GROWTH RESPONSES OF CORN (*ZEA MAYS* L.) INBRED LINES SUBJECTED TO CHILLING STRESS

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Solomon Kibite

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ABSTRACT

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The undesirable aspects of chilling temperatures on young corn seedlings are well known. Chilling temperatures at the time of planting and during seedling growth have been shown to result in poor stands, chlorophyll disturbances, retarded growth, increased infection by diseases and ultimately reduced yield. These undesirable aspects of chilling temperatures occurring at some point in the growing season call not only for a corn genotype that can grow under sub-optimal temperatures, but which is also able to recover rapidly when the chilling stress is removed.

The objectives of this study were: (1) to investigate whether chilling tolerance of an inbred line and the ability of the same inbred line to recover from chilling stress are independent characters or not, and (2) to study some of the metabolic properties expressed before and during chilling stress and to establish whether these metabolic properties are associated with chilling tolerance and the ability to recover from chilling stress, respectively.

When 8-day-old corn seedlings were exposed to an 8-day chilling stress (12/10^{o}C) growth rate was retarded significantly. Chilling stress also reduced the rate of photosynthesis and respiration, and increased

sugar and starch content in the roots as well as in the leaves. Total protein in the leaves declined, while the concentrations of free amino acids and water-soluble proteins in the leaves increased. The reduction in total protein content in the leaves was accompanied by a corresponding increase in total protein, free amino acids and water soluble proteins in the roots. Nitrate reductase activity and nitrate content in the leaves declined, while chilling induced a sharp increase in protease activity. The rate of protein synthesis as indexed by C^{14}-leucine incorporation, and chlorophyll content were also reduced significantly.

When the chilled plants were returned back to favourable temperature ($28/22^{o}C$) they showed very rapid growth rates and tended to catch up with the control plants that had not been subjected to chilling. Some genotypic differences in the rate of recovery were recorded. During recovery the concentrations of soluble carbohydrates dropped, but the recovering plants maintained a more positive carbon balance than the controls. The recovering plants also showed positive nitrogen balance in the leaves and negative nitrogen balance in the roots as compared to the controls.

Simple correlation studies established that there was no relationship between chilling tolerance of an inbred line and its ability to recover rapidly from chilling injury. Rapid recovery from chilling appeared to be more important in determining the final dry matter content than chilling tolerance *per se*. Also, correlation studies showed that the pre-stress level of sugar content in the root was positively and significantly correlated with chilling tolerance while nitrate reductase activity was negatively correlated with chilling tolerance.

These properties may be useful for predicting chilling tolerance in corn. The pre-stress levels of the other properties investigated were not sufficiently correlated with chilling tolerance to be of predictive value.

Metabolites stored in the plant during chilling stress were implicated to be responsible for the rapid growth rate observed when the chilling stress was removed. However, differences in the concentrations of metabolites accumulated during chilling stress were not shown to be responsible for genotypic differences in the speed of recovery. The results also indicated that the size of the root system may have a positive effect on recovery from chilling stress.

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1. INTRODUCTION

The commercial production of a crop on the fringes of its natural habitat is affected by several factors. The most important among these is climate, and prominent among the climatic factors influencing plant growth is temperature.

Corn originated as a heat-loving tropical or subtropical crop, and through genetic modification has become one of the major crops in the warmer sections of the temperate regions. Its movement into the northern portion of the temperate region has been restricted by low temperatures and short seasons. However, as land comes under greater population pressure, there is increasing interest in growing this high yielding crop under relatively cool conditions.

The biggest problem limiting large scale production of corn in the temperate regions is the relatively short duration of the growing season. Owing to the need for extending the growing season, corn in these regions has to be planted early. This practice subjects it to low temperature during germination and early seedling development. Low temperature at the time of planting and during seedling growth results in poor stands, chlorophyll disturbances and retarded growth. In order to avoid these problems farmers are traditionally compelled to delay planting until late spring. Late planting on the other hand, delays the development of the crop and renders it vulnerable to late season frost injury. It also postpones harvest to late fall, when adverse conditions frequently reduce the quality of the crop. These are not the only problems. In addition,

periods of low temperature may occur during the grand period of growth, affecting the final maturity or yield of varieties sensitive to such conditions.

These undesirable aspects of low temperature, occurring at some point in the growing season as it generally does in the northern fringes of corn adaptation, call not only for a corn genotype that is able to grow under sub-optimal temperatures, but which is also well equipped to recover rapidly from injuries caused by sub-optimal temperatures.

While genetic differences among corn lines for low temperature tolerance have been identified, and to a limited extent exploited in breeding programs, a sound physiological basis to account for the genetic differences in cold tolerance upon which in turn a screening technique which would provide a reliable index of field reaction to low temperatures is still lacking. On the other hand, the genetic differences among corn lines for recovery from chilling injury and the associated biochemical changes have not been recognized for their importance, and therefore remain virtually unexplored. An attempt has been made in this study to fill these information gaps concerning the nature and basis of cold tolerance in corn.

The objectives of this investigation were, therefore:

1. to identify corn genotypes which differ in chilling reaction and recovery from chilling injury, and to investigate whether the ability to grow under chilling stress and the ability to recover rapidly from chilling injury are correlated or independent of each other;

2. to study some of the metabolic changes associated with chilling stress and recovery from chilling injury and to determine possible correlations of the observed reactions with the speed of growth and development

during chilling stress and when the chilling stress is removed;

3. to determine whether any of the relationships shown are indicated as useful in screening lines for tolerance to chilling.

2. REVIEW OF LITERATURE

2.1 Chilling Injury

The low temperature injury of crop plants can be apparently classified into two types: freezing injury which occurs at temperatures below $0^{o}C$, and chilling injury which occurs above $0^{o}C$. With regard to the former, many studies have been carried out and the information derived has been reviewed (Levitt, 1951; Levitt, 1956; Mazur, 1969). Studies of the physiological mechanisms of chilling injury, on the other hand, are not as extensive as those concerned with freezing injury.

As early as 1896, Molisch is reported by Lyons (1973) to have cited several early studies demonstrating that a number of plant species are killed at low temperature above the freezing point of water. Since then, this physiological harm has been variously referred to as "chilling injury", "low temperature injury", "cold injury" or "low temperature breakdown". Among these, the term "chilling injury" appears to be the preferred term because it is not easily confused with freezing injury or other phenomena related to cold or winter hardiness (Lyons, 1973).

Chilling sensitive plants appear to have a communality of temperature response. In these plants, the critical temperature below which injury occurs is most often around 10 - $12^{o}C$ (Levitt, 1972). Chilling injury is usually expressed by retarded rate of growth, development of visible symptoms of injury, and generally by alteration of chemical constituents. No quantitative measurement of chilling injury has been developed so far;

therefore the level of injury is usually expressed in terms of severity of symptoms.

2.2 Corn and its Reaction to Chilling Temperatures

Corn is a tropical or sub-tropical crop in origin and requires high temperature for germination and growth. The optimum temperature for the growth of corn is within the range from 65 - 75^{o}F (Wilsie, 1961). Although some genotypes of corn are known to germinate at 4^{o}C (Kalnyn, 1974), at ambient temperatures below 15^{o}C emergence is delayed and growth of seedlings is retarded (Cooper and Taiton, 1968). Retarded seedling growth in turn results in higher incidence of seedling diseases and consequently increases the hazards of crop establishment.

The severity of chilling injury in crop plants is affected by several factors. Prominent among them are: temperature, physiological maturity and the length of the time of exposure (Lyons, 1973). Chilling injury in corn is known to increase progressively with decrease in temperature. Walker (1969) has demonstrated that 1^{o}C change in soil temperature within the range from 12 - 35^{o}C induces changes in the growth and nutritional behavior of corn. With every 2^{o}C increase in soil temperature in the range from 12 - 26^{o}C, he observed that the total seedling dry weight would increase by an average of 20% or more of the weight at the lower temperature level. Extended periods of chilling have been reported to be lethal, whereas shorter periods were found to delay and permanently modify the growth and development of corn (Creencia and Bramlage, 1971).

2.3 Effects of Chilling Temperature on Tissue Components

2.3.1 Changes in Sugar and Starch Content

A number of components have been observed to change when plants are exposed to low temperature. Among them is the large accumulation of sugars at chilling temperatures. Different investigators have presented different views for the expression of high sugar content at chilling temperature. Beevers and Cooper (1964) compared the growth of rye seedlings in controlled environments at 12 and 25°C. They observed that seedlings exposed to low temperature treatment almost ceased to make new growth, and showed a two-fold increase in sugar content. This large effect of chilling on sugar content was not surprising since they also observed a considerable decline in the rate of respiration, accompanied by a less severe reduction in assimilation rate. Therefore, they attributed the increase in sugar content to reduced utilization of assimilates in respiration and new growth. Another school of thought including Parker (1962), Sakai and Yoshida (1968), Siminovitch *et al.* (1968) and Smith (1968) maintain a different view. These investigators observed that the increase in sugar content at low temperature was accompanied by a concommitant reduction in starch content. They ascribed the increase in sugar content to the hydrolysis of starch to sugar. The conversion of starch to sugar, however, does not appear to be a universal phenomenon. In some thermophyllic plants, starch accumulation associated with growth reduction at low temperature has been observed (Hillard and West, 1970; Guinn, 1971; Downton and Howker, 1975).

High sugar content has been associated with chilling tolerance. The principle underlying the importance of sugar in this regard is considered

to be that at chilling temperatures, the uptake of water by the roots is impeded with the result that dehydration appears to be a characteristic effect of chilling stress. Such effects of chilling have been reported in a number of sensitive species including cotton (Arndt, 1937) and corn (Kleindrost and Brouwer, 1970). Sugars, by retaining water, have been implicated as protectants against tissue dehydration and consequently against chilling (Christiansen *et al.*, 1970; Guinn, 1971). High sugar content has been also correlated with freezing tolerance. Evidence presented by Levitt (1966) led to the view that the beneficial effects of sugars on low temperature tolerance is afforded through their capacity to prevent the cryoprecipitation of protein molecules.

2.3.2 Changes in Protein Content

Increase in soluble protein content is another phenomenon frequently associated with acclimation to chilling temperatures. Although there is no investigation, known to the author, correlating soluble proteins with chilling tolerance, there are several investigations on the other hand, suggesting marked relationships between soluble protein content and cold hardiness. Levitt (1956) has discussed that a plant which overwinters, annually goes through a rhythmic rise to maximum followed by a fall to a minimum in freezing resistance. He has presented evidence that in the Summer and in the Spring, it is usually killed by the slightest freeze, while in the Fall, the freezing temperatures required to kill it drop progressively as it becomes more and more adapted to freezing. Implicated with this freezing resistance are the adaptive changes of proteins from insoluble to soluble forms. Jung (1959) and Shih *et al.* (1967) have also observed progressive changes in soluble protein with hardening.

Comparisons between tolerant and non-tolerant species (Jung and Smith, 1961) and tolerant and non-tolerant cultivars within the same species (Jung *et al.* (1967b) have indicated that at any one time during the hardening process, the tolerant lines contained higher levels of soluble proteins than the susceptible lines. Evidence for the presence of a positive correlation between soluble protein content of the tissue and low temperature tolerance of alfalfa plants has been presented by Gerloff *et al.* (1967) and Jung *et al.* (1967a, b). The increase in soluble protein content was preceeded by an increase in RNA (Jung *et al.*, 1967a; Smith, 1968). Levitt (1966) has reviewed earlier works and concluded that the increase in water-soluble protein content during acclimation is not caused by a mere SH → SS interchange. According to him it involves a net hydrolysis of proteins to amino acids followed by a net synthesis. The importance of protease in such processes is apparent.

2.3.3 Changes in Moisture and Mineral Contents

The effects of soil temperature on water absorption by cool and warm season crops was studied by Kramer (1942). He showed that low temperature reduced water uptake in all species, but the reduction was by far greatest in chilling sensitive warm season crops. Kleindrost and Brouwer (1970) found that the growth of corn leaves was closely related to leaf water content, as the latter was varied by changing root temperature. The above two studies appeared to reflect the concept that some degree of turgor pressure was required as a driving force for growth and cell division.

Mineral nutrition of the plant is also one of the many biochemical events severely affected by chilling temperature. In corn Walker (1969)

has indicated that a decrease in root temperature as small as one degree centigrade could result in a marked decrease in tissue mineral content. Tyutynnikov and Yakolev (1975) studied phosphorus uptake by corn seedlings at different temperatures. Their findings indicated the absorption was 3-6 times more under 21/15°C (day/night) temperature than at 12/8°C when day length was 10 hours. Buikan (1962) observed significant differences in nutrient uptake among genetically distinct lines of corn and showed that nutrient uptake at low temperature was slower in normal lines than in cold tolerant ones. The mineral nutrition of a plant at chilling temperature is also affected by nutrient loss from the plant. In a comparative study involving two legumes and two cereal crops, Whatson (1963) observed increased leakage of potassium ion in root tips of beans and corn at chilling temperatures, but no increased leakage in pea or wheat, which are chilling tolerant.

