SELECTION FOR CHILLING AND FREEZING RESISTANCE IN COMMON BEAN

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ABSTRACT

Suboptimal seedbed temperatures (< 15°C) in early spring, and risk of late spring- and early fall-frosts are detrimental to dry bean (*Phaseolus vulgaris* L.) production on the northern prairies. This study: i) determined the effects of suboptimal seedbed temperatures on dry bean seedling emergence and yield; ii) evaluated diverse accessions of cultivated and wild bean for emergence at suboptimal temperatures; iii) investigated freezing resistance in *Phaseolus* species; and iv) obtained interspecific hybrids between frost sensitive *P. vulgaris* and frost resistant *P. angustissimus*.

The mid May planting of dry bean cultivars in 1999 and 2000 resulted in lower emergence (74 to 89%) compared to the late May planting (93 to 95%). However, highest yield was obtained in 1999 with the mid May planting, partly because two indeterminate cultivars, failed to mature prior to the first fall frost, when planted in late May.

When common bean accessions were planted on May 3, 2000, G8823 had the highest emergence at 20 days after planting. The check cultivar CDC Nighthawk was comparable to G8823 at 30 days after planting. A similar trend was observed in 2001 except emergence was higher in later sampling times due to a warmer seedbed. Accessions with low cumulative thermal units to anthesis, however, were not necessarily early in maturity. G8823 was the only accession with consistent early emergence, early anthesis and early maturity in both years.

Leaflets of wild relatives of common bean showed extensive supercooling and their LT_{50} in the presence of external nucleators was 0.5 to 1°C lower compared to the cultivated species. Exposure to cold acclimating temperatures had no effect in enhancing freezing tolerance of *Phaseolus* species. *Phaseolus angustissimus* had the highest seedling survival (55 to 85%) in response to both the fall frost of 2000 and the spring frost of 2001 when the minimum air temperatures were below –5°C.

Frost sensitive common bean was crossed as the female parent with the frost resistant *P. angustissimus* to obtain F_1 interspecific hybrids. In reciprocal crosses, flowers aborted at three days after pollination. Interspecific hybrid plants grew to produce flowers, but failed to set seed.

The mid May planting of dry bean cultivars will result in higher seed yield compared to the late May planting in years with a mid September or earlier frosts. Successful introgression of both resistance to suboptimal seedbed temperatures during emergence (accession G8823) and frost resistance (*P. angustissimus*) into common bean may expand the geographic distribution of bean crop to higher altitudes and latitudes.

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1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a unique legume crop in that all plant parts with the exception of the root are consumed as food. Among legumes, dry bean is the largest crop both in acreage and production, next only to soybean and peanut. In Canada, dry bean production is concentrated in the three prairie provinces and Ontario, with an area of 150,000 ha and a production of 285,000 metric tonnes in 1999 (Food and Agriculture Organization, 2000). In Saskatchewan, area seeded to dry bean was 2,000 ha with a production of 3,400 metric tonnes in 2000 (Saskatchewan Agriculture and Food, 2001). Although dry bean is an economically viable crop, the low acreage in Saskatchewan can partly be attributed to various production constraints particularly, insufficient frost free days in the Dark Brown and Black soil zones of the province. Consequently selection for early flowering, early maturity and high yield are the major objectives of the dry bean breeding program at the Crop Development Centre, Saskatoon. In addition, bean germplasm with superior emergence under suboptimal seedbed temperatures of early spring and resistance to late spring frost, if available, may enable breeders to develop elite dry bean cultivars better adapted to Saskatchewan.

Dry bean is sensitive to chilling temperatures of below 15°C at all stages of plant growth. Germination and emergence is delayed and/or inhibited at seedbed temperatures below 15°C (Dickson, 1971). Dark coloured seed coat (Dickson, 1971), fungicide seed treatment, and seed moisture content above 12% (Roos and Manalo, 1976), to some extent, offset chilling injury by increased vigour of seedlings under cool environments. Genotypic differences for germination and emergence of common bean and its wild relatives under chilling stress have been widely studied (Kooistra, 1971; Dickson and Boettger, 1984; Hucl, 1993; Zaiter et al., 1994). Germination and emergence of bean seeds exposed to suboptimal temperatures, increases rapidly upon onset of favourable temperatures in both controlled environments and field. Hence, chilling sensitivity results in plants being "set back" which causes delayed maturity and poor yield. Chilling tolerant dry bean cultivars may enable early spring planting, and therefore allowing crop

development to maturity prior to the first fall frost. Most chilling injury research is designed to investigate the immediate effects of suboptimal temperatures on germination and emergence, with little reference to the cumulative influence of chilling on subsequent plant growth at above suboptimal temperatures. Time to seedling establishment may affect seed yield through its association with traits such as flowering, maturity, and yield components (Henderson et al., 1998). Alternately, delayed seedling establishment as a consequence of early spring planting may have little or no effect on agronomic traits.

During the growing season, radiation frost on clear, windless nights results in death of bean seedlings. In the presence of extrinsic ice nucleators in both controlled environments and field, bean seedlings are killed at approximately –2°C (Ashworth et al., 1985). In the absence of ice nucleators, however, bean stems avoid freezing by supercooling down to –7°C (Marcellos and Single, 1979). Temperate species, when exposed to cold acclimating temperatures, enhance their freezing tolerance. The importance of acclimation in enhancing dry bean freezing tolerance is not known. Buhrow (1980, 1983) observed fall frost survival of wild relatives of common bean when grown in a germplasm nursery. Seedlings of frost resistant dry bean cultivars may survive late spring frosts. Frost resistance may also expand dry bean production to areas with insufficient frost free growing days and to the high altitudes of the tropics.

Plant breeders often rely on wild relatives as a source of novel and desirable traits for crop improvement. Due primarily to post-fertilization barriers, very few novel traits from wild relatives have been transferred to dry bean cultivars (Singh, 2001). Efficient utilization of wild species requires concerted efforts by taxonomists, plant breeders and researchers in tissue culture.

Suboptimal seedbed temperature in early spring and late spring frost are constraints to dry bean production in northern temperate regions. Therefore, the objectives of this research were to:

1. Compare phenology and yield of selected dry bean cultivars planted under suboptimal and above suboptimal seedbed temperatures in the field,
2. Evaluate common bean accessions and their wild relatives for emergence under suboptimal seedbed temperatures in the field,
3. Investigate freezing resistance of common bean and its wild relatives, and

4. Transfer frost resistance from the resistant wild relative into common bean germplasm.

2. Literature Review

2.1 Common Bean

Common bean (*Phaseolus vulgaris* L.), belongs to the family Leguminosae, subfamily Papilionoideae, tribe Phaseoleae, subtribe Phaseolinae (Debouck, 1991) and section Phaseolus (Delgado-Salinas, 1985). Common bean refers to both cultivars grown solely for immature fleshy pods (garden bean) and for dry seeds (dry bean). However, the largest production and consumption of the crop species is the dry bean type (Singh, 1999).

Evolutionary forces such as mutation, selection, migration, and genetic drift have acted on the viney wild growing common bean in Middle America and the Andes and effected some striking changes in the morphology, physiology and genetic characteristics of present common bean cultivars (Gepts and Debouck, 1991). The wild forms of common bean are distributed over a wide area that extends from northern Mexico (state of Chihuahua) to northwestern Argentina (province of San Luis). Studies on the evolutionary aspect of common bean point to New World origin. The precise centre of domestication (Middle America, the Andes or both) of common bean is however, not known. A comparison between wild and cultivated common bean from the areas of origin (northern Mexico to northern Argentina) indicates a parallel geographic variation for phaseolin type, seed size, bracteole size and seed lectin content. The Middle American genotypes exhibit an "S" phaseolin type, smaller seeds, large oval shaped bracteoles, and lectins, whereas, a majority of the Andean genotypes have a "T" phaseolin type, larger seeds, small triangular bracteoles and lack seed lectins. The "B" phaseolin type was found in small seeded cultivars in the northern Andes, especially Colombia (Gepts and Bliss, 1986). Three centres of domestication are hypothesized for common bean. The first is in Middle America, leading to small seeded (< 25 g/100 seeds) and medium seeded (30 to 50 g/100 seeds) cultivars with 'S' phaseolin pattern. The other two are in South America, one giving rise to large seeded (> 40 g/100 seeds)

cultivars with 'T', 'C', 'H', and 'A' phaseolin patterns and the other giving rise to small seeded cultivars with a 'B' phaseolin pattern (Gepts and Bliss, 1986; Gepts et al., 1986).

Common bean shows a broad range of adaptation to varied climatic conditions and exhibits a tremendous contrast in plant types and length of vegetative period (van Schoonhoven and Voysest, 1991). The production area extends from 52°N latitude to 32°S latitude, from sea level to 3000 m above sea level. The growth of common bean is favoured by a mean growing temperature of 18 to 22°C and well distributed precipitation of 500 to 800 mm during the growing season (Singh, 1992). Common bean genotypes exhibit diverse growth habit. They include determinate bush, indeterminate bush, indeterminate prostrate and indeterminate climbing (CIAT, 1994). The determinate bush genotypes are usually dwarf with a terminal inflorescence and reduced number of nodes on the main stem (Debouck, 1991; CIAT, 1994). The indeterminate bush genotypes have an erect stem with reduced branching; the indeterminate prostrate genotypes have a weak stem with profuse branching; and the indeterminate climbing genotypes have a weak, long and twisted stem with reduced branches. Spaniards and Portuguese took common bean to Europe, Africa and other parts of the Old World (Duke, 1981). Common bean is thus a part of the most diverse production systems in the Americas, Europe, Asia and Africa.

Common bean is the largest of the food legume crops both in acreage and production. Dry bean is grown annually on over 14 million hectares with a production of about 11 million tonnes on a worldwide basis (Singh, 1999). Dry bean serves as a subsistence crop and a major source of protein in Central and South America and in parts of Africa and Asia. In Canada, it is mainly grown for export markets. Approximately 150,000 ha of dry bean are planted in Canada, with a production of 285,000 metric tonnes across the prairie provinces and Ontario (Food and Agriculture Organization, 2000). The Canadian dry bean crop contributes more than $90 million annually to the economy (Statistics Canada, 2001). Diseases, drought, and low soil fertility are among the most widespread and endemic limitations of cultivated common bean in the tropics and subtropics (Singh, 1992). Over 60% of all common bean production areas in the tropics suffer from some sort of drought. At the upper limits of agriculture (i.e., high latitudes and high altitudes), insufficient frost free days and erratic periods of low, but above zero, temperatures during

the growing season are the major abiotic constraints. Other production constraints in Saskatchewan include drought stress and diseases.

2.2 Low Temperature Stress

Biological stress is any environmental factor capable of inducing a potentially injurious strain in living organisms (Levitt, 1980). The environmental factors that inflict stress on plants can be broadly divided into biotic (infection or competition by other organisms) and physicochemical (temperature - low and high; water - deficit and excess; radiation - infrared, visible, ultraviolet and ionizing; chemical - salts or ions, gases, herbicides, pesticides etc.; and wind, pressure, sound, magnetic, electrical etc.). Temperature and photoperiod are probably the most effective forces influencing global crop distribution, while moisture and soil properties determine crop distribution at the regional and subregional levels (Blum, 1988). The temperature regime is critical by way of affecting plant phenology, developmental phases, growth rates, yield components and yield. Growth and development processes follow distinct temperature response curves displaying a minimum, maximum and optimum temperature range. Any temperature outside of the optimum range can be considered as inflicting stress on plants although injury, both internal and external, will become visible only after the threshold stress temperature is reached.

At higher latitudes, low temperatures are of such magnitude that only taxa with special adaptive features grow naturally (Stushnoff et al., 1984). When exposed to low temperatures (< 15°C), plants of tropical and subtropical origin suffer from chilling injury, frequently manifested as inhibited germination, growth, reproduction, wilting, and/or tissue death (Levitt, 1980; Jones, 1992). In contrast to chilling injury, which is more common in tropical and subtropical plants compared to temperate plants, freezing (frost or cryo) injury may occur in all plants when the tissue water freezes. In the absence of acclimation, subzero temperatures (frost) of –1 to –3°C kills most plant tissues. After exposure to cold acclimating temperatures, however, the survival temperature of plants can be lowered considerably below 0°C, depending on the species and tissue. Site of ice formation (intra or intercellular), rate of freezing and thawing, length of time frozen, and repeated freezing and thawing are other factors that determine

the extent of freezing injury. Levitt (1980) categorized plants into six response classes with respect to low temperatures:

Chilling sensitive - sensitive to low temperatures, above 0°C.

Tender - sensitive to light frost or temperatures near 0°C.

Slightly hardy - will survive freezing temperatures to –5°C.

Moderately hardy - will survive freezing temperatures within –5 to –10°C.

Very hardy - will survive freezing temperatures within –10 to –20°C.

Extremely hardy - the most freeze-tolerant species, characterized largely by the ability for supercooling.

A similar classification of plant taxa and physiological changes associated with low temperatures from +10 to –196°C is presented by Stushnoff et al. (1984).

2.2.1 Chilling Stress

Plants of tropical and sub-tropical origin suffer from chilling injury at low, non-freezing temperatures below 10 to 12°C. While the genetic constitution will limit the maximum and minimum temperatures a plant can withstand, the capacity to tolerate a particular temperature is usually governed by the environmental history of the plant. Levitt (1980) classified chilling injury into three types; i) direct injury - leaf discoloration, wilting, death of tissue; ii) indirect injury - solute leakage, starvation, respiratory upset, toxin accumulation, protein breakdown, biochemical lesion; and iii) secondary stress injury - water stress, oxygen deficiency. At the plant level, chilling injury is manifested as poor germination (Greaves, 1996), poor seedling establishment (Auld et al., 1988), stelar lesions (Ashworth and Obendorf, 1980), stunted growth (Auld et al., 1988), wilting (Wolfe, 1991), chlorosis (Kintake, 1998), necrosis (Christiansen, 1964) and poor fruit set (Dickson and Boettger, 1984). Chilling injury is time dependent and is usually reversible if the exposure to chilling temperatures is of short duration. Except for the poor seedling establishment in some field crops and postharvest loss of quality in many fruits, the economic importance of chilling injury is difficult to quantify (Markhart III, 1986). Chilling injury often results in plants being "set back" so that maturation is delayed and this could have a serious consequence on yield and seed quality depending on length of the frost free growing season.

2.2.2 Mechanisms of Chilling Injury and Tolerance

A number of physiological, biochemical and molecular studies suggest that chilling injury is associated with i) changes in membrane properties, such as lipid composition, solute leakage and reduced transport across membranes; ii) malfunction of the mitochondrial respiration; iii) inhibition of photosynthetic activity; iv) induction of active oxygen species; and v) disturbance in the homeostasis of the cytoplasmic pH (Wilson and McMurdo, 1981; Markhart III, 1986; Pearce, 1999; Yoshida et al., 1999).

2.2.2.1 Membrane Properties

Experimental evidence indicates that chilling involves damage to the cell membranes (Jones, 1992). The Lyons-Raison phase change hypothesis states that at a certain critical temperature within the chilling injury range, the membrane lipids of chilling sensitive plants undergo a transition from a liquid crystalline to a solid gel phase (Wilson and McMurdo, 1981). The two main consequences of this transition, which eventually result in injury and death, are an increase in membrane permeability and an increase in activation energy of membrane bound enzymes. A change in the slopes of the Arrhenius plots (ln(rate) against 1/T) at a critical temperature is thought to result from a phase change in the membranes from a relatively fluid form to a more solid gel structure, so that normal physiological activity can only occur above the critical temperature (Blum, 1988; Jones, 1992). This phase change is correlated with the composition of the lipids, with a high proportion of saturated fatty acids occurring in the membranes of chilling sensitive species. Raison et al. (1979) observed that the threshold temperature at which the primary change in membrane lipid structure occurs is correlated with the temperature of the plant's habitat. Levitt (1980) postulated that chilling resistance is due to an ability to maintain the membrane lipids in the liquid crystalline state at chilling temperatures. Cotton and common bean plants become resistant to chilling temperatures ($12^{o}C$) on exposure to temperatures slightly above chilling (Wilson and Crawford, 1974). This hardening is probably due to the observed increase in degree of unsaturation of fatty acids associated with the phospholipid fraction of leaves, which lowers the phase transition temperature below the previously injurious chilling temperature. The membrane phase

transition or membrane lipid unsaturation is not always associated with chilling tolerance. Priestley and Leopold (1980) observed no changes in phospholipids, fatty acids, and free sterols of soybean and pea seeds imbibed at 1 or 20°C for 2 h. In pea, which is capable of cold acclimation, Leheny and Theg (1994) showed that cold block of protein import into chloroplasts was due not to changes in the plastid membrane fluidity, but rather to a decrease in the available energy required for protein import at 5°C. Chloroplasts from both cold hardened and warm acclimated plants were able to import proteins at 5°C, provided that the samples were illuminated during the reaction.

2.2.2.2 Respiration and Photosynthesis

The cold resistant tomato accession PI341988 took 9 days to germinate at 12°C whereas, the cold sensitive line UC82B took 20 days (Leviatov et al., 1994). The difference in days to germination is probably due to a higher respiration rate indicating higher metabolic activity in the cold resistant PI341988 compared to the cold sensitive UC82B. Leviatov et al. (1995) reported that the differences in germination ability between PI341988 and UC82B at both 12 and 25°C is due to higher activity of endomannanase, an enzyme that mobilizes galactomannan in the endosperm cell walls. Low temperature affects nearly all biological processes, including maintenance respiration which is closely associated with overall growth rates (Andrews, 1987).

Photosynthesis is inhibited at chilling temperatures. Wolfe (1991) observed a decrease in water potential, net photosynthesis and stomatal conductance when common bean plants were subjected to an alternating temperature regime of 8/4°C (14/10 h photoperiod, 450 $\mu E\ m^{-2}\ s^{-1}$). Leaves of *Solanum commersonii*, a frost tolerant wild potato, exhibited a marked decrease in leaf area and in total chlorophyll content per leaf area when grown at 5°C compared to 20°C (Griffith et al., 1994). Seedlings of wild tomato accessions from lower altitudes (30 to 2650 m) could not green below 9°C whereas, the accession from 3000 m could green down to 6.5°C (Patterson et al., 1978). Chlorophyll content in the young leaves of chilling sensitive *indica* cultivars of rice was reduced when grown for several days at 15 to 17°C, while no reduction occurred in chilling resistant *japonica* cultivars (Blum, 1988).

2.2.2.3 Other Factors

Activated oxygen species are produced via electron transport systems in mitochondria and chloroplasts as a normal consequence of metabolism (Nykiforuk and Johnson-Flanagan, 1998). Prasad et al. (1994) isolated cDNAs for the mitochondrial catalase3 isozyme from dark grown chilling induced maize seedlings. Hydrogen peroxide increased during acclimation and chilling of nonacclimated maize seedlings. Hydrogen peroxide also increased when seedlings were treated with aminotriazole, a catalase inhibitor. Prasad et al. (1994) therefore, suggested that catalase3 may be an important hydrogen peroxide scavenging enzyme in maize seedlings. Yoshida et al. (1999) reported that H^+-ATPase in chilling sensitive plants is inactivated upon cold exposure resulting in rapid acidification of the cytoplasm and the concomitant alkalization of the vacuoles. In contrast to the H^+-ATPases from hypocotyl of chilling sensitive legumes such as mung, azuki and kidney bean, the H^+-ATPases in chilling tolerant pea and faba bean were very stable for prolonged periods of exposure to cold.

Abscisic acid (ABA) has been related to increased chilling tolerance in a number of species. Pretreatments with water, salt, chilling, or nutrient stresses, or exogenous ABA application, all increased the endogenous ABA and tolerance to a subsequent chilling treatment in cotton (Markhart III, 1986). ABA broadened the temperature range over which the membrane phase transition can occur in liposomes. In soybean seedlings whose roots were maintained at 10°C and shoots at 25°C, ABA was able to overcome the chilling induced resistance to water uptake compared to control seedlings in which the roots were maintained at 10°C but without ABA. Rikin et al. (1983) suggested that destruction of microtubules of the mitotic apparatus and cytoplasm is one cause of chilling injury, and that ABA application to cotton cotyledons stabilized the microtubular network and prevented chilling injury.

The examination of cellular responses to chilling temperatures reveals various disorders such as the loss of turgor, vacuolization, disruption of the cytoplasmic reticulum, vesiculation, reduced protoplasmic streaming, and general disorder of organelles (Blum, 1988). In most plant species, membrane disruption precedes other cellular disorders that ensue as stress intensity and duration increases.

2.2.3 Germination and Chilling Stress

Seed germination has been variously defined as "the protrusion of the radicle", and "appearance of shoot above the ground" depending on one's perspective (Bryant, 1985). Morphologically, germination is the transformation of an embryo into a seedling (Jann and Amen, 1977). Physiologically, germination is the resumption of the metabolism and growth which were earlier depressed or suspended, and the switching on of the transcription of the genome. Biochemically, germination is the sequential differentiation of oxidative and synthetic pathways. Germination includes all processes that are involved in the transformation of a plant embryo into an independent, established seedling. The Association of Official Seed Analysts (AOSA) defines seed germination as "the emergence and development from the seed embryo of those essential structures which, for the kinds of seed in question, are indicative of the ability to produce a normal plant under favourable conditions" (AOSA, 1981; McDonald, 1993).

Jann and Amen (1977) and Bryant (1985) outlined the physiological and morphological events in germination. Imbibition is the first step in germination of quiescent seed, and dormant seeds undergo breakage of dormancy in the re-hydrated state. Early during imbibition, cells near the surface are completely rehydrated, while cells further away from the surface may still be dry. Thus the metabolic activity of the whole seed or whole embryo will increase gradually during the process of water uptake. The renewal of seed metabolism in the imbibition phase is directed towards growth (Simon, 1984). The embryo starts growing and, in most cases, radicle growth precedes epicotyl growth by several hours. In most dicots, the initial growth of the radicle is brought about by elongation of pre-existing cells. In maize and barley, however, cell expansion first occurs in the coleorhiza which first pierces the seed coat (Mayer and Poljakoff-Mayber, 1989). Mobilization of reserves is a relatively late process and occurs two to four days after germination (Bryant, 1985). During mobilization of food reserves, a large increase in hydrolytic enzymes occurs which aids in hydrolyzing carbohydrates, proteins and lipids into smaller, readily transportable, molecules. The mature dry seed contains RNA and DNA. Some of the initial metabolic activity is directed at repairing or replacing the damaged DNA by DNA polymerase and DNA ligase. As the cellular organelles proliferate, there is an increase in the activity of enzymes associated with the

renewal of cell division. An increase in nucleic acid content occurs leading to cell division.

Germination tests are performed under favourable conditions to promote germination and seedling growth (Ferguson, 1993). Germination tests do not relate to field performance under unfavourable conditions. Thus, the concept of seed vigour was introduced to provide additional information on the performance of seeds especially under stress conditions. The AOSA defines seed vigour as "those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of conditions" (AOSA, 1983). The rate of germination, determined for seeds in a standard germination test is generally thought to provide a satisfactory direct measurement of vigour.

2.2.3.1 Factors Affecting Germination and Emergence under Chilling Stress

Temperature, moisture, gases and light are the four major environmental factors affecting germination and emergence. Germination responses under chilling temperatures are probably the most studied in plant species. Seeds of many crop species, mostly of tropical and subtropical origin, are damaged when imbibed at suboptimal temperatures, commonly referred to as imbibitional chilling injury. Imbibitional chilling injury is defined as sensitivity to a combination of low seed moisture and imbibition at cold temperatures (Bedi and Basra, 1993). Poor germination and/or emergence response to suboptimal temperatures has been reported for a range of crop species including lentil (Covell et al., 1986), pea (Tully et al., 1981), chickpea (Chen et al., 1983; Auld et al., 1988), common bean (Hucl, 1993, Zaiter et al., 1994), soybean (Orr et al., 1983; Unander et al., 1986), maize (Greaves, 1996), wheat (Lafond and Baker, 1986), sorghum (Brar and Stewart, 1994), and canola (Nykiforuk and Johnson-Flanagan, 1994).

Based on the onset of chilling injury in relation to the initiation of germination, seeds of chilling sensitive plants can be divided into two categories (Herner, 1986). The first group is not injured by imbibition at suboptimal temperatures, and if temperature rises, germination proceeds normally with little indication of injury. However, once radicle growth has been initiated, injury occurs and typical symptoms include necrosis of radicle tip and damage to root cortex. As in any chilling stress, degree of injury is dependent on

the low temperature itself and duration of exposure. Examples include warm season solanaceous and cucurbitaceous plants. The second group is injured if imbibition begins under suboptimal temperature. Seeds of warm season legumes, sweet corn and cotton are very sensitive during the imbibition period. Physiological studies have suggested that imbibitional chilling injury is associated with i) temperature; ii) interaction between stage of germination and temperature; iii) initial moisture content of the seed at the start of imbibition; iv) rate of imbibition; v) seed coat characteristics or integrity; vi) seed vigour; vii) seed treatments; and viii) cultivar or species (Herner, 1986).

Covell et al. (1986) observed a positive linear relationship between temperature and rate of germination from a base temperature at which the germination rate is zero, to an optimum temperature at which the germination rate is maximal for chickpea, lentil, soybean and cowpea. Hope and Maamari (1994) observed no correlation between germination at a constant temperature of 25°C and an alternating temperature regime of 12/10°C in maize. Time to production of an 1 cm coleoptile was identified as the best predictor of field performance. In canola cultivar Westar, Nykiforuk and Johnson-Flanagan (1999) observed a temporal delay in germination at 10°C that did not affect the overall success of germination. Germination proceeded rapidly if the required equivalent of 16 to 24 degree days occurred before germination. At 6°C however, delay and non-uniform germination due to both thermal and developmental effects was observed.

In an experiment to study the effects of both imbibition rate (slow vs. fast) and temperature (2 vs. 20°C), Chen et al. (1983) observed that the lowest germination in kabuli chickpea cv. Mission resulted from the combination of chilling temperature and fast imbibition. The most chilling sensitive stage was the first 30 min of imbibition at 2°C. When seeds were imbibed at 2°C for 30 min, 1 h or 24 h and then transferred to 20°C, all treatments resulted in approximately 80% germination compared to seeds imbibed at 20°C for 1 h, transferred to 2°C for 23 h, and then transferred to 20°C for 96 h, which resulted in 95% germination. The imbibition rate in soybean cv. Forrest was lower during imbibition at a chilling temperature (5°C) compared to 25°C (Roskruge and Smith, 1997). No difference in potassium leakage was observed between 5 and 25°C temperature treatments for up to 48 h after imbibition. However, greater amounts of amino acids leached from chilled seeds compared to nonchilled seeds. Chilled seeds also

had a significantly higher hydroperoxide level compared to nonchilled seeds. The increase in lipid hydroperoxide levels in chilled seeds and axes after 36 h may be due to the inability of cellular antioxidant defense mechanisms to quench peroxidative free radical damage initiated by some impaired aspect of membrane reorganization during chilling. Decreasing the oxygen available to germinating soybean seeds was more detrimental to the germination process at 25°C than at 10°C (Mortensen et al., 1986). However, the cold tolerant genotypes germinated better and had greater seedling vigour compared to cold susceptible genotypes at both 10 and 25°C and at 2 and 20% oxygen concentration.

