

USE OF CONTROLLED LOW TEMPERATURES IN EVALUATING
THE COLD TOLERANCE OF CORN INBRED LINES

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ABSTRACT

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USE OF CONTROLLED LOW TEMPERATURES IN EVALUATING THE COLD TOLERANCE OF CORN INBRED LINES. Major Professor: Dr. E.N. Larter

The undesirable effects of low air and soil temperatures at the time of seeding and early seedling growth of corn (Zea mays L.) require genotypes which are tolerant to low temperatures during seed germination, seedling emergence and early plant growth. In order to evaluate corn inbred lines for cold tolerance, laboratory methods need to be used as a supplement to field methods since weather conditions in the field are unpredictable for cold tolerance selection. In the present study, an attempt was made to evaluate corn inbred lines under different controlled low temperature conditions and to correlate these responses with field performance. By doing so, it may be possible to establish a screening procedure for cold tolerance in inbred lines of corn.

The cold tolerance responses of 32 early maturing corn inbred lines were evaluated from the following parameters: emergence percentage, emergence index, and seedling dry weight. Evaluations were undertaken in a growth cabinet in which the initial day/night temperatures of 10/10°C were raised to 15/10°C two weeks after seeding. Four weeks after planting, the seedlings were moved to a growth room with ambient day/night temperatures adjusted to 19/10°C. Based upon the results of these evaluations, 12 of the original 32 inbred lines were chosen for in-depth

studies including (1) post-emergence behavior during chilling (7 days 28/18°C C + 7 days 10/10°C), (2) response to low root zone temperatures (10, 14, and 18°C), and (3) to field testing.

Considerable genetic variation was detected for all traits among the 32 corn inbred lines which were evaluated at suboptimal ambient temperatures. Furthermore, heritability estimates for those traits were sufficiently large to permit selection advance for cold tolerance within the population of inbred lines under study. The four best cold tolerant inbred lines based on these tests were CK52, CK62, CK76 and C0255.

When 12 selections of the original 32 inbred lines were seeded at root zone temperatures of 10, 14 and 18°C, differential responses of the inbred lines were noted within each temperature regime. Lines CK64, CK69 and FV212 were sensitive to root zone temperatures of 10 and 14°C, while C0255, RB214 and CH12 exhibited good tolerance to these temperatures.

Despite the adverse effects of chilling on growth, a large amount of genotypic variability among inbreds was observed for both seedling dry weight and leaf area. Part of the variation could be attributed to differences in initial seed weights, but a considerable variation was also found to be related to rate of leaf area expansion.

The field test revealed marked differences among the 12 corn inbred lines with respect to emergence, time to silk, plant stands, yield, and seedling growth. The two best inbred lines tested under field conditions were CK76 and C0255.

Genotypic differences in percent total seed oil and unsaturated/saturated fatty acid ratios were detected among the 12 inbred lines. However, there was no significant association between low temperature tolerance and unsaturated/saturated fatty acid ratios.

The relationship between the different controlled cold-test methods and field performance of the 12 corn inbred lines indicated that

only a root zone temperature of 10° C appeared to be promising in predicting emergence under field conditions. Significant and positive correlations existed, however, between seedling vigor in controlled cold-tests and that observed in the field. Thus, strains with potential for vigorous seedling growth in the field can be identified using either a controlled low root zone temperature of 14°C or suboptimal ambient temperatures (15 days 10/10°C + 15 days 15/10°C + 10 days 19/10°C).

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1. INTRODUCTION

Corn (Zea mays L.) is a warm season crop which possibly had its origin in tropical or subtropical areas. Nevertheless, it has been widely adapted in temperate regions of which Canada is an example. With the advent of earlier maturing hybrids, corn has become a crop of increasing importance throughout Canada, particularly in the provinces of Quebec, Ontario, Manitoba and Alberta. In a 10-year period (1970-1979), the total corn production in the three provinces, Quebec, Ontario and Manitoba, increased from 499,300 to 891,000 hectares (Canada Grains Council, 1981). In Manitoba alone, there has been a steady increase in grain corn production from 1977 to 1982, ranging from 9,700 ha in 1977 to 92,000 ha in 1982 (Manitoba Corn Committee, 1983).

The main constraints to corn production in western Canada are the limited seasonal heat unit accumulation and the low soil temperatures that prevail during the early stages of plant growth. Low air and soil temperatures at the time of seeding result in delayed emergence, poor stands and retarded growth. In order to overcome these problems, planting is usually delayed until the conditions become more favourable. Late planting on the other hand, delays maturity and increases harvest difficulties. Since it also restricts the potential corn heat unit accumulation for that particular cropping season, early maturing genotypes and/or early dates of planting are desirable. Early planted corn ensures early flowering and results in the production of a higher volume of grain per unit leaf area, thus increased efficiency in grain production (Pendleton and Egli, 1969). It has been reported that for each day that planting is delayed beyond the first week of May in Manitoba, there occurs a grain yield reduction of one bushel per acre (Manitoba Corn

Committee, 1983). Such conditions necessitate the identification of genotypes which are tolerant to low temperatures during seed germination, seedling emergence, and early plant growth.

Several researchers have shown that cold tolerance in corn is genetically controlled and is heritable (Haskell and Singleton, 1949; Neal, 1949; Prinnell, 1949; Ventura, 1960; Grogan, 1970; Pesev, 1970; McConnell and Gardner, 1979b). Furthermore, three important traits, all of which are believed to be indicators of cold tolerance, have been identified. These include emergence percentage, emergence index, and seedling dry weight. There appears to exist adequate genetic variation for these traits within today's corn populations (Mock and Eberhart, 1972; Mock and Bakri, 1976; Mock and Skrdla, 1978; Mock and McNeill, 1979).

In order to identify cold tolerant strains, early spring planting has been practised. From predicted selection responses, Mock and Eberhart (1972) indicated that field selection for cold tolerance would be more efficient than growth chamber selection. However, four cycles of selection substantially improved cold germination under laboratory conditions while very little progress in emergence and vigor was made in the field because of very mild weather during the two years of evaluation (McConnell and Gardner, 1979a). Thus, field selection will not be effective unless conditions are conducive for low temperature selection. Both in the laboratory and growth chambers, however, environmental conditions are reproducible and progress can be made in each breeding cycle. Therefore, the appropriate and more economical testing technique that can be used to screen for cold tolerance has to be developed. An improved laboratory method has been used to develop corn genotypes with improved cold tolerance during germination and early seedling emergence at the University of Manitoba (Helgason, 1980). However, a rapid, large scale

screening technique which can be used for post-emergence cold tolerance is lacking.

Gubbels (1974), working with a small number of cultivars, found that emergence time was significantly correlated with weight and oil concentration of the seed. He suggested that a large seed with a high linoleic acid concentration might be a feature of genotypes with superior ability to emergence under cool conditions.

The objectives of the present study were:

1. to examine the behavior of several corn inbred lines grown at suboptimal ambient temperatures in terms of emergence and seedling growth;
2. to study the responses of some of those inbred lines to low root zone temperatures during emergence and seedling growth;
3. to investigate their post emergence behavior by subjecting them to chilling following a period of favorable growth conditions;
4. to determine whether there exists an association between low temperature tolerance and total oil content of the seed and its fatty acid composition; and
5. to determine the relationship between the different controlled cold-test methods and field performance, thereby attempting to establish a rapid inbred line screening procedure for cold tolerance in corn.

2. LITERATURE REVIEW

2.1 THE EFFECT OF LOW TEMPERATURE ON EMERGENCE AND SEEDLING GROWTH IN CORN (Zea mays L.)

2.1.1 Germination and Seedling Emergence at Suboptimal Temperatures

Street and Opik (1977) defined germination as "the resumption of metabolic activity and growth by the seed tissues, involving rehydration, utilization of nutrient reserves, and the gradual development of synthetic systems which enable the young plant to assume an autotrophic existence".

The first step in the germination of corn is water uptake. Various factors such as stage of harvesting, proportion of soft starch in the endosperm, and the protein content of the corn seed affect the rate of water absorption (Dugan, 1924). Temperature also plays an important role in the rate at which water is imbibed. Dugan (1924) found that corn seeds harvested at "milk-stage", "dent-stage" and "mature-stage" exhibited a progressive increase in the amount of water imbibed for each five degrees increase in temperature from 5° to 25°C. However, this increase was not consistently uniform since the quantity of water absorbed at 30° declined for "milk-stage" and "dent-stage" corn. On the other hand, Chaudhary *et al.* (1971) reported that there was an increase in water absorption by corn and wheat seeds from 5° to 35°C with increasing temperature. Maximum water uptake occurred at a temperature of 35°C.

Water absorption promotes the activities of various enzymes in the seed. Some of those enzymes probably were synthesized at the time

of seed maturation and subsequently were maintained in an inactive state during the period of low seed moisture. In contrast, other enzymes were synthesized soon after water imbibition as seen by the presence of components of protein-synthesizing systems such as ribosomes, nucleic acids, and amino acids within 5 to 6 h of water imbibition (Ingle et al., 1964). These intense enzymatic activities result in hydrolysis of storage reserves and translocation of the resulting soluble products to the growing regions. In corn, the early dry weight loss from the endosperm occurs at the expense of lipids and starch. No large change in nitrogen content of the various seed fractions occurs until after protrusion of the radicle. Following protrusion there occurs a rapid redistribution of metabolites between the endosperm, scutellum, aleurone, and axis. These changes are characterized by the breakdown of nutrient reserves (starch, protein and lipid) to soluble compounds such as sugars, fatty acids, amino acids, and inorganic phosphate, calcium, magnesium, and potassium ions. These metabolites are transported to the embryonic axis where they support the growth of the seedling (Ingle et al., 1964).

Although little is known about the influence of low temperature on the changes which occur in the redistribution of metabolites at the time of germination, one can speculate that the slower rate of water uptake at lower temperatures retards metabolic activities in the seed. Murray and Cooper (1967) indicated that in orchardgrass, the time required for endosperm exhaustion, using dry weight loss as a criterion, occurred between 14 and 16 days at 15° and 20°C and between 24 and 26 days at 10°C.

Temperature also affects the rate of germination. Martin et al. (1935) pointed out that the percentage and rate of germination for several sorghum varieties were reduced by soil temperatures below 25°C. Groot (1976) found that germination of maize hybrid seeds was very slow below 14°C.

Seeds fail to germinate if cold soil conditions persist for a long period of time. Cummins and Parks (1961) reported that corn did not germinate at 10°C. However, Dubtez et al (1962) mentioned that although germination occurred at 6°C, the percentage was low.

Plants vary with respect to the temperature requirements at the time of germination. Coffman (1923) found that corn germinated best at temperatures above 10°C with a sharp decrease in germination below 10°C. The minimum, optimum and maximum temperatures for the germination of corn seeds are 10° (Blacklow, 1972), 32° (Groot, 1976) and 46.1°C (Weaver, 1926), respectively.

The adverse effects of sub-optimal temperature on germination is not the only concern in the production of corn. Its influence on seedling emergence is also very important since it is necessary that seedlings emerge and begin photosynthesis as soon as possible in order that vigorous early growth be obtained.

Various factors such as seeding depth, amount of reserve food stored in the seed, seed size, soil disease organisms, and temperature, all influence seedling emergence. Among these, temperature is of particular importance at the time of emergence. Schulz and Bateman (1968) reported that the decrease in emergence of beans, peas, cucumbers, and corn at 5°C pretreatment in the absence of Rhizoctonia Solani was 0, 32, 0 and 76 percent, respectively, while in the presence of the fungus, emergence was decreased by 41, 85, 78 and 91 percent, respectively. At 25°C, in the presence of Rhizoctonia Solani, the decrease in emergence was 3, 22, 30 and 51 percent for beans, peas, cucumbers and corn, respectively. This implies that low temperature does not only reduce percentage emergence, but increases the susceptibility of all four plant genera to injury by Rhizoctonia Solani.

Alessi and Power (1971) found that increasing soil temperatures from 13.3 to 26.7°C reduced the number of days to emergence. The time

required for 80 percent emergence ranged from 4 to 24 days, depending on soil temperature and seed depth. Corn failed to emerge at 6.7°C temperature over a period of 24 days.

Blacklow (1972) measured temperature effects on radicle and shoot elongation of corn seedlings and found that the rates of elongation were greatest at about 30°C and effectively ceased at the two extremes, 9° and 40°C. Hatfield and Egli (1974) reported that the time required for a soybean hypocotyl to reach a 5 cm length decreased as the soil temperature increased from 10° to 32°C. The optimum temperature range for hypocotyl extension was concluded to be between 25° and 32°C.

Seedling emergence varied over a range in temperature; the emergence curve having a maximum value characteristic of the crop. 'Cool' crops, such as wheat, peas and turnips, exhibited emergence maxima at lower temperatures (15 - 20°C) than 'warm' crops, like cotton, sorghum, rice and melons (25 - 30°C). The 'cool' crops showed slow germination at 5°C, and very reduced germination at 35 - 40°C, while 'warm' crops were inhibited only at 40°C (Singh and Dhaliwal, 1972). Low temperatures prolonged the germination period and decreased vigor of seedlings, resulting in lower emergence. Conversely, high temperatures increased respiration rate and metabolic failure occurred.

2.1.2 The Influence of Low Soil Temperature on Corn Root Growth

Based upon their geographical areas of origin, plant species display wide differences in the optimum temperature for root function. Relatively small departures from the optima can depress the rates of nutrient uptake and transport, also permeability to water (Nielsen and Humphries, 1966). In the bean seedling, a shift in temperature above or below the optimum range (25 - 30°C) resulted in suberization of the endodermis closer to the root tip, with accompanying changes in the rate

of xylem exudation and development of symptoms of leaf water stress (Brouwer and Hoogland, 1964).

In his review, Nielsen (1974) mentioned that the optimum root temperature for corn appears to be about 25 - 30°C, depending on other soil environmental factors. Corn plants grown in pots at 20°C for 35 days reached permanent wilting point when placed into a water bath at 5°C (Nielsen et al., 1961). Knoll et al. (1964) reported that corn plants grown at either 20 or 25°C produced significantly reduced dry weight yields of roots when transferred to 15°C from day 14 to day 21 of their growing period.

Duration is important in assessing the adverse effects of low root temperatures on plants. There are irreversible effects induced by low root temperatures (Brouwer and Levi, 1969), but adaptation can compensate to some degree for low temperature damage. When root temperatures are reduced gradually, thereby allowing for some degree of adaptation, the adverse effects due to reduced permeability to water may become much less marked (Kramer, 1969). In rye, pretreatment at 8°C increased the unsaturation of the fatty acids associated with phospholipids in the roots (Clarkson, 1976). These changes might influence transport processes across cell membranes by maintaining them in a fluid condition at the lower temperature (Clarkson and Hall, 1977). Such observations provide some indications as to how roots adapt to temperature gradients.

Soil temperature affects the morphology of roots. In his review, Nielsen (1974) indicated that at cooler temperatures roots are usually whiter, thicker in diameter, and less branched than at warmer temperatures. At higher temperatures, roots become filamentous.

The root distribution pattern is also influenced by soil temperature. Garwood (1968) mentioned that in grasses and clovers, more branching of the root system will occur near the soil surface, decreasing

in numbers with depth but increasing in individual root diameter. The angle of root growth of corn plants was found to be minimum (10°) at 17° C while at temperatures above or below this level ($10 - 30^{\circ}$ C), a more vertical growth occurred (Onderdonk and Ketcheson, 1973).

When young seedlings are exposed to suboptimal temperatures, there will be a reduction in water uptake. Lal (1974) stated that:

restricted water uptake at low root temperature is believed to be due to (i) increased viscosity of water in the root medium, (ii) decreased permeability of the root membrane, (iii) increased viscosity of protoplasm in the roots, and (iv) poor development of the root system.

Low soil temperature influences the rate of release and uptake of nutrients. It also affects the assimilation of the absorbed nutrients and their subsequent translocation to tops (Nielsen, 1974).

The element which is most affected by low temperature is phosphorus. There have been many reports of added P counteracting some of the effects of unfavourably low temperatures (Nielsen and Humphries, 1966). Phosphorus increased yields of corn at low temperatures but had little effect at 26.7° C (Allen and Engelstad, 1963). Ketcheson (1966, 1968, 1970) and others (Knoll *et al.*, 1964a, 1964b) concluded that although phosphorus fertilizer placed with corn seed in a cold soil benefited growth considerably, it did not completely compensate for the harmful effects of low root temperatures, even at higher rates of application.

Phosphorous as a soil constituent is immobile. Sutton (1969) concluded that low soil temperature can reduce the availability of native phosphorus to plants. He suggested that the addition of phosphate fertilizer is beneficial since there is an increase in overall available P and a high concentration can be maintained in a solution at any soil temperature. Soil heating significantly increased phosphorus concentration in corn plants sampled 30 days after seeding (Mederski and Jones, 1963; Jones and Mederski, 1963). This increase in P uptake could

be attributed to the enhancement of root metabolism (Richards et al., 1952), also to the increase in the level of P available in the soil (Nielsen et al., 1961) and to increased transpiration rates with subsequent effects on P uptake (Golden, 1962). However, phosphorus concentration in the plant was not affected by soil temperature at a low soil P level (Nielsen et al., 1961).

Root temperature greatly influences nitrogen uptake by maize (Walker, 1969). Mederski and Jones (1963) reported that in plants sampled 30 days after planting, soil heating increased N concentration by about 25 percent. Moraghan and Porter (1975) pointed out that an inbred maize which was grown in NH_4 and NO_3 nutrient solution at two soil temperatures (14.8 and 28.2°C) produced substantially lower yields of roots in both nutrient solutions at 14.8°C. The yield reduction was greater in the NH_4 nutrient medium, possibly due to NH_4 toxicity. NO_3 concentration in the roots of the inbred line grown in the NO_3 medium was greatly reduced at 14.8°C.

Potassium uptake was studied by Mederski and Jones (1963), and their work showed that K concentration in plants sampled 30 days after seeding was increased appreciably by soil heating. This K uptake may be stimulated by increasing root temperature although available soil K has not been found to increase with soil temperature (Nielsen et al., 1961). Increased water uptake with increasing soil or root temperature has been observed (Nielsen et al., 1961 and Richards et al., 1952). This may account for the large increase in K uptake during early growth.

Soil temperature also influences the uptake of Ca and Mg. Mederski and Jones (1963) indicated that plant samples taken from the heated rows 30 days after planting contained about 25 to 30 percent less Ca and Mg than those samples which were taken from unheated rows. This decrease in Ca and Mg contents can be explained in part by cation competition arising from increased K uptake (Nielsen et al., 1961).

In his summary, Nielsen (1974) suggested that the influence of root temperature on uptake are inseparably linked with its effects on assimilation and translocation. Low root temperature restricts the translocation of nutrients from roots to tops and enhances the accumulation of nutrients on roots.

Low root zone temperatures retards growth processes in the roots and reduces their capacity as sinks for carbohydrates. Hatt (1965) pointed out that translocation of carbohydrates out of the leaves of sugar cane plants grown at a root temperature of 17°C was much less than at a temperature of 22°C.

Plant roots play an important role not only in anchorage and nutrient uptake, but also in the supply of growth substances. Atkin et al. (1973) analyzed xylem sap of 30 to 47-day old corn plants grown in culture solution maintained at 8°, 13°, 18°, 23°, 28° and 33°C. It was revealed that the greatest export of total cytokinin and gibberellin occurred at 29°C, and the lowest export of an unidentified growth inhibitor occurred at 33°C. As the root temperature approached 8°C, the sap contained more inhibitor and less cytokinin. At the lower root temperatures, root mass and branching decreased. Shoot growth was also restricted suggesting that prolonged cold introduced an altered balance between the growth promoters (gibberellin and cytokinin) and the growth inhibitor.

