tuxpeño Sequía C_0†	4.31		
Tuxpeño Sequía C_8†	4.96	0.081	0.081
La Posta Sequía C_0‡	4.85		
La Posta Sequía C_3‡	5.41	0.187	0.093
Pool 26 Sequía C_0‡	4.74		
Pool 26 Sequía C_3‡	5.34	0.199	0.099
Pool 18 Sequía C_0‡	3.85		
Pool 18 Sequía C_2‡	4.14	0.143	0.072
Mean	4.70		0.086
LSD $_{(0.05)}$	0.68		
P(Population)	*		
P(Cycle)	***		
P(Pop.*Cycle)	ns		

*, *** indicates significance at $P < 0.05$ and 0.001; ns indicates differences not significant at $P < 0.10$.

† Full-sib recurrent selection scheme: one cycle requires one year to complete.

‡ S_1 recurrent selection scheme: one cycle requires two years to complete

Further initiatives in improving methodology

a. Lattice and spatial analyses: Throughout our selection and evaluation for drought tolerance and for low N conditions, we have been using alpha 0,1 lattice designs to improve our ability to identify superior progeny. Improvements in efficiency over randomized complete block (RCB) designs are frequently in the range of 20 to 70% for many variables. Covariance analysis has also been valuable in interpreting the results in highly variable low N blocks.

Spatial designs, are also being evaluated. These designs are similar in layout to row and column designs, but can be used to remove error even more efficiently than lattice designs. The technique is a little restrictive in that the experiments need to be laid out in a rectangular form, and that there may be as many as 20 different models to apply to the data. However, in analyses of several drought and international testing trials, the efficiency of the best model of the data was from 40 to 150% more efficient than an RCB. The best data model (2-dimensional auto-regressive) resulted in an improvement in efficiency of almost 130%.

Clustering and AMMI analysis of genotypes and environments: Cluster analysis and AMMI (Additive Main effects and Multiplicative Interaction) analysis were used to analyze the relative performance of 15 genotypes that included different cycles of selection for drought tolerance in the populations and non-drought tolerant checks. Yields ranged from 1.0 to 10.4 t/ha (see above). Analysis of variance of the GxE means matrix across environments showed that GxE interaction sums of squares was almost 3 times that of the contribution of genotype. Only 43% of the GxE interaction was accounted for by linear regression, the rest being in deviations from the regression.

A clustering procedure was used to identify the basis of the GxE interaction. After clustering to the 5 group level we found that the check materials, the earlier drought tolerant materials and the later drought tolerant materials separated from the rest (Chapman et al., 1996). Environments can also be grouped on the basis of genotype performance and resulted in the separation of different types of droughts, and of medium and high yielding environments, and we believe that this type of analysis will be very useful when examining results from the drought testing network. Principal components of the G x E interaction were also examined using the AMMI model. We found that drought and irrigated environments had components that differed in sign, and that it was therefore difficult to select in either of these types of environments and make gains in the other (Chapman et al., 1996). Two of the checks were lower yielding than later cycles of selection for drought tolerance, but were relatively stable. However, another check, a conventionally selected version of La Posta, Population 43, had the highest interaction with the irrigated environments, was directly opposite the droughted environments and had a large negative interaction with those.

Other research

An examination of whether heterosis is a source of stress tolerance: 81 entries, comprising: hybrids; stress-tolerant OPVs; non-stress-tolerant OPVs; OPV x hybrid crosses; stress-tolerant OPVs x non-stress-tolerant OPVs, were grown under several drought regimes. Under drought the 7 highest yielders under drought were all hybrids, varietal crosses or topcrosses. Especially impressive was the varietal cross La Posta Sequía C_3 x Pop. 32. These were followed by TS6 and DTP2, and an outstanding hybrid from Asia, DK888. This hybrid was the highest yielding under well-watered conditions by 1.42 t/ha, (LSD = 1.22 t/ha) and was highly prolific (1.94 EPP). An insect tolerant population, FAW GCA, performed well under drought, and would be a good candidate for introducing insect tolerance into drought materials. Some materials with reputed drought tolerance, such as PNR 473, DTP1 C6 and Pool 26 Sequía C3 were only average under the rather severe conditions of drought imposed at Tlaltizapan. Prolific populations SPE C6 and SPL C6, did not perform well *per se* or in crosses under drought, though the performance of SPL under well-watered conditions was quite reasonable.

Anti-gibberellin seed treatment: A collaborative project with staff of the University of Guelph examined the role of antigibberellin seed treatments (triazoles Paclobutrazol and Ancymidol) applied as an imbibed seed treatment or as topically applied, in determining early seedling growth and survival under drought. Results suggest that while early extension growth and leaf expansion was reduced by triazoles, survival is also reduced and final grain yield was also reduced. We cannot recommend the use of triazoles to improve seedling survival under drought at this stage.

N-fixation in maize: The effectiveness of *Azospirillium* inoculation (from Argentina via Ghana) and inoculation by two strains of *Rhizobium* (from U. of Nottingham, UK) were examined in several genotypes in separate trials under low and high N. This treatment of a free-living N-fixing bacteria was proposed as an adjunct to gains from selection under low N conditions. Yields were generally low, and showed no significant effects of treatments. Treatments with these organisms cannot be recommended at this stage.

Screening of advanced breeding materials from other breeding programs: A number of lines from the main breeding programs have been evaluated under drought and under low N from 1994 through 1996. We do this to determine if there are sources of drought and low N tolerance in elite germplasm products coming from conventional breeding programs. We expect that there will be a low frequency of stress tolerance genes in elite germplasm, since the majority of our stress-tolerant populations were normal elite populations 10-15 years ago. A well, we have conducted several Preliminary Evaluation Trials (PETs) of elite new OPVs from all parts of CIMMYT's program, under low N and drought, and have discovered a number of new sources of stress tolerance in the process. In the past few years we have evaluated around 1,000 such lines under drought, ranging from S_1 to S_9, all arising from headquarters or outreach breeding programs. These also include highland germplasm. As well, we are assisting in managing the droughts tress on several other evaluations of lines and topcrosses for the Lowland Tropical and Subtropical Programs. A total of 169 hybrids from the lowland tropical program were evaluated under low N in 1995.

Transfer of Stress-tolerant germplasm to the stress breeding subprogram:

Tropical Abiotic Stress Tolerant Germplasm: For tolerance to low soil nitrogen, 100 S_3 lines derived from Across 8328 BN C_5 were passed to the stress breeding unit from the physiology subprogram in late 1994. Based on combining ability evaluations and the *per se* performance of the lines in our nurseries, we now have selected 17 S_6 lines. These lines are being further evaluated and crossed to additional elite tropical tester lines. We also have formed a synthetic population using 11 of the superior S_5 lines. Recently, a new set of S_3-S_4 lines derived from Across 8328 BN were received by our unit from the physiology subprogram. Similarly, we are beginning both *per se* and combining ability evaluations with these lines.

Early generation tropical lines derived from two semiprolific white-grained populations developed as a source of general abiotic stress tolerance have been evaluated and advanced in 1995. Approximately 200 S_3 lines from Population SPE (Semi-prolific early) and 101 S_2 lines from SPL (Semi-prolific Late) were received from the Physiology subprogram in early 1995 and evaluated *per se*, and top-crossed to two elite tropical tester lines. The hybrids formed were evaluated in 3 non-stressed locations including one location with the Mexican national program. Hybrid combinations exceeding the yield of the hybrid check by 15-25% have been identified with lines from both SPE and SPL (Table 5.6). We currently have 22 S_7 lines from SPE and 59 S_6 lines from SPL that are being studied and advanced further. Superior materials are being evaluated under drought stress as lines and in hybrid combinations, while the lines are being advanced in our nurseries and top-crossed in new hybrid combinations. Two synthetic populations have been formed using 15 superior lines from SPE and 11 lines from SPL.

Table 5.6 Performance of testcrosses of SPL (Semi-prolific late) C7 S4 lines evaluated over four Mexican locations.

		Yield	Male Flower.	Ear Ht.	Root Lod.	Stalk Lod.	Moisture
Entry	Pedigree	(t/ha)	(days)	(cm.)	(%)	(%)	(%)
55	SPLC7F254-1-2-3-2/CML258	10.2	62.3	130.1	4.3	1.9	24.1
1	SPLC7F254-1-2-3-2/CML 254	9.7	62.6	141.2	11.5	0.7	22.2
73	SPLC7F210-2-3/CML 254	9.6	64.2	152.5	18.8	1.1	24.2
38	SPLC7F254-1-2-3-2/CML264	9.5	62.7	121.1	13.6	0.0	23.3
20	SPLC7F254-1-2-3-2/CML273	9.1	61.8	132.9	15.5	0.5	21.1
82	CHECK1 CML247xCML254	7.9	64.7	132.9	11.5	1.7	26.7
83	CHECK2 CL-G2409xCML48	8.6	64.4	148.9	28.6	1.7	22.4
84	CHECK3 CML61xCML62	7.6	63.3	149.3	42.8	1.9	23.1
	Mean	7.8	63.0	132.6	18.0	1.6	23.1

Various inbred lines developed as sources of drought tolerance in collaboration with the physiology subprogram have been evaluated and advanced during 1995 and 1996. Promising tropical late-maturity white-grained lines have been identified derived from the populations La Posta Sequía C_3 (LPS) and TS6 C_1. Based on *per se* and top-cross evaluations of approximately 100 S_3 lines from each of these populations under normal conditions in 1994 and under drought-stress conditions in early 1995, we have identified a smaller set of promising lines for advance and further evaluation. Twenty-nine S_5-S_6 lines from LPS and 25 S_5-S_6 lines from TS6 have been selected based on their *per se* and top-cross performance in combination with elite tropical single-cross and inbred lines. During the 1995-1996 period, 24 hybrid trials with these materials were conducted in 20 environments including CIMMYT experiment stations and sites in collaboration with the Mexican national program and private seed companies. Additionally, many of these lines are being tested widely in hybrid combination as a part of the drought tolerance network.

Ten superior lines from La Posta Sequía and TS6, along with 7 elite lines from the tropical subprogram are being used in a diallel study as part of a post-doctoral project. Lines and hybrids used in this study have been evaluated in 10 environments including drought stressed, low N stressed and non-stressed conditions. This project has the objectives to: 1) study the genetic control and modes of action for drought tolerance; 2) examine dosage rate effects for drought tolerance in hybrids as well as the role of hybrid vigor in their expression; 3) estimate the relationship between line *per se* and hybrid performance under drought stress and normal conditions; and 4) to identify single crosses for developing second cycle lines that combine several stress tolerance/resistance traits and good agronomic performance. Preliminary findings from this on-going project show that the mode of drought tolerance is largely additive and that dosage effects are important in developing drought tolerant hybrids. We have found that the relative importance of general combining ability effects vs. specific combining ability effects increased as stress level intensified. Several lines, particularly from La Posta Sequía, have demonstrated excellent *per se* and combining ability performance under both drought stressed and non-stressed conditions. Results are shown of the effects of crosses between insect resistant lines and susceptible lines on leaf feeding and relative grain yields (Fig. 5.2), and between tolerant and intolerant lines for grain yield under drought (Fig. 5. 3).

Fig. 5.2 Comparison of crosses among insect resistant (IR) and non-insect-resistant (NIR) inbred lines for leaf feeding damage (1-9 scale, where 1 is more resistant) and grain yield (t/ha) under sugarcane borer infestation in season 96A (SCB96A, GYRED96A) and under south western corn borer infestation in season 96A (SWCB96A, GYRED96A) and in season 1996B (SWCB96B, GYRED96B)

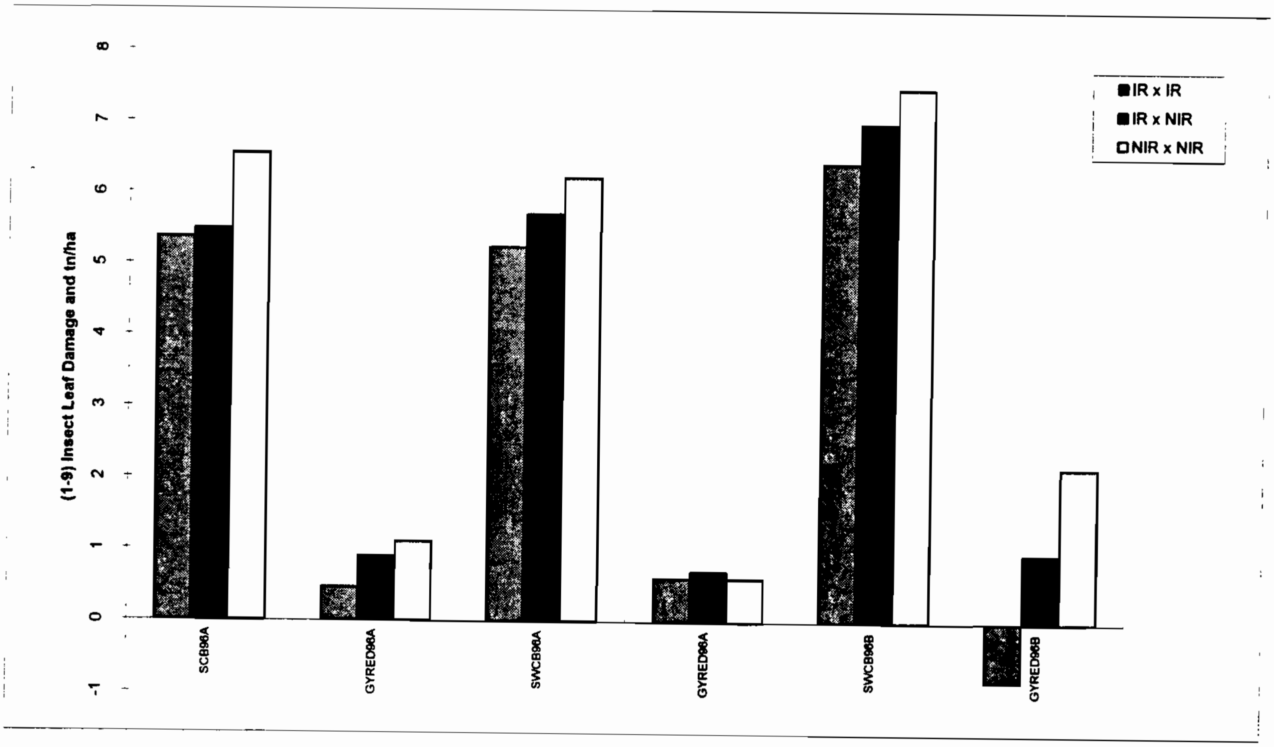
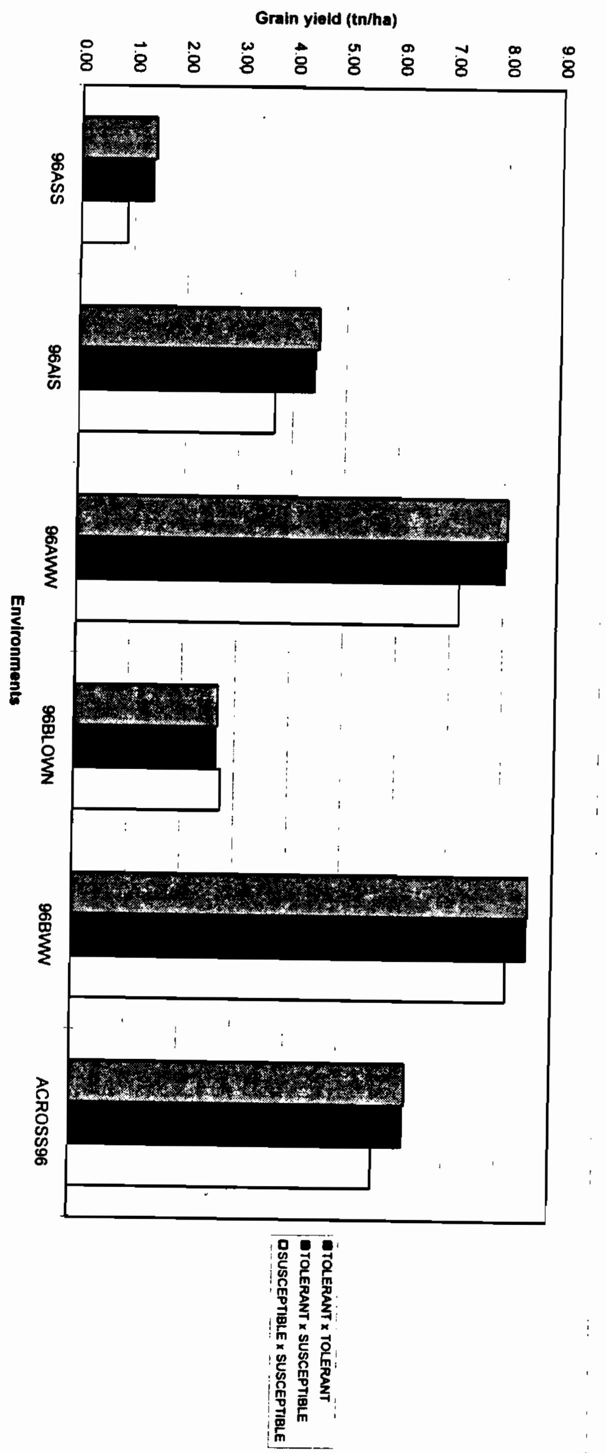


Fig. 5.3 Comparison of grain yield of crosses among drought-tolerant and drought-susceptible lines under well-watered conditions in season 96A (96AWW) and 96B (96BWW), under severe drought stress in season 96A (96ASS), under intermediate drought stress in season 1996A (96ASS), under intermediate drought stress in season 96A (96AIS), under low N stress in season 96B (96BLOWN), and across environments (ACROSS96).



Two synthetic populations utilizing 18 lines from La Posta Sequía and 11 lines from TS6 have been developed. After extensive testing, we now plan to release 5 La Posta Sequía lines and 1 TS6 line in February, 1997 as CMLs (CIMMYT Maize inbred Lines) (Table 3.5). We expect that there will be a tremendous interest world-wide in these drought tolerant lines with proven general combining ability.

Approximately 145 S_3-S_4 yellow-grained lines derived from Pool 26 Sequía C_3 were passed from the Physiology program to the stress breeding unit in 1995. Based on extensive top-cross and *per se* evaluations we have selected 36 S_7-S_8 lines for further evaluations. We plan to release two of the best lines during 1997 as CMLs (Table 3.6); a synthetic population using 10 superior S_5-S_6 lines has been formed. Recently, a new set of S_3 -S_4 lines derived from Pool 26 Sequía were received by our unit from the Physiology subprogram, and we are beginning both *per se* and combining ability evaluations with these lines.

Early maturing drought tolerant inbreds advanced in our nurseries include 176 S_5-S_9 lines derived from Pool 18 Sequía (yellow-grained) and 71 S_4-S_6 lines from Pool 16 Sequía (white-grained). Both *per se* and combining ability evaluations of these materials under both drought and non-stressed conditions are on-going. We hope to release a superior set of these lines in 1998.

<u>Subtropical Abiotic Stress Tolerant Germplasm:</u> Early generation subtropical lines derived from the population Semi-Prolific Mid-Altitude Tropical (SPMAT), developed as a source of general abiotic stress tolerance were evaluated and advanced in our nursery. We started with 102 S_3 white-grained and 102 S_3 yellow-grained lines in early 1995. The lines were advanced by selfing, top-crossed to elite tester lines from the subtropical program, and then evaluated in multi-location trials. Two superior fractions consisting of 25 white-grained and 20 yellow-grained S_7 lines have been selected based on both *per se* and combining ability evaluations. Currently, the selected lines and hybrids formed from them are being re-evaluated under both normal and drought stressed conditions. Additionally, the lines are being further advanced and crossed in new hybrid combinations.

For tolerance to low soil nitrogen, 100 S_3 lines derived from Across 8328 BN C_5 were passed to the stress breeding unit from the physiology subprogram in late 1994. Based on previous combining ability evaluations and the *per se* performance of the lines in our nurseries, we selected 21 S_5 lines. These lines have been further advanced and crossed to various elite tropical tester lines, but this population is proving a difficult material from which to derive inbred lines. We also are forming a synthetic population using 12 of the superior S_5 lines.

A significant accomplishment by the Stress Breeder during 1995 was the establishment of an inbred line database using Microsoft EXCEL software, and data from 1996 and subsequently will be consolidated in this database. We are confident that the stress-tolerant inbred lines identified as described from their performance in hybrid combination under stress, and the information available from this database will provide the breakthrough in the use of stress-tolerant tropical maize germplasm that is needed in the next 5 years.

Chapter 6: Task 5: Development of new germplasm sources for tolerance to drought and low N

Task 5: To develop new germplasm in broad genetic backgrounds as future sources of tolerance to drought or low N. This germplasm will serve to broaden the genetic base of stress tolerance

Output 1

Broad-based populations with tolerance to drought and to low soil N, developed through the following activities:

- Obtain potential sources of drought (or low N) tolerance from germplasm bank, from collaborators throughout the world, and test under stress conditions in Mexico.

- Include in stress tolerant populations components which show differing mechanisms for attaining tolerance and good general performance.

- Following genetic mixing, generate progeny, prescreen in Mexico, and test superior fraction at carefully selected international sites.

- Continue to test genotypes contributed by cooperating national programs; where found superior, include in stress tolerant populations.

I. Drought

a. Rationale for the formation of source populations

A perceived lack of variation for certain drought- or low N-adaptive traits within the elite populations under improvement for tolerance to drought or low N has led to a longer term strategy: the identification of donors of additional adaptive characteristics which would increase production under these stresses at no great cost to performance under well-watered conditions. It was considered necessary to combine these sources into one or two populations so that the unique genetic characteristics of each source material could be exchanged during the recombination phase of population formation. This population could then be used as a vehicle for the transfer of this *reservoir* of variation to national programs. At the outset it was considered unlikely that such a population would be used for direct release because of the virtually impossible task of delivering in final product form an array of traits which a specific environment may require. This is particularly true for disease resistance needed at specific sites. Specific grain texture and color requirements would require that we establish at least a white and yellow population, and perhaps a flint and dent version as well. This was beyond our resources.

In 1986 we formed a single population, DTP1, of mixed color and texture and intermediate in maturity. In order to reduce bias from continuous selection at Tlaltizapán in the dry winter cycle, we have sent S1 progenies of DTP1 to interested collaborators in national programs for screening under the drought conditions they normally encounter (Edmeades et al., 1991).

Characters for which we hope to find additional genetic variability include:

1) Establishment: We are now seeking to determine if there are heritable differences for this characteristic in DTP1 (see Chapter 5).

2) Water use efficiency (WUE) (Reported by Bänziger et al., 1997)

3) Delayed senescence under drought.

4) Osmotic adjustment (limited variation in any maize population - see Bolaños et al., 1991).