Imbibition of maize kernels with 6% moisture content at 5°C resulted in aborted radicle, proliferation of seminal roots and delayed seedling growth (Cal and Obendorf, 1972). The imbibitional chilling injury was partially or totally reversed when the initial kernel moisture was 13 or 16%. Isolated soybean embryonic axes with initial moisture of 6% were also injured by imbibition at 5°C as observed subsequently by reduced growth at 25°C (Ashworth and Obendorf, 1980). Increasing the axis moisture content to 17% decreased the imbibitional chilling injury. Stelar lesions that were observed in embryonic axes was however absent when whole soybean seeds were imbibed in similar conditions, indicating the unsuitability of using embryonic axes to study imbibitional chilling injury. Cultivar and year or location of seed production influenced sensitivity to imbibitional chilling injury. Even at a nonchilling temperature of 20°C, initial seed moisture of 7.5% decreased percent germination and seedling dry weight in faba bean and pea (Rowland and Gusta, 1977). Low seed moisture and low temperature (4 and 10°C) during imbibition reduced seedling growth in faba bean and pea.

Excised soybean embryos imbibed at 10°C leaked solute (Bramlage et al., 1978). Humidifying embryos to between 35 and 50% moisture before imbibition reduced leakage and imparted some resistance to imbibitional chilling injury. The period of profuse leakage was interpreted as time of membrane reorganization. Imposing low temperature stress during this period prolonged the rapid leakage. Bramlage et al. (1978) speculated that low temperature interfered with normal membrane reorganization during imbibition by modifying the physical state of membrane phospholipids. Intact pea seed had a higher germination index than seeds with a nicked seed coat when imbibed at 2°C

(Tully et al., 1981). In contrast, soybean seeds with intact or nicked seed coat had a poor germination index at 2°C. The imbibitional chilling injury in soybean, and pea with split seed coats was attributed to high imbibition rates.

Black seeded genotypes in soybean had a thicker seed coat compared to white seeded genotypes (Tully et al., 1981). Black seeded genotypes also had a lower imbibition rate at 23°C, and a higher germination index compared to white seeded genotypes under both slow and fast imbibition rates at 0°C. The white seeded genotypes were however superior to black seeded genotypes under both slow and fast imbibition rates when imbibition was at 25°C. Legesse and Powell (1992) observed that completely or partially cream/beige cowpea seeds were more susceptible to soil borne fungal pathogens compared to coloured seeds in soils at 25 and 35% moisture content and, at 10 and 20°C. Kelman and Forrester (1999) observed no effect of seed size and ploidy level on germination rate of *Lotus* sp. at 5°C. Large seeded *Lotus* sp. had a higher mean seedling dry weight, stem height and root length compared to small seeded species after 6 weeks of germination/growth at 5°C. In soybean, seeds matured at a low temperature regime (15/10°C) were more tolerant to imbibitional chilling injury compared to seeds matured at a higher temperature regime (24/19°C), as indicated by their germination indices (Orr et al., 1983). The more tolerant cultivars, however, (having imbibed less water at 2°C) retained their tolerance irrespective of the temperature during seed maturation. Unander et al. (1986) also observed that germination index for soybean genotypes were strongly influenced by the genotype and seed source. Lanolin (a hydrophobic seed coating chemical) coated soybean and cotton seeds had a higher percent emergence compared to non-coated seeds when imbibed for 18 h at 2°C (Priestley and Leopold, 1986). The high percent emergence of lanolin coated soybean seed was attributed to its low imbibition rate at 10°C. Addition of gibberellic acid to the germination medium decreased the mean days to germination by 1 day at 25°C and 2 days at 15°C in pepper seeds (Watkins and Cantliffe, 1983). Gibberellic acid may have enhanced the germination rate by triggering some germination process or by increasing embryo growth.

Seed priming is effective in improving germination, seedling emergence, and yield of many early planted small seeded vegetable, fruit and grain crops. Controlled hydration of seeds for a given period followed by dehydration to approximately the original moisture

content is termed priming (Zheng et al. 1994). The major effect of priming is to adjust the osmotic potential of seeds so that they can germinate more readily under stressful conditions. The changes observed during osmotic priming of seeds include i) enhanced metabolic activity and respiration rates, ii) activation of enzymes involved in mobilization of seed reserves, iii) improved capacity for synthesis of RNA and protein, iv) increased accumulation of ATP, v) restoration of membrane integrity and membrane repair or synthesis and vi) leaching of germination inhibitors (Bedi and Basra, 1993).

Watermelon seeds primed with 2 or 3% potassium nitrate solution for 6 days at 30°C without or with 1 or 2 days of drying had the highest emergence under suboptimal temperature regimes (Sachs, 1977). Priming induced rapid and uniform germination of canola seed and rapid emergence of canola seedlings at low temperatures (Zheng et al., 1994). The beneficial effect of priming was most pronounced in a seed lot with low percent germination. Canola seeds primed with either 100 μM ABA or 20% PEG germinated 2 to 3 days earlier compared to control seeds at 8°C (Gao et al., 1999). The final percent germination, however, was not different. Under salt or osmotic stress, the final percent germination was significantly higher for primed seeds compared to control seeds.

Some correlation exists between the environmental requirement for germination and the ecological conditions occurring in the habitat of the plant and the seed (Mayer and Poljakoff-Mayber, 1989). Near the equator, night temperatures fall regularly below 10°C at 2000 m altitude and above, and plants which regularly experience temperatures below 10°C in their native habitat would be expected to be comparatively chilling resistant (Patterson et al., 1978). Wild tomato species *Lycopersicon hirsutum* from high altitudes in the Andes (2100 and 3100 m) had the highest germination in an alternating temperature regime of 15/5°C (12 h/ 12 h). In Kenya, the mean diurnal temperature decreases by 6.5°C for every 1000 m increase in altitude above sea level (Arkel, 1977). Sorghum (*Sorghum bicolor*) plants naturally occurring in the high altitude areas of Ethiopia and Uganda were evaluated in Kenya at three different altitudes of 1920 m, 1860 m and 1850 m. Dry matter yield for accessions from higher altitudes was comparable to, or superior to, maize, millet and sorghum from lowland regions. Although accessions from high altitudes, in general, needed a longer growing season, few

accessions with maturity similar to other check crops were observed. Angosto and Matilla (1994) evaluated the germination response of *Festuca indigesta* seeds collected from 2250 m and 2560 m altitude. Grains of *F. indigesta* from the higher altitude had two aleurone layers whereas, those from the lower altitude had one. Thicker aleurone in grains from higher altitudes might contribute to enhanced degradation of endosperm and consequently to improved germination. This anatomical feature could thus be an adaptive response.

2.2.3.2 Genetics

Knowledge of genetics of traits associated with chilling tolerance would enable plant breeders to efficiently transfer these traits into elite cultivars and advanced breeding lines. Cannon et al. (1973) reported that the ability of the chilling tolerant tomato accession PI341988 to germinate at 10°C was heritable and was governed by a single recessive gene. Ng and Tigchelaar (1973) reported polygenic control of low temperature sprouting ability in tomato with a minimum of three genes. The ability to sprout at 10°C was recessive, the narrow sense heritability was 66%, and the broad sense heritability was 97%. Vos et al. (1981) reported significant additive gene effect with partial dominance for the inability of tomato seeds to sprout at 10°C. Epistatic gene effects were not significant. The narrow sense heritability for low temperature sprouting ability was 69% and the broad sense heritability 85%. In addition, the maternal effect was statistically significant. Cal and Obendorf (1972) also reported a maternal effect for imbibitional chilling injury in maize. Foolad et al. (1998) identified favourable QTL alleles on chromosome 1 of wild tomato *L. pimpinellifolium* accession LA722. The accession LA722 germinates rapidly at 11°C. The cold sensitive tomato line NC84173 had favourable QTL alleles for rapid germination at 11°C on chromosome 4. Cold sensitive trangressive phenotypes were also observed. Identification of QTL(s) with major effects on tomato seed germination under cold stress indicates that rapid response to selection is expected for this trait and also this trait could be improved by marker assisted selection.

Regression analysis of four cycles of recurrent selection indicated that cold germination under laboratory conditions (7.2°C) improved 9% per cycle in a Pioneer cold tolerant synthetic and by 10% per cycle in the Iowa Stiff Stalk Synthetic populations of

maize (McConnell and Gardner, 1979). In the field, however, little improvement was observed for emergence or seedling vigour. The lack of correlation between laboratory and field results for cold-tolerant traits was attributed to mild spring weather during the two year evaluation period. Selection for germination at cold temperatures did not have any detrimental effects on other agronomic traits measured in the two populations. Eagles and Hardacre (1979) reported genetic variation for time to emergence, shoot weight and leaf number in full-sib, paternal half-sib and S1 families derived from a tropical highland population of maize evaluated at 15/10°C. Inbreeding depression of a substantial magnitude occurred for time to emergence, shoot weight, leaf number and chlorophyll concentration. Hence, Eagle and Hardacre (1979) concluded that dominant gene action of varying degree with major effects on these traits must occur in the population.

Patterson et al. (1978) reported that chilling tolerance of tomato seedlings was heritable. Chilling sensitive tomato cv. Rutgers was crossed with two accessions of wild tomato *L. hirsutum* from 3100 m and 30 m in the Andes. The F_1 hybrid seedlings of Rutgers with the high altitude accession had 75% seedling survival after seven days at 0°C whereas Rutgers and its F_1 hybrid with the low altitude accession were killed. Kamps et al. (1987) reported that seedling chilling resistance of intergeneric hybrids between chilling sensitive *Lycopersicon esculentum* cv. Sub-Arctic Maxi and resistant *Solanum lycopersicoides* was dominant.

2.2.3.3 Field Studies

Johnson and Wax (1978) compared several laboratory germination and emergence tests with field emergence and final plant stand in soybean. Laboratory tests correlated with field emergence in favourable seedbed environments compared to unfavourable environments. The cold test showed a consistent high correlation with field emergence and final stand. Unander et al. (1986) however, reported poor correlation between growth chamber germination rating at 10°C and field data in soybean.

McVetty et al. (1986) reported that late April seeding of faba bean resulted in highest seed yield compared to early or late May seeding in Manitoba. Of the yield components, pods plant^{-1} was influenced the most by seeding date. The number of pods plant^{-1}

declined significantly with delay in seeding in both test years. Auld et al. (1988) compared late April, early and late May planted kabuli and desi chickpea cultivars for their stand establishment and yield in the Palouse region of northern Idaho and eastern Washington. Planting in late April produced higher seed yields than planting in late May (34% in 1982 and 5% in 1984), although seedling emergence was slower with the early planting date. Under cool spring conditions (1984), desi chickpea cultivars had higher percent emergence than kabuli types. Auld et al. (1988) recommended that chickpea be seeded in the early spring when average soil temperatures exceed 13°C to ensure optimum seed yield. Henderson et al. (1998) compared seedling establishment and grain yield of amaranth seeded at 15-day intervals from early May to mid June in the northern Great Plain region of the USA. Year-to-year variation in weather influenced the response of amaranth to seeding date for grain yield and other traits. In most years, planting in early June resulted in higher seed yields compared to seeding in May, likely due to better stand establishment. In 1992, however, the crop season was unusually cool and hence, amaranth seeded in early May had the highest yield due to a prolonged growing season while most plants seeded in mid June did not mature prior to the first fall frost. Reduction in yield due to poor plant stand was observed in soybean (Johnson and Wax, 1978).

2.2.4 Germination and Chilling Stress in *Phaseolus*

Upon imbibition at 23/15°C, bean seed swells and the testa shows signs of splitting (Bradbeer, 1988). Between 48 and 72 h after the start of imbibition, the radicle emerges, and between six and eight days, the hypocotyl hook appears near the soil surface and upon unfolding, carries the cotyledons with primary leaves above the soil surface. The hypocotyl elongates rapidly, straightens, and the cotyledons open followed by the unfolding of the primary leaves. The rate of germination and seedling development is, however, temperature dependent within the temperature range considered as optimum for common bean. Ultrastructurally, the storage cells in bean cotyledons are packed with starch grains and protein (Öpik, 1966). Digestion of these reserves starts in cells furthest away from the vascular bundle and is completed in eight days (Belfast New Stringless germinated at 25°C in dark). With the exhaustion of the cotyledonary reserves, the

vascular bundle cells also begin to degenerate. The metabolic activity in the bean seed is at its peak between two and six days of germination. Externally, shriveling of the cotyledons is observed from the fifth day of germination and they drop off once the mobilization of reserves is complete.

Common bean is susceptible to chilling injury. Temperatures below 15°C adversely affect growth and development in bean, causing poor germination, poor vigour, delayed maturity, and poor pollen and seed production (Singh, 1991). Garden bean seed lots imbibed at 15°C for 24 h and germinated at 25°C had a higher percent seed decay compared to seeds from the same seed lot imbibed and germinated at 25°C (Pollock et al., 1969). Vigour as measured by rate of emergence and growth was higher in the seeds imbibed and germinated at the higher temperature. Seed lots with moisture content greater than 12% showed less cotyledon cracking and withstood low temperature imbibition compared to seed lots with 8 or 10% moisture content.

Kooistra (1971) in an extensive evaluation of 280 genotypes of *P. vulgaris* for their germination ability at 9 to 10°C, observed germination mostly in white seeded varieties. In *P. vulgaris* cv. Comtesse de Chambord (white seed coat), all 30 seeds germinated after two weeks. Kooistra (1971) also included accessions of *P. aborigineus* Burkart, *P. ritensis* Jones, *P. calcaratus* Roxb., *P. trilobus* Ait. (now *Vigna trilobus*), *P. coccineus* L., *P. angularis* W.F. Wight, *P. lathyroides* L., and *P. mungo* L. (now *V. mungo*). Above species with the exception of *P. coccineus* and *V. trilobus* showed no germination. All varieties of *P. coccineus* germinated well but considerably slower than Comtesse de Chambord. In general, though the moisture uptake by seeds at 3°C was lower than at 20°C, the former seeds germinated readily when transferred to room temperature. Comtesse de Chambord also required fewer days to 50% emergence compared to Imuna and Vroege Wagenaar. In the cross of Comtesse de Chambord with two snap bean varieties, the F_1 was intermediate between the parents in its response to percent germination at 10°C. Based on the F_6 generation data, Kooistra (1971) concluded that selection for germination capacity was successful although, the germination level of Comtesse de Chambord could not be fully recovered. Dickson (1971) observed that white seeded cold tolerant lines were generally lower in germination compared to the

coloured lines in the field under cool spring conditions. The narrow sense heritability for germination at low temperatures ranged from 33 to 41%.

Roos and Manalo (1976) reported that snap bean seed lots with initial seed moisture content above 12% had higher field emergence than low moisture seed lots particularly, at soil temperatures below 10°C. Using isogenic snap bean lines differing for seed coat colour, Wyatt (1977) demonstrated that white coloured seeds had a thinner seed coat and imbibed water at a higher rate compared to coloured seeds. Slower absorption of water by coloured seeds may permit more uniform swelling of the cotyledons, thereby reducing seed coat and cotyledon cracking, both detrimental factors in snap bean germination and early seedling growth. Dickson and Boettger (1984) studied cold stress tolerance in common bean at four developmental stages - germination, juvenile, blossom set and seed development (as measured by yield). At 8°C, the mean days to radicle emergence was 29 days, at 10°C it was 15 days, and at 12°C it was four days. Also, the emerged radicle at 8°C either rotted and died or was very weak. Thus, if the germination medium is too cold, then bean seeds that are cold tolerant will remain dormant until the germination medium is warm enough (about 10 to 12°C). Dickson and Boettger (1984), also reported that days to bloom at 16°C provides useful information for selection of cold tolerant lines, since earliness to bloom under cool conditions is an essential character for a cold tolerant bean. Taylor and Dickson (1987) studied the susceptibility of a snap bean line with semihard seed characteristic to imbibitional chilling injury. Semihard seeds with greater than 10% moisture content imbibed water at a rate comparable to genotypes without semihard seed coats. However, at 6 and 8% moisture levels, 18 and 6 days were required to complete imbibition, respectively. In general, increased seed moisture content increased the imbibition rate and decreased solute leakage.

In Bulgaria, the soil temperature at seeding of bean is approximately 8°C, and the cultivars take approximately 46 days to emerge and often seeds rot due to soil pathogens (Genchev, 1988). One hundred and eleven genotypes of *P. vulgaris* and one of *P. coccineus* were evaluated for emergence in the field. Variability for days to emergence was observed among *P. vulgaris* genotypes although, they were late in emergence compared to *P. coccineus*, which emerged in 33 days. Hucl (1993) evaluated 16 common bean genotypes at two suboptimal temperatures, 12 and 16°C. Although, the germination

percentage at the higher temperature was almost double that in the lower temperature, significant genotype x temperature interaction that involved change in rank for some genotypes was observed. Genotypes with narrower seeds tended to have high germination, low median germination time and high maximum germination rate. Also genotypes with high seed density were slowest to germinate. Zaiter et al. (1994) evaluated 14 genotypes of common bean at constant temperatures of 8, 10, 12, or 18°C, or at 12 h alternating temperatures of 10/8, 12/8 or 18/8°C. Common bean genotypes differed significantly in their germination response to low temperature, and genotype x temperature interactions was also significant. All genotypes germinated well at 18°C, however, at 12 and 10°C, germination was reduced. The largest germination differences between genotypes were observed at 8°C. The alternating low temperature regime identified two additional cold tolerant genotypes other than those identified as cold tolerant at the constant temperature of 8°C. Zaiter et al. (1994) also showed that during cold treatment, the synthesis of some polypeptides increased in the radicle of certain genotypes while in other genotypes no such difference was observed between cold germination treatment (18°C for 48 h followed by 48 h at 2 or 8°C) and the control (96 h at 18°C in the dark). These proteins likely play an essential role in the development of chilling tolerance. Otubu et al. (1996) reported that additive genetic effects predominated for both percent germination and germination rate in common bean, with no maternal effect.

Snap bean plants grown at 12.5/15 and 12.5/20°C had a relatively lower growth rate compared to plants grown at 20/20°C (night/day temperatures with 15.5 h photoperiod), probably due to a reduced net assimilation rate and specific leaf area (leaf area/leaf weight) (Austin and MacLean, 1972). The reduced specific leaf area may also be an adaptive response to a greater water stress at lower temperatures. Wolfe (1991) also reported that the relative growth rate for common bean was decreased by 40 to 50 % when grown at 18/12°C (14 h photoperiod), due primarily to lower specific leaf area and photosynthetic area compared to control plants grown at 28/18°C. Common bean, pea, spinach and maize had thicker leaves and increased leaf density when grown at 18/12°C. Common bean plants subjected to a chilling stress of 8/4°C (14 h photoperiod) for 24 h, showed a decrease in water potential, visible wilting, and 60 to 80% reduction in net

photosynthesis and stomatal conductance. Kemp (1973, 1978) observed genotypic differences for tolerance to chilling temperature at the time of anthesis and primary leaf stages.

Guye et al. (1987) compared the chilling tolerance of two genotypes of *P. coccineus*, four of *P. vulgaris*, and one of *P. aureus* (now *V. radiata*). Fourteen day old seedlings were subjected to a chilling stress of 5°C for 24 h with a 16 h photoperiod. Loss of leaf pigment, leaf diffusion resistance, relative growth rate recovery, change in leaf water content, and the severity of leaf necrosis were evaluated after the seedlings were returned to 23/18°C. Species tolerance to chilling were *P. coccineus* > *P. vulgaris* > *P. aureus*.

Pardossi et al. (1992) provided evidence for the involvement of endogenous ABA in increasing plant tolerance to chilling induced water stress through its effect on stomata. Bean seedlings were subjected to 3°C for 6 days (0.2 to 0.4 kPa vapour pressure deficit, 100 μmol m^{-2} s^{-1} photosynthetic photon flux density, continuous fluorescent light). During the first 24 h, stomata remained open, and plants wilted rapidly as leaf transpiration exceeded root water absorption. During this phase, ABA did not accumulate in leaves or in roots. After 24 h, ABA content increased in both tissues, leaf diffusion resistance increased, and plants regained turgor. No osmotic adjustment was associated with turgor recovery and stomata remained closed. Application of 0.1 mM ABA to the root system of the plants throughout exposure to 3°C prevented the chilling induced water stress. Feeding ABA to leaves increased leaf diffusion resistance at both 20 and 3°C, however, the amount of ABA needed to elicit a given degree of stomatal closure was higher at 3°C. The above results suggest that ABA may play a role in ameliorating plant water status during chilling.

2.3 Freezing Stress Resistance

Freezing stress resistance is the ability of the plant to maintain its functions and survive freezing temperatures (Palta, 1991). Levitt (1980) defined freezing stress as the freezing potential of the low temperature stress. The freezing potential of a given subzero temperature will differ with plant species, and within a plant, will differ for various organs. In general, the lower the temperature, the greater the amount of ice formed at equilibrium until the temperature is reached at which all the freezable water has

crystallized. The stresses of late spring and early fall frosts, low midwinter minima, and rapid temperature changes cause various types of injury directly and indirectly associated with the freezing of water in plant tissues (Burke et al., 1976). These include crown winter kill in winter cereals, biennials, and herbaceous perennials; sunscald on thin-barked tree species; winter burn to evergreen foliage; blackheart and frost cracking in xylem of trees and shrubs; blossom kill; death of vegetative shoots in late maturing perennial species; death of buds and bark in plants which lose hardiness rapidly during transient warm spells in winter; and death of tender annuals.

During the growing season, radiation frosts on clear, windless nights are the most common type of freezing stress (Chen et al. 1995). Plants lose heat rapidly to the open sky through black body radiation and can cool to temperatures substantially below ambient. Advective cooling can also occur during the growing season due to movement of cold air, and the leaf and air temperatures drop at similar rates. While most warm season crops such as common bean, cucumber and tomato are killed at the moment of ice formation in their tissues, temperate crops such as wheat, barley, and crucifers can survive subzero temperatures either by their inherent freezing tolerance or by acclimation. The mechanisms involved in freezing survival of plants include avoidance and tolerance (Levitt, 1980; Larcher, 1982). Avoidance mechanisms are desiccation, freezing point depression, supercooling and developmental modification. Tolerance mechanisms include tolerance to intracellular and extracellular freezing.

2.3.1 Avoidance

Dehydrated cells (pollen and seeds of plants, and spores of microorganisms) do not freeze at low temperatures due to "bound" or "unfreezable water" and therefore, survive subzero temperatures without any injury. Intact lettuce seeds with 5 to 13% moisture content were not injured by freezing to –196°C, but with 13 to 16% moisture, the threshold of freezing tolerance declined to –40°C (Junttila and Stushnoff, 1977). In seeds containing 20 to 25% moisture, the first exotherm (due to freezing of tissue water) appeared as a single peak when the seed temperature was -10 ± 2°C and another deep supercooling exotherm was also observed. A plateau was reached at 40 to 50% moisture where the mean exotherm of intact seeds remained at -16 ± 2°C irrespective of imbibition

time. Upon radicle emergence, the secondary exotherms disappeared and the first exotherm represented the killing point of the seed (–8°C). Similar exotherms were observed for seeds without seed coat, excised embryos, endosperms, intact seed stuck four times with a needle, and germinated seeds. Therefore, the integrity of the endosperm envelope seems to facilitate deep supercooling which concurrently imparts freezing avoidance to –12 to –18°C in intact, imbibed seeds. Pollen grains with a moisture content of 36% were killed at low temperatures.

Biological systems have two general types of ice nucleators: homogeneous and heterogeneous. In homogeneous ice nucleation, the nuclei form spontaneously in the liquid. In heterogeneous ice nucleation, nucleation occurs as a result of extrinsic nuclei, such as ice crystals (Chen et al., 1995). Potent biological nucleators include bacteria, fungi, plants, and insects. Extrinsic ice nuclei are the major cause of ice formation in tender growing plants. Ice nucleators are ubiquitous, and consequently, the reliability of supercooling as a viable frost survival mechanism is species dependent. Tender plants will readily supercool to varying degrees before tissue water freezes (Li, 1984; Gusta 1985). The extent of supercooling of plant cells depends on i) the presence of ice nucleators, ii) the cell water content, iii) the degree of adaptation (acclimation), and iv) histological and cytological characteristics such as cell size and shape, and hydrophobous barriers. Stem tissue of a range of plants including faba bean, snap bean, pea, alfalfa, rapeseed, potato and wheat were able to supercool to temperatures between –6 and –14°C (Marcellos and Single, 1979). While the difference between cultivars of a species was small for ice nucleus concentration, the difference between species was considerable. Samples of wheat stems contained virtually no active nuclei at –8°C, whereas snap bean contained at least one active nucleus per sample at –8°C, and would have little likelihood of supercooling below it. Using Vali's (1971) differential nucleus spectrum analysis, Marcellos and Single (1979) concluded that snap bean contains nucleators effective in the range –4 to –8°C. Thus, supercooling in the case of frost sensitive plants provides a means of avoiding damaging ice formation during light frost.

The formation of clusters of water molecules able to act as nuclei for the growth of macroscopic ice crystals is a statistical phenomenon which arises from random density and energy fluctuations within the liquid (Franks, 1981). In order to become an active

nucleus, such a cluster must be of a certain minimum size. The lower the temperature, the fewer the number of water molecules needed to produce a critical nucleus. Thus the probability of nucleation increases with decreasing temperature. The rate of nucleation increases by many orders of magnitude over a narrow range of temperature. At –40°C, the number of water molecules required to produce a critical nucleus is in the order of 400. There must be a very high probability that at any given moment, random fluctuations in the liquid will give rise to a molecular arrangement involving 400 molecules which could be recognized as a structure sufficiently close to that of ice onto which the surrounding water molecules can condense. Hence, for plant tissues, spontaneous ice formation without extrinsic ice nucleators occurs between –41 and –47°C (Jones, 1992).

In general, supercooling promotes nonequilibrium freezing, which is more injurious to the tissues compared to initiation of freezing at warmer temperatures. Rapid nonequilibrium freezing that follows significant supercooling leads to a large Gibbs free energy for ice formation, which provides the energy for destruction of tissues at the ice-liquid interface (Chen et al., 1995). The homogeneous nucleation temperature of pure water is –38.5°C (Franks, 1981). The presence of solutes and solvents in water can further depress the spontaneous nucleation point. The freezing point depression due to cell constituents rarely exceeds 2 to 3°C (Larcher, 1982). Therefore, in plants with limited hardiness, freezing point depression could enable plant survival (Burke et al., 1976). Bilanski and Tzeng (1980) reported that diameter of xylem vessels influences the degree of supercooling. Thinner conducting vessels have greater potentials for supercooling. The diameter of the conducting vessels in Festivee, a nonhardy grapevine cultivar, was larger compared to the cold hardy cv. Concord.