2.1.3 Early Growth of Corn Seedlings with Roots Exposed to Cold Soil

Root zone temperature affects seedling growth and consequently is of utmost importance, in the early stages of plant growth. During these early stages of growth and before tassel initiation, root zone temperatures may affect shoot growth directly (Beauchamp and Lathwell, 1966, and Watts, 1971). After tassel initiation and stem elongation, the meristematic regions are elevated above the soil surface. During this

period, shoot growth becomes less influenced by root temperature and more responsive to ambient temperatures (Ketcheson, 1968). When corn plants were exposed to uniform air temperature and a range of root temperatures in sand cultures, Beauchamp and Lathwell (1967) observed highly correlated root and shoot dry matter prior to emergence of the sixth to eighth leaf from the leaf whorl. After this growth stage, the dry matter correlation diminished and plant differentiation was less dependent on root temperature. Earlier, Grobbelaar (1963) observed that shoot primordia had not emerged above the soil surface until after the sixth leaf emerged from the leaf whorl.

The optimum root temperature for maize seedlings was reported to be 20 - 30°C (Grobbelaar, 1963). Walker (1969) pointed out that the growth response of maize seedlings reached maximum at 26°C. Furthermore, he found that as little as one degree increment in soil temperature (ranging from 12° to 35°C) influenced the growth behavior of maize seedlings. With each degree increase in soil temperature from 12 to 26°C, total seedling dry weights were increased by an average of 20 percent. Higher temperatures (e.g., 26° to 35°C) reduced dry weights by an average of 12 percent for each one degree increase (Walker, 1969).

The studies of Brouwer et al. (1970) revealed that when the apical meristem was below ground level, the soil temperature controls the rate of leaf emergence. Thereafter, all other developmental rates up to the tasselling stage were influenced predominately by the air temperatures. Beauchamp and Lathwell (1966) reported a reduction in the rate of leaf initiation and maximum number of leaves finally initiated per plant when a hybrid was grown at cold (15°C) root zone temperatures. From these results, they concluded that root zone temperature has a direct effect on meristematic activity. Coligado and Brown (1974) found that the rate of leaf primordia initiation increased, and time to tassel initiation decreased steadily with increasing soil temperature up to

25°C. Further increase in temperature from 25°C to 30°C had no additional effect. However, they noted that a soil temperature of 30°C caused an appreciable increase in the total number of leaf primordia initiated and hence the final number of leaves produced. Cooper and Low (1977) mentioned that leaf primordia initiation occurs for about four weeks after emergence, and the growing point of the shoot emerges above ground level at nearly five weeks after emergence.

Root zone temperature also influences leaf elongation. Barlow and Boersma (1972) reported that leaf elongation responds rapidly to changes in root temperature. Leaf elongation rate declined within one to two minutes after the soil temperature was decreased from 28° to 10°C (Barlow et al., 1977). This decreased growth rate was ascribed to restricted water uptake resulting from an increase in the viscosity of water which lowered the plant water potential. Similar results have been reported by Kleinendorst and Brouwer (1970), and Kleinendorst (1975). The cause of inhibition in leaf elongation is retardation of cell elongation rather than of cell division (Kleinendorst, 1975).

Low soil temperature can also decrease the rate of leaf elongation by lowering the temperature of the shoot apical meristem region in young corn plants (Kleinendorst and Brouwer, 1970). Beauchamp and Torrance (1969) have shown that when soil temperature is lower than air temperature, the temperature of an apical meristem located 2 to 3 cm above the soil surface tended to remain 1° to 3°C higher than the soil temperature. When the apical meristem temperature was controlled independent of air and soil temperature, leaf elongation decreased uniformly with each unit temperature decrease in the apical meristem region (Barlow et al., 1977). This decrease in leaf elongation is attributed to a decrease in biochemical processes in the meristematic region which reduce cell elongation as well as cell division (Kleinendorst and Brouwer, 1970).

Net photosynthesis and transpiration are less sensitive than leaf elongation to low soil temperature and low shoot apical meristem temperature (Barlow et al., 1977). The greater sensitivity of leaf elongation reduces the photosynthate sink which in turn enhances photosynthate accumulation within the plant (Grobbelaar, 1963; Barlow et al., 1976). The mechanism by which such photosynthate accumulation may influence further photosynthesis is not clear.

In summary, the reduction in leaf enlargement could affect the rate of dry matter accumulation by limiting the photosynthetic area available for light interception. Duncan and Hasketh (1968) stated that "vegetative dry weight growth of maize may be more dependent on the rate of leaf expansion than on the net rate of photosynthesis per unit leaf area". Cooper and Law (1977) concluded that it is the soil temperature during early growth, and hence leaf expansion rate, which is the most important factor in determining the vigor of early growth.

Information regarding the effect of low root temperature on final yield is meager. Cooper and Law (1977) observed a highly significant relationship ($r = 0.94$) between the size of plants at five weeks post emergence and the final grain yield. The rate of total dry matter accumulation was found to be positively correlated with leaf elongation rate (Barlow et al., 1976). Cooper and Law (1977) further mentioned that soil temperature coupled with soil moisture stress accounted for 70 percent of the variation of dry matter and 82 percent of the variation in final grain yield at five weeks post-emergence. Increasing soil temperature hastened the rate of plant development, and produced significant increases in dry matter production and corn yield (Mederski and Jones, 1963). Ketcheson (1968) reported that corn plants grown at low soil temperature required a longer period to the tasselling stage and to maturity. Increasing the soil temperature tended to shorten the period from emergence to silking (Jones and Mederski, 1963).

2.1.4 Growth Responses of Corn Seedlings to Chilling Events

The injurious effects of low temperature above freezing is called chilling. There is a large difference in the relative sensitivity to chilling between C₃ and C₄ species within the Gramineae. In chilling sensitive plants, the critical temperature below which injury occurs is most often around 10 - 12°C (Levitt, 1972). Nevertheless, C₄ plants start to show reduction in photosynthetic rates and growth as well as various physiological disorders when they are exposed to temperatures below about 20°C (Castleberry et al., 1978). The higher degree of physiological reaction of C₄ species to cool temperatures is closely associated to the geographic distribution of these species in temperate ecosystems (Teeri and Stowe, 1976) and may be an important variable limiting the productivity of C₄ grass crop species in cool temperate regions (Mock and Bakri, 1976). In addition, differences in relative sensitivity to low temperature events among C₄ grass species have been demonstrated (Rowley, 1976).

Of the important C₄ grass crop species, corn (Zea mays L.) appears to be the best adapted to various high altitude and latitude areas of the world. Considerable plant breeding efforts have been directed to improve cold tolerance in corn so as to exploit the potential of the crop under short or cool growing seasons (Andrew, 1954; Mock and Eberhart, 1972). Although the crop is grown in many cool temperate environments, corn plants exhibit many metabolic changes at chilling temperatures which are typical of other C₄ grasses. During exposure to ambient temperatures below 15°C, emergence is delayed and growth of seedlings is retarded (Cooper and Taiton, 1968). Significant reduction in growth rates resulting from chilling stress (12/10°C) has also been reported by Kibite (1977). Chilling stress reduces the rate of photosynthesis (Taylor and Rowley, 1971; Kibite, 1977; Teeri et al., 1977) with a concomitant drop in C₄ photosynthetic pathway enzymes (Taylor et

al., 1974; Stamp, 1980) and a loss of correspondance between rate of photosynthesis and stomatal conductance (Raschke, 1970). Chilling temperatures have been found to inhibit chlorophyll synthesis (McMillan and Naylor, 1967; Kibite, 1977; Teeri et al., 1977). The inhibition in chlorophyll synthesis has a direct effect on photosynthesis since net photosynthetic rate on a leaf area basis was closely correlated with leaf chlorophyll content (Teeri et al., 1977). Kibite (1977) summarized the literature regarding the influence of low temperature on changes in sugar and starch contents. In his findings, chilling stress increased sugar and starch content in both the roots and the leaves. An increase in respiration rate has been observed when corn plants were exposed to chilling (Creencia and Bramlage, 1971). However, Kibite (1977) has reported that chilling stress reduced the rate of respiration. There was an increase in the concentrations of free amino acids and water-soluble proteins in the leaves as well as in the roots when corn plants were subjected to chilling (Kibite, 1977). On the other hand, total protein declined in the leaves although an increase had been observed in roots. Chilling temperature has also been found to increase nutrient loss from the plant by increasing ion leakage (Creencia and Bramlaga, 1971).

There exists a wide range of genotypic variability in sensitivity to chilling. For example, Duncan and Hesketh (1968) evaluated 22 races of maize (Zea mays L.) including one selection of teosinte (Euchalaena mexicana Schrad) in glasshouses where the day/night temperatures were maintained from 15/10°C to 36/31°C. They found that high altitude races had relatively higher leaf growth rates and dry weights at low temperatures than low altitude races. These responses of low temperature tolerant genotypes could be due to a changed rate of development due to temperature and an altered temperature sensitivity of metabolic processes. Duncan and Hesketh (1968) indicated that when earlier germination was coupled with large initial seedling size and a more rapid leaf

growth rate, there were dramatic differences in plant sizes among the 22 races of maize grown at the lower temperatures. These differences in the size of seedling were correlated with seed weights.

Detailed studies have not been made regarding changes in metabolic processes for genotypes that do well under cool temperature situations. Hadzi-Taskovic Sukalovic and Jelenic (1980) reported that an increase in soluble protein content of the leaves and a decrease in nitrate reductase activity were associated with tolerance to low temperature. Kibite (1977) pointed out 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". In the lower leaves of young corn plants subjected to low temperature, chlorophyll and carotenoid contents and the activities of photosynthetically active enzymes were depressed by low temperature to a greater extent in the cold sensitive line than in the tolerant one (Stamp, 1980).

A decrease in temperature brings about a decline on leaf number (Duncan and Hesketh, 1968; Tollenaar et al., 1979) and leaf area (Castleberry et al., 1978). The rate of leaf area expansion is related to the area of adaptation and maturity (Castleberry et al., 1978). The leaf area, in turn determines seedling size since leaf area is directly related to dry-weight growth (Duncan and Hesketh, 1968). On the other hand, the duration of the period from planting to silking is associated with both the rate of leaf growth and number of leaves per plant (Tollenaar et al., 1979).

2.2 GENETIC AND ENVIRONMENTAL FACTORS AFFECTING COLD TOLERANCE RESPONSES OF CORN (Zea mays L.)

Most early cornworkers considered disease organisms to be the primary causes of poor seed germination and poor plant stands in the field. Seventeen fungal organisms were reported to be the cause of

seedling blight of maize under early spring suboptimal conditions. These organisms include Pythium, Gibberella zea, Diplodia zea and several species of Helminthosporium (Andrew, 1954). Pythium species live in almost all soils and are considered to be the principal fungi involved in seed rotting under soil conditions (Hoppe, 1955, 1956).

Andrew (1954) obtained poor stand establishment when untreated seeds of corn were planted in non-sterile soil at 50°F for a long period after which the temperatures was raised to an optimum level. On the other hand, a nearly perfect stand was obtained when treated seeds were placed in sterile soil under the same temperature conditions.

In most of these early studies, there was great emphasis on the development of genetic resistance to seed-rotting microorganisms. Nevertheless, with the development of effective chemical seed treatment, efforts directed along these lines have declined (Mock and Eberhart, 1972). At present, the breeding of genotypes with genetic potential to germinate and emerge rapidly at low air and soil temperatures is receiving high priority.

Aside from the influence of soil disease organisms, a number of non-genetic factors are involved in stand establishment when seeds are germinated at low temperatures. There is adequate evidence that the amount of pericarp injury in a given seed lot is highly correlated with low stands obtained in the field under adverse conditions (Brown, 1920; Meyers, 1924; Alberta, 1927; Koehler, 1936). Tatum and Zuber (1943) revealed that injuries in the germ area of the seed were more important in stand reduction than those occurring in other regions of the kernel. Improper processing methods were considered to be responsible for considerable unnecessary injury to commercial seed corn (Kaerwer, 1953).

Immature seed with a high moisture content at harvest is less cold tolerant than that which has reached full maturity. There is a progres-

sive increase in germination percentage and good stand establishment with advancing seed maturity (Neal, 1949; Andrew, 1954).

Age of seed also influences its cold tolerance. There is a progressive decline in cold tolerance and seedling vigor with increasing age of seed (Neal, 1949; Andrew, 1954). Unlike high quality seeds, weak seeds produce slowly emerging, less vigorous seedlings resulting in less productive plants (Funk et al., 1962). Although the rate of loss of viability varies with the strain, high moisture in the seed, high humidities and high temperature during storage enhance the rate of deterioration (Andrew, 1954).

Seeds damaged by frost usually have reduced cold tolerance which varies with the moisture content of the seed at the time of damage, also the extensiveness of the frost damage (Andrew, 1954). Neal (1949) and Koehler (1954) have also mentioned that seeds exposed to frost prior to harvest are susceptible to low temperatures at the time of germination.

While these environmental considerations are important to the understanding and improvement of cold tolerance, hereditary differences are of major interest to a plant breeder. Several researchers have shown that extensive genetic variation exists in the ability of corn genotypes to germinate at low temperatures (Haskell and Singleton, 1949; Neal, 1949; Pinnell, 1949; Andrew, 1954; Grogan, 1970; Mock and Eberhart, 199972; Kushibiki, 1973; Eagles and Hardacre, 1979a). Miedema (1979) examined maize and Phaseolus vulgaris for their ability to germinate at low temperatures. The results showed that greater genetical variation exists for the capacity to germinate at low temperatures than for seedling growth at the same low temperature.

Large genotypic variances have also been found for percentage emergence, emergence rate, and seedling dry weight among the S1 lines from two U.S. corn belt breeding populations (Mock and Eberhart, 1972). Furthermore, Mock and Skrdla (1978) obtained high values for heritability

and genetic variances when they used the three traits described by Mock and Eberhart (1972) to evaluate 144 maize introductions for cold tolerance. Fakirede and Ojo (1980) indicated that high genotypic variances were observed for emergence percentage, emergence index, dry matter accumulation, and relative growth rate among 36 maize populations evaluated under tropical conditions. High levels of variability for the three cold tolerance traits has also been obtained among 34 inbred lines adapted to various latitudes in North America (Mock and McNeill, 1979).

Along with the presence of genetic variation, information on gene action is essential in order to draw conclusions concerning the choice of breeding material and the breeding system to be employed to improve cold tolerance. Pinnell (1949) and Grogan (1970) concluded that the genetic nature of cold tolerance in corn is complex because of large maternal effects on germination and early growth. Eagles and Hardacre (1979a) indicated that maternal influence was of overwhelming importance for germination percentage under low temperatures. A hybrid with a cold tolerant female parent and a cold susceptible male parent was found to be cold tolerant while the reciprocal cross resulted in hybrids that were cold susceptible (Andrew, 1954). The maternal effect was in most cases attributed to the double genetic contribution of the female parent to the endosperm and in a few other instances, to either the influence of the pericarp or to cytoplasmic differences (Ventura, 1960).

Ventura (1960) studied the cold test reaction of F₁, F₂, F₃, backcross, and selfed backcross populations of a cross between two inbreds and concluded that the inheritance of cold tolerance was quantitative. Grogan (1970) reported that heterosis favourably influenced germination and growth at cool temperatures and concluded that an additive multiple-factor genetic system conditions cold tolerance in corn. Pesev (1970) considered inheritance of cold tolerance to be rather complex and concluded that the better stand establishment of single crosses

over inbreds was attributable to complementary gene action.

The ability to germinate at low temperatures appears to be heritable and independent of factors controlling germination at higher temperatures (Kushibiki, 1973). The occurrence of low correlations between emergence percentage, emergence index, and seedling dry weight in the "cold" environments, stand and tasseling date in a "normal" environment led Mock and Eberhart (1972) to conclude that the genetic system conditioning cold tolerance in two adapted maize populations are independent of those controlling emergence and maturity under normal planting conditions. Cabulea and Ochesanu (1979) studied diallel crosses involving eight lines and established that additive effects were important in controlling low-temperature response during germination, while non-additive effects were important at the three-leaf stage. However, McConnell and Fardner (1979b) indicated that epistatic gene effects as well as additive and dominance gene effects contributed significantly to the variation observed for germination at 7.2 C in the laboratory and for emergence measured in the field. They also showed that growth after emergence in the field was predominantly conditioned by additive and dominance gene effects. Keim and Gardner (1979) studied selected and unselected populations for cold tolerance in the field and found that dominance variance was important in determining days to 30 percent and 50 percent emergence. Both additive and dominance variance effects were shown to be important regarding emergence index.

In his studies, Bojarczuk (1980) mentioned that flint corn lines were generally more resistant to cold conditions and soil-borne fungus infection during germination than dent corn lines and that hybrids were found to be more resistant than inbreds. His F₂ data from resistant X susceptible crosses revealed that these resistances are conditioned by dominant genes.

In summary, the various studies involving types of gene action indicated the importance of both additive and non-additive gene action in the inheritance of cold tolerance.

Regarding germplasm sources for cold tolerance improvement, Mock and Eberhart (1972) emphasized the use of adapted corn populations rather than unadapted genotypes. However, cold tolerance responses were reported to have no association with geographical areas of adaptation (Mock and Skrdla, 1978; Mock and McNeill, 1979). Furthermore, Eagles and Hardacre (1979b) found substantial genetic variation in the rate of emergence at 10°C in a population consisting of germplasm of highland tropical and temperate origin (CIMMYT Pool5), with some families from the population markedly superior to the U.S. Corn Belt Dent hybrids used as checks. Consequently, the development of cold tolerant genotypes adapted to all latitudes of maize producing areas should be possible (Mock and Skrdla, 1978).

Cold tolerance studies on two U.S. Corn Belt populations indicated the existence of sufficient genotypic variation for improvement to be realized by selection within the populations (Mock and Eberhart, 1972). Mock and Bakri (1976) evaluated the progeny from several cycles of recurrent selection for cold tolerance traits (emergence percentage, emergence index and seedling dry weight) in the two populations used by Mock and Eberhart (1972). They found that in one population (BSSS13) two cycles of selection had improved percentage of emergence and seedling dry weight by 8.5% and 0.6 g per cycle, respectively. In a similar study, McConnell and Gardner (1979a) found that in two populations, CTCG and SSCG, four cycles of selection improved cold germination under laboratory conditions (7.2°C) by 8.8 and 9.9 percent per cycle, respectively. In the field, however, very little progress was observed because of very mild spring weather during the two years of evaluation.

Annonkova (1976) mentioned that from cold tolerant locally adapted homozygous lines, single cross and double cross hybrids were developed which possessed relatively high yield potential and substantial resistance to unfavourable weather conditions. The cold tolerance study in selected and original maize populations revealed that the selected population out-yielded the original population, although high variability for grain yield was prevalent in both populations (Keim and Gardner, 1979). However, no significant differences in yield between C₀ and C₄ cycles have been observed in two U.S. populations (McConnell and Gardner, 1979a).

Three-way crosses involving only cold susceptible dent lines produced highest yields per plot (Bojarczuk, 1980). Furthermore, McConnell and Gardner (1979a) pointed out that warm-season x warm-season crosses resulted in the highest grain yield followed by cold-season x warm-season and cold-season x cold-season crosses.

Index selection using the three cold tolerance traits (emergence percentage, emergence index and seedling dry weight) could improve cold tolerance for those traits since high correlations have been observed among them (Mock and Skrdla, 1978; Mock and McNeill, 1979). Fakorede and Ojo (1981) showed that emergence percentage, emergence index and relative growth rate are reliable indices for seedling vigor. Growth rate by itself was not an effective indicator since it was highly influenced by the environment.

Because of the presence of highly significant genotype X environment interactions, it is highly desirable to undertake cold tolerance tests in more than one environment (Mock and Eberhart, 1972; Mock and McNeill, 1979). Crosbie et al., (1980) suggested that in order to cope with genotype X environment interactions, it is desirable to select for dry weight/plot since the data can be easily collected at several locations. He further stated that,

instead of using indices with the data biased by genotype X environment interactions in a recurrent selection program, greater gain for cold tolerance would likely be realized on the basis of the dry weight/plot data collected at several locations.

2.3 RELATIONSHIP BETWEEN COLD-TEST PROCEDURES AND FIELD PERFORMANCE IN CORN

Unpredictable weather conditions in the field from year to year have prompted breeders to investigate various controlled environment testing techniques which can simulate field conditions, thereby facilitate evaluation of genotypes. Haskell and Singleton (1949) seeded sweet and field corn in untreated soil and subjected them to 10°C for 32 days. The seedlings were then moved to a warm greenhouse to complete germination. A comparison of the final greenhouse germination with germination of early sowings in the field showed significant correlation coefficients of +0.55 for sweet corn and of +0.62 for field corn. Pinnell (1949) also reported that percent stand under field conditions was significantly correlated with simulated cold test percent stand as well as with cold test vigor. Moreover, Miedema (1978) observed that dry matter yield of early sowing was significantly correlated with dry weight in growth chambers at day/night temperatures of 15/10°C, but not with dry weight at either 20/15°C or 25/20°C.

