BARD

FINAL REPORT
PROJECT NO. US-2242-92

**Breeding for Quality in Vegetable Maize Using
Linked Molecular Marker**

J.A. Juvik, T.R. Rocheford, A. Bar-Zur

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C. Abstract

Recently, the vegetable corn industry has shifted from the use of traditional cultivars with the *sugary1* (*su1*) endosperm mutation to newer hybrids homozygous for the *shrunk2* (*sh2*) or *sugary enhancer1* (*se1*) genes. With greater kernel sucrose content, these hybrids are preferred by consumers and retain sugar for longer post harvest periods, providing the industry with more time to market products with superior quality. Commercialization has been hindered, however, by reduced field emergence, and the establishment of stands with heterogeneous uniformity and maturities.

This investigation was conducted to identify key biochemical and physiological characteristics in *sh2* and *se1* maize kernels associated with improved emergence, and stand establishment; and in immature ears at fresh harvest maturity, properties associated with eating quality. The location of genes or QTL controlling these kernel characteristics and other traits were then mapped to specific chromosomal regions by their linkage to molecular markers using two segregating F_{2:3} populations. This database was used to compare the efficiency of marker-assisted selection of key alleles with phenotypic selection for trait improvement. A model designed to uncover and quantify digenic interaction was applied to the datasets to evaluate the role of epistasis in the inheritance of quantitative traits.

D. Proposal Objectives.

Objective 1: Identification of key genes and regions in the genome of maize associated with emergence, stand establishment, and harvested vegetable corn ear quality.

Objective 2: Development of a genetic database and linked flanking marker system using RFLP, RAPD, and cDNA probes for molecular marker-assisted selection of maize qualitative and quantitative trait loci affecting field emergence, stand establishment, harvested ear quality, and yield.

Objective 3: Comparison of breeding progress for improved emergence, stand establishment and harvested ear quality, where selection in a segregating population is based on either: A. field performance of the phenotype (% emergence, seedling growth rates, and stand uniformity); B. flanking molecular markers linked to beneficial alleles or; C. the combination of both.

E. Report of Research Activities funded by BARD grant no. US-2242-92C.

BARD grant no. US-2242-92C is a continuation and expansion of the previously funded BARD project US-1709-89 (1990-93). This earlier project was conducted in collaboration with Drs. Torbert Rocheford (Univ. of Illinois), W. F. Tracy (Univ. of Wisconsin) and A. Bar-Zur (Newe Ya'ar Research Center, Israel) to identify kernel characteristics associated with field germination, stand establishment, and eating quality of sweet corn with the *shrunk2* (*sh2*) and *sugary enhancer1* (*se1*) endosperm mutations. While these endosperm mutations confer improved eating quality over the traditional *sugary1* (*su1*) sweet corn, primarily through enhanced kernel sucrose content, they are also associated with reduced seed vigor and stand establishment. This previous project was designed to determine the physiological basis for this problem. In addition two F_{2:3} populations (one with *se1*, the other *sh2*) segregating for genes controlling these traits were created in an effort to identify genes that improved *sh2* and *se1* seed quality and field emergence. Fresh (20 days after pollination) and mature-dry kernel subsamples of each family in these two populations were assayed in the laboratory for quantitative determination of kernel physiological and chemical parameters associated with seedling establishment and fresh eating quality. The F_{2:3} families were also mapped with sufficient DNA markers to construct two genetic linkage maps of the populations, each of which was sufficiently saturated for linkage analysis with quantitative trait loci (QTL). This provided a data set sufficient to ascertain the number, chromosomal location and magnitude of the effect of qualitative and quantitative trait loci controlling these traits. The mapping and investigations of these two sweet corn populations conducted under BARD grant US-1709-89 provided the foundation for the work described below.

F. Evaluation of the research achievements with respect to the aims of the original research proposal, emphasizing the benefits to agriculture.

As described in the preceding report substantial progress has been accomplished in each of the objectives of the original proposal (see specifics above). Work on each of the objectives has been extended to include new research that goes beyond the original goals of the project. The results of the work described in this document enhances both our basic understanding of the genetics and physiology of the kernel quality in maize, while providing information and developing germplasm that can and has begun to alleviate the problem of poor stand establishment found in mutant endosperm corn. The associations between kernel physiological and biochemical properties provides new information about the processes of kernel development, maturation, and germination, and seedling growth. The scope of the practical results from this proposal goes beyond sweet corn since some of the new specialty field corn lines (*opaque2*, *floury2*, *amylose extender*, *dull and waxy*) have also been shown to suffer from seed quality problems and display reduced emergence.