Exposure of plants to low acclimating temperatures enhance subsequent freezing avoidance and tolerance (Palta, 1991). Most deciduous forest species and fruit trees avoid freezing by supercooling to –40°C in some of their tissues (Burke et al., 1976). In apple, acclimated bark and bud tissues freeze extracellularly after a few degrees of supercooling. Much of the water in the cambium, cortical, and phloem tissues of the living bark migrates to sites in the outer cortex where ice forms in large masses between cells. Stem tissues of hardy apple cultivars which freeze in this manner survive slow

freezing to –60°C in midwinter or immersion in liquid nitrogen if frozen slowly to –30°C first. Deep supercooling is a survival mechanism for some plant tissues, suggesting that the cold acclimation process involves reduction or elimination of ice nucleating centres in cells of supercooling tissues and development of effective barriers to nucleation by ice in or around adjacent cells, or both. Quamme et al. (1982) studied exotherms in nucleated and nonnucleated twigs of *Prunus* species and seven interspecific hybrids using differential thermal analysis. In general, the most susceptible vegetative tissues were the leaf buds and xylem, while the bark was the hardiest. Flower buds were the most freezing susceptible tissue in almost all taxa. The temperature at which injury occurred to the most susceptible tissues was closely related to the average annual minimum isotherm temperature at the northern limit of distribution of those taxa for which the northern distribution was known. Cold acclimation also i) produces rigid cell walls which may prevent cell deformation during extracellular freezing and ii) results in accumulation of antifreeze protein or antifreeze activity in apoplastic sap (Fujikawa et al., 1999).

Thick cell wall and small cell size may be associated with cold hardiness. Levitt (1980) concluded that cell size is probably a minor factor in cold hardiness. Palta and Li (1979) observed no relationship between differences in cell size and intercellular space, and frost hardiness in potato and its wild relatives. The number and thickness of palisade parenchyma layers and the stomatal index on the upper leaf surface were closely related to frost-hardiness in wild relatives of potato. Hardy species and hybrids (frost killing temperature of –4°C or colder) had two palisade layers, and all nonhardy accessions (frost killing temperature of –3.5°C or warmer), except three, had one palisade layer. The palisade parenchyma occupied a larger portion of the leaf cross section in hardy species (63%) compared to nonhardy species (51%). Estrada (1982) also observed a highly consistent relationship between frost survival both in the field and in a controlled environment chamber, and the double or triple layers of palisade parenchyma cells in the wild relatives of potato. Hybrid clones with 2 or 3 layers of palisade parenchyma had frost hardy wild species in their genetic background. Additional parenchyma layers may ensure plant survival after exposure to spring, fall or mid growing season frosts. On average, frost hardy potato species had an adaxial stomatal index of 27% compared to

non-hardy species which had a stomatal index of 9%, probably due to two palisade layers in hardy species (Palta and Li, 1979). Ploidy was not related to leaf anatomy or frost hardiness. Hardy species occurred in the high Andes, whereas nonhardy species grow mostly at lower altitudes. As the altitude increased from 2300 to 3500 m, frost hardiness of habitant species increased from –2.5 to –5.5°C.

Primary aerial surfaces of vascular plants and some bryophytes are covered by a thin, superficial film, the cuticle, which is composed of soluble and polymeric lipids (Holloway, 1982; Jeffree, 1996). The cuticle provides the first line of defense between the plant and its environment, and the cuticular waxes shed rainwater from the plant surface and limit nonstomatal water loss (Post-Beittenmiller, 1996). In addition to the developmental controls, cuticular lipids are synthesized in response to environmental signals such as light intensity, photoperiod, humidity, chilling, and seasonal variation. A casual observation by the author indicated that leaves of *Phaseolus angustissimus*, a wild relative of common bean are thicker, and have a greyish hue when grown in the field or at a light intensity of 1000 μmol m^{-2} s^{-1} compared to 400 μmol m^{-2} s^{-1}. A thick cuticle, if continuous, may be an effective barrier to nucleation by external ice (Burke et al., 1976). Griffith et al. (1985) observed thickening of the wall of epidermal and mesotome sheath cells in winter rye plants grown at 5°C compared to 20°C. An increase in cuticle thickness in epidermal cells and deposition of lamellar layers in mesotome sheath cells was also observed. Epidermal cells from leaves grown at 20°C were univacuolate while those from leaves grown at 5°C were multivacuolate with a dense cytoplasm. Griffith et al. (1985) speculated that deposition of cell wall materials and lipid derived polymers play a role in maintaining the water status of rye plants during the acclimation and freezing process. The increased leaf thickness may also attenuate light levels within the leaf and therefore decreases the susceptibility to photoinhibition.

Developmental modifications may enable plants to avoid death at sub-zero temperatures. Hypogeal germination in cool season food legumes (lentil, pea, chickpea, faba bean and *P. coccineus*) ensures that at least one meristematic node remains under the soil cover. In the event of severe frost and death of the aerial shoot, the cotyledonary nodes produce a new shoot. The height of the cotyledonary node was negatively associated with both latitude of origin and winter survival in a field of *Centrosema*

virginianum, a tropical pasture legume (Clements and Ludlow, 1977). Accession 26259 (collected from 32°N latitude) had hypogeal germination so that even when the soil was frozen to a depth of one cm as in –7°C frost, survival was complete. With increasing severity of frost, accessions with successively lower potential growing points were killed. Planting depth has a marked effect on cotyledon node height, but the relative ranking of the accessions remained the same. For some accessions, deeper planting resulted in seedlings with cotyledonary nodes two cm above the soil surface, while in others, it resulted in seedlings with cotyledonary nodes deeper in the soil. Cotyledon node height is under strong genetic control with a narrow sense heritability of 79%. Acclimation had no effect on frost survival in this species.

2.3.2 Tolerance

In freezing (frost) injury, the damage is caused by the formation of ice crystals in plant tissues at subzero temperatures (Jones, 1992; Chen et al., 1995). Depending on the rate of cooling, the crystallization of ice occurs in two markedly distinct locations within the tissues of most plants (Guy, 1990). If the cooling is rapid, ice may form within the cells. Crystallization of the water inside the cell may occur by internal nucleation or by penetration into the cell by an external ice crystal. In either case, this intracellular freezing is considered to be lethal. The one exception is the cells and tissues that exhibit supercooling. The rate of cooling required for intracellular freezing of leaf protoplasts suspended in a liquid medium is 3 to 16°C min^{-1}, and for intact plants is approximately 3°C hr^{-1} (Guy, 1990). In nature however, atmospheric cooling rates seldom exceed 1°C hr^{-1} (Steffen et al., 1989). Cells can survive intracellular freezing if the freezing and thawing rates are rapid enough (e.g., 1000°C min^{-1}) to form intracellular ice crystals that do not exceed the damaging size (Li, 1984).

In nature, plants are usually exposed to slow freezing, which results in extracellular ice formation. Ice forms on the cell wall in the extracellular space and spreads from the initial locus throughout the extracellular regions of the tissue if the duration of subzero temperatures is extended (Gusta, 1985; Guy, 1990). When the freezing rate is sufficiently slow to allow water to diffuse to ice extracellularly, the volume of cytoplasm gradually decreases, concentrating cell sap, which in turn depresses the freezing point of

the intracellular water. Equilibrium is reached when the chemical potential of the cell water equals the chemical potential of the extracellular ice (Chen et al., 1995). The amount of water that must be removed to achieve equilibrium by cellular dehydration depends primarily on the permeability of the plasma membrane and the surface area available for efflux relative to the cell volume in relation to the cooling rate and temperature (Lyons et al., 1979). The extracellular freezing results in freeze dehydration, in cell volume reduction, and in concentration of cell solutes including salts, which, if too extensive, may injure the membranes (Chen et al., 1995). The ability of the protoplasm to tolerate the strain exerted by extracellular ice formation determines the freezing tolerance of a plant. Freezing tolerance or tolerance to extracellular freezing is therefore a form of avoidance, i.e., avoidance of intracellular ice formation. The availability of free water in conductive elements and in intercellular spaces affects the readiness and speed of ice formation (Blum, 1988). Thus, the spread of ice in water stressed plants may be slow or even inhibited in discrete regions. In woody tissues, rate of ice growth of 60 to 74 cm min^{-1} has been observed.

2.3.2.1 Cold Acclimation

Plants exhibit constitutive freezing tolerance and/or freezing tolerance after cold acclimation. Constitutive freezing tolerance is expressed even when plants are not exposed to low temperature. Constitutive freezing tolerance is an important feature to survive sudden decreases in temperature (Levitt, 1980). Plant response to tissue freezing is affected by hardening (cold acclimation). Levitt (1980) defined cold acclimation as exposure of plants to a low nonlethal temperature that usually results in an acclimation response characterized by a greater ability to resist injury or survive a low temperature stress that otherwise would be lethal. Cold acclimation is therefore an intrinsic component of plant resistance to freezing and winter survival. It is a complex genetic trait induced by low temperature and results in both morphological and molecular changes. The inherent ability of temperate plants to acclimate to cold and their rate of acclimation are two major factors that limit low temperature survival (Chen et al., 1995). As a general rule, only plants having either a dormancy or a vernalization requirement can acclimate to very low levels (Gusta et al., 1996). Winter cereals can acclimate to

–30°C, whereas spring cereals only acclimate to about –9°C. Plants from northern latitudes, which go into dormancy in mid summer, tend to be more winter hardy than southern or coastal species, which enter dormancy in late autumn. Cold acclimation is a reversible process, and it can be lost upon the rise of temperature. In the field, hardening may fluctuate with temperature and other environmental variables. Other than temperature, which is the most important factor in affecting hardening, several other environmental factors such as light intensity, photoperiod, plant-water stress, and nutrients interact to affect hardening. Low temperatures combined with short days provide the ideal conditions for hardening. Changes in membrane composition, exclusion of nucleators, osmotic adjustment, and changes in gene expression are few of the plant responses to cold acclimation.

Cell Water: Water content decreases during cold acclimation. Chen and Gusta (1982) observed a decrease in water content in cell cultures of both spring and winter wheat. The decrease in water content is probably due to an increase in dry matter or loss of water in the extracellular spaces. Swensen and Murray (1983) reported that during cold acclimation, a decline in percent moisture content occurred in winter pea seedlings but not in spring pea seedlings. Percent moisture of pea seedlings and freezing tolerance were inversely related. Dry matter accumulation did not differ among pea genotypes and was not related to freezing tolerance. If water content of wheat crowns was increased by less than 5% from 73%, the LT_{50} was reduced approximately 6°C (Gusta et al., 1982). The additional water is probably confined to the extracellular spaces and therefore, the extracellular ice plays a role in frost injury. Gusta et al. (1975) observed that the fraction of frozen water which was tolerated by nonacclimated winter cereals and by acclimated spring wheat was less compared to acclimated hardy cereals. Chen et al. (1976) observed similar trends in *Solanum* species.

Plasma membrane: Quantitative and qualitative changes in plant membrane lipid composition have been observed during cold treatment, which leads to increased freezing tolerance. Earlier studies were based on the notion that an increase in the fatty acid unsaturation would increase membrane fluidity which could stabilize membrane structure

and function at low temperatures. Palta (1994) compared plasma membrane lipid changes during cold acclimation in *Solanum tuberosum* (cultivated potato is freezing sensitive and unable to cold acclimate) and *S. commersonii* (wild potato is freezing tolerant and able to cold acclimate). Prior to cold acclimation, the wild species, *S. commersonii*, had a lower sterol/phospholipid ratio, lower total membrane lipid per mg membrane protein, a higher unsaturated/saturated fatty acid ratio, higher proportion of acylated steryl glycosides, and a lower proportion of free sterols than *S. tuberosum*. After acclimation, the changes in the acclimating species, *S. commersonii*, included an increase in phosphatidylethanolamine, a decrease in sterol/phospholipid ratio, an increase in linoleic acid, and a decrease in linolenic acid. The above changes were either absent or opposite in the nonacclimating species, *S. tuberosum*. Levels of linoleic acid content in leaf plasma membrane best correlated with the enhanced freezing tolerance after acclimation and cold acclimation capacity in potato and may impart cryostability to plasma membranes (Palta, 1994).

Compatible Solutes: Sugars, sugar alcohols, quaternary ammonium compounds and the amino acid proline are compatible solutes that accumulate in plants under stress. Synthesis of glucose, fructose and glycerol accounted for most of the observed increase in osmotic pressure during cold acclimation in *Opuntia ficus-indica*, a CAM plant that can tolerate up to $-10^{o}C$ upon cold acclimation (Goldstein and Nobel, 1994). Extracellular mucilage and the relative apoplastic water content increased by 24 and 10%, respectively, during exposure to low temperature. These increases apparently favour extracellular nucleation of ice closer to the equilibrium freezing temperature for plants. During cold acclimation of eucalyptus hybrids, increase in soluble sugars was responsible for increased leaf osmotic pressure, which resulted in greater freezing tolerance (Almeida et al., 1994). Increase in sucrose, glucose, fructose and *myo*-inositol during cold acclimation in cabbage leaves resulted in increased freezing tolerance (Sasaki et al., 1996). With the exception of *myo*-inositol and starch, all soluble sugars increased gradually during cold acclimation such that their levels were positively correlated with the degree of freezing tolerance. Bush et al. (2000), however, observed no relationship between total nonstructural carbohydrate accumulation in stolons of carpetgrass

(*Axonopus affinis* Chase) during acclimation and subsequent freezing tolerance. The raffinose family oligosaccharides (sucrose, raffinose and stachyose) may provide cell membrane stabilization during the process of seed maturation and desiccation and again when the seed rehydrates during germination (Koster, 1991; Bernal-Lugo and Leopold, 1995).

Developmental reprogramming of carbon metabolism, leading to the recovery of photosynthesis and the preferential accumulation of soluble sugars, is an essential element for acclimation to low growth temperatures for the winter survival of herbaceous plants (Hurry et al., 1998). Hurry et al. (1998) compared ^{14}C incorporation among three different leaf treatments of *Arabidopsis*. The treatments included leaves developed at 23°C, leaves developed at 23°C and then transferred to 5°C, and leaves developed at 5°C. At an air temperature of 23°C, leaves that developed at 5°C showed a five fold increase in ^{14}C incorporation compared to leaves developed at 23°C or leaves developed at 23°C but transferred to 5°C. At an air temperature of 5°C, cold developed leaves partitioned less than 30% of the newly fixed carbon into starch, and instead increased partitioning of newly fixed carbon into soluble sugars. The primary effect of leaf development at 5°C was to restore a high overall rate of photosynthesis. This was associated with a shift in the emphasis of partitioning towards the soluble carbon sinks.

Hardened citrus trees kept for three weeks at 16/4°C day/night temperatures had a greater freezing survival than nonhardened trees (Kushad and Yelenosky, 1987). Citrus trees responded to hardening with a uniform and substantial increase in proline and spermidine, but less uniform increase in putrescine and spermine. The increase in the above compounds may collectively contribute to maintain tissue viability through osmoregulation, protection of cellular membranes and enzymes, and conservation of energy for post stress growth. Kishitani et al. (1994) compared winter and spring barley lines for betaine accumulation during acclimation and their subsequent freezing tolerance. Spring barley lines consisted of three near-isogenic lines each differing in a single *Sh* gene for the spring type growth habit. Winter barley line and the spring line with the *Sh* gene, accumulated higher levels of betaine than the spring lines with the Sh_3 gene. Barley lines with higher betaine accumulation also had a higher survival when held at –5°C for two days. Nomura et al. (1995) reported that regardless of the vernalization

requirement (winter or spring growth habit), late heading barley cultivars accumulated more betaine in their leaves compared to those that head early. The higher levels of betaine in the late cultivars might have resulted from co-selection for lateness of maturity and freezing tolerance.

Increase in betaine levels both, in spring and winter wheat during cold acclimation was observed by Allard et al. (1998). Winter wheat cv. Fredrick had 30% more betaine than the spring wheat cv. Glenlea prior to cold acclimation and this difference was maintained throughout the cold acclimation period. With exogenous application of betaine to spring wheat, Allard et al. (1998) established that cold acclimation treatment and betaine application acted in an additive manner in increasing freezing tolerance. Betaine application to nonhardened plants and cold acclimation treatment independently induced the expression of two stress-related *Wcor410* and *Wcor413* genes in spring wheat. Rajashekar et al. (1999) showed that cold acclimation in strawberry plants is associated with an increase in the endogenous glycine betaine levels. Glycine betaine levels in strawberry leaves increased nearly two-fold after 4 weeks of acclimation, while the freezing tolerance increased from –6 to –17°C during the same period. Exogenous application of ABA or glycine betaine to nonhardened plants also increased freezing tolerance by 80% of the untreated control. Exogenous ABA resulted in increased levels of glycine betaine in both nonhardened and hardened strawberry plants, however, the increase in glycine betaine was greater in hardened plants.

Abscisic Acid: Abscisic acid is an essential mediator in triggering plant responses to adverse environmental stimuli. Application of exogenous ABA can substitute for the low temperature stimulus in induction of freezing tolerance in a range of plant species (Gusta, 1985). ABA may also have a central role in the cold acclimation processes. Levels of ABA were strongly correlated with freezing tolerance of nonacclimated barley (both spring and winter) cultivars (Bravo et al., 1998). ABA content also increased in acclimated cultivars during the first six days of acclimation and then decreased to a level that was twice that of the control nonacclimated plants. Cold acclimated barley plants showed a biphasic increase in freezing tolerance, with the second increase in freezing tolerance coinciding with the ABA peak observed on the sixth day of acclimation.

Soluble sugars and proline also increased during acclimation following biphasic kinetics. Using ABA-insensitive (*abi*) and ABA-deficient (*aba*) mutants of *Arabidopsis*, Gilmour and Thomashow (1991) showed that ABA and low temperature may regulate the expression of some *COR* (cold regulated) genes through independent mechanisms.

Calcium: Calcium has a vital role in mediating plant responses to external stimuli of both abiotic and biotic origin. Because the cytosolic calcium concentration is very low, small changes in the absolute amount of calcium can create a 10 to 100 fold difference in concentration without upsetting the ionic balance of the cell. This feature makes calcium an excellent second messenger (Palta, 1991). Alfalfa cells cold acclimated for eight days at 4°C developed nearly 20 times greater freezing tolerance compared to nonacclimated cells (Dhindsa and Monroy, 1994). Cold acclimated cells treated with calcium chelator EGTA had only 25% of the freezing tolerance of the cold acclimated but nontreated control. Cells treated with calcium channel blockers lose their ability to develop freezing tolerance. Furthermore, by treating cold acclimated alfalfa cells with differential inhibitors of protein kinases, Dhindsa and Monroy (1994) established that by inhibiting the activity of calcium/calmodulin (calcium binding protein) dependent protein kinase, development of freezing tolerance could be completely inhibited in cold acclimated cells. With protein kinase C inhibitor, freezing tolerance was decreased to 50% of the cold acclimated but nontreated controls. Calcium inhibitors and channel blockers also inhibited the expression of cold acclimation specific genes in alfalfa cells.

Superoxides: Cold, in common with many other stresses, may cause oxidative stress as a secondary factor (Pearce, 1999). Plants have evolved to mitigate and repair the damage by synthesizing enzymes such as superoxide dismutase (SOD), catalases and peroxidases, and free radical scavengers such as carotenoids, ascorbate, tocopherols, and oxidized and reduced glutathione. McKersie et al. (1999) reported that transgenic alfalfa plants overexpressing mitochondrial MnSOD had a higher survival rate in the field after one winter and greater shoot dry matter production in the field than nontransgenic control plants. Wu et al. (1999) investigated superoxide dismutase gene expression to elucidate its role in drought and freezing tolerance in spring and winter wheat. The mitochondrial

MnSOD and the chloroplastic Cu/ZnSOD genes were mapped to the long arms of the homologous group 2 and 7 chromosomes, respectively. Transcripts of both classes of SOD genes increased during natural acclimatization in both spring and winter types. However, transcripts of Cu/ZnSOD mRNA were detected sooner in winter than in spring wheat. Exposure of fully hardened plants to three nonlethal freeze thaw cycles resulted in Cu/ZnSOD mRNA accumulation, however MnSOD mRNA levels declined in spring wheat but remained unchanged in winter wheat. The above results suggest that winter wheat has evolved a more effective stress repair mechanism than spring wheat. Seppänen and Fagerstedt (2000) observed a concurrent increase in SOD activity and freezing tolerance in *Solanum commersonii* (frost tolerant), *S. tuberosum* (frost sensitive), their somatic hybrid and S1 genotypes of somatic hybrids. The increase in freezing tolerance was, however, not statistically significant. While the MnSOD mRNA accumulated to higher levels in *S. commersonii* and somatic hybrid in response to acclimation at 4/2°C, the CuZnSOD mRNA accumulated to higher levels in *S. tuberosum* and somatic hybrid. No difference in cytosolic CuZnSOD was detected among the species.

During thawing, freezing injury can be increased by increasing the rate of thawing (Levitt, 1980). Slow thawing (0.5 to 2°C h^{-1}) enables plants to tolerate a lower freezing temperature than rapid thawing (2 to 4°C min^{-1}) (Gusta and Fowler, 1977). The death of extracellularly frozen plants may not occur until hours or even days after thawing and the injury may sometimes be reversible (repairable). Hence, the thawed plants are usually kept cool (0 to 5°C) for about 24 h prior to evaluation of freezing injury. Deacclimation is dependent on temperature. Potato plants deacclimated less at 10°C compared to 20°C (Chen and Li, 1980). In winter wheat, however, the hardiness of acclimated plants declined to the same level when deacclimated for 6 days at either 10 or 20°C (Gusta and Fowler, 1976).

2.3.2.2 Genetics

Freezing stress is a major concern in the production of warm season crop species in northern latitudes and at higher altitudes. Even a temperate species with the ability to cold acclimate may be susceptible to midseason frost. Genetic variation for freezing

resistance has been studied in several crop species with the main objective of either enhancing the freezing resistance currently available in a species or of transferring freezing resistance into a sensitive species. Sources of freezing resistance have been identified in a range of genera including *Solanum* (Sukumaran and Weiser, 1972), *Phaseolus* (Buhrow, 1980), *Ficus* (Hummel and Johnson, 1985), *Triticum* and *Aegilops* (Limin and Fowler, 1985), *Cicer* (Singh et al., 1990), *Paspalum* (Cardona et al., 1997), and *Lens* (Eujayl et al., 1999).

Genetics of freezing resistance in winter cereals is the most extensively studied. In a six-parent diallel cross of Chinese spring wheat with five winter wheat genotypes differing in frost resistance, Sutka (1981) reported additive and nonadditive gene action in the inheritance of frost resistance. The high general combining ability: specific combining ability ratio indicated a preponderance of additive genetic variance. Similar trends were observed in pea for freezing tolerance in the field and in a controlled environment (Auld et al., 1983a). Maternal effects were not observed and frost sensitivity was partially dominant in wheat (Sutka, 1981). Chromosome substitution analysis indicated that genes responsible for frost resistance of winter wheat cv. Cheyenne were localized on chromosomes 5A, 7A, 4B, 5B, 4D, and 5D. Chromosome 5A of Cheyenne carries a gene that imparts a higher degree of frost resistance, and that of wheat cv. Hope and *T. spelta*, a gene that imparts a lesser degree of frost resistance (Sutka and Veisz, 1988). For both pairs of parent and at all freezing temperatures, the additive gene effect was greater than the dominant gene effect. Sutka and Veisz (1988) observed that at higher (–10°C) freezing temperature, freezing resistance was dominant, and as the temperature decreased (–14°C), freezing susceptibility became dominant.

Limin and Fowler (1984) studied the importance of rye and wheat (*T. aestivum* L. and *T. tauschii*) cytoplasmic effects in conditioning plant cold hardiness. Cold hardiness of octoploid triticale produced by crossing hardy rye with nonhardy wheat was similar to that of the wheat parent, demonstrating a complete suppression of the rye cold hardiness genes. Similar observations were made for wheat-rye amphiploids from reciprocal crosses, indicating that this suppression was not due to cytoplasmic effects. The cold hardiness of the alloplasmic rye with *T. tauchii* cytoplasm was similar to that of the rye parent indicating that the cold hardiness genes of rye have normal expression in the *T.*

tauchii cytoplasm. Limin and Fowler (1984) concluded that the rye genome is suppressed by a gene or genes in the wheat complement.

Limin and Fowler (1988) evaluated several interspecific or intergeneric hybrids and amphiploids of wheat and other members of the tribe Triticeae to assess the potential of alien species as donors of cold hardiness genes. Results indicated that changes in ploidy level, relative to the parents, may influence the cold hardiness potential of an interspecific combination by affecting gene dosage and possibly cell size. Poor expression of cold hardiness genes from very hardy diploid genomes also indicated some degree of suppression, or homeoallelic dominance of wheat cold hardiness genes in amphiploids. Chen et al. (1999) compared freezing tolerance in F_1 interspecific and somatic hybrids of potato using 20 species and 34 different combinations between hardy and sensitive species. Field freezing (fall frost) tolerance of hybrids resembled either that of their parents or the parental mean depending on the species combination and the genomic ratio. A similar trend was observed for constitutive freezing tolerance and acclimation capacity when plant materials were evaluated for their freezing resistance in a controlled environment chamber. In general, the expression of freezing tolerance was higher in hybrids with more genomes from the hardy parent. Freezing tolerance of hybrids produced by parents with equal ploidy were either close to the sensitive parental mean or intermediate, except in some combinations that involved *S. commersonii*.

Stone et al. (1993) demonstrated the independent inheritance of constitutive freezing tolerance and cold acclimation capacity in diploid *Solanum* species. Both traits were partially recessive as indicated by the F_1 and were controlled by few genes. The number of genes controlling these two traits was not determined. Eujayl et al. (1999) studied the inheritance of radiation frost injury in lentil and observed a monogenic control of the trait in the $F_{6:8}$ and $F_{6:9}$ recombinant inbred lines. Transgressive segregation for susceptibility was observed. A random amplified polymorphic DNA marker linked to the locus for radiation frost tolerance (*Frt*) trait at 9.1 cM was identified.

2.3.3 Assays

The success of a screening technique depends on its ability to reproduce the probable conditions of development of the stress in the target environment (Wery et al., 1994).

Screening techniques also require characterization of the most probable stress in its actual position in the plant cycle and its reproduction in conditions where screening of a large number of genotypes can be made. Therefore, control over the stress environment is critical.

Individual plant organs or whole plants have been used for evaluating freezing resistance either in controlled environment chambers or in the target environment. Electrolyte leakage and regrowth from frozen seedlings is the most widely used technique in evaluating freezing injury. Electrolyte leakage on frozen tissue either from the field or the controlled environment chamber has enabled physiologists and breeders to distinguish freeze tolerant from susceptible species (Sukumaran and Weiser, 1972; Gusta and Fowler, 1977). Low temperatures used for hardening the plants affect the photosynthetic apparatus (Blum, 1988). In maize and rice, chlorophyll loss under chilling stress has been reported. Thus, scoring the seedlings for colour has been proposed as a simple technique. Kamps et al. (1987) reported that chlorophyll fluorescence was the most precise assay to quantify chilling injury in tomato seedlings. Differential thermal analysis was used to study the exotherm events during freezing of plant parts (Burke et al., 1976; Philley et al., 1995). Nuclear magnetic resonance was used to quantify liquid water of frozen tissues.

