

OFFICIAL

Cooperative Regional Research Project Proposal

I. Project Number: NE-124 (Revised)

II. Title: Genetic Manipulation of Sweet Corn Quality and Stress Resistance

III. Duration: 1 October 1999 - 30 September 2004

IV. Statement of the Problem:

Critical challenges must be addressed if sweet corn is to remain a viable crop. To expand consumption in both the U.S. and export markets quality must be improved. Better flavor and tenderness, and enhanced nutritional value are traits this project will investigate. To improve quality new endosperm mutants may be used, but these mutants often have difficulty germinating. Seed physiology and its relation to table quality will be addressed. A critical challenge to the sweet corn industry is pest management. While consumers of sweet corn have little tolerance for pest damage, food safety is a concern to both foreign and domestic consumers. Due to changes in agricultural practices and germplasm, kernel and ear rots have been observed in ears for consumption. Some of these ear rotting pathogens produce dangerous toxins. We must identify and incorporate resistance to these pathogens. Consumers are also concerned about pesticide residues and genetically modified organisms. Fortunately, sweet corn shares the same gene pool with the more diverse field corn and naturally occurring resistances can be identified and transferred. Another important challenge is weed control. The industry is rapidly losing older herbicides and few new ones are being registered for sweet corn. We will look for genes that increase sweet corn competitiveness so that lower rates of herbicides can be used. Advances in our basic biological understanding of starch synthesis must be continued and work done by NE-124 participants in this area will both contribute to and benefit from advances in genomics.

V. Justification:

Sweet corn is one of the most important vegetable crops in the U.S. In the U.S. diet, it is an important source of fiber, minerals, and certain vitamins, especially for children. Among vegetable crops, the volume of sweet corn processed is second only to that of tomatoes. Close to one third of the processed product is exported. Sweet corn production for processing in the U.S. occurs on approximately 550,000 acres and constitutes an annual farm value of \$250 million. A multiplier of five gives the approximate added value by processing (\$1.25 billion). Sweet corn is among the top six fresh market vegetables. Approximately 250,000 acres are grown for the fresh market with a farm value approximately equal to the processing farm value. For many U.S. agricultural areas, sweet corn provides an important alternative source of farm income. Sweet corn seed for the world's crop, including that grown in the U.S., Canada, Europe, and the Pacific Rim, is almost entirely produced in the U.S.

To keep the sweet corn crop viable multidisciplinary research is required. Regional research is critical for this important crop, because publicly supported sweet corn researchers are spread across the country. Usually there are only one or two individuals interested in sweet corn at a location. Seldom is there a breeder at the same location as a physiologist or pathologist.

The regional effort now known as NE-124 originally began in 1956 with the formation of NE-32, entitled "Genetics and Breeding of Sweet Corn." This project involved relatively few cooperators from the northeastern states and one or two states beyond. The objectives, diversity of participants, and research were greatly expanded in 1969 when NE-32 became NE-66, "Genetics and Physiology of Sweet Corn Quality and Biological Efficiency." In recognition of the

significant impact pests have on the sweet corn industry, the regional project was again expanded in 1978. The resulting project, "Genetics and Physiology of Sweet Corn Quality, Pest Resistance and Yield" (NE-124), was a natural response to industry needs. The annual meeting of the Regional Research Technical Committee is scheduled jointly with that of the National Sweet Corn Breeders Association. This interaction among academic, government, and industry scientists has led to a unique relationship which benefits all. Through the exchange of knowledge, ideas, and germplasm at a personal level, a cohesive group of scientists has formed.

NE-124 has had significant impacts on the rate of discovery and application of new knowledge in sweet corn genetics, physiology, breeding, and production. Over the years it has been in existence, the NE-124 project has led to important collaborative efforts between Agricultural Experiment Stations (AES), including collaborations among many scientific disciplines and across the range from very basic to very applied research. Additionally, industry scientists have been intimately involved with project evolution from the outset, through three formal representatives who participate in annual project meetings, and the additional 30 to 40 industry scientists who regularly attend these meetings. The resulting synergism among the stations, and between public and private sectors, is becoming increasingly important, particularly in light of decreasing research budgets. With growing pressure on industry researchers to show financial benefits in the short term, public sector research needs to address critical production concerns requiring more than a two or three year effort, and close collaboration between public and private sectors will be needed to effectively implement the results of this research.

VI. Related Current and Previous Work:

A. Germplasm Resources for Sweet Corn Improvement.

Of the hundreds of open-pollinated varieties existing in 1900, today only about 50 remain. Most of the remaining varieties have been evaluated for germination and seedling growth under cold temperatures⁶⁰ and reaction to a number of pathogens⁸⁴. Hotchkiss et al.⁶⁰ found substantial variation for the ability to grow under cold temperatures among 35 varieties. A few of the varieties grew very well equaling the performance of Mexican high altitude sweet corn and exceeding Corn Belt Dent germplasm that had been selected for cold tolerance⁶⁰. Pataky et al.⁸⁴ screened 36 varieties for reaction to Stewart's wilt (*Erwinia stewartii*), common rust (*Puccinia sorghi*), northern leaf blight (NLB) (*Exserohilum turcicum*), and southern leaf blight (SLB) (*Bipolaris maydis*). Modern commercial germplasm was more resistant to all four diseases than were the open-pollinated varieties⁸⁴. A few of the open-pollinated varieties were resistant to certain diseases and may represent novel sources of resistance alleles⁸⁴.