Various versions of the laboratory cold-test were studied in 15 corn inbred lines by Burris and Navratil (1979). Substrates ranged from sterile kimpak to sand-soil mixtures to a test utilizing pure soil. In all tests a 10°C stress for seven days followed by a grow-out period at approximately 25°C was included. Correlation coefficients between laboratory tests and field performance were highly significant. However, the cold-test was not a consistently reliable predictor of early field emergence since the variability inherent in the test made comparison between laboratories difficult.

Mock and Eberhart (1972) reported that predicted selection responses indicated that field selection for cold tolerance would be more efficient than growth chamber selection. However, McConnell and Gardner (1979b) found that four cycles of selection substantially improved cold germination under laboratory conditions while very little progress was made in the field owing to the very mild spring weather during the two years of evaluation. This suggests that field selection will not be effective unless conditions are favorable. Thus, they suggested the use of laboratory procedure since environmental conditions are reproducible and continued progress could be expected.

2.4 THE EFFECT OF SEED SIZE ON THE GROWTH OF CORN SEEDLINGS AT LOW TEMPERATURES

When two hybrids differing in seed size were seeded at three planting dates and depths, they exhibited similar performance in the rate of emergence, final percent emergence, and grain yield (Hunter and Kannenberg, 1972). They also exhibited similar performance in both rate and percent emergence at three temperature regimes. Furthermore, Eagles and Hardacre (1979a) reported that germination percentage, emergence percentage and emergence time for full-sib and S_1 families evaluated at 10°C were not significantly associated with seed weight.

Kemp (1978) found that large seed size was significantly associated with leaf area growth rate. It was also evident that lines with large seed had an initial advantage over lines with small seed. Eagles and Hardacre (1979b) evaluated S_1 and full-sib families at 15/10°C day/night temperatures. Shoot weight and days to pollination were significantly correlated with seed weight for both families. Hawkins and Cooper (1979) reported that the large, medium and small seeds of H613c showed pronounced differences in dry matter production until the stage of tasselling. Despite the differences in plant size, there were no significant differences in yield.

2.5 THE EFFECT OF TOTAL OIL AND FATTY ACID CONTENT OF THE SEED
ON THE GROWTH OF CORN SEEDLINGS AT LOW TEMPERATURE

About 85 percent of the total lipids in a corn kernel occur in the germ. Analysis of 125 corn inbreds of the midwest gave a total oil content between 1.2 and 5.7 percent (Quackenbush et al., 1963). The major fatty acids of corn oil are palmitic (11.1 percent), stearic (2.0 percent), linoleic (61.9 percent), oleic (24.1 percent), and linolenic (0.7 percent).

The degree of unsaturation of seed fatty acids has been shown to relate to the chilling sensitivity of seeds. Gransenkov (1968) reported that:

highest iodine number was found in oil from the cold hardy varieties. Leaves of varieties with a high iodine number had the highest leaf temperatures after exposure for 2 hours to a subzero temperature. Such forms have a higher level of energy metabolism and so withstand low temperatures better.

Similar observations for those fatty acids were also made by Whelan as cited by Gubbels (1974) in cold germination studies with cucumber. Gubbels (1974) indicated that seed size, total oil content, and linoleic acid percentage in the seed were positively correlated with seedling vigor. Bartkowski et al. (1977) also found that cotton cultivars with high levels of fatty acid unsaturation, showed increased emergence at low temperatures.

3. MATERIALS AND METHODS

3.1 GENOTYPES

A total of 32 inbred lines (Appendix Table 2) of corn (Zea mays L.) was initially chosen for the present study (Table 2). The lines were obtained from the University of Manitoba corn breeding program and were chosen on the basis of their response to cool temperature conditions in the field. From the initial 32 lines, 12 were subsequently chosen for more in-depth studies including post emergence behavior during chilling, response to low root zone temperatures, and field evaluation. The selected inbred lines represented a range of responses.

Before embarking on the various tests, seeds from all lines were subjected to germination and emergence tests under favorable day/night temperatures of 28/18°C. All of the seeds used during this study were produced under the same environmental conditions and all lines exhibited an emergence of over 90 percent. Lack of sufficient seed affected the choice of inbred lines.

3.2 CULTURAL PRACTICES AND EXPERIMENTAL DESIGN USED IN CONTROLLED ENVIRONMENTS DURING THE INITIAL TEST OF 32 CORN INBRED LINES

Clay pots (14 cm) were filled with a mixture of soil, sand and peatmoss in a 2:1:1 ratio, respectively. The seeds of the 32 inbred lines were soaked overnight in water containing a fungicide (Thiram) and 20 seeds of each line were potted at a depth of 2.5 cm in the potting mixture and placed in a growth cabinet. The ambient temperature in the cabinet was maintained at a constant 10° C since this temperature is close to the minimum for corn germination and emergence (Blacklow, 1972). After two weeks, the day temperature was raised to 15° C which

is the maximum air temperature during the first two weeks of May in Winnipeg (Coligado et al., 1968). Photoperiod in the cabinet was controlled at 16 h and the light intensity at 800 microeinsteins M⁻². Sec.⁻¹. Thirty days after planting, the seedlings were moved to a growth room in which the ambient day/night temperatures were adjusted to 19/10° C. Once again, the 19° C day temperature was chosen in an attempt to simulate field conditions of maximum air temperature in Winnipeg during the last two weeks of May.

The experiment was arranged as a randomized complete block design with two replications. The number of emerging plants was recorded every other day for 30 days. In each pot, the emerging plants were thinned to three uniform seedlings and an emergence index was calculated from these data using the following formula (Smith and Millett, 1964):

$$EI = \frac{\sum (\text{Plants emerged in a day}) (\text{Days after planting})}{\text{Total no. of plants emerged 30 days after planting}}$$

To obtain an emergence percentage, the total number of seedlings that emerged 30 days after planting was also expressed as a percentage of the total number of seeds planted.

Forty days after planting, data were taken on leaf area (length x width x 0.75) per plant and juvenile plant height (distance from the soil surface to the leaf whorl). On the same day, the above-ground plant tissue was harvested, dried to uniform moisture in an oven at a temperature of 80 C, and weighed. An analysis of variance for a randomized complete block design was performed for each trait. Using the expectations of mean squares from the ANOVA, phenotypic variances were partitioned into environmental variances (σ_e^2) and genotypic variances (σ_g^2). Broadsense heritabilities (h_b^2) were computed for each trait using the formula of Frey and Horner (1955):

$$h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/r}$$

Simple correlations were also calculated among the above-mentioned traits. Inbreds were rated for cold-tolerance responses by a rank-summation index (Mock and Skrdla, 1978). This index was obtained by: (i) ranking means of each of the three cold-tolerance traits for each inbred and (ii), summing the rankings for the three traits for each inbred. On the basis of this index, an inbred that was best (i.e., ranked first) for all three traits would have an index value of 3.0. It was upon the results of these tests that 12 of the original 32 inbred lines were chosen for more detailed evaluation of their cold tolerance.

3.3 EXPERIMENTAL PROCEDURES FOLLOWED WHILE TESTING TWELVE CORN INBRED LINES AT THREE ROOT ZONE TEMPERATURES

Plastic pots, 20 cm long, 9 cm wide, and 20 cm deep were filled with sand and soil mixture in a 2:1 ratio, respectively. The moisture content of the mixture was maintained at 15 to 20 percent by weight throughout the period of testing.

A sample of seed of each inbred line was soaked in water overnight. Ten seeds were then potted at a depth of 2.5 cm in the prewetted mixture. The surface of the mixture was covered with aluminum foil until seedling emergence. After emerging, plants were thinned to three uniform seedlings per pot, the soil surface of each pot was covered with styrofoam chips to minimize the temperature gradients and excessive water loss by direct radiation from fluorescent lamps in the cabinet. The pots were placed in each of three water troughs having thermostatically controlled temperatures of 10, 14 and 18° C, respectively. A temperature of 10° C was chosen as the lowest temperature because it is the minimum temperature required for the emergence of corn seeds. Also, according to Larter (1981), a soil temperature of 10°C is not uncommon for Manitoba soils at the time of seeding and during early seedling growth. Since the growth cabinet ambient temperature during the day was 18°C, a maximum monthly air temperature in Winnipeg for May (Coligado et

al., 1968), the highest root zone temperature was maintained at 18° C. The 14°C temperature was chosen as an intermediate between the lowest and the highest root zone temperatures. Although the minimum air temperature in Winnipeg throughout the month of May is frequently below 10°C (Coligado et al., 1968), for experimentation purposes the ambient night temperature was maintained at 10°C. Photoperiod was programmed for 16 hours with a light intensity of 590 Microeinsteins.M⁻².Sec.⁻¹.

Two weeks after seeding, the initial soil temperatures of 10 and 14° C were raised by 2 and 1° C, respectively, while the control temperature (18° C) was kept constant. This revised temperature regime simulated field conditions since the average soil surface temperature in Manitoba reaches 12° C at about May 15 (Hallem and Woodbury, 1981).

The experiment was arranged in a split plot design with soil temperatures as main-plots and genotypes (inbred lines) as sub-plots. Since space in the growth chamber restricted the experiment to only one replication, the trial was repeated three times in order to produce, in effect, three replications. The procedures followed while recording emergence percentage and emergence index were identical to the ones that were described on page 28 of this thesis for the testing of the original 32 inbred lines under controlled environments.

Leaf area measurements were conducted using the second leaf of each plant (length x width x 0.75) and averaging the readings from three plants. On the same day, the above-ground plant tissue was harvested and the roots were carefully washed with tap water. Individual dry weights of both the shoot and the roots were determined after drying to uniform moisture in an oven at a temperature of 80°C.

Inspection of the data showed that the variances among individual readings of certain sets of data were not homogeneous. Logarithmic transformation stabilized the variances for seedling dry weight, root dry weight, shoot:root ratio, and leaf area data, and subsequent analyses for

those traits were performed using Log_{10} transformed variables. An analysis of variance for a split-plot design was performed for each trait, and using the expectations of mean squares from ANOVA, the phenotypic variance was partitioned into environmental variance (σ_e^2) and genotypic variance (σ_g^2). Whenever the nonsignificant genotype x temperature interaction was obtained, the genotype x temperature sums of square and error sums of square as well as their respective degrees of freedom were pooled in order to determine error variance (σ_e^2). Broad-sense heritabilities (h_b^2) were computed for each trait using the formula (Fakorede and Ojo, 1981):

$$h_b^2 = \frac{\sigma_g^2}{\sigma_e^2 / rt + \sigma_{ge}^2 / r + \sigma_g^2}$$

where

r = number of replications, and

t = number of temperature regimes.

Simple correlations were calculated among eight traits and inbreds were rated for cold tolerance responses by a rank-summation index (Mock and Skrdla, 1978).

3.4 PROCEDURES USED IN POST-EMERGENCE BEHAVIOR STUDIES DURING CHILLING TREATMENTS

Seeds of each inbred line which were treated with a seed dressing chemical (Thiram) were placed at a depth of about 2.5 cm in a mixture of sand, soil and peatmoss (2:1:1, respectively) contained in clay pots. Plants sampled for dry weight on the seventh day post-emergence were grown in 12 cm pots while all other plants were grown in 14 cm pots. The pots were transferred to a growth cabinet operating at 28°C day/18°C night temperatures with 16 hours photoperiod and a light intensity of 800 Microeinsteins.M⁻² Sec.⁻¹. Low temperature treatment (10/10°C) was imposed starting from the seventh day to the fourteenth day post-emergence and sampling of plant material was conducted every seventh day from the

time of emergence. Plants receiving seven days of favourable temperature (28/18 C) followed by seven days of low temperature (10/10°C) are referred to as "chilled".

In order to minimize the effects of environmental factors, all pots used in the experiment were filled on the same day and those which were not required immediately were temporarily stored. The experimental material was watered daily and sampling for seedling dry weight was done in the second half of the light period. For each temperature treatment, the experiment was arranged in a randomized complete block design with three replications.

Leaf area (cm²/plant) were recorded and plants used for dry weight determination were cut at the soil level and dried to uniform moisture in an oven at 80°C. Three plants were sampled per pot and harvesting was done on the seventh and fourteenth day after emergence. Dry weight was expressed as grams of dry matter produced per plant. Beauchamp and Lathwell (1967) stated that dry matter production of plants grown at low temperature (15°C) may not contribute to further plant growth in a manner comparable to that of plants grown at higher temperature (25°C). Specifically, they stated, "the additional dry weights of the shoots of plants which grew at 15°C offered very little to the overall development of the plants". Because of this relationship, absolute growth rates (total dry matter produced per day) were used to compare growth rates at different root zone temperatures instead of relative growth rates (rate of dry matter accumulation per unit dry matter present). In this study, therefore, it was decided to use absolute growth rates to evaluate the influence of favourable and chilling temperatures on dry matter accumulation of young corn seedlings. The formula used to determine absolute growth rates was:

$$\text{Absolute growth rate} = \frac{W_2 - W_1}{t_2 - t_1}$$

where

W_1 and W_2 were the dry matter weights of the plants at t_1 (7 days) and t_2 (14 days), respectively.

An attempt was made to carry out an analysis of variance on the combined data with the two classifications being inbred lines and temperature treatments (7 days 28/18°C, also 7 days 28/18°C + 7 days 10/10°C). The 14-day control 28/18°C) was not included in the combined analysis since its error variance was significantly different from those of the two temperature treatments. Genotype and treatment effects were tested against the genotype x treatment interaction mean square which in turn was tested using the mean sum of squares for pooled error. Simple correlations among all traits studied were also calculated.

3.5.1 Cultural Practices and Experimental Design in Field Trials

Twelve corn inbred lines were seeded at two planting dates over a two-year period, 1982 and 1983, at the University of Manitoba experimental station (Point). The planting dates for the 1982 trial were April 21 and May 5; those for the 1983 trial were April 19 and May 3. Since early May is the "normal" time for corn seeding in Manitoba, dates which were about two weeks earlier were chosen in order to evaluate the performance of inbred lines under cold, wet soil conditions.

The trials were arranged in a split-plot design with dates as main plots and inbred lines as sub-plots. Seed supply limited the trial to two replicates in 1982, however, six replicates were used in 1983. Twenty seeds of each entry were seeded in a row (plot), 5 m in length, spaced 75 cm apart and at a depth of 5 cm per row per entry. The seeds were treated with a fungicide, Thiram. A thermocouple was used to record soil temperatures at a depth of 5 cm twice a day (6 a.m., and 4:30 p.m.). The mean weekly minimum and maximum soil temperature was calculated from these data for a given period in each of the two years as shown in Table 18.

The number of emerging plants was recorded every other day for 30 days in 1982, and for 50 days in 1983. Emergence index, a measure of the rate of emergence, was calculated from these data using the formula of Smith and Millet (1964).

Emergence percentages were determined based on the total number of seedlings that emerged 30 and 50 days after planting in 1982 and 1983 cropping seasons, respectively, and were expressed as percentages of the total number of seeds planted. Dry weights of above ground tissue were determined from three seedlings selected at random from each row. In the 1982 field trial, seedlings were sampled on June 1 for the first date of planting, and on June 16 for the second date of sowing. In the 1983 trial, seedlings of both populations were sampled on the same date (June 24). Seedling dry weights were determined on the basis of oven drying to a constant weight at a temperature of 80°C. Juvenile plant height and leaf numbers/plant were recorded from the same three plants immediately prior to oven drying. Silking date was recorded for each plot when 50 percent of the remaining plants in the plot displayed silk emergence.

Since lines CK52, CK64, and FV212 had fewer than 10 plants per plot in the early date of sowing (April 19), yield samples were obtained on the basis of five plants randomly selected from each plot. After storage in a well ventilated drying room, sampled ears were shelled by hand. For moisture determination a 15-gram sample was taken from each 5-plant bulk and dried to constant weight in a dryer at 120° C. Each moisture sample was used to convert to respective yield figures based on zero percent moisture.

3.5.2 Analysis of Data

Unfortunately, hot dry climatic conditions during the early spring of 1982, adversely affected germination of seed of the first date

of planting. Furthermore, a severe infestation of cutworms further reduced the plant populations, rendering the data from this trial highly unreliable. Therefore, these data are omitted from the present analysis, but are contained in Table 1 of the Appendix of this thesis.

For the 1983 trial, an analysis of variance was performed for each trait, and using the expectations of mean squares from ANOVA, phenotypic variance was partitioned into environmental variance (σ_e^2), genotypic variance (σ_g^2) and genotype x environment interaction variance (σ_{ge}^2). A broadsense heritability (h_B^2) was computed for each trait using the formula of Fakorede and Ojo (1981).

Simple correlations were calculated among all traits for the 1983 data. In addition, means for the three cold tolerance traits, emergence percentage, emergence index, and seedling dry weight, were computed for each inbred line over the two planting dates. The inbreds were then rated for cold-tolerance responses by a rank-summation index (Mock and Skrdla, 1978).

3.5 OIL EXTRACTION PROCEDURES AND FATTY ACID ANALYSIS

Clean seeds were ground using a roller mill. The middle portion of the milled material was taken for extraction in order to minimize the possibility of mixing with residual ground material in the mill although the mill was cleaned out after each sample. The samples were oven-dried and kept in a desiccator until used for extraction.

Oil extraction was made on a 5 g sample of ground material using a Soxhlet extractor. The extraction process was allowed to proceed for a period of 6 h, the oil being dissolved in a beaker containing petroleum ether. The solvent was subsequently evaporated off using a 60°C water bath under a fume hood. Percent oil was calculated using the formula:

$$\frac{((\text{Weight of beaker} + \text{Oil}) - \text{Weight of beaker}) \times 100}{\text{Weight of meal}}$$

In order to induce methanolysis, a sample of oil (0.1 g) was dissolved in 1 ml of methanol, and 1 ml of sodium methoxide (2N) was added. The tube was allowed to stand at 50° C for methanolysis to take place after which the reaction was terminated by addition of 5 ml of distilled water. The hexane layer was removed and placed in a small vial. From this layer, one micoliter was injected into a Gas Chromotograph column and the percent fatty acid composition was recorded.

4. RESULTS AND DISCUSSION

4.1 EARLY GROWTH RESPONSES OF 32 CORN INBRED LINES TO SUBOPTIMAL TEMPERATURES

Mean squares from analysis of variance for five traits of the original population of 32 inbred lines are presented in Table 1. Significant differences occurred among inbred lines for emergence percentage, emergence index, seedling dry weight, leaf area, and plant height.

4.1.1 Seedling Emergence

Mean differences in emergence percentage ranged from 45 to 100 percent (Table 2). Many corn workers have reported similar variability in emergence percentage of maize germinated at low temperatures (Pinnell, 1949; Mock and Eberhart, 1972; Mock and McNeill, 1979; Mock and Skrdla, 1978; Eagles and Hardacre, 1979a; Eagles and Brooking, 1981). Closer examination of the means (Table 2), however, revealed that 28 of the 32 inbred lines were not significantly different from one another in emergence. The relatively high emergence percentage (over 85 percent) and the absence of significant differences among those lines could possibly be a consequence of selection. In Manitoba, genotypes are planted in cold soils during the early spring and since the growing season is short, selection is routinely conducted for improved standability and early maturity. As indicated by Pinnell (1949), standability under both controlled cold tests and field tests was significantly correlated with earliness of maturity.

Among inbred lines, there was substantial genetic variation in emergence index which is an estimate of rate of emergence (Table 2).