5) Grain yield under moderate to severe drought stress.

b. Identification of source materials

We have screened about 200 potential sources of drought tolerance (elite, landrace, hybrid and OPV) under drought and well-watered conditions at Tlaltizapán. These sources included:

1) Landrace collections in the Germplasm Bank

2) Elite selections from other programs (especially those in southern Africa, Thailand, and USA) with reputed drought tolerance

3) Elite selections from CIMMYT's own program with known drought tolerance

Many of the materials screened and the criteria used in their selection are detailed in the Maize Physiology Germplasm Catalog (Edmeades et al., 1995).

c. Description of drought tolerant populations

Sources of germplasm that have shown adaptation to drought in Tlaltizapán and good performance under drought at other locations have been recombined to form the first drought tolerant population or DTP1. This comprises 13 components, and the final percentage of parentage by ecology is:

Lowland tropical	60.1%
Subtropical	21.6%
Temperate	18.3%

By 1991, a sufficient number of new source germplasm with drought-adaptive traits had been identified to allow a major introgression of new material into DTP1. This was achieved by crossing the new and proven source materials (on basis of *per se* performance and on the basis of the performance of the cross between the source and DTP1, evaluated under drought) with the latest version of DTP1, and an elite set of S1 families identified from those under test internationally (but based on Tlaltizapán performance). The new population was named DTP-2 (for details of the components of this materials see Edmeades et al., 1995; Edmeades et al., 1997).

Combining the adaptation of DTP1, which contributed 58% of the germplasm to DTP2, gives the following adaptation breakdown for DTP2:

Lowland tropical	65%
Subtropical	15%
Temperate	20%

We anticipate that the adaptation of the combined populations, DTPW and DTPY will both be around 60% lowland tropical, 20% subtropical and 20% temperate, suggesting that much selection for disease resistance will be needed before useful inbred lines, EVs and synthetics can be derived from either.

Each population has been improved using an initial series of 3-4 half-sib selection cycles to ensure adequate mixing of diverse components, followed by a two-stage S_1 recurrent selection scheme which improves the base population but also identifies superior S_1 families that can form the bases of superior inbred lines. All versions of the DTP populations are generally intermediate in maturity, though an early selection has also performed well in international trials (Table 6.1).

Data from 16 S_1 Progeny Trials (225 entries) of DTP1 were analyzed in 1994 in connection with the second round of testing of S1 progenies and the results from these trials were published in the second network report (Edmeades et al., 1995), Results from the late maturing international variety trial showed that the new drought population, DTP2 yielded 4th out of 16 across 19 sites (Table 6.2). A selected fraction of this population should perform well in trials in the future. Site-specific selections of the older drought population, DTP1, in several cases outyielded TS6. The recombination was of the best S_2 families from each S_1 family under test, divided for color (see below)

Table 6.1. Across-site means of various characteristics observed in 5 early maturing drought tolerant maize genotypes, 2 references genotypes (RE), and 2 local check genotypes. The trial had 12 entries in total.

	Grain yield at sites with stress level:				
	All sites	Little	Moderate	Severe	Anthesis date
	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	d
DTP1 C_5 Early Sel.	2.63	4.63	2.70	0.89	59.4
Pool 18 Sequía C_3	2.53	4.10	2.67	0.99	54.5
Local Check 1	2.50	4.18	2.38	0.94	58.3
Pool 16 C_{20} Syn. 1	2.47	4.05	2.58	1.03	54.2
TEYF Syn. 2	2.31	3.65	2.59	0.86	53.7
Sta. Rosa 8330 RE	2.29	4.18	1.90	1.00	58.7
TEWF Syn. 2	2.27	3.89	2.37	0.79	55.2
Across 8331 RE	2.20	4.12	1.90	0.83	58.5
Local Check 2	2.17	4.09	1.82	0.69	58.3
Mean	2.38	4.11	2.36	0.87	56.8
Number of sites	21	6	7	7	21
LSD (0.05)	0.28	0.51	0.48	0.40	1.8

Table 6.2. Across-site means of various characteristics observed in 7 late-maturing drought tolerant maize genotypes, 2 references genotypes (RE), and 3 local check genotypes. The trial had 20 entries in total.

	Grain yield at sites with stress level:				
	All sites	Little	Moderate	Severe	Anthesis date
	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	d
La Posta Seq. C_3	3.58	6.06	2.95	0.99	64.5
Local check #2	3.56	5.68	3.36	0.88	63.6
Across 8627 RE	3.42	5.53	2.91	1.17	62.8
Ngabu 89DTP1	3.36	5.48	2.86	1.08	61.6
DTP2 C_4	3.35	5.57	2.75	1.08	60.5
Local check #1	3.34	5.23	3.06	1.08	62.8
S. Lagoas 89DTP1	3.34	5.56	2.70	1.13	61.4
TS6 C_1	3.31	5.57	2.99	0.58	64.2
Local check #3	3.25	5.36	2.80	0.92	62.4
F. Ba 8625 RE	3.20	5.51	2.48	0.96	61.4
Pool 26 Seq. C_3	2.93	4.61	2.36	1.37	58.4
Mean	3.30	5.39	2.84	1.01	61.8
Number of sites	19	7	7	5	19
LSD (0.05)	0.32	0.58	0.55	0.39	1.36

Division of DTP1 and DTP2 into two color classes: S_1 families of DTP1 C_6 were advanced to S_2 and separated into yellow and white families, which were screened under drought and heat in Obregon and for general performance and disease reaction in Poza Rica. Based on data of the original S_1 family in international testing, the best S_2 from each selected S_1 was used to recombine DTP1 to form DTP1-Y C_7 or DTP1-W C_7 in early 1995, i.e., the population was split into color classes. In mid- 1995 a new set of 200 S_1 families of C_7 was created in each of these two color classes. At the same time, recurrent improvement of DTP2 continued in Mexico; in early 1995 new sets of 400-500 S_1 families were formed in DTP2-W C_5 and in DTP2-Y C_5. These were screened in Obregon in 1995 and the best 200 identified. At present the 200 S_1 families from each population and color class are being compared under two levels of drought at Tlaltizapan in a replicated trial. On the basis of data collected from these trials the best whites from each and the best yellows from each will be recombined to form the combined populations, DTP-Y C_8 and DTP-W C_8 in 1996.

It seems unlikely that DTPW or DTPY will be improved for drought tolerance by evaluating S_1 progenies in international tests again because of the very long time taken to complete a cycle and because of obvious defects in disease reaction. Rather improvement for drought will be mainly done in Mexico, and the population will be sent to regional centers in the major ecologies (e.g., Thailand, Zimbabwe, India, China) as 300-400 S_1 or S_2 families in an unreplicated observation nursery. Cooperators at those regional centers will advance lines that appear suited to those environments from remnant seed provided with the unreplicated observation trial, and selection will start immediately for regional adaptation and for GCA using adapted testers. Lines have already been sent to Zimbabwe with this procedure in mind.

Evaluation of germplasm sources which could be used to augment drought and disease resistances of Drought Tolerant Populations : In early 1995 we examined 19 sources, primarily drought tolerant materials from Bolivia (supplied by a network collaborator), or crosses of downy mildew resistant sources with DTP2 C_4

These were evaluated under two levels of stress in early 1995 and the data will be used to augment the performance of either DTP-Y or DTP-W once it has been demonstrated that the sources will actually improve the performance of these DTP populations.

In addition, we formed and improved the *Latente* subpopulation. This population is composed of germplasm reputedly carrying the *latente* complex of drought-tolerant characters (that is, an ability to defer developmental events under severe stress and an enhanced capacity to recover from severe drought stress at the vegetative stage. The trait was originally observed in a collection from 1900 masl here in Mexico, namely Michoacan 21). The *latente* population consists mainly of Corn Belt x selections from Michoaan 21 from DeKalb Genetics and from several other sources, and we are now attempting to tropicalize it. During 1995 we grew C_0 in a half sib recombination block (150 families) using a balanced composite of all females as the male pollinator. In mid-1995 an attempt was made to increase the disease resistance of these highly susceptible materials by using a disease-resistant group of S_3 families derived from DTP1-Y C_6 as the pollinator in a half sib block (again 150 families), while at the same time screening all female families under disease pressure in Poza Rica. At present the population bulk (Pool *Latente* C_2) is being examined as a check entry in a number of trials under drought, and performs well where there is very little pressure for foliar and ear diseases.

II. Nitrogen

a. Rationale for the formation of source populations

It might be argued that improved materials could have lost certain adaptive traits for performance on low fertility soils which were present in the landrace materials from which they were derived. In our search for sources of germplasm which perform well under conditions of low N fertilization, we turned to the germplasm bank to examine the performance of accessions which had not undergone much selection under high fertility conditions. Our objectives were twofold: 1. to contrast the strategies of unimproved and improved maize under conditions of low soil N, and 2. to identify accessions to form populations adapted specifically to low N environments. These populations represent a long-term approach to the problem of low soil fertility.

b. Evaluation of bank accessions under different levels of soil nitrogen

A total of 209 bank entries have been evaluated under high and low N. The low N treatment received no fertilizer N while the high N plots received the normal station application of 200 kg N as ammonium sulfate. The evaluations were made over a period of 3 seasons (1987-1988), and a variety of criteria were used to select the accessions:

Year 1 - Entries were selected on the basis of race: entries represented races of maize which are common to low fertility soil groups in Mexico and Central America, and the accessions were collected at elevations below 1000 masl.

Year 2 - Materials of Caribbean origin (where soils are typically infertile) were evaluated.

Year 3 - Entries were selected on the basis a low fertility rating in the collector's notes.

It should be noted that these accessions, while classified as landraces for the purpose of this study, could all have been improved to some extent prior to their collection. In one trial, improved materials were included as well. In these trials characters measured included: plant and ear height, ear leaf area, flowering dates, ear leaf chlorophyll at flowering, grain yield, biomass yield, and Kjeldahl N concentrations in the stover, cob, and grain.

Contrasting behavior of improved and landrace genotypes -- Grain yields of improved entries tended to be greater than the yields of bank accessions under both N levels. Landrace entries tended to have a lower harvest index, but the harvest index of bank materials was less affected by N stress than was that of improved materials, and the nitrogen harvest index of bank materials actually increased with N stress while it decreased in improved entries.

When fertilizer N was applied, the improved materials accumulated considerably more N in the grain than did the bank entries. At low N, however, N partitioning within the plant was similar for the two germplasm groups. The greater dry matter yields in the improved materials under low N was associated with a greater dilution of the grain N, that is, from a lower N concentration in the grain.

With N stress, the leaf area of bank entries was reduced more than in improved materials, but leaf chlorophyll was maintained at higher concentration. This finding suggests that the advantage of the improved materials lay in the production of leaf area of a lower N content, rather than in an improved ability to absorb N from the environment. In summary, it appears that landrace materials show potentially useful genetic variation for:

- High N uptake under N stress
- Maintenance of leaf chlorophyll concentration -N
- Maintenance or increase in HI and NHI with N stress
- Maintenance of grain N concentration with N stress

Negative correlations among these traits were not observed.

c. Formation of populations with specific adaptation to low N environments

Landrace entries were selected independently in each of the three evaluations, and were combined to form an early population (Pool BN Precoz, 32 accessions) and a late population (Pool BN Tardio, 22 accessions). The populations have undergone five cycles of half-sib mixing under low N with mild selection to improve agronomic characters in the last two cycles. This has been followed by two more cycles of recurrent S1 selection. It was obvious during these last two selection cycles that these populations had virtually no history of self pollination, and both lack tolerance to this. These low N populations are tall and suffer from extreme lodging under high N, such that they show little positive response to N application. In their target low N environment, however, their height is not excessive and their yields are fairly good. During their development, however, they have improved in performance markedly, but even so, it seems unlikely that they will provide useful germplasm for infertile conditions without a great deal more work. At present it seems probable that the best sources of tolerance to low N will be found among elite germplasm.

Advanced line development from source populations: Approximately 36 S_3 white-grained lines and 50 yellow-grained lines developed from the Drought Tolerant Population 1 (DTP1) were passed to the Stress Breeding Unit from the physiology subprogram in 1995. All selected lines have been advanced to the S_5 level. The white fraction has been crossed to two elite subtropical tester lines, and evaluated in 4 environments under drought stressed and fully irrigated conditions. Twenty-two white lines have been selected for further evaluation both *per se* and in new hybrid combinations under drought stressed and normal conditions. Top-cross hybrids from the yellow lines in combination with elite subtropical testers have been formed and will be evaluated in 1996.

The broad-based drought-tolerant populations DTP1 and DTP2 represent approximately 25% of the seed requests for stress-tolerant germplasm by national programs through the Physiology sub-Program. They are especially interested in receiving them as inbred families (S_1's or inbred lines) of a specific grain color, and the demand for these is increasing rapidly. Population bulks continue to remain popular as well.

Chapter 7: Task 6: Networking for testing and development of germplasm tolerant to drought and low N

Task 6: To establish and coordinate a network of cooperators at selected national program sites. The network would foster the exchange of germplasm, techniques and information.

Output 1

Two networks, comprising source developers and source testers. Networks will....

- Establish contact with interested national staff through CIMMYT outreach staff; determine source testers and source developers.

- Test and distribute germplasm

- Bring visiting scientists to Mexico for three months of training in methodology

- Hold workshop on drought and low N in Mexico in 1994

Activities: *The Maize Drought and Low N Tolerance Network.*

Other national program-CIMMYT networks

(a) Soil acidity

(b) Regional breeding (Asia, SE Africa, W Africa)

(c) Regional agronomy (C. America, S Africa, E Africa)

(d) The biggest: testing of normal germplasm (>90 countries)

Reasons for the existence of the Drought and low N Tolerance Networks

National program awareness: the basis for these Networks is the sharing of germplasm and methodology among concerned national programs, and with CIMMYT. This will ensure that national programs are fully aware of stress tolerant germplasm being developed, and they will be in a position to utilize it immediately, if they so desire. The networks provide a vehicle by which information and methodology can also be shared. This will be through exchange of technical reports, through the distribution of the Drought Network Report or the Low N Network Report after each round of testing, and through training courses.

National program involvement: By using the network as a means for testing and developing drought or low N tolerant germplasm, national program scientists will have an important stake in the development process. The germplasm so developed will be theirs to use as they think fit. As well, cooperators can contribute germplasm, selection information and general guidance to the network. For example, the 1995 trials that were sent out to Drought Network participants included a hybrid supplied by INIFAP (H-431) and a number of site-specific synthetics developed from DTP1 and based on site-specific performance data.

Broad adaptation of germplasm under development: During the development of the population Tuxpeño Sequía it was apparent that some of the gains in "drought tolerance" (perhaps 15% of the total gains) were due to improved adaptation of this germplasm to the Tlaltizapán site (Byrne et al., 1995). This highlighted the need to develop and test drought tolerant germplasm at a number of sites in our cooperating countries.

Composition of the Maize Drought or Low N Tolerance Networks

The Networks are made up of individuals in national programs (public and private) who share an interest in developing drought tolerant maize germplasm. We recognize that there are two groups of network cooperators: a larger group who are primarily interested in testing synthetics and varieties being developed within the network. Generally they will conduct such tests in the normal rainy season, and will contribute locally adapted drought tolerant varieties for testing across a wider range of sites by other collaborators. We refer to these as **testing cooperators**.

A second, much smaller group of cooperators will concentrate on the development of drought tolerant germplasm through a recurrent testing scheme of S1 (or full-sib) families or topcross progenies and hybrids under conditions of carefully controlled drought. The development of population(s) and the identification of broadly adapted inbred lines will be coordinated from CIMMYT with national program help, and improved populations and F2 populations formed from recycled inbred lines will serve as the repository for genes associated with drought tolerance. We refer to this group as **development cooperators**, and there are only 2-3 national program scientists who fall in this class in each network.

We believe that over time cooperators in the network have become distinguished by:

1) Interest in the problem of drought tolerance or low N tolerance in maize, and a national responsibility to breed for areas affected by these stresses.

2) Access to good testing sites. Such sites should have deep uniform soils typical of the region in which drought occurs, and should have a high probability of a reliable dry period occurring in a growing season which is either the normal maize season or a dry season not very different in temperature regime to the normal growing season; or should have a well-managed and uniformly low level of indigenous soil N.

3) For **drought development cooperators** the capacity to supply supplemental irrigation at the site is important for drought management. The source of water should be reliable all year around, good quality sprinkler/gravity irrigation systems should be available, and drainage of excess water should be uniform and rapid. Sites relying on furrow irrigation should be well-leveled. If possible the site should be close to a meteorological station so that daily rainfall, wind run, temperatures and pan evaporation data can be reported. It is the physical conditions of such sites that have basically limited the development of a larger group of development cooperators.

4) For **all cooperators** the capacity to observe anthesis-silking interval on a family basis, to score leaf loss from below-ear senescence and above-ear firing, and the capacity to recognize tassel blasting when it occurs is important.

5) Some familiarity with instruments which measure plant water potential (pressure chamber, infrared thermometer, porometers) or leaf chlorophyll content (e.g., SPAD 502 photometer) is an asset, but is by no means essential.

6) We prefer to measure shelled grain yields since shelling percentage is often affected by flowering stress. Access to a sheller would therefore be essential. For certain trials, especially those conducted by development cooperators, we also prefer to have weights of 100 kernel samples at oven-dry moisture percentage (or at a moisture content defined by a good electronic moisture tester). Access to moisture testers or balances and ovens will be needed in those cases.

In general the Project has invested very little in seeking to improve facilities available to national program collaborators, but the next phase of this work will require such investments on a regional basis as appropriate. Currently, the cooperating countries in the Drought Network are: Mexico, Nicaragua, Costa Rica, Guatemala, Panama, Haiti, Ecuador, Peru, Bolivia, Brazil, Argentina, Thailand, India, Pakistan, China, Nepal, Indonesia, Ivory Coast, Ghana, Egypt, Malawi, Zambia, Tanzania, Zimbabwe, Kenya, and Ethiopia.

Mechanisms of germplasm sharing and improvement

Use of drought tolerant populations: The populations DTP1 and DTP2 serve as reservoirs of genetic variability for drought tolerance. (For a complete description of the populations, see Chapter 6 Task 5.) Under stress, their performance has been among the best of the elite drought tolerant populations, but at this stage of their development, it has been no better.

Strengths of this approach -- Germplasm from widely different backgrounds can be combined into a single entity, which is then shipped to cooperators in a form which they can use to extract inbred lines, synthetics, etc. The population is open-ended, in that a major introgression is planned each 4-5 years as new source materials are identified and proven to have superior performance. A second major advantage is that it gets around a number of problems of seed quarantine and proprietary rights. The population becomes a Mexican entity, produced here in Mexico, and covered by Mexican quarantine law.

Weaknesses of this approach – It is probably impossible to have a single population that meets the needs of specific countries and sites. This confines the role of these materials to source germplasm, that will be used in a back-crossing program within national programs. A central problem with DTP1 is its disease resistance. In the process of pursuing drought tolerance among sources, little or no attention was paid to the associated disease resistance that the sources carry. DTP1 carries no streak resistance and no downy mildew resistance, nor is it is strongly resistant to *H. maydis, E. turcicum, P. polysora or P. sorghi.* Ear rots will be a problem where disease pressure is high. The adaptation of these populations to specific sites and specific requirements for grain color and texture is a central issue in their further development. Extraction of lines with clean true color was initially a problem, but with the separation of DTP1 and DTP2 into yellow and white fractions this issue has been resolved, and inbred lines with good general combining ability have been identified.

Results from international testing in the drought tolerance network

a. Summary: from the 56 trials requested we received data from 38. Across-site data from the two variety trials are presented in Tables 7.1 and 7.2, and resulted in several requests from cooperators for germplasm observed under test in their environments. A further development of the evaluations of S1 families of DTP1 C5 was the identification of promising lines at the Golden Valley site in Zambia, under the management of a Yugoslav breeder. These lines were further inbred and have proven useful to the Serbian national maize program as sources of drought tolerance for dry locations in the former Yugoslavia. For a detailed report of results see Edmeades et al. (1991).

Table 7.1 Yield, anthesis date and anthesis-silking interval (ASI) of the 16 genotypes that formed part of the early maturing variety trial of the Drought Tolerance Network. Data are means of 19 sites, and testing was from 1990-91. Entries labeled simply as "Entry n" were changed during testing because of seed limitations.

Entry	Yield t/ha	Anthesis d	ASI d
1. Pool 16 Sequía, C_0 F_2	3.11	53.7	1.99
2. Entry 2	3.30	53.8	1.51
3. Pool 16 Seq., "worst all", C_0 F_2	3.15	53.9	1.83
4. Pool 16 Drt Syn, high yld, F_2	2.74	51.9	2.04
5. Pool 16 Drt Syn, good ASI, F_2	2.72	52.4	1.93
6. Pool 16 Across 86, F_3	3.27	53.2	1.98
7. Entry 7	2.51	53.3	2.68
8. Entry 8	3.28	53.3	1.71
9. Pool 18 Sequía, C_0 F_2	2.66	50.5	2.61
10.Pool 18 Sequía, small tass.C_0 F_2	2.81	50.3	2.30
11.Entry 11	2.73	50.3	2.59
12.Pool 18 Sequía, best ASI, C_0F_2	2.46	51.1	2.47
13.Rattray Arnold (1) 8149	3.69	57.8	1.63
14.Across 8530	3.54	54.2	2.15
15.Local check 1	3.64	56.9	1.61
16.Local check 2	3.72	57.8	2.36

Table 7.2 Yield, anthesis date and anthesis-silking interval (ASI) of the 16 genotypes that formed part of the late maturing variety trial of the Drought Tolerance Network. Data are means of 17 sites, and testing was from 1990-91.

Entry	Yield t/ha	Anthesis d	ASI d
1. La Posta Seq, C_1 F_2	3.25	69.6	3.14
2. La Posta Seq, "best all", C_1 F_2	3.15	68.9	1.78
3. La Posta Seq, "worst all, C_1 F_2	2.89	70.8	3.22
4. La Posta Seq, "best sync", C_1 F_2	3.20	69.3	1.65
5. La Posta Seq, "B GY, IS,SS" C_1F_2	2.97	69.5	1.94
6. Pool 26 Seq, C_1 F_2	2.97	66.6	2.62
7. Pool 26 Seq, "best all", C_1 F_2	3.13	66.1	2.90
8. Pool 26 Seq, "worst all", C_1 F_2	2.60	66.5	3.66
9. Pool 26 Seq, "best sync", C_1 F_2	2.92	66.4	2.05
10.Pool 26 Seq, "B GY, IS,SS" C_1F2	3.13	65.8	1.72
11.DTP1 C_4	3.13	63.9	2.42
12.Tuxpeño Sequía C_6	3.21	68.0	1.32
13.Tak Fa 8536	3.33	67.5	2.83
14.Palmira 8425	2.82	67.0	2.59
15.Local check 1	3.56	68.5	2.42
16.Local check 2	3.33	66.0	2.58

A total of 18 sets of the DTP1 S_1 Evaluation trial were prepared and distributed, and usable results were returned from 11 locations (61% return rate). At each site the best 10 families were selected based on data supplied from each site. In general several of these families outyielded the check entries, the major exceptions being Golden Valley, Zambia where the hybrid MM-501 was outstanding, and Tak-Fa, Thailand, where NS 1 yielded very well. Check entries Chorotega B-105 and Lujosa B-106 were also high yielding at La Lujosa, Honduras, and Ac 87TZUTSR-W was the top yielder at Sinematiali, Ivory Coast. At Tlaltizapán, México, the S_1 progeny were grown under three carefully managed stress levels as part of a larger progeny trial. The 10 best families identified at each site have been recombined to form a synthetic. Within each family we attempted to select grain colors appropriate to each of the sites where selection took place. As well, an across-sites selection for recombination was formed based on data from all sites. Synthetics were then retested in the second round of Drought Network testing.

