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Seed maturation and drying in sweet corn (Zea mays L.) endosperm mutants

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Seed maturation and drying in sweet corn (*Zea mays* L.)
endosperm mutants

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Iowa State University, 1992
Seed maturation and drying in sweet corn
(Zea mays L.) endosperm mutants

by

Henry R. Mloza-Banda

A Dissertation Submitted to the
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For the Graduate College

Iowa State University
Ames, Iowa
1992
DEDICATION

Jesus said to them, "Have you never read in the Scriptures: 'The stone which the builders rejected has become the chief cornerstone. This was the Lord's doing, and it is marvelous in our eyes'?"

Dedicated to my mentors: the late Chief Mkangowamdambo Ngamwane (deceased 1974), uncrowned champion against servitude in Malawi; and to the late Paul Hope Alufandika.

And in sympathy to those that died and the suffering in persuant of equal rights, justice, and sovereignty in South Africa, Northern Ireland, the Palestinians, the Native Americans, and the Australian Aborigines.

A LUTA CONTINUA!
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INTRODUCTION

Characterization of the factors that make one genotype mature sooner or later than another, and the subsequent harvest quality, remains a formidable task due to the continuing introduction of new genotypes. For the farmer, grain moisture percentage is the critical measure of maturity. To a plant breeder, the interval from seeding to flowering is important since plants that flower at the same time can be readily crossed. To the agronomist, maximum accumulation of dry matter is a fundamental measure of the relative maturity of genotypes (Gunn and Christensen, 1965). Consequently, the changes which accompany grain development have been subject to increasing attention. This is partly in response to a need to identify those factors involved in the regulation of grain yield and composition (Duffus, 1979), and partly to compare relative kernel maturity and subsequent planting value of lines or hybrids.

Improved uniformity of germination and emergence has been associated with the physiological quality of the seedlot, which is the result of environmental factors, management decisions, and handling procedures (Bennett et al., 1988). There has long been considerable uncertainty as to when seed corn should be harvested in order to give the best performance when planted (Koehler et al., 1934). The accurate prediction of kernel maturity would facilitate harvest decisions, thus avoiding detrimental field weathering as
well as allowing the production of seed with high seedling vigour (Knittle and Burris, 1976).

In dent corn harvested for seed, several indices have been employed in the determination of kernel maturity, including: kernel moisture percentage; maximum kernel dry weight; black layer formation; and kernel respiration. Other studies have attempted to determine the factors associated with differences in rates of maturity and drying. The relationships between kernel, cob, and ear moistures, weather factors, and kernel composition have received attention. Invariably, however, artificial drying has commonly been used for conditioning harvested seed (Seyedin et al., 1984). Artificial drying has been frequently applied to seed corn (a) harvested before natural drying was complete, (b) harvested under weather conditions unfavourable for storage, or (c) damaged by early frost (Gausman et al., 1952).

The situation is not entirely the same in sweetcorn (*Zea mays saccharata*), which exhibits striking differences from normal dent corn in the texture, form, and amount of the endosperm. The sweetcorns are simply described as pop, flint, and dent varieties that have a diminished ability to synthesize starch (East and Hayes, 1911; Creech, 1965). Throughout the world, they are used chiefly as a canning or as a green garden vegetable in regions favoring their cultivation. But as a product, their table use is limited by the relatively short period during which kernels are at peak quality.
The reserve materials of the endosperm rapidly undergo conversion to starch dextrins and various hexoses. Thus any delay in harvest or utilization (processing) can lower the grade and value of the product (Boyer and Shannon, 1983).

The identification of endosperm genes that maintain high sugar levels to extend the period of acceptable kernel quality has led to the use of these genes in the development of improved sweetcorn cultivars (Laughnan, 1953). However, these cultivars have been associated with poor seed quality and seedling vigour (Creech, 1965; Nass and Crane, 1970). Slow variable germination and poor stands often make the high sugar cultivars unacceptable for commercial production (Styer et al., 1980; Wann, 1980). Although the causes of this low germination and low vigour are not understood, genetic background, kernel weight, kernel moisture, maturity and endosperm carbohydrate reserves have all been implicated (Churchill and Andrew, 1984). Identification of seed quality factors has lagged behind improvements in eating quality characteristics such as flavor, texture, and tenderness (Wann et al., 1971).

Beyond these casual observations, maturing maize endosperm is especially appropriate for studies of the effects of genetic interactions on carbohydrate metabolism because of the many gene mutations available that influence carbohydrate properties (Creech, 1965). Current evidence suggests maturation is generally terminated by some degree of drying, which results in a gradual reduction in
metabolism as water is lost from the seed tissues and the embryo passes into a metabolically inactive or quiescent state (Kermode, 1990). It has been suggested that soluble sugars are associated with tolerance to desiccation, which, whether natural or imposed, plays a role in switching seeds from a developmental mode to one that is essential to promote germination. Sweet corn endosperm mutants, known for high sugar levels and moisture retention during maturation, provided a ready made object of study in elucidating the relationship between maturation physiology and seed quality.
REVIEW OF LITERATURE

Sweetcorn

Genetic background

Significant genetic distinctions have been drawn between the endosperm of sweetcorn and that of field corn. The normal gene Su (starchy) at locus 71 on chromosome 4, in field corn, has mutated to a recessive form su (sugary), in sweetcorn. The sweet flavor of the immature kernels of sweetcorn, thus, results primarily from the effects upon metabolism regulated by this recessive gene. It has been shown to prevent the conversion of sugar into starch during the development of kernel endosperm. However, the actual rate of this conversion, and the final degree of sweetness and other flavors, involves additional genes which modify the endosperm composition. The sugary mutation results not only in a higher total sugar content but also in a persistence of the sugary condition of the endosperm to maturity. When the sugary kernel is in the milk stage, from 18 to 20 days after pollination, it is ready for consumption. At optimum edible maturity, high quality sweetcorn contains 70-75% moisture and 25% of the dry matter as simple sugars. But when mature and dry, sweetcorn kernels are usually wrinkled and transluscent, in contrast to the smooth or dented, flinty and/or starchy characters of field corn kernels (Whistler, 1957; Galinat, 1971; Wann et al., 1971).
Endosperm genes have been distinguished depending upon the protein fraction or the carbohydrate fraction of the endosperm which is altered by the genes (Boyer and Shannon, 1983). The genes opaque-2 (o2) and floury-2 (fl2) are known to alter endosperm protein production. The endosperm genes, amylose extender (ae), brittle (bt), brittle-2 (bt2), shrunken (sh), shrunken-2 (sh2), shrunken-4 (sh4), dull (du), and waxy (wx), are known to alter the carbohydrate fractions. Based on mature kernel phenotype, the mutants have further been divided into two classes: starch deficient and starch modified as illustrated in Fig. 1 (after Shannon and Garwood, 1983). Starch deficient or class one mutants, eg., shrunken 1 (sh1), sh2, sh4, bt1, bt2, and sul, reduce starch content and substantially increase sucrose accumulation (with the exception of sh4) in the endosperm. On the other hand, starch modified or class two mutants, eg., ae, du, su2, and wx, have only a slight effect on starch accumulation in the endosperm. However, these mutants alter the normal ratio of amylose to amyllopectin and contain higher than normal levels of sucrose (Creech, 1965).

Biochemical considerations

Endosperm mutants in class one have been shown to result from deficiencies in the enzymes necessary for the conversion of sucrose to the substrates required for starch synthesis, ADP-glucose and UDP-glucose. Class one mutants accumulate sugars at the expense of
Fig. 1. The genetic background and physiology of carbohydrate metabolism in sweet corn endosperm mutants (Shannon and Garwood, 1983)
starch and phytoglycogen. Sucrose is greatly increased, and the reducing sugars glucose and fructose are elevated. At 18 to 20 days after pollination, generally the harvest stage of fresh market sweetcorn, bt, bt2, sh, sh2, and sh4 endosperm types have been shown to contain 2-3 times the sucrose of su endosperm. And equally significant, at 28 days after pollination, when sucrose levels in su cultivars have fallen by more than 50%, class one mutants continue to have high levels of sucrose. Because of these high sugar levels, sweetcorn cultivars based on these genes have been referred to as extra sweet cultivars.

The reduced conversion of sugars to starch is also known to occur after harvest (Boyer and Shannon, 1983). Stored at room temperature for 24 h, kernels of su cultivars generally lose 50% of the sucrose present at harvest. Although refrigeration of su cultivars delays the post-harvest conversion of sucrose to starch, the conversion of sucrose in class one mutants is delayed even without refrigeration.

It is suggested that class two mutants possibly result from lesions in the enzymatic reactions required for starch formation. For instance, the endosperm of the homozygous wx mutant lacks the starch granule bound starch synthase enzyme. Kernels homozygous for the du mutant, however, have reduced levels of two soluble starch synthase enzymes and one of three starch branching enzymes which are present in normal maize endosperm. At 20 days after pollination,
sugar levels of class two mutant kernels are similar to su kernels (Creech, 1956, 1966). But it is speculated that the additive effect of decreased enzyme activity results in significant reductions in starch synthesis with a concomittant accumulation of sugars (Garwood et al., 1976).

In addition to higher sugar levels, su endosperm is reported to contain a polysaccharide similar in structure to animal glycogen, phytoglycogen (Sumner and Somers, 1944). Although the mechanism of phytoglycogen biosynthesis remains unclear, phytoglycogen and related water soluble polysaccharides (collectively called WSP), are important components of sweetcorn quality (Culpepper and Magoon, 1924, 1927; Holder et al., 1974a, 1974b). Characteristics of texture and creaminess are affected by the water soluble fraction and the ratio of soluble to insoluble polysaccharides. Presently, endosperm mutants of class one and two mutants are known to contain low levels of WSP. To achieve high WSP levels, as well as increased sugars, double or triple mutants with su as one of the mutants are often utilized. The two most frequently used commercial mutants are su and sh2, and the se gene, recently discovered, is a recessive modifier of the su gene (Gonzales et al., 1974, 1976; Brink, 1978). Genotypes with the se gene have a sugar content comparable to that of sh2, yet the phytoglycogen content equals that of su genotypes (Gonzales et al., 1976).
Protein levels are of little consequence in determining sweetcorn quality (Boyer and Shannon, 1983). This is because the synthesis of storage protein, such as zein, begins between 16 and 20 days after pollination. Since this is near the typical harvest stage of sweetcorn, little effect on eating quality would be expected. Thus incorporation of o2 and f12 genes would be expected to have minimal effects on protein quality. In contrast, kernels of the class one mutant would be suitable for harvest later in development after more storage protein has accumulated. On a dry weight basis, however, proteins constitute a greater proportion of the total dry weight of su and class one mutant kernels because of reduced levels of polysaccharides (Misra et al., 1975).

**Germplasm background**

Major effects of the various genes remain unchanged when the genes are incorporated into different inbred backgrounds, i.e., carbohydrate (and protein) fractions are altered in similar fashions. However, subtle, yet significant, differences between lines homozygous for different genes and gene combinations have shown distinct differences in plastid and carbohydrate accumulation patterns between a dent inbred background and sweetcorn inbred background (Boyer et al., 1977). Similarly, differences in the starch polysaccharides of mature kernels homozygous for these genes has been demonstrated in dent and sweetcorn inbred backgrounds.
(Boyer et al., 1977). Each inbred may have a modifier complex that affects the resultant kernel quality interaction between genes and germplasm backgrounds for consumption, as well as germination and seedling vigour (Rowe and Garwood, 1978). Development of new sweetcorn genotypes should be from a wide range of genetic backgrounds to obtain optimum results.

Seed Quality

The most prevalent problem associated with the new sweetcorn cultivars based on endosperm genes other than su has been poor seed quality and seedling vigour. Slow variable germination and reduced stands often make the high sugar cultivars unacceptable for commercial production (Hanna and Cantliffe, 1977). Germination is even lower in cold soils. Although the causes of this low germination and seedling vigour are not understood, several factors, such as genetic background, kernel weight, kernel carbohydrate reserves, moisture, and maturity, have been implicated.

Effect of endosperm genes

The effects of the single genes ae, su, and wx on kernel vigour have been investigated. Haskell and Singleton (1949) found no significant difference in the ability of su and sh2 kernels to withstand cold germination conditions and concluded that genetic factors related to cold hardiness appear to be more important than
whether the endosperm is starchy or sugary. However, using F1 seed from crosses between isogenic lines, Nass and Crane (1970), found su endosperm types associated with significantly reduced seedling height, seedling growth rate, and percentage of emergence compared with normal counterparts. Lack of consistent effects of su was interpreted as being due to different experimental techniques, genetic material used, or experimental design (Rowe and Garwood, 1978).

Rowe and Garwood (1978) studied the effect of endosperm mutants ae, du, su, and wx, individually and in combination on seed vigour in an isogenic series from three sweetcorn inbreds. Genotypes with the ae phenotype had poor vigour and exhibited reduced germination and decreased seedling shoot length. In contrast, genotypes with the su phenotype performed consistently better. Rowe and Garwood found large differences among sweetcorn inbreds in their ability to impart good seed vigour to the mutant genotypes. Their work suggested that the problem of low vigour in the ae, du, and wx genotypes might be overcome by selecting vigorous inbreds for seed parents in the production of hybrid seed.

Churchill and Andrew (1984) concluded that germination at low temperatures can be satisfactory for hybrids carrying the sh2 and se genes. They found that sh2 and se hybrids had satisfactory germination at cold temperatures when harvested at a moisture content of 50% and 60%, respectively. Cold temperature germination
was negatively associated with kernel moisture and various sugar fractions. Root, shoot, and total seedling length were positively correlated with cold temperature germination.

Physiological maturity

The determination of kernel physiological maturity has been recognized as essential to quality seed production. Several measurements have been employed as an index to determine kernel physiological maturity.