2.4 Effects of Chilling Temperature on Photosynthesis

Differential photosynthetic response to varied environmental conditions among corn genotypes have been documented (Duncan and Hesketh, 1968; Heichal and Musgrave, 1969). Associations between genetic differences in photosynthetic rates and chilling tolerance, however, have not been reported. In general, exposure of corn plants to low temperature reduces the rate of photosynthesis (Hesketh, 1967; Taylor and Rowley, 1971). Cooper and Taiton (1968) and Hofstra and Hesketh (1969) have shown that photosynthesis of corn at temperatures lower than 15°C is minimal.

Several suggestions have been proposed to explain the poor photosynthetic rates of heat loving plants like corn at low temperature.

Chilling, through its effect on photosynthetic enzyme activities, has been included in the list. Taylor *et al.* (1972b) observed that when corn and sorghum plants were transferred from 25°C to 10°C at moderate light intensity, the levels of most photosynthetic enzymes remained unaffected. However, NADP malate dehydrogenase, pyruvate P_i kinase and catalase were reduced in activity by 50% or more. In crops in which C_4 type photosynthetic CO_2 fixation is operative, the high temperature optimum of phosphoenol pyruvate carboxylase has been implicated to be the main biochemical lesion (Treharne and Cooper, 1969). Supporting evidence comes also from Taylor *et al.* (1972a) who observed that chilling altered the amino acid content of sensitive cereals, causing a sharp decrease in those amino acids closely related to the C_4 pathway. Recently Law (1975) has reported that, in corn seedlings, both the C_3 and the C_4 types of CO_2 fixation are operative with the primary leaves showing the C_3 type and the secondary leaves the C_4 pattern.

The low rate of photosynthesis of chilling sensitive plants at low temperatures can also be attributed to feed-back inhibition of end products. Humphries (1963) and Hillard and West (1970) have suggested that temperature changes, by varying the ability of the plant to utilize assimilates, may partly govern the photosynthetic rate of the plant. The evidence of such a mechanism was reviewed by Neales and Incoll (1968) who concluded that while the hypothesis has a sound physiological basis, its biochemical validation suffers from lack of evidence.

Some workers (Humphries, 1951; Jacobson *et al.*, 1957) have indicated the possibility that the decrease in photosynthetic rate at low temperature

may be partly due to a decreased rate of mineral uptake by, and export from, the roots. Decreased rate of mineral uptake may lower photosynthetic rate either directly (Tsuno and Shimizo, 1962) or indirectly by reducing the rate of carbohydrate flow from the leaves (Tsuno and Fugise, 1965).

Stomatal closure during chilling resulting from lowered water permeability of roots, and thus decreased water potential in the leaves, is another possible cause of reduced photosynthesis at low temperatures (Taylor and Rowley, 1971).

2.5 Recovery from Injuries Caused by Chilling

The reversibility of chilling injury in corn, especially in regard to genetic relationships, has not been investigated intensively.

In *Xanthium* spp., Drake and Salisbury (1972) showed that the stomata in pre-chilled leaves did not open as wide at any subsequent temperature between 5 and 45^{o}C as did stomata in unchilled plants. Such sluggish responses of stomata after chilling have also been reported by Tschape (1972). He observed that under conditions of water saturation, thermophyllic plants showed, as an after effect of chilling, delayed photoactive stomatal opening and reduced CO_2 uptake. In *Xanthium* the associated reduction in net photosynthesis was not due to decreased stomatal conductance because it was observed that the inter-cellular CO_2 concentration in pre-chilled leaves was equal to or greater than in the controls (Drake and Roschke, 1974).

In cotton seedlings, Stewart and Guinn (1969) could demonstrate that a chilling induced decrease in ATP level was reversible after a 24 hour

exposure to 5°C. However, the damage was permanent if the exposure was 48 hours or more. The experiment of Creencia and Bramlage (1971) also demonstrated a similar reversibility of injury in corn seedlings. They showed that, in 7 days old corn seedlings, visual symptoms of leaf injury developed within 36 hours at 0.3°C, but these symptoms disappeared and the leaves returned to normal within 3 days when the seedlings were transferred to 21°C.

Besides these short term experiments, results from long term experiments present interesting findings. The observation of Patseka (1974) showed that cooling the seeds of the double interline hybrid, VIR 25, and all its parental lines resulted in an increase in yield in the hybrid plant. The duration of the effect was two generations. Cooling the plant at the two leaf stage, however, resulted in a yield reduction of the order of 9 - 26% in the hybrid and 14 - 33% in the inbred lines. A contrasting report comes from the work of Gerasimov (1973) who observed in another corn hybrid, Dneprovski 247 MV, subjected at seedling stage to a temperature of 0 - 4°C for 2-6 days produced normal plants with higher chlorophyll content and a yield equal to that, and in some cases better than that, of plants not so treated.

Albergt and Howard (1974) reported that in Florida, U.S.A., strawberry plants chilled for 30 days consistently produced early yields of fruits and the highest April harvest with more vegetative growth and stolon production than the controls.

In all the above works it appears that little is known of the mechanism of recovery or how complete the recovery is.

3. MATERIALS AND METHODS

3.1 Genotypes

Four inbred lines of corn 212-74, K-27, VCO264 and F7-F2 (the first two originated in Manitoba and the last two in France) were evaluated under growth-room conditions. These entries represented a range in response from the lowest to the highest in seedling growth at cool temperatures (12°C) among 32 entries obtained from the University of Manitoba Corn Breeding Program and observed in a series of preliminary tests. These 32 entries (inbred lines) were chosen primarily on the basis of their countries of origin, and their reaction to cool temperature stress under field conditions. Availability of sufficient seeds for the purpose of this experiment was a limiting factor in the choice, but not a serious one. All of the seeds used were produced, harvested and stored under similar conditions.

3.2 Cultural Practices

Seeds were soaked in distilled water overnight and germinated by placing germination boxes containing the seed in a germination chamber set at 22°C. They were allowed to germinate under these conditions until both the radicle and the plumule had emerged. Single germinating seeds were then transplanted to a 1:1:1 sand:soil:peatmoss mixture contained in plastic pots. Plants harvested on the 8th day were grown in 6 inch pots while all other plants were grown in 8 inch pots. The

germinating seeds were planted about 3 cm deep in the soil mixture and the pots were transferred to growth rooms set at 28/22°C (day/night) temperature, 12 hours photoperiod, uncontrolled humidity and 2400 $\mu W/cm^2$ light intensity. Water was applied every second day. Low temperature treatment (12/10°C) was applied commencing on the 8th day through to the 16th day after which the temperature was raised back to 28/22°C for an additional 8-day period. Harvesting was every 8th day starting from emergence. Plants receiving 8 days of favourable and 8 days of chilling temperature and harvested on the 16th day are, henceforth, referred to as "chilled". Those plants which received an alternating temperature treatment of favourable-chilling-favourable in successive 8-day periods and harvested on the 24th day are referred to as "recovering".

3.3 Experimental Design

Only one growth room was available for use in the present experiment. Owing to this, it was not possible to do all the planting at the same time. It was therefore necessary to systematize the planting in such a way that the chilling treatment could be applied after all plants receiving favourable temperature treatment were harvested. In order to do this, plants which received the chilling treatment had to be planted 16 days after the control plants were planted. As a result, the only way the experiment could be laid out was in a completely randomized design. The experiment was repeated two times. In order to minimize the effects of environmental factors on the results of the experiment, it was necessary to eliminate all known sources of environmental variation to the extent possible, and therefore, the following precautions

were taken: All pots necessary for the experiment were filled on the same day and pots not used immediately in the experiment were stored until they were used; all plantings were done during the first half of the photoperiod; a systematic watering frequency of every second day was followed; temperature was always changed at the end of the photoperiod; sampling and harvesting for dry matter determination were done in the second half of the photoperiod and all inbred lines were sampled and treated in the same way. No insecticides were used and there was no power failure during the course of the experiment.

3.4 Laboratory Procedures

3.4.1 Sampling Procedures and Sample Sizes

Samples for the determination of chemical constituents were taken from the top three leaves and the roots. After harvest, the mid-ribs were removed from the leaf samples and the soil was thoroughly washed from the roots in tap water. The samples were immediately frozen to $-20^{o}C$ and stored at that temperature until they were freeze-dried and ground to pass 40 mesh. The freeze-dried ground samples were stored at $4^{o}C$ until they were used for chemical determination. For the assay of nitrate reductase activity and rate of protein synthesis determination, as measured by C^{14}-L-leucine incorporation, fresh tissues were used. Sample sizes varied according to seedling age, temperature treatment and the parameter investigated. For determination of nitrate reductase activity and nitrate content, 5 plants per replication were used. Leaf discs for determination of rate of protein synthesis were collected from 4 plants. Six plants were used for measurements of photosynthesis and

respiration. Rate of dry matter accumulation was determined using 15 plants per treatment combination. For the remaining parameters, a total of 10 - 30 plants per treatment combination were grown adjacent to and at the same time with the plants grown for dry matter content determination.

3.4.2 Dry Matter Yield and Growth Rate

Plants for dry weight determination were cut at soil level and oven-dried to constant weight. A total of 15 plants per treatment combination was sampled. Harvesting was done on the 8th, 16th and 24th day after emergence. Dry matter yield was expressed as grams of dry matter produced per plant. The absolute rate of growth was determined using the formula:

$$\frac{W_2 - W_1}{8}$$

where W_2 is the dry weight on the 24th or the 16th day; and

W_1 is the dry weight on the 16th or 8th day; and it was expressed as grams dry matter per day.

The relative growth rate per day was calculated using the formula given by Milthorpe and Moorby (1974) and was expressed as grams dry matter produced per day per gram dry weight.

3.4.3 Photosynthesis and Respiration

When measuring photosynthetic and respiration rates, independent measurements were taken in the lower three leaves and the remaining top leaves. Photosynthesis and respiration rates of plants grown at 28/22^{o}C were measured at 28^{o}C and of those plants grown at 12/10^{o}C were measured at 12^{o}C. There was an age difference of 4 days between plants measured at 28^{o}C and plants measured at 12^{o}C, but the plants were of the same size. Six plants of each inbred line were measured at each temperature. Before

measurement, leaves of each plant were detached and immediately floated on tap water for about 15 minutes before they were inserted in the leaf chamber. The assay gas (400 ppm of CO_2 in air) was passed through the system at a rate of about 720 ml/min. The leaves in the chamber were allowed to equilibrate for about 2 minutes after insertion and photosynthetic rate was first determined. On completion of photosynthetic rate measurement the chamber was covered with a black polyethylene bag to exclude light, and dark respiration was measured for the same sample. The semi-open type IRGA system was used to measure photosynthesis, and the light intensity at the time of measurement was 2400 $\mu W/cm^2$. The dry matter content of the leaf sample was determined by oven-drying. By knowing the sample dry weight, CO_2 concentration present at the inlet duct, CO_2 concentration at the outlet duct for each run, the amount of CO_2 (in mg) fixed or given off per gram dry weight per hour was calculated from the following formula:

$$E = \frac{\Delta CO_2 \times F \times k}{\text{gram dry weight}}$$

where $E = CO_2$ exchange in mg CO_2/gram dry weight/hour;

$\Delta CO_2 = CO_2$ differential in ppm between intake and exhaust streams;

F = flow rate in liters per hour;

k = approximate conversion factor from liters CO_2 to mg CO_2 (44,000 mg CO_2/mole; 22.4 liters/mole).

3.4.4 Chlorophyll Content

Chlorophyll content was determined using the method of MacKinney (Vishniac, 1957).

3.4.5 Rate of Protein Synthesis

Leaf samples were taken from the 2nd and 3rd leaves from the top of four plants per treatment combination. Leaves were washed in running tap water for 20 minutes, blotted dry and leaf discs were punched out from the fully expanded terminal portion of leaves using a #3 cork borer. Duplicate sets of random samples of 10 leaf discs each were prepared. Each sample was then arranged on filter paper placed in petri-dishes and 3 ml of reaction mixture containing 0.025M citrate phosphate buffer pH 6.5, 25 µg/ml of streptomycin sulfate, 5 ppm kinetin and 13 mM C^{14}-L-leucine was added and the sample incubated for 2 hr at the temperature in which the plants themselves were grown. At the end of the incubation period, the samples were washed for 1 hour in running tap water to remove the unincorporated C^{14}-leucine from the free-space. The leaf discs were blotted dry and placed in 1.6 ml of 80% ethanol and were ground in a homogenizer, centrifuged at 15,000xg for 20 min and the ethanol supernatant saved for counting. The ethanol insoluble residue was washed with two changes of ethanol, once with two ml of TCA and once more with 80% ethanol, each time centrifuged at 2000xg for 10-15 minutes. The protein was solubilized with 1 ml of 0.3N KOH, stoppered with rubber stoppers, incubated at 37°C for 18 hr, and the supernatant saved for counting. One hundred µl aliquots of the ethanol and KOH fractions were counted in 10 ml of aquasol cocktail. The radioactivity of the ethanol and KOH fractions was summed to obtain total uptake. Rate of protein synthesis was expressed as the ratio of:

$$\frac{\text{Radioactivity of KOH fraction (dpm)}}{\text{Total radioactivity (dpm)}}$$

3.4.6 Reducing Sugar Content

Reducing sugars were extracted by stirring 25 mg of freeze-dried ground sample for 15 min in 10 ml of hot (60°C) 80% ethanol in centrifuge tubes. After standing for 5 min at room temperature, the extract was centrifuged for 20 min at 37,000xg. The residue was similarly re-extracted three more times and the supernatant fluids combined. The ethanol was slowly evaporated in a water bath at 70°C by bubbling nitrogen gas through the solution. The residue was re-suspended in 10 ml of distilled water and the concentration of sugars was determined by dinitrosalicylic method (Fisher and Stein, 1961). D-(+) maltose monohydrate (Baker grade, Baker Chemical Company Lot No. 1-5902) was used as the standard and the results expressed as sugar/gram freeze-dry weight.