The use of molecular markers provides the necessary resolution for the dissection of complex polygenic traits into discrete Mendelian factors. The experiments described above show that although fresh and mature-dry seed quality is the result of the interaction of many genes, a relatively small subset of these genes can account for a significant portion of the total genetic variation. Digenic interaction or epistasis was also found to account for a substantial portion of the variation. This research has provided a method to evaluate the importance of epistasis in trait expression in order to design a breeding program that will tap this component of variation for crop improvement. The results of our studies on the reliability and value of using marker-assisted selection are somewhat equivocal. Reproducibility of marker-QTL associations from one cycle of pedigree breeding to the next was poor, with only 2 of the 14 regions showing a similar effect from C_0 to C_1 . In contrast, population improvement using the $F_{2,3}$ families from within each endosperm mutant group based on MAS provided significantly great gains over PS for all the traits tested.

The most immediate contribution from this work will be improved breeding methodology, selection procedures and germplasm that can be of direct benefit to sweet corn plant breeders and the sweet corn seed industry. This germplasm and data sets have been made available to public and private plant breeders and geneticists. Over the course of this and the preceding BARD project, a broad and diverse range of maize germplasm has been exchanged between the University of Illinois and the corn unit at Newe Ya'ar that has provided all the P.I.s with new and unique materials for use in their programs. As just one of many examples, we are now developing *se1* and *sh2* inbreds from the *se1* population that show superior multigenic resistance to Stewart's wilt, northern corn leaf blight, and common rust. The development of a comprehensive sweet corn

physiological and genetic database, including the RFLP/PCR-based marker map is a significant resource to plant scientists and the seed industry. Determination of the linkage to molecular markers of *se1* and other beneficial alleles that are phenotypically difficult to identify provides a reliable system to incorporate these genes into elite sweet corn germplasm. This work is continuing with efforts to finely map the region near the *se1* locus and ultimately clone this commercially desirable gene.

Finally, this project has lead to the training and enhanced knowledge of the investigators themselves. At Illinois, six graduate students have been awarded M.S. (T. Han and A. Kurilich) and Ph.D. degrees (F. Azanza, A. Meyer, S. Wang, and G. Yousef) from the work described in this report. It also provided post-doctoral training and experience for Drs. Azanza, Mao, and Wang, who are now applying this knowledge as research scientists in permanent

G. Description of Cooperation.

As the preceding report and publications indicate, the scientists working on this project developed into a highly cooperative team. Five of the articles that have or will soon be published from this work were co-authored by at least two of the P.I.s. While all three of the PIs have been trained in genetics and plant breeding, each has a unique and complimentary emphasis. Dr. Juvik has specialized in the biochemical and physiological genetics sweet corn quality components. Dr. Bar-Zur's and now Dr. Tadmor's program emphasizes the application of the germplasm and genetic information into the development of elite inbreds and hybrids with improved quality and disease resistance. Dr. Rocheford's area of expertise involves the construction of maize linkage maps with polymorphic molecular probes which can be used to identify and isolate genes controlling maize kernel oil and protein quality. Each of the scientists regularly interacted with each other via correspondence, e-mail, at professional meetings or actual visits to the investigators' research institutions. The P.I.s have met together in the U.S. at least once each year during the course of this project. Dr. Bar-Zur has visited with Drs. Juvik, and Rocheford several times at the University of Illinois. Dr. Juvik spent ten days in Israel in June of 1994 finalizing several of these articles for publication. The P.I.s have freely exchanged valuable germplasm and methodology that is being utilized in each of their breeding programs. The opportunity to conduct multiple location trials and utilize off-season winter nurseries in Israel was invaluable to the goals of the project. The P.I.s have also exchanged trained personnel. Dr. Tadmor, who completed his Ph.D. program at the Weizmann Institute in Rehovet, spent two years as a post-doc working on this project with Drs. Rocheford and Juvik. After his post-doc he was hired to work at Newe Ya'ar by Dr. Bar-Zur and assumed responsibility for this project with Dr. Bar-Zur's retirement. The success of this project is primarily due to the synergistic contributions of each P.I.s unique resources and knowledge.