In nature, winter kill is more a function of duration of exposure to sub-lethal temperatures, rather than exposure to a minimum low temperature for a short duration (Gusta et al., 1997). Screening for frost and winter survival in the field is complicated by spatial and temporal variability (Blum, 1988). An ideal winter for screening of freezing resistance is infrequent. Furthermore, uniform selection pressure in the field is difficult to achieve and rarely occurs. However, field screening has been successfully employed to select for freezing resistance by using appropriate resistant and susceptible check cultivars in wheat (Fowler, 1979), rapeseed (Teutonico et al., 1993), pea (Auld et al., 1983b), chickpea (Singh et al., 1989), lentil (Erskine et al., 1994), and potato (Estrada, 1982). Laboratory screening of whole seedlings has also been used in several species (Fuller and Eagles, 1978; Auld et al., 1983b; Swensen and Murray, 1983; Limin and Fowler, 1985). A major constraint in laboratory evaluation is the number of samples that could be evaluated at one time without compromising spatial uniformity.

Ice formation in intact plants can be readily detected by exotherms during the freezing process. However, the actual site of ice initiation and the temperature at the site of ice nucleation cannot be determined (Wisniewski et al., 1997). Infrared video thermography enables direct observation of ice nucleation, propagation as revealed by the changes in temperature caused by the release of the heat of fusion as water changes phase from a liquid to a solid, effect of plant structure on the freezing process, and relationship between specific pattern of freezing and visual pattern of injury (Wisniewski and Fuller, 1999).

2.3.4 Freezing Resistance in *Phaseolus*

Common bean is freezing sensitive and is killed at the moment of ice formation (–2°C). In the absence of external ice nucleation, the mean freezing temperature of greenhouse grown snap bean stems was –7.1°C (Marcellos and Single, 1979). Ice nucleators were however, effective in the range of –4 to –8°C. Ashworth et al. (1985) used various freezing techniques and tissue mass to determine the ice nucleation temperature of common bean. A 10 µl sample of a 2% homogenate (0.2 mg tissue) froze at –9.8°C (median = –10.5°C), leaf discs of 6.5 mm (4 to 5 mg tissue) froze at –7.5°C (median = –11.0°C), and 1 ml of 2% homogenate (20 mg tissue) froze at –7°C (median = –8°C). Also, seedlings grown in a controlled environment chamber (2.7 mg tissue) cooled at the rate of 1.5°C h^{-1} froze at –1.3°C (median = –3.9°C), and seedlings in the field, in response to natural frost froze at –1.3°C (median = –2.7°C). The number of bean seedlings that froze at a particular sub-zero temperature increased with the duration of exposure, with the nucleation rate being faster at –5°C than at –4°C. Bean seedlings inoculated with *Pseudomonas viridifalva* at a concentration of 10^8 cells ml^{-1} froze at –3.7 ± 0.8°C, while those sprayed with deionized water froze at –3.1 ± 0.2°C. Misted bean seedlings froze at –1.2 ± 0.4°C while the control plants (unsprayed) froze at –4.3 ± 0.7°C.

Buhrow (1980, 1983) first reported frost tolerance in several wild related species of common bean. *Phaseolus acutifolius* var. *tenuifolius*, *P. angustissimus*, *P. filiformis* (syn. *P. wrightii*), and *P. ritensis* (syn. *P. metcalfei*) survived several successive nights of radiation frost when grown in a germplasm nursery in Tuscon, AZ. *Phaseolus wrightii*

(syn. *P. filiformis*) survived 19 nights of frost during which the lowest recorded temperature was –6.7°C. All other species survived 6 nights of frost when the low temperature was –2.8°C. The above species, with the exception of *P. ritensis*, possess drought tolerance (Buhrow, 1981).

2.4 Genetic Resources and Crop Improvement

Success in plant breeding depends on the ability to identify promising parents, to combine desirable attributes through hybridization, and to recognize and select effectively among segregating populations (Stoskopf et al., 1993). Historically, plant breeders used land races for selection and improvement of cultivated species. Utilization of wild species is another method designed to introduce additional variability into cultivated plants (Stalker, 1980). Transfer of genes from wild relatives and land races into cultivated genotypes has played an important role in crop improvement for many decades and has offered great economic rewards (Goodman et al., 1987)). The extent to which the wild relatives are utilized in a breeding program depends on several factors. How wild is the crop? How desperate is the situation? What are the pressures to turn out new cultivars? How available are the wild relatives? How difficult are the wild relatives to use? and Is the breeder interested in using wild relatives? (Harlan, 1976). Wild relatives are primarily used for disease and pest resistance, wider adaptation, new cytoplasm, improved quality, modes of reproduction, short stature, crossability, thornlessness, resistance to abiotic stress and yield.

Barriers to hybridization are frequently encountered when attempting interspecific and intergeneric hybridization. Hadley and Openshaw (1980) and Stoskopf et al. (1993) discussed several external and internal barriers in obtaining successful hybrids. The external barriers include 1) spatial isolation and 2) prefertilization reproductive barriers - ecological isolation, temporal and seasonal isolation, mechanical isolation, gametic incompatibility. The internal barriers include postfertilization reproductive barriers - hybrid inviability or weakness, failure of flowering in the hybrid, hybrid sterility, inviability and weakness of F_2 and later generation segregates.

Knowledge and understanding of the barriers to interspecific and intergeneric hybridization are essential to increasing the chances of success. Various ways to

overcome reproductive isolation barriers were also discussed by Hadley and Openshaw (1980). The prefertilization barriers can largely be overcome i) by selecting appropriate parents, specific genotype or subgroup for crossing, ii) by employing special techniques in manipulation and modification of parental plants in emasculation and pollination procedures, and iii) by attempting bridging crosses. Postfertilization barriers can be overcome by treating the female parent with growth promoting substances, mixed pollination, embryo rescue, ovule culture, pod culture, grafting and producing alloploids. Embryo rescue is the most frequently used technique in obtaining hybrids in otherwise impossible crosses. Its success in aiding plant breeders in developing improved cultivars however, varies with the plant species (Raghavan, 1980; Young et al., 1981; Sharma 1995).

While gene transfer from landraces and wild species is a long and tedious process, gene transfer from newly produced wide hybrids is even more difficult due to decreased homology between parental chromosomes (Duvick, 1990; Sharma, 1995). However, plant breeders have been successful in transferring traits of importance into cultivated species (Duvick, 1990; Amante-Bordeos et al., 1992; Jiang et al., 1994; Sharma, 1995). Use of wild species germplasm holds a dynamic place in crop improvement and will become increasingly important as new variability is required to meet the needs of conventional breeding programs (Stalker, 1980).

2.4.1 Genetic Resources of Common Bean

Plant breeders are interested in the ease and success with which genes can be transferred among plants - within species, among species and occasionally among genera. The ultimate value of a species as a source of desirable traits depends primarily on the feasibility of gene transfer to cultivars of the cultivated species (Fehr, 1993). Harlan and de Wet (1971) proposed three informal categories - primary gene pool, secondary gene pool, and tertiary gene pool for cultivated plants. The gene pool concept provides a biological basis for a sound nomenclatural hierarchy and also provides a useful system for classifying genetic resources (Smartt, 1990). The genus *Phaseolus* has five cultivated species - *P. vulgaris*, *P. lunatus*, *P. coccineus*, *P. polyanthus*, and *P. acutifolius*, and about fifty true wild species (Delgado-Salinas, 1985; Debouck, 1991).

The primary gene pool includes the cultivated and related species of the crop which can be readily crossed. The hybrids are generally fertile with good chromosome pairing, gene segregation is predominantly normal, and gene transfer is generally easy. The biological species usually includes spontaneous races (wild and weedy) and cultivated races. Hence, Harlan and de Wet (1971) further proposed two subspecies - A (includes the cultivated races) and B (includes the spontaneous races). The primary gene pool of *P. vulgaris*, which includes all the genotypes, cultivated and wild is far more complex with some limited barriers to a free flow of genes (Debouck, 1991). Morphological, agronomic, molecular and other available information reveals the presence of two groups of germplasm - Middle American and Andean South American (Singh et al., 1991). Each group of germplasm consists of three races. The Middle America includes races Mesoamerica (gene pool 1 to 4), Durango (gene pool 5), and Jalisco (gene pool 6). The Andean South America includes races Nueva Granada (gene pool 7 to 9), Chile (gene pool 10), and Peru (gene pool 11 and 12). Germplasm belonging to gene pool 4, 6, 8, 10, 11 and 12 is found either in the highlands of Mexico or South America, and is adapted to cool temperatures (Singh, 1989; Singh et al., 1991).

The genetic distance between Middle American and Andean races is considerable (Singh et al., 1991). Various degrees and kinds of hybrid problems are observed in F_1 and subsequent generations. Complimentary dominant, dosage dependent, lethal genes DL_1 and DL_2 cause F_1 hybrid weakness, dwarfism or incompatibility in some Middle American and Andean common bean crosses. When a plant is heterozygous dominant at both loci, growth is severely reduced. When a plant is homozygous dominant, lethal dwarfing occurs. DL_1 is found primarily in germplasm of Mesoamerican origin, while DL_2 occurs mainly in Andean germplasm (Singh, 1989). Crosses between parents across gene pools have been abandoned after F_2 or F_3 generation due to the lack of adaptation of the introgressed germplasm, the low probability of recovering desirable recombinants from a population of manageable size, the stringent seed and/or pod quality requirements of consumers, and the conservative selection methods adopted by the breeders (Singh, 1989). Members of each race and gene pool possess certain desirable traits not found or inadequately expressed in other races and gene pool. Hence all available variation across

races and gene pools must be used in breeding programs for the improvement of bean crop.

In Middle America, small seeded types predominate in the warmer lowlands and medium and large seeded types predominate in the relatively cooler highlands (Singh, 1989). Seed germination and emergence of seedlings are slower at lower temperatures. A relatively large food reserve would be required, and thus larger seeds would have selective advantage in the highlands. At extremely low temperatures prevailing at 2700 m above sea level in the tropics, a large number of gene bank accessions do not flower, and those able to flower (especially bush beans) often produce a relatively large number of small, thin pods with aborted seeds (Singh, 1991). However, under such conditions, the climbing bean landraces from the Andean highlands of Colombia and Ecuador (> 2500 m above sea level) show the highest level of tolerance to low temperatures at all stages of growth, but they take approximately nine months to reach maturity.

The secondary gene pool includes those species from which genes can be transferred to the cultivated species, but with some difficulty (Harlan and de Wet, 1971; Fehr, 1993). Hybrid seed can be obtained, but the F_1 plants may be weak and difficult to maintain, chromosome pairing during meiosis in the hybrids may be poor, and the hybrids tend to be sterile. The recovery of desired types in advanced generations may be difficult. Few attempts have been made in crossing different species within the genus *Phaseolus* (Debouck, 1991). Experimental evidence shows that *P. vulgaris*, *P. polyanthus*, and *P. coccineus* are closely related. Each of these three species could form the secondary gene pool for the other two species (Smartt, 1985; 1990). In Central America, *P. coccineus* occurs at the highest elevations and is adapted to cool temperature conditions (Smartt, 1985). This crop is less sensitive to cool summers and is grown for its tender pods in the United Kingdom and Western Europe (Purseglove, 1982). *Phaseolus coccineus* is very sensitive to high temperature and in hot summers, few if any pods are set (Smartt, 1979). *Phaseolus coccineus* is sensitive to frost (Purseglove, 1982). *Phaseolus polyanthus* occurs as a cultivar or as an escape from Hidalgo (Mexico) to Peru, mainly in humid or subhumid montane forests between 1000 and 2300 m (Delgado-Salinas, 1985). Unlike *P. coccineus*, this species has epigeal germination (personal observation).

The tertiary gene pool includes those species from which the gene transfer to the cultivated species in the primary gene pool requires special techniques (embryo culture, grafting, chromosome doubling, or bridging cross) or may not be possible with the techniques currently available (Harlan and de Wet, 1971; Fehr, 1993). Extensive experimentation is required to determine the gene pool to which a species should be assigned. In *Phaseolus*, very few attempts have been made to date. For now, all species of *Phaseolus* other than those mentioned in the primary and the secondary gene pools can be assigned to the tertiary gene pool. Debouck (1991) and Delgado-Salinas et al. (1999) have presented a list of wild species in the genus *Phaseolus*. *Phaseolus filiformis and P. metcalfei* are resistant to several viral and bacterial diseases (Hubbeling, 1957). *Phaseolus acutifolius* var. *tenuifolius*, *P. angustissimus*, *P. filiformis* and *P. ritensis* are frost resistant (Buhrow, 1980; 1983), and all except *P. ritensis* are drought tolerant (Buhrow, 1981). Phylogenetic analysis of the cultivated and wild species of *Phaseolus* using various molecular marker techniques confirm speciation patterns obtained by intraspecific and interspecific hybridization (Jaaska, 1996; Delgado-Salinas et al., 1999).

2.4.2 Wide Hybridization in Common Bean Improvement

McComb (1975) reviewed intergeneric hybridization in the Leguminosae. Intergeneric crosses among various legume genera produced no viable hybrids. Interspecific hybridization in *Phaseolus* has been extensively reviewed (Smartt, 1979, 1990; Hucl and Scoles 1985; Debouck, 1991). Wall and York (1957) studied the inheritance of cotyledon position in a cross between *P. vulgaris* and *P. coccineus*. The cotyledon position was controlled by several loci. The F_2 and subsequent generations were skewed to the *P. vulgaris* parent for the cotyledon position, probably due to the fact that these individuals tend to be more fertile than the more *coccineus* like plants. The reciprocal cross was not attempted. Coyne (1964) crossed *P. acutifolius* with *P. coccineus* (reciprocal was unsuccessful) and got one hybrid intermediate between parents for several traits. The hybrid was late flowering compared to both parents and was sterile. Backcross of the hybrid reciprocally to both parents was unsuccessful. Coyne (1964) also reported producing a multispecies hybrid - *P. acutifolius* x [*P. vulgaris* var.

Red Kidney x (F_1 of *P. vulgaris* var. Seaway x *P. coccineus*)]. The reciprocal was unsuccessful. The hybrid was not self-fertile.

Al-Yasiri and Coyne (1966) crossed seven species of *Phaseolus*, namely *P. calcaratus*, *P. mungo* (now *V. mungo*), *P. angularis*, *P. lunatus*, *P. coccineus*, *P. acutifolius*, and *P. vulgaris* in all possible combinations and found that *P. vulgaris* x *P. coccineus* was the only compatible cross (hybrid seeds were obtained). Few other crosses were partially compatible (pods collapse in early stages of development) while most crosses were incompatible (pods did not develop). In partially compatible crosses, pods appeared to develop normally for approximately 16 days after pollination, and all pods collapsed by 22 days. Smartt and Haq (1972) treated F_1 stem cuttings of *P. vulgaris* x *P. coccineus* hybrid with colchicine to get amphidiploid hybrids. The pollen stainability was increased by 50% in the C_1 amphidiploid compared to the F_1 hybrid. The pollen stainability also increased in subsequent generations in amphidiploid. The amphidiploid shared with its *P. coccineus* parent, the requirement for rupturing the stigmatic surface prior to germination of pollen and fertilization. In addition to the chromosomal control of fertility, genic control of fertility was also implicated since the C_1 amphidiploids were not at maximum fertility.

Braak and Kooistra (1975) crossed *P. vulgaris* x *P. ritensis* to transfer cold and frost tolerance from the latter to the former species. The cross with *P. vulgaris* as female parent resulted in the production of a limited number of shriveled seeds whose embryos were later rescued. To restore fertility, the rooted F_1 hybrid plant was treated with colchicine. The amphidiploid hybrid was backcrossed to *P. vulgaris* and the resulting embryos had a cotyledon development similar to *P. vulgaris* when cultured *in-vivo*. Braak and Kooistra (1975) observed 13 to 15 univalents. The F_1 hybrid was propagated by stem cutting and treated with colchicine which resulted in one amphidiploid plant that ultimately produced seed. Seeds had hypogeal germination (like *P. ritensis*) and backcrossing it to parental species yielded few seeds whose embryos had to be rescued (seedlings were triploid $2n = 3x = 33$). It is not known whether the seedlings produced by further backcrossing to *P. vulgaris* had cold and frost tolerance. On a positive note, pairing was observed between 8 chromosomes and hence exchange of at least a part of the genes remains possible. Nabhan (1990) reported that above attempts by Braak and

Kooistra (1975) were flawed on three accounts: i) the accessions of wild species, *P. maculatus* Scheele, formerly known as *P. metcalfei* Woot. & Standl. was misidentified as *P. ritensis* Jones (Nabhan et al., 1980); ii) this taxon was assumed to have the greatest tolerance to cold weather and night frosts of any wild *Phaseolus*, even though several other species exceed its tolerance (Buhrow, 1983); iii) neither *P. maculatus* nor *P. ritensis* is as closely related to *P. vulgaris* as are other potential sources of cold tolerance.

Le Marchand and Maréchal (1977) observed chromosome pairing in several interspecific hybrids to determine the phylogenetic distances between the parents. In the *P. vulgaris* x *P. coccineus* and *P. vulgaris* x *P. polyanthus* hybrids, they observed an almost complete pairing. In *P. lunatus* x *P. ritensis* hybrids, they observed at least two univalents, in *P. lunatus* x *P. polystachyus* hybrids, eight univalents, and in *P. vulgaris* x *P. ritensis* hybrids, 14 univalents.

Mok et al. (1978) crossed *P. vulgaris* with *P. lunatus* and *P. acutifolius*. In the *P. vulgaris* x *P. lunatus* cross, the embryo development was poor and the maternal parent influenced its growth. In the reciprocal cross, pods abscised in less than three days after pollination. Leonard et al. (1987) obtained similar result. In the *P. vulgaris* x *P. acutifolius* cross and its reciprocal, embryo growth was influenced by the maternal parent (Mok et al., 1978). Embryos had uneven growth, but attained the cotyledon stage. Addition of glutamine to the culture medium had a beneficial effect on hybrid embryo survival particularly, smaller embryos. Addition of gibberellin did not enhance the survival of hybrid embryos, but seemed to hasten the greening of surviving embryos and the seedlings subsequently obtained, elongated at a faster rate. In a related study, Rabakoarihanta et al. (1980) reported that the average number of univalents observed in the F_1 of *P. vulgaris* x *P. acutifolius* and the reciprocal crosses were seven and six, respectively. The male fertility as observed by pollen staining was 17%. A higher frequency of embryos was obtained by backcrossing the hybrid as female to the *P. acutifolius* parent compared to the *P. vulgaris* parent. Pods resulting from these backcrosses were retained on the hybrid plant for a period of 14 to 26 days after which, embryos were excised and cultured.

Alvarez et al. (1981) crossed *P. coccineus* with *P. acutifolius* and *P. vulgaris* in both directions, and the F_2 (of *P. vulgaris* x *P. coccineus*) with *P. acutifolius*, to study the

possibility of classifying the above three species into a common gene pool. No difference in interspecific and intraspecific pollen tube growth was detected. Embryos were rescued 15 to 20 days after pollination in all crosses except *P. vulgaris* x *P. coccineus*. Embryos with damaged cotyledonary buds formed true leaves faster than embryos with undamaged cotyledonary buds. Alvarez et al. (1981) suggested the use of *P. coccineus* as the bridging parent since, *P. coccineus* x *P. vulgaris* and *P. coccineus* x *P. acutifolius* hybrids seemed fertile enough to produce advanced generations. Park and Dhanvantari (1987) crossed *P. vulgaris* x (*P. vulgaris* x *P. coccineus*) hybrid and successfully transferred bacterial blight (*Xanthamonas campestris* pv. *phaseoli*) resistance into common bean.

Thomas and Waines (1984) crossed several lines of *P. vulgaris* with several lines of *P. acutifolius* in both directions. *Phaseolus vulgaris* when, used as female parent, held pods for about 22 days compared to *P. acutifolius* which held the pods for only about 16 days. The F_1 hybrids were self sterile and were backcrossed to both parents. Few crosses set BC_1 seeds which on selfing showed segregation for several traits including fertility. Pratt et al. (1985) reported that the frequency of interspecific hybrid plants obtained from heterozygous *P. vulgaris* x *P. acutifolius* cultured embryos was nearly three times that obtained from homozygous *P. vulgaris* x *P. acutifolius* embryos. Hybrids were however, sterile. Stockinger and Waines (1986) crossed 23 lines of *P. vulgaris* from Kenya as females to 4 lines of *P. acutifolius* from CIAT to transfer drought tolerance traits from the latter to the former species. Embryo rescue was required to secure the F_1 hybrids, which were sterile. However, backcrossing to *P. vulgaris* restored fertility. Park et al. (1986) crossed *P. vulgaris* with *P. acutifolius* to transfer common bacterial blight resistance to the former species. The F_2 and subsequent generations showed a wide range of resistance to blight. Nikolova et al. (1986) also reported interspecific hybridization between *P. vulgaris* and *P. acutifolius*. The pollen stainability of the F_1 hybrids ranged between nine and 25%.

Petzoldt and Dickson (1987) crossed *P. vulgaris* as female parent with *P. angustissimus*, *P. ritensis*, *P. filiformis*, and *P. acutifolius* in an attempt to transfer frost tolerance from the first three species and heat and drought tolerance from the fourth species to common bean. Embryo rescue was required to secure the interspecific hybrid,

which on culturing, expressed traits intermediate between their parents. The F_1 hybrids were sterile. Successful interspecific hybridization between *P. vulgaris* and *P. filiformis* was reported by Maréchal and Baudoin (1978). The F_1 hybrid was sterile primarily due to the presence of five to six univalents per cell. To overcome sterility, de Tau et al. (1986) doubled the chromosome number by treating the apical bud of the F_1 hybrid seedlings with colchicine. The pollen stainability was increased to 50% in C_1 plants, and some produced pods with mature seeds. The C_2 (amphidiploid) plants were backcrossed to the *P. vulgaris* parent, which resulted in severe pod abortion (de Tau et al., 1987). Lülsdorf and Holl (1991) were also successful in obtaining interspecific hybrids between *P, vulgaris* and *P. filiformis*. Rooted stem cuttings of the F_1 interspecific hybrids had significantly higher nitrogen fixation rates and accumulated more total nitrogen compared to either parent (Lülsdorf and Holl, 1992)

Belivanis and Doré (1986) reported crossing heterozygous *P. vulgaris* plants with dominant male sterile genes with *P. angustissimus*. Pods were collected 13 to 23 days (19 days was found to be optimal) after pollination, and embryos cultured for 26 h and subjected to colchicine treatment and then transferred back to the medium. Sabja et al. (1990) employed pod culture to develop *P. vulgaris* x *P. acutifolius* interspecific hybrids. During pod culture in a modified MS liquid medium, one or two seeds located in the middle of the pods developed to maturity. The same pod culture also promoted precocious germination of early cotyledonary stage hybrid embryos. Pods of *P. vulgaris* x *P. acutifolius* younger than 14 days gave poor results in culture, due to shriveling of embryos after seven or ten days in culture. Sabja et al. (1990) concluded that developmental arrest of the interspecific hybrid embryo *in-vivo* is due to intrinsic deficiencies.

Plant breeders use recurrent or introgressive backcrossing technique for transferring desirable traits from exotic germplasm into cultivated types (Haghighi and Ascher, 1988). Recurrent backcrossing to either parent can overcome hybrid sterility. Difficulties with recurrent backcrossing include linkage of undesirable with desirable traits from the nonrecurrent parent, advanced generation hybrid breakdown, and loss of traits, chromosomes, or even the whole genome of the nonrecurrent parent. Haghighi and Ascher (1988) proposed congruity backcrossing as an efficient breeding method for

introgressing desirable traits from the tertiary gene pool of common bean into bean cultivars. In the congruity backcrossing, hybrids are backcrossed with each of the parent species in alternating generations. In a congruity backcrossing of *P. vulgaris* with *P. acutifolius* var. *acutifolius*, hybrid fertility, as determined by percent stainable pollen anther^{-1} and mean number of seeds pod^{-1}, increased gradually in later generations. Fertility was higher when the congruity backcross hybrid was crossed with *P. vulgaris* parent. Haghighi and Ascher (1988) observed maintenance of traits from both parental species, usually in a form intermediate to that of the parents in all congruity backcross pedigrees, and appearance of new traits (traits present in neither parents) in the second or third and all later congruity backcross generations. Seed size and colour, flower colour, plant height and leaf sizes of some hybrids were different from both parents. Anderson et al. (1996) reported that hybrid breakdown symptoms in *P. vulgaris* x *P. acutifolius* and reciprocal crosses, were eliminated in F_5 congruity backcross hybrid or later generations. Mejía-Jiménez et al. (1994) reported that intermating selected fertile F_2 plants of the third and fifth congruity backcrosses with their respective male sterile F_1s increased the growth of hybrid embryos before rescue, recovery of mature hybrid plants, and the vigour and fertility of F_1 hybrids.

In addition to the conventional breeding methods used for common bean improvement, various transformation protocols have been developed for common bean and *P. acutifolius* (Dillen et al., 1997; Kim and Minamikawa, 1997; Aragão et al., 1998). Most transformation protocols use pre-existing meristems such as embryonic axis, cotyledonary nodes, shoot apical meristem and root meristem as explants to avoid problems associated with the regeneration of transformed cells. Agronomic superiority of transgenic bean plants over bean cultivars developed by traditional plant breeding methods has not been established to date.

3. Effect of Planting Dates on Agronomic Traits of Dry Bean

3.1 Abstract

Seedbed temperatures below 15°C are detrimental to dry bean emergence. This study was conducted to determine the effects of suboptimal seedbed temperatures on dry bean emergence, and the cumulative effects of suboptimal seedbed temperatures during emergence on crop phenology and yield when planted in the field. Selected dry bean cultivars were planted in mid and late May when seedbed temperatures were below and above the suboptimal temperature, respectively at Saskatoon, SK. Emergence, anthesis, maturity, seed yield, yield components and percent frost damaged seeds were compared among planting dates and cultivars in 1999 and 2000. The seedbed temperature during the first week after the mid May planting ranged between 6 and 18°C, and that for the late May planting ranged between 9 and 25°C in 1999 and 2000. The final seedling count at 30 days from planting was between 74 and 89% for the mid May planting and above 90% for the late May planting, suggesting the sensitivity of bean cultivars to suboptimal seedbed temperatures in both 1999 and 2000. Dry bean cultivars planted in mid or late May required equivalent thermal units to anthesis in both 1999 and 2000. Also cultivars required equivalent thermal units to maturity in 2000. Highest yield was obtained in 1999 with the mid May planting, partly because two high yielding but late maturing indeterminate cultivars, CDC Whistler and UI 906, failed to mature prior to the mid September frost when planted in late May. The mid September frost also resulted in higher percent frost damaged seeds for the late May planting. Seed yield and percent frost damaged seeds were not significantly different for planting dates in 2000 when the first fall frost was in late September. The mid May planting of dry bean cultivars will often result in emergence when the risk of a late spring frost is low (in late May), and also result in higher seed yield compared to the conventional late May planting, particularly in years with a mid September or earlier frosts.