Second round of Drought Network testing, 1992-4.

A total of 80 requests for three trials (early variety, late variety and DTP1 S_1 progeny), were received and data for 45 of these were received for processing (for details see Edmeades et al., 1995). In general trial management was much improved over that of the first round of testing.

Among the early maturing varieties, an early fraction of DTP1 C_5 proved exceptional, and the second top performer across sites was Pool 18 Sequía C_3 (Table 7.3). This must be viewed as a pleasing result and generally demonstrates that CIMMYT's early-maturing drought tolerant germplasm has something to offer to national program collaborators.

Late maturing varieties generally performed poorly in low yield sites. Here local checks did well (lowest superiority index) - but several site-specific selections of DTP1 and DTP2 as well as La Posta Sequia were also fully competitive with the local checks (Table 7.4). Surprisingly, TS6 was poor in low yield environments (we do not know why) and Pool 26 Sequia, the earliest entry in the trial, was good in low yield environments (escape) and poor (very) in high yield environments. Following testing at 7 locations. DTP1 C_6 was divided into white and yellow fractions; will be combined with DTP2 C_5, and site-specific selections of 10 best families recombined. Excellent performance of H430 (CIRNO, Mexico) was noted, and because of this it was included in 1995/6 round of trials. Network trial results published and distributed (Edmeades et al., 1995).

Table 7.3 Anthesis date, grain yield, rank in low, intermediate and high yielding sites, and superiority index (small is best) for 12 entries in the Network Trial of early-maturing varieties, DEVT, 1992-3.

Cultivar	Anthesis (d)	Grain t ha^{-1}	Rank: Low yield	Rank: Int. yield	Rank: High yield	Superiority index[a]
DTP1 C_5 Early Sel.	59	2.63	6	1	1	53
Pool 18 Sequia C_3	55	2.53	3	2	6	52
Local check #1	58	2.50	5	8	3	70
Pool 16 C_{20} Syn. 1	54	2.47	1	4	9	58
Pool 18 Seq. C_2 LTBN	56	2.41	4	7	8	67
TIWD Drt. Tol. Pop. C_0	60	2.41	12	5	2	80
Pool 18 Seq. C_2 BASI	55	2.35	10	6	10	68
TEYF Drt. Tol. Syn. 1	54	2.31	7	3	12	92
Santa Rosa 8330 RE	59	2.29	2	10	4	94
TEWF Drt. Tol. Syn. 2	55	2.27	9	9	11	84
Across 8331 RE	59	2.20	8	11	5	107
Local check #2	58	2.17	11	12	7	130
Sites	21	21	7	7	6	21
Mean	57	2.38	0.87	2.36	4.11	80

[a] Lin and Binns, 1988

Table 7.4 Anthesis date, grain yield, rank in low, intermediate and high yielding sites, and superiority index (small is best) for 11 of 20 entries in the Network Trial of late maturing varieties, DLVT, 1992-3.

Cultivar	Anthesis (d)	Grain t ha^{-1}	Rank Low yield	Rank Int. yield	Rank High yield	Superiority index[a]
La Posta Sequía C_3	65	3.58	12	6	1	79
Local check #2	64	3.56	18	1	2	60
Across 8627 RE	63	3.42	2	8	7	82
Ngabu (1) 89DTP1	62	3.36	6	10	9	71
Sinematiali 89DTP1	61	3.36	11	11	6	69
DTP2 C_4	61	3.35	8	15	4	73
Local check #1	63	3.34	7	2	16	86
TS6 C_1	64	3.31	20	4	3	118
Local check #3	62	3.25	16	13	12	102
Farako Ba 8625 RE	61	3.20	14	19	8	98
Pool 26 Sequía C_3	58	2.93	1	20	20	157
No. sites	19	19	5	7	7	19
Mean	62	3.30	1.01	2.84	5.39	90

[a] Lin and Binns, 1988

Third round of Network testing, 1995-97

In 1995 the following trials were announced and distributed to those Network participants who requested them:

1. ***Late lowland tropical white topcross progeny trial (DLTWT):*** 96 topcross progenies identified as superior under drought in CIMMYT trials, plus 4 check entries. Most topcrosses are from La Posta Sequía C_3 and TS6 C_1 background. Demand:: 10 sets

2. ***Late lowland tropical yellow topcross progeny trial (DLTYT):*** 32 topcross progenies identified as superior under drought in CIMMYT trials, plus 4 check entries. Most topcrosses are from Pool 26 Sequía or elite CIMMYT germplasm. Demand: 12 sets.

3. ***Late lowland tropical cultivar trial (DLTC):*** 30 genotypes, white and yellow (3 local checks; one reference entry; 10 site- or color-specific selections from DTP1 or DTP2; 5 elite drought-tolerant OPVs; 2 national program entries; 8 hybrids or elite topcrosses). Demand: 24 sets.

4. ***Early lowland tropical cultivar trial (DETC):*** 25 genotypes, white and yellow (3 local checks; one reference entry; 18 elite drought-tolerant OPVs; 2 CIMMYT elite hybrids). Note: this trial is a duplicate of the standard CIMMYT Early maturing EVT and Trial 9 NETC of the low N tolerant network. Demand: 24 sets.

5. ***Extra-early lowland tropical cultivar trial (DEETC):*** 12 genotypes, white and yellow (2 local checks; 10 elite drought-tolerant OPVs). These cultivars are the earliest to flower in CIMMYT's breeding nursery. They therefore escape drought rather than tolerating it. This is a standard CIMMYT EVT also, but described as drought escapers. Demand: 27 sets.

In 1995 we announced trials of low N-tolerant germplasm for distribution to national programs. The first three trials contain germplasm which showed an increased tolerance to low N in Mexico. The fourth trial is a duplicate of the standard CIMMYT Early-maturing EVT and the early-maturing drought tolerant trial. Trials are:

6. ***Late lowland tropical white topcross progeny trial (NLTWT):*** 53 topcross progenies identified as superior under low N in CIMMYT trials, plus 3 check entries. Most topcrosses are from La Posta Sequia C_3, TS6 C_1 and CIMMYT elite white populations. About half of the entries carry tolerance to both low N and drought. Demand: 3 sets.

7. ***Late lowland tropical yellow topcross progeny trial (NLTYT):*** topcross progenies identified as superior under drought in CIMMYT trials, plus 4 check entries. Most topcrosses are from Across 8328 BN or elite CIMMYT germplasm. About half of the entries carry tolerance to both low N and drought. Demand: 10 sets.

8. ***Late lowland tropical cultivar trial (NLTC):*** 20 genotypes, white and yellow (3 local checks; 17 elite OPVs). Demand: 23 sets.

9. ***Early lowland tropical cultivar trial (NETC):*** 24 genotypes, white and yellow (3 local checks; one reference entry; 18 elite drought-tolerant OPVs; 2 CIMMYT elite hybrids). **Note:** this trial is a duplicate of the standard CIMMYT Early maturing EVT and the early-maturing drought tolerant trial, DETC. Demand: 22 sets.

In summary: Requests by drought network participants total 97 trials, and by low N Network participants total 58 trials, for a total of 155 trials as of January 1, 1996. Since that time a further 24 requests have been received, bringing the total to 179 trials. These have all been supplied to collaborators.

Networking activities in insect resistance (not formally included under this Task): The Insect Resistance Development Network (IRDN): consists of 17 cooperators who have the capacity to mass rear and artificially inoculate with insects that resemble in life cycle and feeding habits those which challenge maize in the tropics. Cooperators are located in the US (6), Canada, India, China, Egypt, Kenya, Mexico (2), Zimbabwe, Philippines, Repub. Sth. Africa, and Iran. Three sets of S_1 progeny trials (Pops. 590; 590B; 390) are shipped to network cooperators about once every three years (the time taken to complete a cycle of S_1 testing that includes international testing), and results are shared with all network participants. Progenies were last distributed in 1994. A new effort at CIMMYT has be the development of sources of resistance for second generation stem borers. The development of sources of resistance has been slow, as several damage parameters need to be considered and damage assessment occurs after flowering. Pop. 391 has been developed for lowland tropical regions and Pop. 591 for mid-altitude regions. Both populations will be ready for international testing later in 1997. Due to the extra resources required to evaluate this germplasm properly, trials will be sent out to NARs who presently work in second-generation resistance (Philippines, Taiwan, China, Ghana, Kenya, Mexico, US). Information and elite germplasm generated from these trials will be freely available to all NARs who express an interest.

__Summary: an assessment of network activities__

- Global in scope
- Collaborators have specific interest in the stress
- Focused on germplasm development and distribution
- Dominated by CIMMYT germplasm (>80%)
- Trials shipped once every 3 years (1989; 1992; 1995-6) on request
- Report issued with each round of testing, but is often rather late
- Periodic distribution of additional information
- Sporadic training activities and meetings (once of each so far)
- No regular newsletter
- No formal means of collaborator feedback

Trends for the future

- Need for regional adaptation: regional devolution of testing seems a must
- Stronger links to regional breeding initiatives and regional testing networks
- Greater investment in site development and management
- Faster turn around of data analysis and reporting
- Sources separated by color and texture
- Joint planning and decision making through effective meetings
- Restricted exchange of inbred and proprietary products will be a feature of all network activities that include hybrids or inbred lines
- Better relations with quarantine authorities are needed to facilitate germplasm exchange
- Improved information exchange should be a feature of future network activities
- Regional donor support is required to ensure that regionally-oriented networks are properly supported

What should CIMMYT's role be in fulfilling network goals?

Regional activities

- Coordination of activities and networks
- Management of regional funds for national program support
- Strategic research on regional problems
- Adaptation of exotic germplasm
- Application of applied biotechnology tools
- Development and distribution of adapted genetic diversity
- Focus for information exchange and reporting
- Interaction with regional private seed companies

Headquarters activities

- Development of highly stress-tolerant sources (which may be poorly adapted)
- Modeling and GIS: better definition of the timing and nature of stresses
- Germplasm distribution to regional centers
- Improving stress tolerance of broadly-based populations and lines
- Higher-risk strategic research on selection methodology and biotechnology

Other Network-related activities

A: The drought tolerance breeding course

Instead of bringing several visiting scientists to CIMMYT, we chose to bring a larger number of collaborators for a shorter period of time. This arrangement allowed us to provide more intensive instruction in the technical aspects of selecting for drought tolerance, and by including a substantial amount of time in the field, still gave participants hands-on experience in stress breeding. The field visits also served to familiarize national program scientists with the populations available at CIMMYT and how they perform under stress, since the course was scheduled to coincide with harvest of the drought nurseries.

The **objectives** of the course were:

1. To familiarize maize drought network collaborators with breeding and selection methodologies, measurement and use of secondary traits, and plot management techniques which could be used to develop locally-adapted drought tolerant germplasm.
2. To exchange germplasm which will may as a source of drought tolerance, and to build a sense of cooperation among national program cooperators.

A more complete description of the course is included in Chapter 10. Nineteen scientists from fourteen countries participated in the course. Several of these participants were cooperators in the first cycle of network testing, and all came from regions where drought is an important constraint to maize production. In addition to class work and field visits to drought nurseries, participants were also provided instruction in use of the computer software used in the stress program, and were given copies of the program modified for use on PC's.

In response to an open question included in the course evaluation, the majority of the participants indicated that they would modify their selection environments and selection criteria as a result of the course. Some mentioned that they realized they had been selecting for drought escape rather than drought tolerance, and that they would alter their selections to include aspects of tolerance. Others were very interested in the work on tolerance to drought at the seedling stage. One encouraging development from the course was a strong commitment on the part of participants to share and test germplasm with others. We have received numerous requests for seed as a result of the course, and several participants suggested varieties from their regions which we plan to test under drought. In addition, several participants plan to exchange germplasm directly among themselves.

B: Visits to national programs

Major visits by program staff in connection with drought and low N network activities: (each visit included consultation with national staff):

Project staff presented the methodology and results of selections for tolerance to low N to participants in the Advanced and regular crop improvement courses. Drs. Scott Chapman and Marianne Bänziger visited Zimbabwe and Mozambique in March-April, and noted many opportunities for germplasm tolerant of low fertility (low N, low P) conditions, especially in Mozambique where fertilizer use is essentially zero. Greg Edmeades also visited national programs in El Salvador, Guatemala and Panama, and observed attempts to maintain soil fertility for maize production through the use of green manure leguminous crops (notably Mucuna and *Canavalia*). Good links have been maintained between UNDP Stress Project staff and CIMMYT staff in the Programa Regional de Maiz in Central America, CIMMYT's station in Harare (where Mr Joe de Vries has been conducting a study of the inheritance of tolerance to low N, using sites in Zimbabwe and Mozambique), and with CIMMYT staff in Thailand, where Across 8328 BN, selected for tolerance to low N, has been particularly impressive as an OPV. Other visits: Dr. G. Edmeades traveled to Colombia, Brazil, Benin, India, Pakistan, as well as within Mexico. Dr. John Mihm traveled to Kenya, Egypt, South Africa, USA and to Nicaragua and Guatemala several times during the project. Dr. D. Bergvinson traveled to India, Thailand and Honduras. Dr. D. Beck traveled to Thailand to participate in a regional training course dealing with hybrid development and seed production. He also spent 9 days in India visiting maize scientists based at 4 different experiment stations to learn more about their efforts in maize stress breeding and to develop further collaborative links.

C: Symposia involving Network Members and National Program collaborators

See Chapter 11 for details on some of these meetings. Network members participated and contributed to each of the following:

a) "Development of Drought- and Low N-Tolerant Maize". Most participants of the Drought and Low N Networks were present at this meeting (See Chapter 11). Total attendance was 110 (including 11 trainees who were at CIMMYT for the Maize Improvement course), and a Proceedings of the 100+ papers will be published in 1997. Editors are : G.O.Edmeades, M. Bänziger, H. Mickelson, and C.B. Peña-Valdivia.

b) International conference "Insect Resistant Maize: Recent Advances and Utilization", held in November, 1994 with 80 attendees, will be published in early 1997 (Editor: J.A. Mihm), and this will increase awareness of germplasm and methodologies related to host plant resistance.

c) *International Symposium on Environmental Stress: Maize in Perspective,* held in Brazil in 1992. In 1995 the proceedings were published, and this Project contributed to their cost. Editors: A. Toledo Machado; R. Magnavaca, S. Pandey and A. Ferreira da Silva. EMBRAPA/CNPMS and CIMMYT/UNDP. 449 pp.

D: Collaboration with Mexican National Program Staff

During 1995-96 we significantly expanded our network of collaborators in Mexico including both the public and private sector. Collaborating institutes, locations, and collaborators who have participated in our testing network of drought tolerant and insect resistant hybrids are listed below.

Location	Institute	Collaborators
Cd. Obregon, Sonora	INIFAP	Dr. Alejandro Ortega C.
Cotaxtla, Veracruz	INIFAP	Mauro Sierra, Octavio Cano
Celaya, Guanajuato	INIFAP	Arturo Terron, Ernesto Preciado
Aguascalientes, Aguas.	INIFAP	Rodolfo Gaytan
Tlaculmulco, Jalisco	Asgrow, Mexicana	Ing. Humberto Gutierez
Nextipac, Jalisco	Semillas Hibridas	Dr. Fernando Gonzalez
Guadalajara, Jalisco	Univ. of Guad.	Dr. Abel Garcia

The contribution of the first cooperator, **Dr. Alejandro Ortega,** cannot be overemphasized. He has collaborated with us throughout the life of the Project, and has staffed the site in Obregon where large numbers of inbred progenies have undergone heat and drought screening under very demanding physical conditions. The Project owes him a very considerable vote of thanks for his assistance over the years. In general we have expanded our collaboration largely through joint testing efforts with both INIFAP and the private sector. During 1995-96 two maize breeders from INIFAP joined the stress breeding program as visiting scientists for 2-5 month periods. M.S. Mauro Sierra, (INIFAP, Cotaxtla, Veracruz) and M.S. Cesar Reyes, INIFAP, Tamaulipas, and participated in all aspects of our program during their stay. In 1996, computer equipment along with various types of field supplies were purchased for the INIFAP maize research programs in Cotaxtla and Celaya.

E: Training of collaborators

Reference has already been made to the Insect Resistance and Drought Tolerance courses (see Chapter 10). Dr. Jim Hawk, University of Delaware, and Mr. J. Ochieng, MS student from Penn State University, attached to KARI, Kenya, worked with Maize Physiology for a week in 1993. One visiting scientist from Somalia spent six months at CIMMYT also in 1993, working with us on the development of drought tolerant germplasm. In March 1996 a group of four visiting scientists worked with us in drought tolerance research for 3-8 weeks each. They were from Colombia, Cape Verde, India, Ethiopia, and have now returned to work in similar activities in their home countries. Later, a maize breeder from INIFAP joined the Stress Breeding program for 6 months of training in hybrid production for stressed environments, and a second INIFAP scientist is presently on the same scheme for a further 3 months. In addition we have had four regular Maize Breeding trainees from Latin America and Africa who have spent 5 months as part of Physiology or Stress Breeding activities.

*Chapter 8: Task 7: Development of maize possessing tolerance to acid soils**

Task 7: To develop and improve productive maize germplasm with tolerance to aluminum toxicity. Increase the level of tolerance to aluminum saturation levels in improved germplasm.

Acid soils cover 1,660 million ha of developing world agricultural areas, and about 43% of tropical soils are classified as acidic. Sixty-four percent of the soils of tropical south America, 38% of tropical Asia, 27% of tropical Africa, and 10% of Mesoamerica are considered acidic. Where pH falls below around 5.5 on weathered laterites, there is often a sharp increase in the proportion of aluminum which occupies the exchange complex of the soil. When this complex is more than 35% saturated by Al, there is a measurable reduction in maize growth. There are large areas (perhaps 11m ha of *llanos* in Colombia alone) with levels of Al saturation exceeding 60%. Liming these soils reduces Al saturation to acceptable levels, but this is often not feasible for logistical or economic reasons. Acid-soil tolerant maizes have the potential to be used on much of this land. Such varieties have the potential to help farmers upgrade their native pastures, similar to the that accomplished via rice-pasture systems the Amazon basin and *llanos*, and can stabilize maize production in the acid soil areas of Asia and Africa. As a consequence there is increasing interest in acid-tolerant maizes from the private sector and from national programs in Latin America, Asia and Africa.

Accompanying this is a rising interest in using molecular markers to facilitate the breeding, and to transfer acid soil tolerance to otherwise elite but acid-soil susceptible inbred lines that are well-adapted to Asia and Africa. This is partly because acid soils are notoriously non-uniform as field environments. There is also much interest in determining mechanisms of acid soil tolerance and of the relationship between phosphorus use efficiency and acid soil tolerance, since soils with a low pH are usually associated with acute P deficiency. There is a strong possibility that acid-soil tolerant lines will also be highly efficient in their ability to extract P from acid soils.

The research reported here was conducted at CIAT, Cali, Colombia, where CIMMYT has had staff based for around 20 years addressing the problem of maize adapted to acid soils, as well as assuming regional responsibilities for germplasm development and for training in the South American region. Because the acid soil tolerance research was fully funded from other sources from 1990 through 1994, Project funds were not needed to maintain momentum in this activity. (Note that one reason why the Project had unexpended funds in July, 1995, and why we were able to use to extend the life of this project through December 1996, was because of BID support for this aspect of project activities.) Project budget support for acid soil tolerance dates from 1995, when BID support stopped. Project funds were supplied at that stage to support a Post-Doctoral Fellow and his research activities at Cali.

Related to our breeding activities was the maintenance, improvement and development of new evaluation sites. The major Colombian sites in use throughout the project were Palmira (the main CIAT

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* Additional detail is provided in Appendix 4.

station) with non-acidic soils) and Santander de Quilichao with highly acid, but also highly variable soils. Starting in August 1993, two new testing locations were made a part of project activities: a 5 ha site at Villavicencio (Colombia) and a 1 ha location at Yurimaguas (Peru).

Every other year throughout the Project there was a meeting of South American maize research directors to monitor the progress achieved during the last two years and to plan regional activities for the next two years (proceedings available). Around 30 scientist from nine South American countries attended these meetings and they were key to the continuing success of this breeding program. Specific research topics in the 90s have also been addressed by four graduate students from National University of Palmira who have completed their dissertation studies with Project staff.

Similarly, every other year throughout the Project there was a meeting where approximately 100 scientists from around the world gathered and held discussions on issues related to maize improvement and production on acidic as well as non-acidic soils. In these meetings, agreements on sharing of germplasm and other collaborative activities among national programs and between these programs and CIMMYT were also reached.

Output 1:

Better germplasm for acid soils with aluminum saturation, developed through the following activities:

Activity 1: *Screen and identify additional sources of germplasm with aluminum tolerance.*

Activity 2: *Cross sources of aluminum tolerance with good yielding and disease resistant material. Select under multiple levels of aluminum saturation.*

Activity 3: *Test selected material in environments for adaptation and performance.*

The original population improvement activities focused around six populations (Table 8.1).

Table 8.1. Populations in recurrent selection for acid soil tolerance and aluminum toxicity tolerance.

Name	Cycle	Adaptation	Maturity	Grain type
SA3	4	Tropical	Late	Yellow
SA4	4	Tropical	Late	Yellow
SA5	4	Tropical	Late	Yellow
SA6	4	Tropical	Late	White
SA7	4	Tropical	Late	White
SA8	4	Tropical	Late	White

These six populations were improved under acid soil conditions using full sib/S_1 recurrent selection, which replaced the half-sib selection scheme originally used on the older of these populations, SA-3. At each cycle of selection new experimental varieties were formed for testing in experimental variety trials distributed throughout the region in the subsequent cropping season.

A reduction in the number of full-season maturity populations from six to four in 1995 enhanced the efficiency of our selection program and has permited us to better exploit heterosis. Population SA-5 was merged with SA-3, and SA-8 with SA-7. For this 338 S_1 lines from SA-3, 368 from SA-4, 365 from SA-5, 352 from SA-6, 324 from SA-7, and 289 from SA-8 were evaluated for yield in acid soils in Colombia, Brazil, and Thailand, from which 96, 100, 47, 140, 73, 121 lines were selected, respectively. The selected lines were crossed with appropriate heterotic populations and the topcrosses were evaluated in replicated trials in both acidic and nonacid environments in Bolivia, Brazil, Colombia, Ecuador, Guatemala, Ivory Coast, Mexico, and Thailand. (Recently Bolivia, Ecuador and Peru have also become full testing partners). Superior S_1 lines were advanced to S_2 in Colombia. Based on the heterotic pattern information, S_2's of selected S_1 lines were intermated during 1996 to complete the process of consolidation of the populations. These populations have been arranged as heterotic pairs (SA-3 x SA-4 (yellows); SA-6 x SA-7 (white)) and are being improved by reciprocal recurrent selection.