Many sweet corn breeders have resisted using non-sweet germplasm, warning against the difficulties in retaining table quality factors and specific raw product characteristics important in sweet corn^{61,72,101}. However, high levels of resistance to many pests is available in non-sweet corn germplasm. Genes for resistance to common rust and maize dwarf mosaic virus from Corn Belt Dent germplasm have been incorporated into sweet corn^{35,37,70}. Sweet corn inbreds with partial resistance to NLB have been developed using tropical corn varieties⁷⁸. Davis et al.³⁶ developed a rust resistant population using 11 *sugary1* (*su1*) inbreds and 17 Latin American varieties. The Corn Belt Dent inbred B52 was used as a source of genes for resistance to European corn borer (ECB) (*Ostrinia nubilalis*)^{37,69} and Latin American germplasm has also been examined as sources of ECB resistance⁷⁴. In developing sweet corn for the tropics, Brewbaker has emphasized tropical

germplasm sources, because most North American sweet corn germplasm lacks the pest resistance and stress tolerance needed to survive under tropical conditions with minimal inputs^{15,16,17,18}.

Common rust causes serious yield and quality reductions throughout much of the sweet corn growing area. Two forms of resistance are available; general or partial, in which the size and number of pustules are reduced, and specific, in which a hypersensitive reaction restricts pustule development¹⁰³. Hybrids with high levels of partial resistance are available^{85,87,91}. Specific or single gene resistance conditioned by the *Rp1* locus is available in many hybrids. The *Rp1* locus is a complex locus consisting of duplicated sequences^{62,65,64,111}. A high frequency of unequal crossing over has generated a large number of alleles^{64,92} and new allele combinations have been fixed and released⁶³. The allele most frequently used today is *Rp1-d*. However, other alleles at *Rp1* as well as the *c* allele at *Rp3* have some utility^{51,88}. Resistance due to alleles at *Rp1* and *Rp3* is usually dominant. A case of overdominance has been reported at another rust resistance locus, *Rp8*³⁸.

Northern leaf blight can cause economic damage in sweet corn throughout the eastern half of the USA. Both partial and single gene forms of resistance exist for NLB⁸⁶. However, races of the pathogen are able to overcome all commonly used single genes^{79,102,103}. The polygenic form of resistance is effective regardless of races present¹⁰³.

Kernel and ear rots caused by *Fusarium*, *Diplodia*, and *Gibberella* are a problem in sweet corn seed production. *Fusarium* kernel rot is often associated with insect damage and differences in susceptibility to *Fusarium* exist among sweet corn inbreds⁵⁵. In supersweets, reduced germination and seedling dieback can result from *Fusarium* infection of the developing kernels⁵⁵. Maternal effects are important in resistance to *Fusarium*, suggesting that the site of resistance may be the pericarp or the silk⁹⁷. Under high humidity ear and kernel rots are intensified. *Gibberella zeae* ear rot has appeared on sweet corn grown for processing^{41,119}. Under certain conditions *G. zeae* can produce significant levels of toxins prior to fresh ear harvest.

A number of synthetic and composite populations have been developed by university and USDA researchers. In developing composite 'AS1R', Rubino and Davis⁹⁴ performed 10 cycles of mass selection for earliness and pest resistance. Three populations have undergone three cycles of full-sib recurrent selection for resistance to common rust with decreases in rust damage per cycle of 8, 12, and 13%¹. MINN11 responded to a divergent selection program for endosperm phenotype^{1,23}. Brewbaker has developed populations for direct use as open-pollinated varieties^{15,16,17,18}. Brewbaker has used *shrunk2* (*sh2*), *brittle1* (*bt1*), and *brittle2* (*bt2*) in different varieties, all of which are adapted to the tropics.

The use of molecular markers for marker assisted selection programs has been suggested by many workers^{42,106}. In sweet corn, RFLPs have been used to classify and evaluate the variation among publicly available sweet corn inbreds⁴⁶, and to identify the chromosomal location of some quantitative trait loci (QTL)^{6,7,47}. RFLPs have been used to map single genes such as *se1*¹¹³. Preliminary data from Juvik's group at Illinois indicates that MAS can be effective in improving germination.

B. Biochemistry and Physiology.

Sweet corn flavor is determined in part by the amount of sugar in the endosperm. Starch synthesis in the corn endosperm has been the subject of much research. Reviews (many by NE-124 members) on starch biosynthesis and the genetic modification of endosperm carbohydrates

have been published^{12,13,14,30,53,81,82}. Most mutants used in sweet corn improvement increase sugar content and decrease starch content. One mutant, *sul*, elevates the level of water soluble polysaccharide (WSP) (phytglycogen) in addition to increasing sugar content^{13,30}. Eight starch synthesis mutants have been used commercially. For all but *sul* the basic science and initial development and commercialization was done by NE-124 cooperators. Most of these genes have been cloned and sequenced and their specific enzymatic lesions are known^{8,11,22,44,48,50,67,90,98,99,100,112}. Starch synthesis mutants are divided into two classes based on their effects on endosperm composition¹³. The class 1 mutants accumulate sugars at the expense of starch and have greatly decreased total carbohydrates in the mature seed^{13,82}. At 18 to 21 days after pollination these mutants have 4 to 8 times the total sugar found in nonmutant corn^{20,30,26,58,59,68,75,82,116}. Due to high sugar levels, class 1 mutants are used independently in sweet corn varieties. For processing, *sh2* is currently the second most widely used endosperm type after *sul*, while for many fresh uses, usage of *sh2* has surpassed that of *sul*.