Aldrich (1943) reported that field corn was mature when maximum kernel dry weight was achieved, which he found occurred at about 65% dry matter. However, because of the mechanical shelling difficulties generally encountered at grain moisture percentages above 35%, grain moisture percentage rather than maximum grain dry weight has normally been the major consideration in determining a suitable harvest date in field corn (Daynard, 1972).

Seed moisture at harvest per se has been reported to influence germination percentage and vigour. Sprague (1936) found that seed of field corn harvested at 30% moisture provided better results than seed harvested at higher or lower moisture contents. He also reported that the moisture of immature seed must be reduced to 25% before normal germination can occur. Using seed of sweetcorn obtained from samples at 5 day intervals from date of first silking through maturity, Culpepper and Moon (1941) found that the most
favourable harvest time for sweetcorn seed appeared to be between 35 and 45 days after silking or at a grain dry matter of 35 to 50%.

However, Bennett et al. (1988) reported that sweetcorn inbred seed harvested at 45 to 54% moisture produced better stands than seed harvested near 35% moisture. Kernel maturity, determined by kernel moisture, had little effect on germination and did not strongly influence early seedling dry weight. Reduced seedling weights obtained from mechanically harvested seed were interpreted as evidence that physical damage during harvesting, drying, shelling, and sorting may be a primary cause of reduced seed vigor in sweetcorn endosperm mutant kernels.

In addition to kernel moisture, pollination or silking dates have also been used as maturity indices. Styer et al. (1980) compared ATP and various seedling vigour measurements with seed germination and seedling vigour in normal and three corn endosperm mutants harvested at 16 to 42 days post-pollination. Germination and seedling vigour measurements of sh2 were significantly lower than those of su in both lab and field tests. Seedling vigour of sh2 appeared to be highest at or before 36 days post-pollination and declined after 42 days. Seeds also germinated better from 16 to 36 days post-pollination than at 42 days.

Black layer formation has been advocated as an additional maturity index (Daynard and Duncan, 1969). This is because of the close coincidence in time between the attainment of maximum kernel
dry weight and black layer formation. Its development has been shown to be a characteristic of many corn types, including sweetcorn, at maturity. However, variation in percentage grain moisture at black layer formation could, for certain purposes, confound harvest determination for a crop that attains harvestable condition at a high moisture.

Knittle and Burris (1976), working with field corn, concluded that seedling vigour was dependent on date of harvest (stage of maturity) and that poor seed quality could result from untimely seed harvest. Correlations between maturity indices (moisture percentage, black layer formation, respiration rate, and seed dry weight and seed vigour measurements) were specific for hybrid and location.

Endosperm carbohydrate reserves

Poor seed vigour has also been related to the amount of endosperm. Wann (1980) compared seed vigour among cultivars with triple recessive endosperm mutant gene combination ae, du, wx, sh, and their su counterparts. He found that low seed vigour in high sugar genotypes apparently was related to their small endosperms, i.e., low carbohydrate content, and not entirely to genetic inferiority of the embryo. In contrast, several investigators have concluded that endosperm carbohydrate content may not be related to sweetcorn vigour.
Styer et al. (1980) in a time course study of 0 to 96 h imbibition using 42-day old seeds found that the ATP content (resulting from carbohydrate breakdown) of sh2 seeds was generally as high as or higher than in the genotypes su, bt, and normal. Thus, it did not appear that energy level was related to poor vigour during the early stages of germination of sh2 seeds.

Churchill and Andrew (1984) also found that cold temperature germination of sh2 and se hybrids as compared to their counterparts, Sh and Se, was not significantly related to kernel weight when seed was harvested at successive stages of maturity. Using a sweetcorn inbred harvested at 10 stages of maturity, between 42 and 103 days post-pollination, Benett et al. (1988), concluded that endosperm weight may not be related to sweetcorn vigour. Borowiski et al. (1991) obtained similar results using seed weight and endosperm:embryo ratio data.

Carbohydrate changes

The effect of endosperm mutants on various kernel carbohydrate level has emphasized the importance of soluble carbohydrates as a means of improving eating quality. For instance, Rosenbrook and Andrew (1970) reported on differences in level and rate of change in kernel moisture and carbohydrates for a range of su lines at 25 days post-pollination (canning stage). While total sugar levels were similar in su and Su endosperms early in development, su endosperms
achieved a two- to three-fold increase by day 15 and decreased thereafter. Additionally, they found that inbreds differed significantly for percent moisture and for each carbohydrate fraction. Moisture content and carbohydrate composition changed at significantly different rates for the eight lines.

Soberalske and Andrew (1978) studied the effect of 10 gene combinations on level, rate of change, and interaction of kernel moisture and sugar level during 4 weeks post-pollination. They used 7 adapted inbreds carrying the genes du, su, wx, and sh2, and one double mutant du-wx each in association with the sugary-1 alleles Su and su. They reported that the sh2 gene conferred improved kernel moisture and total sugars and, more importantly, reduced rates of loss of these components as compared to su genotypes.

High retention of moisture, sugars, WSP, and low water insoluble polysaccharides (WIP) at the eating stage are kernel attributes associated with high consumer quality and are enhanced by the sh2 and se genes. While these genes have tended to be negatively related to germination and seedling vigour, Churchill and Andrew (1984), argued that this need not always be so. They reported that germination of sh2 did not differ significantly from that of its standard counterpart Sh2 after the first harvest, 33 days post-pollination, even though differences for total sugars and water insoluble polysaccharides were significant at all harvests. Similarly, while germination of se was somewhat lower than its
counterpart Se, it varied with each harvest. Higher total sugars were obtained at each harvest yet satisfactory germination was obtained only up to 40 DAP.

In any case, most major studies of corn endosperm measure carbohydrate changes during the early part of kernel development, i.e., up to 28 DAP (Creech, 1965). Thus, few comparable data exist of carbohydrate measurements as kernel development approaches physiological maturity.

Seed Maturation, Drying, and Drying Injury

Harvest moisture and drying temperatures

Hybrid seed corn is normally harvested at seed moistures as high as 40% and then mechanically dried to 11-12% moisture to maintain seed quality (Seyedin et al., 1984). However, under varying situations, this process has been associated with significant reductions in seed quality. Duncan and Marston (1925) noted that seed harvested at earlier stages of maturity, i.e., milk and dough stage, and dried at 44.4 C gave low germination results. Harrison and Wright (1928) found that at harvest moistures ranging between 16 and 27%, corn dried at 40-45 C was not injured whereas corn dried at 50 C was damaged. Kiesselbach (1939) found similar trends but recommended drying temperatures less than 40.6 C when initial seed moisture content was around 50%. At a drying temperature of 44.5 C,
he found no significant difference in drying injury among 26 hybrids ranging in initial moisture content from 16 to 38%.

Other investigators have, however, reported no appreciable damage with higher drying temperatures. Wileman and Ullstrup (1945) reported that seed dried at 48.9°C and 54.4°C showed no appreciable reduction in germination when the initial seed moisture content was 20-25% and 20%, respectively. With initial moisture content of 30%, McRostie (1949) obtained similar results from drying temperatures up to 54.4°C.

Herter and Burris (1989b) suggested that temperature regimes that start with initial drying at low temperature (preconditioning) followed by subsequent drying at high temperature render seed corn less susceptible to drying injury. They preconditioned ears harvested at 48% and 38% kernel moisture, respectively, at 35°C for 1-2 days followed by drying at 50°C to 12% moisture. In another experiment, they preconditioned kernels at different temperature (10, 20, 35°C) and relative humidity (35, 60, 95%) combinations for 1-3 days prior to drying at 50°C to 12% moisture. They found that preconditioning at 35°C rendered high moisture seed tolerant to subsequent drying at 50°C. Ears of ca. 48% kernel moisture required exposure to 35°C for 23-40 hours to gain tolerance. Ears harvested at 38% moisture required 11-37 hours preconditioning or a moisture reduction to 26-32%. Preconditioning treatments that allowed
moderate drying (20-35 C) and low relative humidity were more
effective in hardening samples against subsequent drying at 50 C.

Variations in drying temperatures, duration of drying, and on
initial harvest moisture are apparent in conditioning sweet corn
seed for seed quality determinations as practiced by different
investigators (Styer et al., 1980; Styer and Cantiliffe, 1983a;
Churchill and Andrew, 1984; and Bennett et al., 1988). Seed has been
dried at temperatures ranging from 30-35 C for varying durations at
the discretion of investigators. Bennett et al. (1988) suggested
that careful management of kernel maturity, seed drying
temperatures, and other factors would likely improve the uniformity
and vigour of emerging sweet corn seedlings.

Genotype differences in maturation drying and drying injury

It is generally agreed that differences exist between corn
strains in their rate of drying and drying injury. Crane et al.
(1959) found that some hybrids which silk at the same time and
maintain the same water to dry matter ratio until they reach 45%
moisture may differ in drying rate after that point. Hallauer and
Russell (1962) reported that inbred Oh 45 silks 10-15 days earlier
than inbred B14. At harvest, combinations involving B14 tended to
have a lower moisture content than those involving Oh 45. They
suggested that since maturity was attained at approximately the same
interval after silking, and the early line had the higher grain
moisture at that point, there was a difference in the rate of drying after maturity. They also reported regressions that indicated that the rate of moisture loss was a heritable character. Drying rate was based on percent moisture loss of ears and or grain harvested at intervals after silking and subjected to drying in forced air driers.

Reiss (1944) found that seeds from inbred line R4 were more tolerant to high drying temperature than seeds from inbred WF9 at increasing temperature and harvest moistures. Navratil and Burris (1984) reported that inbred parents differed in tolerance to high drying temperatures. Ears of inbreds A632, B73, and Mol7 were harvested with moisture percentages ranging from 45-50% to approximately 20% over five harvest dates. Mean temperatures of 35, 40, 45, and 50 C were used to dry seed to 12% moisture. They found that A632 was the most tolerant to high drying temperature whereas B73 and Mol7 were relatively intolerant. Further, they reported that significant inbred-parent by harvest-moisture by drying-temperature interactions for germination and cold test emergence percentages precluded simple recommendations of safe harvest moistures and drying temperatures.

Bdliya and Burris (1988) harvested high moisture seed from the diallel crosses and the reciprocals of the inbreds A632 (tolerant of drying injury), A641, B14A, B73, and W64A (intermediate), and Mol7 (intolerant) and dried at 50 C. Greater variation in germination was
observed among lines when used as female parents than when lines were used as males. Using data of general and specific combining ability, they suggested that most of the variability observed among seeds for tolerance to drying injury was associated with maternal and/or cytoplasmic inheritance. Further, they suggested that the contribution to the endosperm from the female gamete at fertilization and/or the quantity and quality of kernel endosperm may have contributed to the maternal effects.

**Pericarp, in relation to maturation and drying**

Though most studies have focused on pericarp thickness and its effect on tenderness, a character affecting sweetcorn processing quality, several investigators have studied its influence on maturation and drying.

Helm and Zuber (1970) measured pericarp thickness from tissues excised from mature dent kernels harvested at physiological maturity, 30% moisture and at 15% moisture. They observed that corn harvested at physiological maturity, 30% moisture and 15% moisture, had similar pericarp thickness. However, they found significant differences in pericarp thickness among hybrids. In subsequent work, Huber et al. (1970) compared 9 endosperm mutants with non-mutant types in inbred backgrounds, B73, and Oh43. They found that the effects of the endosperm type on pericarp thickness were inconsistent between backgrounds. The endosperm mutants did not show
a strong correlation for pericarp thickness with degree of endosperm development of the mutant kernel class.

Conversely, Tracy and Schmidt (1987) found that pericarp thickness was significantly affected by inbreds, endosperm types, endosperm x inbreds interaction, ear and position of measurement on the kernel. However, they reported that the average pericarp thickness of the most widely used endosperm types, sh, was not significantly different than that of standard (su) types. They concluded that the endosperm effects on pericarp thickness were not a major concern when converting su inbreds to sh2 endosperm.

The pericarp has also been associated with differential water uptake and loss by the seed. Styer and Cantiliffe (1983b) harvested seed of sh2 and su from 18-46 DAP and soaked them in water for 24 h at 20 C. They found that mature sh2 seeds had greater leakage, as measured by conductivity, than did su, and they related this to rapid hydration by the sh2 seeds. Cracking of the pericarp was not noticed in either sh2 or su during development, and they reported that leakage generally decreased in all seed types with increasing maturity. They suggested that protective layers became thicker and possibly more lignified with maturity. Compositional changes during development, such as increases in starch in su seeds, might also assist in reducing leakage.

Wann (1986) observed that protein leaching in sh2, ae-du-wx, and su seeds appeared to be related to broken pericarps that were
more pronounced in sh2 seeds. He suggested that seed pericarps (and membranes) in high-sugar genotypes may be more susceptible to damage during harvest and drying than su genotypes.

The effects of endosperm mutants on pericarp characteristics and its relation to seed quality appear to remain contradictory.

Physico-chemical Properties of Maturation Drying

Maturation drying

Membrane structures are regarded as primary sites of drying injury or dessication damage in seeds and other biological systems. Water withdrawal subjects biomembranes to multiple stresses: mechanical stresses are produced by drastic volume changes of cells and cellular compartments during dehydration and rehydration; chemical stresses are produced by increasing cellular solute levels caused by water loss (Schwab and Herber, 1984). Yet seed development and maturation are associated with an overall loss of moisture. Seeds not only survive desiccation, but apparently in many cases, require it as a necessary step in their development (Adams and Rinne, 1980).