Breeding activities also include the evaluation of our materials in different countries of the region (Brazil, Peru and Venezuela), in addition to Colombia. They are also tested outside the region (Ivory Coast, Mexico, Thailand, Indonesia, and Vietnam). In 1994, for example, progenies of populations SA-3, SA-4, SA-5, SA-6, SA-7 and SA-8 were evaluated at 7 acid soil sites in South America, and perhaps 2 sites in Asia; superior progenies were recombined. From this six experimental varieties were developed: CIMCALI 93SA3, CIMCALI 93SA4, CIMCALI 93SA5, CIMCALI 93SA6, CIMCALI 93SA7, and CIMCALI 93SA8, and were evaluated by NARS during 1994-95. At the same time, 10 acid soil experimental varieties developed during the 1992 season were sent for evaluation to 49 sites during 1992-94. Similarly, seven new open-pollinated varieties (OPVs) were developed in 1995, using elite S_1 lines, for evaluation in NARS plots during 1996-1997.

In 1995-6 we evaluated C_0, C_2, and C_4 of SA-3, C_0, C_1, and C_3 of SA-4, SA-5, SA-6, and SA-7, and C_0, C_1, and C_2 of SA-8 in five acidic environments, two nonacidic and low fertility environments, and one nonacidic fertile environment. Across acidic environments, yield improvements ranged between 0.47 and 8.43% per cycle, and averaged 4.75% per cycle. Across the nonacidic low fertility environments, yield improvements ranged between -0.18 and 9.43% per cycle for an average of 3.0% per cycle. In the nonacidic fertile environment, yield improvement ranged between -7.35 and 7.10% per cycle and averaged 1.85% per cycle. The data suggest that we have been effective in improving the performance of the populations across a range of environments, and particularly in acidic soils.

In collaboration with CIMMYT colleagues based in Thailand, downy mildew tolerance is being introduced in CIMCALI 91SA5, CIMCALI 91SA7, and CIMCALI 91SA8, through backcrossing. In addition, superior lines from both programs are being jointly evaluated to identify those that include high levels of tolerance to both soil acidity and downy mildew. Similarly, in collaboration with our colleagues in Ivory Coast, resistance to streak virus is being incorporated In those acidity-tolerant white endosperm varieties that have shown superior performance in the tropical African environments.

In response to a demand for early maturing populations tolerant of acid soils, one early white (61 S_1 lines recombined) population and one early yellow endosperm (120 S_1 lines recombined) population were developed, based on a large number of materials from CIMMYT headquarters, Thailand, Cali, and Indonesia. Consolidation of late populations released resources to continue development of two early-maturity acid soil-tolerant maize populations. For this, 571 lines were evaluated in acidic and nonacidic fields and 15 lines with white and 67 lines with yellow endosperm were selected in 1995 and intermated to develop the two populations.

Lines have also been under evaluation, and can easily be formed from S_1 recurrent selection schemes that are currently in use in the region. For example, in 1994 81 S_2 lines showing high levels of tolerance to acid soils were evaluated in Colombia and Brazil. They were also topcrossed to a susceptible tester and advanced to S_3 during the same season at Cali, and evaluated during the following season for identification of superior lines for further inbreeding and hybrid development. During 1995 a total of 1,802 lines from our program and 305 lines from CIMMYT's program in Mexico were evaluated in acidic and nonacidic fields in Brazil, Colombia, and Thailand, and 36 lines with white and 142 lines with yellow endosperm were selected. Selected lines have been crossed with 5-6 testers and in a Design II mating system to develop approximately 880 hybrids for evaluation in NARS plots during 1996. The results will help us identify superior hybrids for acidic and non-acidic environments and produce information on appropriate testers for use in breeding programs.

Performance of acid soil tolerant germplasm

In trials conducted during 1992-3, the variety Sikuani V-110 (or CIMCALI 91SA3, developed by the project) averaged 45% higher yield than the check in 18 acid sites and 11% higher in five nonacid sites. Further studies will continue along these lines, exploring sustainable cropping systems (including associations and rotations, etc.), in collaboration with CORPOICA and CIAT. Sikuani V-110 will produce around 3 t/ha under hot lowland conditions and on soils with at least 60% Al saturation (see Table 8.2; and Appendix 4 for further details).

Table 8.2 Yield of CIMCALI 91SA3 (i.e., Sikuani V-110) and that of the best check when grown at 20 acid and five non-acid soil sites during 1992-93.

Sites	Number	SA-3	Check	Superiority
		t/ha	t/ha	%
Colombia	18	2.93	1.92	153
Other countries	2	3.11	2.67	117
Non-acid sites	5	7.14	6.45	111

The trials were so successful that CORPOICA released on 29 July 1994, the first soil acidity tolerant maize variety (Sikuani V-110 = CIMCALI 91SA3) for the Colombian savannas.

An experimental variety trial was conducted over 17 sites in 1994 with our newest OPVs. Our best tolerant OPV (93SA-4) yielded 3.11 t/ha, compared to 2.97 tons/ ha of the best NARS checks over 15 acidic environments. The superiority of our best OPV over the best checks was about 9%, but in 11 of the 15 sites NARS had included our previous OPVs as their checks. Across two non-acidic fertile environments, our best OPV (93SA6) yielded 7.49 t/ha, compared to 5.18 t/ha for the best checks.

Cropping systems research

We have started agronomic studies to measure, in economic terms, the advantage of using acid soil tolerant germplasm under such conditions. We have also started the planning of long term studies to evaluate the effects of maize production in potentially fragile environments such as Colombia's Llanos Orientales. Several genetic studies have been completed or are being carried out to elucidate the genetic basis of tolerance to acid soils, Al-toxicity and P-deficiency (theses studies). In conjunction with the Tropical Pastures Program at CIAT, we are developing management methods for sustainable production systems based around pastures and the acid-tolerant cultivar Sikunai V-110. In the process we plan to determine optimal nutritional environments for growth of acid soil tolerant cultivars, and to assess the environmental impact of the use of acid soil-tolerant cultivars. Approximately 15 trials were conducted in the acid savannas of Colombia during 1994, most of them in farmers' fields. They were planted in monoculture and in association with graminaceous and leguminous pastures. The data indicate that maize has strong possibilities in the savannas in monoculture as well as in association with pastures (see Appendix 4).

Genetic and physiological studies

In collaboration with graduate students we have made considerable progress in completing our studies on the genetics of tolerance to soil acidity, including maternal effects. Our genetic studies to date indicate that both additive and dominance gene effects are responsible for high yield in acidic soils, that selection would be most effective if based on yield alone, but based on a large number of environments with differing soil acidities. Studies also showed that genotypes that yield well in acid soils also yield well in non-acid soils. There is no evidence of reciprocal differences in yield, due to either maternal of non-maternal effects.

We are continuing with development of inbred lines for genetic, physiological, and molecular studies. These S_4 lines are showing excellent contrast for tolerance and susceptibility. Tolerant S_4 lines are potential hybrid parents. In an effort to develop a mapping population for Al-tolerance, and with a view to using MAS in the near future, several tolerant and susceptible S_4 lines were crossed with each other during 1994, but mapping of this F_2 population was delayed because of more promising events involving near-isogenic lines (see Chapter 9). In developing these, lines were advanced to S_6 and reevaluated for tolerance to acidity. Sister lines differing in reaction to acid soils formed the set of near isogenics under evaluation. Understanding the biochemical basis of tolerance is important to increase the efficiency of our breeding activities. We have started a cooperative project with a university (Cornell University) to elucidate the biochemical basis of aluminum toxicity tolerance. The tolerant population evaluated (SA3) exudes citrate and itaconate in the presence of toxic levels of Al. Further studies will be performed with S_4 lines mentioned above. Similar physiological studies are planned in collaboration with the University of Hannover (Germany) to identify chemicals and mechanisms that may be easier and cheaper to measure than citric acid. Such information will greatly enhance the efficiency of Al-tolerance breeding programs around the world.

Training, consultation and dissemination of results

A number of publications describing project outputs in acid soil tolerance have been produced (Appendix 1). Harvest seminars, with participation of two scientists each from most participating countries, plus scientists from CIMMYT have been conducted in Colombia to select materials and share information and experiences on breeding and screening methodologies. Several visits were made to national programs in the region each year to consult with them on acid soil research. Such visits normally totalled around 200 person-days per year. Close collaboration was maintained with the acid soil tolerance program developed by EMBRAPA some years ago for maize at Sete Lagoas, and there is frequent exchange of information and views, collaborative testing of progenies and exchange of methodologies between the two programs.

The III Latin American and XVI Andean Regional Maize Meetings were held in Bolivia during 20-24 November, 1995. A total of 88 scientists participated: 12 from Argentina, 40 from Bolivia, eight from Brazil, seven from Colombia, three from Ecuador, one from Italy, one from Guatemala, one from Honduras, one from Nicaragua, three from Mexico, three from Peru, one from Uruguay, three from USA, and four from Venezuela. The participants presented their own research results and benefited from invited talks by distinguished scientists.

Twelve scientists from the region participated in full-service training courses offered in Mexico and in Brazil and four scientists visited CIMMYT-Mexico to work with the colleagues at the head quarter's program. We supported two Colombian students to pursue their M.S. degree at the University of Palmira (Colombia) and one Ecuadorian student to pursue his Ph.D. degree at the University of Piracicaba (Brazil).

Summary

The release of the first acid soil tolerant maize variety, Sikuani V-110, in Colombia has attracted considerable attention in the press, worldwide as well as in Colombia. It has the potential to change the basic pasture-crop rotation of vast areas of the Colombian llanos. Sikuani V-110 was developed from the population SA-3 and has been released for use on the acidic *llanos* of Colombia as an essential component of a stable and sustainable cropping system for this very extensive acid soil area. At present this variety is thought to be grown on 10,000 to 15,000 ha in the *llanos*. Considerable farmer-to-farmer transfer of seed of this variety has occurred, making the extent of its usage difficult to estimate simply from seed sales. Two seed companies in Colombia have undertaken to increase and sell seed. There is little doubt that acid-tolerant varieties such as Sikuani have the potential to increase maize grain yields by 30% or more over local check entries under soil conditions of pH 4-5 and aluminum saturation levels of 60-70%. (see Appendix 4 for details). The original Sikuani cultivar is being released in Peru where it is also expected to have a significant impact. New hybrids are under development which may increase production under acid soil conditions by up to 70% over that achieved by Sikuani. The use of Sikuani in pasture-based rotations in the *llanos* is expected to increase profitability and economic sustainability significantly.

Good and reliable experimental sites with uniform levels of acidity have been located in Colombia for field screening of progenies. At the same time, progress is being made towards the generation of linkage maps and the use of markers to speed the selection of acid soil tolerant genotypes, hopefully in less time and at less cost than in the field, but not as a complete substitute for the field.

Testing of yellow and white acid-soil tolerant genotypes in Asia (especially countries like Indonesia and the Philippines) is proceeding well, and the incorporation of downy mildew resistance into acid soil-tolerant germplasm will greatly extend its area of suitability. Similar work is in progress with our colleagues in Ivory Coast, to incorporate resistance to streak virus in acidity-tolerant white endosperm maize OPVs. The regional network of breeders and agronomists addressing acid soil tolerance is now well-established. Their annual meetings have consistently resulted in well-integrated approaches to selecting for tolerance to this stress which is widely distributed in the older, weathered and usually well-watered soils that are otherwise well-suited for maize production.

Chapter 9: Task 8: Improved laboratory methods for aluminum tolerance

Task 8: Develop and improve a laboratory methodology for selecting aluminum tolerant maize germplasm. More efficient selection of tolerant germplasm is expected through greater precision and testing uniformity.

Output 1:
Review of current laboratory methodology, involving the following:

Activity 1: *Update literature search of methodologies used to screen for aluminum tolerance.*

Several bioassays have been developed for fast screening of tolerance to Al-toxicity and P-deficiency. The most widely used is growing seedlings in nutrient solutions with varying levels of aluminum. The technique has proven to be efficient differentiating tolerant and susceptible materials in different crops. However, it has two major drawbacks: 1) it can only screen for the reaction to just one of the limiting factors for plant growth under acid soils (toxic levels of Al), and 2) the correlation between performance in nutrient solution and field results are not high (around 30%).

A new technique in sorghum and other cereals but not maize was published for rapid screening. It uses acid soils instead of nutrient solution. The technique has the advantage of evaluating the response to several of the limiting factors for plant growth under acid soils and their interactions. We started a project to adapt the technique to maize and evaluate its potential use in breeding for tolerance to acid soils (Urrea et al., 1996).

Output 2:
Comparison of laboratory and field evaluations through the following:

Activity 1: *Evaluate a set of materials in field under different aluminum saturation levels and in laboratory.*

Several experiments have been conducted to adapt the technique to the case of maize. This set of experiments allowed us to fine tune the technique regarding: 1) length of time the seedlings had to grown, 2) the most appropriate level of edaphic stress (measured as% of al-saturation), 3) the best type of check soil (no stress), 4) kind (size and shape) of pots to use, 5) best way to extract the seedlings from the soil, 6) number of seedlings per unit of soil, and most importantly 7) variables to measure. The materials evaluated in these preliminary experiments were also grown in the field with different levels of edaphic stress, in no less than five environments. They were also tested in nutrient solution, so correlation between field, nutrient solution and pot results could be obtained. Two sets of materials were evaluated: 1. A set of diallel crosses and the parental populations (six tolerant and two susceptible). 2. A set of eight varieties (six tolerant and two susceptible). Preliminary results have shown several problems. A major one has been to recover all the roots from the plants. It seems that the most important difference between tolerant and susceptible germplasm is the number of root hairs, and not root length or number of secondary roots. We have now developed a technique that allows recovering most of the root system by washing the soil. A major constraint of this technique is that large amounts of soil are lost in each experiment. Surprisingly the variable that most consistently differentiated tolerant and susceptible germplasm has been the length of the seedling above ground. Results indicate that, when grown in pots with acid soils for about 15 days, highly tolerant cultivars can be distinguished from highly susceptible ones, based on fresh and dry root weights, total seedling dry weight, length of secondary seminal roots and total seedling length. However, the technique is unable to distinguish among genotypes with small differences and, therefore, is not likely to be useful in a population improvement program.

Output 3:
New laboratory screening methodology for aluminum developed through the following:

Activity 1: *Research laboratory conditions which give similar selections with set of materials used for Output 2*

The methodology is still under development. However, the technique is expected to be able to discriminate only between tolerant and susceptible germplasm but not levels of tolerance such as between different families from a tolerant population. Further development of this method will receive a relatively low priority.

Recent collaborative research with scientists at Cornell University staff has indicated a relatively close relationship between tolerance to acid soil conditions and citric acid production by the roots. The citric acid presumably modifies the rhizosphere and provides ligands that enhance the uptake of soil P while protecting the root against the effects of free Al. This is a promising area of future research that could prove almost as useful and as cost effective in screening resistant types as MAS.

A total of 49 lines (29 highly tolerant and 20 highly susceptible), including 20 pairs of near isogenic lines for tolerance and susceptibility, were identified and are currently being studied using the amplified fragment length polymorphism (AFLP) technique to locate molecular markers for tolerance to soil acidity. We are also developing appropriate genetic stocks by crossing two highly tolerant and two highly susceptible lines to tag the genes responsible to acidity tolerance. This molecular information is expected to increase the efficiency of breeding programs, and shorten the time needed to identify and develop acid- tolerant maize cultivars.

Output 4:
Written procedures for laboratory screening of germplasm developed through the following:

Activity 1: *Collation of results and techniques used to screen for aluminum tolerance in Maize.*

As the experiments are finished and are important, they are being published (e.g., Urrea et al., 1996). Our genetic studies to date indicate that both additive and dominance gene effects are responsible for high yield in acidic soils, that selection would be most effective if based on yield alone and on a large number of environments with differing soil acidities, and that genotypes that yield high in acid soils also yield high in nonacid soils. There is no evidence of reciprocal differences in yield, due to either maternal of nonmaternal effects.

Summary: Less progress on the development of laboratory screening techniques has been made than was originally envisaged. In general, this is because the correlation between such tests and performance in the field is low. Field resistance is a complex character involving the ability to accumulate biomass of roots and shoots, and the ability to partition that biomass at flowering in such a way as to establish a large ear. With the development of uniform acid soil screening sites at Villavicencio and Carimagua in the llanos of Colombia, and progress towards MAS as a cost-effective way of discarding 95% of the non-tolerant backcrosses during the process of transferring tolerance, the need for laboratory screening has diminished.

The greenhouse and soil techniques are poorly associated with field performance and could, at best, be used for discarding only the very susceptible segregates. Field screens have improved tremendously in precision in recent years and have taken their place.

Chapter 10: Training activities

I. Informal training

Applied Molecular Genetics: To date, 46 scientists from 21 countries have received short or long-term training in our laboratories. Three students (Chile, Mexico and Peru) have done their M.Sc. theses, and 10 students (Canada, Cuba, Ecuador, France, Germany and Mexico) have, or are completing, their PhD dissertion in the Applied Molecular Genetics Laboratory. In addition, we have hosted and trained 12 Visiting Scientists (Brazil, France, Germany, Mexico, South Africa, Spain, Switzerland, Syria and the UK) for a period of less than a month and 21 Visiting Scientists for a period between one month to a year (Argentina, Australia, Austria, Brazil, Chile, China, Egypt, France, India, Mexico, Peru, Switzerland and the USA).

II. Formal training courses supported fully of partly by UNDP Projects

A: Insect resistance training course

Date: 11 Jan. - 12 Feb., 1993
Location: CIMMYT, El Batan, Mexico
Objectives:

To expose national program entomologists and breeders to the techniques involved in:

1) Establishing and maintaining a laboratory colony of the pest species;
2) Techniques and methods to efficiently mass culture and produce quality insects for field infestation, including: a) developing a suitable economic meridic diet; how to prepare (cook) it; b) selection and use of most efficient larval rearing containers; c) determining optimal physical (temperatures, humidities, and photoperiods) conditions for larval rearing, pupal survival and adult emergence; d) developing an efficient oviposition cage and substrate; e) manipulating egg development and maximizing egg hatching;
3) Choosing germplasm resources that include variation for genetic resistance; identification and proper use of resistant and susceptible check genotypes; developing resistant source populations with adequate environmental adaptation/suitability;
4) How to accomplish an efficient, uniform artificial field infestation in a timely manner; a) Handling and mixing of larvae; b) Actual application of insects in the field;
5) Evaluating (rating) damage resulting from the infestation;
6) Selecting resistant genotypes and breeding to improve insect resistance; a) developing and improving a source population; b) developing inbred lines for conventional hybrid development c) developing experimental varieties and non-conventional hybrids d) utilizing dominance type resistance to convert susceptible varieties/hybrids to resistant, adapted cultivar e) methods for simultaneously improving multiple insect and disease resistance;
7) Conducting resistance verification/demonstration trials, pre- and post-release;
8) Utilizing host plant resistance as a major component of Integrated Pest Management

The course included lectures on theory and practice, including actual demonstration and practical experience in the mass rearing laboratory, field breeding nurseries, evaluation trials and demonstration plots. Emphasis was on identifying entomologist-breeder teams from national programs so that they could return and mount a successful host plant resistance breeding program as a two-person team. **Course coordinators**: Dr. John Mihm and Dr. J.A. Deutsch. Instructors with knowlege of special subject areas will be used as needed. (Dr. D. Jewell, Dr. J. Crossa, among others)

Participants

Paulo Afonso Viana	Brazil
Ved Pal Singh Panwar	India
Shashi Bhushan, Vemuri	India
José Blas Maya Lozano	Mexico
Paulo Evaristo de Oliveira Guimaraes	Brazil
Uma Kanta	India
Danilo Mapaquit Legacion	Philippines
Marcelo Velásquez	Guatemala
Danilo P. Baldos	Philippines
He Kanglai	China
Juan Francisco Pérez Dominguez	Mexico
Ramanujam Sai Kumar	India

B: Drought tolerance training course

Dates: March 8 - April 2, 1993
Location: CIMMYT, El Batan, Mexico

Course objectives:

1. To familiarise maize drought network collaborators with breeding and selection methodologies, measurement and use of secondary traits, and plot management techniques, which could be used to develop locally-adapted drought tolerant germplasm.
2. To exchange germplasm which will may as a source of drought tolerance, and to build a sense of cooperation among national program cooperators.

Course coordinators: Dr. G.O. Edmeades and Dr. H.R. Lafitte. Instructors with knowlege of special subject areas will be used as needed. (Drs. S. Chapman, M. Bänziger, Dr. Hector Barreto and Dr. J. Crossa, among others)

Course content:

- Basic soil-plant-air water relations
- Statistical methods for abiotic stress breeding
- Field techniques and measurements
- Selection procedures for drought tolerance
- Results of drought tolerance breeding at CIMMYT
- Aspects of drought network operation

Participants were directly involved in scoring, harvest, and selection of materials in CIMMYT's drought breeding nurseries. They will also received instruction in the use of computer software for the evaluation and selection of superior families.

List of Participants

José M. Terrazas Vega	Bolivia
Daniel Alarcón Cobena	Ecuador
Hussein Mohammed Ali	Ethiopia
José Luis Zea Morales	Guatemala
Colette Blanchet Zaongo	Haiti
Iqbal Singh	India
Maha Deo Arha	India
Ramanujam Sai Kumar	India
Francis M. Ndambuki	Kenya
Mugo Ngure	Kenya
Essau Mwendo Phiri	Malawi
Alfonso Peña Ramos	México
Gustavo Adrián Velazquez Cardelas	México
Sajjad-ur-Rehman Chughtai	Pakistan
Juan José Morán Mendoza	Perú
Sansern Jampatong	Thailand
Teerasak Manupeerapan	Thailand
Charles Mwambula	Zambia
Herbert Hlebeni Dhliwayo	Zimbabwe

C: Molecular Marker Applications to Plant Breeding

Dates: November 27 - December 8, 1995
Location: CIMMYT Headquarters, El Batán, Mexico
Schedule:

Tuesday Nov. 28 am		DNA quality and digestions
pm	Cloning and transformation Southern transfer and hybridization Probe labeling (all 2-2.5 hrs) MK	
evening	Trainees presentations (1 hr)	
Wednesday Nov. 29 am		Clone transformation Probe preparation Prepare gels for electrophoresis
pm	RAPDs, DNA enrichment for low copy seq. Other pcr-based markers (all 1.5 - 2hrs) XH	DNA electrophoresis
evening	Trainees presentations (1 hr)	

Thursday **Nov. 30** am	NO lectures Only discussions when time permits	Set up RAPDs and STSs Stain gel, Southern transfer Run probes on gel
pm		Blot probe gel
evening	Trainees presentations (1 hr)	
Friday **Dec. 1** am	NO lectures Only discussions when time permits	Detect probes blot Run RAPDs and STSs gel Fix DNA, prehybridize
pm		Stain RAPDs and STSs gel Hybridize, prepare W/D solutions
Saturday **Dec. 2**	NO lectures Only discussions when time permits	Stringency washes and detection Expose blots to X-rays
Sunday **Dec. 3**		Develop X-rays Start bacterial cultures To the pyramids!