Class 2 mutants alter the types and amounts of polysaccharides produced¹³. The alleles *ael*, *dul*, and *wx1* generally result in slightly less starch in the mature kernel than nonmutant types^{13,30,31,82}. These 3 mutants result in small increases in sugar content and do not make acceptable sweet corn when used singly. However, complementary gene action of certain triple combinations of class 2 mutants results in sugar levels equal to those found in class 1 mutants^{29,31,57}. Commercial hybrids having the triple recessive genotype *ael dul wx1* have been released⁴⁵. The *sul* allele results in greatly increased levels of WSP^{82,31}. WSP is a highly branched polysaccharide¹² and gives *sul* endosperm the smooth texture and creaminess, characteristic of traditional sweet corn varieties^{13,32,33,77}. While high levels of WSP appear to be unique to *sul*, elevated levels are maintained when *sul* is combined with either *wx1*, *dul*, or *bt1*^{3,19,29,52}. The *sugary enhancer1 (se1)* allele¹¹³ when in combination with homozygous *sul*, results in sugar levels near those of *sh2* and WSP levels similar to unmodified *sul*^{5,43,49}. This results in a high quality, sweet, creamy endosperm. It appears that a number of recessive modifiers are required to attain high quality *sul se1* hybrids¹¹⁴.

Many important endosperm genes have extensive allelic series^{13,27,52}. Phenotypic expression of these alleles range from smooth seeded, normal appearing kernels to ones so defective as to be lethals. The presence of some normal appearing alleles can only be detected when another gene is present²⁷. It is not known if all the hybrids of a given endosperm type have the same allele at that locus. However, it has been suggested that certain alleles of *sh2* and *bt2* may have better seed quality, while maintaining adequate levels of sugar for acceptable flavor^{52,53}.

Many endosperm mutants have poor germination and seedling vigor^{28,80,89,93}. Combinations of endosperm mutants usually result in even lower germination and seed vigor^{93,95}. Mutants that accumulate high sugar levels have reduced germination relative to normal corn and other mutants, especially in cold soils^{110,118,122}. Hybrids containing *se1* generally have reduced germination relative to *sul*⁴⁰. Understanding the causes of poor field emergence in high-sugar types and developing techniques to improve it are crucial in making high-sugar varieties commercially acceptable.

Genetic background strongly affects emergence, and background by endosperm mutant interactions are also important in determining emergence and seedling vigor^{4,40,93,95,110,118,122}. Significant effects of genetic background on germination indicate improvement of germination is possible through selection. A *sh2* population mass selected for improved emergence and seed weight had increased cold and warm⁹. Similar changes have been observed in a *sul* population²⁴.

Significant background by endosperm type interactions indicate that breeders can not assume that backcrossing a new endosperm mutant into a line with good seed quality will result in a line that germinates well⁷⁷.

Reduced emergence is affected by both genetic and environmental conditions, both during seed production and at planting^{25,109}. Seed weight of *sh2* is 33 to 50% that of *su1*^{96,118} and seed weight is correlated to percent germination^{4,71}. Reduction in seed weight is a function of greatly reduced starch levels relative to other types of corn^{105,107}. Thus, starch levels are related to germination and seed vigor both among endosperm types¹⁰⁷ and within *sh2*⁷¹. On a percent of dry weight basis, *sh2* seed has high levels of sugars^{104,107}. In an extensive study on effects of recurrent selection for improved germination in a *sh2* population, sugar levels were not directly related to germination⁷¹. However, due to the osmotic potential caused by high sugar levels, *sh2* corn dries very slowly and maturing seed is thus more susceptible to frost damage during maturation¹⁰⁴. Slow drydown rates also may be responsible for the higher incidence of seed-borne *Fusarium moniliforme* Sheldon in *sh2* seed¹⁰⁸. Infection by *F. moniliforme* and other pathogens can greatly reduce germination and seed vigor⁵⁵. While slow drydown rates of *sh2* seed may be responsible for increased infection of seed by *F. moniliforme*, increased infection is not directly related to high sugar concentration⁵⁶.

Low carbohydrate concentration of *sh2* results in severe shrinking of the endosperm as it dries, which creates a number of structural problems for the seed^{107,121}, including cracking of the pericarp. The pericarp is a barrier to pathogens and water movement. Damaged pericarp greatly reduces germination in all endosperm types⁷³.

Starch granule degradation in the subaleurone endosperm was significantly less in a *sh2* variety compared with non-isogenic *su1*, *su1 se1*, and normal varieties⁵⁴. Thus, the availability of food for the germinating seed may be reduced. Both respiration rate and adenosine-triphosphate (ATP) levels have been studied and neither can account for the poor seed vigor in *sh2* corn^{110,118}.

