Date of Submission of the report: 31 October 2002
BARD Project Number: US-2874-97R.

Project Title: Discovery and Transfer of Genes from Wild *Zea* Germplasm to Improve Grain oil and Protein Composition of Temperate Maize

**Investigators**

Principal Investigator (PI): Margaret Smith

Co-Principal Investigator (Co-PI): Yaakov Tadmor

Collaborating Investigators: Susan McCouch

Nurit Katzir

**Institutions**

Cornell University

Newe Ya’ar Research center, ARO

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**Keywords** *not* appearing in the title and in order of importance. Avoid abbreviations.

grain quality, molecular markers, quantitative trait locus analysis, genetic diversity

**Abbreviations commonly** used in the report, in alphabetical order:

AB – Advanced Backcross, BC – Backcross, NIL – Nearly Isogenic Line,

SSR – Simple Sequence Repeat, QTL – Quantitative Trait Locus

**Budget:**

- IS: $134,400
- US: $150,000
- Total: $248,400

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Signature

Principal Investigator

Signature

Authorizing Official, Principal Institution

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Publication Summary (numbers)

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Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Danielle Lupold Clark, 214-92-6658

Cooperation Summary (numbers)

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Description of Cooperation:
Cooperation between the American and Israeli groups worked reasonably well, but not as smoothly as hoped. Two visits of YT to Cornell in November of 1998 and again in 2000 (one utilizing BARD funding) focused on evaluating results and defining future studies. A short sabbatical (Sept - Dec/2000) of NK at Cornell with SM and MS (not BARD funded) focused on the application of transposon-based marker technology. During the 3 month visit, NK worked with Postdoc Danielle Lupold-Clark to analyze the teosinte-maize derived populations for introgressions defined by SSRs and MITE-based markers. During the latter half of the project, developments in Israel (unrelated to
BARD) led to the virtual elimination of support for maize research at Newe Ya’ar Research Center. This seriously compromised our ability to evaluate the performance of our lines under field conditions in Israel, and restricted the development of new lines to evaluate the impact of the target teosinte introgression(s) in a high oil background already adapted to Israeli conditions.

**Patent Summary (numbers)**

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Appendix G6a
ABSTRACT

Project Objectives
1. Develop and amplify two interspecific populations (annual and perennial teosinte x elite maize inbred) as the basis for genetic analysis of grain quality.
2. Identify quantitative trait loci (QTLs) from teosinte that improve oil, protein, and essential amino acid composition of maize grain.
3. Develop near isogenic lines (NILs) to quantify QTL contributions to grain quality and as a resource for future breeding and gene cloning efforts.
4. Analyze the contribution of these QTLs to hybrid performance in both the US and Israel.
5. Measure the yield potential of improved grain quality hybrids. (NOTE: Yield potential could not be evaluated due to environmentally-caused failure of the breeding nursery where seed was produced for this evaluation.)

Background
Maize is a significant agricultural commodity worldwide. As an open pollinated crop, variation within the species is large and, in most cases, sufficient to supply the demand for modern varieties and for new environments. In recent years there is a growing demand for maize varieties with special quality attributes. While domesticated sources of genetic variation for high oil and protein content are limited, useful alleles for these traits may remain in maize’s wild relative, teosinte. We utilized advanced backcross (AB) analysis to search for QTLs contributing to oil and protein content from two teosinte accessions: Zea mays ssp. mexicana Race Chalco, an annual teosinte (referred to as Chalco), and Z. diploperennis Race San Miguel, a perennial teosinte (referred to as Diplo).

Major Conclusions and Achievements
Two NILs targeting a Diplo introgression in bin 1.04 showed a significant increase in oil content in homozygous sib-pollinated seed when compared to sibbed seed of their counterpart non-introgressed controls. These BC4S2 NILs, referred to as D-RD29 and D-RD30, carry the Diplo allele in bin 1.04 and the introgression extends partially into bins 1.03 and 1.05. These NILs remain heterozygous in bins 4.01 and 8.02, but otherwise are homozygous for the recurrent parent (RD6502) alleles. NILs were developed also for the Chalco introgression in bin 1.04 but these do not show any improvement in oil content, suggesting that the Chalco alleles differ from the Diplo alleles in this region. Testcross F1 seed and sibbed grain from these F1 plants did not show any effect on oil content from this introgression, suggesting that it would need to be present in both parents of a maize hybrid to have an effect on oil content.