Seeds appear intolerant of drying at certain stages of their development. They are reported to undergo a transition from a desiccation-intolerant to a desiccation-tolerant state at a particular time in the course of their development. Dasgupta et al. (1982) demonstrated that seeds of Phaseolus vulgaris will not
germinate if removed fresh from the pod and placed under imbibition conditions at stages of development prior to 40 (DAP). Seeds at 26-32 days of development were induced to germinate when first dried over silica gel while those dried at 22 days failed to germinate when rehydrated and eventually deteriorated. A similar situation was demonstrated in *Ricinus communis* where Kermode and Bewley (1985) showed that germinability was not achieved until after some 50-55 days of development. However, premature drying promoted the germination of seeds as young as 25 DAP. It is proposed then that drying plays an important role in the switch in cellular activities from a developmental program to a germination/growth oriented one.

The rate at which drying occurs during the tolerant phase of development appears critical for subsequent expression of germinability. Kermode and Bewley (1985) found that while gradual rates of water loss result in germination of castor bean seeds as young as 25 DAP, rapid drying over silica gel was fatal to seeds younger than 55 DAP. It was suggested that gradual water loss allows protective changes to occur and hence increases the seed's resistance to disruption by dehydration. Rapid drying predisposes the seed to imbibitional injury, as indicated by increased rates of solute leakage, a symptom of cellular disruption (Adams et al., 1983). In dent corn seed, this leakage was reduced by preconditioning at 35 C before drying at 50 C (Herter and Burris, 1989a).
The theory that membrane deterioration is an early change in the process of aging that may occur during drying has been supported by three findings. Firstly, Powell and Matthews (1981) and Schoettle and Leopold (1984) reported that vital staining of pea cotyledons and uptake of Evans blue by cells of soybean cotyledons revealed that increased leakage of solutes did not result from dead tissue but was characteristic of membrane damage. Second, reduced turgor during imbibition has also been taken as presumptive evidence for a primary effect on membranes during ageing since ability to develop turgor is directly related to membrane integrity. Thirdly, Berjak et al., (1986) reported that electron microscopy of aged maize caryopses showed deterioration of the mitochondria before germination declined. Initially, deterioration was revealed by little development of cristae, followed by the development of abnormal membrane formations. Later, when germination had fallen to 76%, both the mitochondria and plastids of viable seeds showed considerable disorganization, being almost devoid of inner membrane formations.

Two possible explanations for membrane deterioration have been examined and discussed elsewhere (Powell and Matthews, 1981; Wilson and McDonald, 1986; Chen and Burris, 1991). Firstly, the decline in phospholipid content due to hydrolysis and secondly, peroxidation of lipids leading to the production of free radicals.
**Soluble carbohydrates of dry and maturing seeds and membrane integrity**

When enzymes, structural proteins, nucleic acids, macromolecular complexes etc., are desiccated in their native state, the integrity of the molecules can be retained if some water remains associated with them to prevent the formation of unfavourable conformations. Hence the production or the availability of substances to maintain bound water content could be an important feature of desiccation tolerance (Bewley, 1979). Soluble sugars have been implicated in preventing desiccation damage in model systems and a protective role has been proposed for them in several anhydrous systems.

Crowe et al. (1984) reported that in organisms that survive extreme desiccation, the disaccharide trehalose was found to play a major role in membrane integrity. Studies on phospholipid vesicles indicated that during drying, trehalose interacts with the phosphate group in such a way that phase separation of the different phospholipids (Crowe and Crowe, 1982), fusion (Rudolph and Crowe, 1985), and leakage (Crowe et al., 1986) do not occur. Leopold and Vertucci (1986) suggested that polyhydroxy compounds or sugars may provide this protective effect only if they do not become crystallized during loss of moisture since they must be present to protect the hydrophilic membrane components during drying.
Studies of the crystallization of sucrose and other sugars were conducted by Smythe (1967). He placed pieces of crystalline sucrose into syrups of either sucrose or sucrose with other sugars added and measured the growth of sucrose crystals gravimetrically. He reported that certain sugars suppressed the growth of sucrose crystals; the most effective was raffinose followed by stachyose. It was suggested that the oligosaccharide, raffinose, competitively inhibited sucrose crystallization because of its similarity to sucrose in the fructose-glucose moieties, while its lack of fit onto the surface of the growing crystals was ascribed to the additional galactose moiety. Leopold and Vertucci (1986) proposed, therefore, that desiccation tolerance mediated by trehalose depended in part, on the noncrystallizing characteristic of this sugar. Trehalose however, has not been reported to occur in angiosperm seeds.

Koster and Leopold (1988) studied the relationship between soluble sugar content and loss of desiccation tolerance in the axes of germinating pea, soya, and corn seeds. They monitored ability of seeds to germinate after periods of pre-imbibition by following the rates of electrolyte leakage from dried and rehydrated axes. Sucrose and large oligosaccharides (stachyose and raffinose) were consistently present during the tolerant stages and desiccation tolerance was lost as oligosaccharide content in the seeds decreased. They suggested that during the tolerant stages, oligosaccharides prevented sucrose crystallization allowing the non-
crystalline form (of sucrose) to interact with membrane surfaces, possibly replacing water in the maintenance of membrane structure. Shiroya (1963) reported also that raffinose appeared in cotton seed during ripening but was also formed in detached, unripe, air dried seed suggesting a mechanism for formation of raffinose inside unripe seed.

Ferguson et al. (1979) analyzed endosperm sugars of a sweetcorn inbred with the se gene compared with sugars of other genotypes. The se genotype showed high sucrose content but was distinguished from all other genotypes by its high maltose content during kernel development. Sucrose, fructose, and glucose decreased during kernel development in the se genotype and the other genotypes from 19 DAP until mature. Maltose accumulation was therefore ascribed as the primary factor in the slow drying characteristic and reduced starch content in maturing seeds of the se sweetcorn endosperm mutant.

It remains to be demonstrated whether these findings provide a new insight into the role of sugars in dry and maturing seeds in membrane integrity. Thus, carbohydrate metabolism, particularly in sweet corn which exhibits a preponderance of high sugar levels throughout maturation drying is a useful model system.
Mechanism of dehydration resistance

Of obvious interest was the elucidation of the physical basis of the remarkable effects of trehalose on membranes based on the observation that many dry phospholipids remain in bilayers in the absence of water. Further, the configuration of phospholipids in the presence of small amounts of water was determined from differential scanning calorimetry studies of phospholipid-water mixtures by Chapman et al. (1967) and from x-ray defraction studies by Luzzati and Husson (1962).

Chapman et al. (1967) showed that phosphatidyl cholines decrease their gel to crystalline transition temperature as they go from the dry to the hydrated state, reaching a minimum at about 20 - 25% moisture. Luzzati and Husson (1962) showed that isolated brain phospholipid went through a lamellar to hexagonal II phase change below 20% water. Based on this early work, it is expected that membranes at less than 20% water content would be disturbed to the extent of forming non-bilayer phases, such as hexagonal II phase crystals. Crowe and Crowe (1982, 1984) provided evidence based on freeze fracture and P-nuclear magnetic resonance that such phase separations of membrane proteins and transition of phospholipids to hexagonal II phase occur during dehydration of a membrane in the absence of trehalose. They proposed that an increase in the presence of trehalose inhibited some initial event in the transition of
phosphatidylcholine to gel phase, and thus prevent elevated membrane damage from dehydration.

Based on x-ray defraction studies, Luzzati and Husson (1962), and Simon (1974) proposed that membrane lipids in seeds form a hexagonal phase below 20% moisture. According to Simon's model, dehydration induces an alteration in the hydrophobic-hydrophilic interaction within the membrane so that the structure of the cellular membranes below 20% hydration is different from that above 20%. Consequently, water uptake by desiccation-tolerant seeds reinstates the original structure of the cellular membrane, whereas the membranes of desiccation-sensitive seeds are unable to reform completely.

The possibility that cellular membranes form a hexagonal phase as a result of dehydration was investigated by McKersie and Stinson (1980) by repeating Luzzati and Husson's experiment using phospholipid extracted from birdsfoot trefoil seeds. They argued that phospholipid and fatty acid composition of seed extracts compared to human brain extracts might differ and that hexagonal phases as detailed by Luzzati and Husson had only been observed in bulk phospholipid. In their experiment, McKersie and Stinson found no evidence for the presence of hexagonally packed phospholipid at any moisture content examined in lipid extracts from birdsfoot trefoil seeds. They concluded that membrane damage, and leakage of
cytoplasmic solutes from seeds cannot be explained by the formation of a hexagonal phase by membrane phospholipids.

The presence of hexagonal phase phospholipids has not been demonstrated in organisms that normally survive desiccation. Crowe and Crowe (1986a), however, suggested an alternative explanation, that initial leakage from desiccation-tolerant systems when placed in water may occur because of hydration-induced phase transitions in the bilayers. Using liposomes as a model system, they demonstrated that trehalose was effective against fusion of lyophilized liposomes, and that (trehalose) and maltose were the most effective in preventing leakage during dehydration. They proposed that trehalose acted as a spacer, preventing contact between the collapsing membranes during drying thus inhibiting lipid fusions that result in membrane lipid phase change. Such effects are yet to be demonstrated in seeds.

Bewley (1979) argued that even though membrane structures may be protected against desiccation damage by sugars, desiccation would not occur without exhibiting cellular changes some of which may be regarded as quite extensive. He stated that the critical features of desiccation tolerance are the abilities (a) to limit damage during desiccation, (b) maintain physiological integrity in the dry state so that metabolism can resume quickly upon rehydration, and (c) provide a repair mechanism upon rehydration, in particular, to regain integrity of membranes and membrane bound organelles. These
features are subject to genotypic and environmental manipulation and therefore are integral to this investigation.
OBJECTIVES

This work addressed the following objectives:

1. To examine the progressive maturation of kernels of three popular sweetcorn hybrids with the same germplasm background but with different endosperm genes

2. To measure germination, seedling vigour, and conductivity of hybrids when
   a) harvested at successive stages of maturity
   b) dried at three different temperatures

3. To measure changes in soluble carbohydrates at successive stages of maturity

4. To examine germination, seedling vigour, and conductivity as influenced by corresponding carbohydrate changes at successive stages of maturity
MATERIALS AND METHODS

Seed Production

Sweet corn seed used in this work was produced in 1989 and 1990 at the Bruner Farm of Iowa State University. Two seed crops, an early planted and a later planting after two weeks, were established. The hybrids grown in 1989 were the su and sh2 versions of Jubilee, commercially known as Jubilee and Sucro, respectively, and obtained from Rogers Brothers Co., Boise, Idaho. In 1990, a third genotype, an se-gene version of Jubilee was added. Genotypes were grown open pollinated in 2-row plots, 75 cm apart, and 100 m in length. To ensure good pollination, the seed parents were alternated with two rows of pollinator.

Harvest and Drying

At 4-day intervals beginning 35 days after anthesis through mature kernel stage, 15 random ear samples per plot were harvested to obtain at least 5 harvests. At each harvest, ear samples were brought to the laboratory and husked. Seed moisture and dry weight were determined from 20 seeds shelled from a 2.5-cm mid-section of each ear. Seed dry weight was obtained following the oven method (103 C, 48 h).

Five-ear samples were separately hand-shelled (tip and base kernels discarded), and seed were immediately frozen in liquid
nitrogen and stored at -20 C until analysis. The remaining 10 ears were bagged separately and placed in thin-layer experimental dryers described by Navratil and Burris (1982). Dryers were operated at 35 C, 40 C, and 45 C, to dry the seed to approximately 12% (w/w) moisture. The dried ears were placed in paper bags in cold storage at 10 C and approximately 50% relative humidity.

Evaluation of Seed Quality and Membrane Integrity

Standard warm and cold germination tests were performed as described by Loeffler et al., (1985) on Captan ([trichloro-methyl] thio]-4-cyclohexene-1,2-dicarboximide) treated kernels. Test samples consisted of 50 kernels per replication and 10 replications per treatment combination (harvest x temperature x genotype). The warm germination test was evaluated after 7 d at 25 C (AOSA, 1985). Shoots and roots from seedlings were removed for dry weight determination following the oven method (103 C, 48 h) and shoot to root ratios were computed. The soil-free cold germination test was evaluated after 14 d, 7 d at 10 C followed by 7 d at 25 C.

The conductivity test was performed on non-treated seed from cold storage. In 1989, individual seed conductivity and sugar leakage were determined by soaking 100 kernels of each sample (harvest x temperature x genotype) in soaking trays for 24 h in distilled and deionized water. Conductivity was measured by ASA-610 automatic seed analyser (Neogen., Ann Arbor, MI.) as microamps
In 1990, conductivity was measured by ASA-1000 automatic seed analyser at 6, 12, and 24 h soaking periods, respectively. In both years, seed were removed from soaking trays and tested for standard warm germination (Loeffler et al., 1985). From each of the wells in soaking trays, 0.1 ml of leachate was bulked for measurement of sugar leakage.

Soluble Sugar Analysis

Soluble carbohydrate composition was determined on samples consisting of 10 excised embryos and endosperms from kernels stored at -20 C following methods described by Chen and Burris (1990). Sugars were extracted by homogenizing 10 embryos or endosperms in 10 ml of 85% ethanol with a Brinkmann homogenizer (Brinkmann Instruments Co., Westbury, NY.) and centrifuging at 16,000 x g for 10 minutes. The ethanol supernatant was vacuum evaporated in a rotary evaporator. Distilled and deionized water was added to the residue to a final volume of 3.5 ml per extract. The extract was cleared by filtration through a 0.2 micrometre filter. Sugars were separated by high performance liquid chromatography (HPLC) with a refractive index detector (LC-6A, Shimadzu Corporation, Kyoto, Japan). Separation was through a SupelCosil LC-NH2 column (Supelco, Bellefonte, PA.) under 1 ml per minute of an acetonitrile:water (3:1) mobile phase. Composition was determined by integration of peak areas of interest with those of the individual sugar standards.
according to preliminary calibrations. Fig. A-1 shows a representative chromatograph for soluble sugars as separated by a SupelCosil LC NH2 column.