Effects of soil-borne pathogens on germination of *sh2* seed and the need for chemical seed treatments were recognized early in the development of *sh2* as a crop^{10,21}. Reliable fungicides are required for high-sugar corns to be a viable crop. Fungicide combinations have been observed to increase germination under cold test performance three to four fold relative to the untreated check¹¹⁵. Three to five different fungicides plus an insecticide are commonly applied to commercially treated sweet corn seed^{77,120}.

C. Insects, Diseases, and Weeds.

As a result of concerns related to human health, nontarget organisms, and the environment, the reduction of pesticide use in US agriculture is an important goal. A means to achieve this goal is through the development and adoption of the integrated pest management (IPM) strategy, an ecologically based strategy that promotes the use of non-chemical control tactics. Pesticides are a key component of IPM, but they are only used when all other options fail to control the pest. The continued availability of pesticide options is in jeopardy due to the development of resistant pests and changes in pesticide regulations. The recent enactment of the Food Quality Protection Act may result in the loss of many classes of certain pesticides. Alternatives be developed for sweet corn.

The ECB is the most serious insect pest in the major processing states, while the corn earworm (*Heliothis zea*) and fall armyworm (*Spodoptera frugiperda*) cause significant damage in other regions. Inheritance of resistance to these pests has been the subject of intense investigation

in field corn³⁹, and breeding for ECB and corn earworm resistance is a major effort in public sweet corn programs^{2,34,117}. However, the genetic variability for resistance within elite sweet corns is low⁷². The development of sweet corn possessing the *Bacillus thuringiensis* (*Bt*) toxin has the potential to revolutionize control of lepidopteran pests of sweet corn^{66,76}. Key to the long-term effectiveness of *Bt* sweet corn however, is the development and adoption of a resistance management program. NE-124 cooperators are well positioned to address this need⁸³. Other insect pests of sweet corn include the corn leaf aphid (*Rhopalosiphum maidis*), corn flea beetle (*Chaetocnema pulicaria*, the vector of Stewart's wilt), and sap beetles (Nitidulidae). With the availability of genetically engineered *Bt* sweet corn, infestations of pests previously controlled by insecticides applied for control of ECB may become more common.

Herbicides constitute 60 to 70% of the pesticide use in U.S. agriculture. With enactment of the Food Quality Protection Act the availability of a wide spectrum of herbicides may be in jeopardy. Efforts need to be made to evaluate and optimize existing and new herbicides and find cost-effective alternatives. Numerous strategies for herbicide reduction are being researched across the country. Of particular importance to sweet corn, weed management tactics such as cultivation, reduced tillage, banded applications, and cover crops need to be developed. While published literature includes a significant amount of efficacy and phytotoxicity information on the individual herbicides in field corn, there is little published on sweet corn and nothing that relates to planning a total herbicide program. Sweet corn is planted at different times than field corn, emerges slowly and at the same time as many of the summer annual weeds, and is therefore far less competitive than field corn. Need for effective weed control is more critical in sweet corn than in field corn if high yields are to be maintained. An additional problem with sweet corn is the number of varieties, both for fresh market and processing. There is documented evidence of increased herbicide injury due to the extreme differences in vigor between the traditional, sugar-enhanced, and supersweet varieties. Research needs to be conducted to determine a screening strategy for herbicide tolerance particularly in the sugar-enhanced and the supersweet varieties and to develop economical weed management programs that are not based on atrazine, or which minimize its use.

VII. Objectives:

- A. Genetics and Plant Breeding: Genetics and Plant Breeding: germplasm acquisition, enhancement, and distribution, identification of new genes or novel allelic combinations useful in sweet corn improvement, and utility of marker assisted selection.**
- B. Molecular Biology, Biochemistry and Physiology: Determine the genetic, biochemical and physiological mechanisms regulating carbon flow, and seed and food quality.**
- C. Crop and Pest Management: Reduce environmental impacts of sweet corn production while maintaining or improving product quality.**

VIII. Procedures and Research Plan:

Cooperators: This regional research committee will investigate quality and stress resistance in sweet corn, by examining underlying mechanisms and genetics conditioning these traits. Researchers participating in the project include specialists in biochemistry, molecular biology, genetics, physiology, pathology, entomology, crop production, food science and plant breeding.

Approaches from these diverse disciplines will be integrated, with the goal of improving sweet corn productivity and quality, and minimizing the negative environmental impacts of sweet corn production. To carry out the objectives of this project, 11 State Agricultural Experiment Stations (13 locations) and many cooperators in private industry will be involved.

Communication and Planning: The current NE-124 committee uses email to plan collaborative experiments, exchange data, and submit annual reports. We also plan experiments and joint nurseries and discuss issues regarding germplasm utilization at our annual meeting. One of the reviewers suggested the development of a website for posting goals, data, and accomplishments of the committee. We believe that this is an excellent suggestion and will develop and maintain such a site.

Cooperation: Details of multistate cooperative programs are usually developed at the annual meeting and refined via email. For most cooperative programs an individual coordinator is identified

Objective A. Genetics and Plant Breeding: Genetics and Plant Breeding: germplasm acquisition, enhancement, and distribution, identification of new genes or novel allelic combinations useful in sweet corn improvement, and utility of marker assisted selection.

Obj. A.1. Sweet corn germplasm acquisition, enhancement, and distribution. Commercial sweet corn germplasm is derived from a narrow genetic base. NE-124 cooperators have developed and implemented a number of strategies to broaden sweet corn germplasm but much remains to be done.