3.2 Introduction

Dry bean (*Phaseolus vulgaris* L.) has been an integral part of pulse crop production in Saskatchewan since the mid 1990s. Dry bean seeds of various commercial classes such as black, pinto, navy, red etc., are either canned or packaged as dry seed for the domestic and export markets. Dry bean is a warm season crop and can be severely affected by temperatures below 15°C at all stages of plant growth (Dickson and Boettger, 1984; Ashworth et al., 1985; Zaiter et al., 1994). Suboptimal seedbed temperatures, whether in the field or in a controlled environment chamber, inhibit and delay dry bean emergence resulting in poor and nonuniform seedling stand. Dry bean planting in the Dark Brown and Black soil zones of Saskatchewan is delayed until late May when the soil temperature at the seeding depth approaches 20°C. Delayed planting ensures uniform seedling emergence and helps avoid late spring frost. Delayed planting may, however, make the dry bean crop more vulnerable to early fall frost, resulting in poor seed yield and quality. To effectively use the short growing season, early to mid May planting of dry bean may be an alternative, provided the seed yield is not compromised due to poor emergence, seedling death or chilling injury that the crop may suffer when planted in suboptimal seedbed temperatures of early spring. The risk of economic losses due to a possible spring frost injury must be weighed against the risk of economic losses caused by early fall frost.

In the early spring, seedbed temperature and moisture are probably the crucial abiotic factors that influence germination and emergence of viable seeds. Scully and Waines (1987) reported the optimum temperature range for common bean germination and emergence under a controlled environment as 20 to 30°C. However, radicle emergence in bean seed was observed at 8°C in controlled environments (Dickson and Boettger, 1984; Zaiter et al., 1994), and when mean minimum soil temperature was 4°C in the field (Dickson, 1971). Germination increased when bean seeds imbibed and germinated at a suboptimal temperature of 9°C were transferred to optimum temperature (Kooistra, 1971). Similar observations in the field, in late spring, with increasing air and soil temperatures (personal observation), indicate that suboptimal temperatures cause a temporary setback without severely affecting bean seed viability.

Germination and emergence responses of diverse bean genotypes under suboptimal temperature has been widely studied (Kooistra, 1971; Dickson and Boettger, 1984; Hucl, 1993; Zaiter et al., 1994). Dickson (1971) observed that seed colour and resistance to seed decay by soil organisms were strongly associated with superior germination at suboptimal temperatures. Treating white coloured seeds with fungicide enhanced their germination in the field under cool spring conditions although, cold tolerant lines with white seed coat were generally lower in germination compared to lines with colored seed coat. Using isogenic snap bean lines differing for seed coat colour, Wyatt (1977) demonstrated that white seeds have thinner seed coats and imbibe water at a higher rate compared to coloured seeds. Slower absorption of water by coloured seeds may permit more uniform swelling of the cotyledons, thereby reducing seed coat and cotyledon cracking, both detrimental factors in snap bean germination and early seedling growth. Roos and Manalo (1976) reported that snap bean seed lots with initial moisture content above 12% have higher field emergence compared to low moisture seed lots at soil temperatures below 10°C.

Preliminary work identified dry bean cultivars with superior emergence under suboptimal temperature in controlled environments. Chilling tolerant genotypes belonged to either navy or black bean commercial classes with a 100-seed weight < 25 g. Pinto and great northern genotypes (100-seed weight = 35 g) had poor emergence. Chilling tolerant dry bean cultivars may enable early spring planting on the prairies without compromising seedling stand. Furthermore, planting date may affect seed yield through its association with seedling stand, anthesis, maturity (Henderson et al., 1998), and yield components. Most chilling injury research in dry bean crop is designed to investigate the immediate effects of suboptimal temperature on germination and emergence, with little reference to the cumulative influence of suboptimal seedbed temperatures during emergence on subsequent plant growth at above suboptimal temperatures. The objective of this study was to compare the phenology and seed yield of selected dry bean cultivars planted under suboptimal and above suboptimal (>20°C) seedbed temperature conditions in the field.

3.3 Materials and Methods

Field experiments were conducted at Saskatoon (52° 07' N, 106° 38' W, elevation 501 m), Saskatchewan, during 1999 and 2000. Two navy bean cv. AC Skipper and CDC Whistler, and two black bean cv. UI 906 and CDC Nighthawk were grown. AC Skipper has a determinate bush type growth habit, and all other cultivars are indeterminate bush type. Seeds of above cultivars grown at Outlook, SK in 1996 were the source seed for both years. Seeds were treated with the fungicide Apron FL @ 0.046 L per 100 kg of seed, two days prior to seeding. Moisture content of the seed was between 10 and 12%. Soil type at the trial site was a Dark Brown Chernozem clay loam. Mean annual precipitation at Saskatoon is approximately 347 mm, with an average of 110 frost-free days. Soil N, P and K were adequate for dry bean production except that N level was below 57 kg ha^{-1}. Granular inoculant (MicroBio RhizoGen Co., Saskatoon) was seed placed at the rate of 9 kg ha^{-1} in both years.

The experimental design was a randomized complete block in a split-plot treatment arrangement with four replicates. Planting dates were mainplot and cultivars were subplot factors. Two planting dates were selected and targeted for mid May and late May when temperatures at the seeding depth of five cm were below 15°C and above 20°C, respectively. The actual planting dates were May 17 in 1999 and 2000 (correspond to mid May), and June 5 in 1999 and May 30 in 2000 (correspond to late May). Seeds of all cultivars were planted at a depth of approximately five cm with a hoe-type-opener drill to give a plant population of approximately 60 plants m^{-2}. Plots were 3.7 m long and consisted of four rows spaced 30 cm apart. Plots were hand-weeded. Percent emergence at 20 and 30 days after planting was determined. A 3 m section of the two centre rows was used to measure all other traits. Time to anthesis was calculated as the number of days from planting to first anthesis. Time to physiological maturity was calculated as the number of days from planting to when 50% of the plants had pale yellow pods (buckskin stage). Cumulative thermal units (CTUs) in degree Celsius from planting to first anthesis or physiological maturity of 50% of plants was calculated using the formula CTU = $\Sigma[(T_{max} + T_{min}/2) - T_{base}]$ (Boote and Gardner, 1998). The T_{max} and T_{min} are maximum and minimum air temperatures, respectively at one m height in the field. The base temperature (T_{base}) was 8°C (Hall, 2001). Number of pods $plant^{-1}$ and number of seeds

pod^{-1} were determined from a random sample of five plants. Plants were hand harvested and counted. Plants were air-dried, threshed and seeds were stored in an airtight container for two weeks before determination of yield on a dry weight basis. A random sample of 100 seeds was taken to determine the 100-seed weight (dry weight basis) and percent frost damaged seed. Soil temperature at planting depth (5 cm) was monitored using a datalogger (Campbell Scientific Canada Corp., Edmonton, AB) from mid May to early June. Air temperature at one m height from the soil surface was monitored throughout the growing season.

Planting date and cultivar were considered as fixed effects, and year and replication as random effects. Percent emergence at 20 and 30 days after planting, and percent frost damaged seed were subjected to analysis of variance using SAS (SAS Institute, Inc. 1987). Data for all other traits were subjected to analysis of covariance using number of plants harvested as a covariate, except for yield m^{-2} for which, the number of plants m^{-2} was the covariate. However, separate analyses were performed for each year for the following reasons, i) planting dates were not consistent over years for five traits including percent emergence at 20 and 30 days after planting, CTUs to physiological maturity, seed yield m^{-2} and percent frost damaged, ii) Bartlett's test detected heterogeneous error variance for percent emergence at 30 days after planting and percent frost damaged seed, and iii) a mid September frost in 1999 severely affected yield m^{-2} and percent frost damaged seeds. Mean separation was done using an *F*-protected LSD at the 5% probability level.

3.4 Results

Both 1999 and 2000 growing seasons were relatively normal with respect to maximum and minimum air temperatures (Table 3.1). A large deviation from the average was however observed for precipitation during May in both years (Table 3.2).

Table 3.1 Mean monthly maximum and minimum air temperature during the 1999 and 2000 growing seasons and long-term average at Saskatoon.†

	Temperature									
	May		June		July		August		September	
Year	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
	°C									
1999	16.5	4.8	20.7	8.4	22.7	10.0	25.2	10.4	18.1	2.4
2000	18.2	2.7	21.3	7.2	25.3	12.0	24.2	9.4	19.3	4.0
30-yr average	18.4	4.5	23.0	9.4	25.4	11.7	24.5	10.3	17.7	4.6

† Air temperature and average (1961-1990) values at Saskatoon International Airport (Environment Canada, 1999; 2000).

Table 3.2 Total monthly precipitation during the 1999 and 2000 growing seasons and long-term average at Saskatoon.†

	Precipitation				
Year	May	June	July	August	September
	mm				
1999	114.8	58.9	80.4	43.4	19.7
2000	16.4	49.8	82.8	42.0	27.0
30-yr average	44.2	63.4	58.0	36.8	32.1

† Precipitation and average (1961-1990) values at Saskatoon International Airport (Environment Canada, 1999; 2000).

3.4.1 Seedling Emergence and Stand Establishment

Precipitation was 71 mm above the long term average in the May of 1999 growing season. Approximately 80 mm of the total precipitation was received on or after the mid May planting on May 17, and was characterized by intermittent showers thus, delaying the late May planting to June 5. In 2000, 14 mm of the total precipitation was received on or after May 17. Emergence was first observed at 14 days after planting from the mid May planted seeds and at 10 days after planting from the late May planted seeds. Delayed emergence of mid May planted bean seeds in both years is due primarily to minimum seedbed temperatures below 10°C during the first seven days after planting (Figure 3.1). Planting date main effect was significantly different for percent emergence at 20 and 30 days after planting in both 1999 and 2000 (Table 3.3). The late May planting consistently resulted in percent emergence greater than 90, indicating good seed quality (Table 3.4). Although, percent emergence in mid May planting did not exceed 90% during the sampling period in both years, several plants with slender stems, and with

or without pods were observed at the time of hand harvest, indicating the possibility of delayed emergence of these seedlings. The increase in percent emergence of mid May planted bean seeds over time (65% vs. 74% in 1999 and 77% vs. 89% in 2000 at 20 and 30 days after planting, respectively) in both years indicates a small effect of suboptimal seedbed temperatures on bean seed viability.

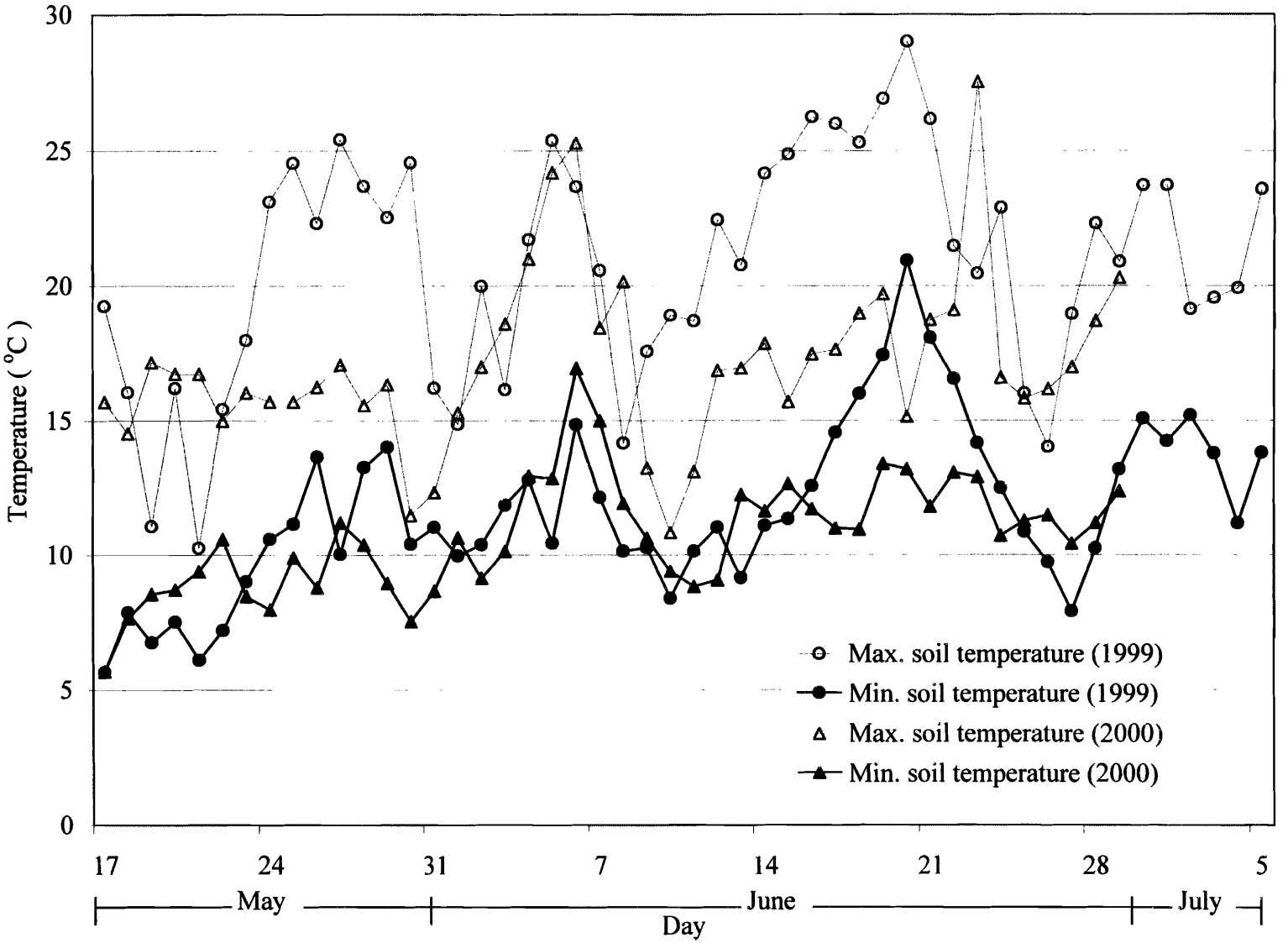

Figure 3.1 Maximum and minimum daily soil temperature (^{o}C) at 5 cm depth during the spring of 1999 and 2000 at the Preston field plot, Saskatoon.

Table 3.3 Mean squares from analysis of variance for percent emergence at 20 and 30 days after planting (DAP) of four dry bean cultivars grown at Saskatoon, SK, during 1999 and 2000.

Source of variation	df	Mean square			
		1999		2000	
		Percent emergence at 20 DAP	Percent emergence at 30 DAP	Percent emergence at 20 DAP	Percent emergence at 30 DAP
Block (B)	3	144.8	92.1	33.3	9.2
Planting date (PD)	1	7149.4*	3443.9*	373.6*	180.4**
B*PD	3	166.1**	102.1**	26.9	1.8
Cultivar (C)	3	65.7	142.0**	1334.9**	230.4**
C*PD	3	18.6	73.3*	505.4**	72.5**
Error	18	21.4	16.0	15.7	4.9
Total	31				
C.V. (%)		5.8	4.7	4.9	2.4

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

Table 3.4 Means for percent emergence at 20 and 30 days after planting (DAP) of four dry bean cultivars planted in mid May and late May at Saskatoon, SK, during 1999 and 2000.

Year	Cultivar	Percent emergence at 20 days after planting			Percent emergence at 30 days after planting		
		mid May planting	late May planting	**Mean**	mid May planting	late May planting	**Mean**
		%			%		
1999	AC Skipper	61.3	93.0	**77.2**	68.1	94.3	**81.2**
	CDC Whistler	62.5	93.7	**78.1**	69.0	93.5	**81.2**
	UI 906	65.3	96.6	**80.9**	76.9	96.6	**86.8**
	CDC Nighthawk	70.8	96.2	**83.5**	83.3	96.0	**89.7**
	Mean	**65.0**	**94.9**		**74.3**	**95.1**	
	LSD (0.05)	**14.5†**		**NS‡**	**11.4**		**4.2**
2000	AC Skipper	84.3	83.8	**84.0**	92.8	94.8	**93.8**
	CDC Whistler	89.5	87.7	**88.6**	91.7	94.9	**93.3**
	UI 906	46.4	77.1	**61.8**	76.1	89.7	**82.9**
	CDC Nighthawk	89.4	88.5	**89.0**	93.6	93.8	**93.7**
	Mean	**77.4**	**84.3**		**88.5**	**93.3**	
	LSD (0.05)	**5.8**		**4.2**	**1.5**		**2.3**

† Least significant difference to compare planting dates.
‡ Least significant difference to compare cultivars.

The cultivar effect was significant for percent emergence at 20 days after planting in 2000, and at 30 days after planting in 1999 and 2000 (Table 3.3). CDC Nighthawk consistently had a higher emergence compared to AC Skipper, CDC Whistler and UI 906 during the sampling period (Table 3.4). The cultivar x planting date interaction effect was significant for percent emergence at 20 days after planting in 2000 and at 30 days after planting in 1999 and 2000 (Table 3.3). The cultivar x planting date interaction for percent emergence at 20 and 30 days after planting in 2000 was of the noncrossover type, indicating no change in ranking of the cultivars in both mid and late May plantings (Table 3.4). However, in 1999, the cultivar x planting date interaction was significant for percent emergence at 30 days after planting primarily due to differences in emergence among cultivars in the mid May planting, while no difference was observed among cultivars in the late May planting.

3.4.2 Cumulative Thermal Units to Anthesis and Maturity

Planting date main effect was significant only for CTUs to maturity in 1999, indicating similar CTUs were required by bean cultivars to anthesis in both years, and to maturity in 2000 at both planting dates (Table 3.5). In 1999, all four plots of CDC Whistler and two of UI 906 in the late May planting failed to mature prior to the first fall frost, which occurred on September 13 and 14 (minimum air temperature of -1.6 and $-2.6^{\circ}C$, respectively). The significant difference among planting dates for the CTUs to maturity in 1999 could partly be attributed to the mid September frost (Table 3.6). The average minimum air temperature was $2.2^{\circ}C$ below normal in September of 1999 (Table 3.1). Therefore, cooler air temperatures may have also delayed the maturity of late May planted CDC Whistler and UI 906 in 1999. In 2000, the first fall frost was on September 23 ($-3.8^{\circ}C$) by which time, cultivars in both planting dates had passed physiological maturity.

Cultivar effect was significant for both CTUs to anthesis and to maturity in 1999 and 2000 (Table 3.5). AC Skipper, a determinate bush type cultivar, required fewer CTUs to anthesis and to maturity compared to the indeterminate bush cultivars CDC Whistler, CDC Nighthawk and UI 906 (Table 3.6). Little or no difference for CTUs to anthesis and to maturity was observed between CDC Whistler and UI 906, both of which were

relatively late to flower and to mature, compared to AC Skipper. The indeterminate cultivar, CDC Nighthawk was either similar to CDC Whistler and UI 906 for CTUs requirement to anthesis and to maturity (as in 1999) or intermediate between CDC Whistler and UI 906, and AC Skipper for above traits (as in 2000). The covariate (plant count) was not significant for either trait (Table 3.5). The cultivar x planting date interaction was significant for CTUs to anthesis in 1999 due primarily to the higher CTU requirement of CDC Whistler in the late May planting compared to the mid May planting (Table 3.6).

Table 3.5 Mean squares from analysis of covariance for cumulative thermal units (CTUs) to anthesis and to maturity of four dry bean cultivars grown at Saskatoon, SK, during 1999 and 2000.

	Mean square						
	1999				2000		
Source of variation	df	CTUs-Anthesis	df	CTUs-Maturity	df	CTUs-Anthesis	CTUs-Maturity
Block (B)	3	224.7	3	54	3	91.0	412.4
Planting date (PD)	1	63.6	1	22856**	1	970.4	6103.1
B*PD	3	571.4	3	38	3	178.8	891.9**
Cultivar (C)	3	2536.6**	3	915**	3	4439.4**	8568.3**
C*PD	3	1538.8*	2	41	3	321.1	246.2
Plant count	1	6.6	1	18	1	93.2	65.5
Error	17	300.0	12	47	17	250.3	119.4
Total	31		25		31		
C.V. (%)		3.5		0.7		3.2	1.1

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

Table 3.6 Means for cumulative thermal units (CTUs) to anthesis and to maturity (°C d) of four dry bean cultivars planted in mid May and late May at Saskatoon, SK, during 1999 and 2000.

Year	Cultivar	CTUs-Anthesis			CTUs-Maturity		
		mid May planting	late May planting	**Mean**	mid May planting	late May planting	**Mean**
		°C d					
1999	AC Skipper	481.9	447.6	**464.8**	960.5	887.2	**923.8**
	CDC Whistler	480.6	513.5	**497.1**	1004.6	†	‡
	UI 906	499.9	509.4	**504.6**	970.4	899.6†	**935.0**
	CDC Nighthawk	496.2	500.2	**498.2**	962.2	898.6	**930.4**
	Mean	**489.7**	**492.7**		**974.4**	‡	
	LSD (0.05)	**NS§**		**22.2¶**	**‡#**		**‡#**
2000	AC Skipper	468.2	454.5	**461.4**	940.1	909.9	**925.0**
	CDC Whistler	506.1	502.5	**504.3**	1001.2	984.4	**992.8**
	UI 906	516.4	514.3	**515.3**	1011.0	966.8	**988.9**
	CDC Nighthawk	497.8	468.1	**482.9**	963.1	931.1	**947.1**
	Mean	**497.1**	**484.8**		**978.9**	**948.1**	
	LSD (0.05)	**NS**		**16.9¶**	**NS**		**11.7¶**

† All four plots of CDC Whistler and two of UI 906 failed to mature prior to the first fall frost.

‡ Mean non-estimable

§ Least significant difference to compare planting dates. The standard error of differences was obtained from the 'estimate' statement of the analysis of the covariance in the SAS program.

¶ Least significant difference to compare cultivars. The mean standard error of differences was calculated from the 'estimate' statement of the analysis of the covariance and was used to calculate LSD.

Mean squares from the analysis of covariance indicated that both planting date and cultivar effects were significantly different.

3.4.3 Seed Yield and Yield Components

Except for the 100-seed weight, the planting date effect was significant for other traits including number of pods plant^{-1}, number of seeds pod^{-1}, seed yield and percent frost damaged seed in 1999 (Table 3.7). In 2000, planting date effect was not significant for any trait (Table 3.8). In 1999, the mid May planting had a higher number of pods plant^{-1} compared to the late May planting (Table 3.9). The covariate effect (plant count) was significant for number of pods plant^{-1} (Table 3.7), indicating a possible effect of lower plant stand in the mid May planting. Planting date effect on number of seeds pod^{-1} was

small, but significant (Table 3.9). Due to the mid September frost in 1999, seeds of late May planted bean cultivars were severely affected due to their later maturity (Table 3.10). As a consequence of an increased number of pods plant^{-1}, seeds pod^{-1} and reduced percent frost damaged seed, grain yield for the mid May planting was twice that of the late May planting in 1999 (Table 3.10). Both planting dates resulted in similar 100-seed weights (Table 3.11). In 2000, both planting dates matured prior to the first fall frost (September 23) and no difference was observed for seed yield and yield components (Table 3.8 to 3.11).

Table 3.7 Mean squares from analysis of covariance/variance for seed yield and yield components of four dry bean cultivars grown at Saskatoon, SK, during 1999.

Source of variation	df	Mean square				df	Mean square
		No. of pods plant^{-1}	No. of seeds pod^{-1}	100-seed wt.	Yield m^{-2}		Percent frost damaged seed
Block (B)	3	7.5	0.08	1.0	2895	3	404.9
Planting date (PD)	1	65.8*	0.83*	1.9	218238**	1	10046.5*
B*PD	3	1.7	0.04	0.4	844	3	294.5**
Cultivar (C)	3	2.0	3.69**	46.6**	692	3	2015.5**
C*PD	3	4.3	0.11	0.2	862	3	610.0**
Plant count†	1	98.6**	0.01	0.0	1242		
Error	17	3.3	0.07	0.2	839	18	33.8
Total	31					31	
C.V. (%)		13.0	6.5	2.8	10.3		26.1

*, ** Significant at 0.05 and 0.01 levels, respectively.
† Plant count m^{-2} was used as a covariate for yield m^{-2}.

Table 3.8 Mean squares from analysis of covariance/variance for seed yield and yield components of four dry bean cultivars grown at Saskatoon, SK, during 2000.

Source of variation	df	Mean square				df	Mean square
		No. of pods plant^{-1}	No. of seeds pod^{-1}	100-seed wt.	Yield m^{-2}		Percent frost damaged seed
Block (B)	3	5.2	0.15	1.2	2997	3	1.03
Planting date (PD)	1	0.2	1.02	1.2	420	1	0.03
B*PD	3	1.4	0.36	0.2	11140**	3	3.28
Cultivar (C)	3	10.7	2.42**	29.6**	2361*	3	66.78**
C*PD	3	5.1	0.13	0.0	54	3	9.20**
Plant count†	1	0.2	0.25	0.4	8773**		
Error	17	3.8	0.11	0.3	577	18	1.32
Total	31					31	
C.V. (%)		15.5	9.8	3.3	9.0		54.9

*, ** Significant at 0.05 and 0.01 levels, respectively.
† Plant count m^{-2} was used as a covariate for yield m^{-2}.

Table 3.9 Means for number of pods plant^{-1} and number of seeds pod^{-1} of four dry bean cultivars planted in mid May and late May at Saskatoon, SK, during 1999 and 2000.

Year	Cultivar	No. of pods plant^{-1}			No. of seeds pod^{-1}		
		mid May planting	late May planting	**Mean**	mid May planting	late May planting	**Mean**
1999	AC Skipper	15.3	14.0	**14.7**	3.5	2.9	**3.2**
	CDC Whistler	14.4	12.3	**13.4**	4.1	3.7	**3.9**
	UI 906	15.8	12.3	**14.0**	5.1	5.1	**5.1**
	CDC Nighthawk	17.0	11.7	**14.3**	4.3	3.9	**4.1**
	Mean	**15.6**	**12.6**		**4.3**	**3.9**	
	LSD (0.05)	**1.6†**		**NS‡**	**0.2**		**0.3**
2000	AC Skipper	14.4	13.7	**14.0**	2.8	2.6	**2.7**
	CDC Whistler	11.5	13.2	**12.3**	3.7	3.0	**3.4**
	UI 906	13.4	11.6	**12.5**	4.2	4.0	**4.1**
	CDC Nighthawk	10.5	12.0	**11.2**	3.8	3.3	**3.6**
	Mean	**12.4**	**12.6**		**3.6**	**3.2**	
	LSD (0.05)	**NS**		**NS**	**NS**		**0.4**

† Least significant difference to compare planting dates.
‡ Least significant difference to compare cultivars.

Table 3.10 Means for yield m^{-2} (g) and percent frost damaged seed of four dry bean cultivars planted in mid May and late May at Saskatoon, SK, during 1999 and 2000.

Year	Cultivar	Yield m^{-2}			Percent frost damaged seed		
		mid May planting	late May planting	**Mean**	mid May planting	late May planting	**Mean**
		g			%		
1999	AC Skipper	370.7	204.9	**287.8**	1.0	28.0	**14.5**
	CDC Whistler	396.1	183.9	**290.0**	15.8	74.3	**45.0**
	UI 906	363.2	201.5	**282.3**	1.5	39.8	**20.6**
	CDC Nighthawk	351.4	184.5	**267.9**	0.0	18.0	**9.0**
	Mean	**370.3**	**193.7**		**4.6**	**40.0**	
	LSD (0.05)	**35.0†**		**NS‡**	**19.3**		**6.1**
2000	AC Skipper	249.4	235.7	**242.5**	0.0	0.0	**0.0**
	CDC Whistler	288.2	279.1	**283.7**	4.8	7.5	**6.1**
	UI 906	271.5	270.4	**270.9**	3.5	1.0	**2.3**
	CDC Nighthawk	269.2	260.9	**265.0**	0.0	0.0	**0.0**
	Mean	**269.6**	**261.5**		**2.1**	**2.1**	
	LSD (0.05)	**NS**		**25.7**	**NS**		**1.2**

† Least significant difference to compare seeding dates.
‡ Least significant difference to compare cultivars.