Week 2 (December 4 - December 10)

	Lectures **(Sasakawa)**	**Practice** **(room B-116)**
Monday **Dec. 4 am**		Plasmid mini-preps Stripwash blots **(Wheat Pathology Training Lab)**
pm	HyperMapdata (30-45 min) MK *Group picture in front of main bldg at 3pm*	RFLP data entry and verification
evening	Trainees presentations (1 hr)	
Tuesday **Dec. 5** am	Genetic segregation, X2 tests Populations used in mapping Creating maps: linkage groups, assigning link. gps to chromosomes (1.5-2 hrs) DH	Map construction
pm	QTL mapping: simple ANOVA, Interval mapping, Composite interval mapping (2 hrs) CJ	
evening	Trainees presentations (1 hr)	
Wednesday **Dec. 6 am**		ANOVA for QTL analysis MAPMAKER/QTL for QTL maps
pm	Gene tagging: bulk segregant analysis (1 hr) XH Marker-assisted selection: schemes and strategies (1hr) DGL CIMMYT's MAS projects: ASI (JMR), SWCB (MW), triticale (BS) (1.5hr)	
evening	Trainees presentations (1 hr)	
Thursday **Dec. 7 am**	Introduction to the DataBases (1.5 hrs) JDB	Exercises on DB

pm	Fingerprinting and applications (1.5 hrs) DGL	Demonstrate HyperBlot HyperSelect: graphical genotypes, do selections
Friday Dec. 8 am	Questions/Answers session (1.5 hrs) All staff	More excercises on DB
pm	Evaluation of the course (0.5-1 hr) Graduation (0.5-1 hr)	
evening	Farewell dinner: Noche Mexicana	
Saturday, Sunday Dec. 9 & 10	Departures	

Trainees presentations: evenings after dinner (7:30 - 8:30 pm), 3 per session starting the first Tuesday evening

DH Dave Hoisington, MK Mireille Khairallah, XH Xueyi Hu, CJ Chianjian Jiang, JMR Jean-Marcel Ribaut, MW Martha Willcox, BS Bent Skovmand, JDB John Barnett

Participants

Mr. Hermann Buerstmayr	Austria	Univ. of Agriculture	Vienna	
Mr. Claudio Brondani	Brazil	EMBRAPA/CNP	Passo Fundo	presently PhD student at Univ. c Brasilia
Dr. David Gavin Humphreys	Canada	Ag. Canada	Winnipeg	
Ms. Doris Angela Prehn Roth	Chile	Universidad Catolica de Chile	Santiago	
Mr. Ahmed Abd-Rabboh Mahmoud	Egypt	Genet. Eng. Inst.	Agric Res Center	Egypt presently PhD student at Univ. of Missouri
Dr. Maruthi Prasanna Boddupalli	India	Indian Agri. Res. Inst.	New Delhi	
Mr. Suresh Venktesh Naik	India	Agharkar Res. Institute	Maharashtra	India
Dr. Ratan Tiwari	India	Directorate of Wheat Res.	Karnal	
Mr. Clemente Villanueva Verduzco	Mexico	Chapingo University	Mexico	
Dr. Ricardo Ernesto Preciado-Ortíz	Mexico	INIFAP	Campo Exp. Bajío	Mexico
Dr. José Antonio Serratos Hernández	Mexico	INIFAP	Campo Exp. Valle de Mexico	Mexico
Dr. Zhang Aimin	P.R. China	Beijing Agric. University		
Mr. Lei Zhensheng	P.R.China	Henan Acad. of Agri. Sci.		
Dr. Desirée Menancio-Hautea	Philippines	Inst Plt Breed	Univ. of Philippines at Los Baños	
Dr. Chokechai Aekatasanawan	Thailand	Nat Corn Sorghum Res Center	Kasetsart University	
Mr. Ahmet Yildirim	Turkey	Gaziosmanpasa Univ.	Tokat	Turkey presently PhD student at Wash. State Univ.
Ms. Dragana Ignjatovic	Yugoslavia	Maize Research Institute	Belgrade	
Dr. Pangirayi Bernard Tongoona	Zimbabwe	Univ. of Zimbabwe		

Repeat course: Molecular Marker Applications to Plant Breeding

Dates: October 14 - November 1, 1996
Location: CIMMYT Headquarters, El Batán, Mexico

Participants

Ms.	Formica, María Beatriz	Inst. Nac. Tecn. Agropecuaria	Argentina	Córdoba
Mr.	Tadeu Sibov, Sergio	Universidade Estadual de Campinas	Brazil	Campinas
Ms.	Landjeva, Svetlana	Bulg. Acad. Sciences	Bulgaria	Sofia
Ms.	Shen, Xiaorong	Jiangsu Acad. Agric. Sciences	China	Nanjing
Dr.	Shi, Yong Gang	Sichuan Acad. Agri. Sciences	China	Chengdu
Dr.	Tadesse, Nigatu	presently visiting scientist at CIMMYT	Ethiopia	
Dr.	Ramesh, Bandarupalli	C.C. Singh University	India	Meerut
Dr.	Ortegón Pérez, Jesús	Univ. Auton. Agra. Antonio Narro	Mexico	Saltillo
Dr.	Luna Ruíz, José de Jesus	Univ. Auton. Aguascalientes	Mexico	Aguascalientes
Mr.	Pinto do Nascimento, Mário	University of Tras-os-Montes e Alto Douro	Portugal	Vila Real
Mr.	Giura, Aurel	Research Institute for Cereals and Industrial Crops	Romania	Fundulea
Ms.	McGregor, Cecilia	Veg. & Ornam. Plt Inst.	S. Africa	Pretoria
Ms.	Román del Castillo, Belén	ETSIAM	Spain	Córdoba
Dr.	Abdennadher, Mourad	Inst. Nation. Rech. Agron.	Tunisia	Ariana
Dr.	Penner, Greg*	Agriculture and Agri-Food Canada	Canada	Winnipeg
Dr.	Nelson, Clare*	Cornell University	USA	Cornell

* Instructors from outside CIMMYT

Workshop on In situ Hybridization and Detection

Dates: May 23- May 31, 1994
Location: CIMMYT, El Batan, Mexico

Course objectives: to train a selecetd group of CIMMYT staff and national program collaborators from Mexico in the technques associated with *in situ* hybridization and detection of alien chromosome fragments.

Course and Participants: Dr. Nurul Islam-Faridi conducted an 8-day course entitled "*In situ* Hybridization Training". This course trained Ing. Alejandro Cortez (from Dr. Mujeeb Kazi's Lab); Mr. Daniel Grimanelli and Mr Olivier LeBlanc of Dr. Yves Savidan's laboratory; and a colleague of Dr. Nina Ortega (who stood in for her at the last minute) of the Colegio de Postgraduados, Chapingo. The course was well run, and those who attended succeded in learning and using Dr. Faridi's technique with excellent results. The technique has this been placed in the hands of a several CIMMYT and national staff, and will continue to be used in Project-related and apomixis research.

Documentation: A full course outline decribing the technique, and an album of outstanding microscope photographs were filed with the Project Coordinator.

Chapter 11: Technology transfer and impact

Seed shipments

PHYSIOLOGY PROGRAM

YEAR	COUNTRIES	NO. OF GENOTYPES
1991	16	140
1992	16	107
1993	32	328
1994	31	235
1995	22	167
1996	21	153

ENTOMOLOGY PROGRAM

YEAR	COUNTRIES	NO. OF GENOTYPES
1991	15	107
1992	7	22
1993	8	25
1994	5	31
1995	9	69
1996	13	158

STRESS BREEDING PROGRAM

YEAR	COUNTRIES	NO. OF GENOTYPES
1991	14	102
1992	8	62
1993	25	175
1994	6	332
1995	16	618
1996	22	1,665

Devolution of methods: The Swiss Development Cooperation (SDC) have recently funded a 5 year project in southern Africa which fosters the development of drought- and low N-tolerant maize varieties and hybrids from within germplasm locally adapted to the region. This project is essentially a devolution of the UNDP Stress Project, with emphasis on application of principles of stress tolerance improvement developed over the past 6 years under this project and less on methods development.

Appendix 1: Publications

Draft list of UNDP Stress Publications 1990-1996, plus manuscripts in preparation or in press for 1997 publication.

General:

Edmeades, G.O. and J.A. Deutsch (Editors). 1994. Development of New Stress-Resistant Maize Genetic Resources. Briefing Book prepared for the 1993 mid-term review of the the Project. Published as a Technical Publication by CIMMYT, El Batan, Mexico.

Machado, A.T., R. Magnavaca, S. Pandey, and A.F. da Silva. 1995. Proc. International Symposium on Environmental Stress: Maize in Perspective. Belo Horizonte, Brazil, 8-13 March, 1992. EMBRAPA/CNPMS and CIMMYT/UNDP. 449 pp.

1. Molecular marker technology

Bohn, M.; Acevedo, F.; Deutsch, J.A.; Gonzalez de Leon, D.; Hoisington, D.A.; Jewell, D.C.; Khairallah, M.M.; Mihm, J.A. 1993. Mapping of yield components and morphological QTLs involved in tropical maize populations. Agron. Abstr. p. 172. ASA, Madison, WI.

Bohn, M., M.M. Khairallah, D. González-de-León, D.A. Hoisington, H.F. Utz, J.A. Deutsch, D.C. Jewell, J.A. Mihm and A.E. Melchinger. 1996. QTL mapping in tropical maize: I. Genomic regions affecting leaf feeding resistance to sugarcane borer and other traits. Crop Sci. 36:1352-1361.

Bohn, M., M.M. Khairallah, D.C. Jewell, D.A. Hoisington, D. González-de-León, H.F. Utz, C. Jiang, and A.E. Melchinger. 1996. QTL mapping of insect resistance in tropical maize. Paper presented at the XVII Conference on Genetics, Biotechnology and Breeding of Maize and Sorghum, EUCARPIA, October 20-25 1996, Thessaloniki, Greece.

Bohn, M., M.M. Khairallah, C. Jiang, D. González-de-León, D.A. Hoisington, H.F. Utz, J.A. Deutsch, D.C. Jewell, J.A. Mihm and A.E. Melchinger. 1997. QTL mapping in tropical maize: II. Comparison of genomic regions for resistance to *Diatraea* spp.. Submitted to Crop Sci.

González-de-Léon, D., F. Acevedo, J. Alarcón, M. Bohn, J. Deutsch, D. Jewell, M. Khairallah, J. H. Mihm and D. Hoisington. 1994. Mapping of QTL involved in resistance to corn borers, yield components and morphological characters in tropical maize. Poster presented at Plant Genome II, Jan. 24-27 1994, San Diego, California, USA.

González de León, D., C. Jiang, M. Khairallah, J-M Ribaut, M. Bohn, M. Willcox, & D. Hoisington. 1995. QTL for the anthesis-silking interval (ASI) and insect resistance in tropical maize - perspectives on marker-assisted selection. Paper presented at the VII Congreso Nacional de Bioquímica y Biología Molecular de Plantas/ 1er. Simposium México-Estados Unidos. Cocoyoc, Morelos, Mexico.

González de León, D., M. Khairallah, J-M Ribaut, C. Jiang, M. Willcox, & D. Hoisington. 1995. Uso práctico y masivo de marcadores moleculares en cereales. Apuntes y perspectivas del CIMMYT. Conferencia de Planificación — Programa Nacional de Biotecnología Agropecuaria y Forestal, Oct. 95, Chillán, Chile.

Groh, S., M. Bohn, C. Jiang, M. Khairallah, A. Melchinger, D. Hoisington and D. González-de-Léon. 1996. Comparison of QTL for insect resistance and plant height in F_2 and RIL populations of tropical maize. Poster presented at Plant Genome IV, Jan. 14-18 1996, San Diego, California, USA.

Groh, S., M. Khairallah, C. Jiang, D. González-de-León, and A.E. Melchinger. 1996. Mapping QTL for corn borer resistance and its components in tropical maize. Memorias del XVI Congreso de Fitogenética. J.S. Castellanos, P. Ramírez Vallejo y F. Castillo González (Compiladores). Sociedad Mexicana de Fitogenética, A.C., Montecillo, Mexico. p.236.

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Stress Breeding

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Appendix 2: Workshops, Symposia and Conferences

A: International Symposium

Insect Resistant Maize: Recent Advances and Utilization.

CIMMYT, El Batan. 27 November - 3 December, 1994

An international symposium hosted by CIMMYT in November, 1994, this event covered advances in worldwide research on the mechanisms and bases of insect resistance in maize; the genetics of resistance; on the biotechnological manipulation of resistance; on techniques for the mass rearing of pests, for scoring damage, for conducting bioassays, and for detecting resistance mechanisms; and on the verification and use of resistance. It also carried reports on maize insect pests and related research in specific countries and regions.

The conference had approximately 80 participants and involved 60 scientific papers. Many developing country scientists contributed with presentations and posters and participated in discussions.

All contributions are being incorporated into a bound proceedings edited by Dr. John A. Mihm and that will be printed and distributed in May, 1997. The initial mailing will target some 500 cooperators and key libraries worldwide. Given the reduced and specialized nature of research on insect resistant maize, particularly for the tropics, the proceedings should serve as a useful guide, training resource material, and classroom text for years to come.

B: Symposium: Developing Drought- and Low N-Tolerant Maize. March 25-29, 1996

Goals of the meeting were to bring together researchers having an active role in investigating and improving drought- and low N-tolerance of maize, and to share their insights on: distribution, timing and intensity of these stresses; traits related to tolerance to drought and low N; selection methodologies and results from selection experiments; field techniques that improve efficiency of selection; sources of tolerant germplasm; crop management techniques complementing tolerant germplasm; mechanisms for more effective NARS-CIMMYT collaboration in abiotic stress tolerance.

The 4.5 day-long meeting was attended by 121 participants, broken down by origin as: national program invitees: 68; trainees at CIMMYT from national programs: 10; invited experts from US and Australia: 9; representatives from other IARCs: 3; CIMMYT staff from outreach: 9; CIMMYT staff from headquarters: 22. Regional representation was: Latin America and the Caribbean: 40; Africa 24; Asia 14, for a total of 41 countries. Most participants were from public sector institutions, but three were from the private sector, and several others had private sector experience. Absent was representation from NGOs.

The program included 36 oral presentations and 65 posters. About 80% of participants made presentations. The talks were in general of a high standard and simultaneous translation was provided throughout. The central part of the Borlaug Building was transformed into a poster display area, often with excellent results. The first two days of the meeting were devoted to scientific presentations, and were followed by a field visit for about 90 people to the Tlaltizapan Experiment station where participants observed harvests of demonstration materials, explanations of experimental procedures and an overview of CIMMYT's maize breeding program.

The latter third of the meeting took a different tone: the papers were focused mainly on methodologies of direct use to national programs, and a whole day was devoted to consultation with national programs on the best way in which CIMMYT could help them meet their goals. Conference participants were divided into 5 large groups (Asia, lowland Africa, mid-elevation Africa, highland Latin America, lowland Latin America) and into small groups within each of these. CIMMYT staff served as resources in each group. Pre-selected issues were discussed for 2.5 hours, and each main group reported to the plenary session next day. Topics addressed the extent and nature of stresses encountered by maize by region; types of products CIMMYT can best provide; global versus regional organization of maize improvement; germplasm exchange; key regional testing sites; organization and sharing of work ; and possible sources of donor support. The conference concluded with a panel discussion on issues affecting the transfer of technology related to stress-tolerance to the maize fields of small-scale farmers.

Outcomes from the symposium can be summarized as an acceptance that effective selection methodologies for tolerances to these stresses exist, but depend on managed levels of stress for efficient execution; that CIMMYT can best serve national programs by establishing regional breeding programs focused on improvement of regionally-adapted germplasm; and that networks are an acceptable mechanism for regional collaboration provided all participants are treated as equals in planning and execution phases. The Proceedings will be printed and distributed in October, 1997.

Major sponsors were UNDP, Rockefeller Foundation, with assistance from Ciba Seeds and Mahyco India. Conference organizers were Marianne Bänziger, Anne Elings and Greg Edmeades, with extensive help from CIMMYT's Maize Program and Administration.

Appendix 3: Comparison of marker-based and conventional selection for Southwestern Corn Borer resistance

<u>Special Report:</u> Comparison of first generation leaf damage by Southwestern Corn Borer *(Diatraea grandiosella)* and topcross grain yield in tropical maize lines derived from the same population by marker-assisted backcrossing or by conventional backcrossing.

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Introduction

Marker-assisted selection (MAS) is being used increasingly for the rapid and effective transfer of simply inherited traits from one genotype to another with good success, but success has been less frequent for more complexly inherited traits. On the other hand, conventional backcrossing for transfer of simply inherited traits is a routine activity in breeding programs, and its successes in transfer of quantitatively inherited traits are notable and too numerous to report here. One of the major limitations to conventional backcrossing, though, has been linkage drag.

Little information is available about the comparative advantages of MAS over conventional selection. Rapid changes in molecular marker technologies make evaluations difficult, because the effectiveness and efficiency of MAS. Moreover, the selection intensity used in either method will determine its relative advantage. This study was initiated not as comparison of the two methods in order to prove one superior to the other, but rather as a determination of the relative advantages of both methods to aid in designing strategies which blend the best of the two for maximum selection efficiency.

CIMMYT has a long history of breeding maize for host-plant resistance to tropical insects. Several lines have been released which have high levels of resistance to insects, particularly the lepidopteran pests, Southwestern corn borer (SWCB, *Diatraea grandiosella)* and sugarcane borer (SCB, *D. saccharalis).* Unfortunately, these lines have been of little utility for breeders, due to their severe agronomic shortcomings (particularly, poor yield). This study compares BC_2F_3 lines derived from the same original population through two selection methods, marker-assisted or conventional, for their resistance to first generation leaf feeding damage from SWCB. In addition the yield of BC_2F_2 lines as topcross hybrids was evaluated at four locations to determine which method produced superior yielding lines.

Materials and Methods

<u>Population Development</u>

The lines compared in this study were selected from the same BC_1F_1 population [(CML 204/CML 67)/ CML 204] either through marker-assisted backcrossing or conventional backcrossing, where CML 204 is the elite susceptible, recurrent parent and CML 67 is the donor source of resistance to SWCB (see characteristics of parental lines below). The selection procedures for the two methods have been described previously in other reports. CML 204 is an elite line from the CIMMYT maize breeding program in Harare, Zimbabwe, and has agronomic characteristics and disease resistances desirable for southern African. CML 67 is highly resistant to first generation leaf feeding by SWCB but produces low yielding hybrids which are susceptible to several of major diseases of the subtropics. CML 67 is derived from Antigua germplasm characterized by an indistinct heterotic pattern, further limiting the use of the line in hybrid-oriented breeding programs.

Characteristics of parental lines

CML 67	CML 204	
Agronomic quality	poor	good
Grain color	yellow-red	white
Grain type	semident	dent
SWCB resistance[†]	4.6	7.6
MSV resistance[*]	9	2
E. turcicum resistance[§]	susceptible	2
P. sorghi resistance[§]	susceptible	2
Heterotic group[‡]	?	B

† Southwestern Corn Borer resistance: 1 = no damage, 10 = dead
* Maize streak virus: 1 = immune, 9 = totally susceptible
§ 1 = resistant, 5 = susceptible
‡ CIMMYT's heterotic groups are normally described as Tuxpeño (A) or non-Tuxpeño (B)

SWCB-infested trial of BC_2F_3 lines selected by conventional methods or by using molecular markers

Trials were conducted under artificial infestation with SWCB in the winter and summer cycles of 1996 to evaluate lines selected for resistance to first generation leaf feeding damage by SWCB using conventional methods or molecular markers. Both seasons of the trial were conducted at the CIMMYT experiment station in Tlaltizapán, Morelos, Mexico at an elevation of 940 m. The trial comprised 49 entries: 10 were the two parental checks repeated five times (CML 204 or CML 67); 5 were conventionally-selected BC_2F_3 lines; 10 were conventionally selected BC_2F_4 lines derived from the selected BC_2F_3 lines; three were marker-selected BC_2F_3 lines which were homozygous for the resistant genotype (CML 67) at 3 QTL; 6 were individuals homozygous for the CML genotype at only one QTL; 6 were individuals homozygous for the resistant genotype at two regions; and the remainder were individuals homozygous for the resistant genotype at two of the three regions, with the third region being heterozygous.

The 1996A trial was a row-column design (7 rows x 7 columns) with 3 replications, and was infested with 45 neonate larvae of SWCB at the 6-leaf stage. The 1996B trial was conducted as an alpha (0,1) lattice (7 blocks x 7 entries per block) with 3 replications and was infested with 35 neonate larvae of SWCB at the 6-leaf stage. Leaf damage ratings were taken on a 1-10 rating scale (Mihm, 1985), where 1 represents no visible damage and 10 represents a dead plant. Plants were rated on two occasions at approximately 21 and 28 days after infestation.

<u>Yield trial to evaluate topcross hybrids of BC_2F_2 lines selected using conventional methods or molecular markers</u>

Six BC_2F_2 lines selected conventionally and six BC_2F_2 lines selected with molecular markers were crossed with two testers used in the CIMMYT-Zimbabwe breeding program to separate lines into heterotic groups. Tester CML 206 represents the A heterotic group (or Tuxpeño type), and tester CML 202 represents the B (or non-Tuxpeño) heterotic group. The recurrent parent, CML 204, and four BC_2F_3 lines selected conventionally and derived from the conventional BC_2F_2 entries were crossed to the two testers. The two testers were also crossed to each other. Crosses to produce these hybrids were made in 1995A and B cycles in Tlaltizapán. The crossing block for the 1995A cycle was in a particularly calcareous part of the experiment station. Lines CML 204, CML 202, and CML 206 are adapted to southern Africa where soil pH is usually below pH 5, and the soils in Tlaltizapán are around pH 8. Some of the lines suffered severe lime-induced iron chlorosis in the 1995A cycle and sufficient seed was not produced. A subset of the crosses were remade in 1995B. Seed of the cross CML 204/CML 206 was in particularly short supply, so seed of this cross produced at the CIMMYT experiment station in Harare, Zimbabwe, was used for the two Zimbabwe locations. Seed produced at Tlaltizapán was used for the locations at Tlaltizapán and Petit, South Africa.