Acquisition and characterization: We will continue to acquire and characterize U.S. sweet corn germplasm and expand our activities to include the acquisition of internationally developed germplasm that has been developed for direct consumption. A second effort will be directed at the characterization of non-sweet corn germplasm that could be useful in sweet corn improvement programs. An important source of germplasm is the vegetable corns of Latin America. Non-sweet germplasm can be used in two ways: desirable traits or genes from non-sweet germplasm can be introgressed into sweet corn; and non-sweet germplasm can be converted to one or more of the sweet corn endosperm types. Before introgression or conversion begin, preliminary characterization of the potential utility of the germplasm is required. We will evaluate germplasm at multiple locations (Florida, Hawaii, Illinois, Oregon, New York, & Wisconsin) with each location concentrating on specific traits important in that environment.

Enhancement: NE-124 cooperators are located in a wide range of environments allowing NE-124 to evaluate and select germplasm in environments characterized by a wide array of abiotic and biotic stresses. Additionally, NE124 cooperators are developing improved germplasm for a number of different markets This diversity of expertise ensures that germplasm will be evaluated for multiple traits in multiple environments. The range of environments also allows us to implement recurrent selection schemes that evaluate material in multiple environments and use tropical locations for recombination. A selection cycle can be completed in one year. NE124 cooperators will select for resistance to ear, seed, and seedling rots. Our approach is convergent-divergent selection. Composites of diverse genotypes with varying levels of resistance to root and

seedling rots will be bulked and random mated for three cycles at the Florida site, then sent to Hawaii, Wisconsin, Oregon, New York, and Illinois for selection under the soil-borne pathogen pressure common to each environment. Seed from selected plants will be returned to Florida and random mated prior to another dispersal for selection. NE-124 cooperators will focus on developing resistance to foliar diseases including common rust, southern rust (*Puccinia polysora*), NLB, SLB and two significant systemic diseases, Stewart's wilt and maize dwarf mosaic (MDMV). Each station will also work on diseases specific to production regions or individual research interests. Populations developed by cooperators (Florida, Hawaii, Illinois, Minnesota, New York, Pennsylvania, Wisconsin) will be exchanged among stations and evaluated and selected for appropriate disease resistances. All populations will also be selected for quality factors. Selected families will be recombined and reselected. To increase diversity and stability of resistance, we will also select for and incorporate simply inherited resistance genes.

"Bt" genes have been incorporated into sweet corn and will become important in certain sweet corn markets. However, due to the widespread deployment of Bt in field corn it is likely that some of the major sweet corn pests will evolve resistance to Bt. Thus NE-124 cooperators will continue to incorporate other sources of resistance. Of primary interest among NE 124 cooperators are the corn earworm, ECB, and fall armyworm. Although these insects occur in different regions of the country, many of the control strategies are similar, and a cooperative approach will yield useful information and germplasm. Germplasm largely consisting of sweet by insect resistant non-sweets has been developed independently at the various research stations. We will evaluate and select these materials at multiple locations and recombine selected material in tropical or subtropical environments.

Obj. A.2. Identification of new genes or novel allelic combinations useful in sweet corn improvement.

Novel sources of resistance to diseases and insects: Novel sources of resistance to various pests have been identified. Cooperators identify and locate new genes or new alleles at loci already known to have resistance. For disease resistance, New York will continue efforts to extract and identify resistance to anthracnose found in teosinte, while Wisconsin will focus on the identification of at least six alleles found in high-altitude maize that confer resistance to common rust. In Florida and Hawaii genes have been found that confer resistance to northern leaf blight. The Hawaiian gene has tentatively been named *Etu*, confers an immune reaction, and was derived from the field corn line 'Suwan-1, while the Florida gene is found in the proprietary *sh2* inbred 'UFISH 8072' and confers only partial resistance. The experiment stations in Hawaii and New York have discovered novel sources of resistance to aphids and ECB, respectively. The aphid resistance has tentatively been identified as a recessive gene from tropical sweet corn germplasm. As individual cooperators identify new alleles they will be distributed to others for rapid incorporation into adapted germplasm.

Novel Allelic Combinations in the Endosperm: The interaction of near normal *sul* alleles, *sul-66*, *sul-am*, and *sul-P*, are being investigated in combination with *dul* and *su2*. Characterization of intermediate *sul* alleles, *sul-Bn2* and *sul-st*, will continue. Carbohydrate analysis will include sugars and phytylglycogen. Other new combinations to be evaluated includes *sul* and

intermediate alleles at *bt1* and *sh2*. We will also evaluate the potential of *bt1-mutable* for sweet corn improvement.

Obj. A.3. Investigation of the utility of marker assisted selection.

Marker-assisted selection to facilitate germplasm enhancement and development. In previous NE-124 studies, two segregating F_{2:3} sweet corn populations (one *se1* and the other *sh2*) were mapped to near saturation using DNA markers (primarily RFLP) and assayed for genes influencing a number of traits (stand establishment, eating quality, yield, disease resistance, kernel chemical composition, etc.) These populations are the source material for several experiments designed to evaluate selection gain using marker-assisted selection (MAS) and phenotypic selection (PS) to enhance economically important traits in sweet corn that are quantitatively inherited. Marker-facilitated genotypic selection will be based on the polymorphism of five RFLP markers linked to the major QTL associated with significant effects on the various traits in the F_{2:3} generation for each population. The 20% of the families in each population (C₀) with the highest and lowest genotypic scores and F_{2:3} phenotypic performance values have been selected to constitute the MAS and PS C₁ composites, respectively. The C₀, C₁, and additional selection cycles will be evaluated for several traits (yield, eating quality, and stand establishment) over years and environments using NE-124 cooperators.