Table 1. Mean squares from analysis of variance for various traits of 32 corn inbred lines grown at suboptimal ambient temperatures (15 days 10/10°C + 15 days 15/10°C + 10 days 19/10°C).

| Source | df | Emergence (%) | Emergence Index (days) | Seedling Dry Weight (g) | Leaf Area Per Plant (cm ²) | Plant Height (cm) |
|---------|----|---------------|------------------------|-------------------------|--|-------------------|
| Blocks | 1 | 0.391 | 2.262 | 0.022 | 761.926* | 1.351 |
| Inbreds | 31 | 286.076*** | 1.929* | 0.145*** | 1,156.493*** | 8.602*** |
| Error | 31 | 45.552 | 0.878 | 0.017 | 143.942 | 0.417 |
| C.V. % | | 7.340 | 5.600 | 23.688 | 18.980 | 7.562 |

*, *** Significant at 0.05 and 0.001 level, respectively.

Table 2. Rank-summation-index values and means of various traits of 32 corn inbred lines grown at sup-optimal temperatures (15 days 10/10°C + 15 days 15/10°C + 10 days 19/10°C).

| Inbred | Index Value | 100-seed Weight (g) | Emergence (%) | Emergence Index (Days) | Seedling Dry Weight (g) | Leaf Area (cm ² /plant) | Juvenile Plant Height (cm) |
|--------|-------------|---------------------|----------------------|------------------------|-------------------------|------------------------------------|----------------------------|
| CK52 | 9 | 28.22 | 100.0 a ¹ | 15.70 defg | 1.034 abc | 102.06 bc | 12.50 b |
| CK62 | 11 | 26.12 | 100.0 a | 14.70 g | 0.672 defg | 72.82 defgh | 9.00 cdefg |
| CO255 | 13 | 24.30 | 100.0 a | 15.15 efg | 0.757 cdef | 85.29 bcde | 9.75 cd |
| CK76 | 26 | 24.18 | 97.5 a | 16.51 abcdefg | 1.132 ab | 107.32 b | 14.00 a |
| RB214 | 26 | 30.18 | 100.0 a | 16.80 abcdefg | 0.768 cdef | 73.32 defgh | 9.50 cde |
| CK29 | 32 | 21.01 | 100.0 a | 16.70 abcdefg | 0.538 defgh | 52.37 fghij | 6.50 jk |
| CK65 | 34 | 25.70 | 97.5 a | 16.38 bcdefg | 0.585 defgh | 67.92 defghi | 7.75 fghij |
| CH12 | 35 | 32.28 | 85.0 ac | 15.03 fg | 0.844 bed | 90.76 bcd | 12.00 b |
| CK26 | 35 | 24.86 | 97.5 a | 16.20 bcdefg | 0.442 ghi | 64.10 defghij | 9.50 cde |
| CK47 | 36 | 36.24 | 97.5 a | 16.06 bcdefg | 0.430 ghi | 59.28 efghij | 6.50 jk |
| VC0264 | 40 | 40.60 | 95.0 ab | 17.21 abcdef | 1.193 a | 137.79 a | 12.40 b |
| CK31 | 41 | 28.84 | 97.5 a | 15.08 efg | 0.314 hij | 59.80 efghij | 8.75 defg |
| CK74 | 41 | 23.58 | 100.0 a | 16.30 bcdefg | 0.187 ij | 35.62 jk | 5.60 k |
| CK64 | 42 | 22.31 | 100.0 a | 16.35 bcdefg | 0.216 ij | 48.91 ghij | 7.00 hijk |
| CK27 | 43 | 19.78 | 97.5 a | 16.57 abcdefg | 0.438 ghi | 56.79 efghij | 8.50 defgh |
| FV212 | 44 | 15.60 | 100.0 a | 17.55 abcd | 0.458 fghi | 45.49 hij | 8.25 defghi |
| D14 | 46 | 34.96 | 97.5 a | 18.25 ab | 0.797 cde | 65.71 defghi | 8.50 defgh |
| CK36 | 49 | 24.83 | 95.0 ab | 15.89 cdefg | 0.379 ghij | 56.20 efghij | 8.25 defghi |
| CK82 | 49 | 21.31 | 90.0 ab | 16.70 abcdefg | 0.637 defg | 79.06 cdef | 8.50 defgh |
| CK84 | 49 | 21.34 | 87.5 abc | 16.51 abcdefg | 0.611 defgh | 72.97 defgh | 9.25 cdef |
| D121 | 52 | 26.08 | 92.5 ab | 17.48 abcd | 0.982 abc | 77.58 cdefg | 10.50 c |
| CK77 | 55 | 24.47 | 100.0 a | 18.70 a | 0.392 ghij | 39.56 ij | 7.50 ghij |
| CK79 | 55 | 23.90 | 95.0 ab | 17.30 abcdef | 0.505 efghi | 48.55 gkij | 6.75 ijk |
| CK83 | 59 | 17.90 | 97.5 a | 17.39 abcde | 0.385 ghij | 45.26 hij | 7.50 ghij |
| CK54 | 65 | 27.70 | 80.0 b | 16.30 bcdefg | 0.373 ghij | 42.80 ij | 7.00 hijk |
| CM72 | 66 | 16.24 | 95.0 ab | 17.58 abcd | 0.429 ghi | 50.16 fghij | 8.50 defgh |
| CK71 | 71 | 24.64 | 87.5 abc | 16.80 abcdefg | 0.364 ghij | 40.62 ij | 7.50 ghij |
| CK25 | 72 | 22.56 | 90.0 ab | 17.06 abcdef | 0.312 hij | 51.28 fghij | 9.25 cdef |
| CK69 | 72 | 19.87 | 87.5 abc | 17.38 abcde | 0.387 ghij | 46.55 hij | 7.75 fghij |
| CK22 | 75 | 23.10 | 65.0 d | 18.34 ab | 0.550 defgh | 78.56 cdef | 8.00 efghij |
| CK67 | 82 | 21.63 | 45.0 e | 18.08 abc | 0.428 ghi | 54.58 fghij | 7.10 hijk |
| CK59 | 85 | 22.62 | 72.5 cd | 17.33 abcdef | 0.101 j | 13.75 k | 4.00 l |
| Mean | | 24.90 | 92.0 | 16.73 | 0.551 | 63.21 | 8.54 |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test.

Mean values for emergence index ranged from 14.7 days for CK62 to 18.7 days for CK77. On the average, the inbred lines emerged in about 17 days after seeding. The results of Mock and Skrdla (1978), Eagles and Hardacre (1979a, 1979b), and Eagles and Brooking (1981) support the present findings in that these workers also observed genotypic differential in the rate of emergence at lower temperatures.

4.1.2 Seedling Dry Weight, Leaf Area, and Juvenile Plant Height

Marked differences were observed among inbred lines for seedling dry weight. Mean values for this trait ranged from 0.101 g for CK59 to 1.193 g for VC0264 (Table 2). The differences in 100-seed weight contributed to the variation in seedling dry weight. This was confirmed by the significant correlation found between 100-seed weight and seedling dry weight (Table 3). However, closer examination of the means indicated that lines with similar seed weight exhibited significant differences in seedling dry weight. CK52 had a significantly higher seedling dry weight than CK31 although both were derived from the same cross (W15.M13.CM7.CM14) and had similar seed weights. Similar differences existed between CK76 (24.18 g) and C0255 (24.30 g). Therefore, while increased seed weight may contribute to larger seedlings in some lines, in others, seedling vigor could be due to tolerance to suboptimal temperatures.

Differences in chlorophyll concentration might have also contributed to the genetic variation in seedling dry weight. In this study, while some inbred lines remained green throughout the growing period (CK52, CK62, CK65, CK69, CK79, CK84, C0255, D121 and VC0264), others initially exhibited chlorosis (pale-green, yellow, even white) at day/night temperatures of 15/10°C (Figure 1). Those lines that exhibited chlorosis as late as harvest time included CK47, CK59 and RB214. It is evident from previous studies that the photosynthetic capacity of chlorotic leaves is strongly reduced (Alberda, 1969). Furthermore, photosynthetic rate is correlated with chlorophyll concentration at low temperatures (Terri *et al.*, 1977). Therefore, chlorotic seedlings might lag behind normal seedlings in growth although most of the seedlings gradually recovered with time (Miedema *et al.*, 1982).

Figure 1. Observed genotypic differences among corn inbred lines with respect to sensitivity to chlorosis at day/night temperatures of 15/10°C.

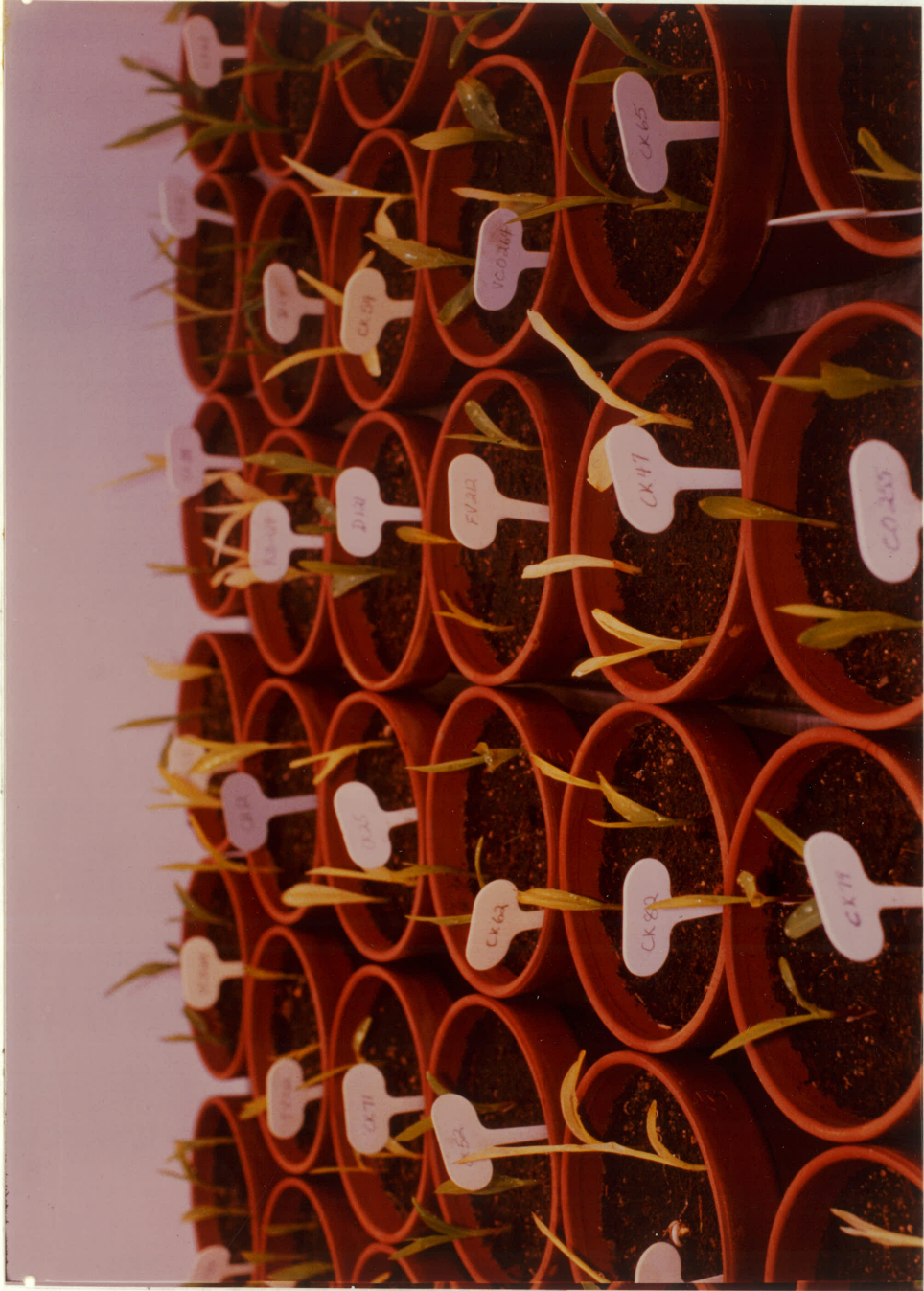


Table 3. Simple correlations among six traits of 32 inbred lines of corn grown for 40 days at suboptimal temperatures (15 days 10/10°C + 15 days 15/10°C + 10 days 19/10°C).

| | Emergence Index | Seedling Dry Weight | Plant Height | Leaf Area | 100-seed Weight |
|---------------------|-----------------|---------------------|--------------|-----------|-----------------|
| Emergence % | -0.355* | 0.186 | 0.235 | 0.181 | 0.117 |
| Emergence Index | | -0.112 | -0.277 | -0.286 | -0.234 |
| Seedling dry weight | | | 0.849*** | 0.853*** | 0.487** |
| Plant height | | | | 0.812*** | 0.340 |
| Leaf area | | | | | 0.527** |

*, **, ***Significant at 0.05, 0.01 and 0.001 level, respectively.

The genetic variation among inbred lines in leaf area was quite striking (Table 2). Mean values for this trait ranged from 13.75 cm²/plant for CK59 to 137.79 cm²/plant for VC0264. Leaf area was significantly correlated with 100-seed weight (Table 3). Kemp (1978) indicated that "lines with large seed, more probably large embryonic leaves, have an initial advantage over lines with small seed". This relationship might be reflected in a greater leaf area. Nevertheless, lines with similar seed weight exhibited significant differences in leaf area. CK52 had a significantly greater leaf area than CK31 in spite of their similarity in seed weight and identical pedigree (W15.M13.CM7.CM14). Similar differences existed between CK82 (21.31 g) and CK64 (22.31 g). Thus, while seed weight might contribute to larger leaf area in some lines, in others, greater leaf area could be attributed to low temperature tolerance (Kemp, 1978).

Juvenile plant height is also an indicator of seedling vigor. Considerable genetic variability existed within inbred lines for this trait (Table 2). Mean values for plant height ranged from 4 cm for CK59 to 14 CM for CK76. Unlike seedling dry weight and leaf area, plant height was not significantly correlated with 100-seed weight (Table 3).

4.1.3 Heritability Estimates, and Correlations Among Various Inbred Traits

Heritability estimates calculated from mean squares were 84.1, 54.5, 88.2, 95.2 and 87.6 percent for emergence percentage, emergence index, seedling dry weight, juvenile plant height, and leaf area, respectively. This clearly shows that there was adequate genetic variability for cold tolerance within this group of inbred lines. Since these estimates were obtained in one environment, genotype x environment interactions could not be estimated. Thus, parameter estimates are probably biased upward. However, the results of Mock and Skrdla (1978), and Mock and McNeill (1979) support the present findings in that these workers also observed high heritability values for emergence percentage, emergence index, and seedling dry weight at low temperatures.

Simple correlations among six traits of the 32 corn inbred lines grown for 40 days at suboptimal temperatures is presented in Table 3. A

high percentage of emergence was significantly associated with a rapid rate of emergence. This result agrees with earlier reports from both early field planting and controlled low temperature studies (Mock and Eberhart, 1972; Mock and Skrdla, 1978; Eagles and Hardacre, 1979b; Mock and McNeill, 1979). Emergence percentage and emergence index determined in the present study were not significantly correlated with seedling dry weight. Inbred lines with similar emergence percentages and emergence indices (Table 2), but with different seedling dry weights, suggested the involvement of two separate genetic systems. Similarly, Maguire (1962) also found genotypes with comparable germination potential but with different post germination growth rates. Furthermore, Kemp (1978) observed some lines of Phaseolus vulgaris L. with high rates of germination at 10° C, but which were unable to maintain that advantage at a later growth stage. In the present study, neither emergence percentage nor emergence index was significantly associated with 100-seed weight (Table 3).

Seedling dry weight was highly correlated with plant height and leaf area. The high positive correlations suggest that seedling vigor can be improved by simultaneously selecting for those traits.

4.1.4 Multiple Regression

Multiple regression analysis with seedling dry weight as a dependent variable was carried out in the present study. The purpose of the analysis was to single out the most important variable for predicting seedling dry weight.

The maximum R^2 improvement technique (Helwig and Council, 1979) was employed for inclusion or exclusion of specific variables. In contrast to the stepwise procedure in which a single best model is sought, the maximum R^2 criterion is used to depict a series of best models producing highest R^2 values.

The F values for all regression equations (Table 4) were significant at the 0.001 level indicating that some portion of the variability in seedling dry weight was explained by the independent variables in each

Table 4. Stepwise multiple regression of five characters on seedling dry weight for 32 corn inbred lines (using maximum R² criterion).

| Dependent Variable: Seedling Dry Weight | | | |
|---|--|----------------|----------|
| No. of Variables | Variable(s) | R ² | F |
| 1 | Leaf Area | 0.728 | 80.25*** |
| 2 | Leaf Area, Plant Height | 0.800 | 59.96*** |
| 3 | Leaf Area, Plant Height, Emergence Index | 0.826 | 44.19*** |
| 4 | Leaf Area, Plant Height, Emergence Index, 100-seed weight | 0.838 | 35.03*** |
| 5 | Leaf Area, Plant Height, Emergence Index, 100-seed weight, Emergence per- centage | 0.840 | 27.33*** |

***Significant at 0.001 level.

regression equation. It was observed that leaf area was the best predictor of seedling dry weight, accounting for 72.8 percent of the variability. The inclusion of plant height to the equation raised the R^2 value from 72.8 to 80 percent. At the three variable level, the addition of emergence index to the model accounted for 82.6 percent of the variability in seedling dry weight. Similarly, including the fourth independent variable, 100-seed weight, explained 83.8 percent of the variability in seedling dry weight while the addition of emergence percentage increased the R^2 value by only 0.2 percent.

In short season environments, assimilate supply (source) apparently limits grain yields of corn (Hunter, 1980). One way of enhancing assimilate supply is by increasing leaf area per plant (Tollenaar and Daynard, 1978; Hunter, 1980). Increased leaf area per plant can be accomplished by the selection of genotypes for greater leaf number and/or larger leaves (Hunter, 1980). This in turn may produce plants with improved assimilate supply and hence higher yields (Hunter, 1980). Therefore, when screening genotypes for breeding purposes, measurement of leaf area should be valuable since this trait appears to be the most useful character for predicting seedling dry weight.

In summary, the relative ranking of cold tolerance responses of all 32 inbred lines (Table 2) using rank-summation-index values (Mock and Skrdla, 1978) indicated that CK52, CK62, CK76 and C0255 were the four best inbred lines, while CK22, CK59, CK67 and CK69 ranked poorest. A considerable genotypic variation was detected for five traits among the 32 corn inbred lines. Selection for these traits, therefore, can improve the general level of performance at suboptimal temperatures. As proposed by Mock and Eberhart (1972), selection for improved cold tolerance should involve simultaneous improvement in percentage emergence, emergence index, and seedling dry weight. Based on present results, leaf area should also be included in the selection procedure. Probably, a selection index can be used to achieve significant selection advance. For example, Crosbie et al. (1980) reported that a rank summation index was one of the indices which gave best predicted results for emergence percentage, emergence index, and seedling dry weight. This index was "not seriously

affected by unequal variances among traits and combined 1) simplicity of use, 2) freedom from need to estimate genetic parameters, and 3) good selection differentials and predicted gains in each trait and in the aggregate genotype". The procedures in this study might be used to screen genotypes not only for a high emergence percentage and a rapid rate of emergence, but also for reduced sensitivity to chlorosis and for a faster rate of growth.

4.2 EARLY GROWTH OF CORN SEEDLINGS WITH ROOTS EXPOSED TO LOW ROOT ZONE TEMPERATURES

This phase of the study involved 12 of the original 32 inbred lines chosen for a more in-depth study of their cold tolerance characteristics. Special attention was devoted to the differential response of these lines to three root zone temperature regimes, i.e., 10, 14 and 18°C. Visual observations revealed that the lines exhibited different reactions to suboptimal root zone temperatures. Of all the lines, CH12, CO255, and CM72 developed red pigmentation in their leaves at a root temperature of 10 C while others showed a yellowing of the leaves. The inbred line RB214 particularly, had poorly developed chlorophyll at this temperature (10 C). All the lines had green leaves at 14 C except CO255, CM72 and CH12. The growth of all lines at 18°C was rated satisfactory.

Table 5 shows mean squares from the analysis of variance for various traits of the 12 corn inbred lines. Differences among temperatures were highly significant for all traits as were genotypic differences. Seedling dry weight, juvenile plant height and root dry weight showed significant genotype x temperature interaction, suggesting that not all corn inbred lines responded similarly to changes in root zone temperature.