The resulting hybrid entries were evaluated in a trial designed solely to evaluate yield potential. It was conducted at four locations without artificial infestation. These were: Harare, Zimbabwe; Rattray Arnold Farm, Zimbabwe; Petit, South Africa; and Tlaltizapán, Mexico. The trial consisted of 42 entries in two replications. Plots consisted of two 5 m-long rows. Row-column (6 columns, 7 rows) designs were used in Tlaltizapán and Harare, and alpha (0,1) designs (7 blocks, 6 entries per block) were used at Rattray Arnold Farm and Petit. Data were taken on grain yield, male and female flowering dates, root and stalk lodging, and number of ears per plant.

Results

Results from the infested line trials are shown in Tables 1, 2 and 3. The leaf damage ratings of marker-selected and conventionally-selected lines are averages of the leaf damage ratings from both the 1996A and 1996B cycles. Also shown in Table 1 are the average leaf damage ratings for the two parental lines, CML 204 (recurrent) and CML 67 (donor). Statistical contrasts in Table 2 were performed by comparing the three lines selected with molecular markers which were homozygous for the three QTL regions, and the three best lines selected conventionally, based on their leaf damage ratings as BC_2F_2 families (Table 2). The means of the leaf damage ratings of conventional and marker-selected lines were not significantly different (Table 2). However, both conventional and marker-selected lines had significantly less leaf damage than the original recurrent line (Table 2). Post facto QTL analysis (Table 3), based on the genotypic data of the 24 marker-selected lines and their leaf damage ratings for the two repetitions of the trial showed the region on chromosome 9 as having a highly significant effect in lowering leaf damage ratings. Regions on chromosomes 7 and 10 significantly lowered leaf damage ratings ($P > 0.10$ and $P > 0.05$, respectively). The relative importance of the QTL regions changed between cycles A and B (Table 3), with the region on chromosome 7 having a greater effect in the winter season (96A) and the region on chromosome 9 having a greater effect in the summer season (96B). Analysis included two-way interactions between the three QTL regions, made possible by the fact that several of the entries were homozygous for the donor parent in two of the three QTL regions. Significant epistatic interactions among QTL were observed (Table 3) and were in all cases positive, meaning that interactions between QTL actually increased leaf damage ratings. Particularly noteworthy is the interaction between regions on chromosomes 9 and 10 which raised the leaf damage rating by +0.9 scoring units. This is an increase in leaf damage above the individual effect of the region on chromosome 10 of lowering leaf damage (-0.7). Thus the leaf damage rating of a maize line containing the donor genotype in both regions 9 and 10 would be expected to be 1.3 less than a line containing the recurrent genotype in these two regions. This expected rating is due to the individual effects of the two regions (-1.5 and -0.7) plus the epistatic effect between the two regions (+0.9).

Yield data averaged across the three locations in southern Africa, shown in Table 4, indicate that the marker-selected lines performed better as hybrids, with a yield advantage significant at $P < 0.10$ for combined testers and for tester B. There was no significant difference in yield performance between conventional and marker-selected lines when crossed with tester A (Table 4), the preferred tester for the recurrent parent, CML 204. Hybrids involving lines from both selection methods were compared with hybrids from the recurrent parent, CML 204 (Table 5). Both methods produced hybrids which yield significantly more than the recurrent parent, when the crosses involved tester A or when compared with the combined yields of testers A and B (Table 5). Hybrids involving tester B showed no significant difference in yield between the two selection methods and their recurrent parent (Table 5). Average yields for CML 204 with tester A are usually much higher in southern Africa than those observed in this trial (K. Pixley, pers. comm.). One explanation for the poor performance of CML 204/CML 206 hybrids could be poor seed quality. The fact that there were no significant differences between the experimental lines crossed with CML 206 versus the hybrid CML204/CML 206 at the two Zimbabwe locations (Tables 9 and 11) where the seed source was from the Harare experiment station indicates that seed of this cross, produced at Tlaltizapán, was of lower quality. The seed of CML 204/CML 206 produced at Tlaltizapán appeared normal but had poor germination. As can be seen from the yields of CML 204/CML 206 in Tlaltizapán, Mexico and Petit, South Africa, poor germination caused a significant drop in yield for this hybrid in these locations (Tables 7 and 13). Yields could not be adjusted for plant stand at the Petit site, as stand counts were not available. Lack of adjustment in yield for poor plant stand in this location accounts for the rather large differences between the selected lines and the recurrent parent when averaged across the southern African locations (Table 5).

Table 1. Leaf damage ratings of infested BC_2F_3 lines selected conventionally or with molecular markers (data are an average of two trials conducted in 1996 at Tlaltizapán, Mexico).

Conventionally-selected lines		Marker-selected lines	
38-4-4	6.69	218-40-57	6.71
82(1)-5	6.50	218-40-175	6.84
95-11-3	6.63	218-39-31	6.98
Mean	6.61	Mean	6.84
Parental lines	CML204	7.46	
	CML 67	4.73	

Table 2. Comparison of leaf damage ratings for infested BC_2F_3 lines selected conventionally or with molecular markers and recurrent parent CML 204. Data are means of two trials conducted at Tlaltizapán, Mexico, in 1996.

Lines compared	Leaf damage rating	$P > \|T\|$
Conventional vs marker-selected	6.61 vs. 6.84	0.32 ns
Conventional vs CML 204	6.61 vs. 7.46	0.0001 **
Marker-selected vs CML 204	6.84 vs. 7.46	0.0036 **

Table 3. Effect of QTL regions on leaf damage ratings in BC_2F_3 lines selected using molecular markers.

	Decrease in leaf damage rating from donor genotype		
Region	1996A	1996B	Combined, 1996
Chromosome 7	-0.8 †	-0.4 ns	-0.6 †
Chromosome 9	-0.8 ns	-2.2 **	-.1.5 **
Chromosome 10	-0.5 ns	-0.8 ns	-0. 7 *
Chrom 7 x Chrom 9	+0.1 ns	+0.4 ns	+0.3 ns
Chrom 7 x Chrom 10	+0.4 ns	+0.2 ns	+0.3 ns
Chrom 9 x Chrom 10	+0.5 ns	+1.4 *	+0.9 **

†, *, ** , ns: $P < 0.10$, $P < 0.05$, $P < 0.01$, and $P > 0.10$, respectively

Table 4. Comparison of yields of BC_2F_2 lines selected conventionally or with molecular markers (MAS) and evaluated as topcross hybrids. Data are averages of three southern African locations, 1996.

Hybrids compared	Yield (kg/ha)	*P* > |T|
Conventional vs. MAS, testers A & B hybrids	8070 vs. 8633	0.07 †
Conventional vs. MAS, tester A hybrids	8616 vs. 8959	0.43 ns
Conventional vs. MAS, tester B hybrids	7524 vs. 8306	0.08 †

†, ns: $P < 0.10$ and $P > 0.10$, respectively
Tester A = CML 206; Tester B = CML 202.

Table 5 Comparison of yields of topcross hybrids of the recurrent parent, CML 204, with BC_2F_2 lines selected by conventional or molecular marker selection (MAS). Data are means of three trials conducted at southern African locations in 1996.

Hybrids compared	Yield (kg/ha)	*P* > |T|
Conventional vs. CML204, testers A & B	8070 vs. 6936	0.0001 **
MAS vs. CML204, testers A & B	8633 vs. 6936	0.0001 **
Conventional vs. CML 204, tester A	8616 vs. 5944	0.0001 **
MAS vs. CML 204, tester A	8959 vs. 5944	0.0001 **
Conventional vs. CML 204, tester B	7524 vs. 7928	0.29 ns
MAS vs. CML 204, tester B	8306 vs. 7928	0.32 ns

**, ns: $P < 0.01$ and $P > 0.10$, respectively
Tester A = CML 206
Tester B = CML 202

Table 6 Comparison of yields of BC_2F_2 lines selected conventionally or with molecular markers (MAS), evaluated as topcross hybrids, Tlaltizapán, Mexico, 1996 winter cycle.

Hybrids compared	Yield (kg/ha)	*P* > |T|
Conventional vs. MAS, testers A & B hybrids	8224 vs. 7453	0.06 †
Conventional vs. MAS, tester A hybrids	8047 vs. 8571	0.38 ns
Conventional vs. MAS, tester B hybrids	8401 vs. 6335	0.002 **

†, **, ns: $P < 0.10$, $P<0.01$, and $P > 0.10$, respectively
Tester A = CML 206
Tester B = CM L202

Table 7 Comparison of yields of topcross hybrids of recurrent parent, CML 204, with BC_2F_2 lines selected by conventional or molecular marker selection (MAS), Tlaltizapán, Mexico, 1996 winter cycle.

Hybrids compared	Yield (kg/ha)	$P > \|T\|$
Conventional vs. CML204, testers A & B	8224 vs. 4749	0.0000 **
MAS vs. CML204, testers A & B	7453 vs. 4749	0.0000 **
Conventional vs. CML 204, tester A	8047 vs. 2434	0.0000 **
MAS vs. CML 204, tester A	8571 vs. 2434	0.0000 **
Conventional vs. CML 204, tester B	8401 vs. 7063	0.017 *
MAS vs. CML 204, tester B	6335 vs. 7063	0.19 ns

*, ** , ns: $P < 0.05$, $P < 0.01$, and $P > 0.10$, respectively.
Tester A = CML 206
Tester B = CML 202

Table 8 Comparison of yields of BC_2F_2 lines selected conventionally or with molecular markers (MAS), evaluated as topcross hybrids, Harare, Zimbabwe, 1996.

Hybrids compared	Yield (kg/ha)	$P > \|T\|$
Conventional vs. MAS, testers A & B hybrids	8050 vs. 8452	0.22 ns
Conventional vs. MAS, tester A hybrids	8718 vs. 8908	0.67 ns
Conventional vs. MAS, tester B hybrids	7381 vs. 7997	0.18 ns

ns: not significant
Tester A = CML 206
Tester B = CML 202

Table 9 Comparison of yields of topcross hybrids of recurrent parent, CML 204, with BC_2F_2 lines selected by conventional or molecular marker selection (MAS), Harare, Zimbabwe, 1996.

Hybrids compared	Yield (kg/ha)	$P > \|T\|$
Conventional vs. CML204, testers A & B	8050 vs. 8573	0.06 †
MAS vs. CML204, testers A & B	8452 vs. 8573	0.68 ns
Conventional vs. CML 204, tester A	8718 vs. 9008	0.45 ns
MAS vs. CML 204, tester A	8908 vs. 9008	0.81 ns
Conventional vs. CML 204, tester B	7381 vs. 8138	0.06 †
MAS vs. CML 204, tester B	7997 vs. 8138	0.72 ns

†, ns: $P < 0.10$ and $P > 0.10$, respectively
Tester A = CML 206
Tester B = CML 202

Table 10. Comparison of yields of BC_2F_2 lines selected conventionally or with molecular markers (MAS), evaluated as topcross hybrids, Rattray Arnold Farm, Zimbabwe, 1996.

Hybrids compared	Yield (kg/ha)	*P* > |T|
Conventional vs. MAS, testers A & B hybrids	7151 vs. 7583	0.43 ns
Conventional vs. MAS, tester A hybrids	6838 vs. 7687	0.27 ns
Conventional vs. MAS, tester B hybrids	7464 vs. 7479	0.98 ns

ns: not significant
Tester A = CML 206
Tester B = CM L202

Table 11. Comparison of yields of topcross hybrids of recurrent parent, CML 204, with BC_2F_2 lines selected by conventional or molecular marker selection (MAS), Rattray Arnold Farm, Zimbabwe, 1996.

Hybrids compared	Yield (kg/ha)	*P* > |T|
Conventional vs. CML204, testers A & B	7151 vs. 7415	0.57 ns
MAS vs. CML204, testers A & B	7583 vs. 7415	0.72 ns
Conventional vs. CML 204, tester A	6838 vs. 6869	0.96 ns
MAS vs. CML 204, tester A	7687 vs. 6869	0.22 ns
Conventional vs. CML 204, tester B	7464 vs. 7962	0.45 ns
MAS vs. CML 204, tester B	7479 vs. 7962	0.47 ns

ns: not significant
Tester A = CML 206
Tester B = CML 202

Table 12. Comparison of yields of BC_2F_2 lines selected conventionally or with molecular markers (MAS) evaluated as topcross hybrids at Petit, South Africa, 1996A.

Hybrids compared	Yield (kg/ha)	*P* > |T|
Conventional vs. MAS, testers A & B hybrids	9010 vs. 9871.	0.21 ns
Conventional vs. MAS, tester A hybrids	10296 vs. 10365	0.94 ns
Conventional vs. MAS, tester B hybrids	7724 vs. 9377	0.09 †

†, ns: $P < 0.10$ and $P > 0.10$, respectively
Tester A = CML 206
Tester B = CML 202

Table 13. Comparison of yields of topcross hybrids of recurrent parent, CML 204, with BC_2F_2 lines selected by conventional or molecular marker selection (MAS), Petit, South Africa, 1996.

Hybrids compared	Yield (kg/ha)	$P > \|T\|$
Conventional vs. CML204, testers A & B	9010 vs. 4789	0.00 **
MAS vs. CML204, testers A & B	9871 vs. 4789	0.00 **
Conventional vs. CML 204, tester A	10296 vs. 1915	0.00 **
MAS vs. CML 204, tester A	10365 vs. 1915	0.00 **
Conventional vs. CML 204, tester B	7724 vs. 7663	0.94 ns
MAS vs. CML 204, tester B	9377 vs. 7664	0.05 *

** , ns: P<0.01, and P > 0.10, respectively
Tester A = CML 206
Tester B = CML 202

Conclusions

Both marker-assisted backcrossing and conventional selection produced lines improved for resistance to first generation leaf damage of SWCB compared with line CML 204. The lines derived from the two methods were not statistically different from each other with respect to their leaf damage ratings averaged across two seasons and it must be said, therefore, that both methods were effective at improving line CML 204 for insect resistance. For future marker-assisted selection for SWCB resistance, the region on chromosome 9 could be transferred as a single region. This region certainly has the greatest effect in lowering leaf damage from first generation SWCB. The interaction between regions on chromosomes 9 and 10 makes the region on chromosome 10 not worthwhile for transfer to other lines. The region on chromosome 7 has a null to slightly positive effect, when its main effects and interactions are taken into account. It would be of slight benefit to transfer this region to a susceptible line, but the cost of moving two regions versus one would have to be weighed against its rather small benefit.

Marker-assisted lines generally gave higher yielding hybrids than the conventionally-selected lines, when evaluated as hybrids in the environment where the recurrent parent, CML 204, was properly adapted. This difference was not significant at any single location, though it was when data were combined across the three southern African location (P < 0.10). The only location where conventionally-selected lines gave rise to higher yielding hybrids was Tlaltizapán, Mexico, the site where the conventional selection was conducted. This was not an entirely unexpected result, since the soil type of the Mexican experiment station where the conventional selection and nursery work took place is very different from the southern African locations, particularly for pH. Undoubtedly there was selection in the conventional scheme for vigor under the alkaline soil conditions prevailing at Tlaltizapán. More vigorous plants tend to outgrow leaf feeding damage more quickly, resulting in a lower leaf damage rating. Marker-assisted selection, on the other hand, allows an unbiased recovery of the recurrent parent in all but the QTL regions. This would be an advantage in a situation such as that often encountered in CIMMYT, where we often, because of our global mandate and limited facilities, find it necessary to conduct selection in environments which differ substantially from the target environment.

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Appendix 4: Impact of Acid Soil-Tolerant Germplasm in Colombia: Sikuani, a component of sustainable agriculture in acid soils in the tropics

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Introduction

Lack of sufficient food on a global scale requires the utilization of appropriate technology, both to increase crop productivity and to use new cropping areas such as those characterized by low pH (<5.5), high Al saturation (>40%), and low P content (<8 ppm). Areas with acid soils cover 1,660 million ha, located mainly in the savannas (460 million ha) and the tropical rainforest (1,200 million ha), and representing 40% of tropical soils of the world. In the humid tropics 64% of these acid soils are found in South America, 38% in Asia, 27% in Africa, and 10% in Mexico and Central America.

To increase the maize producing area in the tropics, the development of appropriate technology is required to increase maize production on acid soils, since most of the fertile soils have already been planted to high productivity crops. This technology must be based both on improvement soil management and in generating cultivars tolerant to stresses present in these environments. To achieve the latter objective, it is required to use available knowledge in plant breeding to get fast and positive results, backed up by appropriate policies relating to input and markets in each of the countries affected by these types of soils. Maize can often be a key part of production systems on these soils because of its wide range of adaptation and its near-universal utilization as food and feed.

CIMMYT, in partnership with a number of NARS, has been generating maize-based technology aimed at the rational utilization of acid soils in the tropical areas of the world, through the CIMMYT- South American Regional Maize Program (SARMP). One important component of this has been the development of acid soil-tolerant maize open-pollinated varieties. This report describes the evidence of the utilization and dissemination of one such acid soil-tolerant cultivar, "Sikuani V-110", released in 1995 by ICA (Instituto Colombiano Agropecuario) and CORPOICA (Corporación Colombiana de Investigación Agropecuaria) official agencies of the Colombian government. Sikuani was derived from the genetically broad-based population SA3.

Collaborative research

Even though there is a research network in acid soils that includes developing countries in South America, Asia, and Africa, the results described here are basically the outcome of work done in collaboration with South American countries.

Bolivia

Activities have been established in coordination with the "Instituto Boliviano de Tecnología Agropecuaria" (IBTA), in testing improved acid soil tolerant cultivars, in farmers' fields. The research area is located in the tropical rainforest of the Amazon basin, in Cochabamba and Beni provinces, with soils pH 5.3 and Al saturation from 56 to 81% (Table 1). Under these conditions Sikuani has a grain yield of 3.0 t/ha, which is similar to that of the best check (Table 2). Due to its better plant and grain type, this cultivar has arosed much interest among farmers. A selection program with Sikuani under local conditions will allow the identification and selection of best families with better adaptation to the local stress conditions.

Brazil

Even though Brazil has been actively involved in CIMMYT's acid soil research activities, their interest is more directed towards hybrid development. Several sources of germplasm derived from acid soil-tolerant selections from CIMMYT-Cali have been made available to various research institutions in the country. Among these are acid soil-tolerant inbred lines derived from Populations SA3, SA4, and SA5. Recent observations by CIMMYT regional staff indicate that over 15% of inbred lines derived from these populations show a high level of resistance to *Phaeosphaeria* leaf spot, a disease of great concern for maize production in Brazil.

Colombia

The headquarters of CIMMYT's acid soil research program are established in this country, where improved germplasm is generated and activities are coordinated. Results obtained in coordination with CORPOICA, the Federación Nacional de Cerealistas (FENALCE), and the Centro Internacional de Agricultura Tropical (CIAT) are described below.

Evaluation of new germplasm in collaboration with CORPOICA is done in farmers' fields in the eastern savannas. For the last several years trials have been planted in soils of riversides, savanna, and *vegón*. Riverside and *vegón* are areas located between the riverside and the savanna. The savanna is characterized by lower fertility than riverside and *vegón* areas (Table 3). Table 4 describes the average grain yields of Sikuani and the farmers' variety under two production technologies evaluated at four locations. The differences between these two production technologies are plant density and use of fertilizer. The improved technology included 50,000 plants/ha and 100-60-60 kg NPK, while farmers' technology was 37,000 plants/ha and half the recommended level of fertilizer. Results indicated that by planting Sikuani there was an increase in grain yield under both sets of growing practices. The grain yield of the local variety planted under recommended technology was similar to the yield of Sikuani under farmers' technology. The improved technology increases variable costs by a factor of two; consequently, it would be advisable to use the Sikuani improved cultivar with the farmers' technology (1.91 t/ha as compared to 1.4 t/ha for the local check), since this would generate a net benefit of US$290 as compared to US$217 when the local check is grown (see Table 4).

FENALCE has evaluated maize cultivars produced by public and private institutions both in riverside and savanna conditions in the eastern Colombian savannas (Table 5). Grain yields in riverside locations are twice those obtained in the savanna soils. There was little information available on soil characteristics where the trials were planted, and even though results show that the grain yield of Sikuani is lower than expected under savanna conditions, the inter-institutional coordination that was shown in the execution of these trials is an important positive step towards the identification and dissemination of better cultivars for farmers growing maize in these conditions.

Since 1994 CIAT has generated information on the rational agricultural use of the Colombian savannas and has included Sikuani as one component of an agro-pastoral system (Culticore Project). The native savanna has approximately 80% Al saturation. In the first year of the project two tons of dolimitic lime were applied to the soil to allow more normal development of crops like maize. This was followed afterwards by a renewal of pasture with other crops. Three production systems in the savanna were studied: maize monocrop; maize in rotation with soybean; and maize in rotation with a green manure crop. After three years no differences were observed in maize productivity among these three systems.

Due to low plant density (37,000 plants/ha), maize grain yields were lower in the first year, while production in the second year increased to approximately 4.5 t/ha, making the maize crop an economically feasible alternative. Information generated in future trials will hopefully explain the decrease in grain yield recorded in the third year of these project (Table 6).

The above experiences indicate that from the agro-economic standpoint, maize can be an important component in the agro-silvo-pastoral production system in the acid soil savannas, due both to its range of adaptation and to its varied uses as food and feed. To promote changes in the management of the native savanna and its extensive use in a semi-intensive system which includes improved pastures and various annual crops, scientists at CIAT have evaluated Sikuani in the rotation systems in the savannas. In the Colombian savannas land prices of improved savannas have increased to 2.5 times over those of native savanna, thus providing an incentive for the use by farmers who demand new production alternatives while adopting available technologies. The advantages of renewing pastures are large: renewed pasture supports an increased stocking rate as well as an increased weight gain in cattle.

Examples of this research are described below. In the native savanna (Case 1), the stocking rate was 0.35 animals/ha, while in the non-renewed pastures (Case 2), which includes another crop (i.e, rice or maize) and a late establishment of pastures (*Brachiaria dictyoneura*) after rice, stocking rate was increased to 1.5 animals/ha. Case 3 included native savanna plus, a rice or maize crop, and establishment of *Brachiaria dictyoneura* for four years, followed by another rice crop resulting in an increase of 2.2 animals/ha in stocking rate. These results are even more encouraging when weight gains are considered: gains were 74, 71, and 302 gr/animal/day for Cases 1, 2 and 3, respectively. When a legume is included in the process renewal, the stocking rate is higher than in cases 1 and 2, and the weight gains increase considerably (552 gr/animal/day) (Table 7). Results indicate that the value of the maize crop in a pasture renewal system in the Colombian savannas covers the costs involved in the establishment of improved pastures (Table 8). Surveys on the dissemination of Sikuani in the Colombian savannas (Mallesteros pers. comm.), indicate that more than 90% of farmers in the Meta province in the eastern savannas have utilized Sikuani.

Other studies carried out at CIAT indicate that even though Sikuani is tolerant to acid soils, its main root biomass formation (76%) and root length (75%) are in the top 10 to 20 cm layer of soil (Fig. 1), an area where higher concentrations of available P (20 ppm) and exchangeable Ca (1.4 cmol/kg) are found.

