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Evaluation of progress from selection for cold and freeze tolerance in maize

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EVALUATION OF PROGRESS FROM SELECTION FOR COLD AND FREEZE TOLERANCE IN MAIZE

by

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Evaluation of Progress from Selection for Cold and Freeze Tolerance in Maize

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GRADUATE COLLEGE
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EVALUATION OF PROGRESS FROM SELECTION FOR COLD AND FREEZE TOLERANCE IN MAIZE

Kevin D. Eichelberger, Ph.D.
University of Nebraska, 1990

Advisor: C. O. Gardner

In 1972, a full-sib recurrent selection program for improved cold tolerance was initiated in the maize (Zea mays L.) synthetic population NS, a Nebraska version of Iowa Stiff-Stalk Synthetic. Selection was based primarily on percent emergence and visual vigor-rating under controlled (11.1/4.4°C day/night) conditions. Visual selection was also practiced for agronomic traits. After an early freeze in the 1976 nursery, selection was initiated to form a freeze-tolerant subpopulation.

The first objective of this study was to evaluate progress from selection for cold tolerance, and to examine changes in agronomic traits. Materials evaluated included cycles 0, 4, 8, and 12 of the cold-tolerant population (NS-CT-C0, C4, C8, and C12) and cycles 8 and 12 of the freeze-tolerant subpopulation (NS-CT-FT-C8 and C12). In general, much more improvement in cold tolerance was noted in the growth chamber than in the field. Grain yield of NS-CT-C12 and NS-CT-FT-C12 was increased by 1.10 and 0.85 Mg ha^{-1}, respectively, relative to NS-CT-C0. The NS-CT and NS-CT-FT selections also flowered earlier, were lower in plant and

ear height, and had lower grain moisture at harvest than NS-CT-C0.

The second objective was to measure genotypic variation for cold tolerance in the aforementioned entries, under cool conditions in the growth chamber and in an early planting in the field. In general, NS-CT-C0 displayed more genotypic variation for cold tolerance than did the selected populations. Selection appeared to have acted in eliminating highly suceptible genotypes in the early cycles. Genetic associations among cold tolerance traits were generally low, indicating that they are controlled by different genetic systems. Correspondence between performance in the growth chamber and in the field was low.

The third objective was to evaluate freeze tolerance of the above entries in the field and in the growth chamber. No appreciable level of freeze tolerance was noted in either environment. Nor was any differential regrowth after freezing noted. Much more damage was caused to plants by temperatures of -3.0 versus -2.5^{o}C.

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INTRODUCTION

Improving tolerance to adverse environmental conditions is an important goal of many maize (Zea mays L.) breeders. Cold tolerance, defined as the ability to germinate, emerge, and grow relatively normally in cool, wet soils early in the growing season, is a trait that is desirable under a number of circumstances. Early planting of maize by producers in temperate regions is deemed advantageous in order to distribute the workload more evenly over the growing season. Early planting also makes earlier flowering possible, thereby reducing risk of heat and drought stress during pollination, and may allow for more efficient use of early season precipitation. Higher grain yields are often obtained with early planting (Pendleton and Egli, 1969; Benoit et al., 1965; Imholte and Carter, 1987; Eckert, 1984). Early planting may also result in lower grain moisture at harvest (Imholte and Carter, 1987; Eckert, 1984), and increased stalk strength (Pendleton and Egli, 1969; Imholte and Carter, 1987). In high latitude or altitude regions, early planting is often essential for maximum production and for avoidance of damaging frosts before maturity. However, early planting of maize often results in poor stand establishment and reduced seedling vigor, which may decrease the yield potential of the crop. Cold tolerance would be especially useful in conjunction with reduced tillage or no-till cultural practices, where plant residues left on the soil

surface result in lower soil temperatures (Mock and Erbach, 1977) and higher moisture levels (Blevins et al., 1971).

A prerequisite for genetic improvement of any trait is the existence of heritable genetic variation. Numerous researchers have detected significant genetic variation for cold tolerance in maize. Grogan (1970) concluded that cold tolerance was conditioned primarily by additive effects, but also found dominance, epistasis, and maternal effects to be important. Pesev (1970) concluded that maternal effects were important and that the genetic control of cold tolerance is complex. Mock and Eberhart (1972) observed significant genetic variation for percent emergence and rate of emergence among S_1 lines from two breeding populations adapted to the U.S. Corn Belt. Eagles and Hardacre (1979a) found dominance genetic effects to be more important than additive effects for percent emergence and time to emergence at 10°C in a highland tropical maize population. In a generation means analysis involving three "cool season" and three "warm season" inbred lines, McConnell and Gardner (1979a) found additive, dominance, and epistatic gene effects to be important for germination at a constant 7.2°C, as well as for percent emergence and seedling vigor rating in the field. These and other studies indicate that sufficient genetic variation exists to improve maize cold tolerance via selection.