Implications, both Scientific and Agricultural
The Diplo region identified increases oil content by 12.5% (from 4.8% to 5.4% oil in the seed). Although this absolute difference is not large in agronomic terms, this locus could provide additive increases to oil content in combination with other maize-derived loci for high oil. To our knowledge, this is the first confirmed report of a QTL from teosinte for improved grain oil content in maize. It suggests that further research on grain quality alleles from maize wild relatives would be of both scientific and agricultural interest.
Achievements

Significance of Main Scientific Achievements

Three chromosomal regions with putative QTLs for maize grain oil and protein content from teosinte were investigated. These regions were identified based on evaluation of BC2 testcrosses (crosses between Diplo or Chalco-introgressed RD6502 lines and maize inbred tester B73). Data on BC4S2 NILs indicate a significant effect of the Diplo genotype for the chromosome 1 target (bin 1.04) on oil content in homozygous sib-pollinated seed. Plants that are homozygous Diplo in this region had 12.5% higher oil content than their counterpart RD6502 controls (P<0.01). This difference was not apparent in plants carrying the Chalco genotype in this region, suggesting that the Chalco alleles may be distinct from the Diplo alleles in this region, and either not different or not better than the alleles in RD6502.

The BC4S2 NILs identified are referred to as D-RD29 and D-RD30. They are homozygous for the RD6502 genotype at most loci, but carry the Diplo allele in bin 1.04 (extending partially into bins 1.03 and 1.05) and remain heterozygous in bins 4.01 and 8.02. Heterozygosity in bins 3.04, 4.07-4.10, 8.03, 8.04-8.06, 8.09, and 9.07 that was present in the BC2 used for initial QTL detection was eliminated during NIL development. These two lines are available for further study, as are a series of Chalco-derived BC3S2 NILs carrying the Chalco genotype in the target bin 1.04 region (C-RD2, C-RD4, C-RD5, C-RD6).

Although the putative QTLs targeted for study in this project were identified by screening testcrosses with BC2 lines, it is notable that the NILs described above did not show improved oil content when grain from their testcrosses was evaluated. The same tester was used for both generations, so this result was not initially expected. The influence of different evaluation environments may be part of the reason for this difference. However, it seems even more likely that genetic background effects are the cause, given that the BC2s originally testcrossed had a number of background introgressions from teosinte that are no longer present in the BC4S2 NILs. One or more of these background introgressions may have
influenced the grain quality in the earlier generation testcrosses and that influence would have been eliminated in the NILs. In fact, the BC3S2 parent of the two Diplo NILs described above did carry an introgression in the 8.04-8.06 region that remains a candidate QTL for grain quality based on our initial screening of the BC2 population. Parent seed from this heterozygous BC3S2 generation (D-RD110) is available and a different set of NILs could be selected from it to test the influence of the chromosome 8 putative QTL on grain oil content.

To our knowledge, this is the first confirmed report of a QTL from teosinte that improves maize oil content. While teosintes should represent a significant reservoir of genetic diversity for maize improvement, commercial maize breeding efforts have made little (if any) use of these wild materials. The ABA methodology used in this research was successful at identifying a region of the teosinte genome that benefits maize grain quality, despite the poor agronomic performance of the early generations of this population. Thus the ABA method provides a means to extract useful specialty trait alleles from teosinte and a feasible means of utilizing teosinte more broadly in maize improvement. This increases the diversity of germplasm sources that are readily accessible to maize breeders.

Agricultural and/or Economic Impacts of the Research Findings

The increase in oil content of 12.5% detected in our NILs is of limited direct use (oil content of the NILs is 5.4%, whereas producers seeking high oil maize hybrids are aiming for 7% oil content or higher). However, since this QTL originates from *Z. diploperennis* teosinte, which does not readily cross with maize under field conditions, it is probably new to the commercial maize germplasm base. As such, it would be valuable to introgress it into existing high oil germplasm to determine whether it could provide an additive increase in oil content. Its use will require that it be introgressed into both parents of a hybrid, since it did not show dominance in testcross combinations. The market for specialty grain traits in maize is growing and it has proven difficult to improve oil content without negatively affecting yield. Novel genetic variation for improved oil content constitutes a significant resource for maize improvement.
Details of cooperation

The relationships established between all four partners were fruitful, although the collaboration did not develop as thoroughly as we would have liked. The major cooperation was through field evaluations. Progenies carrying putative QTLs were developed at Cornell and evaluated for field performance in both Israel and the US. Quality attributes (oil and protein content) were routinely measured at Newe Ya’ar, while US external laboratories were utilized for validation of results and in cases where they were more convenient than overseas shipping of large amounts of grain. Molecular marker work was done at Cornell as planned.