**Statistical Analysis**

A randomized complete block design in a $2 \times 5 \times 3 \times 2$ factorial with 8 replications in 1989, and a $2 \times 5 \times 3 \times 3$ factorial with 10 replications in 1990 were used. Analysis of variance using the Statistical Analysis System (SAS) were performed and treatment means were separated by the Least Significant Difference (LSD) method. Only significant main effects and interactions were used for mean separation and correlation analysis.
RESULTS

Seed Maturation, Drying, and Quality Assessment:
1989 Growing Season

Harvest moisture

Time of planting had a significant effect on harvest moisture with the crop from the second planting having a 4% higher moisture content than the first planting at similar days after pollination (DAP). Averaged across genotypes, Jubilee and Sucro, moisture content declined by 20% during maturation (35 to 51 DAP). The largest decrease in moisture of 13% for Jubilee and 7% for Sucro, occurred at 43 and 47 DAP, respectively. In general, Jubilee maintained a higher moisture content throughout maturation (Fig. 2).

Seed viability

Normal seedlings In general, the first planting produced seed that had a higher proportion of normal seedlings i.e., there was a reduction in viability associated with later planting. Jubilee viability decreased from 75% to 51%, while Sucro viability declined from 57 to 51%.

Although the harvest x genotype interaction was not significant, Jubilee had superior germination at all stages of maturity with the highest germination obtained at 47 DAP. Sucro achieved its highest viability (67%) at 51 DAP. Both genotypes
Fig. 2. Moisture content of two sweet corn genotypes, Jubilee and Sucro, at successive stages of maturation.
showed an improvement in germination of 30% through the maturation period.

Drying at 45 °C reduced seed viability by 27% and 18% as compared to drying at 35 and 40 °C, respectively. And drying at 40 °C reduced viability by 10 percentage points compared to 35 °C. Jubilee exhibited higher germination results at all drying temperatures (Fig. 3). In terms of kernel maturity and drying temperature (Fig. 4), the difference in viability between seed dried at 35, 40, and 45 °C declined with maturity through 43 DAP. There was no significant difference in the germination of seed harvested between 47 and 51 DAP dried at any of the three temperatures. However, seed dried at 35 °C produced relatively higher germination at all stages of maturity. Correlation between standard germination test and harvest moisture was low (r = -0.37).

Abnormal seedlings There was no significant difference in the proportion of abnormal seedlings between the two planting dates. There were, however, more abnormal seedlings early in maturation. Furthermore, none of the drying temperatures significantly influenced abnormal seedling development and there was no significant genotype x temperature interaction.

Germination percentage and abnormal seedlings were negatively correlated (r = -0.5) and the relationship between harvest moisture and abnormal seedlings was low (r = 0.3).
Fig. 3. Standard germination of two sweet corn genotypes, Jubilee and Sucro, as influenced by drying temperatures.

Fig. 4. Standard germination of two sweet corn genotypes, Jubilee and Sucro, as influenced by drying temperatures and stage of maturity.
**Shoot:root ratio**  Shoot to root ratio was not significantly affected by any of the main effects except for the effect of genotypes, i.e., Jubilee had a higher shoot:root ratio than Sucro. There was a very low correlation \((r = 0.27)\) between germination and shoot:root ratio and correlation coefficients between shoot:root ratio with harvest moisture and abnormal seedlings were negligible.

**Cold germination test**

**Normal seedlings**  Overall, the first planting resulted in seed that produced a higher proportion of normal seedlings (Fig. 5). Although Jubilee exhibited superior performance overall compared to Sucro, the effect was only evident in seed from the first planting. There was no significant difference between Jubilee and Sucro seed from the second planting.

The highest seedling vigor for Jubilee was obtained after 47 DAP with the largest increase in vigor of 26% occurring between 43 and 47 DAP (Fig. 6). Sucro exhibited the highest vigor in seed produced 51 DAP with the largest increase in vigor (36%) occurring between 47 and 51 DAP. During maturation, Jubilee exhibited the largest improvement in vigor of 88%, while Sucro exhibited a 73% improvement. Drying seed at 45 C compared to drying at 35 C and 40 C, reduced vigor by 48% and 32%, respectively. Drying at 40 C compared to 35 C reduced vigor by 23%. Jubilee exhibited higher vigor than Sucro at all drying temperatures (Fig. 7). Irrespective
Fig. 5. Cold-temperature germination and proportion of abnormal seedlings of two sweet corn genotypes, Jubilee and Sucro, as influenced by date of establishment.

Fig. 6. Cold-temperature germination of two sweet corn genotypes, Jubilee and Sucro, as influenced by stage of maturity.
Fig. 7. Cold-temperature germination of two sweet corn genotypes, Jubilee and Sucro, as influenced by drying temperatures.
of drying temperature, seed from the first planting produced higher cold test values than seed from the second planting and there were no significant harvest x temperature interactions.

The cold and standard germination tests exhibited a high correlation \( r = 0.70 \) while that with harvest moisture was low \( r = -0.42 \) and with shoot:root ratio was very low \( r = 0.17 \).

**Abnormal seedlings** Overall, there was no significant difference in the proportion of abnormal seedlings between the first and second planting dates (Fig. 5). However, Jubilee produced a higher proportion of abnormal seedlings. There was no significant difference in the proportion of abnormal seedlings between 35 and 47 DAP for Sucro. Jubilee had the largest number of abnormal seedlings early (35-43 DAP) during maturation. Drying temperature had no significant effect on the number of abnormal seedlings produced nor did kernel maturity, genotype, harvest x temperature, or genotype x temperature interactions. Correlation coefficients between abnormal seedlings from the cold test with other variables were very low.

**Conductivity Test**

**Electrolyte leakage after 24 h of soaking** After 24 h of soaking, seed from the second planting dates exhibited a 16% higher conductivity than that from the first planting. Seed of Jubilee from both plantings leaked 38% more than Sucro. Conductivity of leachate decreased with maturity in both genotypes (Fig. 8). The largest
Fig. 8. Conductivity of leachate from seed of two sweet corn genotypes, Jubilee and Sucro, during 24-hr imbibition at 25 C, as influenced by stage of maturity.
reduction in leakage of 45% for Jubilee occurred between 43 and 47 DAP while a reduction of 24% occurred between 47 and 51 DAP for Sucro. Jubilee had the greatest reduction in leakage of 54% through maturation while Sucro exhibited a 41% reduction.

Drying at 45 C resulted in a 19% and 9% increase in leakage compared to drying at 35 and 40 C, respectively. Drying at 40 C compared to 35 C resulted in a 12% increase in leakage, and Jubilee exhibited more leakage than Sucro (Fig. 9). In the harvest x temperature interaction (Fig. 10), differences in conductivity between seed dried at 35, 40 and 45 C declined with maturation until 43 DAP after which there were no significant differences.

Electrolyte leakage was highly correlated with harvest moisture (r= 0.72) but exhibited very low correlations with standard germination (r= 0.29), cold test (r= -0.29) and germination after soak (r= 0.24). Slightly higher correlations were obtained between electrolyte leakage and abnormal seedlings from the viability and vigor tests with r values of 0.34, 0.39, and 0.44, respectively.

**Soluble carbohydrates in seed leachate.** The levels of sugars (fructose and sucrose) in seed leachates were highest early in maturity and declined to negligible levels by 51 DAP (Fig. 11). Overall, Jubilee had 52% more leachate sugar than Sucro. Drying seed at 45 C resulted in 45% and 24% more leachate sugar than seed dried at 35 and 40 C, respectively. In Fig. 12, differences in the levels
Fig. 9. Conductivity of leachate from seed of two sweet corn genotypes, Jubilee and Sucro, during 24-hr imbibition at 25 C, as influenced by drying temperatures.

Fig. 10. Conductivity of leachate from seed of two sweet corn genotypes, Jubilee and Sucro, during imbibition at 25 C, as influenced by drying temperatures and stage of maturity.
Fig. 11. Soluble sugars (sucrose and glucose) leached from seed of two sweet corn genotypes, Jubilee and Sucro, during 24-hr imbibition at 25 C, as influenced by stage of maturity.

Fig. 12. Soluble sugars (sucrose and glucose) leached from seed of two sweet corn genotypes, Jubilee and Sucro, during 24-hr imbibition at 25 C, as influenced by drying temperatures and stage of maturity.
of sugar from seed dried at the three temperatures were maintained up to 39 DAP and declined beginning 43 DAP.

Soluble carbohydrates in seed leachate were moderately correlated with harvest moisture ($r = 0.58$) and leakage ($r = 0.7$) but less so with standard germination ($r = -0.42$), cold test ($r = -0.5$), germination after soak ($r = -0.41$) or abnormal seedlings from these tests.

**Germination test after 24 h soaking**

**Normal seedlings** Overall, Jubilee had 36% higher germination than Sucro with the highest results obtained at 39 and 47 DAP for Jubilee and at 51 DAP for Sucro (Fig. 13). Drying seed at 45°C reduced viability by 32% and 22% after the 24-h soak compared to seed dried at 35 and 40°C, respectively. Seed dried at 40°C versus 35°C exhibited a reduced viability of 14% (Fig. 14). Differences in germination due to drying temperatures declined as maturity advanced (Fig. 15). At 47 DAP, there were no significant differences in viability between seed dried at 35, 40, and 45°C.

High correlation coefficients were exhibited between germination after soak and standard germination test ($r = 0.78$) and the cold test ($r = 0.76$). Correlations with harvest moisture ($r = -0.26$) and conductivity ($r = -0.24$) were very low.

**Abnormal seedlings** Although the tendency was for a decrease in the proportion of abnormal seedlings as maturity advanced, there
Fig. 13. Standard germination after 24-hr soaking of two sweet corn genotypes, Jubilee and Sucro, as influenced by stage of maturity.

Fig. 14. Standard germination after 24-hr soaking of two sweet corn genotypes, Jubilee and Sucro, as influenced by drying temperatures.
Fig. 15. Standard germination after 24-hr soaking of two sweet corn genotypes, Jubilee and Sucro, as influenced by drying temperatures and stage of maturity.
were no significant differences in the number of abnormal seedlings between 43 and 51 DAP. Seed drying temperature, and the interaction between temperature with kernel maturity or genotype, did not significantly affect abnormal seedlings after the 24 h of soaking.

Correlation coefficients between abnormal seedlings with harvest moisture (r= 0.41), conductivity (r= 0.44), and germination after soak (r= -0.28), were very low. Correlations between abnormal seedlings and shoot:root ratio were not significant.

Seed Maturation, Drying, and Quality Assessment:

1990 Growing Season

Harvest moisture

Time of planting did not have a significant effect on harvest moisture across genotypes. However, genotypes exhibited a protracted period of high moisture retention (Fig. 16). From 35 to 51 DAP, the ss-genotype, Jubilee, and Sucro declined in moisture content by 26%, 20%, and 15%, respectively. The largest moisture decrease occurred at 47 DAP for all genotypes. Overall, Jubilee subtended a higher moisture content throughout maturation followed by Sucro.

Seed viability

Normal seedlings In general, the second planting produced seed that resulted in a higher proportion of normal seedlings and represented an improvement in viability of 4% compared to the first
Fig. 16. Moisture content of three sweet corn genotypes, Jubilee, se, and Sucro, at successive stages of maturity.
planting (Fig. 17). Viability improved by 14% and 8% for Jubilee and Sucro, respectively. However, the second crop of the se-genotype exhibited a decline in viability to 81%, compared to the first planting at 87%. The se-genotype had superior germination at all stages of maturity with the highest values obtained 51 DAP (Fig. 18). The highest germination for Jubilee was also obtained at 51 DAP. Jubilee exhibited the largest improvement in viability from 13% at 35 DAP to 77% at 51 DAP. The largest portion of the increase, 22%, occurred between 39 and 43 DAP. Harvestable ears from the first planting of Jubilee at 51 DAP were not available which could confound the combined results obtained for this harvest date. Sucro exhibited the smallest increase in viability of only 16% during maturation. The largest portion of that increase, 8%, occurred between 43 and 47 DAP. There was no significant effect of harvest date on viability between 47 and 51 DAP seed yield which showed the highest viability for Sucro.

Overall, the 45 C drying temperature reduced viability 20% and 12% compared to seed dried at 35 C and 40 C, respectively (Fig. 19). Compared to 35 C, drying at 40 C resulted in the least decline in viability of only 8%. There was a 20-33% difference in viability between seed of the se-genotype compared to seed of Sucro and Jubilee dried at 35 C.

Except for Sucro, where there were no significant differences between the two planting dates, the second crop of the se-genotype
Fig. 17. Standard germination and proportion of abnormal seedlings of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by date of establishment.

Fig. 18. Standard germination of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by stage of maturity.
Fig. 19. Standard germination of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by drying temperatures.
and Jubilee produced seed that had higher viability across all
drying temperatures. In terms of maturity and drying temperatures
(Fig. 20), seed of all genotypes harvested between 47 and 51 DAP and
dried at either 35 C or 40 C outperformed seed of the same maturity
dried at 45 C or seed of an earlier maturity and dried at 35 or 40
C. The difference in viability due to drying temperatures declined
with increasing maturity.

Seed viability was negatively correlated with harvest moisture
\( r = -0.52 \).

**Abnormal seedlings** There were no significant differences in
abnormal seedlings between the two planting dates (Fig. 17). There
was a higher proportion of abnormal seedlings produced early during
maturation. There was no significant difference in the proportion of
abnormal seedlings produced at either 35 or 40 C compared to drying
at 45 C. And, at all drying temperatures, Jubilee seed produced a
higher percentage of abnormal seedlings compared to Sucro or the se-
genotype. Irrespective of drying temperature, the number of abnormal
seedlings was higher early in maturity but quickly declined as
maturity advanced.

Abnormal seedlings and seed viability were negatively
correlated \( r = -0.58 \) while the correlation between abnormal
seedlings and harvest moisture was positive but low \( r = 0.44 \).