Recurrent selection for cold tolerance in maize has been attempted. Mock and Bakri (1976) evaluated several

cycles of selection for cold tolerance in two U.S. Corn Belt-adapted populations. Selection was based on an index integrating percent emergence, rate of emergence, and seedling dry weight. Two cycles of recurrent selection increased percent emergence and seedling dry weight by 8.4% and 0.6 dg plant^{-1}, respectively, in one population (BSSS13(SCT)). The other population (BSS2(SCT)) showed no response after three cycles of selection. Rate of emergence showed no response to selection in either population. Hoard and Crosbie (1985) evaluated five cycles of selection in BSSS13(SCT) and BSSS2(SCT) in the field. Percent emergence increased at a rate of 2.4 and 2.2% cycle^{-1} in the two respective populations. Increases in seedling dry weight and vigor rating, but not in rate of emergence, were reported for both populations. McConnell and Gardner (1979b) practiced recurrent selection for rapid germination at a constant 7.2°C in two U.S. Corn Belt-adapted populations. Four cycles of selection resulted in significant increases in percent germination of 8.8 and 9.9% cycle^{-1} for the two populations in the laboratory at a constant 7.2°C. Little improvement was noted in percent emergence or vigor in early planted field experiments. This lack of response in the field was attributed to mild spring weather during the 2-year test period.

Plants in the field may also occasionally be subjected to frost in late spring. Frost can result in considerable damage to leaves or even death of the seedling. Even when

plants are not killed, frost damage may seriously affect the yield potential of the crop. Arny and Upper (1973) found that frost-damaged plants in the field yielded 29 percent less than their undamaged counterparts. Frost-damaged plants also had delayed maturity and higher grain moisture compared to undamaged plants.

The study of genetic aspects of freeze tolerance in maize has not received much attention. Dhillon et al. (1988) observed significant genotypic differences among 64 maize inbred lines for freezing injury in the growth chamber and the field. Gardner et al. (1987) reported that a population which had undergone several cycles of recurrent selection for cold and freeze tolerance was considerably more tolerant of freeze damage than the original population. However, Brar et al. (1987) found no significant genotypic variation in maize for tolerance to freezing temperatures. Miedema (1982) concluded that genetic variation for freeze tolerance in maize is low.

In 1972, a recurrent selection program for improved cold tolerance was initiated in a Nebraska version of Iowa Stiff-Stalk Synthetic at the University of Nebraska-Lincoln. Selection was based primarily on percent emergence and a visual rating of vigor after 30 days in the growth chamber under 11.1/4.4^{o}C day/night conditions. After an early-season frost in the nursery in 1976, selection was initiated to form a freeze-tolerant subpopulation. The objectives of this study were, 1) to assess progress from

selection for cold tolerance, under conditions in the field and in the growth chamber; 2) to measure genetic variation and genetic relationships among cold tolerance traits in selected cycles of the cold- and freeze-tolerant populations in the field and in the growth chamber, and to compare results in the two environments; and 3) to evaluate freeze tolerance of original and selected populations in the field and in the growth chamber.

LITERATURE REVIEW

Physiological Aspects of Chilling Sensitivity in Maize

Maize belongs to the chilling sensitive (CS) category of plant species, which includes those species which are sensitive to low, nonfreezing temperatures up to about 10-12^{o}C (Lyons, 1973). Exposure of CS plants to chilling temperatures results in a number of visual injury symptoms, including reduced germination and emergence, retarded growth and development, wilting, chlorosis, and eventually, reduced vigor or even premature death (McWilliam, 1983; Blum, 1988). At the cellular and subcellular levels, exposure to chilling temperatures causes such disturbances as reduction or cessation of protoplasmic streaming, leakage of cell solutes and loss of turgor, disruption of cellular membranes, and inhibition of chloroplast development (Lyons, 1973). The extent and severity of damage depends on the intensity and duration of the stress, as well as on the developmental and physiological state of the plant. However, these visual symptoms are secondary manifestations of injury at a more basic level of organization.

The primary events or lesions induced by exposure of CS plants to chilling stress are not fully understood. Possible mechanisms of chilling injury include changes in the physical state of biological membranes; metabolic imbalances due to differences in the activation energies (Ea) between different enzymes or enzymatic systems; and

low-temperature-induced denaturation or dissociation of proteins, leading to loss of essential activity.