N. Katzir spent a three-month sabbatical in the laboratory of S. McCouch. Y. Tadmor visited Cornell twice (once utilizing the BARD funding and a second time utilizing other funding). During Tadmor’s first visit we jointly evaluated field trials at Cornell and discussed future collaboration. Tadmor’s second visit occurred shortly after N. Katzir’s arrival at Cornell, allowing for a special meeting in which all four collaborators, the post-doc, and students involved in the project discussed interpretations of the results and future directions. These visits and the sabbatical exchange enhanced interactions among the project team.

During the latter half of the project, developments in Israel that were completely unrelated to BARD led to the virtual elimination of support for maize research at Newe Ya’ar Research Center. This seriously compromised our ability to evaluate the performance of our lines under field conditions in Israel, and restricted the development of new lines to evaluate the impact of the target teosinte introgression(s) in a high oil background already adapted to Israeli conditions.

List of Publications

At present there are no reviewed publications from this project, however these will be forthcoming. Several abstracts of presentations at scientific meetings and of Ph.D. dissertations related to this work are included in the Appendix.
Appendix

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  Abstracts of Conference Presentations A13 – A15
  Abstracts of Dissertations A16 – A19
Unpublished Data

Saturated molecular maps were developed for both the Diplo-introgressed (Fig. 1) and Chalco-introgressed (Fig. 2) BC2 populations. The original AB analysis revealed QTLs for oil and protein content on chromosome 1 in both the Diplo and Chalco populations, and on chromosome 5 in the Chalco population (see Fig. 3 for an example of the chromosome 1 oil QTL from Diplo). A clearly distinguishable marker was identified for each putative QTL (Fig. 4) and BC4 (Diplo) or BC3 (Chalco) plants carrying the target QTL but having a minimum of other background introgressions were selected. Fig. 5 and Fig. 6 depict graphical genotypes for the parent plants of the NILs developed for the chromosome 1 QTL in Diplo and Chalco, respectively. From these progenies, individual BC4S1 plants (Diplo) and BC3S1 plants (Chalco) were genotyped and homozygous NILs with and without the target QTL from the teosinte parent were selected (see Fig. 7 for an example of marker data used for selection of the Diplo chromosome 1 NILs).

Once identified, NILs were self pollinated and simultaneously crossed to the B73 tester. Selfed and testcrossed progenies were grown in two replications and sib pollinated to produce grain from both the homozygous NIL and from the testcross hybrid. In addition, testcrosses were made again to produce testcross F1 seed from the same environment. All three sets of seed (sibs, F1 seed, and testcross grain) were analyzed for protein and oil content. Results showed significant effects from the Diplo chromosome 1 QTL on oil content in the sibbed homozygous seed (Table 1). The Chalco chromosome 1 introgression in this same region showed no significant difference compared to controls. Figure 8 shows the distribution of values for oil content in the homozygous sibbed seed of NILs for the Diplo, Chalco, and RD6502 genotypes at this target QTL.

There were statistically significant effects of this same QTL on protein content in the sibbed homozygous seed and the testcross grain (Table 1), however the increase in protein was of very limited agronomic significance – from 11.4% protein in RD6502 to 12.2% protein in the Diplo-introgressed NIL homozygous sibbed seed, and from 11.1% in B73 x RD6502 grain to
11.5% in B73 x Diplo-introgressed NIL grain. Interestingly, the testcross F1 seed showed decreased protein in the presence of the Diplo QTL. The effect in F1 seed would not be of concern to maize producers, since their crop consists of the grain from this F1 seed. In the F1 seed, any change in protein level would come only from xenia, so it is not surprising that an increase in protein was not detected. However, the apparent decrease in protein is fairly large (from 12.2% in the B73 x RD6502 seed to 10.7% in the B73 x Diplo-introgressed NIL seed) and difficult to explain. Given the lower overall significance (P<0.05) of these results compared to those for oil content, the relatively small increase in protein detected in the homozygous and testcross grain, and the odd behavior of the F1 seed in this case, we chose not to highlight the effects of this QTL on protein content in our Abstract and Achievements sections, however these effects are worthy of further study.