**Shoot:root ratio** Seed from the first planting produced
seedlings with a higher shoot:root ratio than the second planting.
Fig. 20. Standard germination of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by drying temperatures and stage of maturity.
There was no significant effect of kernel maturity or drying temperature on shoot:root ratio. However, Sucro had a relatively lower ratio of shoot to root dry weight throughout maturation.

Correlation coefficients between shoot:root ratio with harvest moisture or abnormal seedlings were not significant while that with seed viability was low (r= 0.44).

**Cold germination test**

**Normal seedlings** The second planting date produced seed that resulted in a higher proportion of normal seedlings under cold germination conditions (Fig. 21). However, for the se-genotype there was no significant difference in cold germination between the first and second planting date. While, Jubilee and Sucro exhibited an improvement of 11 and 4%, respectively.

The se-genotype exhibited superior cold germination performance throughout maturation (Fig. 22). The highest germination was obtained at 43 DAP and it subsequently declined. The largest increase was 26% between 35 and 39 DAP. Jubilee had the largest improvement in cold germination of 54% from 5% at 35 DAP to 59% at 51 DAP. The largest part of this increase (25%), occurred between 39 and 41 DAP, and the highest cold germination was obtained at 51 DAP. Sucro showed the least increase in cold germination of only 5% during maturation which occurred between 35 and 39 DAP. There was no significant difference in cold germination after 39 DAP.
Fig. 21. Cold-temperature germination and the proportion of abnormal seedlings of three sweet corn genotypes, Jubilee, \textit{se}, and Sucro, as influenced by date of establishment.

Fig. 22. Cold-temperature germination of three sweet corn genotypes, Jubilee, \textit{se}, and Sucro, as influenced by stage of maturity.
There were no significant effects of drying temperature on seed from either of the two plantings or on seed harvested at different maturity stages. Drying at 45 C produced seed that exhibited a drop in cold germination of 23% and 12% compared to drying at 35 C and 40 C, respectively, across genotypes and kernel maturities (Fig. 23). Seed of the se-genotypes dried at 35, 40, or 45 C outperformed seed of Jubilee and Sucro dried at 35 C by 18-44%.

Correlation between cold and warm germination was very high ($r = 0.82$) while that between cold test and shoot:root ratio was negligible ($r = 0.15$). Correlations between cold test with harvest moisture and abnormal seedlings were low, $r = -0.46$ and -0.44, respectively.

**Abnormal seedlings** There were no significant differences due to planting dates in the proportion of abnormal seedlings (Fig. 21). For Sucro and the se-genotype, fewer abnormal seedlings were produced as maturity advanced and there were no significant differences between the genotypes in the number of abnormal seedlings beginning 39 DAP throughout maturation. For Jubilee, the number of abnormal seedlings peaked at 43 DAP and then declined. Jubilee had the highest number of abnormal seedlings from 39 DAP through maturation.

There were no significant differences in the number of abnormal seedlings produced when seed was dried at 40 C compared to drying at 45 C. Drying at 35 C, however, produced 13% fewer abnormal
Fig. 23. Cold-temperature germination of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by drying temperatures
seedlings. At any of the drying temperatures, Jubilee had the highest number of abnormal seedlings. Lower drying temperatures (35 C or 40 C) early in maturity resulted in a large number of abnormal seedlings. However, there was little difference in the proportion of abnormal seedlings at later stages of maturity due to drying temperature.

Correlation coefficient between abnormal seedlings and the cold germination test was very low (r= -0.19) as was with harvest moisture (r= 0.28), shoot:root ratio (r= 0.20) or abnormal seedlings from the standard germination test (r= 0.43).

Conductivity test

Total leakage after 6, 12, 24 h of soaking There was no significant difference in leachate conductivity due to planting date after the 6-h soak. After 12 and 24 h of soaking, seed from the second planting leaked slightly less (Fig. 24). However, seed of the se-genotype from the second crop, leaked more by 11, 7, and 4% after 6, 12, and 24 h of soaking, respectively.

Seed of Jubilee, from each stage of maturation and during the entire soak period, leaked more compared to Sucro while the se-genotype leaked the least (Fig. 25). Leakage decreased with increasing stage of kernel maturity at all soak periods by a mean of 46, 30, and 19% for the genotypes se, Sucro and Jubilee, respectively. The greatest decline in leakage occurred between 39-43,
Fig. 24. Conductivity of leachate from seed of three sweet corn genotypes, Jubilee, se, and Sucro, during 6-hr (A), 12-hr (B), and 24-hr (C) imbibition at 25 C, as influenced by date of establishment.
Fig. 25. Conductivity of leachate from seed of three sweet corn genotypes, Jubilee, se, and Sucro, during 6-hr (A), 12-hr (B), and 24-hr (C) imbibition at 25 C, as influenced by stage of maturity.
43-47, and 47-51 DAP at all soak periods for the genotypes se, Sucro, and Jubilee, respectively.

Drying at 45 C increased leakage compared to seed dried at either 40 or 35 C, particularly for Sucro and Jubilee as there were no significant differences in leakage at 45 or 40 C for the se-genotype at any of the soak periods. The interaction of drying temperature and genotype was highly significant after 6 h soaking. Jubilee, followed by Sucro exhibited the highest conductivity at all drying temperatures after 6 h of soaking. This trend was not evident at either 12 or 24 h. The se-genotype ranked lowest in conductivity at all soak periods (Table 1).

The interactions between drying temperature and kernel maturity on leakage showed that although leakage declined with maturation when seed was dried at any of the three temperatures, the effect of each drying temperature at any stage of maturity was consistent (Fig. 26). Total leakage after 6, 12, and 24 h of soaking was negatively correlated with the standard and cold germination tests and germination tests after soaking. Correlations increased with increases in duration of soaking but were identical following 12 and 24 h soaking. The r values after 6, 12, and 24 h of soaking were, respectively: -0.55, -0.63, and -0.63 for the standard test; -0.53, -0.57, and -0.58 for the cold test; and -0.58, -0.62, and -0.63 for germination after soak. Correlation coefficients between total leakage and harvest moisture were fairly high being r= 0.55,
Table 1. Changes in leachate conductivity of genotypes following drying treatments and soaking

<table>
<thead>
<tr>
<th>Soaking time (hr)</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
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<tr>
<td>Mean&lt;sup&gt;a&lt;/sup&gt;</td>
<td>T</td>
<td>G&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Mean</td>
</tr>
<tr>
<td>75.2 a 3 1</td>
<td>91.0 a 3 1</td>
<td>101.7 a 3 1</td>
<td></td>
</tr>
<tr>
<td>73.8 a 2 1</td>
<td>89.1 ab 2 1</td>
<td>101.1 bc 3 3</td>
<td></td>
</tr>
<tr>
<td>71.3 b 1 1</td>
<td>87.9 b 3 3</td>
<td>100.0 c 2 1</td>
<td></td>
</tr>
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<td>96.6 d 1 1</td>
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<tr>
<td>55.9 e 1 3</td>
<td>72.1 e 1 3</td>
<td>84.6 f 1 3</td>
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<tr>
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<td>67.5 f 2 2</td>
<td>77.6 g 3 2</td>
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<tr>
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<td>67.5 f 3 2</td>
<td>77.2 g 2 2</td>
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<tr>
<td>49.4 g 1 2</td>
<td>60.5 g 1 2</td>
<td>70.5 h 1 2</td>
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<td>LSD</td>
<td>1.4</td>
<td>1.5</td>
<td>1.5</td>
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<sup>a</sup>Means within a column followed by the same letter are not significantly different from each other at P < 0.05. All values are units of current expressed in microamps.

<sup>b</sup>Abbreviations: T = drying temperature (+ = 35°C, Z = 40°C, 3 = 45°C); G = genotypes (1 = Jubilee, 2 = se, 3 = sucro)
Fig. 26. Conductivity of leachate from seed of three sweet corn genotypes, Jubilee, se, and Sucro, during 6-hr (A), 12-hr (B), and 24-hr (C) imbibition at 25 C, as influenced by drying temperatures and stage of maturity.
0.61, and 0.62 after 6, 12, and 24 h of soaking. Correlation between total leakage and abnormal seedlings from the viability and vigor tests were low (r= 0.27 to 0.47) though positive.

**Rate of leakage between soak periods** Increasing kernel maturity reduced leakage between the three soak intervals (Fig. 27). Kernel maturity had a subtle effect on leakage of Sucro and an insignificant effect on the leakage of Jubilee. Overall, Sucro expressed the largest difference in leakage between soak periods followed by Jubilee. The largest difference in conductivity was expressed during 6-12 h, and was less evident during the 12-24 h period.

There were no significant differences in the interactions between drying temperatures and genotypes or between kernel maturity and leachate between conductivity readings. Correlation coefficients for the rate of leakage with standard germination, cold test, and germination after soak were negative and very low. The r values for the tests and leakage were -0.34, -0.26, and -0.29, respectively, at 6-12 h period which exhibited the highest coefficients compared to the 12-24 or 6-24 h intervals. Correlations between rate of leakage during the 6-12 h period with total leakage after 6, 12, and 24 h of soaking were positive but low with r values of 0.08, 0.45, and 0.47, respectively.
Fig. 27. Change in rate of leakage of seed of three sweet corn genotypes, Jubilee, se, and Sucro, between 6-12 hr (A), 12-24 hr (B), and 6-24 hr (C) periods during imbibition at 25 C, as influenced by drying temperatures and stage of maturity.
Germination after 24 h soaking

**Normal seedlings**  In general, seed from the second planting exhibited a 7% increase in viability over the first planting following the 24 h of soaking. However, viability of the se-genotype decreased by 6% in seed from the second planting. The se-genotype exhibited superior germination at all stages of maturation with the highest germination obtained from seed harvested at 47 and 51 DAP (Fig. 28). The highest germination values for Jubilee and Sucro were obtained from seed harvested at 51 and 47 DAP, respectively.

Overall, drying at 45 C produced seed with lowered germination compared to drying at either 35 or 40 C (Fig. 29). Seed of the se-genotype dried at any temperature exhibited superior germination compared to seed of the other genotypes dried at 35 C. In terms of kernel maturity and drying temperature (Fig. 30), differences in germination remained between drying temperatures as maturation advanced, until 51 DAP when there were no significant differences between seed dried at 35 and 40 C.

Germination values after soaking were highly correlated with standard germination ($r = 0.83$) and the cold test ($r = 0.85$) while that with harvest moisture was fair ($r = -0.50$) and with shoot:root ratio, negligible ($r = 0.15$).

**Abnormal seedlings**  Seed from the first planting date gave more abnormal seedlings after 24 h soaking than seed from the second
Fig. 28. Standard germination after 24-hr soaking of seed of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by stage of maturity.

Fig. 29. Standard germination after 24-hr soaking of seed of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by drying temperatures.
Fig. 30. Standard germination after 24-hr soaking of seed of three sweet corn genotypes, Jubilee, se, and Sucro, as influenced by drying temperatures and stage of maturity.
planting. While Jubilee had the highest number of abnormal seedlings and the se-genotype the least, the number of abnormal seedlings declined with maturity. Drying temperature had little effect on number of abnormal seedlings after 24 h soaking although in general, drying at 45 C produced a higher proportion of abnormal seedlings.

Low correlation coefficients were exhibited between abnormal seedlings with harvest moisture (r= 0.37) and germination after soaking (r= -0.35) or with values of abnormal seedlings from the standard germination test (r= 0.43) and the cold test (r= -0.34).

Soluble Carbohydrates During Maturation:
1990 Growing Season

Fructose

Seeds from the first planting exhibited significantly higher levels of fructose than those from the second planting. The embryo fraction exhibited a sharp increase in the level of fructose between 43 and 47 DAP with the highest fructose peak at 47 DAP (Fig. 31A). A similar trend was observed for total fructose and fructose from endosperm fractions (Fig. 31B, 31C). However, the level of fructose at 47 DAP was not significantly different from that earlier in maturity. When embryo fructose was expressed as a function of total fructose, there were no significant differences in the ratio between 47 and 51 DAP and between 35 and 43 DAP (Fig. 31D).
Fig. 31. Fructose, embryo (A), endosperm (B), total (C), and embryo fructose : total fructose (D) contents of seeds of three sweet corn genotypes, Jubilee, se, and Sucro, harvested at successive stages of maturity.
<table>
<thead>
<tr>
<th></th>
<th>FRUCTOSE (EMBRYO) (mg/ml)</th>
<th>FRUCTOSE (ENDOSPERM) (mg/ml)</th>
<th>FRUCTOSE (TOTAL OF SEED) (mg/ml)</th>
<th>FRUCTOSE (EMBRYO):TOTAL OF SEED</th>
</tr>
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<tbody>
<tr>
<td>DAYS AFTER POLLINATION</td>
<td></td>
<td></td>
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<tr>
<td>35</td>
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<tr>
<td>51</td>
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</tr>
</tbody>
</table>

FRUCTOSE (EMBRYO) (mg/ml):
- **JUBILEE**
- **SE**
- **SUCRO**

FRUCTOSE (ENDOSPERM) (mg/ml):
- **JUBILEE**
- **SE**
- **SUCRO**

FRUCTOSE (TOTAL OF SEED) (mg/ml):
- **JUBILEE**
- **SE**
- **SUCRO**

FRUCTOSE (EMBRYO):TOTAL OF SEED:
- **JUBILEE**
- **SE**
- **SUCRO**
In general, there were no significant differences in fructose levels between Jubilee and Sucro throughout maturation. The se-genotype had the lowest levels of fructose. The ratio of embryo fructose to total fructose showed Jubilee with the lowest ratio while there was no significant difference between Sucro and the se-genotype. The harvest x genotype interactions were not significant as describing the ratio of embryo fructose to total fructose, total fructose, and embryo fructose (Table A-1).