H. List of Publications (in order as presented in section E).

Refereed publications (published, in press, submitted, or in preparation)

Objective 1

1. Mao, Z., R. Paiva, A. L. Kriz, and J. A. Juvik. 1995. Dehydrin gene expression in normal and *viviparous* embryos of *Zea mays* during seed development and germination. *Plant Physiol. Biochem.* 33: 649-653.
2. Juvik, John A., Chunlin Xiao, and Z. Mao. Correlation of kernel physiological characteristics in *Zea mays* during embryogenesis with mature-dry seed germination. In preparation for submission to *Plant Physiol. Biochem.*
3. Young, T. A., J. A. Juvik, and D. DeMason. 1997. Changes in carbohydrate composition and α -amylase expression during germination and seedling growth of starch deficient endosperm mutants of maize. *Plant Science* 129:175-189.
4. Meyer, Annette C., A. Lane Rayburn, Richard L. Mulvaney, and John A. Juvik. Cell cycle parameters, DNA replication, and sweet corn stand establishment. In preparation for submission to the *J. Amer. Soc. Hort. Sci.*
5. Azanza, F., A. Bar-Zur, and J. A. Juvik. 1996. Sweet corn stand establishment and variation in kernel characteristics associated with eating quality. *Euphytica* 87:7-18.
6. Y. Tadmor, J. A. Juvik, T. Han, F. Azanza, T. R. Rocheford, W. F. Tracy, and A. Bar-Zur. QTL influencing kernel physiology and seedling stand establishment in maize with the *shrunk2* and *sugary enhancer1* endosperm mutations. In preparation for submission to the journal, *Theor. Appl. Genet.*
7. Tadmor, Y., F. Azanza, T. Han., T. R. Rocheford, and J. A. Juvik. 1995. RFLP mapping of the *sugary enhancer1* gene in sweet corn. *Theor. & Appl. Genet.* 91:489-494.
8. Katzir, N., G. Tzuri, A. Meir, T. Goldman, T. Chemke, J. Juvik, A. Bar-Zur and Y. Tadmor. Chromosomal regions affecting sugar accumulation and kernel color in *sugary enhancer1(sel)* sweet corn. In preparation for submission to the *J. Amer. Soc. Hort. Sci.*
9. Wong, A. D., J. M. Swiader, and J. A. Juvik. 1995. Nitrogen and sulfur fertilization influences on aromatic flavor components in *shrunk2* sweet corn kernels. *J. Amer. Soc. Hort. Sci.* 120:771-777.
10. Azanza, F., B. P. Klein, and J. A. Juvik. 1996. Sensory characterization of sweet corn lines differing in physical and chemical composition. *J. Food Sci.* 61:253-257.
11. Azanza, F., Y. Tadmor, B. P. Klein, T. R. Rocheford, and J. A. Juvik. 1996. QTL influencing chemical and sensory characteristics of eating quality in sweet corn. *Genome* 39:40-50.
12. Shultz, J., Y. Tadmor, A. Bar-Zur, T. Rocheford, and J. Juvik. Identification of QTL controlling ear quality characteristics in sweet corn. In preparation for submission to the journal, *Euphytica*.

13. Juvik, J. A., A. F. Brown, and J. K. Pataky. Quantitative trait loci associated with partial resistance to Stewart's bacterial wilt, Northern Corn Leaf Blight, and Common Rust in *sugary1* maize. In preparation for submission to the Journal of Phytopathology .
14. Kurilich, A. C. and J. A. Juvik. Quantitative trait loci influencing antioxidant carotenoid and tocopherol composition in sweet corn. In preparation for submission to the Journal of the American Society for Horticultural Science.

Objective 2

15. Wang, S. and J. A. Juvik. A two-locus model for measuring epistasis in the F₂ and the F_{2:3} generations. In preparation for submission to the journal, Genetics.
16. Wang, S. and J. A. Juvik. Epistasis of QTL associated with eating quality and field emergence in two sweet corn F_{2:3} populations. In preparation for submission to the journal, Crop Science.

Objective 3

17. Wang, S. and J. A. Juvik. Reproducibility of marker-QTL associations over two cycles of a pedigree breeding program. In preparation for submission to the Journal of the American Society for Horticultural Science.
18. Yousef, G. G. and J. A. Juvik. Marker-assisted selection for improved emergence and eating quality in sweet corn. In preparation for submission to the journal, Theoretical and Applied Genetics.

Published abstracts or reports.