Table 1. Significance levels from t-tests comparing NIL effects on oil and protein content of sibbed seed of homozygous NILs, F1 seed of their B73 x NIL testcrosses, and testcross grain from the F1s.

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<tr>
<td></td>
<td>9.3% 3.9 - protein</td>
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<td>1.04-1.05 Chalco</td>
<td>7.2% 2.8 - protein</td>
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<td>-</td>
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<tr>
<td>5.05-5.06 Chalco</td>
<td>4.1% 1.6 - oil</td>
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* Probability value in parentheses indicates that the teosinte introgression reduced (rather than increased) grain quality.
Figure 1. Map of BC2 population derived by introgression of Diplo teosinte into RD6502 elite inbred line of maize. Chromosomes 1-3.
Figure 1, continued. Map of BC2 population derived by introgression of Diplo teosinte into RD6502 elite inbred line of maize. Chromosomes 4-6.
Figure 1, continued. Map of BC2 population derived by introgression of Diplo teosinte into RD6502 elite inbred line of maize. Chromosomes 7-10.
Figure 2. Map of BC2 population derived by introgression of Chalco teosinte into RD6502 elite inbred line of maize.

- QTLs identified by Doebley, et al.
- Skewed towards the recurrent parent
- Skewed towards Chalco
Figure 3. Graphical representation of the effect of individual chromosome intervals on chromosomes 1 and 5 from Diplo teosinte on oil content in a Diplo-introgressed BC2 testcross population.
Figure 4. Marker chosen to follow the Diplo introgression on chromosome 1. The far right lane is the RD6502 recurrent parent, the second lane from the right is the RD6502 x Diplo F1, and the two left lanes are BC4 plants identified as heterozygotes for the target introgression. The individual in the third lane from the right was chosen for NIL development, and its graphical genotype is represented in Figure 5.
Figure 5. Graphical genotype of Diplo-introgressed BC4 plant selected for NIL development. Hashed areas represent heterozygous regions; solid areas are homozygous RD6502.
Figure 6. Graphical genotype of Chalco-introgressed BC4 plant selected for NIL development. Hashed areas represent heterozygous regions; solid areas are homozygous RD6502; white areas are missing data.
Figure 7. Marker data for RD6502 (right lane), RD6502 x Diplo F1 (second lane from right), and several BC4S1 plants derived from the BC4 individual depicted in Figure 5. The two left lanes are plants that were self-pollinated to generate D-RD29 and D-RD30, selected as NILs for the chromosome 1 introgression that increased oil content in sibbed homozygous seed.

Figure 8. Mean values (horizontal lines) and 95% confidence intervals (diamonds) for percent oil content of chromosome 1 teosinte-derived introgressions vs. RD6502 control (designated RD).
ADVANCED BACKCROSS QTL ANALYSIS AND INTROGRESSION OF PERENNIAL TEOSINTE (Zea diploperennis) ALLELES TO MAIZE

CARLOS E. HARJES, M.E. Smith, T.G. Ruff, Y. Tadmor, S.D. Tanksley, S.R. McCouch

A molecular breeding approach successfully utilized in tomato and rice is currently being evaluated in several maize backcross populations to determine the potential of this breeding strategy for hybrid maize improvement. Preliminary results for a Maize / Zea diploperennis Bc2 population indicate that the potential value of the advanced backcross QTL approach to improve performance in this population are highly dependent on the trait evaluated. While the frequency of favorable alleles from the donor teosinte was only 10% for testcross yield QTL, donor alleles for grain quality characters such as increased oil and protein were superior to the elite alleles at 80% to 90% of QTL detected. In addition to the reduced frequency of positive putative QTL for yield from Z. diploperennis, the latter showed a strong genotype by environment interaction. Confirmation and more precise characterization of putative QTL, including genotypic interactions, via replicated field trials of marker assisted selection-derived nearly isogenic line testcrosses, is currently being pursued at various locations. These results indicate a duality in the applicability of advanced backcross QTL in this maize population, possibly determined by the reproductive biology of maize and the potential for gene flow between maize and its wild relatives throughout the domestication process of this crop.