Ecuador

In Ecuador a special block has been selected within the Payamino Expt. Station where acid soil tolerant germplasm is evaluated and selected. In trials under farmers' conditions over the last three years, Sikuani outyielded the local check 2.20 to 1.49 t/ha, while having a better plant type (Table 9).

Peru

In Peru, two locations have been chosen to select acid soil tolerant germplasm, one in the savanna (Alto Mayo) and the other one in the tropical rainforest (Pucallpa). The main characteristics in these locations are described in Table 10. In these locations, plantings have also been established in farmers' fields where Sikuani and a local check were evaluated under two production technologies, namely "improved" and the farmers' technologies. Farmers' technology does not include fertilizer, while the improved technology includes the use of 90-60-0 kg NPK. Results obtained indicate that if the farmer only adopted improved Sikuani cultivar, he would obtain higher grain yields regardless of the production technology (Tables 11 and 12). Higher yields (4.01 t/ha) were obtained with Sikuani grown under the improved technology in Alto Mayo (Table 12). A similar response was observed as in Colombia in 1995 (Table 4), where grain yield of the local variety with the improved technology was equivalent to the yield of Sikuani grown with farmers' technology.

Sikuani shows a higher grain production efficiency as compared to the local cultivar. Evidence indicates that before harvest, the farmer normally assumes that his cultivar will outyield Sikuani due to the more "lush" development of his cultivar. However, Sikuani, with its shorter plant stature and less foliar development, outyields the local cultivar. Based on two year results (1995 and 1996) obtained in farmers' field conditions, the Peruvian government has decided to also release Sikuani in the second semester of 1997. Activities were initiated when 40 kg of basic seed of Sikuani were sent by the CIMMYT-SARMP to the Instituto Nacional de Investigación Agropecuaria (INIA). This seed was planted and mildly selected. INIA is now increasing the seed in five separate 1 ha blocks. In these activities, INIA, the Fundación para el Desarrollo del Alto Mayo (FUNDAM) and CIMMYT have been actively involved.

The cultivar Sikuani is an important milestone of national programs and CIMMYT in the overall strategy aimed at developing sustainable production technologies for acid soils. It is important for the future of this program to continue generating new, improved acid soil-tolerant cultivars. Data recently collected in the Acid Soil IV trial planted in locations throughout South America indicate that, under acid soil conditions, several new nonconventional hybrids (variety x line) show a 50% higher yield over Sikuani (Table 13). In the case of conventional single cross hybrids, results indicate there are yield increases of 64, 70, and 68% over Sikuani (Table 14).

Conclusions

Sikuani V-110 is presently being grown on an estimated 10,000 to 15,000 ha of savanna land in the *llanos* of Colombia. Because it is an open-pollinated variety, farmer-to-farmer transfer of seed has occurred extensively, and actual seed sale statistics have little meaning. Two seed companies in Colombia have undertaken to increase and sell seed. There is little doubt that acid-tolerant varieties such as Sikuani have the potential to increase maize grain yields by 30% or more over local check entries under soil conditions of pH 4-5 and aluminum saturation levels of 60-70%. The original Sikuani cultivar is being released in Peru where it is also expected to have a significant impact. The use of Sikuani in pasture-based rotations in the *llanos* is expected to increase profitability and economic sustainability significantly. New hybrids are under development which may increase production under acid soil conditions by up to 70% over that achieved by Sikuani.

Table 1. Soil characteristics in three farmers' plots that were used in the evaluation of acid-soil, aluminum tolerant germplasm. IBTA, Bolivia, 1995.

Locations	Senda B	Lauca	Litoral
pH	5.5	5.3	5.3
Al sat. (%)	56	61	81

Table 2. Grain yield (t/ha) of Sikuani and two local checks planted at three sites in forest marginal lands. IBTA, Bolivia, 1995.

	Sites			
Cultivar	Senda B	Lauca	Litoral	Mean
Sikuani	4.48	3.33	1.18	3.00
Across LT-102	4.42	3.67	1.15	3.08
Choclero LJ-101	4.18	2.92	1.16	2.75

Table 3. Soil characteristics of farmers' fields where demonstration plots were planted. CORPOICA, Colombia, 1995.

	Site and Soil type			
	Granada		Villavicencio	Guamal
Soil characteristic	Riverside	Savanna	Savanna	Vegón
pH	5.5	4.8	4.2	5.0
P (meq/100 gr)	65	4	49	3
Al (sat. %)	15	68	64	63
Organic matter (%)	3.5	3.6	2.5	4.6

Table 4. Yield, variable costs and net benefits for Sikuani and a local check evaluated in farmers' fields under two technologies (improved vs. farmers), CORPOICA, Colombia, 1995.

Cultivar	Technology	Grain yield (t/ha)	Variable costs US$	Net benefits US$
Sikuani	Improved	2.82	251	365
	Farmer's	1.91	128	290
	Mean	2.36	190	238
Local check	Improved	1.99	232	208
	Farmer's	1.48	112	217
	Mean	1.74	172	212
	LSD (0.05)	0.56	-	-

1 US=1001 Col. Pesos

Table 5. Yield (t/ha) of cultivars evaluated at two sites in the Colombian *Llanos*. FENALCE, Colombia, 1996.

	Site and soil type	
	Granada	San Martín
Cultivar	Riverside	Savanna
ICA V 156	0.78	1.56
AG 612	4.48	1.45
Sikuani	3.12	1.20
ICA V 305	3.58	0.71
Pioneer 3018	4.02	2.67
Mean	3.20	1.52

Table 6. Grain yield (t/ha) of maize in maize-based systems. Culticore, 1994-96.

	Year		
System	**1994**	**1995**	**1996**
Maize monoculture	2.57	4.41	3.29
Maize-soybean rotation	2.78	4.69	3.44
Maize-green manure rotation	2.53	4.38	4.14

Table 7. Stocking rate and weight gains of cattle under different farming systems.

		Brachiaria dictioneura		
Variable	**Savanna**	**Non-renovated[1]**	**Renovated grass alone[2]**	**Renovated grass-legume[2]**
Stocking rate (AU/ha)	0.35	1.5	2.17	1.79
Weight gains (gr/an/d)	74	71	302	552

1/ Established with rice in 1989

2/ Established with rice in 1989 and renovated with rice in 1993.

After: Sanz, J. 1995. CIAT Working document No. 151

Table 8. Economics (US$/ha) of two different farming systems involving maize and pastures in savanna. Colombia, 1994.

Variables	**Maize monoculture**	**Maize + Graminae pastures**
Cost of production	380	416
Maize yield (t/ha)	2.9	3.1
Value of maize	493	527
Value of pastures	-	175
Net benefits	113	286

(1 US=1.001 Col. pesos)

Table 9. Grain yield (t/ha) of Sikuani and other cultivars evaluated at four sites under farmers' conditions. Ecuador, 1995.

	Sites				
Cultivars	El Heno	La Punta	San Carlos	Payamuno	Mean
CIMCALI 93SA5	1.00	3.01	1.80	1.58	1.78
Sikuani	1.59	3.26	1.54	2.17	2.20
INIAP 526	0.49	2.45	1.36	1.92	1.41
Tusilla	0.69	1.89	1.06	1.75	1.49

Table 10. Soil characteristics for two locations where trials were planted in farmers' fields. INIA, Perú 1995-96.

	Site	
	Alto Mayo (Savanna)	Pucallpa (Forest Margins)
pH	4.8	4.4
P (ppm)	8.5	0.4
Al sat (%)	78	51
Organic matter (%)	3.5	1.2

Table 11. Grain yield and plant height for Sikuani and a local check planted under two technologies (improved versus farmers). INIA, Perú, 1995.

Cultivar	Technology	Grain yld (t/ha)	Plt. ht (cm)
Sikuani	Improved	2.10	144
	Farmer's	1.13	130
	Mean	1.62	137
Check	Improved	1.43	165
	Farmer's	0.81	155
	Mean	1.12	160
	LSD (0.05)	0.58	

Table 12. Grain yield, plant and ear heights for Sikuani and the local check planted under two technologies at two sites. Alto Mayo, Perú, 1996.

Cultivar	Technology	Yield (t/ha)	Height (cm) Plant	Height (cm) Ear
Sikuani	Improved	4.01	195	143
	Farmer's	2.41	187	94
	Mean	3.40	212	118
Check	Improved	2.80	215	112
	Farmer's	2.78	208	96
	Mean	2.60	191	104

Table 13. Grain yield of Sikuani, the best variety, and the best hybrid (variety x line) evaluated under acid soil (Alto Mayo, Perú) and normal soil conditions (Turipaná, Colombia), 1996B.

	Alto Mayo		Turipaná		Across sites	
Cultivar	(t/ha)	(%)	(t/ha)	(%)	(t/ha)	(%)
Sikuani	2.5	100	3.9	100	3.2	100
Best variety	3.1	124	4.0	102	3.4	106
Best hybrid	4.4	176	5.9	151	5.2	162

Table 14. Yield of Sikuani and the best conventional single cross hybrid at three acid soil sites, Colombia, 1996B.

	Sites					
	SQ		Villavicencio 1		Villavicencio 2	
Cultivar	(t/ha)	(%)	(t/ha)	(%)	(t/ha)	(%)
Sikuani	4.20	100	4.20	100	3.80	100
Best hybrid	6.89	164	7.14	170	6.40	168

Appendix 5: Impact of Insect-Resistant Germplasm in Farmers' Fields and in National Breeding Programs

Utilization of sources of resistance in maize for management of Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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Summary

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) is a significant pest of maize in the Americas. Host plant resistance (HPR) in maize to FAW could constitute a very important component of integrated pest management (IPM), because of its economic viability, environmental safety and compatibility with other pest management tactics. The objective of this study was to examine FAW resistance and agronomic traits of certain hybrids made from crosses between and among insect resistant lines and agronomically elite lines developed at CIMMYT. The insect resistant lines used belonged to two categories: 1) insect resistant CIMMYT maize lines (CML-IR) with high level of inbreeding (S_7 level or greater): CML67, CML139, CML135, CML69, and 2) CML-IR lines with low level of inbreeding (S_2 level) derived from Population 390: examples: 94171, 94172, 94072, 94164 and 94060, or no inbreeding as in the case of populations GCA-FAW and GCA-SCB. The agronomically elite but non-insect resistant (CML-AG) lines used were: CML247, CML254, CML285, CML287 and CL00331. The following three categories of hybrids were examined in four different experiments: 1) CML-AG x CML-AG; 2) CML-AG x CML-IR (high inbreeding); and 3) CML-AG x CML-IR (low inbreeding). Checks included CML67 x CML135 (resistant check), Ki3 x CML69 (susceptible check), and *Criollo* (local populations maintained by farmers). In the first experiment, the hybrids (Ki3 x CML131, CML135 x CML139 and CML67 x CML135) infested at the 6-leaf stage suffered significantly higher leaf feeding damage by FAW than plants infested at the 4-leaf stage. Grain yield losses suffered by the hybrids were the same at both phenological stages at infestation. In the second experiment, 24 hybrids were evaluated for leaf feeding resistance and CML-IR lines suffered from lower leaf feeding. The hybrids (CML-AG x CML-AG) suffered considerable damage by FAW but still produced the highest grain yield. Leaf feeding resistance in the CML-AG x CML-IR hybrids did not significantly reduce yield losses. Leaf feeding damage by FAW also did not cause any reduction in the growth of the CML-AG x CML-AG hybrids except that of CML247 x CML254. Growth of CML-AG x CML-IR hybrids was, however, significantly reduced. It is concluded that agronomic traits, plant growth and FAW resistance are governed by independent sets of genes. **On-farm trials showed that although CML-AG x CML-IR hybrids suffered less damage by FAW than CML-AG x CML-AG hybrids, grain yield of agronomically elite hybrids was nonetheless significantly higher under this moderate level of insect attack, where around 30% of the plants showed symptoms of leaf damage. Had the insect attack been rather more severe, the improved resistance of the IR sources would likely have resulted in higher yields.** Farmer interest in the source hybrids that showed less damage was, nonetheless, high. It is suggested that through conventional backcrossing procedures, inbred lines with desired levels of resistance and agronomic traits can be developed for utilization in breeding programs to produce FAW resistant hybrids with high yields.

Introduction

Host plant resistance (HPR) in maize has been demonstrated against several species of insects (Anonymous, 1989; Kumar, 1997). The International Maize and Wheat Improvement Center (CIMMYT) has developed several sources of resistance in maize against an array of field pests which include stem borers, fall armyworm and diseases prevalent in Asia, Americas and Africa (Smith et al., 1989; Mihm, et al., 1996). The most important sources of resistance include CIMMYT maize inbred CMLs derived from Antigua sources; i.e., CML 67, CML139, and lines derived from populations 390 (MIRT) and 590 (MBR). Resistance in these sources has been reported by others (Mihm, et al., 1988; Smith et al., 1989; Kumar, 1994; Kumar and Mihm, 1995). Antibiosis resistance in these sources is heritable and has been reported to be expressed in the hybrids between resistant and susceptible lines (Kumar and Mihm, 1995; 1996a).

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera:Noctuidae) is an economically important pest of maize in the Americas. The magnitude of yield losses caused by FAW in farmers' fields is not well documented, but is thought to be high. According to van Huis (1981), yield losses from FAW can be 40-50% under experimental conditions. In order to reduce these losses, host plant resistance is a favored strategy because it is environmentally safe, economically viable and compatible with other pest management tactics.

To quantify levels of resistance in maize to FAW, previously reported studies have relied mainly on visual ratings of leaf feeding damage, larval survival and growth on different genotypes (Wiseman et al., 1966; Widstrom et al.,1972; Williams et al., 1978; Wiseman et al., 1986; Williams at al., 1989). The relationship between leaf feeding, resistance of maize genotypes and their grain yield has not been reported. Attempts to develop germplasm that possesses resistance to insects have often not emphasized a corresponding improvement in the grain yield of these resistant sources. Generally, experience at CIMMYT has shown that a high level of resistance in a genotype has not been associated with high grain yield in the absence of insects (such germplasm will henceforth be referred to as "CML-Insect Resistant," or CML-IR). High yielding hybrids/varieties developed by CIMMYT, though not showing antibiosis reaction to insects, possess a good level of tolerance against stem borers and fall armyworms, and these genotypes produce high yields under moderate insect attack. Inbred lines showing these traits are referred to as CML-agronomically good (CML-AG) (Kumar, 1994). About 80% of the inbred lines released by CIMMYT belong to this category. No systematic effort has been made to examine the performance of hybrids of the type CML-IR x CML-AG, despite the potential usefulness of this information for national program and seed company breeders, who must determine whether further intensification of antibiosis resistance is justified in their selection program and what the role of resistant source germplasm may be. The objective of the present study was to examine hybrids of this type for FAW resistance and agronomic characteristics.

Materials and Methods

Experiments were conducted during 1995-1996 at the following locations :

- CIMMYT's lowland tropical experimental station at Poza Rica (60 masl; 21 °N)
- A farmer's field at Zapotalillo, Veracruz, situated 50 km NW of Poza Rica station at 60 masl.
- A farmer's field at Buena Vista, Veracruz, situated 40 km north of Poza Rica station at 60 masl.

For this study CML-AGs comprised CML247 (white), CML254 (white), CML285 (yellow), CML287 (yellow), and CL00331 (yellow). These lines and hybrids CML247 x CML254; CML247 x CML274; CL00331 x CML287; CML264 x CML258; and CML264 x CML273 were developed by Dr. S.K. Vasal, CIMMYT's lowland tropical maize breeder.

Lines in the CML-IR class were: **Category 1**: Five S_2 lines derived from population 390 (MIRT): 94171, 94172, 94072, 94164 and 94060 selected on the basis of nine CIMMYT International Insect Resistance Progeny Trials (IRPT) conducted in 1994, and Populations GCA-FAW and GCA-SCB developed for resistance against fall armyworm and sugarcane borer. **Category 2 lines were** highly inbred with known resistance to stem borers and FAW (Kumar and Mihm, 1995; 1996a; 1996b):, namely: CML67, CML139, CML135 and CML69. The lines were planted in the summer cycle of 1994 at Poza Rica station. Reciprocal crosses were made among and between CML-AG and CML-IR lines, resulting in the hybrids listed in Table 1.

Experiment 1: For this study, one susceptible (Ki3 x CML131) and two resistant (CML67 x CML135 and CML135 x CML139) hybrids were used (Kumar and Mihm, 1995; 1996a). The hybrids were planted 10 d apart in two adjacent blocks to have two early plant growth stages for simultaneous infestation with FAW. Plot size was two rows 5m in length . The row-to-row and plant-to-plant spacings were 75 and 25 cm, respectively, and the design was an RCBD with three replications. On the day when the plants in the first and second block were at the 4- and 6-leaf stage, respectively, the first 10 plants were infested with 40 FAW larvae per plant using a bazooka (Kumar and Mihm, 1995). The plants behind those infested were protected with the insecticide, Ambush (FMC Corp., Mexico), @ 2 kg a.i./ha, for yield comparisons. The plots were fertilized at 50 kg P_2O_5/ha before planting and 150 kg N/ha in split doses, with half applied before planting and half six weeks later. Two weeks after infestation, plants in both the blocks were rated for leaf feeding damage by FAW on a scale of 0-9 (0 = no damage; 9 = extensive damage leading to the death of plant) (Davis et al., 1989; Mihm, 1989b). At harvest, ears of infested and protected plants in plot were harvested, shelled grain weighed and adjusted for moisture, and the percent loss due to insect damage calculated from the infested and protected plots.

Experiment 2: This experiment was conducted at the Poza Rica station with 24 hybrids (see Table 1). Three trials were conducted during 1995-1996. All were planted on seed beds prepared with conventional tillage. For each trial, hybrids were planted in a split plot design with three replicates, where hybrid was the main plot and treatment (protected/infested) the subplot. Plots consisted of 4 rows 75 cm apart and 2.5 m long, with plants spaced 25 cm apart. Fertilizer was applied as before. Two rows of each 4-row plot were protected with Ambush and the other two rows were artificially infested with FAW larvae, obtained from a laboratory culture maintained on artificial diet (Mihm, 1989a). When the plants reached the 5- to 6-leaf stage, each infested plant received 50-60 FAW larvae. For infestation, the larvae were mixed with corn cob grits and placed in the leaf whorl with a bazooka.

Twenty days after infestation the plants in the infested and protected plots were rated for leaf feeding damage by FAW on the 0-9 scale described above. On the basis of leaf damage ratings, hybrids were categorized for their resistance/susceptibility against FAW as highly susceptible (8-9), susceptible (7-8), moderately resistant (5-7), resistant (3-5), highly resistant (0-3) and immune (0). At harvest, plant height for each hybrid was measured from the base to the flag leaf. Ears from all plants in infested and protected subplots of each hybrid were harvested separately and weighed; a sample was shelled to determine shelling percentage, and yields were adjusted for grain moisture and shelling percent. Grain weights were classified as high (> 8 t/ha), medium (5- 8 t/ha), and low (< 5 t/ha). Data were subjected to analyses of variance within and across experiments.

Experiment 3: This was conducted in the farmers fields at Zapotalillo during 1995 and 1996 under rainfed conditions. During each season, 13 hybrids were grown in an RCBD with two replicates under zero tillage. Each plot consisted of two rows, 5 m long, 75 cm between rows, and plots were overplanted by hand into moist soil and later thinned to 2 plants per hill in hills 50 cm apart. Six weeks after planting, plots were fertilized with 90 kg N/ha. Weeds were controlled by herbicide, and plants were exposed to the prevailing natural populations of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) and stem borer, *Diatraea lineolata*. Six weeks after planting, the numbers of plants damaged by FAW and stem borer in each plot were recorded. At harvest, plant height was recorded, and grain yield measured as in Experiment 2. Stems of 10 randomly selected plants from each plot were split open and the number of tunnels made by the stem borers in each hybrid was measured. Data were analyzed by year and across years.

Experiment 4: One trial was planted at Buena Vista in 1995, but some entries were destroyed by rabbits. In 1996, two adjacent trials were planted under zero tillage -- one with a maize stalk residues mulch and one without. In each trial, 16 hybrids were planted (Table 1) in an RCBD with two replicates. Plot practices, data recorded and analytical procedures were as for Experiment 3.

Results

Experiment 1: When the three hybrids Ki3 x CML131, CML67 x CML135, and CML135 x CML139 were infested with FAW at the 4- and 6-leaf stages, respectively, during 1995 and 1996, leaf damage caused by larvae differed significantly between the two phenological stages, seasons, and among hybrids. When infested at the 6-leaf stage, the susceptible hybrid Ki3 x CML131 suffered significantly greater damage (damage rating = 8.0) from FAW than the resistant hybrid CML67 x CML135 (damage rating 6.0) or CML135 x CML139 (damage rating = 6.4). Leaf damage rankings were the same when infestation was done at the 4-leaf stage (damage ratings = 5.4-5.9), but significantly less than at the 6-leaf stage (damage rating = 7.0). This difference was consistent over seasons, and the hybrid x season interaction for damage at these stages was non-significant. The phenological stage of infestation did not affect the percent yield loss suffered by hybrids; both stages suffered losses of 16.0-19.2%. There was significant crop phenology x season interaction; during the first season losses were the same (15-20%) regardless of growth stage at infestation, but in the second season infestation at the 6-leaf stage reduced yields more than infestation at the 4-leaf stage.

Experiment 2: Infestation of 24 hybrids with FAW at Poza Rica station for three seasons resulted in consistent significant differences between infested and protected sections of the plots, and among hybrids, for leaf feeding damage, plant height, ear and grain weight and shelling percentage (ANOVA not shown). There were significant interactions between hybrid, season, and infested vs. protected treatments, so seasons were analysed separately (ANOVAs not shown). Again, traits differed significantly between infested and protected plots. The interaction between hybrids and protected/infested plots was generally not significant across seasons except for leaf feeding damage by FAW. During Season 1 leaf feeding damage, ear and grain weights, shelling percentage, and plant height varied significantly among hybrids (Table 6). Leaf feeding damage by FAW was high (7-.9) on hybrids between CML-AG lines (CML247 x CML254, CML247 X CML274, CL00331 x CML287) and between CML-AG and materials belonging to the first category (*Criollo* and Ki3 x CML69, susceptible check), CML285 x Ki3, MIRT94171 x CML254, and CML247 x MIRT94172 or MIRT94072). Leaf feeding damage was moderate (5-7) on CL00331 x GCA-SCB, CL00331 x CML287, *Criollo*, CML247 x MIRT94171, and MIRT94164 and CML285 x GCA-SCB. Leaf feeding damage was low (3-5) for CML287 x CML139; CML135 x CML139 (resistant check), CML264 x CML258, CL00331 x CML139, CML285 x CML67, and CML287 x CML67 (Table 6). Ear weights for most hybrids were moderate (3-4 kg/plot) except for the hybrids CML264 x CML258, CML247 x CML254, CML247 x CML274, CML264 x CML273, which had high (> 4 kg/plot) ear weights (Table 2). Plant height in the infested plots was significantly lower than that in the protected plots. FAW infestation caused a significant reduction in the ear or grain yield across hybrids as depicted in percent grain losses due to infestation (Fig. 1).