3.4.7 Starch Content

The residue from the sample used for reducing sugar determination was resuspended in 4 ml of distilled water and the starch gelatinized by placing the centrifuge tubes in vigorously boiling water for 2 min. The samples were then cooled rapidly at 30°C, 5 ml of sodium phosphate buffer (pH 6.9) was added, and they were placed in a 30°C water-bath. 1.0 ml (15 mg) of α-amylase [(diastase) Bacterial crude type III-A from *B. subtillis*, free of foreign extenders, Sigma Chemical Company Lot 80C-1170] was added and the incubation carried on for 2 hours after which the reducing sugars produced were determined by the method of Fisher and Stein (1961). Linter starch (Fisher Laboratory Chemicals, Lot No. 742804) was used as a standard and the result was expressed as mg starch/gram freeze-dry weight.

3.4.8 Total Protein

Total protein was determined by the standard Kjeldahl method and was expressed as percent protein on oven dry weight basis.

3.4.9 Water-Soluble Proteins

Water-soluble proteins were extracted following the procedure of Perez *et al.* (1973) using 10 ml of double-distilled water. The amount of protein in the extract was determined by the method of Lowry *et al.* (1951). Water-soluble protein was expressed as mg/gram freeze-dry weight.

3.4.10 Free Amino Acids

Free amino acids were extracted by stirring 0.2 gram of freeze-dried ground sample in 5 ml of distilled water at 0-4°C for 30 min. The homogenate was treated as described by Cruz *et al.* (1970) and analyzed for amino acid by using the ninhydrin method described by Moore (1968). L-leucine was used as the standard and the results expressed as mg amino acids per gram freeze-dry weight.

3.4.11 Nitrate Reductase Activity

The method of nitrate reductase activity determination was essentially the same as described by Hageman and Hucklesby (1971), except for the following modifications: casein (3% w/v) was added to stabilize the enzyme (Schrader *et al.*, 1974); 0.2 ml of 1M zinc acetate was added to remove the excess NADH which would otherwise interfere with the color development (Scholl *et al.*, 1974); and before homogenization the samples were frozen to -20°C for 3 min (the shearing action of the ice crystals so formed and the partial cell disruption due to membrane breakage facilitates better extraction of the enzyme). Nitrate reductase activity was expressed as μM KNO_2 produced per gram fresh weight per hour.

3.4.12 Nitrate Content

A portion of the leaf samples used for nitrate reductase activity determination was freeze-dried, ground to 40 mesh and used for tissue

nitrate content determination. A sample of 0.1 gram of freeze-dried powder was stirred vigorously for 10 min in 10 ml of deionized distilled water. The resulting solution was treated with charcoal to remove the green color and filtered through Whatman #4 filter paper before the nitrate content of the solution was determined by the method of Kamphake *et al.* (1967). Appropriate dilution was necessary to bring the concentration of the nitrate within a working range. Nitrate content was expressed as µg/ 0.1 gram dry weight (1 gram fresh weight equivalent).

3.4.13 Protease Activity

Extraction of protease was carried following the procedure of Perez *et al.* (1973), except that 10 ml of the extraction mixture was used. Protease activity was determined by the method of Cruz *et al.* (1970) except that hemoglobin (freeze-dried salt free) was used as the substrate. Protease activity was determined in the sample used for nitrate determination and was expressed as $\Delta O.D._{280}$/hr/gram freeze-dry weight.

3.5 Statistical Analysis of the Data

One way analysis of variance (ANOVA) was carried for each parameter in two ways: (1) the ANOVA pooled over all inbred lines was used for comparing any two adjacent treatment means, (2) separate ANOVA for each of the four inbred lines was used for comparing treatment effects within inbred lines. For the ANOVA of dry matter content each plant was used as a replicate.

A 4 x 20 (4 classes and 20 treatment combinations, i.e., 4 inbred lines x 5 treatment per inbred line = 20 treatment combinations) contingency Chi-square test of independence on frequency distribution of the

dry matter content data was executed to determine if the dry matter content data collected had uniform distribution from treatment to treatment.

Simple correlation analyses were made between growth rate (both absolute and relative) and the levels of the other properties (metabolites and metabolic processes) in three different ways: (1) the pre-stress levels of plant properties versus growth rate during chilling stress; (2) the levels of plant properties expressed during chilling stress versus growth rate during chilling stress, and (3) the levels of plant properties expressed during chilling stress versus growth rate during recovery.

4. RESULTS

4.1 Visual Observations

The growth of the corn plants in the controlled environment room was fairly satisfactory. During the 24 days of growth at favourable temperature, the plants attained a rate of growth almost comparable to that expected in their natural environment. On the 24th day of growth at favourable (28/22^{o}C) temperature, inbred lines 212-74 and VCO264 were described as vigorous and larger leaved with well developed root systems; while F7-F2 and K-27 were less vigorous, narrow leaved and had smaller root systems.

Upon exposure to chilling stress, red pigmentation, presumably anthocyanins, developed in the leaves of the chilling tolerant inbred lines, VCO264 and F7-F2. The amount of anthocyanin appeared to be higher in VCO264 than in F7-F2. Within the plant profile, the lower leaves seemed to produce more anthocyanin than the upper leaves. Production of anthocyanin was also observed in some plants of 212-74, but such individuals formed only a minor segment of the population. K-27 did not produce any visible level of anthocyanin, but showed yellowing of leaves that were fully exposed to light. Most leaves of this inbred line showed drooping orientation.

4.2 Experimental Observations

The effect of two temperature regimes, a constant favourable (28/22°C day/night temperature) versus an alternating (8 days 28/22°C + 8 days 12/10°C + 8 days 28/22°C) temperature on 18 parameters was evaluated. The mean square ratios (F-values) as influenced by inbred line, seedling age and temperature treatments (confounded as treatment effect) are presented in Appendix Table 1. For all parameters, the treatment effects were highly significant. When the total sum of squares was sub-divided among the four inbred lines, the treatment mean square ratios were again highly significant for all parameters and in all inbred lines.

In the presentation of more detailed results which follow, the various parameters are grouped into six classes, viz., (1) changes in dry matter content and growth rates during chilling stress and during recovery from chilling stress, (2) changes in the rates of photosynthesis respiration, and chlorophyll content due to chilling stress, (3) changes in soluble carbohydrate metabolism during chilling stress and during recovery from chilling stress, (4) changes in the concentrations of total protein, water-soluble protein and free amino acids during chilling stress and during recovery from chilling stress, (5) changes in nitrate reductase activity, nitrate content, protease activity and rate of protein synthesis during chilling stress and during recovery from chilling stress, and (6) association between the levels of plant properties with chilling tolerance and recovery from chilling stress. The results of each of these classes will be presented separately.

4.2.1 Change in Dry Matter Content and Growth Rates During Chilling Stress and During Recovery from Chilling Stress

Changes with time in the rates of dry matter accumulation in the stover of four corn inbred lines of known reaction to chilling temperature were followed at a constant favourable (28/22°C) and at alternating (8 days 28/22°C + 8 days 12/10°C + 8 days 28/22°C) temperature conditions. Fifteen plants of each inbred line were harvested at 8-day intervals to determine the dry matter weights.

Before any further statistical analysis and interpretation of the data were undertaken, a Chi-square test of independence on the raw data of dry matter yield was executed. The objective was to determine if the dry matter data obtained were random samples with normal distribution, and if the frequency of distribution was uniform from treatment to treatment. To do this, the observations of each treatment combination were classified into four frequency classes using one standard deviation as a class interval. A 4 x 20 contingency table (4 classes and 20 treatment combinations, i.e., 4 inbred lines x 5 treatments per inbred line = 20 treatment combinations) was constructed and a test of independence was executed. The Chi-square value obtained, 10.27 with 57 degrees of freedom (Appendix Table 2), was not significant, indicating that the distribution of dry matter yield within a treatment had the same form regardless of genotype, seedling age and temperature treatment received.

The dry matter weights of the four inbred lines at different stages of growth and at different temperature conditions is presented in Table 1. From the data in Table 1, the absolute and relative rates of growth per day during chilling stress and during recovery from chilling stress were

TABLE 1. Dry matter weights (in grams) of four corn inbred lines grown at favourable (28/22°C) and alternating (8 days 28/22 + 8 days 12/10 + 8 days 28/22) temperature conditions and harvested at 8-day intervals.

Inbred line	8 days	16 days			24 days			LSD[1]	
	Control	Chilled		Control	Recovering		Control	5%	1%
212-74	0.13	0.28	(11.2)[2]	2.51	4.58	(42.2)	10.85	0.30	0.36
K-27	0.12	0.32	(15.2)	2.10	2.60	(36.7)	7.09	0.21	0.26
VCO264	0.12	0.44	(20.0)	2.20	6.15	(62.0)	9.92	0.21	0.26
F7-F2	0.11	0.34	(19.4)	1.75	4.50	(62.2)	7.24	0.21	0.26
MEAN	0.12	0.34		2.14	4.46		8.78		

CV = 11.38%.

LSD for comparing treatment means between two inbred lines:

5% = 0.23 grams
1% = 0.29 grams

[1] LSD for comparing two treatment means within an inbred line.

[2] Figures in parenthesis are dry matter contents of chilled plants expressed as percentage of dry matter content of control plants of comparable age.

calculated. These growth rate values are presented in Table 2.

Exposure of eight-day old corn seedlings to chilling temperature resulted in a considerable reduction in both absolute and relative growth rates. The greatest reduction in growth rate occurred in 212-74 and the least in VCO264 (Table 2).

On the 16th day, the dry matter weight of chilled plants of all inbred lines was significantly less than that of the respective controls. On this day, the dry matter weight of chilled plants expressed as percentage of the dry matter weight of unchilled controls in the four inbred lines ranged from 11.2 - 20.0% (Table 1). Comparisons were also made between 8-day old unchilled seedlings and 16-day old chilled plants of each inbred line using the appropriate least significant difference (LSD) values for comparing treatment effects within inbred lines. These comparisons indicated that inbred lines VCO264 and F7-F2 had accumulated significant quantities of dry matter during the 8 days of growth at chilling temperatures, whereas in the inbred lines 212-74 and K-27, the amount of dry matter accumulated during this period was not significant. From these LSD comparisons, from the growth rate data presented in Table 2, and also from the dry matter ratios of chilled/unchilled plants on the 16th day (Table 1), the reactions of the inbred lines to chilling stress were apparent. Inbred line 212-74 and K-27 were designated as chilling sensitive and VCO264 and F7-F2 were described as chilling tolerant. The terms "tolerant" and "sensitive", however, are used in a rather restricted sense; the term "tolerant" denoting the ability of an inbred line to gain a statistically significant quantity of dry matter within eight days of growth at chilling temperature.

TABLE 2. Absolute growth rate (grams dry matter produced per day) and relative growth rate (gram dry matter produced per day per gram dry matter) of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred line	GROWTH RATES							
	During chilling				During recovery			
	Absolute		Relative		Absolute		Relative	
	Chilled	Control	Chilled	Control	Chilled	Control	Chilled	Control
212-74	0.019 (6.4%)[1]	0.298	0.096	0.370	0.538 (51.6%)	1.042	0.349	0.183
K-27	0.025 (10.0%)	0.248	0.123	0.358	0.285 (45.7%)	0.624	0.262	0.152
VCO264	0.040 (15.4%)	0.260	0.162	0.364	0.714 (84.0%)	0.965	0.330	0.189
F7-F2	0.029 (11.6%)	0.250	0.141	0.346	0.520 (75.8%)	0.686	0.323	0.178
MEAN	0.028	0.253	0.130	0.360	0.514	0.829	0.316	0.176

[1] Figures in parenthesis are absolute growth rates of chilled plants expressed as percentage of control plants of comparable age.

When the chilled plants were returned back to favourable temperature a relatively rapid growth rate was observed in all inbred lines (Table 2). As a result of this rapid growth rate, the chilled plants tended to catch-up with the control plants that had not been subjected to chilling. As is shown in Table 1, the gap between chilled plants and their controls narrowed from 11-20% (dry matter content of chilled plants expressed as percentage of dry matter content of the controls) at the termination of the chilling stress (i.e., on the 16th day) to 36.7 - 62.2% within eight days of exposure to favourable temperature (i.e., on the 24th day). The data in Table 2 also show that inbred lines varied widely in the extent of their recovery from chilling stress. Relative growth rate during recovery was most rapid in 212-74 (0.349 grams dry matter/day/gram dry matter weight) and least rapid in K-27 (0.262 grams dry matter/day/gram dry matter weight).