Table 3.11 Means for 100-seed weight (g) of four dry bean cultivars planted in mid May and late May at Saskatoon, SK, during 1999 and 2000.

Cultivar	1999			2000		
	Mid May planting	late May planting	**Mean**	mid May planting	late May planting	**Mean**
	g					
AC Skipper	18.0	17.9	**17.9**	18.6	18.3	**18.4**
CDC Whistler	13.3	12.6	**12.9**	15.1	14.5	**14.8**
UI 906	13.1	12.3	**12.7**	14.2	13.8	**14.0**
CDC Nighthawk	14.7	14.2	**14.5**	16.6	16.2	**16.4**
Mean	**14.8**	**14.2**		**16.1**	**15.7**	
LSD (0.05)	**NS†**		**0.5‡**	**NS**		**0.6**

† Least significant difference to compare seeding dates.
‡ Least significant difference to compare cultivars.

Dry bean cultivars were significantly different for number of seeds pod^{-1}, 100-seed weight, and percent frost damaged seed in 1999 and 2000 (Table 3.7 and 3.8). An inverse relationship was observed between the 100-seed weight (and hence seed size) and number of seeds pod^{-1}. The black bean cultivar UI 906 with the lowest 100-seed weight

had the highest number of seeds pod^{-1} in both years (Table 3.9 and 3.11). In contrast, the navy bean cultivar AC Skipper with the highest 100-seed weight had the lowest number of seeds pod^{-1} in both years (Table 3.9 and 3.11). In general, the 100-seed weight and number of seeds pod^{-1} of CDC Nighthawk and CDC Whistler were intermediate between AC Skipper and UI 906. CDC Nighthawk and CDC Whistler had similar numbers of seeds pod^{-1}, although the 100-seed weight of the former was significantly higher compared to that of the latter.

The cultivar effect was also significant for seed yield in 2000 (Table 3.8). The covariate factor (plants m^{-2}) was significant only for seed yield (Table 3.8). Seed yield of AC Skipper was significantly lower compared to CDC Whistler, CDC Nighthawk or UI 906 in 2000 (Table 3.10). The covariate factor (plants m^{-2}) was significantly different indicating a probable effect of plant density on yield. The two early flowering and early maturing cultivars, AC Skipper and CDC Nighthawk, had the lowest percent frost damaged seed compared to UI 906 and CDC Whistler in both years (Tables 3.6 and 3.10). CDC Whistler and UI 906 failed to mature prior to the first fall frost in 1999 and had between 40 and 74% frost damaged seeds (Table 3.11). The cultivar x planting date interaction effect was significant for percent frost damaged seed in both years (Tables 3.7 and 3.8). The interaction was of the crossover type that involved changes in ranking only among the two early maturing cultivars, AC Skipper and CDC Nighthawk.

3.5 Discussion

3.5.1 Effect of Planting Date

Planting dates partition a growing season into several subseasons resulting in different growing conditions for plant growth and development (Henderson et al., 1998). Crop response in a season/subseason is highly influenced by environmental variables including temperature and precipitation. Chilling sensitivity of dry bean cultivars for emergence was evident by relatively poor seedling establishment when planted in mid May compared to late May in 1999 and 2000 (Table 3.4). Although the seedbed temperature during the first week after the mid May planting ranged between 6 and 17°C in both 1999 and 2000 (Figure 3.1), percent emergence of the mid May planting at both 20 and 30 days after planting was lower in 1999 compared to 2000 (Table 3.4). Saskatoon received 59

mm of precipitation within 24 h of the mid May planting in 1999, subjecting imbibed bean seeds to stress by flooding. The greater than expected precipitation on a single day far exceeds the long-term monthly average for May, which is 44 mm (Table 3.2). Excess water during imbibition of seeds has deleterious effects (anoxia) on both the resulting plant and more importantly, on the germination process itself in common bean (Orphanos and Heydecker, 1968). The excess water suffocates the embryonic axis, leading to the death of the embryo either prior to or after the emergence of the radicle. Suboptimal seedbed temperatures combined with excess precipitation may have resulted in the reduced seedling stand at both 20 and 30 days after planting in the mid May planting of 1999 compared to 2000 (Table 3.4).

Initial seed moisture content below 12% and soil pathogens decreased common bean emergence at soil temperatures below 10°C (Dickson, 1971; Roos and Manalo, 1976). Seeds used in this study had moisture content in the range of 10 to 12%, and this may have had a small effect in reducing emergence of the mid May planting. The confounding effect of soil pathogens on decreased emergence was minimized in this study by treating seeds with a fungicide.

Chilling injury during imbibition is dependent on both temperature and duration of exposure (Herner, 1986). For mid May planted bean seeds, with increasing air and seedbed temperatures over time, emergence increased from 65% at 20 days after planting to 74% at 30 days after planting in 1999, and from 77 to 89% in 2000. Hence in 2000, the overall emergence of dry bean cultivars was not severely affected by suboptimal seedbed temperatures at the time of the mid May planting. Non-uniform and decreased emergence of mid May planting was evident by random barren patches in the field plots in both years. This may pose severe weed control problems in commercial field. Increasing the seeding rate may enable better ground cover and efficient use of radiation, but will also lead to a high input cost, and in wet years may pose problems with foliar diseases particularly, at the time of flowering. Late May planting of dry bean seeds resulted in a uniform and high percent emergence (> 90%) by mid June (i.e., 20 days after seeding), due primarily to warm seedbed temperatures (> 20°C) (Table 3.4, Figure 3.1). High percent emergence in late May planted bean seeds indicates good seed quality.

Reduction in time to anthesis and maturity of amaranth as planting date progressed was primarily due to warmer temperatures and accelerated accumulation of heat units associated with planting later in the growing season (Henderson et al., 1998). Similar trends were observed in this study with dry bean in both years. Time to anthesis ranged between 62 and 64 days for the mid May planting and about 55 days for the late May planting in both years. Time to maturity ranged between 105 and 110 days for the mid May planting, and between 97 and 100 days for the late May planting in both years. Due to the possible influence of differences in days to emergence between the two planting dates on time to anthesis and to maturity, CTUs were determined using 8°C as the base temperature (Table 3.6). Time to anthesis in terms of CTUs was a more efficient measurement in assessing variation for this trait compared to days to flowering in lentil (Tullu et al., 2001). Scully and Waines (1988) reported a base temperature of 8°C for tepary bean (*Phaseolus acutifolius* A. Gray) and 2°C for common bean for vegetative development in the field. Dapaah et al. (1999) reported a base temperature of above 7°C for all developmental stages of dry bean in the field. Kapitsimadi (1988) evaluated several common bean cultivars for their growth over a range of temperatures and reported that the base temperature was near 10°C. In general, the base temperatures for warm season species range between 8 and 10°C (Hall, 2001).

Except for the CTUs to maturity in the frost affected 1999, CTUs to anthesis and maturity in 1999 and 2000 were not significantly different for planting dates, indicating that cultivars flowered or matured upon accumulation of a certain number of thermal units. Nykiforuk and Johnson-Flanagan (1999) observed a similar trend, but for germination in the canola cv. Westar. At 10°C, germination proceeded rapidly if the required equivalent of 16 to 24 degree days occurred before germination. The analysis of covariance indicated that CTUs to maturity was significantly different for planting dates in 1999 (Table 3.5). The mid May planting required a CTU of 974.4°C d while the late May planting required a cumulative thermal unit of below 900°C d (Table 3.6). The estimate for the late May planting, however, includes only two replicates of UI 906 and does not include CDC Whistler, both of which failed to mature prior to the first fall frost. Hence, the CTU mean for the late May planting date in 1999 is biased downwards.

Although the initial seedling stand in 1999 was relatively lower in the mid May planting compared to the late May planting (Table 3.4), a higher yield was obtained (Table 3.10) due, in part, to the high yield compensating ability of dry bean, and to the longer growing season. The yield compensation of dry bean plants was primarily through an increased number of pods plant^{-1} with the mid May planting (Table 3.9). Furthermore, the covariate factor (plant count) was also significant, indicating that reduced plant stand probably decreased plant-to-plant competition for resources and therefore enabled higher pod production per plant. Increased yield in late April seeded faba bean (*Vicia faba* L.) was attributed to higher number of pods plant^{-1} (McVetty et al., 1986). However, it is not clear whether increased pods plant^{-1} with a late April planting was due to a reduced faba bean plant stand compared to early or late May planting. Though differences between planting date for number of seeds pod^{-1} were significant (Table 3.7, Table 3.9), the differences were too small to account for the large difference in seed yield among the planting dates. The late May planting had a higher proportion of frost damaged seeds compared to the mid May planting (Table 3.10). The 100-seed weight however, was not significantly different among planting dates (Table 3.11). Either the late May seeded plants were at physiological maturity at the incidence of the first fall frost or, the shrivelled and shrunken seeds of the late May seeded plants were lost during threshing. In 2000, planting dates did not differ significantly for the yield component traits (Table 3.8). Furthermore, dry bean plants in both planting dates had reached physiological maturity at the time of the first fall frost on September 23. No difference in seed yield or percent frost damaged seed was observed between seeding dates (Table 3.10).

3.5.2 Effect of Cultivar

The overall emergence of navy bean cultivars AC Skipper and CDC Whistler was comparable to that of black bean cultivar CDC Nighthawk in 2000, probably due to the fungicide seed treatment (Table 3.4). In 1999, however, the mid May planted seeds were subjected to both chilling and flooding stresses that severely affected the percent emergence of navy bean cultivars compared to black bean cultivars (Table 3.4). Consistent low percent emergence of UI 906 at 30 days after the mid May planting in both years indicate its sensitivity to suboptimal seedbed temperatures. In a related study

(chapter 4) in which dry bean cultivars and accessions from the gene bank of the International Center for Tropical Agriculture were planted on May 3 and their emergence observed for up to 50 days after planting, the emergence of UI 906 increased gradually from 4% on May 23 (i.e., 20 days after planting) to 95% on June 22 (i.e., 50 days after planting). In this study however, the emergence was last observed on June 16 for the mid May planting, hence, it is possible that emergence in UI 906 may have continued after this period but was unaccounted.

Dry bean cultivars with a determinate growth habit are more stable in their response across short season environments compared to cultivars with indeterminate growth habit (Nleya et al., 1999). Accordingly, AC Skipper with a determinate bush growth habit was the earliest to flower and to mature in both years at all planting dates (Table 3.6). Among the indeterminate cultivars, CDC Whistler and UI 906 are relatively late to flower and to mature in any given growing season. Due to a relatively early first fall frost in 1999 compared to 2000, both cultivars failed to mature when planted in late May. The cultivar x planting date interaction was significantly different for CTUs to anthesis in the 1999 growing season (Table 3.5). CDC Whistler which, along with AC Skipper required the least CTUs to anthesis (481°C d) with the mid May planting, required the highest CTUs to anthesis (514°C d) with the late May planting (Table 3.6). CDC Nighthawk, although indeterminate, required either an equivalent or fewer CTUs to anthesis and to maturity compared to CDC Whistler or UI 906 in both years (Table 3.6). Also, the difference in CTU requirements to anthesis and to maturity between CDC Nighthawk and CDC Whistler was greater with the late May planting compared to the mid May planting in 2000. Possible reasons include, CDC Nighthawk (i) has a short vegetative growth period in response to warm temperatures after planting in late May, (ii) has a short flowering period, (iii) is able to respond more efficiently to decreasing air temperatures in the fall and hence early maturity, and/or (iv) has a short seed fill duration. Indeterminate cultivars have a greater positive response to a more productive environment compared to determinate cultivars (Nleya et al., 1999). Therefore, compared to determinate cultivars, indeterminate cultivars may be more suited for production under rainfed environment, or where the production environment is unpredictable. Under Saskatchewan dryland conditions with typical short growing season, indeterminate cultivars such as CDC

Nighthawk may be desirable for their relatively early maturity and inherent plasticity in their response to the environment.

Indeterminate cultivars have more growing points for potential pod formation compared to determinate cultivars and therefore, may have a greater yield potential (Beaver, 1999; Nleya et al., 2001). The higher yield potential of indeterminate cultivars, particularly CDC Whistler and UI 906, and their ability to efficiently use the longer growing season, when available, is indicated by their high yield with the mid May planting in both years (Table 3.10). Similar results were observed with the late May planting in 2000 when the first fall frost was delayed until September 23. Earlier anthesis, earlier maturity, and relatively lower yield of AC Skipper compared to CDC Whistler and UI 906 is reflected in its low levels of frost damaged seeds at all planting dates in both years (Table 3.6; Table 3.10). Except for the number of pods plant^{-1}, which was not significantly different among cultivars in both years, the yield component compensation (Adams, 1967) is evident when comparing CDC Whistler or UI 906 with AC Skipper. Both CDC Whistler and UI 906 had a lower number of pods plant^{-1}, higher number of seeds pod^{-1} and lower 100-seed weight compared to AC Skipper. In general, the indeterminate CDC Nighthawk presents a balance between the early anthesis, early maturity, low yield and high seed quality of determinate cultivars, and the late anthesis, late maturity, high yield and poor seed quality of indeterminate cultivars when bean cultivars are planted in late May.

3.6 Conclusions

Achieving high yield in regions with short growing seasons requires making use of as much of the season as possible (Henderson et al., 1998). Planting early extends the potential length of the growing season, thus enabling complete crop development for cultivars with relatively late maturity. The mid May planting in both 1999 and 2000 resulted in poor initial seedling establishment likely due to the sensitivity of dry bean cultivars to suboptimal seedbed temperatures during planting. Depending on the year, good final seedling stand was attained with increasing air and soil temperatures. Since dry bean is sensitive to frost injury, delayed seedling establishment with suboptimal temperatures is an adaptive feature that would normally enable bean seedlings to escape

late spring frosts. However, this study indicates that for a mid May planting, when seedbed temperature is below 15°C, dry bean seedlings will often emerge after May 23, the average date for a late spring frost. With the exception of CTUs to maturity in 1999, the cumulative effects of suboptimal temperatures during bean seedling establishment did not affect anthesis and maturity as indicated by similar CTU requirements for both planting dates. Anthesis and maturity of dry bean planted in mid May occurred earlier compared to those planted in late May. The mid May planting resulted in high seed yield and quality, especially in the growing season with the mid September frost. Even in years with a late September frost, seed yield and quality of mid May planted bean was equivalent to that of late May planting. Although, the late May planting may be advantageous for uniform seedling establishment and reduced risk of seedling death due to late spring frosts, the risk of crop loss may be increased in growing seasons that are cool or experience a mid September frost. Therefore, the mid May planting of dry bean cultivars will often result in emergence when the risk of a late spring frost is low (late May), and also result in higher seed yield compared to the conventional late May planting in years with a mid September or earlier frosts. The mid May planting may also avoid late season drought stress to the bean crop. In the future, further risk reduction in regions with short growing season may be achieved through reducing the CTU requirements for flowering and maturity for indeterminate cultivars of dry bean.

4. Low Temperature Emergence of Common Bean Accessions

4.1 Abstract

Suboptimal seedbed temperatures limit common bean emergence in early spring. The objective of this study was to evaluate diverse accessions of cultivated and wild common bean for emergence under suboptimal temperatures in the field. Common bean accessions and check cultivars were planted on May 3, at Saskatoon, SK in 2000 and 2001. Percent emergence, cumulative thermal units to 50% anthesis and to 50% maturity, and yield were determined. The seedbed temperature during the two weeks after planting ranged between 1 and 18°C in 2000, and 5 and 17°C in 2001. At 20 days after planting (i.e., May 23), accession G8823 (from the Netherlands) had a significantly higher percent emergence than the check cultivars in 2000. CDC Nighthawk, a black bean check cultivar was comparable in percent emergence to G8823 at 30 days after planting (i.e., June 2). A similar trend was observed in 2001 with the exception that emergence was higher at 30, 40 and 50 days after planting due to warmer seedbed. Emergence of UI 906 was delayed in both years until the seedbed temperature exceeded 15°C. Accessions with a significantly lower cumulative thermal unit requirement to anthesis, compared to the check cultivars, were not necessarily early in maturity. CDC Nighthawk was among the earliest to mature although it required a significantly higher cumulative thermal unit to anthesis compared to accessions from northern Europe. G8823 was the only accession with a consistent early emergence, early anthesis and early maturity in both years. G8823 should serve as a parent to develop elite bean cultivars with the ability to emerge under suboptimal temperature conditions.

4.2 Introduction

Dry bean (*Phaseolus vulgaris* L.) acreage in Saskatchewan amounted to 2,000 ha with a production of 3,400 metric tonnes in 2001 (Saskatchewan Agriculture and Food, 2001).

In comparison to other pulse crops, the relatively low dry bean acreage (0.1% of the total pulse acreage) is due partly to the abiotic constraints associated with the production of this crop. In the Dark Brown and Black soil zones of the province, abiotic constraints include poor emergence in the cool wet soils of early spring, and the risk of late spring and early fall frosts. Seedbed temperatures of below 15°C are suboptimal for germination and emergence of dry bean (Scully and Waines, 1988). The mean daily soil temperature for Saskatoon (Dark Brown soil zone) from 1977 to 1984 indicates that optimum seedbed temperature (20 to 30°C) is usually achieved after May 21, thus delaying dry bean planting to late May. Delayed planting, although ensuring good seedling establishment, makes the dry bean crop vulnerable to early fall frost. The short growing season (105 to 110 frost free days) on the prairies requires dry bean cultivars with either early maturity to enable late May planting or the ability to emerge under suboptimal temperatures to enable early May plantings.

Selection for early maturity has been successful in dry bean breeding programs (White and Izquierdo, 1991). Early maturity, however, may reduce yield potential during favourable growing seasons (Egli, 1998; Beaver, 1999). Izquierdo and Hosfield (1983) observed significant positive correlations between the effective seed filling duration and seed yield, seeds pod^{-1}, and seeds m^{-2} in dry bean genotypes with differing growth habits. White and Izquierdo (1991) reported a significant positive correlation between yield and days to maturity in common bean. In the dry bean Cooperative and Regional trials in Saskatchewan, time to anthesis ranged from 47 to 50 days for early maturing cultivars, and 50 to 60 days for medium and late maturing cultivars (Saskatchewan Agriculture and Food, 2002). Cultivars with early maturity, with the exception of a pinto bean cultivar CDC Pintium, were relatively lower yielding compared to cultivars with medium or late maturity. The above studies indicate a positive relationship between maturity and seed yield. It is safe to conclude that selection for early anthesis, early maturity and high seed yield will be a viable breeding strategy as long as cultivars with an optimum combination of the above traits can be effectively selected. By virtue of late May planting, such cultivars would minimize risk with regards to poor seedling establishment due to cool seedbed temperatures and death due to late spring frosts.

Kooistra (1971) evaluated 280 common bean lines and identified one navy bean line with 100% germination at 9°C. Dickson (1971) selected several common bean lines from a population segregating for germination and emergence at temperatures below 10°C both in a controlled environment and field. Dickson (1971), also observed that seed coat colour and resistance to seed decay by soil pathogens were strongly associated with good germination at suboptimal temperatures. Dickson and Boettger (1984) studied the response of 20 common bean lines to suboptimal temperatures at germination, juvenile and flowering stages. Common bean genotypes with radicle emergence at 8°C were very weak and their seeds rotted. When planted in the field in mid April, these genotypes were less vigorous with poor seed yield. In contrast, genotypes with radicle emergence only at 10 or 12°C were vigorous with high seed yield. Hucl (1993) observed significant changes in genotype ranking for percent germination among diverse common bean genotypes germinated at 12 and 16°C. Zaiter et al. (1994) reported that difference among common bean genotypes for percent germination was highest at the low temperature (8°C). Furthermore, alternating temperature regimes of 10/8, 12/8 or 18/8°C (12 h at each temperature) identified two additional genotypes with superior emergence compared to a constant temperature of 8°C, indicating differential response of genotypes to the temperature treatment.

Correlation exists between the environmental requirement for germination and the ecological conditions occurring in the habitat of the plant and the seed (Mayer and Poljakoff-Mayber, 1989). Near the Equator, night temperatures fall regularly below 10°C at altitudes of 2000 m and higher (Patterson et al., 1978). Plants that regularly experience temperatures below 10°C in their native habitat would be expected to be comparatively chilling resistant. *Lycopersicon hirsutum*, a wild relative of the cultivated tomato from high altitudes of the Andes (2100 and 3100 m) had higher germination at an alternating temperature regime of 15/5°C (12 h at each temperature) compared to those from lower altitudes. The accession from 3100 m also exhibited the lowest temperature for greening of cotyledons. In Kenya, the mean diurnal temperature decreases by 6.5°C for every 1000 m increase in altitude above sea level (Arkel, 1977). Dry matter yield for sorghum (*Sorghum bicolor*) accessions from higher altitudes was comparable to or superior to maize, sorghum and Bulrush millet (*Pennisetum typhoides*) from lowlands. Although

accessions from high altitudes needed a longer growing season, few accessions with a growing season length similar to check crops were observed. Wild relatives of common bean *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus* have been collected from altitudes of up to 2270 m in the Sierra Madre in Central America and 2950 m in the Andes in South America, respectively. The mean growing temperature in the highlands of Central America is 18°C and that in South America is 16°C (Singh, 1989). Wild common bean accessions from the high altitudes of the Sierra Madre and the Andes may have the ability for germination and emergence under suboptimal temperatures.

In a related study (chapter 3), dry bean cultivars were planted in mid and late May under suboptimal and optimal seedbed temperatures, respectively. In years with a mid September frost, the mid May planting resulted in a higher seed yield and quality. Even in years with a late fall frost (i.e., in late September), seed yield and percent frost damaged seed of mid May planted cultivars were equivalent to that of late May planting. A relatively low seedling establishment in a mid May planting compared to a late May planting (65% vs. 95% at 20 days after planting in 1999, and 77% vs. 84% in 2000) was primarily due to sensitivity of current dry bean cultivars to suboptimal seedbed temperatures. Nonuniform seedling stand may be a concern in efficient utilization of radiation, weed management and harvest. Furthermore, common bean germplasm with resistance to suboptimal temperatures during germination and emergence may enable mid May or earlier plantings and thus, increase the effective growing period. The objective of this study was to evaluate the cultivated and wild common bean accessions for their emergence under suboptimal temperature in the field.

4.3 Materials and Methods

4.3.1 Seed Increase

Wild and cultivated common bean accessions were obtained from the International Center for Tropical Agriculture (CIAT), Cali, Colombia in the summer of 1998 and seed was increased in the phytotron facility of the College of Agriculture, University of Saskatchewan. Plants were grown at 26/18°C with a 12 h photoperiod. Seed from the above increase was used in further studies. Approximately 300 accessions were planted on June 3, 1999 at Saskatoon (52° 07' N, 106° 38' W, elevation 501 m), Saskatchewan,

along with a dry bean tester set. The tester set included agronomically superior dry bean lines from several dry bean breeding programs across the World. Thirty-six seeds per accession were planted at approximately five cm of soil depth with a hoe-type-opener drill in 1.8 m row. Seeds of wild common bean accessions were scarified by nicking the seed coat with a scalpel. Growth habit, time to first anthesis and time to maturity were determined.

4.3.2 Field Evaluation in the 2000 Growing Season

A field experiment was conducted at Saskatoon in 2000. Accessions that flowered earlier than the tester set in 1999 and that had between 50 and 100 seeds were included in this study. Accessions from altitudes of 2000 m and higher from the Sierra Madre and the Andes were also included. In total, seeds of 180 accessions and three check cultivars (CDC Whistler, CDC Nighthawk and UI 906) from the phytotron seed increase were used. Seeds of wild common bean accessions were scarified by nicking the seed coat with a scalpel. Seeds were treated with the fungicide Apron FL @ 0.046 L per 100 kg of seed, two days prior to seeding. Moisture content of the seed was between 10 and 12%. Soil type at the trial site was a Dark Brown Chernozem clay loam. Mean annual precipitation at Saskatoon is approximately 347 mm, with an average of 110 frost-free days.

The experiment used an Augmented design (Petersen, 1994) with six blocks. Each block consisted of 30 accessions and three check cultivars. Seeds of all genotypes were planted on May 3, 2000 at a depth of five cm with a hoe-type-opener drill. Plots were 1.2 m long and consisted of four rows spaced 30 cm apart. In addition to hand weeding when needed, a foliar application of Basagran @ of 0.71 L per 0.4 ha was performed when bean seedlings were at the third trifoliolate leaf stage to control broad leaf weeds. Appearance of the hypocotyl hook at the soil surface was observed at about 15 days from planting. Hence, percent emergence at 20, 30, 40 and 50 days after planting were determined. A one m section of the two centre rows was used to determine other traits for those accessions, which were initially planted using 100 seeds. Time to anthesis was calculated as the number of days from planting to when 50% of the plants had at least one opened flower. Time to physiological maturity was calculated as the number of days

from planting to when 50% of the plants had pale yellow pods (buckskin stage). Cumulative thermal units (CTUs) in degree Celsius from planting to 50% anthesis or physiological maturity of 50% of plants was calculated using the formula $CTU = \sum[(T_{max} + T_{min}/2) - T_{base}]$ (Boote and Gardner, 1998). The T_{max} and T_{min} were maximum and minimum air temperatures, respectively, at one m height in the field. The base temperature (T_{base}) was 8°C (Hall, 2001). Plants were hand-harvested and counted. Harvested plants were air-dried, threshed and seeds were stored in an air-tight container for two weeks. Yield was determined on a dry weight basis and a random sample of 100 seeds was taken to determine the 100-seed weight (dry weight basis) and percent frost damaged seed.

Soil temperature at the planting depth of five cm was monitored using a datalogger (Campbell Scientific Canada Corp., Edmonton, AB) from the first day up to 50 days after planting. Air temperature at one m height from the soil surface was monitored throughout the growing season. Trait means for check cultivars were subjected to analysis of variance appropriate to a randomized complete block design (RCBD) to estimate the experimental error, which was then used to construct standard error of differences for the various comparisons. Adjustments of entry means for block differences were based on the difference between the mean of the check cultivars in a given block and the mean of the check cultivars over the entire trial (Petersen, 1994). Mean separation was done using the LSD. Block and cultivar in the analysis of variance were considered as random and fixed effects, respectively.

4.3.3 Field Evaluation in the 2001 Growing Season

Five accessions with the highest seedling emergence at 20 days after planting (G7551, G8090, G8823, G9345, G9430), five accessions with no emergence at 20 days after planting (G746, G991, G5024, G19504, G19899) and the three check cultivars were planted on May 3, 2001 to confirm emergence results observed in the previous growing season. The experimental design was a RCBD with two replicates. Fifty Apron-treated seeds per accession per replicate were planted. Plots were similar to the previous growing season except that they had three rows instead of four. Percent emergence over time was determined as described earlier (Section 4.3.2). A one m section of the centre

row was used to determine all other traits. Soil and air temperatures were monitored as described in section 4.3.2.