4.2.1 Seedling Emergence

Seedlings emerged after approximately 19, 12 and 7 days at root zone temperatures of 10, 14 and 18°C, respectively (Table 6). Lower temperatures significantly delayed seedling emergence. Compared to the control (18°C), about 12 and 5 more days were required for emergence at

Table 5. Mean squares from analysis of variance for various traits of 12 corn inbred lines subjected to different root zone temperature treatments.

| | df | Emergence | Emergence Rate | Seedling Dry Weight | Leaf Area | Juvenile Plant Height | Root Dry Weight | Shoot:Root Ratio |
|-----------------|-----|------------------------|-------------------------|---------------------|---------------------|-------------------------|---------------------|---------------------|
| | | (%) | (days) | (g) | (cm ²) | (cm) | (g) | |
| Blocks | 2 | 56.482 | 0.738 | .251 | 0.154 | 73.55 | 0.012 | 0.152 |
| Temperature | 2 | 1,225.926 [#] | 1,276.643 ^{**} | 6.161 ^{**} | 7.721 ^{**} | 2,145.553 ^{**} | 2.500 ^{**} | 1.085 ^{**} |
| Error (a) | 4 | 28.704 | 11.738 | 0.217 | 0.172 | 83.736 | 0.035 | 0.049 |
| Inbreds | 11 | 562.290 ^{**} | 7.354 ^{**} | 0.054 ^{**} | 0.123 ^{**} | 45.153 ^{**} | 0.026 ^{**} | 0.023 ^{**} |
| Inbreds x Temp. | 22 | 72.391 | 1.038 | 0.009 ^{**} | 0.014 | 6.096 [#] | 0.007 [#] | 0.005 |
| Error (b) | 66 | 78.367 | 0.976 | 0.003 | 0.045 | 3.250 | 0.004 | 0.004 |
| TOTAL | 107 | | | | | | | |
| C.V. % | | 10.043 | 7.803 | 0.407 | 1.532 | 13.872 | 0.448 | 0.4951 |

[#], ^{**}Significant at 0.05 and 0.01 level, respectively.

Table 6. Means of emergence index (days) for 12 corn inbred lines as a function of root zone temperature.

| Inbred | Initial Temperature (°C) | | |
|--------|--------------------------|------------|----------|
| | 10 | 14 | 18 |
| CH12 | 18.67 abcde ¹ | 9.75 a | 6.10 ab |
| CK25 | 19.15 bcdef | 12.16 bcd | 8.53 c |
| CK 27 | 17.27 a | 11.00 abc | 5.93 a |
| CK29 | 20.34 efg | 11.50 abcd | 7.90 bc |
| CK52 | 18.15 abcd | 11.80 bcd | 7.87 bc |
| CK64 | 19.95 defg | 12.53 cd | 7.73 abc |
| CK69 | 20.90 g | 12.05 bcd | 7.20 abc |
| CK76 | 19.54 cdefg | 11.90 bcd | 7.73 abc |
| CM72 | 18.92 abcdef | 11.64 bcd | 7.40 abc |
| CO255 | 17.42 ab | 10.53 ab | 6.27 ab |
| FV212 | 20.57 fg | 13.23 d | 8.37 c |
| RB214 | 17.90 abc | 11.33 abc | 6.47 ab |
| Mean | 19.06 a | 11.62 b | 7.29 c |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test.

10 and 14°C, respectively. In accordance with the present results, slower emergence rates with decreasing soil temperatures were also observed by Dubtez *et al.* (1962); Alessi and Power (1971); Cal and Obendorf (1972); and Singh and Dahaliwal (1972). The increase in the number of days required for emergence with decreasing temperature could possibly be due to reduced enzymatic activities in the seed which caused both a delayed germination and a reduced growth rate of the radicle and coleoptile (Blacklow, 1972; Hatfield and Egli, 1974).

Among inbred lines, significant differences in emergence index were observed within each temperature regime. At a temperature of 10°C, CK27 was the fastest emerging line, while CK69 was the slowest. At a temperature of 18°C, CK27 again emerged about three days earlier than the latest emerging line (CK25), while at 14°C, the earliest emerging line was CH12.

Table 7 shows the means of emergence percentages for the 12 corn inbred lines seeded at suboptimal root zone temperatures. Lowering the temperature, reduced emergence percentage significantly although within each temperature regime, significant differences among inbred lines were noted. At a temperature of 10°C, CK27 exhibited 100 percent emergence, while both CK69 and CK64 had only 67 percent emergence. At the 14°C regime, all lines with the exception of FV212 and CK69 exhibited over 80 percent emergence while at 18°C, all lines displayed over 85 percent emergence.

In this study, the failure of some seedlings to emerge could be attributable to reduced vigor of the seedling resulting from a prolonged germination period (Sing and Dhaliwal, 1972). Also, seed germination and emergence of corn was retarded and its susceptibility to disease increased as a result of low temperatures (Pinnell, 1949). Furthermore, Eagles and Brooking (1981) reported the occurrence of fungal infection on most of their experimental seedlings which failed to emerge, despite sterilized potting mixtures and fungicidal treatments.

4.2.2 Seedling Dry Weight, Leaf Area, and Juvenile Plant Height

Mean dry weights (g) of inbred seedlings as a function of root zone temperatures are given in Table 8. The determinantal effect of low

Table 7. Means of emergence percentage for 12 corn inbred lines seeded at suboptimal root zone temperatures.

| Inbred | Initial Temperatures (°C) | | |
|--------|---------------------------|--------|-------|
| | 10 | 14 | 18 |
| CH12 | 80 ab | 80 ab | 87 a |
| Ck25 | 77 ab | 90 abc | 93 a |
| CK27 | 100 c | 96 bc | 100 a |
| CK29 | 93 bc | 100 c | 100 a |
| CK52 | 83 ab | 90 abc | 97 a |
| CK64 | 67 a | 90 abc | 90 a |
| CK69 | 67 a | 76 a | 93 a |
| CK76 | 90 bc | 86 abc | 100 a |
| CM72 | 80 ab | 83 abc | 90 a |
| C0255 | 90 bc | 90 abc | 100 a |
| FV212 | 70 ab | 76 a | 90 a |
| RB214 | 90 bc | 100 c | 100 a |
| Mean | 82 a | 88 b | 95 c |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test.

Table 8. Means of seedling dry weight (g) for 12 corn inbred lines as a function of root zone temperature.

| Inbred | Initial Temperature (°C) | | |
|--------|--|---------------------|-----------|
| | 10 | 14 | 18 |
| CH12 | 0.375 ² b ¹ (4.6) | 2.130 f (26.2) | 8.139 ef |
| CK25 | 0.228 ab (2.8) | 1.386 de (16.7) | 8.288 ef |
| CK27 | 0.102 ab (1.8) | 0.767 b (13.8) | 5.578 bc |
| CK29 | 0.122 ab (2.1) | 0.946 bcd (16.0) | 5.919 bcd |
| CK52 | 0.166 ab (2.1) | 1.072 bcd (13.8) | 7.743 ef |
| CK64 | 0.063 a (1.1) | 0.804 b (14.1) | 5.712 bc |
| CK69 | 0.131 ab (2.8) | 0.786 b (16.7) | 4.708 b |
| CK76 | 0.265 ab (2.8) | 1.713 ef (18.3) | 9.339 f |
| CM72 | 0.139 ab (2.1) | 0.881 bc (13.2) | 6.681 cde |
| C0255 | 0.206 ab (2.8) | 1.321 cde (17.8) | 7.416 def |
| FV212 | 0.135 ab (4.2) | 0.386 a (11.9) | 3.240 a |
| RB214 | 0.188 ab (2.2) | 1.947 f (22.4) | 8.701 f |
| Mean | 0.175 a (2.7) | 1.123 a (17.1) | 6.568 b |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test, and groupings were done on Log₁₀ transformed means.

²Figures in parenthesis are seedling dry weights expressed as percentage of control (18°C).

temperature on dry weight was pronounced. Forty-two days after seeding at 14°C, seedling dry weight of the 12 inbreds was 11.9 to 26.2 percent of the control (18°C) but only 1.1 to 4.6 percent of the control at the 10°C temperature. Although there was a progressive decline in seedling dry weight with decreasing temperature, the 10°C soil temperature treatment did not differ significantly from the 14°C treatment. These differences existed notwithstanding the existence of an ambient day-night temperature of 18/10°C during the experiment. Cal and Obendorf (1972) also reported that seedling dry weights of four hybrids decreased with decreasing soil temperatures although the ambient temperature was maintained at 20°C during the growing period. Watts (1973) reported that relative rates of leaf area increase was closely associated with mean daily soil temperatures at a soil depth of 5 cm. Therefore, soil temperature appeared to exert more influence than air temperature during early seedling growth.

Plants grown at lower temperatures exhibited less blade area (Table 9). For the 12 lines grown at 14°C, leaf areas were 20.8 to 33 percent of the control (18°C), compared to a range of 8.6 to 26.2 percent for plants grown at 10°C.

Retardation of leaf growth at lower soil temperatures is due to the influence of soil temperature on leaf elongation (Barlow and Boersma, 1972). Before the apical meristem is elevated above ground, both rate of leaf emergence and leaf expansion are greatly influenced by soil temperature (Cooper and Law, 1977). Barlow et al. (1977) reported that by reducing the soil temperature below 28°C, leaf elongation was decreased in young corn plants. The decrease in leaf expansion with decreasing temperature was ascribed to lower water uptake (Kleinendorst, 1975; Barlow et al., 1977) which reduced cell elongation. In addition, as a result of lower temperature surrounding the apical meristem, biochemical processes in the meristematic region are retarded (Kleinendorst, 1975). It was concluded, therefore, that suboptimal soil temperatures affect corn seedling growth primarily by decreasing leaf elongation (Barlow et al., 1977).

Table 9. Means of leaf area (cm²) for 12 corn inbred lines as a function of root zone temperature.

| Inbred | Initial Temperature (°C) | | |
|--------|---|---------------------|-----------|
| | 10 | 14 | 18 |
| CH12 | 5.891 a ¹ (10.1) ² | 12.577 ab (21.5) | 58.390 b |
| CK25 | 7.297 a (13.1) | 14.839 ab (26.7) | 55.524 b |
| CK27 | 4.786 a (26.2) | 6.036 a (33.0) | 18.266 a |
| CK29 | 4.647 a (13.4) | 10.566 ab (30.4) | 34.722 ab |
| CK52 | 3.794 a (8.6) | 9.179 ab (20.8) | 44.168 ab |
| CK64 | 3.691 a (8.7) | 9.946 ab (23.5) | 42.274 ab |
| CK69 | 4.501 a (12.5) | 8.007 ab (22.3) | 35.931 ab |
| CK76 | 6.611 a (13.0) | 14.388 ab (28.4) | 50.682 b |
| CM72 | 4.490 a (10.0) | 11.660 ab (26.0) | 44.899 ab |
| C0255 | 5.305 a (11.1) | 11.704 ab (24.6) | 47.604 b |
| FV212 | 3.355 a (11.9) | 8.291 ab (29.3) | 28.280 ab |
| RB214 | 7.569 a (13.9) | 16.613 b (30.6) | 54.342 b |
| Mean | 4.989 a (12.2) | 10.740 b (26.2) | 41.020 c |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range test, and groupings were done on Log₁₀ transformed means.

²Figures in parenthesis are leaf areas expressed as percentage of control (18°C).

Lowering the root zone temperature in the present study reduced juvenile plant height significantly (Table 10). The variation among lines within temperatures (14 and 18°C) was also significant. The heights of lines which were grown at 14°C ranged from 44.9 to 71.5 percent of the control (18°C), whereas at a root zone temperature of 10°C, the heights were only 21.9 to 32.4 percent of the control. Cal and Obendorf (1972) observed similar responses of four hybrids grown at low root zone temperatures.

4.2.3 Root Growth and Shoot:Root Ratio

Table 11 shows means of root dry weight (g) for 12 corn inbred lines grown at suboptimal root zone temperatures. Even though a reduction in mean root dry weight was observed with decreasing temperature, differences in weight between the 10 and 14°C temperature regimes were non-significant. At a root zone temperature of 14°C, the root dry weights for all lines ranged from 12.5 to 25.2 percent of the control (18°C), while at 10°C, the range was from 7.8 to 17.6 percent. Cal and Obendorf (1972) observed similar responses of four hybrids at low root zone temperatures.

Suboptimal temperature restricts root extension and reduced root mass and degree of branching. It also increases the viscosity of water and decreases the permeability of the root membranes (Lae, 1974), thereby reducing overall water and nutrient uptake. Furthermore, the response of plants to various soil temperatures has been related mainly to changes in the metabolic activity of the root (Nielsen, 1974). Consequently, the overall development of the plant is retarded at suboptimal root zone temperatures.

Table 10. Means of juvenile plant height (cm) for 12 corn inbred lines as a function of root zone temperature.

| Inbred | Initial Temperature (°C) | | |
|--------|--|----------------------|-----------|
| | 10 | 14 | 18 |
| CH12 | 7.67 a ¹ (29.1) ² | 15.67 f (59.5) | 28.33 f |
| CK25 | 6.67 a (30.8) | 15.50 f (71.5) | 21.67 bcd |
| CK27 | 4.27 a (21.9) | 10.17 abc (52.2) | 19.50 abc |
| CK29 | 5.50 a (31.1) | 9.00 ab (50.9) | 17.67 a |
| CK52 | 5.17 a (22.2) | 12.00 bcde (51.4) | 23.33 def |
| CK64 | 4.23 a (22.7) | 10.83 abcd (58.0) | 18.67 ab |
| CK69 | 4.33 a (26.5) | 8.50 a (52.0) | 16.33 a |
| CK76 | 6.70 a (26.3) | 14.67 ef (57.5) | 25.50 ef |
| CM72 | 6.00 a (26.8) | 12.50 cdef (55.7) | 22.43 cde |
| CO255 | 6.33 a (27.9) | 13.67 def (60.3) | 22.67 cde |
| FV212 | 5.23 a (29.3) | 8.00 a (44.9) | 17.83 a |
| RB214 | 6.97 a (32.4) | 14.83 ef (69.0) | 21.50 bcd |
| Mean | 5.76 a (27.3) | 12.11 b (57.3) | 21.12 c |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test.

²Figures in parenthesis are juvenile plant heights expressed as percentage of control (18°C).

Table 11. Means of root dry weight (g) for 12 corn inbred lines as a function of root zone temperatures.

| Inbred | Initial Temperature (°C) | | |
|--------|---|---------------------|-----------|
| | 10 | 14 | 18 |
| CH12 | 0.492 a ¹ (13.7) ² | 0.843 bc (23.4) | 3.603 de |
| CK25 | 0.270 a (9.5) | 0.523 abc (18.3) | 2.855 bcd |
| CK27 | 0.277 a (11.6) | 0.510 abc (21.4) | 2.388 bc |
| CK29 | 0.268 a (10.1) | 0.484 ab (18.2) | 2.665 bcd |
| CK52 | 0.350 a (10.2) | 0.708 bc (20.7) | 3.415 cde |
| CK64 | 0.326 a (7.8) | 0.524 abc (12.5) | 4.196 ef |
| CK69 | 0.276 a (11.1) | 0.484 ab (19.4) | 2.495 bc |
| CK76 | 0.387 a (9.3) | 0.646 bc (15.6) | 4.153 ef |
| CM72 | 0.280 a (10.4) | 0.450 ab (16.8) | 2.684 bcd |
| C0255 | 0.330 a (10.9) | 0.761 bc (25.2) | 3.017 bcd |
| FV212 | 0.284 a (17.6) | 0.252 ab (15.6) | 1.617 a |
| RB214 | 0.351 a (6.6) | 0.950 c (17.8) | 5.344 f |
| Mean | 0.321 a (10.4) | 0.588 a (19.0) | 3.093 b |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test, and groupings were done on Log₁₀ transformed means.

²Figures in parenthesis are root dry weights expressed as percentage of control (18°C).

The shoot:root ratio which is an indicator of an increase in total dry matter production as it relates to size of the assimilatory system, was also analyzed in the present study. Table 12 shows means of shoot:root ratio for the 12 corn inbred lines as a function of root zone temperature. Significant variation existed among inbred lines within each temperature regime. Increased soil temperatures were accompanied by increased shoot:root ratios. Similarly, David and Lingle (1961) observed that a 15°C root temperature caused a lower shoot:root ratio compared to a 25°C temperature. This response may be attributed to an excess of assimilates at low temperatures which promotes greater root growth relative to shoot growth (Brouwer, 1962).

Across the three temperature regimes used in this study, CK25 had highest shoot:root ratios, suggesting that it was more efficient in total dry matter production. In contrast, line CK64 had the lowest shoot:root ratio of 12 inbreds tested.

4.2.4 Heritability Estimates, and Correlations Among Various Inbred Traits

On the basis of broadsense heritabilities (h_b^2) using the formula of Fakoureda and Ojo (1981), heritability estimates were calculated for each of the seven traits investigated in this study. The following values were obtained: for percentage emergence, 86.3%; emergence index, 86.6%; seedling dry weight, 84.8%; juvenile plant height, 87.3%; leaf area, 69.2%; root dry weight, 75.2%; and shoot:root ratio, 80.6%. These estimates were sufficiently high to suggest that there was considerable genetic variability for those traits within the population of 12 inbred lines.

Because of a significant temperature x inbred interaction for some traits, simple correlations among all traits were calculated separately for each root zone temperature (Table 13). A rapid rate of emergence was

Table 12. Means of shoot:root ratio for 12 corn inbred lines as a function of root zone temperatures.

| Inbred | Initial Temperature (°C) | | |
|--------|--------------------------|------------|-----------|
| | 10 | 14 | 18 |
| CH12 | 0.755 b ¹ | 2.519 bcd | 2.270 bc |
| CK25 | 0.835 b | 2.668 cd | 2.918 c |
| CK27 | 0.376 ab | 1.674 abc | 2.361 bc |
| CK29 | 0.453 ab | 1.950 abc | 2.237 bc |
| CK52 | 0.467 ab | 1.538 a | 2.302 bc |
| CK64 | 0.199 a | 1.553 a | 1.372 a |
| CK69 | 0.480 ab | 1.612 a | 1.903 ab |
| CK76 | .691 b | 2.979 d | 2.278 bc |
| CM72 | 0.506 ab | 1.761 ab | 2.526 bc |
| C0255 | 0.645 b | 1.765 ab | 2.504 bc |
| FV212 | 0.485 ab | 1.502 a | 2.039 abc |
| RB214 | 0.609 b | 2.141 abcd | 1.648 ab |
| Mean | 0.531 b | 1.938 a | 2.170 a |

¹Mean followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test, and groupings were done on Log₁₀ transformed variables.

Table 13. Simple correlations among the various traits at 10°C (top), 14°C (middle) and 18°C (bottom).

| | Emergence Index | Seedling Dry Weight | Plant Height | Leaf Area | Shoot:Root Ratio | 100-seed Weight | Root Weight |
|------------------|-----------------|---------------------|--------------|-----------|------------------|-----------------|-------------|
| Emergence % | -.664* | .140 | .247 | .370 | .135 | .239 | .067 |
| | -.229 | .206 | .174 | .253 | -.004 | .279 | .376 |
| | -.349 | .544 | .170 | .085 | .133 | .337 | .422 |
| Emergence Index | | -.230 | .291 | -.334 | -.161 | -.455 | -0.262 |
| | | -.643* | -.512 | -.200 | -.287 | -.610* | 0.697* |
| | | -.186 | -.262 | .004 | .094 | -.411 | -.245 |
| Seedling Dry Wt. | | | .870** | .604* | .860** | .654* | .792** |
| | | | .881** | .793** | .748** | .853** | .868** |
| | | | .827** | .803** | .299 | .724** | .711** |
| Plant Height | | | | .745** | .829** | .582* | .616* |
| | | | | .821** | .762** | .681* | .730** |
| | | | | .701* | .389 | .634* | .450 |
| Leaf Area | | | | | .708** | .516 | .301 |
| | | | | | .780** | .516 | .589* |
| | | | | | .142 | .674* | .640* |
| Shoot:Root Ratio | | | | | | .378 | .380 |
| | | | | | | .385 | .340 |
| | | | | | | -.109 | -.445 |
| 100-Seed Wt. | | | | | | | .802** |
| | | | | | | | .925** |
| | | | | | | | .732** |

*, **Significant at 0.05 and 0.01 level, respectively.

significantly associated with a high percentage emergence at 10°C. This result agrees with earlier findings from both field planting and from controlled low temperature studies (Mock and Eberhart, 1972; Mock and Skrdla, 1978; Eagles and Hardacre, 1979 a; Mock and McNeill, 1979). It also agrees with results from the present study involving 32 corn inbred lines grown at suboptimal ambient temperatures. However, at temperatures of 14 and 18°C, emergence index was not significantly associated with emergence percentage. Possibly, the desirable association between emergence percentage and emergence index is a widespread phenomenon in corn at lower temperatures (Eagles and Brooking 1981), but may not persist at higher temperatures. In contrast to previous reports (Mock and Skrdla, 1978; Mock and McNeil, 1979), emergence percentage and emergence index as determined in the present study were not significantly associated with seedling dry weight. For the rather restricted sample of inbred lines used, the genetic system conditioning both emergence percentage and emergence index might be different from the genetic system controlling seedling growth. Neither emergence percentage nor emergence index was significantly associated with shoot:root ratio, and root dry weight. While small kernel size was associated with a high emergence of inbreds (Pinnell, 1949), no such significant association was observed in the present study. Furthermore, emergence index was not significantly associated with seed weight.