As a result of the variability in the rate of recovery, differences in the ranking of the inbred lines at the two different stages of comparison, i.e., on the 16th day and on the 24th day, were observed. On the basis of the sensitivity of dry matter increase to chilling stress measured on the 16th day, the four inbred lines could be placed on a tentative tolerance sequence: VCO264 > F-7-F2 > K-27 > 212-74. On the 24th day, that is, after the chilled plants had received 8 days of favourable temperature, this sequence was changed to: F7-F2 $\geq$ VCO262 > 212-74 > K-27. These changes in the ranking of the inbred lines at the two different stages of comparison suggested that the ability of an inbred line to grow at chilling temperature may be controlled independently of the ability to recover rapidly from chilling stress. This fact was further substantiated by correlation analyses. In the control

plants that were grown at favourable temperature only, the growth rate between days 16 and 24 was significantly correlated with the growth rate between days 8 and 16 ($r = +.751$; $P > 0.01$). A similar correlation analysis run on the chilled plants, on the other hand, suggested that chilling tolerance was not significantly correlated with the speed of recovery ($r = +.493$).

Once the independence of the rate of recovery from chilling stress was established, it was of practical interest to know which one of the two traits was more important in determining the final dry matter content expressed on the 24th day. A stepwise multiple regression analysis of the form:

$$Y = f(x_1, x_2, x_3)$$

where Y = the dry matter content on the 24th day;

x_1 = dry matter content of 8 days old seedlings;

x_2 = relative growth rate between days 8 and 16; and

x_3 = relative growth between days 16 and 24; was run.

This analysis was applied both to plants subjected to chilling stress and to the control series. The stepwise multiple regression analysis (Table 3) indicated strongly the importance of rapid growth rate between days 16 and 24 in determining the final dry matter yield on the 24th day in both the chilled plants and in the unchilled controls. It alone accounted for 54.7% and 48.2% of the variability in the dry matter content in the chilled plants and in the control plants, respectively. Growth rate between days 8 and 16 resulted in a reduction of the residual sum of squares by a further 34.5% and 30.3% in chilled plants and control plants, respectively. If seedling chilling tolerance was more important

TABLE 3. Stepwise multiple regression analysis of mean dry matter yield of chilled and unchilled corn plants on relative growth rates at different stages in ontogeny.

Variable added to equation	Total reduction in residual sum of squares (%)	Regression coefficients b_1	b_2	b_3	Multiple correlation coefficient (R)
		Chilled plants			
RGR[1] days 16-24	54.7	0.333			0.739**
RGR days 8-16	89.2	0.330	0.378		0.935**
Seedling weight on the 8th day	98.9	0.375	0.443	31.7	0.992**
		Control plants			
RGR days 16-24	48.2	0.988			0.694**
RGR days 8-16	78.5	1.082	0.893		0.802**
Seedling weight on the 8th day	99.8	0.818	0.905	74.09	0.999**

N = 16.

[1]RGR = Relative growth rate.

** Significantly different from the previous R at the 1% level.

than the speed of recovery, obviously, a higher reduction in the residual sum of squares would have been obtained for relative growth rate between days 8 and 16 (i.e., during chilling stress) than between days 16 and 24 (i.e., during recovery).

Thus, limited findings of the present experiment established that: (1) corn genotypes vary, both in seedling chilling tolerance, and in their ability to recover quickly from chilling stress, (2) the ability to recover rapidly is not correlated with seedling chilling tolerance *per se*, and (3) when corn plants were subjected to an 8-day chilling stress, the rate of recovery was more important than seedling chilling tolerance *per se*.

4.2.2 Changes in the Rate of Photosynthesis, Respiration and Chlorophyll Content Associated with Chilling Stress

4.2.2.1. Effects of chilling temperature on photosynthesis. It was considered logical to determine the extent to which changes in the rates of dry matter accumulation resulting from exposure to low temperature were a reflection of changes in the rate of photosynthesis, and to assess genotypic differences in photosynthesis in relation to chilling.

The rate of photosynthesis in the "top leaves" and the "lower leaves" of the four corn inbred lines as measured at 28°C and 12°C is presented in Table 4. Highly significant genotypic differences in the rates of photosynthesis, both in the top and lower leaves were noted when measurements were taken at 28°C. At this temperature, the chilling tolerant inbred lines, VCO264 and F7-F2, exhibited significantly higher photosynthetic rates than the chilling sensitive inbred lines. Measurements at 12°C showed no significant differences in photosynthetic rates among

TABLE 4. Photosynthesis and respiration rates (mg CO_2 per gram dwt per hour) in the "top leaves" and "lower leaves" of four corn inbred lines as affected by temperature.

Inbred lines	Lower leaves		Top leaves	
	12°C	28°C	12°C	28°C
	Photosynthesis (mg CO_2/gm dwt/hr)[1]			
212-74	36.1	64.8	24.7	65.8
K-27	38.5	73.8	29.3	67.1
VCO264	38.1	99.3	20.6	82.2
F7-F2	39.5	100.1	21.0	91.0
MEAN	38.0	84.5	23.9	76.5
	Respiration (mg CO_2/gm dwt/hr)			
212-74	3.8	6.5	3.6	7.3
K-27	4.1	7.4	4.4	13.0
VCO264	3.2	10.6	2.3	10.7
F7-F2	4.0	8.2	3.4	14.2
MEAN	3.8	8.2	3.4	11.3

CV (photosynthesis) 13.0%; CV (respiration) 18.7%.

LSD:

	5%	1%
Between two photosynthetic rate values	8.3	11.0
Between two respiratory rate values	1.4	1.9

[1] mg CO_2/mg dwt/hr = milligram carbon dioxide per gram leaf dry weight per hour.

the four corn inbred lines. Averaging the effects over the four inbred lines and two leaf positions, chilling resulted in a highly significant reduction in the rate of photosynthesis. The magnitude of reduction varied from genotype to genotype, mainly depending on the photosynthetic efficiency of the inbred lines at 28°C, but was of the order of about 62%.

Comparisons within the plant profile showed the lower leaves to have higher photosynthetic rates as compared to the top leaves at both 28°C and 12°C. The lower leaves exceeded the top leaves by about 10% when measurements were taken at 28°C and 37% when measured at 12°C. These relationships also indicated that photosynthesis in the top leaves was more sensitive to chilling (reduced by an average of 69%) than is photosynthesis in the lower leaves (reduced by an average of 55%).

4.2.2.2. Effects of chilling temperature on respiration. The rate of respiration of the four inbred lines, as affected by temperature and leaf positions, is presented in Table 4. Like photosynthesis, respiration was significantly reduced by chilling temperature. When inbred line and leaf position effects were disregarded, the rate of respiration at chilling temperature was reduced by an average of about 63%. Unlike photosynthesis, the rate of respiration at 28°C was higher in the top leaves than in the lower leaves. The rate of respiration in the lower leaves at this temperature was about 72% that of the top leaves. At 12°C, this trend was reversed and the lower leaves exhibited a 9% higher respiration rate than the top leaves. Consequently, the degree of reduction in the rate of respiration at chilling temperatures appeared to be much higher in the top leaves (reduced by 70%) than in the lower leaves (reduced by 54%). The results of both photosynthesis and respiration experiments indicated that the top leaves were more sensitive

to chilling stress than the lower leaves.

4.2.2.3. Effect of chilling temperature on total chlorophyll content. Unlike the measurements of photosynthesis and respiration, chlorophyll content was determined in the top leaves only. Also, sampling for chlorophyll content was carried out at eight-day intervals and concomitantly with the sampling for dry matter content.

Exposure of eight-day old seedlings to low temperatures for eight subsequent days resulted in about 40% reduction in chlorophyll content (Table 5). This reduction in chlorophyll content was highly significant in all inbred lines.

When chilled 16-day old plants were returned to 28°C and allowed to grow at this temperature for eight days, a sharp increase in chlorophyll content in the top three leaves was observed. This increase in total chlorophyll content, about 22% over and above that of the appropriate 24-day old unchilled controls, was significant in all inbred lines. It was assumed to have resulted from improved internal plant nutrition, generated by remobilization of metabolites from sites of storage to the actively growing top leaves.

The total chlorophyll content was partitioned into its major components, chlorophyll$_a$ and chlorophyll$_b$, in an attempt to identify which of these two very important chlorophyll types was most severely affected by chilling temperature. Since all the energy trapped by chlorophyll$_b$ must be passed through chlorophyll$_a$, a disproportionate amount of the two at chilling temperatures may also affect photosynthesis. A knowledge of the relative changes in these components was therefore desirable. The chlorophyll$_a$ to chlorophyll$_b$ ratio (Table 5) did not show significant

TABLE 5. Changes in total chlorophyll content (mg/gram freeze-dry wt) and chlorophyll$_a$/chlorophyll$_b$ ratio of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred lines	8 days	16 days		24 days	
	Controls	Chilled	Control	Recovering	Control
		Total chlorophyll content			
212-74	1.32	0.58	1.09	0.88	0.75
K-27	1.13	0.72	1.20	1.03	0.83
VCO264	1.11	0.82	1.11	0.95	0.86
F7-F2	1.12	0.57	1.01	0.92	0.67
MEAN	1.17	0.67	1.10	0.94	0.78
		Chlorophyll$_a$/chlorophyll$_b$ ratio			
212-74	1.79	1.63	1.62	1.89	1.56
K-27	1.35	1.54	1.53	1.54	1.66
VCO264	1.63	1.56	1.78	1.57	1.61
F7-F2	1.63	1.62	1.64	1.57	1.65
MEAN	1.69	1.59	1.64	1.64	1.62

CV (Total chlorophyll content) 3.0%; CV (Chlorophyll$_a$/chlorophyll$_b$) 13.5%.

LSD:	5%	1%
Between two total chlorophyll content values	0.04	0.06
Between two chlorophyll$_a$/chlorophyll$_b$ ratios	0.37	0.50

changes as the inbred lines were subjected to different temperatures. Therefore, it was concluded that the increase or decrease in total chlorophyll content was not due to any type of chlorophyll being preferentially affected, but due to a general increase or decrease of both types of chlorophyll molecules simultaneously.

4.2.3 Changes in Soluble Carbohydrate Metabolism During Chilling Stress and During Recovery from Chilling Stress

The growth and development of plant cells depend on soluble carbohydrates for energy production and as a building block for storage and structural compounds. Besides these basic functions, soluble carbohydrates, sugars in particular, have been reported to provide protection against freezing (Levitt, 1966) and chilling (Christiansen *et al.*, 1970) injuries. Such relationships of sugars with chilling tolerance in corn, however, have yet to be established. A knowledge of soluble carbohydrate metabolism as affected by changes in temperature, and especially, the relationships of soluble carbohydrates and chilling tolerance in corn was therefore deemed necessary in order to better understand the effects of chilling and favourable post-chilling temperatures on the growth and development of corn. The following observations were noted.

4.2.3.1. Changes in sugar content. When eight-day old seedlings of the four inbred lines were subjected to chilling temperature, highly significant accumulations of sugars in the leaves, as well as in the roots, were recorded (Table 6). The amount of sugar accumulated in the leaves during chilling stress was on the average about 2.8 times greater than the amount contained in 16-day old unchilled controls. When compared to the pre-stress level (i.e., on the eighth day after emergence)

TABLE 6. Changes in sugar content (mg/gram freeze-dry weight) in the leaves and roots of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred lines	8 days	16 days		24 days	
	Control	Chilled	Control	Recovering	Control
		In the leaves			
212-47	97.2	151.3	65.5	56.2	72.4
K-27	109.8	209.0	69.2	88.2	56.8
VCO264	125.4	207.8	93.4	107.5	68.9
F7-F2	101.2	268.9	73.8	75.6	62.6
MEAN	108.4	209.2	75.5	81.9	65.2
		In the roots			
212-74	-	93.7	64.0	87.9	80.7
K-27	57.4	243.5	52.5	141.8	106.1
VCO264	114.5	147.3	56.2	79.9	71.2
F7-F2	72.7	156.0	56.0	121.4	51.1
MEAN	81.5	160.1	57.2	107.8	77.3

CV (leaf sugar content) 11.4%; CV (root sugar content) 10.6%.

LSD:	5%	1%
Between two leaf sugar content values	22.8	30.2
Between two root sugar content values	15.0	19.9

the amount of sugar in the 16-day old chilled plants was found to be increased by two-fold. Incidentally, the extent of increment of root sugar content at chilling temperatures was proportionally very similar to that observed in the leaves.

Returning corn seedlings to favourable temperature after exposing them to an 8-day chilling stress resulted in appreciable declines in the concentration of sugars from the level observed at chilling temperature. In spite of this significant decline in sugar content, the recovering 24-day old plants continued to maintain a more positive carbon balance in the leaves, as well as in the roots, compared to the 24-day old controls.

4.2.3.2. Changes in starch content. The trends of change in starch content appeared to parallel changes in sugar levels. The results of the present experiment indicated that, concomitant with the increase in sugar content, chilling also resulted in a significant increase in starch content in the leaves and roots of all inbred lines (Table 7). The amount of starch that accumulated during chilling stress varied depending upon the genotype and plant part. However, averaging over the four inbred lines, it was observed that the level of starch in the chilled plants exceeded that of the 16-day old controls by about 204% in the leaves and 114% in the roots.