4.4 Results

The 2001 growing season was relatively warmer and received less precipitation compared to the 2000 growing season and the long term average (Tables 4.1 and 4.2). The maximum air temperature during the 2001 growing season was 1 to 5°C warmer than the long term average (Table 4.1). Precipitation was lower than the long term during May and June of both 2000 and 2001 with a large deviation from the average during August and September of 2001 (Table 4.2).

Table 4.1 Mean monthly maximum and minimum air temperature during the 2000 and 2001 growing seasons and long-term average at Saskatoon.†

	Temperature									
	May		June		July		August		September	
Year	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
	°C									
2000	18.2	2.7	21.3	7.2	25.3	12.0	24.2	9.4	19.3	4.0
2001	20.9	3.9	22.8	8.0	27.4	11.9	28.8	10.7	22.3	5.4
30-yr average	18.4	4.5	23.0	9.4	25.4	11.7	24.5	10.3	17.7	4.6

† Air temperature and average (1961-1990) values at the Saskatoon International Airport (Environment Canada, 2000; 2001).

Table 4.2 Total monthly precipitation during the 2000 and 2001 growing seasons and long-term average at Saskatoon.†

	Precipitation				
Year	May	June	July	August	September
	mm				
2000	16.4	49.8	82.8	42.0	27.0
2001	21.6	38.3	52.2	6.0	7.6
30-yr average	44.2	63.4	58.0	36.8	32.1

† Precipitation and average (1961-1990) values at the Saskatoon International Airport (Environment Canada, 2000; 2001).

4.4.1 Seedling Emergence and Stand Establishment

Seedbed temperature during the first two weeks after planting ranged between 1 and 18°C during the 2000 growing season, and between 5 and 17°C during the 2001 growing

season (Figure 4.1). Precipitation during May was also lower compared to the long term average in both 2000 and 2001. However, with increasing seedbed temperature (Figure 4.1) and precipitation (Table 4.2) in June, bean seedling emergence of greater than 80% was observed.

In 2000, the check cultivars CDC Whistler, CDC Nighthawk and UI 906 were significantly different for percent emergence at all sampling times except at 20 days after planting (Table 4.3). At 20 days after planting (i.e., May 23, the conventional planting date for dry bean on the Dark Brown and Black soil zones of the province), two navy bean accessions, G8823 (the Netherlands) and G9345 (USA) had a significantly higher percent emergence compared to the check cultivars (Table 4.4). Accessions G8823 and G9345 also had the highest emergence (> 75%) at 30 days after planting (i.e., June 2) although they were not significantly different from CDC Nighthawk (Table 4.4). Starting June 4, the maximum and minimum seedbed temperatures were above 15°C and 10°C, respectively (Figure 4.1), hence by 40 days after planting (i.e., June 12), most accessions had some degree of emergence (Appendix 1). By 50 days after planting (i.e., June 22), all but one accession (G8855) had emerged. Emergence however was below 50% for several accessions including the check cultivar CDC Whistler (Table 4.4, Appendix 1). The two accessions G8823 and G9345 with the highest percent emergence early in the season, had an emergence of 89% and 93%, respectively, at 50 days after planting, while the check cultivars CDC Nighthawk and UI 906 had an emergence of 91% and 94%, respectively. In general, common bean accessions with some degree of emergence early in the season (i.e., 20 and 30 days after planting) belonged to either small or medium seed size classes. With the exception of G23554A from the highlands of Mexico and G23450 from the highlands of Peru, accessions of wild relatives of common bean had no emergence at 20 and 30 days after planting. Accession G8855 did not emerge during the growing season probably due to poor seed quality.

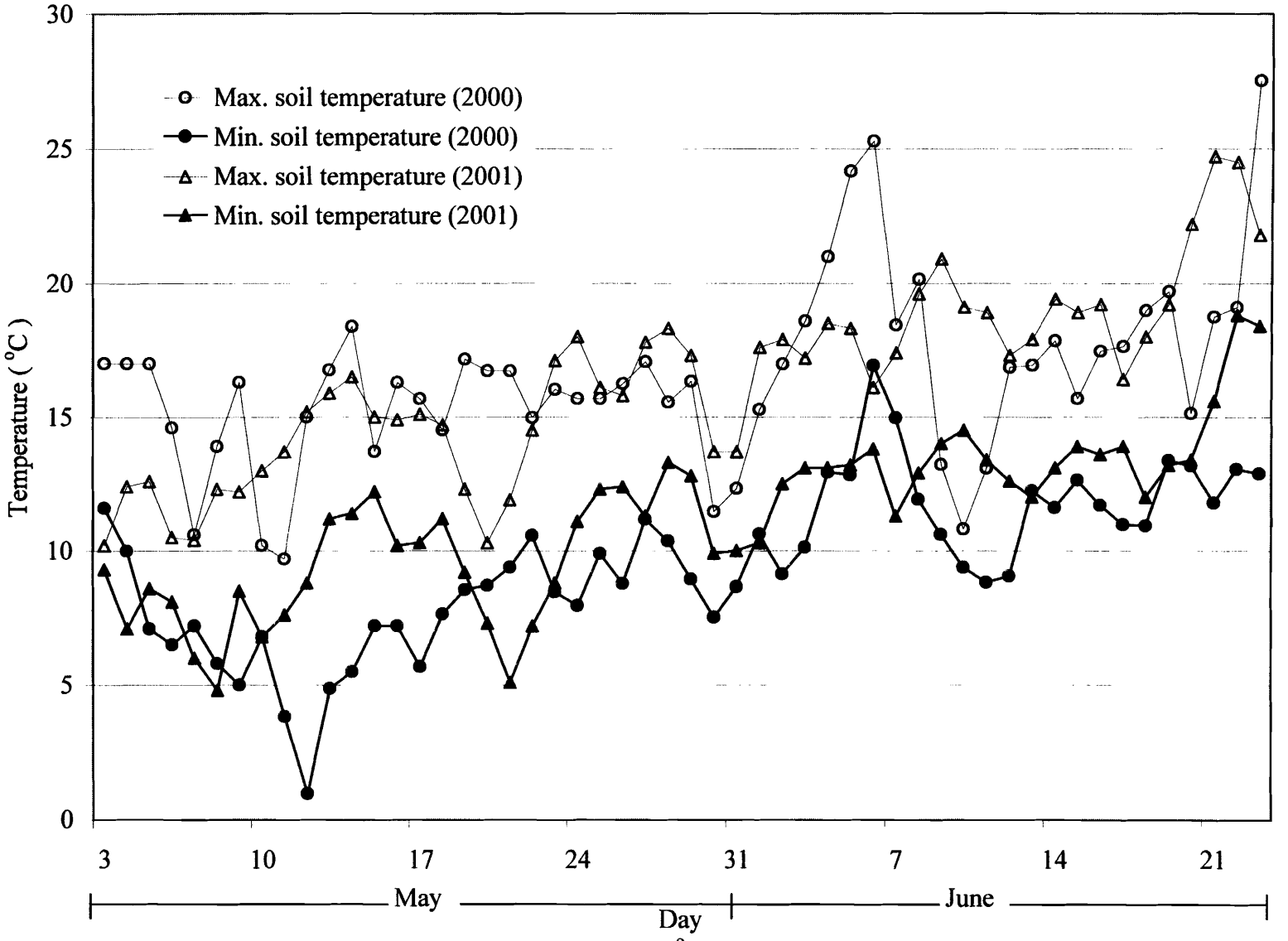

Figure 4.1 Maximum and minimum daily soil temperature (°C) at 5 cm depth during the spring of 2000 and 2001 at the Preston field plot, Saskatoon.

Table 4.3 Mean squares from analysis of variance for percent emergence at 20, 30, 40 and 50 days after planting (DAP) of three dry bean check cultivars grown at Saskatoon, SK, during 2000.

Source of variation	d.f.	Mean square			
		Percent emergence at 20DAP	Percent emergence at 30DAP	Percent emergence at 40DAP	Percent emergence at 50DAP
Block	5	20.2	104.6	38.2	33.0
Check cultivar	2	72.2	5134.5**	3313.6**	3651.6**
Error	10	41.1	138.0	41.9	48.8
Total	17				
C.V. (%)		183.2	31.6	8.7	8.9

** Significant at 0.01 level.

Table 4.4 Means for percent emergence at 20, 30, 40 and 50 days after planting of common bean cultivars and accessions grown at Saskatoon, SK, during 2000 and 2001.

Cultivar/accession	Emergence at							
	20 days		30 days		40 days		50 days	
	2000	2001	2000	2001	2000	2001	2000	2001
	%							
CDC Whistler	1.3	11.0	24.2	84.0	47.7	90.0	49.7	90.0
CDC Nighthawk	1.7	17.0	70.7	95.0	89.3	98.0	91.0	98.0
UI 906	7.5	0	16.7	32.0	87.3	97.0	93.7	97.0
G7551	7.8	0	49.5	83.0	67.5	94.0	75.1	94.0
G8090	5.2	1.0	32.5	63.0	62.8	78.0	64.4	78.0
G8823	30.2	25.0	75.5	85.0	83.8	94.0	89.4	94.0
G9345	49.8	25.0	84.5	81.0	91.5	92.0	93.1	92.0
G9430	8.8	0	50.5	71.0	74.8	94.0	77.1	96.0
G746	0	0	3.9	36.0	69.1	97.0	80.8	99.0
G991	0	0	0	47.0	61.1	96.0	88.8	97.0
G5024	0	0	38.5	70.0	89.8	98.0	89.8	99.0
G19504	0	0	0	64.0	80.8	97.0	87.4	97.0
G19899	0	0	0	9.0	69.8	92.0	81.4	94.0
LSD (0.05)†	17.8	13.4	32.6	27.9	18.0	7.8	19.4	6.6

† Due to the augmented experimental design, the LSD in the year 2000 is to compare an accession with a check cultivar.

A subset of common bean accessions and the check cultivars planted in 2001 were significantly different for percent emergence at all sampling times (Table 4.5). The emergence trend in 2001 was very similar to that observed in 2000, except that the warmer seedbed in late May and early June of 2001 enhanced the percent germination at 30, 40 and 50 days after planting (Figure 4.1, Table 4.4). The cumulative thermal units at

20, 30, 40 and 50 days after planting in 2000 were 67, 110, 179 and 261°C d, respectively, compared to 62, 123, 201 and 291°C d, respectively, in 2001. At 20 days after planting, the two navy bean accessions, G8823 (the Netherlands) and G9345 (USA), again, had the highest percent emergence, although they were not significantly different from the check cultivar CDC Nighthawk (Table 4.4). Due to a warm seedbed, at 30 days after planting, all genotypes had greater than 60% emergence with the exception of UI 906, G746, G991 and G19899. The check cultivar UI 906, although low in percent emergence early in the season, had 97% emergence at 40 days after planting. Three accessions, G746, G991 and G19899 with no emergence at 30 days after planting in 2000, had between 9 and 47% emergence in 2001 due primarily to a warmer seedbed. At 50 days after planting, all genotypes with the exception of G8090 had an emergence of greater than 90%.

Table 4.5 Mean squares from analysis of variance for percent emergence at 20, 30, 40 and 50 days after planting (DAP) of common bean cultivars and accessions grown at Saskatoon, SK, during 2001.

Source of variation	d.f.	Mean square			
		Percent emergence at 20DAP	Percent emergence at 30DAP	Percent emergence at 40DAP	Percent emergence at 50DAP
Block	1	3.9	74.5	67.8*	67.8*
Cultivar/accession	12	196.8**	1274.8**	56.8*	62.1**
Error	12	37.8	164.5	12.8	9.2
Total	25				
C.V. (%)		101.2	20.3	3.8	3.2

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

4.4.2 Anthesis, Maturity and Seed Yield

In 2000, the check cultivars were significantly different for all traits except CTUs to 50% anthesis (Table 4.6). Due to photoperiod sensitivity, only 115 of the 183 genotypes evaluated flowered prior to August 10. The CTUs to 50% anthesis for the check cultivars ranged from 527°C days for CDC Nighthawk to 549°C days for CDC Whistler (Table 4.7, Appendix 2). The CTUs to 50% anthesis of common bean accessions ranged from 374°C days for G9509, an accession from Belgium to 797°C days for G5478, an accession from the USA (Appendix 2). Among the two accessions with early emergence,

G8823 required significantly less CTUs to 50% anthesis compared to check cultivars (Table 4.7). G19504 from the Peruvian highlands (2140 m) was the only accession from high altitudes that flowered within the sampling period (Appendix 2). All but 30 accessions flowered earlier than the early flowering check cultivar CDC Nighthawk.

Of the 115 genotypes that flowered prior to August 10, 60 matured prior to the first fall frost on September 23 (minimum air temperature = $-3.8^{\circ}C$) (Appendix 3). Among the check cultivars, CDC Nighthawk required the least number of CTUs to 50% maturity (Table 4.7, Appendix 3). The CTUs to 50% maturity for the accessions ranged from $950^{\circ}C$ days to $1141^{\circ}C$ days (Appendix 3). The CTU requirement to 50% maturity for accessions with early emergence G8823 and G9345 were not significantly different from that of CDC Nighthawk (Table 4.7).

In 2001, genotypes differed significantly for CTUs to 50% anthesis and to 50% maturity, and 100-seed weight (Table 4.8). Of the three check cultivars and 10 accessions evaluated, G746 and G19899 failed to flower, while G991 and G19504 failed to mature prior to the first killing frost on October 5 (minimum air temperature = $-13^{\circ}C$). The above four accessions failed to emerge at 20 days after planting in both 2000 and 2001 (Table 4.4). Accession G7551 (the Netherlands) required fewer CTUs to 50% anthesis in both years (Table 4.7). With the exception of UI 906 and G5024, accessions were comparable to the check cultivar CDC Nighthawk in their CTU requirements to 50% anthesis (Table 4.7). With the exception of accessions G9345, G9430 and G5024, that required significantly higher CTUs to 50% maturity, other genotypes were comparable to CDC Nighthawk for maturity.

In 2000, the seed yield of the check cultivars ranged from 187 g m^{-2} for CDC Whistler to 450 g m^{-2} for CDC Nighthawk (Table 4.9, Appendix 3). No accession was significantly higher yielding than CDC Nighthawk (Appendix 3). Accessions G8823 and G9345 with early emergence were not significantly different from CDC Nighthawk for seed yield, number of plants m^{-2}, 100-seed weight and percent frost damaged seed (Table 4.9). Snap bean accession G9519 (the Netherlands) had the highest percent frost damaged seed (Appendix 3). In 2001, genotypes did not differ significantly for yield, plants m^{-2} and percent frost damaged seed (Table 4.9).

Table 4.6 Mean squares from analysis of variance for cumulative thermal units to 50% anthesis and to 50% maturity, seed yield, number of plants m^{-2}, seed weight and percent frost damaged seed of three dry bean check cultivars grown at Saskatoon, SK, during 2000.

Source of variation	d.f.	Mean square					
		Cumulative thermal units to 50% anthesis	Cumulative thermal units to 50% maturity	Yield m^{-2}	No. of plants m^{-2}	100-seed weight	Percent frost damaged seed
Block	5	180.7	916.9	6435	104.0	0.3110	5.7
Check cultivar	2	778.4	18851.7**	106895**	3903.2**	7.4038**	113.2**
Error	10	340.0	476.1	5174	73.1	0.6180	5.2
Total	17						
C.V. (%)		3.4	2.2	21.7	16.8	5.1	80.2

** Significant at 0.01 level.

Table 4.7 Means for cumulative thermal units to 50% anthesis and to 50% maturity (°C d) of common bean cultivars and accessions grown at Saskatoon, SK, during 2000 and 2001.

Cultivar/accession	Cumulative thermal units to 50% anthesis		Cumulative thermal units to 50% maturity	
	2000	2001	2000	2001
	°C d			
CDC Whistler	548.8	529.7	1070.0	1032.0
CDC Nighthawk	527.3	529.7	958.5	1008.4
UI 906	544.6	623.1	1004.2	1059.9
G7551	380.6	425.5	1011.3	945.3
G8090	491.6	516.4	1015.4	1026.9
G8823	435.1	503.1	978.0	1093.9
G9345	522.8	516.4	1012.4	1120.2
G9430	546.5	583.2	1046.1	1176.8
G5024	609.3	644.1	1078.4	1124.7
LSD (0.05)†	51.2	60.6	60.6	96.2

† Due to the augmented experimental design, the LSD in the year 2000 is to compare an accession with a check cultivar.

Table 4.8 Mean squares from analysis of variance for cumulative thermal units to 50% anthesis and to 50% maturity, seed yield, number of plants m^{-2}, seed weight and percent frost damaged seed of common bean cultivars and accessions grown at Saskatoon, SK, during 2001.

Source of variation	d.f.	Mean square					
		Cumulative thermal units to 50% anthesis	Cumulative thermal units to 50% maturity	Yield m^{-2}	No. of plants m^{-2}	100-seed weight	Percent frost damaged seed
Block	1	546.7	3629.5	3898.4	2.4	0.002	3.6
Cultivar/ Accession	8	8848.2**	10011.4*	2289.9	115.7	27.0**	2.0
Error	8	691.6	1741.0	1987.9	115.0	1.4	2.6
Total	17						
C.V. (%)		4.9	3.9	33.7	28.1	6.6	159.9

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

Table 4.9 Means for seed yield (g), number of plants m^{-2}, seed weight (g) and percent frost damaged seed of common bean cultivars and accessions grown at Saskatoon, SK, during 2000 and 2001.

Cultivar/accession	Yield m^{-2}		No. of plants m^{-2}		100-seed weight		Percent frost damaged seed	
	2000	2001	2000	2001	2000	2001	2000	2001
	g				g		%	
CDC Whistler	187.0	145.1	21.4	33.4	15.3	16.6	7.8	0.5
CDC Nighthawk	449.8	119.6	65.0	35.0	16.6	18.0	0	0
UI 906	358.7	165.9	66.1	48.3	14.4	13.3	0.7	2.5
G7551	547.8	169.8	67.0	46.7	25.5	24.5	13.2	1.0
G8090	231.3	87.4	37.5	23.3	16.7	16.6	5.9	1.5
G8823	260.9	144.1	74.1	36.7	17.5	19.0	1.9	0
G9345	505.8	143.2	73.6	36.7	16.3	16.6	1.2	1.0
G9430	249.1	70.0	55.8	43.3	18.8	15.6	7.9	2.5
G5024	403.2	144.4	60.8	40.0	24.2	23.6	0	0
LSD (0.05)†	199.9	NS	23.8	NS	2.2	2.8	6.3	NS

† Due to the augmented experimental design, the LSD in the year 2000 is to compare an accession with a check cultivar.

4.5 Discussion

4.5.1 Seedling Emergence and Stand Establishment

Planting in early May (i.e., May 3) ensured that common bean seeds were subjected to suboptimal temperatures for germination and emergence (Figure 4.1). At temperatures below 10°C, Kooistra (1971) observed little or no response in common bean germination for about 14 days. In this study, large variation in emergence was observed among common bean accessions (Table 4.4, Appendix 1). Emergence for most accessions was poor at 20 days after planting, but increased with warming of the seedbed in mid June, indicating good seed quality and viability under suboptimal seedbed temperatures. Increase in germination and emergence with increasing seedbed temperature was observed in both controlled (Kooistra, 1971) and field environments (Dickson, 1971). Accessions with poor emergence after 50 days from planting were observed in 2000. This was likely due to a relatively colder seedbed in 2000 compared to 2001 (Figure 4.1). Emergence in the above accessions may have continued after the last emergence count in this study. Standard germination tests were not conducted prior to planting in the field. Furthermore, below average precipitation in May and June of both years of evaluation may have also reduced percent emergence of common bean cultivars and accessions. Hucl (1993) reported water potential of –0.8 MPa hinder common bean germination even at an optimum temperature of 22°C. At both 22 and 26°C, the decrease in water potential from 0 to –0.4 MPa, decreased percent germination of common bean by 10 percentage units. Germination response of common bean genotypes at suboptimal seedbed temperature and low water potential is not known.

Two navy bean accessions, G8823 from the Netherlands and G9345 from the USA, were superior in emergence when seeded under suboptimal temperatures in both years (Table 4.4). The accession G8823 was consistent in its emergence (30% in 2000 vs. 25% in 2001) compared to G9345 (50% in 2000 vs. 25% in 2001) at 20 days after planting. Kooistra (1971) identified a white seeded bean cultivar (Comtesse de Chambord - G1514) that germinated in two weeks at 9°C. The above cultivar however was not included in our study and hence its field emergence could not be evaluated. Dickson (1971) in common bean and Legesse and Powell (1992) in cowpea observed poor germination of white/light coloured seeds, with suboptimal temperatures compared to

their dark coloured counterparts, primarily due to the susceptibility of the light coloured seeds to soil borne fungal pathogens. Fungicidal seed treatment in our study may have reduced the confounding effect of soil pathogens on poor emergence of genotypes with light coloured seeds. The check cultivar CDC Nighthawk was comparable to the two early emerging accessions for percent emergence at 30 days after planting in 2000 and at 20 days after planting in 2001.

Cold tolerant maize and soybean lines are able to initiate germination and seedling establishment by using an alternative respiratory (CN insensitive) pathway not present in cold sensitive lines (McDonald, 1994). In tomato, the cold resistant genotype with a higher germination rate at 9°C had a higher respiration rate indicating higher metabolic activity compared to the cold sensitive genotype (Leviatov et al., 1994). The cold resistant genotype also had a higher activity of endomannanase, an enzyme that mobilizes galactomannan in the endosperm cell walls at both 12 and 25°C compared to the cold sensitive genotype (Leviatov et al., 1995). The mechanism of chilling resistance in dry bean accessions G8823 and G9345 is not known.

Dickson and Boettger (1984) observed that common bean radicles that emerged at 8°C were either weak or rotted and died compared to those radicles that emerged at 10 or 12°C. On this basis, Dickson and Boettger (1984) argued that cold tolerant lines will remain dormant until the germination medium is at least 10 to 12°C. In this study however, common bean seeds were subjected to seedbed temperatures of below 10°C in the field (Figure 4.1) for varying lengths of time. Accessions G8823 and G9345, and the check cultivar CDC Nighthawk had more than 80% emergence at 50 days after planting in both years, indicating little or no effect of suboptimal temperatures on final plant stand establishment (Table 4.4). However, this could not be said for the check cultivar UI 906 and for those accessions that required 40 days or more for plant stand establishment. At 30 days after planting, UI 906 had an emergence of 17% in 2000 and 32% in 2001 when the soil temperatures were at or below 15°C (Figure 4.1). With an increase in soil temperature in mid June, emergence in UI 906 increased to 87% (Table 4.4). The temporal delay in emergence in UI 906 and in accessions with a similar emergence pattern may be due to their requirement for high temperature maximum or an accumulation of certain degree days prior to start of germination and/or emergence. In

canola cv. Westar, Nykiforuk and Johnson-Flanagan (1999) observed a temporal delay in germination at 10°C that did not affect the overall success of germination. The germination proceeded rapidly if the required equivalent of 16 to 24 degree days occurred before germination. At 6°C, however, delay and non-uniform germination due to both thermal and developmental effects was observed. The temporal delay in germination in cold soils of early spring may be an adaptive feature in dry bean, which is sensitive to light frost. Common bean genotypes with emergence responses similar to G8823, G9345, and CDC Nighthawk can be classified as chilling tolerant while that of UI 906 as chilling sensitive. The extent of delay and non-uniformity of emergence under sub-optimum temperatures could not be established since the accessions were not seeded under optimal temperature regimes in the field.

Unlike wild tomato species (Patterson et al., 1978), the wild relatives of common bean *P. vulgaris* var. mexicanus and *P. vulgaris* var. *aborigineus* from the high altitudes of the Sierra Madre and the Andes, respectively, showed poor or no emergence under suboptimal temperatures in the field (Appendix 1). Percent emergence at 50 days after planting was poor in accessions such as G23554A (1950 m, Mexico) and G23450 (2840 m, Peru). Seed coat integrity has been implicated in reducing imbibitional chilling injury in pea seeds (Tully et al., 1981). Seeds of the wild relatives of common bean were scarified by nicking the seed coat to overcome seed coat induced dormancy. Hence the poor emergence in these accessions may partly be attributed to the enhanced chilling injury due to loss of seed coat integrity. The confounding effect of imbibitional chilling injury on early spring emergence needs to be overcome before any decision can be made on the utilization of wild relatives in improving suboptimal temperature emergence in common bean. The imbibitional chilling injury could partly be overcome by slow imbibition of seeds (Chen et al., 1983).

4.5.2 Anthesis, Maturity and Seed Yield

Delayed germination and plant stand establishment results in a delayed harvest date (Guy, 1994). Large year to year variation was observed in this study for anthesis, maturity and yield of common bean accessions. Due to the augmented experimental design used in 2000, trait means for common bean accessions were based on one

replicate after adjusting for the block variation. In 2001, the primary objective of the field trial was to confirm the emergence pattern of selected common bean accessions observed in the previous growing season. Due to limited seed availability, not all accessions were evaluated. Hence, the inference for the above traits is conservative. CDC Nighthawk required 527 to 530°C days to 50% anthesis in both growing seasons (Table 4.7). Accessions, primarily from northern Europe, flowered earlier than the early flowering check cultivar CDC Nighthawk (Appendix 2). Earliness in flowering among northern European accessions may have resulted from intense selection pressure from a short growing season or due to interaction of cool night temperatures with their photoperiod requirement. Cooler night temperatures enhance growth responses in common bean (Masaya and White, 1991). Early anthesis would also indicate a shorter vegetative growth period, which may become source limiting unless rapid establishment of leaf area occurs (White and Izquierdo, 1991).

CDC Nighthawk was among the earliest to mature, although it required a significantly higher CTU to anthesis compared to accessions from northern Europe (Table 4.7, Appendix 2). The relatively short period of reproductive growth may be due to its short seed-fill duration or its ability to hasten the maturity process in response to decreasing temperatures in the fall. If total vegetative growth determines crop yield, a genotype which flowered late but filled pods rapidly would be preferred for regions with a short growing season (White and Izquierdo, 1991). It is not known if the above holds true in CDC Nighthawk. Accession G8823 was early to emerge and to flower, but has CTUs to 50% maturity and yield similar to CDC Nighthawk (Table 4.4, 4.7 and 4.9). G8823 was the only accession with a consistent early emergence, early anthesis and early maturity in both years. Accessions G11285 from Sweden and G9256 from the Netherlands were the earliest to flower but failed to mature prior to the first fall frost, due probably to a longer seed fill duration compared to accessions with similar CTU requirement for anthesis. Except for G19504, accessions from the high altitudes of the Sierra Madre and the Andes did not flower during the growing season due primarily to their photoperiod sensitivity (Singh, 1989).