In the second season, the leaf damage ratings remained low and most hybrids exhibited resistance to FAW (Table 7). The resistant check, CML135 x CML139, and the *Criollo* displayed little leaf feeding damage comparted with other hybrids. Higher ear weights were reserved for CML285 x GCA-SCB, CML264 x CML273, CL00331 x CML287, CML254 x MIRT94171 (Table 3). Grain yield of the following 12 hybrids was moderate (3-4 kg/ha): CML285 x GCA-SCB, CML264 x CML273, CML264 x CML258, CL00331 x CML287, CML247 x CML274, CML254 x MIRT94171, CML254 X GCA-FAW and CML285 x KI3 (Table 7), while that of remaining 12 hybrids was low (1-3 kg/ha). The difference in plant height of hybrids between infested and protected plots was significant. FAW-infested plots had significantly lower yield than protected plots (Fig. 2).

During the third season leaf feeding damage was high on the hybrids between CML-AG lines (CL00331 x CML287, CML247 x CML254, CML264 x CML258, CML264 x CML273, CML247 x CML274) and between CML-AG and resistant sources belonging to the first category (CML247 x MIRT94172, CML247 x MIRT94171, CML247 x MIRT94072, CML247 x MIRT94164, CML285 x GCA-SCB, Ki3 x CML69, CML285 x Ki3) (Table 8). The remaining hybrids were moderately resistant to FAW (Table 8). Ear and grain weights of almost all hybrids were low (1-3 kg/plot) (Table 4).

In order to examine whether degree of variability among hybrids was significant or not, an analysis of covariance was conducted using percent yield loss as a dependent variable and yield of protected plots as the independent variable. The analysis indicated that the percent yield loss suffered by the high yielding hybrids was greater than that by the low yielding hybrids. Such a relationship was expected because parents of hybrids with high yields were developed principally for good agronomic traits and not necessarily for insect resistance.

Experiment 3: When 13 hybrids were planted in Zapotalillo during 1995-1996, the percentage of plants damaged by insects, leaf damage ratings, ear weights, and plant heights did not differ significantly between the two seasons (Table 5). Number of tunnels made by the stem borers in the stalks of hybrids, grain weights and shelling percentages did, however, differ between seasons. Hybrids differed significantly for all traits measured except leaf damage ratings (Table 5). Hybrid x season interaction was not significant for leaf feeding damage or ear and grain weight, but was significant for number of borer tunnels, shelling percentage, and plant height (Table 5).

The percentage of plants damaged by stem borers and FAW was the highest for the hybrids between CML-AG lines (CML247 x CML274, CML247 x CML254) and the lowest for the hybrids between CML-AG and resistant sources (CML287 x CML139, CML285 x CML139 or CML67) (Table 6). Yields were high (> 8 t/ha) for the hybrids between CML-AG lines (CML247 x CML274 and CML247 x CML254), moderate (5-8 t/ha) for the remaining hybrids, but low for (< 5 t/ha) for CML135 x CML139 (resistant check and for the *criollo*. No stalk damage was observed during harvest in the first season. During the second season, the stems of most hybrids were moderately tunneled (2-4 tunnels/stem) by the stem borer, *D. lineolata* (Table 7).

Experiment 4: When 16 hybrids were planted at Buena Vista in the presence and absence of mulch, the percentage of plants showing leaf feeding damage or stalk rot, ear and grain weights, and plant height differed significantly between the two mulch treatments (Table 8), but the hybrid x mulch interaction was not significant, so data were combined. Leaf feeding damage caused by FAW and stem borers was low (< 3) (Table 9). The incidence of stalk rot was high (40-60%) on the hybrids between CML-AG and insect resistant (CML285 x CML139, CML287 x CML67, CML285 x CML67), but moderate (20-40%) on CL00331 x GCA-SCB, CML135 x CML139, and CML69 x CML139, and low (0-20%) on hybrids between CML-AG and insect resistant sources belonging to the first category (Table 13). Tunneling by stem borers was high (> 4 tunnels/stem) on *Criollo*, CML287 x CML139, CL00331 x CML287, CML287 x CML67, CL00331 x CML67, CML69 x CML139, CML247 x CML274/ MIRT, CML247 x CML254, CML247 x CML274, and CML247 x MIRT172, and moderate on the remaining hybrids. Yields of the 13 hybrids were moderate (5-8 t/ha) while those of Ki3 x CML69, CML285 x CML139 and CML135 x CML139 were low (< 5 t/ha).

Discussion

Our data show that although hybrids infested at the 6-leaf stage suffered higher leaf feeding damage by FAW than plants infested at the 4-leaf stage, yield losses were the same at both phenological stages at infestation. Hybrids were clearly classed into leaf feeding categories from resistant to susceptible by infestation at the 6-leaf stage, but this did not correlate with grain yield losses. Hybrids may sustain high leaf feeding damage by FAW with little or no yield reduction. Thus, in a breeding program for FAW resistance, grain yield-infestation relationships must be clearly understood so that both traits can be improved simultaneously.

Our data show that the antibiosis resistance in the CML-IR lines was clearly manifested in CML-AG x CML-IR hybrids. Resistance genes in the highly inbred lines derived from Antigua germplasm (CML67, CML139) were much more effective than those derived from Population 390 (MIRT) in inhibiting FAW leaf feeding damage in hybrids. Inbred lines derived from Antigua germplasm possess a high frequency of genes for resistance against insects (Bohn et al., 1996) and by crossing such lines with those having a high frequency of genes for agronomic traits (Vasal et al., 1994), leaf feeding resistance and good yield can be obtained. For example, when either CML287 or CL00331were crossed with a FAW resistant source (CML67), the resulting hybrid was relatively undamaged by FAW. In spite of improvement for resistance, the grain yield of the resistant hybrids remained lower than that of CML287 x CL00331.

Leaf feeding resistance among the hybrids did not cause a significant improve yields under FAW infestation. The grain yield losses suffered by the hybrids involving CML67 (highly resistant to FAW) did not differ from those of the susceptible hybrid (Ki3 x CML69) and hybrids among CML-AG lines. The hybrid CML247 x MIRT94072 suffered considerable damage by FAW but suffered little grain yield loss. Percent grain yield reduction was positively correlated with FAW damage on the susceptible Ki3 x CML69, CML264 x CML258 and CL00331 x CML287. The inbred line CL00331 combined well with resistance sources, giving good leaf feeding resistance and little grain yield reduction. FAW damage on high yielding hybrids caused a greater absolute grain yield reduction than that on low yielding hybrids such as CML285 x CML139 and CL00331 x CML139, but the high yielding hybrids still outyielded the those made from IR lines.

The hybrids CML254 x CML247, CML247 x CML274, CML264 x CML258, and CML264 x CML273 yielded the best under conventional tillage and irrigation on station, but yields of these hybrids were equally high under zero tillage and rainfed conditions in farmers' fields. CIMMYT hybrids proved superior to the *Criollo* in insect resistance, plant height and grain yield. The farmers at both locations (Buena Vista and Zapotalillo) displayed keen interest in CIMMYT germplasm because hybrids were higher yielding and less susceptible to lodging due to a shorter plant type. Hybrids grown in the presence of mulch at Buena Vista were taller and higher yielding than under no mulch, though they suffered more stalk rot. Although hybrids involving CML67 or CML139 as one of the parents displayed leaf feeding resistance against FAW, stalk rot incidence was high. The *criollo* which has been grown by the farmers for many years had little stalk rot, though its grain is known to be highly susceptible to larger grain borer, *Prostephanus truncatus* (Kumar, unpublished data).

In conclusion, resistant populations, lines, and hybrids developed at CIMMYT can be used effectively as source germplasm to reduce damage and grain yield reductions caused by FAW and stem borers. Quite effective insect control and yield can be obtained by crossing IR and AG lines. Data from these studies suggest that it is unlikely that IR materials will be released directly for use by farmers, except possibly for areas where maize is grown primarily as a forage crop and insect pests pose a serious threat to biomass production. Breeding strategies will have to be devised to develop lines, hybrids and varieties with high grain yield *and* high levels of antibiosis against FAW and stem borers. Through conventional backcrossing procedures and newer marker-assisted methods, inbred lines with desired levels of insect resistance and agronomic traits can be developed for use in hybrid breeding programs. Such breeding strategies should be supported by careful experimentation in farmers' fields to determine the desirable level of resistance needed to keep FAW populations below economic injury level. Information of this kind will assist in delivering hybrid products with good yield potential and stable production in areas prone to FAW and stem borer attack.

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Table 1. Hybrids and varieties used for various experiments

Hybrid / variety
CML 247 X CML 254
CML 247 X CML 274
CML 264 X CML 273
MIRT 94171 X CML 254
Ki3 X CML 69 (susceptible check)
CLOO331 X MIRT 94060
CML 254 X GCA-FAW
CML 247 X MIRT 94072
CML 247 X MIRT 94172
CML 285 X Ki3
CL00331 X GCA-SCB (topcross)
CL00331 X CML 287
Criollo (Local check)
CML 247 X MIRT 94171
CML 247 X MIRT 94164
CML 285 X GCA-SCB (topcross)
CML 287 X CML 67
CML 287 X CML 139
CML 135 X CML 139 (resistant check)
CML 264 X CML 258
CL00331 X CML 139
CML 285 X CML 67
CML 285 X CML 139
CL00331 X CML 67
CML67 x CML135
CML69 x CML139
CML247 x CML274/MIRT

Table 2. Leaf feeding damage, grain weight, shelling percentage and plant height of 24 hybrids-varieties infested with FAW in Season 1.

Hybrid/Pedigree	Leaf[a] damage ratings	Grain weight (kg/plot)	Shelling percentage	Plant height (cm)
CML 247 X CML 254	8.0	3.4	81	213
CML 247 X CML 274	8.0	3.0	80	231
CML 264 X CML 273	7.9	3.3	79	228
MIRT 94171 X CML 254	7.6	2.9	77	239
Ki3 X CML 69	7.4	2.4	78	215
CL00331 X MIRT 94060	7.3	2.3	80	213
CML 254 X GCA-FAW	7.3	3.0	79	231
CML 247 X MIRT 94072	7.1	2.9	74	233
CML 247 X MIRT 94172	7.1	2.5	74	216
CML 285 X Ki3	7.0	2.8	80	218
CL00331 X GCA-SCB	6.9	2.5	79	216
CL00331 X CML 287	6.8	3.0	78	243
Criollo (Local Check)	6.8	2.6	82	306
CML 247 X MIRT 94171	6.8	2.6	73	220
CML 247 X MIRT 94164	6.6	2.6	78	230
CML 285 X GCA-SCB	6.4	3.1	81	228
CML 287 X CML 67	6.3	2.3	77	225
CML 287 X CML 139	6.1	3.1	86	254
CML 135 X CML 139	6.0	2.9	86	214
CML 264 X CML 258	5.7	3.5	80	226
CL00331 X CML 139	5.6	2.7	85	229
CML 285 X CML 67	5.3	2.5	80	221
CML 285 X CML 139	5.2	2.5	84	242
CL00331 X CML 67	5.1	2.3	78	201
LSD at P = 0.05	1.4	0.4	1.5	9

[a]Based on a scale of 0-9; [b]Yield of 4- row plot (2 rows infested and 2 rows protected)

Table 3. Leaf feeding damage, grain weight, and shelling percentage of 24 maize hybrids combined over FAW-infested and insecticide-protected plots in season 2.

Hybrid/Pedigree	Leaf damage ratings	Grain weight (kg/plot)	Shelling percentage
CML 264 X CML 258	4.7	3.3	85
CML 247 X MIRT 94172	4.4	2.9	81
CML 264 X CML 273	4.4	3.5	84
Ki 3 X CML 69	4.3	2.8	84
CML 247 X CML 254	4.1	3.0	79
CML 285 X CML 67	4.0	2.3	84
CML 247 X CML 274	3.9	3.2	83
CML 247 X MIRT 94171	3.8	2.8	80
CML 287 X CML 67	3.8	2.4	81
CML 247 X MIRT 94164	3.7	2.5	81
CL00331 X CML 287	3.7	3.3	81
CL00331 X CML 67	3.7	2.1	82
CML 285 X GCA-SCB	3.7	3.6	85
CML287 X CML 139	3.7	2.9	89
CML285 X CML 139	3.6	3.1	89
CL00331 X CML 139	3.6	2.7	90
CL00331 GCA-SCB	3.5	2.6	83
CML 247 X MIRT 94072	3.5	3.0	80
CML 254 X GCA-FAW	3.5	3.2	83
MIRT 94171 X CML 254	3.5	3.3	82
CML 285 X Ki3	3.4	3.2	85
CL00331 X MIRT 94060	3.3	2.7	84
CML 135 X CML 139	2.5	2.3	87
Criollo (Local Check)	2.1	3.1	87
(LSD at P=0.05)	0.8	0.4	3.2

Table 4. Leaf feeding damage and grain weight of 24 hybrids combined over FAW-infested and insecticide-protected plots in season 3.

Hybrid/Pedigree	Leaf damage ratings	Grain weight (kg/plot)
CML 247 X MIRT 94172	8.0	1.6
CL00331 X CML 287	8.0	1.7
CML 247 X CML 254	7.7	2.1
Criollo (Local Check)	7.7	0.7
CML 264 X CML 258	7.7	1.9
CML 247 X MIRT 94171	7.7	1.7
Ki X CML 69	7.7	1.6
CML 264 X CML 273	7.3	1.8
CML 247 X MIRT 94072	7.3	1.7
CML 247 X CML 274	7.3	1.9
CML 247 X MIRT 94164	7.3	1.7
CML 285 X Ki3	7.0	1.5
CML 285 X GCA-SCB	7.0	1.5
CML 254 X GCA-FAW	7.0	1.7
CML 254 X MIRT 94171	6.7	1.6
CL00331 X GCA-SCB	6.7	1.7
CML 285 X CML 139	6.3	1.7
CML135 X CML 139	6.3	1.7
CL00331 X CML 67	6.0	1.4
CL00331 X MIRT 94060	6.0	1.6
CL00331 X CML 139	6.0	1.6
CML 285 X CML 67	5.7	1.4
CML 287 X CML 67	5.7	1.5
CML 287 X CML 139	5.7	2.0
LSD at P = 0.05	0.9	0.4

Table 5. Combined analysis of variance for damage ratings and counts, and yield of 13 maize hybrids grown under natural infestation in Zapotalillo in farmers' fields over two seasons.

Source	df	Percent damaged plants	Leaf damage rating	Number of tunnels	Grain yield
Season (S)	1	4.3 NS	15.9NS	72.6**	25.9*
Error mainplot	2				
Hybrid (A)	12	2.6*	1.6NS	2.3*	3.0*
S x A	12	1.5NS	0.8NS	2.3*	0.8NS
Error subplot	24				

*, ** = significant at P = 0.05 and P = 0.01, respectively. NS = non-significant.

Table 6. Percentage of plants damaged and yield of 13 maize hybrids over two seasons in farmers' fields at Zapotalillo, Mexico.

Hybrid/pedigree	% plants showing leaf damage	Grain yield (kg/ha)
CML 247 X CML 274	44.3	6987
CML 247 X CML 254	41.5	6591
CML 254 X GCA-FAW	37.0	5104
Criollo	35.2	4836
CML 287 X CL00331	34.7	5380
Ki3 X CML 69	32.1	5550
CL00331 X GCA-SCB	31.4	5526
CML287 X CML 67	30.4	5304
CL00331 X CML 67	24.7	5059
CML 285 X CML 139	21.3	5893
CML 285 X CML 67	20.7	5300
CML 287 X CML 139	17.3	5815
CML 135 X CML 139	12.1	4852
LSD at P = 0.05	18.4	1027

Table 7. Plant height, shelling percentage, and number of tunnels made by *Diatraea lineolata* larvae on 13 maize hybrids in farmers' fields at Zapotalillo, Mexico, in 1995 (Season 1) and 1996 (Season 2).

	Plant height		No. tunnels/stem[a]	
Hybrid/Pedigree	Season 1	Season 2	Season 1	Season 2
CML 247 X CML 274	174	169	0	2.3
CML 247 X CML 254	156	167	0	1.4
CML 254 X GCA-FAW	164	173	0	1.6
Criollo	271	238	0	3.8
CML 287 X CL00331	190	188	0	2.9
Ki3 X CML69	163	166	0	2.1
CL00331 X GCA-SCB	165	168	0	2.9
CML 287 X CML 67	168	165	0	1.3
CL00331 X CML 67	145	160	0	2.8
CML 285 X CML 139	166	173	0	2.3
CML 285 X CML 67	146	171	0	3.1
CML 287 X CML 139	159	189	0	3.2
CML 135 X CML 139	142	158	0	2.5
LSD at P = 0.05	141.5	158.0	89.7	84.0

[a] Damage under natural infestations of stem borers.

Table 8. Analysis of variance for leaf damage, stalk damage, plant height and yield for 16 hybrids grown with and without mulch under natural conditions in farmers' fields at Buena Vista, Mexico.

Source of variation	df	% plants damaged	% plants with stalk rot	No. tunnels/ plant	Grain yield
Mulch (M)	1	24.2**	35.6**	0.3NS	3.5*
Error mainplot	2				
Hybrid (A)	15	2.6*	7.2**	2.9*	4.3**
A x M	15	1.8NS	1.1NS	0.5NS	0.7NS
Error subplot	30				

Table 9. **Damage by *D. lineolata*, stalk rot, grain yield and plant height of 16 hybrids combined over mulch and without mulch in farmers' fields at Buena Vista, Mexico.**

Hybrid /Pedigree	% plants with leaf damage (6 wk post-planting)	% plants stalk rot (harvest)	No. tunnels/ plant (harvest)	Grain yield (kg/ha)	Plant height (cm)
CML 247 X CML 274	2.3	12.5	4.3	4665	133
CML 247 X CML 254	2.3	10.0	4.5	5218	142
CML 254 X GCA-FAW	2.3	5.0	4.6	4893	146
Criollo	2.5	2.5	7.8	4225	193
CML 287 X CL00331	2.3	5.0	7.4	5045	151
Ki3 X CML 69	2.4	40.0	3.9	3434	144
CL00331 X GCA-SCB	2.3	20.0	3.6	4431	143
CML 287 X CML 67	2.3	50.0	5.2	4437	156
CL00331 X CML 67	2.3	40.0	4.6	3950	135
CML 285 X CML 139	2.5	60.0	3.9	3670	139
CML 285 X CML 67	2.4	45.0	3.6	3926	132
CML 287 X CML 139	2.4	2.5	7.5	4162	160
CML 135 X CML 139	2.4	30.0	3.0	4013	129
CML 69 X CML 139	2.4	27.5	5.1	4612	153
CML 247 X CML274/MIRT	2.3	2.5	6.0	4335	143
CML 247 X MIRT 172	2.4	17.5	5.0	4494	138
LSD at P = 0.05	0.2	20.5	2.2	639	12

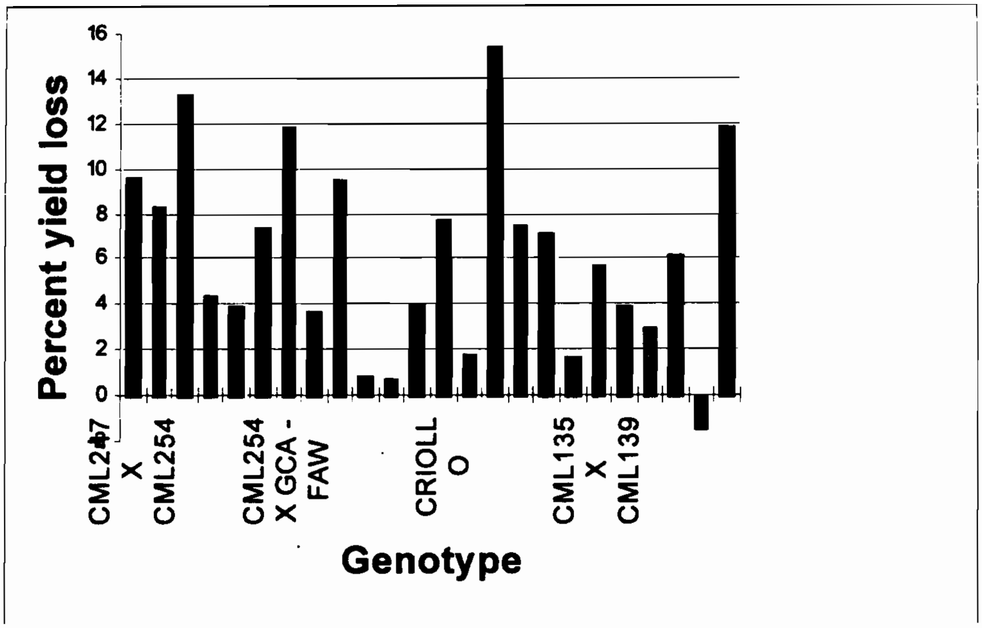

Figure 1. Percent yield reduction for infested and insecticide-protected plots of 24 experimental CIMMYT hybrids under infestation with fall armyworm, *Spodoptera frugiperda*.

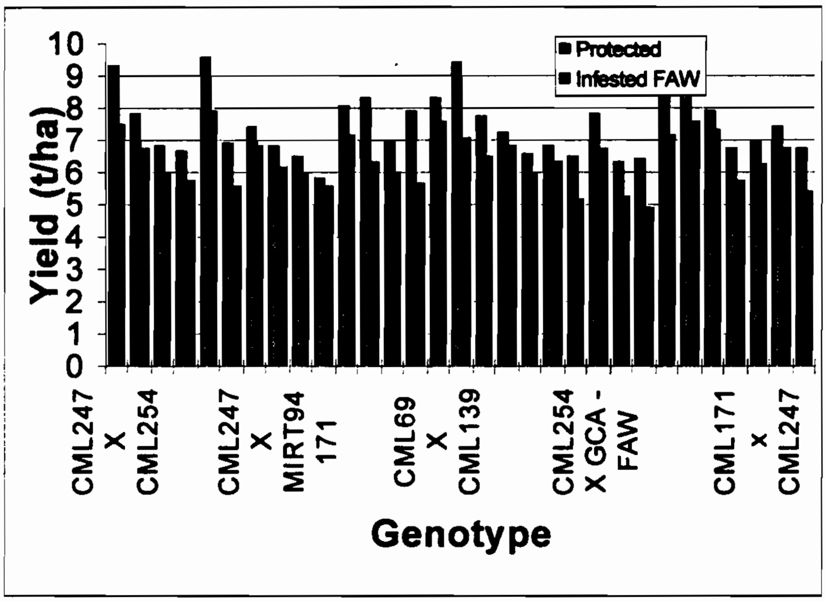

Figure 2. Yield of experimental CIMMYT hybrids under insecticide protection and artifical infestation with fall armyworm, *Spodoptera frugiperda*. The least significant difference (P=0.05) between means is 0.98 t/ha.

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