Marker-assisted backcrossing to improve seedling emergence in *sh2* hybrids. There is limited evidence that desirable QTL influencing traits identified in one population can exert a similar effects in other genetic backgrounds. A protocol to evaluate a set of candidate QTL alleles identified in one genotype for their effect in a range of genetic backgrounds would be valuable to target key genes for use in crop improvement programs. A recombinant inbred sweet corn line (1657-90) generated from a previously mapped *sh2* F_{2:3} population (Ia453*sh2* X IL451*bsh2*) was found homozygous for alleles at three marker loci, bn19.08 (chromosome 8), umc139(chromosome 2), and p200689 (chromosome 1) linked to favorable QTL that increase percent seedling emergence. This line has been used as a donor parent for introgression of the desirable alleles into three commercial inbreds (donated by a seed company collaborating with the NE-124 project) with relatively poor seedling emergence by marker-assisted backcrossing. BC₄S₁ families from each of the three recurrent parents homozygous for different combinations of the desired marker alleles will be crossed to each of the recurrent inbreds. This will generate three sets of 'hybrid' seed lots whose performance will be compared with the three near isogenic hybrids generated from the unmodified inbreds in trials conducted cooperatively with other NE-124 scientists.

Objective B. Molecular Biology, Biochemistry, and Physiology: Determine the genetic, biochemical and physiological mechanisms regulating carbon flow, and seed and food quality.

B.1. Seed Quality and Physiology: New endosperm mutants of sweet corn have highly desirable marketing and consumer qualities, but can present seed quality and physiology challenges. An understanding of seed development and seedling establishment issues will permit

reliable crop production under a wide range of environments. Field studies will subject a number of sweet corn hybrids with different genetic backgrounds (*su1*, *se1*, and *sh2*) to three types of seed treatments (protectant only, protectant + systemic, and untreated). Idaho will coordinate and continue the multi-location seed treatment trial (18 sites in 1998, including most NE-124 cooperators) based on deliberations with the NSCBA Seed Treatment Committee and NE-124 cooperators. Research into the species identification of the major pathogens associated with sweet corn seed (mainly species of *Penicillium*, *Fusarium* and *Rhizopus*) and characterization of their fungicide sensitivity in vitro is planned.

Solid-matrix priming and row cover evaluations will be conducted by with the goal of improved early season establishment of low vigor sweet corn cultivars. A drum priming system will be used to apply biological seed treatments to sweet corn, in addition to hydrating the seeds (to 20-25% seed moisture content) prior to planting. The saturated salt accelerated aging (SSAA) test, using NaCl to modify relative humidity levels, will be used to assess cultivars of several endosperm types. Results from SSAA rankings will also be compared to cold tests for prediction of seedlot performance under field conditions. Field study of precision planting options using GPS/GIS for *sh2* sweet corn will also be expanded.

B.2. Endosperm carbon flow. Genetic investigations will be designed to further define the changes in seed carbohydrates in single and multiple gene combinations of naturally occurring mutations. Emphasis will be placed on the formation of phytyglycogen and the accumulation of neutral sugars. Studies will be designed to evaluate differences in carbohydrates among inbreds, hybrids, and varieties within the same endosperm class. Differences will be related to seed quality and food quality in companion experiments. Inbreds will be shared among cooperators. Individual investigators will provide field locations to evaluate environmental variation. In addition, materials will be harvested and distributed to different laboratories for biochemical analysis. Similar strategies previously have been employed in NE-124 to make optimal use of individual laboratory strengths. NE-124 laboratories are characterizing the genes encoding enzymes involved in starch synthesis. Probes, clone libraries, and genetic lines are shared among laboratories and cooperative investigations will characterize the biochemical lesions of various mutations affecting seed carbohydrates. We will study the interactions of near-normal *su1* alleles, *su1-66*, *su1-am*, and *su1-P*, with *dul* and *su2*. Characterization of intermediate *su1* alleles, *su1-Bn2* and *su1-st*, will continue. WSP accumulation and structure will be examined in non *su1* genotypes including *dul wx1* and *ae1 dul wx1*.

B.3. Genetic and physiological attributes of food quality and carbon partitioning in sweet corn.

A selectable marker for the sugary enhancer1 gene in maize.

Development of new *se1* hybrids has been hampered by the lack of an efficient and reliable method to select for this mutation in sweet corn breeding programs. To address this problem we propose to use recent DNA marker technology (AFLPs and SSRs) on isolines and segregating populations to identify probes tightly linked to the *se1* locus on chromosome 2 to use as selectable markers.

We will isolate and clone *Se1* via transposon tagging with Robertson's mutator to investigate the role of the *Se1* gene product in wild type maize endosperm development.