Seedling dry weight was highly correlated with plant height, leaf area, shoot:root ratio, root dry weight and 100-seed weight. Similarly, juvenile plant height was also significantly correlated with leaf area, shoot:root ratio, 100-seed weight at 18°C, and root dry weight at both 14 and 18°C temperatures. The shoot:root ratio exhibited no significant relationship with either 100-seed weight or with root dry weight. The 100-seed weight was significantly associated with root dry weight.

In summary, the relative ranking of tolerance of the 12 inbreds to cold soils (10 and 14°C) can be postulated using rank-summation-index (Mock and Skrdla, 1978). At 10°C, the ranking was as follows: C0255 RB214 > CK27 > CH12, CK76 > CK52 > CK25 > CM72 > CK29 > FV212 > CK69, CK64. At 14°C, the following ranking was obtained: RB214 > C0255 > CH12 > CK29 > CK52, CK27 > CK25 > CK76 > CM72 > CK64 > CK69 > FV212. The three best inbred lines which showed good tolerance to low root zone temperatures (10 and 14°C) were C0255, RB214 and CH12, while FV212, CK64 and CK69 showed poor tolerance at both temperatures.

4.3 GROWTH RESPONSES OF CORN INBRED LINES TO CHILLING STRESS

Twelve corn inbred lines were evaluated at favourable (28/18°C) and chilling (7 days 28/18°C + 7 days 10/10°C) day/night temperatures. Nine plants from each inbred line were harvested at seven-day intervals to determine seedling dry weight and leaf area (cm²/plant).

Before undertaking any combined analysis of variance, error variances of temperature treatments (7 days 28/18°C, 7 days 28/18°C + 7 days 10/10°C, and 14 days 28/18°C) were tested for homogeneity (LeClery et al., 1962). Since the chi-square values obtained from the tests of error variances of both transformed (Log10) and untransformed variables were significant, it was necessary to exclude the error variance of one of the temperature treatments, viz. 14 days 28/18°C. This treatment produced an error variance much larger than the error variances of the other two temperature treatments while testing for homogeneity.

The resulting chi-square values for seedling dry weight and leaf area were non-significant (2.56 and 2.31, respectively; 1 d.f.), indicating that the individual error mean squares might be considered as estimates of the same population variance. Therefore, the error terms for the two temperature treatments (7 days 28/18°C, and 7 days 28/18°C +

7 days 10/10°C) of both seedling dry weight and leaf area were sufficiently homogeneous to permit the combined analysis of variance.

The chilling treatment resulted in some visible effects on all plants. For example, all genotypes exhibited necrosis of the leaf margins. Those inbred lines with the greatest amount of leaf necrosis included CK27, CK29, CK52, CK69 and CK64. Taylor and Craig (1971) also reported that the transfer of corn or sorghum plants from 25 to 10°C at a moderate light intensity, caused severe necrosis of exposed leaves within four to five days.

Highly significant differences between temperature treatments for both seedling dry weight and leaf area were found, suggesting that substantial increases in leaf area expansion and dry matter accumulation occurred during chilling (Table 14). Significant differences among inbred lines were also observed for seedling dry weight and leaf area. Moreover, significant temperature x inbred interactions implied that there were differences in the rate of dry matter accumulation and leaf area expansion during chilling. This was apparent from the means for both leaf area and seedling dry weight as shown in Tables 15 and 16. Seven-day-old plants of CK27 and CM72 which were grown at a favourable temperature (28/18°C) were significantly lower in seedling dry weight and leaf area than the inbreds CK25, CK76 and C0255. However, 14-day-old plants of CK27 and CM72 which were subjected to a seven-day chilling treatment were not significantly different from chilled plants of CK25, CK76 and C0255 in both leaf area and seedling dry weight. Thus, CK27 and CM72 grew more rapidly than CK25, CK76 and C0255 during chilling.

Exposing the seven-day-old seedlings to chilling temperature of 10°C resulted in a marked reduction in seedling dry weight (Table 15). Chilling treatment reduced seedling dry weight by an average of 71 percent although the magnitude of reduction varied according to genotype.

Table 14. Mean squares from analysis of variance combined over two temperature treatments (7 days 28/18°C (day/night) versus (7 days 28/10°C + 7 days 10/10°C) for two traits in 12 corn inbred lines.

| Source | df | Seedling Dry Weight (g) | Leaf area cm ² /plant ⁺ |
|---------------------------------|----|-------------------------------|--|
| Blocks in Temperature Treatment | 4 | .00067 | .00368 |
| Temperature Treatments | 1 | .10746** | .18011** |
| Inbreds | 11 | .00668** | .05826** |
| Temp. Trets. x Inbreds | 11 | .00059* | .00613* |
| Error | 44 | .00029 | .00279 |
| C.V. % | | 10.13 | 3.01 |

⁺Analysis of variance was carried out on Log₁₀ transformed variables.

Table 15. Mean seedling dry weights (g/plant) and absolute growth rates (grams dry matter produced per day) for 12 corn inbred lines grown at a favourable (28/18°C) and a chilling (7 days 28/18°C + 7 days 10/10°C) temperature condition and harvested at 7-day intervals.

| Inbred Line | 7-day Favourable (Control) | Seedling Dry Weight 14 days | | Absolute Growth Rate (14 days) | |
|-------------|----------------------------|---|------------|--------------------------------|------------|
| | | Chilled | Favourable | Chilled | Favourable |
| RB214 | .1714 f | .2491 e ¹ (31.5) ² | .7913 bc | .0111 (12.5) | .0886 |
| CH12 | .1692 f | .2678 e (28.2) | .9479 c | .0141 (12.6) | .1112 |
| CK52 | .1643 ef | .2536 e (27.8) | .9127 c | .0128 (12.0) | .1069 |
| CO255 | .1474 de | .2226 d (29.2) | .7631 bc | .0107 (12.0) | .0890 |
| CK25 | .1352 cd | .2196 cd (26.8) | .8180 bc | .0121 (12.4) | .0975 |
| CK76 | .1327 cd | .1985 c (26.4) | .7508 bc | .0094 (10.6) | .0883 |
| CK64 | .1209 bc | .1739 b (25.3) | .6875 b | .0076 (9.4) | .0809 |
| CM72 | .1092 b | .2171 cd (31.3) | .6942 b | .0154 (18.4) | .0836 |
| CK27 | .1079 b | .2018 cd (28.0) | .7528 bc | .0147 (16.0) | .0921 |
| FV212 | .1071 b | .1743 b (39.3) | .4430 a | .0096 (20.0) | .0480 |
| CK69 | .1049 b | .1543 ab (33.3) | .4633 a | .0071 (13.9) | .0521 |
| CK29 | .0843 a | .1400 a (31.2) | .4487 a | .0090 (17.3) | .0521 |
| Mean | .1295 | .2068 | .7060 | .0111 | .0824 |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test.

²Figures in parentheses are seedling dry weights and absolute growth rates of chilled plants expressed as percentages of control plants of comparable age.

The seedling dry weight of chilled plants expressed as a percentage of the dry weight of controls in the 12 inbred lines ranged from 25.3% for CK64 to 39.3% for FV212. Closer examination of the means (Table 15), however, revealed that the inbred lines, FV212, CK69, and CK29, which were less vigorous than CH12, CK25, and CK52 exhibited a relatively high dry matter ratios of chilled/unchilled plants. This reaction of FV212, CK29, and CK69 might be associated with their slow rate of growth at a favourable temperature. Chilling temperature also resulted in a considerable reduction in absolute growth rates. The greatest reduction in growth rate occurred in CK64 (9.4%) and the least in FV212 (20%). In spite of these adverse effects of chilling treatment on seedling growth, the seven-day-old seedlings accumulated about 60 percent of their total dry matter during chilling.

Exposure of seven-day old corn seedlings to chilling temperature resulted in a substantial reduction in leaf area (Table 16). Leaf area expansion at chilling temperatures was reduced by an average of approximately 73 percent although the magnitude of reduction again varied from one inbred line to another. The leaf area of chilled plants expressed as percentage of the leaf area of controls in the twelve inbred lines ranged from 21.6 percent for CK29 to 31 percent for CK69. Similarly, Miedema et al. (1982) reported that prolonged exposure to temperatures of 10°C, or lower, steadily decreased the leaf extension rate resulting in damage to the leaves. In the present study, the seven-day-old plants increased their leaf area expansion during the chilling period by an average of 26 percent. Thus, the leaf area expansion was affected more than dry matter accumulation by the chilling treatment. The observed changes in leaf area expansion apparently occurred prior to the observed changes in dry matter production (Castleberry et al., 1978).

Table 16. Mean leaf areas (cm²/plant) for twelve corn inbred lines grown at favourable (28/10°C) and a chilling (7 days 28/18°C + 7 days 10/10°C) temperature condition and harvested at 7-day intervals.

| Inbred | 7 days Favourable (control) | 14 days | |
|--------|-----------------------------------|----------------------------------|-----------|
| | | Chilled | Favorable |
| RB214 | 70.05 e ¹ | 79.48 efg (27.3) ² | 291.4 cd |
| CK52 | 69.77 e | 80.84 fg (29.2) | 277.0 bcd |
| CH12 | 64.14 de | 89.22 g (30.0) | 297.3 d |
| CK76 | 59.03 d | 62.70 cd (25.2) | 249.1 bcd |
| CO255 | 58.07 d | 65.91 d (23.6) | 279.7 bcd |
| CK25 | 56.22 d | 72.90 de (29.6) | 245.9 bc |
| CK64 | 47.87 c | 55.39 bc (23.7) | 233.9 b |
| CM72 | 45.85 bc | 68.06 de (27.8) | 244.5 bc |
| FV212 | 41.09 b | 49.95 b (30.4) | 164.2 a |
| CK27 | 39.74 ab | 72.38 def (30.9) | 234.2 b |
| CK69 | 39.34 ab | 50.35 b (31.0) | 162.7 a |
| CK29 | 34.34 a | 40.28 a (21.6) | 186.1 a |
| Mean | 52.12 | 65.62 | 238.8 |

¹Means followed by the same letter are not significantly different at the 5 percent level by Duncan's Multiple Range Test.

²Figures in parentheses are leaf areas of chilled plants expressed as percentage of leaf areas of control plants of comparable age.

Simple correlations calculated for various traits of the 12 corn inbred lines grown at a favourable (28/18°C) and a chilling temperature condition (7 days 28/18°C + 7 days 10/10°C) are presented in Table 17. Seed weight (100-seed weight) was significantly correlated with dry matter and leaf area of both chilled and unchilled plants. This finding supported the results of Cooper and MacDonald (1970) that partial removal of endosperm from seeds of equal embryo size resulted in reduced root and shoot growth as well as leaf area. Thus endosperm reserves are important not only with respect to providing energy during early seedling growth, but also subsequently providing for a more optimal leaf area, thus a more rapid plant growth rate (Cooper and MacDonald, 1970).

Correlations between leaf areas and dry matter weights of both chilled and unchilled plants were highly significant, indicating that dry matter accumulation was dependent on rate of leaf expansion. Dry matter weight and leaf area of seven-day-old seedlings were significantly correlated with those of chilled and unchilled (14 days) plants. This relationship suggests that vigorous plants at the initial stage of growth generally produced vigorous seedlings at harvest irrespective of the temperature treatment. The dry matter weight and leaf area of chilled plants were also positively associated with those of unchilled (14 days) control plants. This implied that those genotypes that did well at suboptimal temperature also did well at favourable temperature and vice versa. As expected, the absolute growth rates of both chilled and unchilled (14 days) plants were significantly associated with dry matter and leaf area since the absolute growth rate of a plant at any given time is proportional to its size. This suggested that the potential of the plant to synthesize new material (and hence increase dry weight) was clearly dependent upon its leaf area.

Table 17. Simple correlations among various traits of 12 corn inbred lines grown at favourable (28/18°C) and a chilling (7 days 28/18°C) + 7 days 10/10°C) temperature conditions.

| | Dry Matter of 7-day control | Leaf Area of 7 day control | Dry Matter of 14-day chilled | Leaf Area of 14-day chilled | Dry Matter of 14-day control | Leaf Area of 14-day control | Absolute growth rate of chilled | Absolute growth rate of control |
|------------------------------------|-----------------------------------|----------------------------------|------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|---------------------------------------|---------------------------------------|
| 100-seed wt. | .874*** | .836*** | .702* | .697* | .737** | .780** | .145 | .656* |
| Dry mat. of 7- day control | | .972*** | .886*** | .842*** | .835*** | .863*** | .301 | .767** |
| Leaf area of 7- day control | | | .851*** | .796** | .828*** | .860*** | .285 | .764*** |
| Dry Mat. of 14- day chilled | | | | .977*** | .915*** | .898*** | .706* | .870*** |
| Leaf area of 14- day chilled | | | | | .934*** | .867*** | .723** | .890*** |
| Dry matter of 14- day control | | | | | | .932*** | .614* | .982*** |
| Leaf area of 14- day control | | | | | | | .545 | .907*** |
| Absolute growth rate of chilled | | | | | | | | .625* |

*, **, ***Significant at 0.5, 0.01 and 0.001 level, respectively.

In general, chilling temperature had a detrimental effect on the growth of all corn inbred lines. One of the immediate effects of chilling stress is considered to be on the fluidity of membrane lipids. Lyons (1973) suggested that for thermophilic plant species, lipids undergo a phase change from fluid to solid at temperatures ranging from 10 to 12°C resulting in a conformational change and a higher activation energy of membrane bound enzymes. This, in turn, results in an imbalance in the plant's metabolism and disfunction of its membranes. However, the physical basis of this hypothesis has been criticized by Bishop et al., (1979) who argued that membrane lipids would not solidify above 10°C in either chilling sensitive or chilling resistant species. Although the mechanism of chilling injury is still unclear, there is some evidence which suggests that inactivation of C₄-pathway enzymes may reduce the photosynthetic activity and growth of certain genotypes under certain conditions (Miedema, 1982).

There are also many reports of various metabolic changes in thermophilic plant species when exposed to chilling stress. Chilling stress reduces the rate of photosynthesis (Raschke, 1970; Taylor and Rowley, 1971; Kibiti, 1977; Terri et al., 1977) with a concurrent reduction in the activity of C₄ photosynthetic pathway enzymes (Taylor et al., 1974); Stamp, 1980), reduced stomatal aperture (Taylor and Rowley, 1971) and reduced chlorophyll content (McWilliam and Nylor, 1967; Kibiti, 1977; Terri et al., 1977). Nevertheless, reduction in photosynthetic rate does not seem to be the primary process affecting growth rate during chilling stress. Chilling stress has been shown to result in increases in starch and sugar contents (Kibiti, 1977). Thus, growth is not limited by the rate of photosynthesis during the period of chilling stress. Further evidence comes from observations that growth rates were more

sensitive to chilling stress than photosynthesis (Kibiti, 1977; Meidema, 1982).

In his review of effects of low temperature on corn, Miedema (1982) recognized that temperature curves for relative growth rates were similar to those of leaf extension rates. In the present investigation, there was a very high association between absolute growth rate and leaf area of both chilled and unchilled (14 days) control plants (Table 17). There also occurred a sharp decline in growth rate (80-91 percent) and a reduction in leaf area (69-78 percent) during chilling stress. These data suggest that the effect of low temperature on dry matter accumulation is associated with the leaf expansion rate.

Considerable genotypic variation existed in this sample of inbred lines for seedling dry weight and leaf area after chilling stress (10°C day/10°C night). Similarly, several other reports show genetic variation in dry matter production and leaf extension of seedlings exposed to low temperature (Duncan and Hesketh, 1968; Terri et al., 1977; Castleberry et al., 1978; Lee and Estes, 1982). Part of the variation in seedling dry weight during chilling stress could be attributed to initial seed weight, but considerable variation was also related with leaf extension rate (Miedema et al., 1982).

The mechanism which regulates the differential responses of various genotypes to chilling stress is still unknown. However, an early maturing corn hybrid has more stable chloroplast structure following exposure to chilling temperatures than does a late maturing hybrid (Lee and Estes, 1982). The better performance of the early maturing hybrid during chilling stress and its superior seedling vigor in the field appears to be associated with a more stable chloroplast structure. Moreover, the improved performance of chilling tolerant genotypes at

lower temperature could possibly be due to their ability to stabilize cold-sensitive proteins at low temperatures (Graham and Patterson, 1982).

The seedling dry weight of chilled plants expressed as a percentage of control plants of comparable age was not an indicator of chilling tolerance since the percentage was dependent on seedling vigor. Thus, absolute growth rates being quite sensitive to chilling might be considered a measure of chilling tolerance. The relative ranking of chilling tolerance of the 12 inbred lines used in this study was, therefore; CM72 > CK27 > CH12 > CK52 > CK25 > RB214 > C0255 > FV212 > CK76 > CK29 > CK64 > CK69.

4.4 FIELD EVALUATION OF TWELVE CORN INBRED LINES FOR COLD TOLERANCE

Mean weekly minimum and maximum 1983 soil temperatures at the University of Manitoba test site (Point) for the period April 19 to May 30 are given in Table 18. The mean minimum temperature from April 19 to May 23 ranged from 3.9 to 8.2°C. For the same period, the mean maximum temperatures ranged from 8.0 to 12.8°C. Thus, the seeds were exposed to 35 and 21 days of low soil temperatures in the first and second dates of planting, respectively. Each planting environment, therefore, was cold enough to permit detection of differential cold tolerance responses among inbred lines.