During recovery from chilling stress, depletion of starch in the leaves as well as in the roots was observed. The depletion appeared to be more severe in the leaves than in the roots. This was apparently due to the emergence of new leaves during recovery. However, on the 24th day, the recovering plants were still maintaining a more positive starch

TABLE 7. Changes in starch content (mg/gram freeze-dry weight) in the leaves and roots of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred lines	8 days	16 days		24 days	
	Control	Chilled	Control	Recovering	Control
		In the leaves			
212-74	172.7	252.1	127.2	141.3	122.5
K-27	156.3	250.6	118.7	144.5	124.4
VCO264	156.3	261.0	118.7	111.7	116.4
F7-F2	144.5	231.9	123.9	122.5	104.2
MEAN	157.4	248.9	122.1	130.0	116.9
		In the roots			
212-74	-	176.5	137.0	171.8	121.1
K-27	146.4	166.6	141.3	185.9	136.6
VCO264	153.9	169.9	148.3	186.3	138.0
F7-F2	151.1	188.7	184.4	184.4	159.6
MEAN	150.5	174.2	152.8	183.3	138.5

CV (leaf starch content) 10.8%; CV (root starch content) 9.3%.

LSD:	5%	1%
Between two leaf starch content values	23.9	31.7
Between two root starch content values	20.9	27.9

balance compared to their controls. On this day, the recovering plants exceeded their controls by about 11% in leaf starch content and by 30% in root starch content.

4.2.4 Changes in Total Protein, Water-Soluble Protein and Free Amino Acid Concentrations During Chilling Stress and During Recovery from Chilling Stress

Results from preliminary experiments indicated that inbred lines which were chilling tolerant were characterized by exhibiting nitrogen deficiency when they were subjected to cool temperatures, while their chilling sensitive counterparts did not show this symptom. An investigation on nitrogen metabolism was therefore initiated. This section deals with the effects of chilling and favourable post-chilling temperatures on the concentrations of total protein, water-soluble protein and free amino acids in leaf and root tissues. Changes in leaf nitrate reductase activity, nitrate content, protease activity and rate of protein synthesis during chilling and favourable post-chilling temperatures are dealt with in the next section.

4.2.4.1. Changes in total protein content. The concentration of protein in the leaves and roots, like most other metabolites, declined during ontogeny (Table 8). When grown at favourable temperatures, the four inbred lines showed a consistent trend in leaf total protein content with K-27 showing the highest and VCO264 showing the lowest level of leaf total protein content. This trend in total protein content, however, was distorted when the inbred lines were subjected to chilling temperature. Unlike the leaves, the roots showed no consistent trend in root total protein among the four inbred lines.

Exposure of 8-day old seedlings to chilling temperature for 8 days

TABLE 8. Changes in total protein content (on percent oven dry weight basis) in the leaves and roots of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred line	8 days	16 days		24 days	
	Control	Chilled	Control	Recovering	Control
		In the leaves			
212-74	32.13	24.87	28.20	25.17	22.83
K-27	36.07	25.00	32.53	26.23	27.13
VCO264	28.07	18.77	25.23	23.40	21.97
F7-F2	32.07	21.23	27.60	23.57	22.13
MEAN	32.08	22.47	28.36	24.59	23.52
		In the roots			
212-74	-	21.10	17.45	17.10	16.50
K-27	27.55	25.80	19.30	15.90	20.70
VCO264	23.10	19.50	18.90	18.20	20.60
F7-F2	24.70	25.40	21.05	16.60	18.80
MEAN	25.12	22.80	19.32	16.95	19.15

CV (leaf total protein content) 4.77%;
CV (root total protein content) 5.80%.

LSD:	5%	1%
Between two leaf total protein content values	2.06	2.76
Between two root total protein content values	1.94	2.85

resulted in a significant reduction in leaf total protein content. The extent of reduction in leaf total protein content during chilling stress, was on the average, about 21% as compared to 16-day old unchilled controls, and was matched by a corresponding increase in total protein content in the roots. Root total protein content of the chilled 16-day old plants exceeded that of the unchilled 16-day old controls by an average of 18%.

Returning 16-day old chilled plants to favourable temperature resulted in an increase (about 9.4%) in leaf total protein content within 8 days. During the same period, there was a concomitant reduction (about 25.6%) in root total protein content. However, the recovering 24-day old plants in general showed a more positive nitrogen balance in the leaves and a negative nitrogen balance in the roots as compared to the 24-day old unchilled controls (Table 8).

4.2.4.2. Change in water-soluble protein content. Chilling resulted in an increase in water-soluble protein content (Table 9). Averaging over the four inbred lines, the concentration of water-soluble protein in the leaves of chilled 16-day old plants exceeded that of the 16-day old controls by about 54.6%. Except in 212-74, the level of water-soluble protein in the roots of the chilled 16-day old plants of the remaining inbred lines was also significantly higher than the 16-day old unchilled controls. At chilling temperature, the small rooted inbred lines, K-27 and F7-F2, showed higher concentrations of water-soluble proteins in the roots than the larger rooted inbred lines.

When the chilled plants were returned to favourable temperature, the level of water-soluble protein in the leaves of the recovering plants

TABLE 9. Changes in water-soluble protein content (mg/gram freeze dry wt) in the leaves and roots of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred lines	8 days	16 days		24 days	
	Control	Chilled	Control	Recovering	Control
		In the leaves			
212-74	74.99	87.89	74.30	46.35	47.18
K-27	88.96	85.17	61.74	64.10	52.86
VCO264	85.41	93.78	55.34	60.32	52.39
F7-F2	83.28	85.76	50.73	49.90	47.42
MEAN	83.16	88.15	60.53	55.17	49.96
		In the roots			
212-74	-	73.65	73.96	76.75	64.04
K-27	100.02	94.75	62.18	101.26	60.93
VCO264	78.30	87.92	52.25	86.37	58.45
F7-F2	97.54	106.53	73.34	91.02	80.19
MEAN	91.95	90.71	65.43	88.85	66.05

CV (leaf water-soluble protein content) 4.82%;
CV (root water-soluble protein content) 7.50%.

LSD:	5%	1%
Between two leaf water-soluble protein content values	4.43	5.89
Between two root water-soluble protein content values	8.06	10.71

declined to a level lower than what it was at chilling temperature. Water-soluble proteins in the roots, on the other hand, remained at about the same level as during chilling stress. However, comparisons between recovering 24-day old plants and 24-day old controls showed that the recovering plants contained on the average about 10.4% and 35.4% more water-soluble protein in the leaves and roots, respectively.

4.2.4.3. Changes in content of free amino acids. Chilling treatment begun on 8-day old seedlings resulted in a highly significant accumulation of free amino acids in the leaves and roots of all inbred lines (Table 10). The degree of change in free amino acid content, induced by lowering the temperature from 28/22°C to 12/10°C, was calculated as percentage change from the value of 16-day old unchilled controls. Averaging over the four inbred lines, free amino acids were increased by 63.7% and 82.0% in the leaves and roots, respectively.

The composition of free amino acids in the leaves of the four inbred lines at chilling and favourable temperatures is given in Table 11. Taylor *et al.* (1972a) have reported that a 1.5 day chilling stress resulted in the reduction of free amino acids related to the C_4 pathway in chilling sensitive species. This finding was not supported by the observations of the present experiment, since an increase in the content of all free amino acids was recorded. The conflicting results of the present experiment with that of Taylor *et al.* (1972a) might have been due to differences in the length of the chilling stress as an 8-day chilling treatment was applied in the present investigation while Taylor *et al.* used a chilling stress of only 1.5 days. Also, proline did not appear to provide the type of protection against stress reported by Bates *et al.* (1973), Taylor *et al.*

TABLE 10. Changes in free amino acid content (mg/gram freeze-dry wt) in the leaves and roots of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred lines	8 days	16 days		24 days	
	Control	Chilled	Control	Recovering	Control
		In the leaves			
212-74	18.92	43.42	18.37	14.70	18.15
K-27	39.01	38.39	30.40	24.34	21.70
VCO264	13.56	21.58	13.83	15.98	13.41
F7-F2	18.83	24.67	15.61	14.66	14.33
MEAN	22.58	32.02	19.55	17.42	16.90
		In the roots			
212-74	-	31.77	19.47	11.90	23.69
K-27	36.92	36.55	24.43	18.56	25.16
VCO264	32.69	38.39	15.24	13.15	22.78
F7-F2	28.10	36.92	19.84	13.70	17.08
MEAN	32.57	35.91	19.74	14.33	22.18

CV (free amino acids in the roots) 3.35%;
CV (free amino acids in the leaves) 3.47%.

LSD:	5%	1%
Between two leaf free amino acid content values	0.89	1.27
Between two root free amino acid content values	1.35	1.97

TABLE 11. Effects of chilling temperature on free amino acid composition in the leaves of four corn inbred lines.

Temperature was lowered from 28/22°C to 12/10°C when seedlings were 8 days old. Samples were taken after the seedlings were exposed to the chilling temperature for 8 days. Data given are for 16-days old chilled plants and 16-days old controls.

	Amino acid concentration (mg/gm dry wt)							
	212-74		K-24		VCO264		F7-F2	
	CHLD[1]	CONT[2]	CHLD	CONT	CHLD	CONT	CHLD	CONT
Aspartic acid	0.66	0.39	0.66	0.70	0.61	0.46	0.46	0.61
Threonine	0.69	0.24	0.65	0.44	0.22	0.19	0.38	0.25
Serine	5.60	1.30	4.25	2.62	1.45	0.62	2.35	0.80
Glutamic acid	2.68	0.64	2.40	0.77	2.05	1.03	1.86	1.59
Proline	0.17	0.13	0.20	0.15	0.04	0.04	0.15	0.11
Glycine	7.50	0.56	4.85	1.56	2.34	0.47	1.91	0.30
Alanine	7.14	3.83	6.79	5.50	2.55	2.42	3.78	3.09
Valine	0.23	0.13	0.23	0.15	0.22	0.08	0.15	0.12
Isoleucine	0.09	0.06	0.13	0.05	0.09	0.03	0.08	0.02
Leucine	0.12	0.10	0.19	0.13	0.14	0.04	0.13	0.06
Tyrosine	0.24	0.10	0.29	0.20	0.22	0.09	0.26	0.12
Phenylalanine	0.05	0.03	0.08	0.07	0.07	0.02	0.10	0.04

[1] CHLD = Chilled 16 days old plants.

[2] CONT = Control 16 days old plants.

(1972a) and Barnett and Naylor (1966) as the extent of its increment was by far lower than the level reported by these workers.

During recovery from chilling stress, the leaves of the chilled 24-day old plants contained, on the average, about the same level of free amino acids as the 24-day old controls (Table 10). On the other hand, recovering plants of all inbred lines had significantly lower free amino acids in the roots compared to the 24-day old unchilled controls. On the average, roots of the recovering 24-day old plants had about 35.4% less free amino acids than the 24-day old controls.

4.2.5 Changes in Nitrate Reductase Activity, Nitrate Content, Protease Activity and Rate of Protein Synthesis During Chilling Stress and During Recovery from Chilling Stress

Table 12 contains data on changes in nitrate reductase activity, nitrate content and protease activity in the leaves of the four corn inbred lines analyzed as affected by temperature treatment.

When 8-day old seedlings were subjected to chilling temperature, the level of nitrate reductase activity declined significantly in all inbred lines. Averaging over the four inbred lines, the magnitude of reduction in nitrate reductase activity was about 30.5% and 35.5% as compared to 8-day old controls and 16-day old controls, respectively. Under chilling temperature, the tolerant lines exhibited significantly lower nitrate reductase activity than the susceptible lines.

In contrast to changes in nitrate reductase activity, the level of protease activity showed a steep increase when corn plants were exposed to chilling. The amplification of protease activity was significant in all inbred lines, but was much more intense in the chilling-tolerant

TABLE 12. Changes in nitrate reductase activity, nitrate content and protease activity in the leaves of four corn inbred lines during chilling stress and during recovery from chilling stress.

Inbred lines	8 days	16 days		24 days	
	Control	Chilled	Control	Recovering	Control
		Nitrate reductase activity ($\mu g\ KNO_2$/gram fresh wt/hr)			
212-74	7.48	4.84	6.71	4.70	2.69
K-27	5.32	5.08	6.10	3.43	1.95
VCO264	4.41	2.43	6.12	3.04	2.51
F7-F2	5.45	3.36	5.44	1.08	1.22
MEAN	5.66	3.93	6.09	3.06	2.09
		Nitrate content (µg/gram fresh wt)			
212-74	2470	1245	1670	1705	830
K-27	2625	885	2695	850	1060
VCO264	2625	960	1835	1285	720
F7-F2	2635	640	1460	915	1010
MEAN	2589	932	1915	1189	905
		Protease activity ($\Delta O.D._{280}$/hr/gram freeze dry wt)			
212-74	2.8	3.5	1.4	1.8	2.4
K-27	4.0	3.8	1.1	2.3	2.2
VCO264	2.1	5.2	1.5	1.6	1.2
F7-F2	4.0	4.2	0.6	2.1	2.2
MEAN	3.2	4.2	1.2	2.0	2.0

CV (nitrate reductase activity) 7.53%; CV (nitrate content) 18.75%; CV (protease activity) 7.23%.

LSD:	5%	1%
Between two nitrate reductase activity values	0.37	0.53
Between two nitrate content values	500	734
Between two protease activity values	0.6	0.9

inbred lines, F7-F2 and VCO264, than in the sensitive inbred lines. When compared to 16-day old unchilled controls, protease activity was increased by 2.5; 3.3; 3.5 and 7.3 fold in chilled 16-day old plants of 212-74, K-27, VCO264 and F7-F2, respectively.