Primary Physiological Events During Chilling Stress

A widely accepted hypothesis explaining the damaging effect of chilling temperatures on CS plants holds that the primary event during chilling is a low-temperature-induced phase transition of the biological membranes. According to this hypothesis, membrane lipids of CS species undergo a thermotropic change from a fluid, liquid-crystalline state to a more rigid gel state at some critical low temperature. This hypothesis was first proposed by Lyons and Raison (1970) and was later presented in a more refined form by Lyons (1973). The hypothesis was based in part on the observation that Arrhenius plots of succinate oxidation in mitochondria isolated from three CS species showed a marked discontinuity (increase in slope) at the temperature that coincided with the onset of visual chilling injury symptoms in these plants. Succinate oxidase activity in mitochondria from three chilling resistant (CR) species exhibited no such discontinuity between 0 and 25^{o}C (Lyons and Raison, 1970). Further evidence for this hypothesis was found in discontinuities of Arrhenius plots of other membrane-bound enzymatic systems at the same critical temperature (Lyons, 1973). It was postulated that a phase transition of the membranes would lead to an increase in the permeability of membranes and to an increase in activation energy (Ea) of many membrane-bound enzymes or

enzymatic systems. The increase in permeability would lead to an inability to maintain the proper ionic balance within the cell. The increased Ea of membrane-bound enzymes would lead to metabolic imbalances which in turn could eventually lead to a buildup of toxic metabolic intermediates. For example, it was hypothesized that the activity of mitochondrial membrane-bound enzymes of the TCA cycle would be more affected than would the soluble enzymes of glycolysis. This could lead to the accumulation of toxic levels of acetaldehyde and ethanol in plant cells (Lyons, 1973). If the chilling stress was alleviated in time, the plants would recover; if not, they would suffer irreversible injury or death.

The phase transition hypothesis has been tested in a large number of species using a variety of techniques, with mixed results. Earlier studies using electron spin resonance (ESR) spectroscopy seemed to indicate a reasonably close relationship between visual chilling sensitivity and occurence of a phase transition of the membranes. However, the use of ESR spectroscopy to detect phase transitions has been criticized (Schreier et al., 1978), as has the interpretation of the data obtained using this technique (Wolfe and Bagnall, 1979). More recent results have not been as consistent. For example, Raison and Orr (1986) used three different techniques - ESR, differential scanning calorimetry (DSC), and fluorescence techniques using the fluorescent probe trans-parinaric

acid- to examine chilling-induced phase changes in thylakoid polar lipids of oleander, mung bean, and tomato, all CS species. All three techniques detected a phase change at virtually the same temperature within each species. On the other hand, O'Neill and Leopold (1982) were unable to detect a phase transition above 0°C in phospholipid vesicles of mitochondrial membranes of CS soybeans using DSC. Similarly, Dalziel and Breidenbach (1982) were unable to detect a phase transition above 0°C with either DSC or ESR in two ecotypes of wild tomato which differed in chilling sensitivity.

The question of whether certain membrane systems of CS plants undergo a phase transition in the range of 0-12°C has not been fully resolved. Although it is an attractive hypothesis, its confirmation awaits further study.

An important part of the phase transition hypothesis is the expected increase in Ea of membrane-bound enzymes or enzymatic systems. Lyons and Raison (1970) hypothesized that a phase transition might affect lipid-protein interactions, thereby increasing the Ea of membrane-associated proteins. Their hypothesis was based on the observation that Arrhenius plots of the rate of succinate oxidation in mitochondria from CS tomato, cucumber fruit and sweet potato roots exhibited a deviation from linearity (interpreted as an increase in Ea) at about 9-12°C. No such deviation was found in mitochondria from CR cauliflower buds, potato tubers, or beet roots. This was

interpreted as a phase transition-induced partial inactivation of the enzyme. While an increase in Ea of a membrane-bound enzyme may not be harmful in itself, it was thought that such effects may result in metabolic imbalances, leading to a buildup of toxic metabolic intermediates (Lyons, 1973).

Other membrane-bound enzymes or enzymatic systems have also shown abrupt changes in Ea upon chilling. Murata et al. (1975) noted a break in the Arrhenius plot of photosynthetic activity above 0^{o} C in chloroplasts from the CS blue-green alga Anacystis nidulans, which correlated with a phase transition event as detected by ESR. They found no temperature-induced break in Arrhenius plots of photosynthetic activity for CR spinach or lettuce. Shneyour et al. (1973) noted a temperature-induced break in the Arrhenius plot of photoreduction of $NADP^{+}$ in isolated chloroplasts from CS tomato and bean at about 12^{o}C. No discontinuity in the Arrhenius plot was seen for CR lettuce or pea. More recently, Yoshida et al. (1986) noted a break at 6.7^{o}C in the Arrhenius plot of Mg^{2+}-stimulated ATPase activity in highly purified plasma membrane fractions from CS mung bean. However, membrane-bound enzymes do not always exhibit sharp drops in Ea at temperatures where changes in the molecular ordering of lipids is indicated by ESR or other techniques. For example, Patterson et al. (1979) were unable to detect a break in the Arrhenius plot of O_2 uptake above 0^{o}C in pollen membranes from CS tomato.