Mean squares for the analysis of variance are shown in Table 19. Date mean squares were highly significant, and constituted the largest components of variation for all traits except for juvenile plant height and yield. Differences among inbred lines were also highly significant for all traits. Although mean squares for inbred lines ranged from 1.7 to 28.9 times larger than those for the interaction components, the significant interactions for almost all traits suggested that field tests

Table 18. Mean weekly minimum and maximum soil temperatures at a depth of 5 cm for the period, April 21 to May 18 in 1982, and April 19 to May 30 in 1983.

| Week | Soil Temperature (°C) | | | |
|---------------------|-----------------------|------|--------------|------|
| | Mean Minimum | | Mean Maximum | |
| | 1982 | 1983 | 1982 | 1983 |
| April 19 - April 25 | | 4.5 | | 10.5 |
| April 21 - April 27 | 5.15 | | 12.75 | |
| April 26 - May 2 | | 3.9 | | 8.5 |
| April 28 - May 4 | 8.71 | | 14.53 | |
| May 3 - May 9 | | 6.5 | | 11.5 |
| May 5 - May 11 | 6.41 | | 10.81 | |
| May 10 - May 16 | | 4.4 | | 8.0 |
| May 12 - May 18 | 10.59 | | 14.51 | |
| May 17 - May 23 | -- | 8.2 | -- | 12.8 |
| May 24 - May 30 | -- | 10.2 | -- | 14.8 |

Table 19. Means squares from analysis of variance for various traits of 12 corn inbred lines planted at two dates (April 19, and May 3) in 1983, at the Point (University of Manitoba).

| Source | df | Emergence % | Emergence index (Days) | Seedling dry weight (g) | Leaf number/plant | Juvenile plant height | 50% Silk emergence (days) | 5-Plant yield (g) |
|-------------|-------------------|--------------|------------------------|-------------------------|-------------------|-----------------------|---------------------------|-------------------|
| Blocks | 5(2) ¹ | 155.49 | 12.65 | 2.804* | 1.128* | 22.41* | 5.597** | 13,068.67 |
| Date | 1 | 14,460.06*** | 8,079.46*** | 24.408*** | 6.250** | 58.78*** | 4,125.347*** | 18,368.06 |
| Error(s) | 5(2) | 37.55 | 16.37 | 0.264 | 0.183 | 2.34 | 6.597 | 2,295.38 |
| C.V. % | | 7.69 | 10.37 | 18.836 | 6.746 | 9.64 | 7.140 | 16.30 |
| Inbreds | 11 | 1,202.54*** | 31.06*** | 14.742*** | 2.998*** | 120.16*** | 16.741** | 41,503.92*** |
| Inb. X Date | 11 | 721.96*** | 6.25 | 1.977*** | 0.311 | 7.40*** | 2.287** | 1,435.96 |
| Error(b) | 110(44) | 107.06 | 2.08 | 0.327 | 0.213 | 2.18 | 0.824 | 3,273.69 |
| C.V. % | | 12.99 | 3.69 | 20.978 | 7.273 | 9.30 | 0.983 | 19.47 |

*, **, ***Significant at 0.05, 0.01 and 0.001 level, respectively.

¹Figures in parenthesis are degrees of freedom for 50 percent silking and 5-plant yield.

for cold tolerance response should be carried out in more than one environment.

4.4.1 Seedling Emergence

Average emergence percentage of the early sown inbred lines was significantly lower (22.3 percent) than the late sown lines (Table 20). Nonetheless, the mean emergence of five lines, CK76, CO255, RB214, CK29, and CM72, was not significantly lower than those in the late sown trial. Emergence percentage for the twelve lines ranged from 35.83 percent for CK52 to 89.17 percent for CK76 in the early planting. In the late sown test, all with the exception of CK69 and CK64, showed over 80 percent emergence; CK69 and CK64 exhibited 77.5 percent and 79.2 percent emergence, respectively.

During the field test, the failure of some seedlings to emerge could be attributed to various factors. Although the seeds were treated with fungicide, brown lesions appeared on the radicle and the first internode of most of the seedlings which failed to emerge in this trial. In addition, considerable seed rotting was observed. Miedema (1982) suggested that "the protective action of the fungicide is restricted to the seed and its environment". Thus, "cold conditions after germination may result in seedling blight". Some seedlings also showed seedling malformations before emergence. Miedema et al. (1982) reported that in a field experiment in which seedling emergence was reduced by prolonged exposure to low temperature, about 15 percent of the non-emerged seedlings showed breakage in the region of the coleoptile, resulting in disoriented shoot growth and abnormal seedling development. In general, corn seedlings which are subjected to temperatures below 6°C before emergence are injured or killed by chilling (Miedema, 1982).

Table 20. Rank-summation-index values and means of cold tolerance traits for 12 corn inbred lines planted at two dates (April 19, and May 3), University of Manitoba, 1983.

| Inbred | Index Value | Emergence % | | Emergence Index | | Seedling Dry Weight | |
|--------|-------------|-----------------------|----------------------|-----------------|-----------|--|----------|
| | | April 19 | May 19 | April 19 | May 3 | April 19 | May 3 |
| CK76 | 5 | 89.17 f ⁺ | 93.33 c ¹ | 42.60 a | 30.52 ab | 4.800 g ⁺ (106.9) ² | 4.489 h |
| CO255 | 7 | 87.50 ef ⁺ | 95.83 c | 44.68 b | 29.40 a | 4.823 ef (66.4) | 4.249 gh |
| CH12 | 14 | 60.83 c | 85.000 abc | 45.62 bcd | 30.14 ab | 3.348 f (77.3) | 4.332 h |
| CK25 | 14 | 65.00 cd | 90.83 bc | 46.03 bcd | 30.35 ab | 2.995 ef (68.3) | 4.387 h |
| RB214 | 14 | 85.83 ef ⁺ | 93.33 c | 45.31 bc | 31.40 bc | 3.002 ef ⁺ (84.2) | 3.567 fg |
| CK29 | 17 | 88.33 ef ⁺ | 95.00 c | 46.38 bcd | 31.11 abc | 2.070 cd ⁺ (94.7) | 2.186 cd |
| CK27 | 20 | 73.33 d | 94.17 c | 46.86 cde | 31.47 bc | 2.425 de ⁺ (88.6) | 2.737 de |
| CM72 | 21 | 75.83 de ⁺ | 86.33 abc | 47.08 cdef | 30.71 ab | 1.368 abc (43.4) | 3.149 ef |
| CK52 | 27 | 35.83 a | 92.50 c | 48.38 efg | 31.26 abc | 1.546 bc (38.5) | 4.014 gh |
| FV212 | 29 | 55.00 bc | 93.55 c | 48.99 g | 32.86 cd | 1.220 ab ⁺ (64.2) | 1.900 bc |
| CK69 | 31 | 65.83 cd | 77.50 a | 47.21 def | 33.32 d | 1.434 abc ⁺ (97.8) | 1.466 ab |
| CK64 | 35 | 53.33 b | 79.17 ab | 48.79 fg | 35.62 e | 0.747 a ⁺ (63.1) | 1.183 a |
| Mean | | 69.65 a | 89.69 b | 46.50 b | 31.51 a | 2.315 a | 3.138 b |

¹Means followed by the same letter are not significantly different at 0.05 level by Duncan's Multiple Range Test.

²Figures in parenthesis are seedling dry weights expressed as percentages of the second date of sowing (May 3).

⁺Means of the first date of planting are not significantly different from means of the second date of planting at 0.05 level by Duncan's Multiple Range Test.

The average date of emergence for the 12 inbred lines seeded on April 19, and May 3 were June 4, and 3, respectively (Table 20). While means for emergence index in the first date of sowing (April 19) ranged from 42.60 days for CK76 to 48.99 days for FV212, those in the second date of sowing (May 3) ranged from 29.40 days for C0255 to 35.62 days for CK64 only.

A longer period was required for emergence in the early date of sowing relative to the later seeding date due to slow germination and low rate of shoot growth (Miedema, 1982) and radicle development (Blacklow, 1972).

4.4.2 Seedling Dry Weight, Leaf Number and Juvenile Plant Height

Seedling dry weight was significantly reduced in the first date of sowing (Table 20). On the average, the seedling dry weight of the first sowing was 26.2 percent below the seedling dry weight of the second sowing. The additional two weeks of exposure to low temperature had an adverse after-effect on seedling growth. Miedema *et al.* (1982) observed a similar reduction in shoot dry weight when early sowing was followed by cold weather. Although almost all inbreds exhibited reduced seedling growth, there was a marked genotypic variation in the degree of the effect. The seedling dry weights of the 12 inbred lines in the first sowing expressed as percentages of those in the second sowing ranged from 38.5 percent for CK52 to 106.9 percent for CK76. Mean seedling dry weights of CK76, RB214, CK29, CK27, FV212, CK69 and CK64 in the early sowing were not significantly lower than those in the late sowing. The physiological basis of this adverse after effect is not known (Miedema, 1982).

Leaf number/plant was also significantly reduced in the first date of sowing (Table 21). The average leaf number of the first sowing was 7.6 percent below the leaf number of the second sowing. All inbred

Table 21. Inbred means for four traits in 12 corn inbred lines planted at two dates (April 19 and May 3), University of Manitoba, 1983.

| Inbred | Leaf number/plant | | Juvenile plant height (cm) | | 50% Silk emergence (days) | | 5-plant yield (g) | |
|--------|--|----------|--|-----------|---------------------------|---------|-------------------------------|----------|
| | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 |
| CK76 | 7.5 d ¹ (104.2) ² | 7.2 e | 22.00 _f (103.9) ² | 21.17 g | 97.33 a | 84.00 b | 348 de (95.6) ² | 364 cdef |
| CO255 | 6.2 bc (92.5) | 6.7 bcde | 17.17 de (90.4) | 19.00 ef | 101.33 f | 86.00 d | 373 e (80.0) | 424 f |
| CH12 | 6.5 c (97.0) | 6.7 bcde | 18.75 e (100.4) | 18.67 de | 98.67 bc | 84.00 b | 261 bcd (85.6) | 305 bcde |
| CK25 | 6.5 c (92.9) | 7.0 de | 17.25 de ⁺ (83.8) | 20.58 fg | 98.67 bc | 82.00 a | 363 de (93.8) | 387 def |
| RB214 | 6.3 bc (92.6) | 6.8 cde | 16.08 cd (97.9) | 16.42 bc | 98.00 ab | 84.00 b | 282 bcde (108.5) | 260 bc |
| CK29 | 5.8 b (92.1) | 6.3 bc | 12.17 ab (99.4) | 12.25 a | 99.33 cd | 84.67 c | 96 a (88.9) | 108 a |
| CK27 | 6.0 bc (92.3) | 6.5 bcd | 16.00 cd (101.1) | 15.83 b | 98.000 ab | 84.00 b | 205 bc (75.6) | 271 bc |
| CM72 | 6.0 bc ⁺ (89.6) | 6.7 bcde | 13.17 b ⁺ (77.5) | 17.00 bcd | 100.00 de | 84.67 c | 307 cde (104.1) | 295 bcd |
| CK52 | 6.0 bc ⁺ (85.7) | 7.0 de | 15.00 c ⁺ (84.9) | 17.67 cde | 101.67 f | 84.00 b | 330 de (81.7) | 404 ef |
| FV212 | 6.0 bc (96.8) | 6.2 b | 12.75 b (82.3) | 15.50 b | 103.00 g | 86.67 d | 291 bcde (85.6) | 340 cdef |
| CK69 | 5.8 b (93.5) | 6.2 b | 11.50 ab (98.5) | 11.67 a | 103.00 g | 88.00 e | 289 bcde (84.8) | 341 cdef |
| CK64 | 5.0 a (90.9) | 5.5 a | 10.75 a (88.3) | 12.17 a | 100.67 ef | 86.00 d | 190 ab (86.8) | 219 b |
| Mean | 6.1 a | 6.6 b | 15.22 a | 16.49 b | 99.97 b | 84.83 a | 278 a | 310 a |

¹Means followed by the same letter are not significantly different at 0.05 level by Duncan's Multiple Range Test.

²Figures in parenthesis are leaf number, juvenile plant height, and grain yield expressed as percentages of the second date of sowing (May 2).

⁺Means of the first date of planting are not significantly different from means of the second date of planting at 0.05 level by Duncan's Multiple Range Test.

lines, with the exception of CK76, showed reduced leaf number although there was some genotypic variation in the degree of the effect. Leaf numbers of the 12 inbred lines in the first sowing expressed as percentages of those in the second sowing varied from 85.7 to 104.2 percent. CM72 and CK52 were the only inbreds in the early sown trial which expressed a lower leaf number than inbreds of the late sowing. The extra two weeks of exposure to low temperatures appeared to reduce leaf number. Duncan and Hesketh (1968) observed that a lowering of the growth temperature from 36/31 to 15/10°C (day/night) resulted in a decrease of the average leaf number from 26 to 19 in 22 races of maize. A similar but less pronounced effect was found in hybrids adapted to higher latitudes (Hesketh et al., 1969). Juvenile plant height of the early sown inbreds was significantly reduced (7.7%) below the mean plant height of those sown later (Table 21). Mean juvenile plant heights of CK25, CM72 and CK52 in the early sowing were the only inbreds which were significantly lower than those in the late sowing.

4.4.3 Days from Planting to 50 Percent Silk Emergence, and Grain Yield.

Early sowing followed by cold weather significantly increased the number of days required from planting to 50 percent silk emergence (Table 21). Days from planting to 50 percent silk emergence for the 12 lines ranged from 97.33 to 103 days in the early sowing, and from 82 to 88 days in the late sowing.

Early sowing followed by cold weather also reduced grain yield of the inbred lines, although the reduction was not statistically significant (Table 21). The average grain yield of the first sowing was 10.3 percent below that of the second sowing. Even though most of the inbred lines exhibited reduced grain yield, there was a marked genotypic variation in the degree of the effect. Yields of the 12 inbred lines in the

first sowing expressed as percentages of those in the second sowing ranged from 75.6 to 108.5 percent.

4.4.4 Heritability Estimates, and Correlations among Various Inbred Traits

Heritability estimates calculated from mean squares were 77.8, 89.3, 94.3, 91.9 and 96.8 percent for emergence percentage, emergence index, seedling dry weight, leaf number/plant, and juvenile plant height, respectively. Clearly, there was adequate genetic variation for cold tolerance within this group of inbred lines. Mock and Skrdla (1978) and Mock and McNeill (1979) also reported high heritability values for emergence percentage, emergence index, and seedling dry weight in their studies.

Simple correlations between emergence traits (Table 22) indicated that emergence index was negatively and significantly correlated with emergence percentage, seedling dry weight, juvenile plant height and leaf number/plant. Thus, simultaneous selection for a high emergence percentage, a rapid rate of emergence, and increased seedling dry weight, should be possible.

Seedling dry weight was significantly correlated with leaf number/plant, and juvenile plant height for both sowing dates. The correlations between 100-seed weight and the seven traits were non-significant.

Emergence index was positively correlated with 50 percent silk emergence, i.e., a rapid rate of emergence was associated with early silk emergence. Early silk emergence was also correlated with increased seedling dry weight, leaf number/plant, and juvenile plant height. Similarly, Lee and Estes (1982) observed an inverse relationship between seedling growth of 20 hybrids grown at an optimum planting data and their maturity ranking. Furthermore, MacLean and Donovan (1973) detected an inverse relationship between the rate of early growth and heat unit

Table 22. Simple correlation coefficients among various traits of 12 corn inbred lines planted at fifteen day interval starting from April 19, at the Point (University of Manitoba): April 19 values top, May 3 values bottom.

| | Emergence Index | Seedling Dry Weight | Leaf Number | Juvenile Plant Height | 100-Seed Weight | 50% Silk Emergence | 5-Plant Yield |
|--------------------|-------------------|----------------------|--------------------|-----------------------|-----------------|--------------------|----------------|
| Emergence % | -.755** -.625* | .555 .494 | .242 .542 | .334 .471 | -.056 .071 | -.513 -.480 | -.093 .097 |
| Emergence index | | -.933*** -.857*** | -.669* -.841*** | -.823** -.746** | -.392 -.316 | .654* .546 | -.315 -.352 |
| Seedling dry wt. | | | .759** .907*** | .942*** .933*** | .496 .581* | -.727** -.690* | .344 .550 |
| Leaf Number | | | | .810** .848*** | .087 .393 | -.355 -.647* | .503 .534 |
| Juvenile Plant ht. | | | | | .519 .360 | -.645* -.660* | .517 .659* |
| 100-Seed Weight | | | | | | -.388 -.375 | .136 .128 |
| 50% Silk Emergence | | | | | | | .106 -.044 |

requirements of hybrids as an index of maturity at controlled soil temperatures of 10 and 16°C. The correlations between grain yield and seven other traits in the present study were generally low and non-significant. Since the inbred lines were severely affected by drought, and heavy infestation with stalk borer, it was difficult to correlate grain yield with other parameters.

The relative ranking of cold tolerance responses of the 12 inbred lines (Table 20) using rank-summation-index values (Mock and Skrdla, 1978) indicated that CK76 and C0255 were the two best inbred lines in the 1983 field trial.

4.5 THE CORRELATION BETWEEN SEED CONSTITUENTS AND SEEDLING VIGOR VARIABLES OBTAINED FROM VARIOUS LOW TEMPERATURE TESTS OF CORN INBRED LINES.

Total oil was extracted from seeds of each corn inbred line and the fatty acid compositions were analyzed using a gas chromatograph. Percentage of total seed oil, fatty acid compositions, and unsaturated/saturated fatty acid ratios of corn inbred lines are shown in Table 23. There were significant differences among inbred lines for percent total seed oil and all fatty acids. Palmitic, oleic, and linoleic acids accounted for more than 95 percent of the total dry seed fatty acids. Saturated fatty acid consisted of palmitic, and stearic, while unsaturated fatty acids included oleic, linoleic, and linolenic acids. The unsaturated/saturated fatty acid ratios of cornseed lipids also showed significant differences.

Percent total seed oil and unsaturated/saturated fatty acid ratios were not significantly associated with emergence and seedling vigor variables which were obtained from various tests (Table 24). These results were contrary to what have been reported by Gerasenkov (1968), Gubbels (1974), and Bartkowski (1977). These data, therefore, suggest

Table 23. Percent total seed oil, fatty acid compositions and unsaturated/saturated fatty acid ratios of corn inbred lines

| Inbreds | % of total seed oil | Fatty acids expressed as a percentage of total | | | | | |
|---------|---------------------|--|----------------------|---------------------|-------------------------|------------------------|--|
| | | Stearic Acid (sat.) | Palmitic Acid (sat.) | Oleic Acid (unsat.) | Linolenic Acid (unsat.) | Linoleic Acid (unsat.) | Unsaturated/saturated fatty acid ratio |
| CH12 | 5.4 a ¹ | 2.09 a | 12.82 cd | 38.33 a | 0.58 i | 45.20 k | 5.64 f |
| CM72 | 3.9 ef | 1.59 abc | 8.86 f | 22.70 f | 1.08 f | 64.74 c | 8.47 b |
| RB214 | 3.4 gh | 1.32 c | 10.20 e | 18.29 i | 1.73 a | 67.43 a | 7.59 c |
| CO255 | 3.6 g | 1.86 ab | 16.58 a | 28.40 d | 0.69 h | 51.35 j | 4.36 h |
| CK25 | 4.2 d | 1.17 c | 8.06 g | 21.32 g | 1.53 c | 66.71 b | 9.70 a |
| CK52 | 4.6 b | 1.35 bc | 13.41 c | 29.34 c | 0.99 g | 54.24 h | 5.73 f |
| FV212 | 3.9 ef | 1.69 abc | 15.62 b | 22.82 f | 0.68 h | 58.06 g | 4.71 g |
| CK27 | 4.0 e | 1.58 abc | 10.26 e | 27.57 e | 0.93 g | 58.64 f | 7.36 cd |
| CK29 | 3.8 f | 1.67 abc | 10.56 e | 32.91 b | 1.15 de | 52.55 i | 7.08 d |
| CK69 | 3.5 gh | 1.37 bc | 10.50 e | 21.33 g | 1.18 d | 64.70 c | 7.35 cd |
| CK76 | 3.3 h | 1.31 bc | 12.68 cd | 22.63 f | 1.65 b | 60.84 e | 6.08 e |
| CK64 | 4.3 c | 1.50 bc | 12.17 d | 20.52 h | 1.12 ef | 63.89 d | 6.26 e |
| Mean | 4.0 | 1.54 | 11.81 | 25.51 | 1.11 | 59.03 | 6.70 |
| C.V.(%) | 2.3 | 14.50 | 3.00 | 0.76 | 2.40 | 0.40 | 2.31 |

¹Means followed by the same letter are not significantly different at 0.05 level using Duncan's Multiple Range Test.