Normally, high nitrogen fertilization followed by any factor that restricts plant growth would be expected to result in the accumulation of nitrate in the tissues through its effect on nitrate reductase activity. This has not been the case with chilling stress. Chilling reduced leaf nitrate content by an average of about 51.1% (Table 12). In all inbred lines, the reduction in tissue nitrate content was significant and presumed to be due to the inability of the plants to absorb nitrate when exposed to chilling stress.

The rate of protein synthesis as indexed by C^{14}-leucine incorporation in the leaves of corn plants grown at chilling and favourable temperature and measured at $12^{o}C$ and $28^{o}C$, respectively, is presented in Table 13. Although symptoms of protein degradation in the leaves were recorded when young corn seedlings were exposed to chilling temperature (Tables 8, 10 and 11) the data in Table 13 showed that protein synthesis was taking place at the same time. However, there was a marked decline in the rate of C^{14}-leucine incorporation due to chilling. The magnitude of decline ranged from 39% to 49% and was greater for the vigorous inbred lines, 212-74 and VCO264, than for the less vigorous inbred lines, F7-F2 and K-27. The rate of protein synthesis as determined by this method did not appear to reflect the original ranking of the inbred lines with respect to chilling tolerance or growth rate at favourable temperature. Also, from the results of this experiment, no evidence was obtained

TABLE 13. Incorporation of C^{14}-L-leucine into protein by corn leaf discs obtained from chilled and unchilled plants of four corn inbred lines and measured at 12°C and 28°C, respectively.

Inbred lines	Chilled plants			Unchilled plants		
	Total uptake (dpm)[1]	Incorporation into protein (dpm)	Percent incorporation	Total uptake (dpm)	Incorporation into protein (dpm)	Percent incorporation
212-74	24,840	1,790	7.23	63,225	8,950	14.20
K-27	21,360	2,585	12.11	62,998	12,260	19.50
VCO264	30,510	2,760	8.05	57,635	12,035	17.80
F7-F2	22,528	1,440	6.39	70,980	7,380	10.47

[1] dpm = disintegration per minute.

indicating that rapid rate of protein synthesis had an important influence on the rate of growth at chilling temperature.

When the chilled plants were returned to favourable temperature, the level of nitrate reductase activity of the recovering plants exceeded that of the controls by about 46.4%. In all inbred lines the differences between the recovering plants and the 24-day old control were statistically significant. During recovery, changes in protease activity and tissue nitrate content were also observed. Protease activity decayed back to its normal level and no significant differences were observed between the recovering plants and the 24-day old controls. With regard to tissue nitrate content, an average increase of the order of about 31% was recorded when the means of the recovering plants and the 24-day old controls were compared. The larger rooted inbred lines, 212-74 and VCO264, contained significantly higher contents of nitrate as compared to the 24-day old controls. Recovering plants of the mediocre rooted inbred lines, K-27 and F7-F2, on the other hand, contained lower levels of nitrate as compared to the 24-day old controls. These differences, however, were not statistically significant.

4.2.6 Association Between the Levels of Plant Properties with Chilling Tolerance and Recovery from Chilling Stress

One of the primary objectives of this study was to determine whether any relationship exists between the metabolic properties of an inbred line and its chilling tolerance or its ability to recover rapidly from chilling stress. Any such relationship would be a useful aid in screening parental lines, hybrids and segregating populations in order to develop new varieties that would perform well particularly in the cooler corn

growing areas. Simple correlations between growth rate (both absolute and relative) and the levels of the remaining parameters were, therefore, calculated in three different ways: (1) the pre-stress levels of plant properties *versus* growth rate during chilling stress; (2) the level of plant properties expressed during chilling stress *versus* growth rate during chilling stress; and (3) the levels of plant properties expressed during chilling stress *versus* growth rate during recovery. The results of these correlation studies are presented in Table 14.

Out of 19 plant properties investigated, only the pre-stress levels of sugar content in the roots and nitrate reductase activity in the leaves were significantly correlated with the level of chilling tolerance (Table 14). Sugar content in the roots was positively correlated with chilling tolerance whereas nitrate reductase activity was negatively correlated with chilling tolerance. The levels of the following properties expressed at chilling temperature – total protein in the leaves, free amino acids in the leaves, nitrate reductase activity in the leaves – were negatively correlated with chilling tolerance, whereas water-soluble protein in the leaves, free amino acids in the roots and protease activity in the leaves were significantly and positively correlated with chilling tolerance. Also, the levels of root sugar content and root protein content expressed at chilling temperature were both negatively and significantly correlated with the rate of recovery from chilling stress. The significance and implications of these correlations will be discussed in a subsequent section.

TABLE 14. Associations of the levels of some plant properties exhibited before chilling stress and during chilling stress with chilling tolerance and ability to recover rapidly from chilling stress (Chilling tolerance and ability to recover rapidly from chilling stress were represented by asbolute and relative growth rates).

Plant property		Correlation I[1]		Correlation II[2]		Correlation III[3]	
		ABS[4]	REL[5]	ABS	REL	ABS	REL
		Correlation coefficients					
Sugar content	(leaf)	-.001	+.291	+.044	+.423	+.058	+.100
	(root)	+.999**	+.820*	+.117	+.131	-.707*	-.946**
Starch content	(leaf)	-.002	+.278	+.454	+.079	+.091	-.304
	(root)	+.620	+.185	+.255	-.005	-.307	-.191
Total protein	(leaf)	-.532	-.590	-.746*	-951**	-.556	-.397
	(root)	-.781	-.690	-.477	-.339	-.811**	-.777*
Water-soluble protein	(leaf)	+.517	+.539	+.802*	+.862**	+.251	-.215
	(root)	-.734	-.857	+.310	+.405	-.268	-.478
Free amino acids	(leaf)	-.434	-.399	-.845**	-.899**	-.578	-.104
	(root)	-.204	-.417	+.836**	+.825**	+.135	-.454
Nitrate content		+.067	-.054	-.289	-.365		
Nitrate reductase activity		-.799**	-.858**	-.812*	-.832*		
Protease activity		-.471	-.254	+.967**	+.889**		
Chlorophyll content		-.629	-.684	+.698	+.615		
Photosynthesis	(top leaf)	+.491	+.269	-.603	-.605		
	(lower leaf)	+.536	+.144	+.481	+.678		
Respiration	(top leaf)	-.361	-.597	-.779	-.677		
	(lower leaf)	-.671	+.117	-.735	-.559		
Protein synthesis		-.250	-.592	+.086	+.043		

[1] Pre-stress level of properties versus chilling tolerance.

[2] The level of properties during chilling stress versus chilling tolerance.

[3] The level of properties during chilling stress versus recovery from chilling stress.

[4] ABS = Absolute growth rate.

[5] REL = Relative growth rate.

* Significant at the 5% level of significance.

** Significant at the 1% level of significance.

5. DISCUSSION

5.1 Growth and Metabolic Changes During Chilling Stress

5.1.1 Effect of Chilling on the Synthesis and Metabolism of Carbohydrates

There are many reports of changes in the synthesis and metabolism of carbohydrate when sensitive plant species are subjected to chilling stress. These have shown that the rates of photosynthesis (Hesketh, 1967; Cooper and Taiton, 1968; Hofstra and Hesketh, 1969; Taylor and Rowley, 1971) and respiration (Beevers and Cooper, 1964) are reduced significantly and that the concentration of sugars increase slowly (Parker, 1962; Beevers and Cooper, 1964; Smith, 1968; Hillard and West, 1970; Guinn, 1971). This study provides additional evidence supporting the observations previously reported. In the present investigation, photosynthetic and respiratory rates were reduced by about 60% and two-fold increases in sugar and starch contents were recorded. The inhibition of plant growth rate by chilling temperature was more severe than that of photosynthesis and respiration in all observations.

Experimental findings of some investigators working with crops other than corn (Parker, 1962; Sakai and Yoshida, 1968; Siminovitch *et al.*, 1968; Smith, 1968) led to the view that low temperatures promote the hydrolysis of starch to sugar. This view has not been supported by the findings of the present experiment. When corn plants were exposed to chilling stress concomitant increases in both sugar and starch contents

were recorded. This observation lent support to the conclusion that, in corn, the excess accumulation of sugars at chilling temperatures was not a result of starch hydrolysis, but a direct product of current photosynthesis.

Although the chilling tolerant lines showed significantly higher photosynthetic rates than the chilling sensitive lines when they were grown at favourable temperatures, these apparent differences diminished at chilling temperatures. This should not be surprising since factors such as improper nitrogen nutrition (Ryle and Hesketh, 1969; Tsuno and Shimizo, 1962), reduced stomatal aperture (Taylor and Rowley, 1971), reduced chlorophyll content, and feed-back effect of accumulated carbohydrates (Upmeyer and Koller, 1973; Neales and Incoll, 1968), all of which were observed during chilling stress, can impose restriction on photosynthesis and as such mask the true potential of the genotype.

The exact mechanism by which chilling causes reduction in growth rate is not clearly known. Nevertheless, reduction in photosynthetic rate does not appear to be the primary biochemical lesion affecting growth rate during chilling stress. Support for this contention is obtained from the observation that more than two-fold increases in starch and sugar content were recorded during chilling stress. This would indicate that deficiency of carbohydrates, and therefore, photosynthesis, was not limiting growth. Further evidence comes from the observation that growth rate averaged over an 8-day period, was more sensitive to chilling stress (reduced by 85 - 94%) than photosynthesis (reduced by about 60%). The greater sensitivity of growth rate than photosynthetic rate to chilling may indicate that chilling primarily affects some other factor controlling growth, and its effect on

photosynthesis is secondary. Since the leaves of the chilled plants showed drooping orientation and lowered nitrate uptake (Table 12), it seems probable that the primary effect of chilling was to limit water and nutrient uptake from the soil.

Reduced water and nutrient uptake are crucial in the life of the plant when it is exposed to chilling stress. For example, reduced water uptake would be expected to bring about loss of cell turgor pressure. One of the immediate effects of loss of cell turgidity is the alteration of membrane structure. Altered membrane structure may not only increase the activation energy of membrane-bound enzyme systems, but will also elicit an imbalance with non-membrane bound enzyme systems, consequently leading to reduced growth rate (Lyons, 1973). In addition, turgor presure is crucial in cell enlargement, supplying the necessary push or pressure from inside the cell (Hsiao, 1973; Kleindrost and Brouwer, 1970) and as such may assume a profound role in plant growth rate. Reduced turgor pressure in the guard cells of stomata may bring about a reduction in stomatal aperture, the consequence of which would be reduced CO_2 diffusion into the leaves and ultimately reduced photosynthetic rate. Since nutrient elements are necessary as cofactors to promote the formation of -C-C- chains, for manufacturing a wide spectrum of enzymes and for the synthesis of chlorophyll molecules, reduced nutrient uptake would also be expected to retard photosynthesis and growth. Thus, the direct effects of turgor change and nutrient uptake can be considered as the primary factor responsible for causing other indirect effects such as accumulation of carbohydrates and reductions in photosynthetic and respiratory rates.

The significant increase in soluble carbohydrates, particularly that

of sugars, appears to be a protective mechanism designed to minimize injuries caused by chilling. The increase in osmotic potential of the plant will reduce the extent of tissue dehydration (Christiansen *et al.*, 1970; Guinn, 1971) and possibly, sugars may also serve to stabilize protein molecules (Levitt, 1966).

5.1.2 Effect of Chilling on the Synthesis and Metabolism of Proteins

When the corn seedlings were subjected to chilling stress, total protein in the leaves declined by about 20% while the levels of water soluble proteins and free amino acids increased. The activity of the enzyme nitrate reductase and the concentration of its substrate nitrate declined, whereas a sharp increase in protease activity was recorded. At the same time, the rate of protein synthesis, as indexed by C^{14}-leucine incorporation, was reduced to about 50%. The reduction in total protein content in the leaves was accompanied by a corresponding increase in total protein, free amino acids and water soluble proteins in the roots.

It is not clearly known whether the effects described above were essentially aspects of senescence, or whether they were representing yet another manifestation of the working out of a genetically determined adaptive mechanism as envisaged by Levitt (1966) and other workers. However, it is noteworthy that the latter view was supported by the observations of the present study. The basis of this hypothesis is that these symptoms were in general more accentuated in the chilling tolerant than in the chilling sensitive lines. This is clearly evidenced by the correlation studies (Table 14), which revealed that at chilling temperature leaf total protein, leaf free amino acids and nitrate reductase activity

were negatively correlated, while the levels of free amino acids in the roots and protease activity in the leaves were positively correlated, with the rate of growth at chilling temperature. These correlations appeared to point to the fact that greater mobilization of nitrogenous metabolites from the leaves to the roots was a characteristic property of chilling tolerant lines. The correlation analysis presented in Table 14 also revealed a strong positive relationship between water soluble protein in the leaves at chilling temperature and the level of chilling tolerance. Thus, formation of higher quantities of water soluble proteins may be considered as an additional character differentiating chilling tolerant corn genotypes from chilling sensitive genotypes. When put together, all these observations led to the hypothesis that the concomitant changes in nitrogenous metabolites were indicative of the fact that protein hydrolysis followed by formation of water soluble proteins was taking place as an adaptive mechanism. Examples from the works of Li *et al.* (1965) and Kohn and Levitt (1966) illustrate this view and show that this type of change in protein metabolism is not essentially a matter of senescence.