Furthermore, some CR species exhibit discontinuities in Arrhenius plots of membrane-bound enzymes at temperatures above 0°C, contrary to expectation. For example, Pomeroy and Andrews (1975) detected a break in the Arrhenius plot of plasma membrane ATPase activity at 11°C in wheat, a species which is completely chilling resistant.

In the past, an increase in Ea of membrane-bound enzymes has usually been ascribed to changes in the molecular ordering of membrane lipids. This change supposedly affects hydrophobic interactions between acyl portions of membrane lipids and hydrophobic amino acids of membrane proteins, resulting in a change in protein conformation and therefore a change (usually a decrease) in activity. However, chilling may have a direct effect on enzyme activity, independent of lipid-protein interactions. Conformational changes in proteins due to low temperatures (cold lability) are a distinct possibility (Lyons et al., 1979). In fact, some enzymes which are not associated with membranes show breaks in Arrhenius plots of activity versus temperature. For example, Graham et al. (1979) noted a discontinuity in the Arrhenius plot for PEP carboxylase from CS tomato at about 10°C. PEP carboxylase from CR wheat showed no break in the Arrhenius plot above 0°C. Similarly, Uedan and Sugiyama (1976) noted an increased Ea of CS maize PEP carboxylase at 11-12°C.

Photosynthesis

Photosynthesis (Ps) in CS plants such as maize is

greatly inhibited at low teperatures. Duncan and Hesketh (1968) examined the effect of temperature on net photosynthetic rates of 21 races of maize and a modern single-cross hybrid (OH45 x K4). Average net photosynthetic rates were reduced 52 and 25%, respectively, at 15/10 and 18/13°C day/night temperatures compared to values measured at 30/25°C. Alberda (1969) observed negligible rates of Ps eight days after seedlings growing at 20°C were shifted to 10°C. Bird et al. (1977) also found photosynthetic rates in maize to be negligible at low temperature (13/10°C, day/night).

Several explanations are possible for the observed decrease in Ps at low temperatures. One is that certain photosynthetic enzymes are inhibited at some critical low temperature. As mentioned previously, Uedan and Sugiyama (1976) noted an increase in Ea of maize PEP carboxylase at 11-12°C. Stamp (1981a), on the other hand, found only small differences in PEP carboxylase activity of 28 maize inbreds when grown at low versus high (15/12.5°C versus 25/22.5°C day/night) temperature, while the activity of ribulosebisphosphate carboxylase declined 73% at the low temperature regime. Taylor et al. (1974) found that exposure of maize seedlings to 10°C for 3 days under high light reduced PEP carboxylase activity only slightly relative to 25°C-grown controls, while NADP-malate dehydrogenase and pyruvate dikinase activities declined by 39 and 50%, respectively. No loss in activity was observed

under lower light levels, however, indicating a light dependence of loss of activity.

Stomatal resistance to CO_2 uptake may also be important in reducing Ps at low temperatures. Low temperatures may reduce the hydraulic conductivity of root membranes of CS plants. In addition to reduced water uptake, the conductivity of the tonoplast of stomate guard cells may be reduced, rendering them less responsive to reduced leaf water potential at low temperatures. Bean (Phaseolus vulgaris L.) plants exposed to 5°C night temperatures had severely reduced levels of Ps the following day (Crookston et al., 1974). This effect was paralleled by a decrease in leaf water potential and an increase in stomatal resistance to CO_2 uptake. When shoots (but not roots) were cooled, Ps, leaf water potential, and stomatal CO_2 resistance were similar to control levels.

Chilling CS plants in strong light enhances inhibition of Ps, and can lead to irreparable damage of the photosynthetic apparatus (Oquist and Martin, 1986). Long et al. (1983) studied the combined effect of high light intensity and low temperature on CO_2 assimilation rate in maize. Chilling young maize plants from 20° to 5°C resulted in a 93% reduction in CO_2 assimilation rate. Upon return to 20°C, plants chilled in the dark regained 90% of their CO_2-assimilating capacity, compared to 50% for plants chilled in bright light.

Chlorosis is one of the most commonly reported