Glucose

The first planting exhibited a higher level of glucose than the second crop. Like fructose, embryo fractions exhibited peaks at 47 DAP and levels subsequently declined (Fig. 32A). The highest levels of total glucose or endosperm glucose were obtained at 47 DAP (Fig. 32B, 32C). However, glucose levels at 47 DAP were not significantly different from that earlier in maturity at 39 DAP. The ratio of embryo glucose to total glucose revealed that across genotypes, the highest ratio was at 51 DAP (Fig. 32D).

There were no significant genotype differences or harvest x genotype differences in the level or ratio of glucose between seed fractions (Table A-1).
Fig. 32. Glucose, embryo (A), endosperm (B), total (C), and embryo fructose:total fructose (D) contents of seeds of three sweet corn genotypes, Jubilee, se, and Sucro, harvested at successive stages of maturity.
GLUCOSE (EMBRYO) (mg/ml)  

GLUCOSE (TOTAL OF SEED) (mg/ml)  

GLUCOSE (ENDOSPERM) (mg/ml)  

GLUCOSE (EMBRYO):TOTAL OF SEED
Sucrose

There were no significant differences in the level of sucrose between the two seed crops. Except for the se-genotype, there was a sharp increase in the level of sucrose in the embryo fractions between 43 and 47 DAP with a peak at 47 DAP (Fig. 33A). There were no significant differences in embryo sucrose levels at 47 and 51 DAP. Endosperm sucrose and total sucrose exhibited similar trend without significant peaks during maturation (Fig. 33B, 33C).

There were no significant differences between Jubilee and Sucro in endosperm sucrose levels and total sucrose levels which were significantly higher than that of the se-genotype. Sucro and Jubilee also did not significantly differ in the ratio of embryo sucrose to total sucrose while the se-genotype exhibited higher ratio throughout the maturation period (Fig. 33D). The harvest x genotype interaction for sucrose was not significant for endosperm sucrose or total sucrose (Table A-1). But unlike fructose and glucose, the interaction was significant for embryo sucrose and, more importantly, for the ratio of embryo sucrose to total sucrose.

Correlation coefficients between the ratio of embryo sucrose to total sucrose with total sucrose levels, or embryo and endosperm sucrose levels. However, the ratio varied with endosperm fructose (r= 0.52) and total maltose (r= 0.73).
Fig. 33. Sucrose, embryo (A), endosperm (B), total (C), and embryo fructose:total fructose (D) contents of seeds of three sweet corn genotypes, Jubilee, se, and Sucro, harvested at successive stages of maturity.
SUCROSE (EMBRYO) (mg/ml)

SUCROSE (TOTAL OF SEED) (mg/ml)

SUCROSE (ENDOSPERM) (mg/ml)

SUCROSE (EMBRYO):TOTAL OF SEED
Maltose

Date of planting had no significant influence on the level of maltose in seed fractions. The level of maltose was variable between fractions at different stages of maturity. Embryo fractions showed a maltose peak at 47 DAP while the ratio of embryo maltose to total maltose suggested no significant difference between 47 and 51 DAP (Fig. 34A, 34D). The level of total maltose was highest at 47 DAP while there were no significant differences in endosperm maltose levels between 43 and 47 DAP (Fig. 34B, 34C).

The se-genotype had the highest levels of total and endosperm maltose up to 47 DAP, but they subsequently declined. Further, the se-genotype had the lowest level of maltose from embryo fractions and the lowest ratio of embryo maltose to total maltose. Jubilee and Sucro exhibited lower levels of endosperm and total maltose which increased through 47 DAP and subsequently declined. There were no significant differences in level of maltose in embryo fractions between Jubilee and Sucro throughout maturation though Sucro had the highest ratio of embryo maltose to total maltose between 35 and 47 DAP. Unlike sucrose, the ratio of embryo maltose to total maltose was not significant. But unlike sucrose, glucose and fructose, there was a significant harvest x genotype influence over the level of maltose in both embryo and endosperm fractions (Table A-1).

Maltose:sucrose ratio There were no significant differences in the maltose to sucrose ratio arising from the two planting dates.
Fig. 34. Maltose, embryo (A), endosperm (B), total (C), and embryo fructose:total fructose (D) contents of seeds of three sweet corn genotypes, Jubilee, se, and Sucro, harvested at successive stages of maturity
The ratio of maltose to sucrose increased with maturity reaching a peak at 47 DAP and subsequently declined (Fig. 35). There were no significant differences in maltose:sucrose ratio between Jubilee and Sucro across the maturation period. The se-genotype however, differed significantly from the other genotypes in exhibiting the highest ratio throughout maturation. As expected the harvest x genotype interaction was significant.

Significant but weak correlations were exhibited between the ratio of maltose to sucrose with embryo maltose (r = 0.58) and embryo sucrose (r = 0.47). There were no significant correlations between maltose to sucrose ratio with the ratio of embryo sucrose to total sucrose, total maltose, or endosperm maltose.

Raffinose

Date of planting had no significant influence on the level of raffinose in seed fractions. The level of raffinose differed between seed fractions at different stages of maturity and between genotypes. For all genotypes, raffinose was not detected in seed fractions until 39 DAP following which there was an increase in raffinose level with a peak at 51 DAP.

For Sucro, the level of raffinose in embryo fractions reached a plateau at 47 DAP when it was the highest among the genotypes (Fig. 36A). The se-genotype had the lowest levels of embryo raffinose at 47 DAP but following that date, the level increased
Fig. 35. The ratio of maltose (total) to sucrose (total) contents of seeds of three genotypes, Jubilee, se, and Sucro, harvested at successive stages of maturity.
Fig. 36. Raffinose, embryo (A), endosperm (B), total (C), and embryo fructose:total fructose (D) contents of seeds of three sweet corn genotypes, Jubilee, se, and Sucro, harvested at successive stages of maturity.
RAFFINOSE (EMBRYO) (mg/ml)

RAFFINOSE (ENDOSPERM) (mg/ml)

RAFFINOSE (TOTAL OF SEED) (mg/ml)

RAFFINOSE (EMBRYO):TOTAL OF SEED
sharply and peaked at 51 DAP. There were no significant differences in the levels of endosperm and total raffinose between Sucro and Jubilee throughout maturation (Fig. 36A, 36B). The se-genotype, however, exhibited significantly higher amounts of raffinose beginning 43 DAP through 51 DAP. When raffinose from embryo fractions was expressed as a function of total raffinose, the highest ratio was at 51 DAP (Fig. 36D). Sucro exhibited the highest ratio throughout maturation while the se-genotype and Jubilee did not differ in the ratio of embryo raffinose to total raffinose and the harvest x genotype interaction was significant (Table A-1).

**Raffinose:sucrose ratio**  The raffinose to sucrose ratio was not meaningful until 43 DAP (Fig. 37). Although raffinose peaked at 51 DAP, there were no significant differences in the proportion of raffinose to sucrose between 47 and 51 DAP. The se-genotype exhibited the highest raffinose to sucrose ratio compared to Sucro and Jubilee which showed no significant differences throughout the maturation period.

**Correlation coefficients between raffinose:sucrose ratio with maltose:sucrose ratio, or with sucrose levels in both endosperm and embryo fractions, were not significant. However, raffinose:sucrose ratio varied with endosperm raffinose ($r = 0.75$) and the ratio of embryo sucrose to total sucrose ($r = -0.50$).**
The ratio of raffinose (total) to sucrose (total) contents of seeds of three genotypes, Jubilee, Se, and Sucro, harvested at successive stages of maturity.
DISCUSSION

Production Environment

Seasonal and climatic factors through their influence on the rate at which the corn plant develops and matures play an important part in determining kernel development. The seasonal climatic conditions under which the crop was grown are shown in Fig. A-2 and A-3 which give temperature, precipitation, and evaporation data of part of the growing season of 1989 and 1990 at Bruner Farm of Iowa State University.

Outstanding features that mark the two seasons include higher temperatures around tasseling time followed by small fluctuations through the silking stage and a sharp decline during the later part of harvesting. It is noted that these ordinary temperature readings do not furnish a basis for a quantitative comparison of the temperature effect in reference to maturation. Various methods have been proposed for interpreting temperature observations in different localities and seasons with reference to plant growth and development (Appleman and Eaton, 1921). Evaporation data followed a similar trend in both years while precipitation was relatively evenly spread in 1990 than in 1989. In 1989, there was very low precipitation following silking (mid-July) through the first part of the harvest period followed by a few days of high precipitation.
towards the end of harvesting for the first planting and mid-harvest for the second planting.

The results obtained in this study, while falling short of a persuasive testimony, are however indicative of the influence of environmental factors on seed development and quality. Harvest moistures were higher in 1989 than in 1990. This parallels higher temperatures and evaporation during harvest period in 1990 compared to that of 1989. In addition, very high precipitation levels at the end of the harvest period of the first planting and the mid of the second planting were observed. High seed moistures and precipitation could have contributed to severe ear rots *(Fusarium moniliforme)* that precluded harvesting the fifth sample for Jubilee in both plantings in 1989. Styer et al. (1983a) reported that the decrease in germination and seedling vigor of sh2 kernels harvested later in development was related to an invasion of the kernels by pathogens. This was based on the observation that seed maturity of sh2 was slower than su thereby exposing the genotype to a longer period to damage.

The prevailing weather conditions during the first planting and second planting, respectively, were expected to have a very different influence on maturities as has been observed in other studies (Culpepper and Magoon, 1924). In 1989, seed from the first planting exhibited better quality as measured by germination tests and conductivity while in 1990, the second planting was only
marginally better than the first. Deterioration of seed following precipitation during the harvest period and relatively low field temperatures and evaporation could have contributed to poor seed quality of the second planting in 1989. In 1990 however, genotype differences influenced seed quality more than did environment. Differences in seed quality due to date of establishment as measured by germination tests and conductivity, were marginal. Both Jubilee and Sucro exhibited better seed quality when planted later while the first planting of the se-genotype exhibited better seed quality.

The effect of date of establishment on soluble carbohydrates was too variable for any conclusions to be drawn.

Moisture Content and Seed Quality

Grain moisture has been widely used by researchers for measuring both relative and actual maturities. Culpepper and Magoon (1924) working on sweet corn reported that the percentages of moisture and dry matter in the kernels constituted a fair index of endosperm maturity. In this study, seed moisture content was independent of viability \( (r = -0.37) \) and vigor \( (r = -0.42) \) although high germination values in the standard and the cold test were generally obtained 42 DAP when significant moisture decline had taken place. All genotypes exhibited a protracted period of moisture retention ranging from 51-60% at 35 DAP to 25-40% at 51 DAP. Since the endosperm, is in large part of maternal origin, a divergence in
moisture was not expected because the genotypes were from the same su (Jubilee) background. Nevertheless, Jubilee subtended high moisture content throughout maturation as observed in other su genotypes (Nass and Crane, 1970). Jubilee and Sucro had greater moisture contents at all harvests in comparison to the se-genotype. These results are in contrast to the slow drying characteristic of the se-genotype line (Illinois 677a) studied by Gonzales et al. (1978).

Bennett et al. (1988) found that the mean seed moisture percentages of a sweet corn inbred decreased steadily throughout the sampling period (42-100 DAP) but the difference between the maximum and minimum values increased from 60 DAP to 104 DAP. The authors cautioned against using kernel moisture to rank maturity because of the large range between minimum and maximum values at a given harvest. Culpepper and Moon (1941), argued that moisture percentage in the kernel could be an absolute index of maturity only provided the embryo and pericarp showed the same changes in moisture content as did the endosperm.

The lack of correlation between moisture content and germination and seedling vigor observed in this study confirmed observations that seed moisture is a poor indicator of harvest maturity vis-a-vis germination and vigor. It is suggested that date at 50% silking be used in combination with harvest moisture as aids in determining harvest dates/maturity. It is recognized though that
the use of inferior seed, differences in cultural practices, meteorological conditions, and other factors may be responsible for certain variations in rate of silking (Culpepper and Magoon, 1924).

Seed Viability and Seed/Seedling Vigor

Production environment had little effect on the seed viability potential of the genotypes. In 1989, the first planting dates produced seed that exhibited high germination potential while in 1990, with the exception of the se-genotype, the second planting produced seed with a higher germination potential. Jubilee seemed to utilize the growing season to improve seed viability potential by 30% in 1989 and 63% in 1990 and exhibited the highest germination values at 46 and 51 DAP, respectively. The se-genotype had the highest germination at 51 DAP while Sucro had the lowest overall germination with its highest value obtained at 47-51 DAP.

Seed production environment exhibited subtle effects on cold germination although it did vary with genotypes. Jubilee was the second best in cold germination in 1990; in both years, Jubilee exhibited the greatest improvement in germination over the maturation period. The highest cold germination values were obtained after 47 DAP in both years. The se-genotype exhibited superior cold germination throughout maturation with the maximum occurring at 43 DAP. Sucro exhibited a 73% improvement in cold germination during maturation in the first year with the highest germination occurring
at 51 DAP. In the following season, Sucro exhibited only a 5% improvement and the highest values were at 39 DAP. In 1989, the maturity stage during which there was the greatest improvement in cold germination was coincident with the highest values, but, in 1990, the highest values occurred much later. In contrast to other studies, these results indicate that germination at low temperatures may not be satisfactory for hybrids carrying the \textit{sh2} gene. In addition, genotype differences and production environment are among the primary factors responsible for differences in viability and vigor. High correlations between viability and vigor tests obtained in this study indicate that the tests can be used to distinguish genotype and environment differences.