The association of water soluble protein with chilling tolerance has not been previously reported in the literature. However, formation of water soluble proteins in plants exposed to hardening temperatures has been consistently found to reflect both genetic and environmentally induced variation in cold (freezing) tolerance (Jung *et al.*, 1967a, b; Jung and Smith, 1961; Shih *et al.*, 1967). Also, the concentration of water soluble proteins was associated with cold tolerance when top growth

was regulated with chemicals (Shih and Jung, 1971). The role of water soluble proteins in chilling tolerance is not clear from the results of the present investigation. Since several properties of water soluble proteins, including their capacity to bind water and to protect enzymes when the enzymes are subjected to freezing and thawing (Gerloff *et al.*, 1967; Herber, 1968) have lent support to the relationship between water soluble proteins and freezing resistance, it is possible that the same principles could hold true regarding chilling tolerance. However, this hypothesis is largely speculative and the matter requires further investigation.

In recapitulation of the preceeding discussions, it would be appropriate to state that corn plants subjected to chilling stress show an array of metabolic changes seemingly designed to minimize injuries caused by chilling. Among these, the most important metabolic changes are; a dramatic increase in the osmotic potential of the plant, formation of water soluble proteins and mobilization of nitrogenous metabolites from the leaves to the roots. Given that the chilling stress is not so severe as to cause death to the plant, these metabolic changes occur in both tolerant and susceptible genotypes alike; but the extent to which different genotypes manifest these changes vary widely. It appears that the reactions of corn to chilling environments are under its genetic constitution and from the multiplicity of the metabolic changes observed it can be inferred that there must be many genes directly or indirectly affecting the level of chilling tolerance. Thus, chilling tolerance can be regarded as a quantitative rather than a qualitative trait. Even after the necessary simplifications have been

attempted in the present study, it remains evident that the whole phenomenon is still very complex. In view of this fact, it should be emphasized that the potentiality for chilling tolerance may be expressed in different ways by different genotypes.

5.2 Growth and the Associated Metabolic Changes During Recovery from Chilling Stress

The virtual cessation of growth as soon as environmental stress is imposed followed by a rapid growth rate upon relief of stress is a phenomenon frequently referred to in literature dealing with drought and heat stress (Gates, 1968; Hsiao, 1973). The same reaction was obtained in corn subjected to chilling stress in this investigation. A transitory phase of acclerated rate of growth immediately following the release from chilling stress was recorded in all inbred lines (Table 2). This response may have been due to the accumulation of cell metabolites caused by the physical constriction of cell enlargement, and the degradation and translocation of proteins and carbohydrates. Obviously, such phenomena cannot be expected to occur when the chilling stress is so severe as to preclude the accumulation of metabolites or so prolonged as to cause death to meristematic tissues.

It is evident from the results here reported that a number of important physiological changes accompany recovery from chilling. A brief review of these metabolic changes may illustrate the types and direction of changes one may expect during recovery.

Following recovery from chilling, the concentration of soluble carbohydrates dropped to a level lower than that during chilling stress. However, after 8 days of recovery at normal temperature, the chilled

plants were showing a more positive carbon balance than their unchilled controls.

With respect to nitrogenous metabolites, the following changes were observed during recovery. The levels of total protein and nitrate in the leaves increased as compared to the levels of the same metabolites during chilling stress. On the other hand, the levels of water soluble proteins, free amino acids and protease activity in the leaves; and total protein and free amino acids in the roots, decreased as compared to the levels of the same at chilling temperature. The level of changes in nitrate reductase activity in the leaves and water soluble protein in the roots appeared to depend on the genotype.

When comparisons were made between the recovering plants and the 24-day old controls, the levels of total protein and water-soluble protein in the roots of recovering plants were found to be higher than in the controls. Meanwhile, the levels of free amino acids in the leaves and roots of the recovering plants were found to be lower than in the controls. Comparisons with respect to nitrate reductase activity, nitrate content and protease activity showed that the changes in these parameters were dependent on the genotype. In general, these observations were in agreement with the findings of Omarman *et al.* (1971), who found that the incorporation of amino acids into protein was greatly stimulated following recovery with the result that the metabolic processes of the plant, including protein synthesis, were increased.

Some investigators (Barnett and Naylor, 1966; Taylor *et al.*, 1972a; Bates *et al.*, 1973) have observed a several-fold increase in free proline content when plants were exposed to stress conditions and have suggested

that free proline may act as a storage compound for carbon and nitrogen when the synthesis of both starch and protein is inhibited. These workers attribute the accelerated growth upon relief of the stress to the utilization of such a storage pool, and have emphasized strongly that selection for the potential to synthesize high levels of proline should be given special attention in plant breeding. The results of this investigation, however, do not support the hypothesis that proline was an important factor in rapid recovery from chilling stress. The following evidence supports the contention that the role of proline with regard to chilling stress was not profound: (1) the level of increase in free proline content observed at chilling temperature was very low compared to the several-fold increase reported by the above cited workers; (2) evidence for starch synthesis during chilling stress was observed in the present experiment indicating that metabolites were stored as heavy molecular weight compounds; and (3) the free amino acids that accumulated during chilling stress, including proline, appeared to be largely the result of protein hydrolysis rather than newly synthesized amino acids.

In addition to showing that the growth of plants following recovery is rapid, the results of the present experiment showed that inbred lines varied markedly in their response to favourable temperatures following chilling. However, no evidence was obtained indicating that these genotypic differences in the rate of recovery were direct reflections of the differences observed in the concentration of metabolites that accumulated during chilling stress. This aspect will be discussed in more detail elsewhere in this report.

Rapid recovery from chilling stress has, to this time, not been well understood, nor has the importance of it been recognized by plant-breeders. It may have been overlooked due to the expectation that such traits would be correlated with seed or seedling characteristics. The indication of the statistical test in the present experiment is that the ability to recover rapidly from chilling injury is a character independently controlled from seedling chilling tolerance. Also, evidence was obtained indicating that when the chilling stress was mild and of short duration, the ability to recover rapidly from chilling stress was more important in determining the final plant development than seedling chilling tolerance *per se*. These considerations indicate an urgent need for plant breeders to take advantage of the neglected capacity for rapid recovery from chilling stress. It is likely that, with further knowledge of the process involved, it might be possible to tap this unused potential for the breeding of fast growing genotypes.

Rapid recovery may have several advantages in providing flexibility and stability of grain yield under moderately cool temperature conditions. For example, if a rapid rate of recovery is not achieved when high temperatures recur, this is likely to be reflected in smaller final size of plant with the yield consequently reduced. Lack of rapid recovery from chilling stress may also delay flowering and grain filling, and by so doing make the crop vulnerable to late season weather and insect injury. Rapid recovery, unless initiated as early as possible, may overlap with inflorescence development. Such overlapping of rapid growth rate with inflorescence development may induce the production of smaller ears and ultimately reduce crop yield (Milthorpe and Moorby, 1974).

5.3 The Association of Metabolic Properties with Chilling Tolerance and Recovery from Chilling Stress and Their Utilization as Criteria of Selection

The task of selecting for chilling tolerant genotypes would have been much simpler had knowledge advanced to a point where the basic principles concerned in the genetic control of chilling tolerance been clear. Unfortunately, this stage has not yet been reached. There is no shortage of schemes and hypotheses to set beside a mountain of observational and experimental data, but the unifying thread which might allow the plant breeder to identify the chilling tolerant genotype from among a heterogenous population is still lacking. For example, based on the findings of many experimenters, Lyons (1973) has presented a schematic pathway of events leading to chilling injury in sensitive plant tissues. His model shows that every process or event has its cause, and each produces its consequences, out of which flow other events or processes. Models of this kind have some attraction in seeking to explain the behavior of a plant when it is subjected to chilling, but they have nothing to offer a plant breeder by way of proposing selection criteria for chilling tolerance. From a plant breeding point of view, it would have been desirable if a single controlling response or master reaction differentiating the tolerant genotypes from the sensitive genotypes was available, and more so if the level of chilling tolerance could be predicted before the occurrence of the stress. One of the primary objectives of the present study was, therefore, to identify a character that is expressed at favourable temperatures and which is correlated with chilling tolerance with such consistency that corn genotypes can be

screened under field conditions without the need for subjecting the plants to chilling stress. In order to arrive at this objective it was necessary to correlate the pre-stress levels of all parameters studied in the present experiment with the rate of growth at chilling temperatures.

Findings of the present experiment indicated that out of the 19 parameters investigated only the pre-stress levels of sugar content in the roots and nitrate reductase activity in the leaves were significantly correlated with chilling tolerance (Table 14). The correlation of chilling tolerance with sugar content was positive; and its correlation with nitrate reductase activity was negative. No other parameters studied appeared promising as selection criteria to be useful in a corn breeding program.

A difficulty lies in utilizing root sugar content as a criterion of selection for chilling tolerance. Sampling roots is not only an expensive venture, but is also destructive, and therefore its use as a selection criterion in a segregating population is virtually impossible. It would have been desirable had such a characteristic of chilling tolerance been expressed in the leaves where sampling would be easier, less expensive and non-destructive. Although there was a good correlation between sugar content in the leaves and sugar content in the roots ($r = +.654$, $P > 0.01$), there was no correlation between sugar content in the leaves and chilling tolerance. The reason why there was no correlation between sugar content in the leaves and the level of chilling tolerance could not be easily explained. One possible reason is that each pooled sample of leaf consisted of leaves which were at different stages of

development and this might have distorted the correlations. In contrast, the whole root system was harvested for sugar determination.

The nature of this experiment did not permit the definition of the mechanism by which high levels of sugars in the roots affect the rate of growth at chilling temperatures. However, the hypothesis that sugars, by providing readily available energy for growth at chilling temperatures would help the plant to withstand chilling, was quickly dispelled when it was learned that there was an excess accumulation of sugars during chilling stress. The opinion of Christiansen *et al.* (1970) is that sugars, by retaining moisture, may protect the plant from dehydration and indirectly from chilling. Because there was a good correlation between the pre-stress levels of sugars in the roots and the level of chilling tolerance, and there was no correlation between the level of sugars that accumulated in the roots during chilling stress and the level of chilling tolerance (see Table 14), the conclusion is unavoidable that the level of sugars present before chilling stress, and not the level that accumulated during chilling stress was the important factor in chilling tolerance. If so, the main differences in growth rates among the four inbred lines should be attributed to the differences in the amount of dry matter that accumulated in the first few days the plants were exposed to chilling. Further studies are obviously needed to prove this hypothesis.

The negative correlation between the pre-stress levels of nitrate reductase activity and the level of chilling tolerance also deserves further discussion. Several workers (for example, Bolaria, 1956: Smith 1968; and Palmertree, 1972) have indicated that increased nitrogen

nutrition predisposes the plant to cold injuries. Nitrate reductase is a substrate inducible enzyme and the level of its activity has been shown to be rate limiting in nitrogen metabolism (Hageman and Hucklesby, 1971). In the present experiment, nitrate reductase activity was significantly correlated with leaf total protein content ($r = +.651$, $P > 0.01$). The lack of significant correlation between leaf total protein content and chilling tolerance might again be attributed to the inefficiency of the sampling method. For the determination of nitrate reductase activity, only the second and the third leaves from the top were used. In contrast, leaf total protein content was determined on samples collected from the top three leaves. The inefficiency of the latter sampling method is apparent as the topmost leaves may vary widely in the stage of their maturity, and as such may introduce more sampling error into the experiment.

Many workers (for example, White, 1973; Smith and Leinweber, 1971; Gates, 1968; Bates *et al.*, 1973; Barnett and Naylor, 1966) have proposed that storage compounds accumulated before or during an environmental stress are used in the early initiation and formation of new growth when the stress is removed. With the premise that the speed of recovery from chilling stress may be a reflection of the amount of metabolite accumulated in the plant during chilling stress, an attempt was made to correlate the level of metabolites expressed at chilling temperature with the rate of recovery. The correlation analysis (Table 14) indicated that utilization of metabolites that have accumulated during chilling stress did not contribute in a major way to the observed genotypic difference in the rate of recovery. In fact, significant negative correlations were obtained for the levels of sugar content and total protein

content in the roots. In view of the hypothesis around which the correlation studies were made, these negative correlations appeared very strange when they were first noticed. A careful examination of the data (Tables 6 and 8), however, revealed that the small rooted genotypes, K-27 and F7-F2, contained higher concentrations of sugars and total protein when they were exposed to chilling stress. It is therefore assumed that under conditions which result in mobilization of metabolites to the roots, genotypes with smaller root systems would accumulate higher concentrations of metabolites than genotypes with larger root systems. If this is true, then the negative correlations of total protein and sugar contents in the roots with the rate of recovery from chilling stress were indicating the primary importance of larger root systems for more rapid growth during recovery. This suggestion is largely confirmed by the data on tissue nitrate content (Table 12) which indicated that the uptake of nitrate during recovery was significantly higher in the leaves of the larger rooted genotypes, VCO264 and 212-74, than in the leaves of the smaller rooted genotypes. It is, therefore, proposed that metabolite reserves may affect the rate of recovery for only a few days, and after initial regrowth, other factors such as photosynthesis and nutrient uptake influence plant growth rate more than metabolite reserves.