Table 24. Simple correlation coefficients between seed oil constituents and seedling vigor variables obtained from (1) a controlled root zone temperature study, (2) a suboptimal ambient temperature study, (3) a field trial, (4) and a chilling temperature study.

| | (1) ROOT ZONE TEMPERATURE STUDY | | | | | | | | | (2) SUBOPTIMAL AMBIENT TEMPERATURE STUDY | | |
|--|---------------------------------|-------|-----------------|-----------------|---------------------|-------|-------------------------------|------|---------------------|--|-----------|---------------------|
| | Emergence % | | | Emergence index | | | Seedling dry weight | | | Emergence index | Emergence | Seedling dry weight |
| | 10°C | 14°C | 18°C | 10°C | 14°C | 18°C | 10°C | 14°C | 18°C | | | |
| % total seed oil | -.211 | -.194 | -.602* | -.139 | -.352 | -.094 | .386 | .192 | .072 | -.422 | -.507 | .024 |
| Unsaturated/saturated fatty acid ratio | .016 | .258 | -.031 | .045 | .117 | .194 | -.136 | .038 | .185 | -.339 | .552 | -.448 |
| | (3) FIELD TRIAL | | | | | | (4) CHILLING STUDY | | | | | |
| | Emergence % | | Emergence index | | Seedling dry weight | | Seedling dry weight (chilled) | | Leaf Area (chilled) | | | |
| | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 | | | | | | |
| % total seed oil | .341 | -.032 | -.137 | .174 | -.016 | -.055 | .476 | | | .515 | | |
| Unsaturated/saturated fatty acid ratio | .152 | -.182 | -.030 | -.043 | .009 | .010 | -.037 | | | .083 | | |

*Significant at 0.05 level.

that the degree of fatty acid unsaturation in cornseed lipid may not be related to the mechanism by which seedlings resist injury at suboptimal temperatures.

4.6 THE RELATIONSHIP BETWEEN THE DIFFERENT CONTROLLED COLD-TEST METHODS AND FIELD PERFORMANCE IN 12 CORN INBRED LINES

The correlations between emergence traits which were obtained from the root zone temperatures study and the field trial exhibited consistent trends (Table 25). Emergence percentage at the three root zone temperatures were positively correlated with field emergence, as were the three seedling vigor variables, seedling dry weight, leaf number/plant, and plant height, but was negatively correlated with emergence indices from the field. In contrast, emergence indices at the three root zone temperatures were negatively correlated with field emergence as were the three seedling vigor variables, but was positively correlated with emergence indices from the field. Most of the correlations were non-significant, however, and hence are of little practical significance to the plant breeder. Of more importance were the significant correlations between emergence percentage at a root zone temperature of 10°C and field emergence. This relationship indicated that the responses of genotypes which were evaluated at a root zone temperature of 10°C appeared to be similar to field performance under cold, wet conditions.

Significant negative correlations existed between seedling dry weights at the three root zone temperatures and emergence indices in the field. Thus, greater seedling dry weights at all root zone temperatures were associated with rapid field emergence. The correlations between seedling dry weights at all root zone temperatures and seedling dry weights from the field were positive and highly significant. Seedling dry weights at all root zone temperatures were also positively and significantly correlated with juvenile plant heights from the field, and

Table 25. Correlation coefficients between various traits obtained from a controlled root zone temperature study and those from the field test.

| ROOT ZONE TEMPERATURE STUDY | FIELD TEST | | | | | | | | | |
|-----------------------------|-------------|--------|-----------------|---------|---------------------|---------|-------------------|--------|-----------------------|---------|
| | Emergence % | | Emergence Index | | Seedling Dry weight | | Leaf number/plant | | Juvenile plant height | |
| | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 |
| Emergence % at | | | | | | | | | | |
| 10° C | .592* | .757** | -.557 | -.631* | .549 | .467 | .272 | .503 | .502 | .325 |
| 14° C | .391 | .516 | -.214 | -.171 | .184 | .142 | -.282 | .112 | .071 | -.033 |
| 18° C | .566 | .677* | -.494 | -.347 | .418 | .249 | .127 | .372 | .308 | .122 |
| Emergence index at | | | | | | | | | | |
| 10° C | -.142 | -.478 | .296 | .546 | -.322 | -.611* | -.055 | -.481 | -.480 | -.506 |
| 14° C | -.328 | -.148 | .499 | .629* | -.468 | -.532 | -.195 | -.347 | -.475 | -.303 |
| 18° C | -.323 | -.030 | .323 | .281 | -.268 | -.196 | -.083 | -.068 | -.275 | -.046 |
| Seedling dry weight at | | | | | | | | | | |
| 10° C | .100 | .129 | -.611* | -.659* | .746** | .785** | .678* | .640* | .788** | .721** |
| 14° C | .318 | .134 | -.730** | -.548 | .783** | .744** | .375 | .588* | .747** | .590* |
| 18° C | .291 | .260 | -.732** | -.606* | .758** | .853*** | .458** | .750** | .774** | .727** |
| Juvenile Plant height at | | | | | | | | | | |
| 10° C | .360 | .311 | -.672* | -.721** | .720** | .796** | .499 | .665* | .712** | .722** |
| 14° C | .214 | .165 | -.654* | -.601* | .710** | .862*** | .433 | .672* | .772** | .803** |
| 18° C | .069 | .237 | -.591* | -.658* | .680* | .887*** | .623* | .727** | .827*** | .843*** |

*, **, ***Significant at 0.05, 0.01 and 0.001 level, respectively.

positively correlated with leaf number/plant with correlations in the second planting date being significant. Thus, one could use controlled root zone temperature of 14°C for example, to identify strains which would also exhibit vigorous seedling growth under field conditions.

The correlations between juvenile plant heights at all root zone temperatures and emergence indices from the field were negative and significant. Thus, increased juvenile plant heights at all root zone temperatures were associated with rapid rate of seedling emergence in the field. Positive and highly significant correlations existed between juvenile plant heights at all root zone temperatures and seedling dry weights as well as juvenile plant heights from the field. Positive correlations were also observed between juvenile plant heights at all root zone temperatures and leaf number/plant from the field, with correlations in the second planting date (May 3) being significant. Thus, these results support the previous conclusion that strains with vigorous seedling growth in the field can be identified using the low root zone temperature regimes.

Correlation coefficients between various traits obtained from low ambient temperature studies and those from the field test are shown in Table 26. In general, the correlations between emergence traits which were obtained from the suboptimal ambient temperatures study and the field trial were quite low and non-significant.

Seedling dry weight, leaf area/plant, and juvenile plant height at suboptimal ambient temperatures were significantly associated with seedling dry weights and juvenile plant heights from the field. While seedling dry weight, and leaf area/plant at suboptimal ambient temperatures were significantly associated with leaf number/plant in the second date of sowing, juvenile plant height at suboptimal ambient temperatures was significantly ($p = 0.01$) associated with leaf number/plant in both sowing

Table 26. Correlation coefficients between various traits obtained from low ambient temperature studies and those from the field test.

| | FIELD TEST | | | | | | | | | |
|---|-------------|-------|-----------------|--------|---------------------|--------|--------------------|--------|-----------------------|--------|
| | Emergence % | | Emergence index | | Seedling dry weight | | Leaf number /plant | | Juvenile plant height | |
| | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 | April 19 | May 3 |
| SUBOPTIMAL AMBIENT TEMPERATURE STUDY | | | | | | | | | | |
| Emergence % | .136 | .533 | .132 | .159 | -.189 | -.163 | -.301 | -.137 | -.178 | -.133 |
| Emergence index | .080 | -.160 | .296 | .363 | -.345 | -.505 | -.169 | -.197 | -.451 | -.337 |
| Seedling dry wt. | .125 | .438 | -.571 | -.562 | .637* | .695* | .557 | .709* | .705** | .578* |
| Leaf area/plant | .039 | .345 | -.575 | -.519 | .651* | .750** | .556 | .663* | .760** | .642* |
| Juvenile plant height | -.071 | .258 | -.545 | -.516 | .688* | .795** | .723** | .764** | .837** | .783** |
| CHILLING STUDY | | | | | | | | | | |
| Seedling dry wt. of chilled plants | -.182 | .211 | -.241 | -.531 | .380 | .764** | .196 | .614* | .580* | .674* |
| Leaf area of chilled plants | -.210 | .107 | -.231 | -.469 | .390 | .718** | .197 | .583* | .578* | .632* |
| Absolute growth rate of chilled plants | -.067 | .287 | -.056 | -.580* | -.142 | .614* | .157 | .508 | .346 | .502 |

*, **Significant at 0.05, and 0.01 level, respectively.

dates. These results indicated that those inbred lines which were vigorous at suboptimal ambient temperatures were also vigorous in the field. Therefore, this testing technique can be effectively used to identify genotypes with vigorous seedling growth at suboptimal temperatures.

Simple correlations between various traits obtained from the field trial and chilling study were calculated and are shown in Table 26. The absolute growth rate which was considered to be an indicator of chilling tolerance was significantly correlated with a rapid rate of field emergence, and significantly correlated with seedling dry weight of lines from the second date of planting (May 3). The seedling dry weight and leaf area of chilled plants were also significantly correlated with the seedling dry weight, leaf number/plant, and plant height of the second planting date. The absence of significant correlations between chilling tolerance and seedling growth in the early date of sowing suggested that post emergence seedling vigor after prolonged cold germination and chilling tolerance are independent traits.

Findings of the present study indicate that correlations between emergence traits obtained from the controlled cold-tests and the field trial were generally low. Only a root zone temperature of 10°C appeared to be promising in predicting field emergence. The absence of close associations between emergence traits in the two environments could be attributed to the adverse effects of long-term exposure of germinating seeds to low temperature under field conditions.

Significant and positive correlations between early vigor variables, seedling dry weight, juvenile plant height, leaf number and/or leaf area, were obtained from both controlled tests (root zone temperatures, and suboptimal ambient temperatures) and under field conditions. This relationship suggested that controlled cold-tests could be effective in identifying vigorous inbred lines which would show similar vigor under

field conditions. Moreover, the results from these same tests were positively and significantly associated with early vigor variables at both planting dates, indicating that selection for vigorous seedlings made at suboptimal temperatures would result in selected populations exhibiting similar vigor at favourable temperatures.

5. SUMMARY AND CONCLUSIONS

Unpredictable weather conditions in the field from year to year have prompted breeders to investigate various testing techniques which can facilitate the evaluation of genotypes for cold tolerance. In the present study, various methods were employed to evaluate corn inbred lines for their tolerance to low temperatures. In addition, an attempt was made to correlate the responses of those lines with their field performance, thereby to establish a rapid screening procedure for cold tolerance in corn. The following observations were recorded:

1. Considerable genotypic variability was detected for emergence percentage, emergence index, seedling dry weight, leaf area/plant and juvenile plant height among the 32 corn inbred lines which were evaluated at suboptimal ambient temperatures. Furthermore, heritability estimates for those traits were large, suggesting that there was sufficient genetic variation for cold tolerance within this group of inbred lines. Simple correlations among the three cold tolerance traits indicated that a high percentage emergence was significantly associated ($p = 0.05$) with a rapid rate of emergence, but not with increased seedling dry weight. However, there were some genotypes which did combine satisfactory emergence characteristics with greater seedling dry weight. Thus, simultaneous improvement of the three traits should be possible. Although seedling dry weight and leaf area were positively correlated ($p = 0.01$) with seed weight, some lines with similar seed weight exhibited significant differences in both leaf area and seedling dry weight, suggesting that increased seed weight might contribute to large seedlings in some lines,

while in others, seedling vigor could be attributed to tolerance to low temperature. Also, genotypic differences were detected with respect to susceptibility to chlorosis. Multiple regression analysis using seedling dry weight as a dependent variable showed that leaf area was the best predictor of seedling dry weight, accounting for 72.8 percent of the observed variability. The correlations between the performance of the 12 lines at suboptimal ambient temperatures under both growth cabinet and field conditions revealed that the association between emergence traits was generally low and non-significant. Nevertheless, post-emergence seedling vigor at suboptimal ambient temperatures was positively and significantly correlated ($p = 0.05$) with that of the field test.

2. When 12 of the original 32 inbred lines were tested at three root zone temperatures (initial temperatures of 10, 14, and 18°C), the two low root soil temperatures of 10, and 14°C were detrimental to emergence and seedling growth of all the lines. However, differential responses of inbred lines were noted within each temperature regime. Heritability estimates for cold tolerance traits, emergence percentage, emergence index, and seedling dry weight, were sufficiently high to suggest that effective selection could be made for these traits in the population of corn inbred lines used in this study. Simple correlations among the three cold tolerance traits indicated that early emergence was significantly correlated ($p = 0.05$) with high percentage stand, but not with increased seedling dry weight. The correlations between emergence traits obtained from root zone temperature tests conducted in the growth cabinet and those from the field test were non-significant. Only a root zone temperature of 10°C appeared to be promising in predicting field emergence. However, post-emergence seedling vigor at low root zone temperatures was positively associated with that in the field.

3. Chilling stress had a detrimental effect on the growth of 12 of the original 32 lines. Despite the adverse effects of chilling on growth, the inbred lines increased their leaf areas and dry weights during the chilling period. Sufficient genotypic variation was observed for both seedling dry weight and leaf area during the period of chilling. The positive correlation between dry weight and leaf area of chilled plants with those of unchilled control plants, suggested that selection for vigorous seedlings made at chilling temperatures would result in selected populations exhibiting vigor at favourable temperatures. Although significant associations were observed between seedling vigor of the late field planting and the vigor of chilled seedlings, similar associations were lacking for the early-sown field test.

4. Although genotypic differences in percent total seed oil and unsaturated/saturated fatty acid ratios were detected among the inbreds in the present study, these parameters were not significantly associated with low temperature tolerance.

5. Field trials revealed that early seeding followed by prolonged cold weather delayed emergence and silking, reduced stand and yield, also retarded post-emergence seedling growth. However, marked differences were detected among the 12 corn inbred lines with respect to emergence, early seedling growth, 50 percent silk emergence, and grain yield. Heritability estimates were also sufficiently large to suggest that effective selection for cold tolerance could be made within the population of inbred lines tested. A rapid rate of emergence was associated with a high emergence percentage, increased seedling growth, and early silk emergence. Early silking was also correlated with increased seedling dry weight, leaf number/plant, and juvenile plant height.

On the basis of the above observations, the following conclusions were reached:

1. Under various tests, considerable genotypic variability was observed for emergence percentage, emergence index, and seedling dry weight. Furthermore, heritability estimates for those traits clearly showed the existence of sufficient genetic variation for cold tolerance within this population of corn inbred lines.

2. A rapid rate of emergence exhibited a consistent association with high emergence percentage, but not with increased seedling dry weight. Thus, the genetic system conditioning emergence appeared to be different from the system controlling post-emergence seedling growth. Nevertheless, the significant correlations between a rapid rate of emergence and increased seedling dry weight in the field trial, and the presence of a similar relationship for these two parameters from field and growth cabinet studies, suggests that simultaneous improvement in emergence percentage, emergence index, and seedling dry weight might be possible. Therefore, rank-summation-index can be used to achieve significant selection advance for cold tolerance.

3. The field trial revealed that generally, early seeding followed by prolonged cold weather delayed emergence and silking, reduced stand and yield, and retarded post emergence seedling growth. However, marked differences were detected among inbred lines for these traits under the field conditions for the present study.

4. The absence of a significant association between low temperature tolerance and unsaturated/saturated fatty acid ratios suggests that the degree of fatty acid unsaturation in cornseed lipids may not be related to the mechanism by which seedlings resist injury at suboptimal temperatures.

5. The correlations between emergence traits obtained from the controlled cold-tests and the field trial were generally low. Only a

root zone temperature of 10°C appeared to be promising for predicting field emergence.

6. Post-emergence seedling vigor from controlled cold-tests was positively and significantly associated with that from the field trial. Thus, strains with vigorous seedling growth in the field can be identified using either a low root zone temperature of 14°C or suboptimal ambient temperatures (15 days 10/10°C + 15 days 15/10°C + 10 days 19/10°C). The use of low root zone temperatures in evaluating the cold tolerance of a large number of genotypes for breeding purposes, however, may not be economically feasible. While screening a large number of genotypes for cold tolerance, therefore, the use of suboptimal ambient temperatures should be considered since large growth rooms can be used for this purpose. Furthermore, the soil used in such studies should be unsterilized in order to simulate field conditions with respect to the presence of soil pathogens. With this modification, the use of suboptimal ambient temperatures may facilitate the identification of genotypes not only with a high emergence percentage and a rapid rate of emergence, but also with reduced susceptibility to soil borne pathogens.

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Appendix Table 1. Means of four traits for 12 corn inbred lines planted at fifteen days interval starting from April 21, 1982, at the Point

| Inbred | Emergence % | | Emergence Index (days) | | Seedling dry weight (g) | | Juvenile plant height (cm) | |
|--------|-------------|-------|------------------------|-------|-------------------------|-------|----------------------------|-------|
| | April 21 | May 5 | April 21 | May 5 | April 21 | May 5 | April 21 | May 5 |
| RB214 | 86.5 | 93.5 | 24.27 | 20.56 | .938 | 1.194 | 7.25 | 9.40 |
| CK52 | 87.0 | 86.5 | 27.38 | 18.52 | .505 | 0.856 | 7.25 | 9.75 |
| CK25 | 76.5 | 96.5 | 25.54 | 20.12 | .527 | 1.061 | 6.75 | 11.75 |
| C0255 | 63.5 | 100.0 | 26.32 | 20.06 | .657 | 1.271 | 7.20 | 11.00 |
| CK27 | 73.0* | 90.0 | 27.45* | 20.08 | .518* | 0.916 | 6.81* | 9.15 |
| CH12 | 70.0 | 87.0 | 24.42 | 19.84 | .602 | 1.364 | 7.50 | 11.75 |
| CK29 | 50.0 | 96.5 | 28.27 | 21.10 | .240 | 0.674 | 4.25 | 7.10 |
| FV212 | 43.0 | 96.5 | 28.82 | 20.88 | .241* | 0.650 | 6.81* | 8.50 |
| CK64 | 53.5 | 83.5 | 26.92 | 20.16 | .200 | 1.297 | 6.25* | 10.25 |
| CK76 | 56.5 | 93.6 | 26.66 | 21.18 | .388* | 1.528 | 6.00* | 12.25 |
| CK69 | 50.0 | 63.5 | 26.16 | 20.60 | .331* | 0.580 | 5.00* | 7.25 |
| CM72 | 76.5 | 83.5 | 25.84 | 19.79 | .305 | 0.788 | 6.25 | 9.40 |

*Values were obtained from a single observation.

Appendix Table 2. Pedigree and origin of 32 corn inbred lines used in the study.

| Inbred | Pedigree | Approximate number of years of selfing as of 1983 | Origin |
|--------|------------------------|---|------------------|
| CH12 | | | Switzerland |
| CK22 | (WD.ND255)V3 | 20 | Canada--Winnipeg |
| CK25 | CabP1.V3 | 20 | Canada--Winnipeg |
| CK26 | C0136.ND203 | 20 | Canada--Winnipeg |
| CK27 | B14.SWF | 20 | Canada--Winnipeg |
| CK29 | ND203.ND284 | 20 | Canada--Winnipeg |
| CK31 | W15.M13.CM7.CM14 | 20 | Canada--Winnipeg |
| CK36 | 63.385xKF _a | 18 | Canada--Winnipeg |
| CK47 | KN2.W153R | 19 | Canada--Winnipeg |
| CK52 | W15.M13.CM7.CM14 | 17 | Canada--Winnipeg |
| CK54 | Haapala101 | 16 | Canada--Winnipeg |
| CK59 | A556.CM37 | 15 | Canada--Winnipeg |
| CK62 | Cold Tol. Syn. | 16 | Canada--Winnipeg |
| CK64 | A495.WD | 16 | Canada--Winnipeg |
| CK65 | A556.CK29#2 | 17 | Canada--Winnipeg |
| CK67 | C0203 Sel. | 17 | Canada--Winnipeg |
| CK69 | A639.CK27 | 12 | Canada--Winnipeg |
| CK71 | A639.CK27 | 12 | Canada--Winnipeg |
| CK74 | Early Sun. | 12 | Canada--Winnipeg |
| CK76 | Z019-1.CK22 | 12 | Canada--Winnipeg |
| CK77 | F7.F2 | 13 | Canada--Winnipeg |
| CK79 | A639.CK27 | 12 | Canada--Winnipeg |
| CK82 | A495.WD | 11 | Canada--Winnipeg |
| CK83 | DeKalb 22 | 12 | Canada--Winnipeg |
| CK84 | (CabP1.V3) M552 | 11 | Canada--Winnipeg |
| CM72 | | | Canada--Morden |
| C0255 | INRA258 | 10 | Canada--Ottawa |
| D14 | | | Germany |
| D121 | | | Germany |
| FV212 | | | France |
| RB214 | | 10 | France |
| VC0264 | | | France |