Culpepper and Moon (1941) using open pollinated ears harvested at 5-day intervals between 30-70 days from silking found that the most favourable time of harvesting sweet corn for seed was between 35-45 days after silking. Styer et al. (1980) reported that \textit{sh2} kernels had higher germination rates and increased seedling vigor when the kernels were harvested 16-36 DAP, whereas germination and seedling vigor of normal, \textit{su}, and \textit{bt} improved with longer kernel development. Styer and Cantiliffe (1983a) reported that the stage of development at which seeds were harvested did not greatly affect germination under optimal conditions. Field and greenhouse grown 18-day old seeds germinated as well as mature seeds as did greenhouse \textit{sh2} produced seeds 22 DAP and older. Churchill and Andrew
using F1 seed found that warm and cold germination for both sh2 and se were always greater than 90% at 40-47 DAP indicating good viability and vigor in the genotypes used.

Germination/Seedling Vigor After Soak

In early spring plantings, sweet corn seed must remain viable in cold wet soils until germination begins at 8 or 10 C. The seed must tolerate anoxia prior to germination and then grow at suboptimal temperatures. Thus when seeds were first soaked for 24 h in a conductivity test and subsequently planted in a germination test, conductivity and vigor could be compared on the same seeds. In terms of absolute germination values, the results of this test were closer to the standard germination test than the cold test. However, in both years, this test correlated very well with both the germination test and cold test (r= 0.73 to 0.85). In both 1989 and 1990, harvest dates with the highest germination values were coincident with those found for the standard germination. The effect of soaking following drying treatments did not severely depress germination as did low temperature and wet conditions in the cold test.

There are no comparable data on germination following soaking in sweetcorn but Herter and Burris (1989a) working with dent corn originating from drying-injured seed lots that were soaked for 24 h for a conductivity test before being germinated found that the
correlation between conductivity and cold test results averaged slightly lower than that between conductivity and the standard germination test. They suggested that running the conductivity test at 10 C might have resulted in a higher correlation between conductivity and cold test results. However, Perry and Harrison (1970) found no evidence that leakage was different at 20 C compared with 10 C as would have been expected if the mechanism of active transport was responsible for leakage.

Shoot/Root Dry Weights and Abnormal Seedlings

Seedling dry weight determinations and the number of abnormal seedlings are often used for growth analysis during the early phases of seedling development to characterize early growth potential. Rowe and Garwood (1978) found that kernel vigor measurements of vegetative weight gave more consistent results across sweet corn inbreds than did either germination percentage or shoot length. Gausman et al. (1952) found that drying high moisture corn promoted radical growth but delayed plumule growth. In contrast, Navratil and Burris (1984) reported that root development was more susceptible to drying injury than shoot development. Wellington and Bradnock (1964) studied the damage sustained by barley and wheat seed heated in sealed tubes and those allowed to dry whilst being heated. They demonstrated that damage from heating with evaporation was characterized by an increase in the number of grains that germinated
abnormally. Further tests using tetrazolium chloride indicated that seeds heated without evaporation were damaged in the region of the radicle while seeds with evaporation were damaged in the centre of the scutellum.

In this study, in both 1989 and 1990, none of the main effects of date of planting, seed maturity, or drying temperature exhibited significant effects and correlation coefficients on shoot:root dry weights except for the effect of genotypes. Sucro exhibited the lowest shoot:root ratio compared to the other genotypes. The shoot to root ratio was obtained following the warm germination test. It is suggested that the ratio, computed from seedling dry weights under stressful conditions, as in the cold germination test could have separated the effects of maturity as reported by Bennett et al. (1988) or those of drying temperatures.

In general, the environment in which the seed crops were produced did not affect the number of abnormal seedlings. In both years, there were fewer abnormal seedlings as maturity progressed, suggesting that maturity enhances seed viability and seed/seedling vigor.

In 1989, drying temperatures exhibited little effect on seed viability and vigor while in 1990, drying at 45 C generally produced more abnormal seedlings, although there were overlaps in the effect of drying temperature between 35 and 40 in the standard test and between 40 and 45 C in the cold germination test. In addition, low
drying temperatures (35 and 40 C) exhibited a higher number of abnormal seedlings in the cold test early in maturation. It appears that the effect of drying temperatures was not the main determinant of the abnormal seedlings.

Genotype effects played an important role with Jubilee exhibiting the highest number of abnormal seedlings in both 1989 and 1990 despite being second to the se-genotype in viability and vigor. The highest numbers of abnormal seedlings in percentage points were 32, 26, and 20 in 1989, and 23, 26, and 16 in 1990 for the cold test, the standard test, and the germination test after soaking, respectively. The standard germination after soaking was expected to exhibit poorer results owing to leakage of metabolites and anoxia and that seeds were not treated prior to the soaking treatments. The number of abnormal seedlings was therefore considered a poor indicator of drying injury, physiological maturity, or vigor and viability in the material studied.

Maturation and Drying

The embryo is morphologically mature approximately 45 DAP except for some additional development of the seedling leaves and a slight increase in size. In the endosperm, meristematic activity ceases about 48 DAP (Randolph, 1936). In this and other studies (Culpepper and Moon, 1941; Gonzales et al., 1976; Churchill and Andrew 1984 and Bennett et al., 1988) stage of maturity at which the
highest germination and vigor were obtained appeared coincident with embryo maturity. However, satisfactory germination was also obtained at earlier stages of maturity as reported by several investigators (Styer et al., 1980; Styer and Cantliffe, 1983; Borowiski et al., 1991).

It has been established that seeds undergo a transition from desiccation-intolerance to desiccation-tolerance before completion of major developmental events. Furthermore, coincident with the acquisition of tolerance, whether natural or imposed, they gain the capacity to germinate upon subsequent rehydration (Kermode and Bewley, 1985). In this study, the effects of drying temperature on seed viability were similar in 1989 and 1990 with the higher drying temperature (45 C) reducing germination by two- to three-fold compared to drying at 35 or 40 C. In the first year, Jubilee exhibited higher germination following drying while in the second year, the se-genotype exhibited germination superior to Sucro and Jubilee by a margin of 20 to 30%. Sucro was the poorest in both years. In terms of kernel maturation and drying temperature, across years, seed of all genotypes harvested between 47 and 51 DAP and dried at either 35 or 40 C outperformed seed of the same maturity dried at 45 C, or seed of earlier maturity and dried at 35 C or 40 C. In addition, the difference in viability due to the three temperatures declined with increasing maturity. In the cold germination test, Jubilee was superior to Sucro at all drying
temperatures in the first year while the se-genotype dried even at 45 °C outperformed seed of Jubilee and Sucro dried at 35 °C by an 18-44% margin. In both years, cold germination declined with increasing drying temperatures but the effect of drying temperature on the reductions in seed/seedling vigor in the cold test were twice as severe in 1989 compared to 1990. In both years, the harvest x temperature interaction for cold germination was not significant.

There are no comparable data on drying temperature regimes in sweet corn, but results obtained in dent corn have shown that high drying temperatures of 45 and 50 °C adversely affect seed germination and seedling vigor (Navratil and Burris, 1984). In this study, the high germination and seedling vigor exhibited by 35 °C dried seed, and the observation that differences in viability of seeds dried at the three temperatures narrow with maturity seem to support the hypothesis that the capacity to withstand desiccation brought about by high temperature drying is not acquired by seed until it is nearly mature. The lack of significant effects of drying temperature on cold germination of seed harvested at different stages of maturity is contrary to findings in dent corn which showed that seedling vigor is highly dependent on date of harvest (Knittle and Burris, 1976).
Conductivity

The relationship between electrolyte leakage and pre-emergence mortality under field conditions was first reported by Flentje and Saksena (1964) working with peas. This correlation has been extended to other crops including sweet corn (Waters and Blanchette, 1983) where it has been suggested that the extent of leakage provides a reliable indicator of potential field emergence.

In the present study, production environment failed to influence leakage potential of the seed from genotypes planted at two different dates in both 1989 and 1990. In the first year, the second planting exhibited higher conductivity while in the second year the difference between the two crops was marginal. Overall, leakage declined with maturity but there was no consistency among genotypes. Simon (1974) argued that leakiness appeared not to be an inherited character but that it depended upon the precise conditions under which the seed developed. The time of harvest, moisture content of the seed, conditions during harvesting, and storage environment may all affect conductivity.

In this study, in 1989, Jubilee seed exhibited the greatest leakage as well as the largest decline in leakage throughout maturation compared to Sucro. In the second year, when conductivity was measured at 6, 12, and 24 h after soaking, Jubilee seed at each stage of maturity and during the entire soak period exhibited a high conductivity up to 47 DAP and exhibited the least decline in
conductivity. These results are in contrast to studies that have shown that genotypes with \textit{sh2} gene leak more electrolytes than those with \textit{su} gene (Styer and Cantiliffe, 1983b; Wann, 1986). The difference has been attributed to smaller seed size, high sugar-to-starch ratio, and thinner protective layer on the \textit{sh2} seed that was used in their studies. The \textit{se}-genotype, which exhibited the lowest conductivity in 1990 also showed the greatest decline in leakage much earlier in maturity at 39-43 DAP. Jubilee and Sucro exhibited their major decline at 43 and 47 DAP in 1989, and at 47 and 43 DAP in 1990, respectively.

Leakage increased with increase in drying temperature but also varied with genotype. There were no significant differences in conductivity between seed dried at 35 and 40 C and 40 and 45 C, for Jubilee in 1989 and the \textit{se}-genotype in 1990, respectively. The genotypes exhibited significant effects of drying temperature for the 6 hours of soaking than for 12 or 24 hours. This observation is in contrast to work on dent corn (Herter and Burris, 1989a) which showed that differences in conductivity between different drying treatments were more evident after 12 and 24 h of soaking. Simon (1974) reported that when conductivity measurements were taken at shorter time intervals, the rate of leakage declined dramatically even during the first few minutes of immersion.

In both years leakage declined with maturation, but the effect of drying temperature at each stage of maturity remained large.
These observations are in contrast to the results obtained in the viability and vigor tests where germination of seed dried at the three temperatures increased with increasing seed maturity. Simultaneously, however, the magnitude of the differences in germination between seed drying temperatures narrowed with increasing maturation instead of remaining constant as in the conductivity test. The conductivity test may respond to the physical effects of drying on individual seed while tolerance to desiccation will increase with maturity; the magnitude (low to high temperature regime) of this effect may be independent of physiological changes associated with maturity. The viability and vigor tests deal with a population of seeds whose numbers would increase with maturity. As seeds dried at the higher temperature regime become tolerant to injury the magnitude of the temperature effect on viability and vigor decreases as maturity advances.

The concentration of soluble carbohydrates in the steep water has also been shown to correlate with emergence (Matthews and Bradnock, 1967). Data obtained in the present study showed that the level of sugars (fructose and sucrose) were highest early in maturity and declined to negligible quantities by 51 DAP. Jubilee exhibited 50% more leachate sugar than Sucro and the level of sugar increased with increases in drying temperatures. Correlation between soluble sugars in leachate and standard germination test and the cold test were low being $r= -0.42$ and $-0.5$, respectively. Takayangi
and Murakami (1968) developed simple viability tests based on the concentration of sugar in the leachates. But the validity of such tests was questioned by Abdul-Baki and Anderson (1970) who found that the correlation broke down in rapidly aged seeds. Furthermore, they suggested that leachable sugar may be related to internal concentration or rate of utilization of sugars during germination rather than changes in membrane permiability of dry seeds.

Few data are available on the relationship between drying temperatures and seed leakage in sweet corn. Using optical measurements of leached materials, Hottes and Huelsen (1927) found that the quantity of leached material increased in relation to the degree of injury to the protoplasts as affected by temperature and the period of exposure. Styer and Cantliffe (1983b) found that during maturation, sh2 seeds generally leaked more electrolytes, including carbohydrates, than did su seeds. Leakage decreased in all seed types with increasing maturity. Waters and Blanchette (1983) reported that the ASA 610, which measures the conductivity of the leachates from individual seeds, gave a higher correlation with field performance of sweet corn than did bulk-seed conductivity tests. Adjusting the bulk conductivity test for seed weight did not improve the correlations, suggesting that seed size failed to influence the amount of electrolyte leaked. In dent corn, Seyedin et al., 1984) showed that electrolyte leakage was significantly increased after drying at high temperatures.
When differences in conductivity readings, or increases in leakage, were calculated between the three soak intervals of 6-12, 12-24, and 6-24 h., all genotypes exhibited 1.5x increase in leakage between the 6-12 h soak period compared to the 12-24 h period. In contrast to observations for total leakage, Sucro expressed the largest increase during all soak periods. In the 6-12 h period, maturation widened the differences in leakage between genotypes with the se-genotype exhibiting the largest decline. Kernel maturity and genotype were only relevant at 51 DAP in the 12-24 h soak period. The rate of leakage between conductivity readings has been suggested to be a better measure than total leakage after a specified time because the complicating factor of initial leakage is removed (Herter, 1987).

In this study, however, rate of leakage did not correlate strongly with standard germination (r= -0.34), cold test (r= -0.26), or germination after soaking (r= -0.29) compared to total leakage which exhibited higher correlation coefficients of -0.55, -0.53, and -0.58 for the same tests. This may be expected because total leakage and germination percentages are absolute values. In addition, Jubilee exhibited a decline in rate of leakage as maturity advanced while Sucro maintained a high rate of leakage even at a stage of maturity where germination was optimal. Under field conditions, leachate in the germination environment could be a predisposing
factor for microbial invasion and subsequent reductions in viability and vigor.