19. Azanza, F. and J. A. Juvik. 1993. Genetic variability for eating quality attributes in sweet corn. HortScience 28:566.
20. Meyer, A. C., R. L. Mulvaney, J. A. Juvik, and A. L. Rayburn. 1993. *In vivo* labeling of maize DNA with nitrogen-15. 1993 Agron. Abstr. 85:118.
21. Wong, A., J. M. Swiader, and J. A. Juvik. 1993. Effect of nitrogen and sulfur fertilization on the chemical components of flavor in *shrunk2* sweet corn. HortScience 28(abst.):532.
22. Azanza, F., Y. Tadmor, B. Klein, T. Rocheford, & J. Juvik. 1994. Mapping genes associated with human flavor preferences in sweet corn. Abst. of the Plant Genome Annual Meeting.
23. Juvik, J. A. 1995. Genetic approaches to improving seedling vigor and stand establishment. Proc. Fourth National Symposium on Stand Establishment of Horticultural Crops. April 23-26, 1995, Monterey, Calif. pp. 1-10.
24. Azanza, F., Y. Tadmor, T. Han, S. Wang, T. Rocheford, and J. Juvik. 1995. Assigning physiological and biochemical roles to genes controlling quantitative traits. Gordon Research Conference: Quantitative Genetics and Biotechnology. Ventura, California.
25. Faver, M. K., J. M. Coons, and J. A. Juvik. 1995. Seed vigor and sugar leakage for isolines of sweet corn with traditional versus *sh2* endosperm. HortScience (abst.) 30:870.

26. Wang, S. S. and J. A. Juvik. 1995. Gene on chromosome 8 of maize that improves emergence of *shrunk2* sweet corn. 1995 Agron. Abst. 87: 276.
27. Juvik, J. A. 1996. Integration of classical and molecular genetic approaches to vegetable quality improvement. Proceedings of International Symposium on Life Sciences Toward the 21st Century, pp. 103-115. Kon-Kuk University, May 14-15, 1996, Seoul, Korea.
28. Juvik, J. A. 1996. Sweet corn as a model system for the genetic improvement of stand establishment in large seeded horticultural crops. Proceedings of the 1996 Korean Horticultural Society Meetings, p. 28.
29. Kurilich, A. C., S.-S. Wang and J. A. Juvik. 1996. *Sugary enhancer1 (se1)* gene endosperm dosage and sweet corn carbohydrate content. HortScience (abst.) 31: 625.
30. Wang, S.-S., K. Tadmor, T. R. Rocheford, and J. A. Juvik. 1996. Use of covariance for marker-QTL associations. 38th annual Maize Genetics Conference, Program and Abstracts. St. Charles, Illinois, p. 68.
31. Wang, S.-S. and J. A. Juvik. 1997. A model to estimate epistasis in the F₂ and the F_{2:3} generations. Abstract of the Maize Genetics Cooperative 1997 Annual Meetings, Florida, March 13-16.
32. Juvik, J. A. 1997. A genetic approach for investigating the chemistry of cooked flavor and aroma in sweet corn. HortScience (Abst.) 32:555.
33. Juvik, J. A. 1997. Genetic improvement of vegetables for enhanced cancer chemoprevention. HortScience 32(abst.):555.
34. Kurilich, A. C. and J. A. Juvik. 1998. Survey of carotenoid and tocopherol content in sweet corn. HortScience (Abstr.) 33:471.
35. Yousef, G. G. and J. A. Juvik. 1998. Marker-assisted selection for improved emergence and eating quality in sweet corn. HortScience (Abstr.) 33:472.

Theses.

36. Azanza, Fermin. 1994. Genetics, physiology and sensory perception of sweet corn eating quality. Ph.D. dissertation, University of Illinois. 213 p.
37. Han, Tae-ho. 1994. Investigations into the physiology and genetics of sweet corn seedling emergence and vigor. M.S. dissertation, University of Illinois. 164 p.
38. Wong, Adamson. 1994. A study of kernel composition affecting the quality of *shrunk2* sweet corn. Ph.D. dissertation, University of Illinois. 123 p.
39. Meyer, Annette C. 1995. Cell cycle parameters and sweet corn emergence and seedling vigor. Ph.D. dissertation, University of Illinois. 116 p.
40. Wang, Shyh-Shyan. 1997. Epistasis and other factors influencing the detection of marker-QTL associations. Ph.D. dissertation, University of Illinois. 183 p.
41. Kurilich, Anne C. 1998. Genetic investigations of carotenoid and tocopherol variability in sweet corn. M.S. dissertation, University of Illinois. 107 p.