B.4. Investigations into sweet corn phytochemicals that promote human health. In addition to providing dietary nutrients for normal metabolic activity, sweet corn contains components that contribute additional health benefits. These components are generally referred to as phytochemicals and have been associated with the chemoprevention of cancer and cardiovascular disease. Two categories of these phytochemicals are carotenoids (vitamin A precursors) and tocopherols (vitamin E analogs). These and other compounds (primarily phenolics) act as antioxidants in metabolic systems by preventing or terminating oxidation reactions which produce free radicals. Free radicals in biological systems can cause damage to cells, tissues, and DNA which is believed to lead to the induction of cancer. Corn is a substantial source of both carotenoids and tocopherols and could be used as a vehicle for increasing the amount of these health-promoting compounds delivered to consumers in their diet.

We surveyed 44 sweet and dent corn inbreds to assay the existence of qualitative and quantitative variability in kernel carotenoid (lutein, zeaxanthin, b-cryptoxanthin, a-carotene, and b-carotene) and tocopherol (a-, d- and g-tocopherol) content. There is substantial variability among inbreds for the dominant antioxidants (b-carotene, lutein, and g-tocopherol). We propose to investigate the genetics controlling the chemical form and concentrations of these compounds in sweet corn using several segregating populations developed from this set of inbreds. We will identify and characterize key qualitative and quantitative trait loci influencing carotenoid, tocopherol and phenolic content in segregating F_{2:3} populations with linked molecular markers. We are currently assaying one population previously mapped and will be evaluating a second population by the year 2000. This information and germplasm can they be used to develop sweet corn genotypes with potentially enhanced health promotion.

Objective C. Crop and Pest Management: Reduce environmental impacts of sweet corn production while maintaining or improving product quality.

C.1. Integrated Pest Management.

Disease management: Disease screening nurseries will be established at Florida, Hawaii, Illinois, Minnesota, New York, and Wisconsin to identify current levels of host plant resistance and facilitate identification and further development of new sources of resistance. Symptomatic host plant responses to common rust, smut, Stewart's wilt, NLB, SLB, anthracnose, maize dwarf mosaic virus, bacterial leaf blight, and yellow leaf blight will be evaluated in both commercial hybrids and select populations of sweet corn at Illinois.

Seed-borne fungi associated with seed rot and seedling diseases in different sweet corn genotypes will be characterized. Fungi will be isolated from developing ears, dried seed, and symptomatic seedlings. Seed lots from hybrids of different genetic backgrounds (*su1*, *sh2*, *se1*) will be examined. Selected seed treatments (3-4) will be evaluated in greenhouse and field using seedlots from these hybrids for their effectiveness in increasing the stand. Representative isolates of the major pathogens associated with seed and seedling diseases will be characterized as to their sensitivity in vitro to selected systemic and protectant fungicides, using the poison agar plate technique. Multi-location evaluation of seed treatments (chemical and biological) will be conducted with the participation of sweet corn industry personnel and NE-124 cooperators (Florida, Minnesota, New York, Ohio, Oregon, Wisconsin). Selected seedlots will be subjected to

a set of promising seed treatments and the treated seed will be planted in different sweet corn growing regions.

Insect management: Significant strides have been made in sweet corn insect management, specifically through the development of composite populations having resistance to many lepidopteran insects. The mechanisms of resistance will be investigated to aid in directing breeding efforts. In addition, the development and adoption of genetically engineered Bt sweet corn will greatly improve management of lepidopteran pests, especially ECB. Small-plot and on-farm efficacy evaluation of new Bt sweet corn hybrids, for ECB and corn earworm will continue in Minnesota, Florida, Illinois, and Wisconsin. The use of Bt sweet corn as a practical "in-field" resistance monitoring tool for ECB and corn earworm, for both the benefit of the field and sweet corn industries.

The long-term sustainability of Bt sweet corn for control of lepidopteran pests is dependent on management of resistance to the Bt toxin. Recommendations for resistance management in Bt field corn, as developed by members of Regional Project NC 205, will be adapted to fit the needs of the sweet corn industry. Insecticide rates for control of lepidopteran species will be reduced through the establishment of resistant hybrid-specific action thresholds (Minnesota) and investigation of alternatives to chemicals (Minnesota, New York).

Weed management: Research will be conducted to determine an early season screening technique for evaluating sweet corn tolerance to a range of registered and soon-to-be registered herbicides. This work will be done with assistance of several commercial seed producers. It is estimated that 10-15 lines will be screened for tolerance to members of various herbicide families. Following establishment of a screening strategy, additional lines will be evaluated in the subsequent years. Using widely grown sweet corn varieties, non-atrazine based herbicide programs will be evaluated (Illinois, Minnesota, New York, Wisconsin). Determining the economics as well as the efficacy of these programs will be an essential component of the research. Research will explore banding herbicides, coupled with cultivation and interseeded legumes. This work will eventually be expanded to determine the competitiveness of the less vigorous high sugar types when grown with interseeded cover crops.

C.2. Genetic improvement of nutrient use efficiency. Agricultural fertilizers, especially nitrogen and phosphorous, are non-point source pollutants in surface and ground water. However, these elements are essential for crop production. Sweet corn germplasm contains genetic variation for nutrient use efficiency, defined as the ability to produce high yields with reduced fertilizer. In some cases, these genotypes also have higher nutrient levels in the kernels, which may offer seedling vigor and crop establishment benefits when the kernels are seeded in nutrient deficient soils. Work toward this objective will focus on nitrogen and phosphorus use efficiency. NE-124 researchers have identified sweet corn inbreds which grow well at greatly reduced levels of phosphorus and nitrogen. Plots low in N and P have been established and we will exchange breeding germplasm for selection in different environments. We will also identify additional sources of phosphorus and nitrogen stress tolerance.