The observation that the 6-12 h period distinguished treatment effects better may be related to the cause of leakage itself. Perry and Harrison (1970) suggested that leakage is caused by a broaching of cell membranes by the inrush of water. Simon (1974) argued that while it is clear that the rapid inflow could rupture the plasma membrane and tonoplast allowing cell contents to diffuse out, it was not likely that membrane fragments could reassemble in a few minutes to prevent further leakage in the face of continued inflow of water. Simon and Raja Harum (1972) found that rapid imbibition was not necessarily accompanied by profuse leakage of electrolytes and postulated that high conductivity levels in soak water resulted from damaged seed membranes which allowed greater leakage of electrolytes. The current hypotheses about leakage and membranes have been discussed in the literature (Simon, 1974; McDonald, 1980 and Simon and Mills, 1982). It is envisaged that membranes are disorganized in dry seeds, no longer forming an intact barrier around the cytoplasm. They regain their normal semi-permeable condition during imbibition. It is proposed that there would be a short period at the start of imbibition when the membrane constituents in each cell were going through a phase of reorganization and solutes could leak out. Increased leachate conductivity is thus ascribed to the loss of ability to reorganize
cellular membranes rapidly and completely. The greater rate of leakage exhibited by Sucro during the 6-12 h, 12-24 h, and 6-24 h periods indicate that membrane reorganization or repair was slower or incomplete in Sucro seed. Similarly, membrane reorganization or repair may account for the greater rates of leakage observed during the 6-12 h period in this study.

Soluble Carbohydrates

Nass and Crane (1970) proposed that drying rate is regulated in part by hydrophilic compounds in the endosperm of corn kernels and suggested that role for the sugar or other carbohydrates components in sweet corn endosperm mutants. Comparing their results with other studies, they postulated that a positive relationship existed between per cent reducing sugars, water soluble polysaccharides, and drying rate.

Gonzales et al. (1976) noted a slow drying as well as enhanced sucrose accumulation in Illinois 677a, a genotype with an se gene. Ferguson et al. (1978) ascribed the slow drying nature of Illinois 677a to osmotic retention of moisture caused by the unusually high levels of soluble carbohydrates. The authors proposed that high maltose content, which distinguished Illinois 677a from other genotypes may be a factor in the slow drying of the genotype.

In the present work, the relationship between soluble carbohydrates and drying was explored. In general, there were no
significant differences in endosperm glucose and total glucose levels among the three genotypes. In terms of fructose and sucrose levels, the se-genotype exhibited the lowest endosperm and total sucrose and fructose levels while Jubilee and Sucro exhibited similar levels. The trend for embryo sugars in Jubilee and Sucro, with the exception of raffinose peaked at 47 DAP and declined. The se-genotype followed a similar pattern but at 47 DAP, it exhibited significantly lower levels of fructose (and maltose) while sucrose levels declined at 43 DAP and peaked again at 51 DAP. When embryo sugars were expressed as a fraction of total sugars, the harvest by genotype interaction for sucrose was the only significant statistic with the se-genotype exhibiting a high ratio of embryo sucrose to total sucrose.

In related studies, Culpepper and Magoon (1924, 1927) in a study of sweet and starchy sweet corn varieties during the first 30 DAP, reported that a rise and then a fall in the level of sucrose present and that endosperm sucrose levels were greater than levels of reducing sugars. In non-sweet corn, disappearance of reducing sugars was gradual and essentially complete at maturity while in the sweet types, a small amount of sugar, mostly sucrose, remained in the mature endosperm. Laughnan (1953) studied the effects of the genes sh2 and sul in the distribution of endosperm carbohydrate reserves. It was found that almost 20% of the dry weight of the shrunken kernels was composed of sugars, 16% of which was sucrose.
Whistler et al. (1957) using 7-56 day-old *sh2* seeds found that fructose and glucose were equal earlier in maturity, showing peaks at 14 DAP and at 35 DAP. In *su* seeds, glucose and fructose peaks were at 28 DAP. Using a sweet corn genotype with the *se* gene, Ferguson et al. (1979) reported that glucose, sucrose, and fructose continually decreased from 19 DAP to dry mature stage. Glucose level in *su* genotypes was 3.5-4x higher than that of IL 677a while sucrose levels were 5x higher in *se* than *su* genotype.

Creech (1968) reported thesis data of Jordan (1965) who found significant differences in fructose and glucose levels between certain sweet corn mutants and reported striking differences between mutants for sucrose and maltose levels at 20 DAP. The kernels of *sh2* were high in sucrose and had trace levels of maltose while no maltose was found in *su* genotypes. Triple recessives (none of which involved *sh2* and *sul*) were 13x higher in maltose than normal indicating the influence of gene interactions. Ferguson et al. (1979) found large quantities of maltose associated with the mutant gene *se* in a breeding line IL677a. During kernel development, maltose content of IL677a increased to 3.28% at 40 DAP and remained high. The authors suggested that the slow drying characteristic of the *se* genotype was related to maltose accumulation. In this study the *se*-genotype had the highest levels of endosperm maltose and total maltose and the least levels of embryo maltose.
Some workers have questioned the natural occurrence of maltose since it can readily arise through autolysis, by hydrolysis of starch during extraction of plant tissues, or be derived from enzymatic breakdown of starch between harvest and storage (Whistler et al. 1957; Creech, 1968). Avigad (1982) also reported that the presence of very high maltose concentrations in starch forming tissues, or their extracts, could be indicative of amylosis by fungal or bacterial enzymes. However, Dickinson et al. (1983), established that it was unlikely that the observed increase in maltose resulted from premature action of amylases on starch and phytoglycogen during seed maturation.

After sucrose, the next most abundant oligosaccharide in plants is raffinose. Raffinose is known to accumulate in storage organs, particularly seeds where it only appears during maturation and disappears during germination (Miller, 1973). Whistler et al. (1957) working with 7-56 day-old su and sh2 seeds did not detect any raffinose in seeds. In the present study, raffinose was not detected in seed fractions until 39 DAP following which there were variations in the level of total seed raffinose among genotypes during maturation but the highest levels were at 51 DAP in all genotypes. The se-genotype exhibited higher levels of raffinose throughout maturation while Sucro showed the highest ratio of embryo raffinose to total raffinose.
Sucrose is suggested to be the principal agent of membrane stabilization with the larger oligosaccharides that include raffinose, serving to keep the sucrose from crystallizing (Leopold and Vertucci, 1986; Caffrey et al., 1988; Koster and Leopold 1988). Chen and Burris (1990) studied field corn harvest maturity and seed drying as related to membrane behaviour and carbohydrate metabolism using a preconditioning process that induces high temperature drying tolerance without a substantial loss in moisture. Conductivity and composition of leachate were used to measure the condition of membranes as related to reductions in seed quality associated with drying. The authors found that the percentage composition of sucrose and raffinose increased significantly during preconditioning while soluble sugar concentration decreased.

In the present study, the harvest x genotype interaction was only significant for the ratio of sucrose in embryo fraction to total sucrose. And when the ratios of maltose and raffinose to sucrose were calculated, the se-genotype exhibited high ratios throughout maturation. There were no significant differences between Jubilee and Sucro in the ratios of maltose and raffinose to sucrose at successive stages of maturation. It is probable that the phenomena of membrane protection observed by Koster and Leopold (1988) and Chen and Burris (1990) may be prevalent in the material used in this study, particularly in the se-genotype, which exhibited the highest viability and seedling vigor following drying. The work
of Koster and Leopold (1988), however, was on germinating axes, while Chen and Burris (1990) measured soluble sugars following artificial drying. In the present study, soluble sugars were obtained from freshly harvested seed prior to drying treatments. There are few comparable data in sweet corn, but in a related study, Culpepper and Moon (1941) reported that the "power" of germination was not directly related to sugar content. They found that germination increased with age up to 22 to 25 days after pollination but the sugar content increased to about the 16- or 18-day stage and then decreased. They concluded that satisfactory germination was not obtained until the sugar content had declined considerably from its maximum. Chen and Burris (1990) found a significant correlation between sugar ratios (raffinose/sucrose) with viability and conductivity measurements and concluded that soluble compositional relationship rather than absolute content may be important to membrane stabilization. It is probable that the ratios of maltose and raffinose to sucrose found in this study, other than total sugar content (Culpepper and Moon, 1941) or maltose content (Ferguson et al., 1979) may be related to maturation drying and desiccation tolerance. However, owing to the observation that the ratio of maltose to sucrose declines after 47 DAP, and that there was no significant correlation between it and the ratio of raffinose to sucrose, it is suggested that these ratios may be responsible for different functions.
While the correlations between the ratio of raffinose/sucrose and seed conductivity and sugar leakage were associated with the protection of membranes in high-moisture seed (Chen and Burris 1990), other investigators have suggested that leachable sugar may also be related to internal concentration (Abdul-Balki and Anderson, 1970). Doehlert and Kuo (1990) observed that the failure of kernels to utilize sucrose in sh2 seeds appeared to result in its accumulation in all parts of the kernel, with higher levels occurring in the pericarp than the rest of the kernel. In this study, Sucro was shown to exhibit the highest rate of leakage between soak periods of 6-12, 12-24, and 6-24 h and also exhibited the least shoot to root ratio, viability and vigor. While this may be explained by the paucity of sucrose in the kernels and germination conditions, it is probable that inability for membrane repair, following imbibition after desiccation, may play a role owing to a very low maltose and raffinose to sucrose ratio in Sucro as observed in this study. Furthermore, it possible that sucrose levels in embryo fractions are a necessary component in maturation drying and desiccation tolerance. The se-genotype exhibited the highest ratio of embryo sucrose to total sucrose than did Sucro and Jubilee.
SUMMARY AND CONCLUSIONS

It has been suggested (Rowe and Garwood, 1978) that the problems of seed quality associated with the various endosperm genes of sweet corn are not inherent to the genes themselves. Significant inbred x genotype interactions for germination, shoot length, and seedling weight have been observed. However, Boyer and Shannon (1983) argued that although considerable improvement in seed quality may be obtained through selection programs, cultivars based on genes or gene combinations that produce high sugars and low starch kernels will have reduced kernel energy reserves needed for germination.

The subject of carbohydrate reserves limiting viability and vigor has been supported by various investigators (Wann, 1980; He, 1991). However, there is a paucity of information suggesting that endosperm carbohydrate content may not be related to sweet corn vigor (Styer et al., 1980; Churchill and Andrew, 1984; Bennett et al., 1988). Further, it has been suggested that it is not total carbohydrate content per se that influences viability and vigor, but percentage composition of oligosaccharides as they influence membrane integrity during artificial or natural drying.

The high rate of electrolyte leakage, that included soluble sugars, in Sucro (sh2 genotype) as observed in this study, may predispose seeds/seedlings to root rots and seedling blights under germination conditions in the field. But the premises that leakage of soluble sugars may deplete the limited substrates available to
the embryo for germination was not supported in this study. Germination following 24 h soaking of seeds was superior to the cold test and as good as the standard warm germination test. In addition, correlation coefficient between rate of leakage, or soluble carbohydrates in leachate, with germination after soak or with other viability and vigor tests were very low. Doehlert and Kuo (1990) related inability of sh2 seeds to utilize sucrose to the localization of the sugar, predominantly in the pericarp tissue.

This study has demonstrated that leakage can be reduced and viability and vigor improved if appropriate drying temperatures and harvest maturity dates are sought. Based on the proposed relationship between soluble sugars and membrane integrity during drying, later stages of maturity, viz., 47 DAP, during which oligosaccharides are suggested to protect membranes, were coincident with high germination values and reduced leakage when seed was dried at 35 C. In addition, the effect of high (45 C) temperature drying on leakage and viability and vigor declined considerably.

These findings, while pointing to opportunities for improving the understanding of sweet corn maturation and germination physiology, are based on material from the same genotypic and germplasm (Jubilee) background. The relationship between endosperm genes and metabolic processes that culminate in germination and vigor could be affected by the common background. The relationship between sucrose, maltose, and raffinose in embryo fractions suggest
that the level of investigation towards understanding the role of sugars in maturation drying should include metabolic characterization that encompass enzyme systems and localization of their products in seed tissues.

There is a wealth of information which discusses organic compounds and processes associated with redirecting metabolism from a developmental mode to a germinative mode. These processes include; the relationship between abscisic acid and ribonucleic acids at the gene level, soluble protein synthesis, stress-induced proteins, changes in phospholipid composition or conformation, and organic solutes such as sugars. Invariably, it is an appropriate intricate balance of these properties that result in the adaptive changes in membranes, at the organelle or tissue level, during maturation drying that is mirrored by resumption of metabolic activity.
LITERATURE CITED


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Fig. A-1. Representative chromatograph for soluble sugars in seeds as separated by a SupelCosil LC NH2 column.
Table A-1. Analysis of variance means for levels/ratios of soluble sugars in seed fractions as influenced by genotypes and kernel maturity

<table>
<thead>
<tr>
<th>Seed Fractions</th>
<th>Fructose</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>Raffinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo</td>
<td>0.86ns*</td>
<td>1.35ns</td>
<td>795.35*</td>
<td>10.65**</td>
<td>7.68***</td>
</tr>
<tr>
<td>Endosperm</td>
<td>253.39*</td>
<td>216.50ns</td>
<td>24367.50ns</td>
<td>348.39***</td>
<td>23.72***</td>
</tr>
<tr>
<td>Total</td>
<td>255.11ns</td>
<td>222.02ns</td>
<td>31952.41ns</td>
<td>.227***</td>
<td>27.68***</td>
</tr>
<tr>
<td>Embryo: Total</td>
<td>.005ns</td>
<td>.002ns</td>
<td>.048***</td>
<td>.028ns</td>
<td>.010ns</td>
</tr>
</tbody>
</table>

*ns = not significant  
* significant at 0.05 level  
** significant at 0.01 level  
*** significant at 0.001 level
Fig. A-2. Temperature, precipitation, and evaporation data for the 1989 growing season based on 5-day averages covering the period of the tests including: 50% silking date for the first (a) and second (b) planting; first harvest date for the first (c) and second (d) planting; and last harvest date for the first (e) and second (f) planting.
Fig. A-3. Temperature, precipitation, and evaporation data for the 1990 growing season based on 5-day averages covering the period of the tests including: 50% silking date for the first (a) and second (b) planting; first harvest date for the first (c) and second (d) planting; and last harvest date for the first (e) and second (f) planting.