The interpretation of the results presented above should by no means be considered conclusive. This has to be emphasized, not only because of the complexity of the problem, but also because of the limitation imposed by the small number of inbred lines used in the present study. Because of limited growth room facilities available and

the large number of parameters investigated, it was not possible to handle more than four inbred lines. It is, therefore, strongly suggested that this experiment be repeated, using a large number of genotypes and perhaps only those parameters that appeared promising as selection criteria in the present study. If the results are confirmed with adequate certainty they may have an important bearing on the breeding and selection of genotypes suitable for the cooler corn growing regions.

6. SUMMARY AND CONCLUSION

Two chilling tolerant and two chilling sensitive inbred lines were selected for intensive investigation based on preliminary evaluation of 32 inbred lines for reaction to chilling. The time course changes in dry matter accumulation in the stover of these selected inbred lines, namely, 212-74, K-27, VCO264 and F7-F2 were followed at a constant (28/22^{o}C) and alternating (8 days 28/22 + 8 days 12/10 + 8 days 28/22^{o}C) temperatures by harvesting 15 plants of each inbred line at 8-day intervals. In a similar manner, the time course changes in the contents of soluble carbohydrates, nitrogenous metabolites and enzymes were studied. The carbohydrates investigated were reducing sugars and starch, while the nitrogenous metabolites were total protein, water-soluble protein, and free amino acids. Independent determinations of these parameters were made in the top three leaves and in the roots. Also, chlorophyll content, protease activity, nitrate reductase activity and nitrate content in the top three leaves were determined. In separate experiments, the rates of photosynthesis, respiration and protein synthesis as indexed by C^{14}-leucine incorporation were measured.

An attempt was made to correlate the pre-stress levels of all these properties with the rate of growth at chilling temperature with the hope of identifying biochemical criteria suitable for predicting chilling tolerance in corn. Also, attempts were made to correlate the levels of these properties expressed during growth at chilling termperature with the rate of growth at chilling temperature and also with the rate of

growth during recovery from chilling stress. The idea behind these correlation studies was to identify biochemical changes that may provide protection against chilling injury; and to assess the contributions, if any, of these biochemical changes to the rate of recovery from chilling injury. The following observations were recorded.

1. Subjecting eight-day-old corn seedlings to chilling temperatures for a period of eight days resulted in severe reductions in growth rates. The extent of reductions in growth rates varied from 86 - 94% depending on the genotype.

2. When the chilling stress was removed, the recovering plants started to grow at a much faster rate than the control plants that had not been exposed to chilling temperatures. By so doing they at least partially compensated for the loss in dry matter accumulation brought about by chilling. Some genotypic differences were recorded among the four inbred lines with regard to their response to favourable temperature following chilling.

3. The levels of all properties (metabolites, enzymes and metabolic processes) investigated changed significantly during chilling stress and during recovery from chilling stress. Most of the metabolic changes expressed during chilling stress appeared to have been designed to minimizing injuries caused by chilling.

4. Out of 18 parameters investigated, only the pre-stress levels of sugar content in the roots and nitrate reductase activity in the leaves were significantly correlated with the level of chilling tolerance. Sugar content in the roots was positively correlated with chilling tolerance whereas nitrate reductase activity was negatively correlated

with chilling tolerance. The levels of the following properties expressed at chilling temperature — total protein in the leaves, free amino acids in the leaves, nitrate reductase activity in the leaves — were negatively correlated with chilling tolerance, whereas water-soluble protein in the leaves, free amino acids in the roots and protease activity in the leaves were significantly and positively correlated with chilling tolerance. Also, the levels of root sugar content and root protein content expressed at chilling temperature were both negatively and significantly correlated with the rate of recovery from chilling stress.

Based on the above observations, the following conclusions are reached:

1. The ability to recover rapidly from chilling injury in corn is a different and an independently controlled character from the ability to grow at chilling temperature. In much the same way as there are genotypic differences in chilling tolerance, there appear to exist differences among corn inbred lines in their ability to recover rapidly from chilling injury. Rapid recovery from chilling injury appeared to be more important in determining the final dry matter content than the ability to grow at chilling temperatures.

2. Rates of photosynthesis, respiration, protein synthesis and chlorophyll content are not the primary biochemical lesions affecting growth rate at chilling temperature, and therefore, selection for these characters would not help to identify chilling tolerant lines in a breeding program.

3. Based on the correlation studies and visual observations, it is suggested that chilling tolerant lines can be identified by the

following characters:

a) before chilling stress, the leaves of chilling tolerant lines express low levels of nitrate reductase activity, while the roots show high levels of sugars.

b) during chilling stress, the leaves of the tolerant lines contain high levels of anthocyanins, water-soluble proteins, protease activity, and low levels of total protein, free amino acids and nitrate reductase activity. The roots of the tolerant lines also contain high levels of free amino acids.

4. Rapidly recovering inbred lines appeared to be characterized by having large and well developed root systems.

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APPENDIX TABLE 1. Mean square ratios (F-values) for dry matter content and 17 other parameters of 4 corn inbred lines gorwn under different temperature regimes and harvested at 8-day intervals.

SOURCE OF VARIATION	D.M. Content	Sugar Content		Starch Content		Total Prot. Cont.		Water S. Prof. Cont.	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Treatments (Pooled)[1] Within Inbred Lines	1,358.26**(19)[2]	13.28**(19)	97.0**(18)	36.83**(19)	7.39**(18)	36.67**(19)	64.20**(18)	112.67**(19)	69.38**(18)
212-74	1,420.93**(4)	18.18**(4)	5.49**(3)	35.47**(4)	25.05**(3)	19.21**(4)	12.60**(3)	74.84**(4)	2.45**(3)
K-27	1,110.28**(4)	10.31**(4)	199.14**(4)	34.49**(4)	7.67**(4)	49.52**(4)	14.76**(4)	104.05**(4)	53.81**(4)
VCO264	2,531.46**(4)	11.77**(4)	67.38**(4)	69.96**(4)	5.11**(4)	31.80**(4)	9.20**(4)	97.81**(4)	49.45**(4)
F7-F2	1,279.61**(4)	21.28**(4)	56.06**(4)	39.38**(4)	5.79**(4)	35.03**(4)	44.56**(4)	267.12**(4)	18.91**(4)

[1] Separate analysis of variance were made for each inbred line.

[2] Numbers in brackets are degrees of freedom for treatments.

** F-value significant at the 1% level of significance.

APPENDIX TABLE 1 - Continued

SOURCE OF VARIATION	Amino Acid Content		N.R.A.	NO_3	Proteinase Activity	Chlorophyll Content	Photo-synthesis	Respira-tion	Protein Synth.
	Leaf	Root							
Treatments (Pooled) Within Inbred Lines[1]	595.36**(19)	342.82**(18)	138.84**(19)	13.02**(19)	17.85**(19)	185.36**(19)	89.9**(15)	54.4**(15)	66.01**(7)
212-74	1,013.14**(4)	147.03**(3)	627.11**(4)	143.57**(4)	20.17**(4)	504.18**(4)			
K-27	428.85**(4)	303.36**(4)	1,483.56**(4)	6.01**(4)	9.98**(4)	107.78**(4)			
VCO264	80.20**(4)	631.03**(4)	412.17**(4)	236.55**(4)	36.48**(4)	66.40**(4)			
F7-F2	121.62**(4)	200.26**(4)	54.41**(4)	174.28**(4)	30.39**(4)	415.76**(4)			

APPENDIX TABLE 2. 4 x 20 Contingency Chi-square test of independence on the frequency distribution of dry matter yield data.

TREATMENT COMBINATIONS			INPUT DATA, EXPECTED FREQUENCIES AND CONTRIBUTION OF EACH CELL TO CHI-SQUARE				
				Class interval: Standard deviations above or below the mean			
				-2	-1	+1	+2
8-day old controls	212-74	OB FREQ		3.	4.	6.	2.
		EX FREQ		2.55	4.65	4.95	2.85
		CELL X2		0.00	0.00	0.06	0.04
"	"	K-27	OB FREQ	3.	2.	7.	3.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.99	0.49	0.04
"	"	VCO264	OB FREQ	4.	3.	5.	3.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.35	0.28	0.04	0.04
"	"	F7-F2	OB FREQ	1.	8.	3.	3.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.43	1.75	0.42	0.04
16-day old chilled	212-74	OB FREQ		3.	4.	5.	3.
		EX FREQ		2.55	4.65	4.95	2.85
		CELL X2		0.00	0.00	0.04	0.04
"	"	K-27	OB FREQ	2.	5.	4.	4.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.00	0.04	0.15
"	"	VCO264	OB FREQ	3.	5.	5.	2.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.00	0.04	0.04
"	"	F7-F2	OB FREQ	2.	4.	7.	2.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.00	0.49	0.04
16-day old controls	212-74	OB FREQ		2.	5.	5.	3.
		EX FREQ		2.55	4.65	4.95	2.85
		CELL X2		0.00	0.00	0.04	0.04
"	"	K-27	OB FREQ	3.	3.	5.	4.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.28	0.04	0.15
"	"	VCO264	OB FREQ	2.	6.	3.	4.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.16	0.42	0.15
"	"	F7-F2	OB FREQ	2.	6.	5.	2.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.16	0.04	0.04
24-day old Recovery	212-74	OB FREQ		3.	4.	5.	3.
		EX FREQ		2.55	4.65	4.95	2.85
		CELL X2		0.00	0.00	0.04	0.04
"	"	K-27	OB FREQ	2.	6.	5.	2.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.16	0.04	0.04
"	"	VCO264	OB FREQ	3.	4.	6.	2.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.00	0.06	0.04
"	"	F7-F2	OB FREQ	4.	4.	4.	3.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.35	0.00	0.04	0.04
24-day old Control	212-74	OB FREQ		3.	4.	5.	3.
		EX FREQ		2.55	4.65	4.95	2.85
		CELL X2		0.00	0.00	0.04	0.04
"	"	K-27	OB FREQ	3.	5.	3.	4.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.00	0.42	0.15
"	"	VCO264	OB FREQ	1.	5.	7.	2.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.43	0.00	0.49	0.04
"	"	F7-F2	OB FREQ	2.	6.	4.	3.
			EX FREQ	2.55	4.65	4.95	2.85
			CELL X2	0.00	0.16	0.04	0.04
Column Totals				51.	93.	99.	57.
Grand Total				300.			

Chi-square = 10.20 with 57 degrees of freedom.

Theoretical Chi-square = 75.62 - 5%; 84.74 - 1%.

Chi-square was adjusted via Yate's Correction for Continuity Factor.

APPENDIX TABLE 3. Pedigree, mean rate of growth per day (inches) at 75°F, mean rate of growth under 55°F and comparative rate of growth (55°F/75°F ratio) of the 32 inbred lines included in Preliminary Experiment I.

Ser. No.	Pedigree	Mean rate of growth (inches/day)		
		75°F	55°F	55/75°F
1	K-25	0.766	0.207	0.270
2	K-26	0.724	0.182	0.251
3	K-27	0.553	0.043	0.078
4	K-29	0.522	0.147	0.282
5	K-48	0.504	0.129	0.256
6	K-51	0.647	0.219	0.338
7	K-52	0.755	0.182	0.241
8	CM-25	0.744	0.186	0.250
9	CM-72	0.769	0.139	0.181
10	CM-108	--	--	--
11	Spooner W329 A	0.569	0.205	0.360
12	French FNO 3	0.639	0.196	0.308
13	" RB 214	0.785	0.232	0.296
14	" F7-F2	0.793	0.229	0.289
15	" UV 113915	0.679	0.184	0.271
16	" VC 0264	0.866	0.234	0.270
17	" F 101	0.595	0.231	0.388
18	Smith C.L.T. 12-71	0.796	0.239	0.300
19	Haa Pala 101 T-22-71	0.707	0.181	0.256
20	212-74	0.568	0.152	0.268
21	SWF x B14	0.750	0.217	0.289
22	" "	0.655	0.170	0.260
23	63-326 x KN 11^3	0.740	0.214	0.289
24	A495 x D	0.696	0.180	0.257
25	" "	0.808	0.204	0.252
26	2019-1 backcrosses	0.973	0.225	0.231
27	Guatemala Grupo 30-1A	0.680	0.157	0.231
28	A639 x K27 S_1 1971	0.732	0.168	0.230
29	A639 x K27 " "	0.559	0.011	0.020
30	K25-T	0.797	0.186	0.233
31	K27-T	0.615	0.063	0.102
32	K29-T	0.56	0.156	0.291

APPENDIX TABLE 4. Nitrogen and phosphorus contents (% dry weight) in the leaves of 10 corn inbred lines grown at 28/22°C (day/night temperature) and 12/10°C temperature.

Inbred Line	Phosphorus		Nitrogen	
	12/10°C	28/22°C	12/10°C	28/22°C
A639 x K-27S$_1$-1971	0.28	0.37	3.2	4.8
K-26	0.26	0.28	3.3	3.8
F7-F2	0.18	0.37	2.4	3.5
VCO264	0.23	0.45	2.5	3.0
Smith C.L.T. 12-71	0.20	0.22	2.4	2.6
K-27-T	0.24	-	3.2	3.2
212-74	0.25	0.53	3.4	3.2
SWF x B14	0.20	0.32	2.2	2.5
K-27	0.22	0.24	3.1	3.5
CM-72	0.24	0.39	2.6	2.7