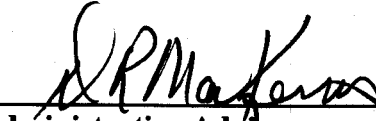
IX. Expected Outcomes:

- Knowledge and germplasm generated by this project will result in more flavorful, nutritious sweet corn that will be produced with fewer synthetic inputs. This should increase consumption both at home and abroad benefiting both farmers and processors.
- Identification and characterization of germplasm useful to sweet corn breeders.
- Sweet corn germplasm with improved pest and stress resistance and table quality.
- New genes for pest resistance and endosperm traits will be identified and incorporated into elite sweet corn germplasm.
- Evaluation of marker assisted selection will provide information as to when and under what conditions marker-assisted selection is justified for use in a sweet corn breeding program.
- A useful molecular marker for the valuable gene *se1* will be identified.
- Identification of physiological and biochemical factors important in starch biosynthesis and ultimately affecting germination and table quality.
- Identification of improved seed treatments.
- Applying GIS technology to seed production will assist in determine factors affecting seed quality.
- Identification of genes affecting phytochemicals that promote human health and incorporation of these genes into useful germplasm.
- Improved pest management systems will result in the production of higher quality products with fewer chemical inputs.
- Development of commercially acceptable germplasm with high nutrient use efficiency will result in sweet corn production with reduced fertilizer and reduced nutrient leaching and runoff, and fertilizer costs.

X. Organization:

The technical committee will be organized according to the procedures outlined in the Manual for Cooperative Regional Research. An annual meeting, authorized by the Administrative Advisor, will be held for the purposes of evaluating current work, discussion, and planning future work and regional publications. To help promote the regional aspects of this project, sub-committees will be appointed by the technical committee chairperson to coordinate activities of researchers under each major objective. Within each subcommittee project leaders may be appointed to coordinate activities for specific objectives. The officers will consist of the chairperson and secretary, to be elected annually by the technical committee.


Regional Project Title: Genetic Manipulation of Sweet Corn Quality and Stress Resistance



Administrative Adviser
AA - W. Ronnie Coffman (NIC) *for AA*

Aug 16, 1999

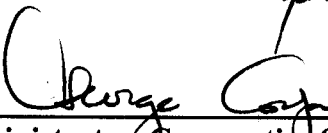
Date



Chair, Regional Association of Directors
for NERA

Aug 16, 1999

Date



**Administrator, Cooperative State Research,
Education, and Extension Service**

8/26/99

Date

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Attachments:**A. Project Leaders:**

State (SAES)	Project Leader(s)	Specialization
Florida (Belle Glade)	B. T. Scully *	Genetics and Breeding
Florida (Gainesville)	D. J. Cantliffe	Physiology
	L. C. Hannah	Molecular Genetics
Hawaii	J. L. Brewbaker*	Genetics and Breeding
Idaho	K. Mohan*	Plant Pathology
Illinois	J. A. Juvik*	Genetics and Breeding
	B. Klein	Food Science
	J. K. Pataky	Plant Pathology
Indiana	D. V. Glover*	Genetics and Breeding
Minnesota (St. Paul)	J. V. Groth	Plant Pathology
	Roger Becker	Weed Science
	W.D. Hutchison	Entomology
Minnesota (Waseca)	V. A. Fritz*	Physiology
New York (Ithaca)	M. E. Smith*	Genetics and Breeding
	M. Hoffman	Entomology
	D. W. Wolfe	Physiology
	R. Bellinder	Weed Science
	T. A. Zitter	Plant Pathology
New York (Geneva)	R. Straub	Entomology
	S. Reiners	Crop Production
Ohio	M. Bennett	Physiology
Oregon	C. D. Boyer*	Genetics and Breeding
	J. Myers	Genetics and Breeding
Pennsylvania	J. E. Ayers	Plant Pathology
	J. C. Shannon*	Physiology
Wisconsin	W. F. Tracy*	Genetics and Breeding

*=voting member of the technical committee

Administrative Advisor:

W.R. Coffman, Cornell University

CREES Advisor:

C. Stushnoff, Colorado State University.

Industry Advisory Personnel:**National Sweet Corn Breeders Association:**

Current NSCBA President

National Food Processors Association:

R. Teyker, DelMonte Corp. Rochelle, IL

Seed Producers Representative:

D. Plaisted, Novartis, Nampa, ID

B. Scientist years (SY), professional years (PY), and technical support years (TY) committed by each cooperating state:

State	SY	PY	TY
Florida	1.15	0.9	1.5
Hawaii	0.5	0.5	
Idaho	0.2	0.6	
Illinois	0.6	0.75	0.5
Indiana	0.2		0.5
Minnesota	1.4	2.2	1.2
New York	0.4	0.3	0.4
Ohio	0.3		0.5
Oregon	0.1		0.1
Pennsylvania	0.4		0.6
Wisconsin	0.5	0.7	0.4
Total	4.4	4.1	5.7