4.6 Conclusions

Accessions G8823 and G9345 had between 25% and 50% of their seedlings emerged at 20 days after planting (i.e., May 23), when the risk of late spring frost is very high. For CDC Nighthawk and UI 906, however, fewer than 20% of seedlings had emerged by May 23. Even at 30 days after planting (i.e., June 3), emergence for UI 906 was less than 20% in 2000 when the seedbed was relatively cold, and less than 40% in 2001 when the seedbed was relatively warm (Table 4.4). Our earlier experiment with dry bean cultivars indicated that in years with a mid September frost, mid May planting results in higher seed yield and quality compared to planting on a conventional planting date. The option of mid May or earlier planting of dry bean on the prairies depends on a developing frost resistant dry bean cultivar that will minimize risks from late spring and early fall frosts on seedling stand, and seed yield and quality, respectively. The above however is a long term strategy. In the short term, breeding dry bean cultivars with an emergence similar to UI 906 and CDC Nighthawk would enable early May and mid May planting, respectively of dry bean cultivars. This strategy would enable germination but prevent emergence of seedlings until after the threat of late spring frost has receded. Scattered emergence of CDC Nighthawk and UI 906 may pose a problem in the field management of early planted dry bean crop in commercial fields, but will ensure survival of 60% or more seedlings in the event of a late spring frost. G8823 can be used as a parent to develop elite bean cultivars with the ability to emerge under suboptimal temperature for regions with a low risk of frost at the bean seedling stage and in the high altitudes of the tropics. Also the above trait can be transferred into frost resistant bean cultivars when those are developed.

5. Freezing Resistance in *Phaseolus* Species

5.1 Abstract

Frost resistance, if successfully introgressed into common bean germplasm, may enable early spring planting of dry bean cultivars on the Canadian prairies and also expand the geographic distribution of the bean crop, possibly to higher altitudes in the tropics. This study was conducted to determine the frost resistance in the wild relatives of common bean. Leaflets of common bean and its wild relatives in the primary and tertiary gene pools were subjected to subzero temperatures with and without nucleation to determine the levels of tolerance and avoidance, respectively. Freezing tolerance of plants after exposure to cold acclimating temperatures was determined. Resistance of common bean and its wild relatives to spring and fall frosts were also determined on seedlings transplanted to the field. The LT_{50} for leaflets of the tertiary gene pool species *P. filiformis*, *P. angustissimus*, *P. ritensis* and *P. acutifolius* var. *tenuifolius* was 0.5 to 1°C lower compared to the primary gene pool species *P. vulgaris*, *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus*. Leaflets of the tertiary gene pool species were characterized by extensive supercooling compared to leaflets from the primary gene pool species. Under the experimental conditions used, cold acclimation had little or no effect in enhancing freezing tolerance of *Phaseolus* species. In the field study, *P. angustissimus* had the highest seedling survival in response to both fall frost in 2000 and spring frost in 2001, when the minimum air temperatures were –5 and –7°C, respectively. The primary gene pool species succumbed to frost injury and showed no recovery with the return of favourable temperatures.

5.2 Introduction

Subzero temperatures in late spring and early fall are detrimental to dry bean (*Phaseolus vulgaris* L.) production on the northern prairies. Dry bean plants are killed at

the moment of ice formation (–2°C) within tissues (Ashworth et al., 1985). Due to the risk of late spring frost, dry bean is usually planted in late May on the Canadian prairies. Delayed planting, however, decreases the effective growing period, and increases the risk of late season drought stress and frost. Also, an earlier study (chapter 3) showed that in years with a mid September frost, seed yield and quality of the late May planted dry bean cultivars are severely affected compared to the mid May planted dry bean cultivars. Furthermore, a subsequent study (chapter 4) identified a dry bean accession (G8823) with superior emergence under suboptimal seedbed temperature. Availability of bean germplasm with improved emergence in cool soils, and improved seed yield and quality associated with the mid May planting makes early spring planting an attractive option for bean producers. Hence, frost resistant dry bean cultivars, if available, may reduce the risk of seedling death with early spring planting and thus expand the area of bean production.

Freezing stress resistance in plants involves avoidance and tolerance (Levitt, 1980). Freezing avoidance is the ability to avoid subzero temperatures, to avoid ice formation by lowering the freezing point, or to avoid intracellular freezing where extracellular freezing is tolerated (Clements and Ludlow, 1977). Avoidance is achieved by desiccation in seeds, and by freezing point depression, supercooling (Chen et al., 1995) and/or developmental modification in plants (Burke et al., 1976; Clements and Ludlow, 1977; Palta and Li, 1979). Plants will readily supercool to varying degrees in the absence of both intrinsic and extrinsic nucleators (Gusta, 1985). Greenhouse grown plants including snap bean are able to supercool to temperatures between –6 and –14°C (Marcellos and Single, 1979). In snap bean stems, however, ice nucleators were effective in the range of –4 to –8°C. Thus, supercooling in frost sensitive plants provides a means of avoiding damaging ice formation during light frost (Gusta, 1985). Supercooling however promotes nonequilibrium freezing which leads to symplastic ice formation and is considered lethal (Gusta and Fowler, 1977).

Developmental modifications may also enable plants to avoid freezing death. In the absence of intrinsic nucleators, a thick continuous cuticle may serve as an effective barrier to nucleation by external ice (Burke et al., 1976). Estrada (1982) observed a highly consistent relationship between frost survival under field and controlled

environment conditions, and the double or triple layers of palisade parenchyma cells in the wild relatives of potato. The hypogeal germination in cool season legumes such as lentil, pea, chickpea, and faba bean ensures that at least one meristematic node remains under soil cover. In the event of frost and subsequent death of the aerial shoot, the cotyledonary nodes produce new shoots. In *Centrosema virginianum*, a tropical herbaceous legume, the height of the cotyledonary node was negatively associated with both latitude of origin and winter survival in the field (Clements and Ludlow, 1977).

Freezing tolerance is the ability to tolerate ice formation in tissues without injury (Clements and Ludlow, 1977). Tolerance of freezing stress is by extracellular freezing. Depending on the rate of cooling, the crystallization of water occurs in two markedly distinct locations within the tissues of most plants (Guy, 1990). Under rapid cooling, ice formation may occur intracellularly and is invariably lethal. The rate of cooling required for intracellular freezing of intact plants is approximately $3^{\circ}C\ hr^{-1}$ (Guy, 1990). In nature, however, atmospheric cooling rates seldom exceed $1^{\circ}C\ hr^{-1}$ (Steffen et al., 1989) which usually results in extracellular freezing. Plants exposed to low but nonlethal temperatures develop an acclimation response characterized by a greater ability to resist injury or survive a low temperature stress that would otherwise be lethal (Levitt, 1980). The inherent ability of temperate plants to acclimate to cold and their rate of acclimation are two major factors that limit low temperature survival (Chen et al., 1995). Winter cereals can acclimate to $-30^{\circ}C$, whereas spring cereals only acclimate to about $-9^{\circ}C$ (Gusta et al., 1996). Acclimation decreased the LT_{50} of winter pea from $-3^{\circ}C$ to $-9^{\circ}C$ (Swensen and Murray, 1983). Common bean seedlings grown under controlled environment conditions and cooled at the rate of $1.5^{\circ}C\ h^{-1}$ froze at $-1.3^{\circ}C$ (median = $-3.9^{\circ}C$), while seedlings in the field, in response to a natural frost, also froze at $-1.3^{\circ}C$ (median = $-2.7^{\circ}C$) (Ashworth et al., 1985).

Buhrow (1980, 1983) reported frost resistance in several wild species of *Phaseolus*. *Phaseolus acutifolius* var. *tenuifolius*, *P. angustissimus*, *P. filiformis* (syn. *P. wrightii*), and *P. ritensis* (syn. *P. metcalfei*) survived several successive nights of radiation frost when grown in a germplasm nursery at Tucson, AZ. The mechanism of freezing resistance and the acclimation response in *Phaseolus* species has not been studied to date. Furthermore, screening of *Phaseolus* species for frost resistance in the field will enable

breeders to decide on appropriate parents and breeding strategies to introgress this trait into dry bean cultivars. The objectives of this study were to investigate i) the mechanism of freezing resistance in common bean and its wild relatives, ii) the ability of *Phaseolus* species to acclimate as a means of increasing freezing tolerance, and iii) their freezing resistance in the field.

5.3 Materials and Methods

Black bean cv. CDC Nighthawk, two wild relatives in its primary gene pool *P. vulgaris* var. *mexicanus* (G11031 from 2270 m altitude of the Sierra Madre) and *P. vulgaris* var. *aborigineus* (G23457 from 2940 m altitude of the Andes), and four species in its tertiary gene pool *P. filiformis* (Unknown), *P. angustissimus* (PI535272), *P. ritensis* (PI494138) and *P. acutifolius* var. *tenuifolius* (PI535248) were included in this study. Seed grown in a uniform environment (controlled environment chamber at 23/18°C with a 12 h photoperiod) was the seed source. Seeds of all accessions, except CDC Nighthawk, were scarified by nicking the seed coat with a scalpel.

5.3.1 Mechanism of Freezing Resistance

Leaflets from a Controlled environment chamber:

Plants were grown in two controlled environment chambers under an alternating temperature regime of 23/18°C (8 h/16 h) and a 16 h photoperiod (PPFD = 1000 μmol m^{-2} s^{-1}). The low temperature (18°C) overlapped both light and dark photoperiods. Plants were watered and fertilized as needed. Starting 30 days after planting, leaflets were harvested from plants in the two controlled environment chambers alternatively to determine freezing tolerance and avoidance. The experiment consisted of six replications.

Freezing Tolerance: Leaflets were washed in double deionized water (dd-water), blotted dry, and two leaflets per accession/cultivar were placed in a 15 cm glass test tube that had a wet paper towel in the bottom to initiate freezing. Sufficient leaflets were harvested so that one tube per accession could be removed from the glycol bath at hourly intervals for each subzero temperature. Also, one tube per accession was kept at 4°C as a control. The tubes were placed in a glycol bath at 0°C and the bath temperature decreased to –2°C

over 30 min. The samples were nucleated by spraying with ice crystals and held at $-2^{\circ}C$ for 1 h. One set of samples was removed to the control and the bath temperature was decreased at a linear rate of $1^{\circ}C\ h^{-1}$ down to $-5^{\circ}C$. Samples were removed at hourly intervals and placed with the control samples at $4^{\circ}C$. After 12 h at $4^{\circ}C$, viability was assessed by electrolyte leakage as follows: leaflets removed at each subzero temperature were shaken in 20 ml of dd-water for eight h and the conductivity of the decanted leachate determined using a CDM3 conductivity meter (Bach-Simpson Ltd., London ON). The original leachate was poured back and flasks with sample were frozen at $-80^{\circ}C$ for 12 h. The samples were then shaken for four h and the final leachate measured. Percent electrolyte leakage ratio (PELR) was determined as the ratio of conductance of the initial leachate after freeze test and the conductance of the final leachate and expressed as a percentage.

Freezing Avoidance: Leaves tended to nucleate spontaneously in test tubes and hence a new method was used to determine the extent of freezing avoidance. Six leaflets per accession were randomly laid on their adaxial surface on a polythene film inside plastic containers. Nine containers were prepared and placed in the dark in a controlled environment chamber set at $0^{\circ}C$. The air temperature was decreased to $-2^{\circ}C$ and subsequently decreased at a linear rate of $1^{\circ}C\ h^{-1}$ down to $-10^{\circ}C$. Samples were removed at hourly intervals and placed in a chamber maintained at $4^{\circ}C$. After 12 h at $4^{\circ}C$, the LT_{50} (lethal temperature at which 50% of the population is killed) was determined as the first subzero temperature at which three leaflets were killed.

Leaflets from Field:

Bean accessions were also grown on the Preston field plot at Saskatoon during the summer of 1999. Starting 78 days after planting (i.e., August 23), stem sections with leaves were harvested and brought to the chamber with cut ends dipped in water to prevent leaf wilting. The freezing tolerance and avoidance of field samples were determined as described in the freezing avoidance section of leaflets from a controlled environment chamber study. To determine freezing tolerance, leaflets were sprayed with dd-water at $0^{\circ}C$ to facilitate nucleation at subzero temperatures. Four leaflets per

accession were used and the experiment consisted of four replications. The LT_{50} for the field samples was the first subzero temperature at which two leaflets were killed.

Data were subject to analyses of variance appropriate to a randomized complete block design. In the freezing avoidance study, for the purpose of statistical analysis, an LT_{50} of $-10^{o}C$ was assumed for those species in which the LT_{50} was not achieved even at $-10^{o}C$. Heterogeneity of error variance was also determined.

5.3.2 Acclimation and Freezing Tolerance

Plants were exposed to cold acclimating temperatures either in the natural environment or in a controlled environment chamber prior to freezing tolerance determination. Plant materials were the same as described in section 5.3 except that in the latter study, *P. vulgaris* var. *aborigineus* accession G23559 (2900 m altitude) was used instead of G23457 (2940 m altitude).

Acclimation in the Natural Environment

Plant growth conditions were the same as described in section 5.3.1 (leaflets from a controlled environment chamber). Single seedlings per 102 mm pot filled with Redi-earth® (W.R. Grace and Co. of Canada Ltd., Ajax, ON) were grown in a controlled environment chamber for 21 days. Plants were watered and fertilized regularly. Sufficient plants were grown so four seedlings per accession could be removed at hourly intervals for each subzero temperature. At 21 days after planting, plants were in the V3 growth stage (the first trifoliolate leaf opens and the second and third trifoliolate leaf appears) and were moved to the outdoors (April 28, 2000) for 14 days with the exception of the control plants. Air temperature at plant height was monitored using a Hobo datalogger (Onset Computer Corporation, Bourne, MA). Plants were covered with a white, transparent plastic dome if frost was forecasted for a particular night. Freezing tolerance of the plants was evaluated at weekly intervals as follows: Four plants per accession were randomized in plastic trays. Four trays were prepared and placed in the in a controlled environment chamber set at $0^{o}C$. Plants were sprayed with water kept at the same temperature as the chamber. The air temperature was decreased to $-2^{o}C$ and subsequently decreased at a linear rate of $1^{o}C\ h^{-1}$ down to $-5^{o}C$. Plants were removed at hourly intervals and placed in a chamber maintained at $4^{o}C$. After 12 h at $4^{o}C$, the $LT_{50}K$

(lethal temperature at which 50% of the population is killed) was determined as the first subzero temperature at which two seedlings were killed. Plants were then moved to a controlled environment chamber at 23/18°C (12 h/12 h) with a 12 h photoperiod for 14 days. The $LT_{50}G$ (lethal temperature at which 50% of the population regrew) was determined as the lowest subzero temperature at which two seedlings showed regrowth. Plants that escaped freezing injury were not considered for $LT_{50}K$ and $LT_{50}G$ determination.

Data were subject to analysis of variance appropriate to a split plot design with two replicates. Acclimation treatment was the mainplot and accession/cultivar was the subplot. Contrast coefficients were used to partition acclimation mean squares into linear and quadratic mean squares. Both acclimation treatment and accession/cultivar were considered as fixed effects. Heterogeneity of error variance was also determined.

Acclimation in a Controlled Environment Chamber

Accessions/cultivars were planted on July 5, 2000 in Redi-earth® (W.R. Grace and Co. of Canada Ltd., Ajax, ON) in 102 mm plastic pots and grown outdoors for 26 days. Watering and fertilizer applications were as described above. At 26 days after planting (i.e., July 31), accessions were in the V3 growth stage except for *P. filiformis* and *P. vulgaris* var. *mexicanus*, which were in the V4 growth stage (the third trifoliolate leaf opens and buds on the lower nodes produce branches). Except for the control plants that were immediately subjected to a freezing test, other plants were moved to a controlled environment chamber maintained at 7/5°C (8 h/16 h) with a 16 h photoperiod (PPFD = 50 $\mu mol\ m^{-2}\ s^{-1}$). The low temperature (5°C) overlapped both light and dark photoperiods. The chamber temperature was decreased from 7/5°C to 5/2°C and 2/0°C at three day interval. Air temperature at plant height was monitored. At the end of each temperature regime, the freezing tolerance of plants was evaluated as described above. Statistical analysis was the same as described above.

5.3.3 Resistance of Seedlings to Fall Frost

Plant materials were the same as described in section 5.3, except for *P. vulgaris* var. *aborigineus*, accession G23454D (2460 m altitude) was used instead of G23457. Plants

were grown in Jiffy pots (Jiffy-7®, Jiffy Products (N.B.) Ltd., Shippagan, NB) for 15 to 20 days in a controlled environment chamber at 23/18°C (8 h/16 h) with a 16 h photoperiod (PPFD = 300 μmol m^{-2} s^{-1}). The low temperature (18°C) overlapped both light and dark photoperiods. One hundred seedlings per accession per replication were hand transplanted in the Preston field plot on August 10 and 29, 2000. Two replicates per transplanting date were used. Transplants were watered every day in the field until they were well established. The number of seedlings that survived transplanting shock were determined. At the time of transplanting, seedlings were at the V2 growth stage (fully opened primary leaves). At the time of incidence of the first fall frost on September 23, the seedlings transplanted on August 10 were at the V4, R5 (preflowering) or R6 (flowering) growth stage. In contrast, seedlings were at the V3 growth stage for those transplanted on August 29. Air temperature at plant height was monitored using Hobo dataloggers. Number of surviving plants on the second day after the first fall frost, and number of plants that survived and showed regrowth from the aerial shoot on the seventh and 14th day after the first fall frost were determined. Data were subjected to chi-square test of independence of proportions to determine if the mean number of surviving plants was independent i) of the growth stage and ii) of the species. The above experiment was repeated in the fall of 2001.

5.4 Results

5.4.1 Mechanism of Freezing Resistance

Leaflets of *Phaseolus* species from a controlled environment chamber and the field were significantly different in their levels of freezing tolerance and avoidance (Tables 5.1 and 5.2). The leaflets of the tertiary gene pool species of common bean from a controlled environment chamber suffered less than 50% damage when nucleated and frozen at –2°C compared to the primary gene pool species (Figure 5.1 and Table 5.3). At –3°C, however, all species had greater than 75% electrolyte leakage ratio (Figure 5.1). This was confirmed by the freeze tolerance test on the leaflets from the field in which, the LT_{50} was 0.5 to 1°C lower for the tertiary gene pool species compared to the primary gene pool species (Table 5.3).

Leaflets of *Phaseolus* species from a controlled environment chamber showed more extensive supercooling compared to leaflets from the field (Table 5.3). Leaflets of the primary gene pool from a controlled environment chamber froze at –7°C while those of the tertiary gene pool froze at –9°C or below. In the case of leaflets from the field, the primary gene pool species froze at –4°C while those of the tertiary gene pool species froze at –5°C or below. On average, the accession of *P. acutifolius* var. *tenuifolius* used in this study showed a response that was intermediate between the accessions of the primary and tertiary gene pool species.

Table 5.1 Mean squares from analysis of variance for percent electrolyte leakage ratio (PELR) and LT_{50} of leaflets of *Phaseolus* species from a controlled environment chamber.

Source of variation	d.f.	Mean square	
		Tolerance	Avoidance
		PELR (–2°C)	LT_{50}
Block	5	480.5	0.8000
Species	6	1211.5**	7.0397**
Error	30	202.7	0.3444
Total	41		
C.V. (%)		32.8	7.1

** Significant at 0.01 level.

Table 5.2 Mean squares from analysis of variance for LT_{50} of leaflets of *Phaseolus* species from a field.

Source of variation	d.f.	Mean square	
		Tolerance	Avoidance
		LT_{50}	LT_{50}
Block	3	0.1310	5.8*
Species	6	0.8690**	17.5**
Error	18	0.0754	1.6
Total	27		
C.V. (%)		11.1	21.8

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

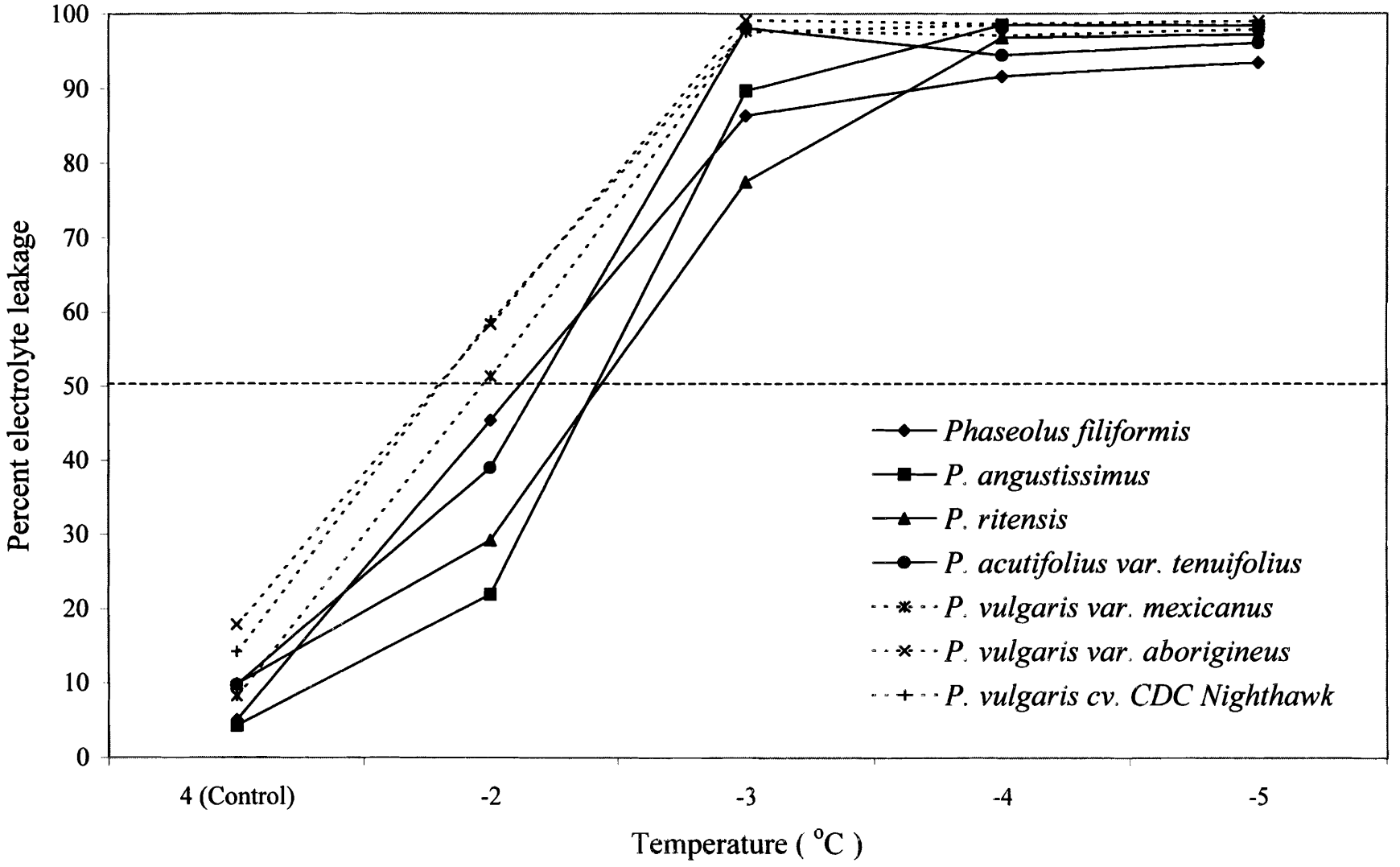

Figure 5.1 Relative freezing tolerance of *Phaseolus* species grown in a controlled environment chamber maintained at 23/18°C and a 16 h photoperiod.

Table 5.3 Means for percent electrolyte leakage ratio (PELR) and LT_{50} (°C) of leaflets of *Phaseolus* species from a controlled environment chamber and field.

Species	Tolerance†		Avoidance†	
	Controlled environment chamber	Field	Controlled environment chamber	Field
	PELR (–2°C)	LT_{50}	LT_{50}	LT_{50}
		°C		
Phaseolus filiformis	45.3bcd	–3.0a	–9.8a	–8.3a
P. angustissimus	21.9a	–2.8ab	–8.8b	–8.5a
P. ritensis	29.2ab	–3.0a	–9.0b	–6.8ab
P. acutifolius var. *tenuifolius*	38.9bc	–2.5b	–8.7b	–5.0bc
P. vulgaris var. *mexicanus*	51.3cd	–2.0c	–7.7c	–3.0d
P. vulgaris var. *aborigineus*	58.3d	–2.0c	–7.0c	–4.3cd
P. vulgaris cv. CDC Nighthawk	58.8d	–2.0c	–7.0c	–4.8cd
SE(diff.)	8.2	0.2	0.3	0.9

† Means followed by the same letter are not significantly different at 0.05 level.

5.4.2 Acclimation and Freezing Tolerance

Exposure of plants to cold acclimating temperatures either in natural or controlled environment did not affect the $LT_{50}K$ and $LT_{50}G$ traits (Table 5.4 and 5.5) indicating little or no acclimation in response to low temperature regimes (Figures 5.2 and 5.3). Seven days after the transfer of plants to a natural environment, the $LT_{50}K$ mean decreased from –1.9°C to –3.1°C, but the decrease was not statistically significant (Table 5.6). Although maximum and minimum air temperatures were controlled efficiently in a controlled environment chamber, freezing tolerance did not increase during acclimation (Tables 5.5 and 5.8).

Both $LT_{50}K$ and $LT_{50}G$ were significantly different for *Phaseolus* species under natural or controlled environment conditions (Tables 5.4 and 5.5). With the exception of *P. acutifolius* var. *tenuifolius*, species of the tertiary gene pool had a lower $LT_{50}K$ and $LT_{50}G$ compared to species of the primary gene pool (Tables 5.6, 5.7, 5.8 and 5.9). Acclimation x species interaction was significant for $LT_{50}G$ when *Phaseolus* species were acclimated under natural or controlled environment conditions (Tables 5.4 and 5.5). The above interaction was of the crossover type in which species ranking was inconsistent across acclimation treatments. Change in rank primarily involved the tertiary gene pool species *P. angustissimus* and *P. ritensis*, which were not significantly

different from *P. acutifolius* var. *tenuifolius* and the primary gene pool species in the control but differed in the acclimation treatments (Tables 5.7 and 5.9).

Table 5.4 Mean squares from analysis of variance for $LT_{50}K$ (kill) and $LT_{50}G$ (regrowth) for *Phaseolus* species when acclimated under a natural environment.

		Mean square	
Source of Variation	d.f.	$LT_{50}K$	$LT_{50}G$
Replication	1	0.60	0.00
Acclimation	2	4.67	0.31
Acclimation Linear	1	1.29	0.32
Acclimation Quadratic	1	8.05	0.30
Replication x Acclimation	2	1.24	0.50
Species	6	4.71**	3.48**
Acclimation x Species	12	0.28	1.20*
Error	18	0.47	0.39
Total	41		
CV (%)		27.9	23.8

*, ** Significant at the 0.05 and 0.01 levels, respectively.

Table 5.5 Mean squares from analysis of variance for $LT_{50}K$ (kill) and $LT_{50}G$ (regrowth) for *Phaseolus* species when acclimated under a controlled environment.

		Mean square	
Source of Variation	d.f.	$LT_{50}K$	$LT_{50}G$
Replication	1	5.16*	0.02
Acclimation	3	0.78	1.73
Acclimation Linear	1	1.29	2.23
Acclimation Quadratic	1	0.88	2.16
Acclimation Cubic	1	0.18	0.80
Replication x Acclimation	3	0.35	0.21
Species	6	4.21**	1.61**
Acclimation x Species	18	0.89	0.40**
Error	24	0.47	0.12
Total	55		
CV (%)		28.9	24.5

*, ** Significant at the 0.05 and 0.01 levels, respectively.

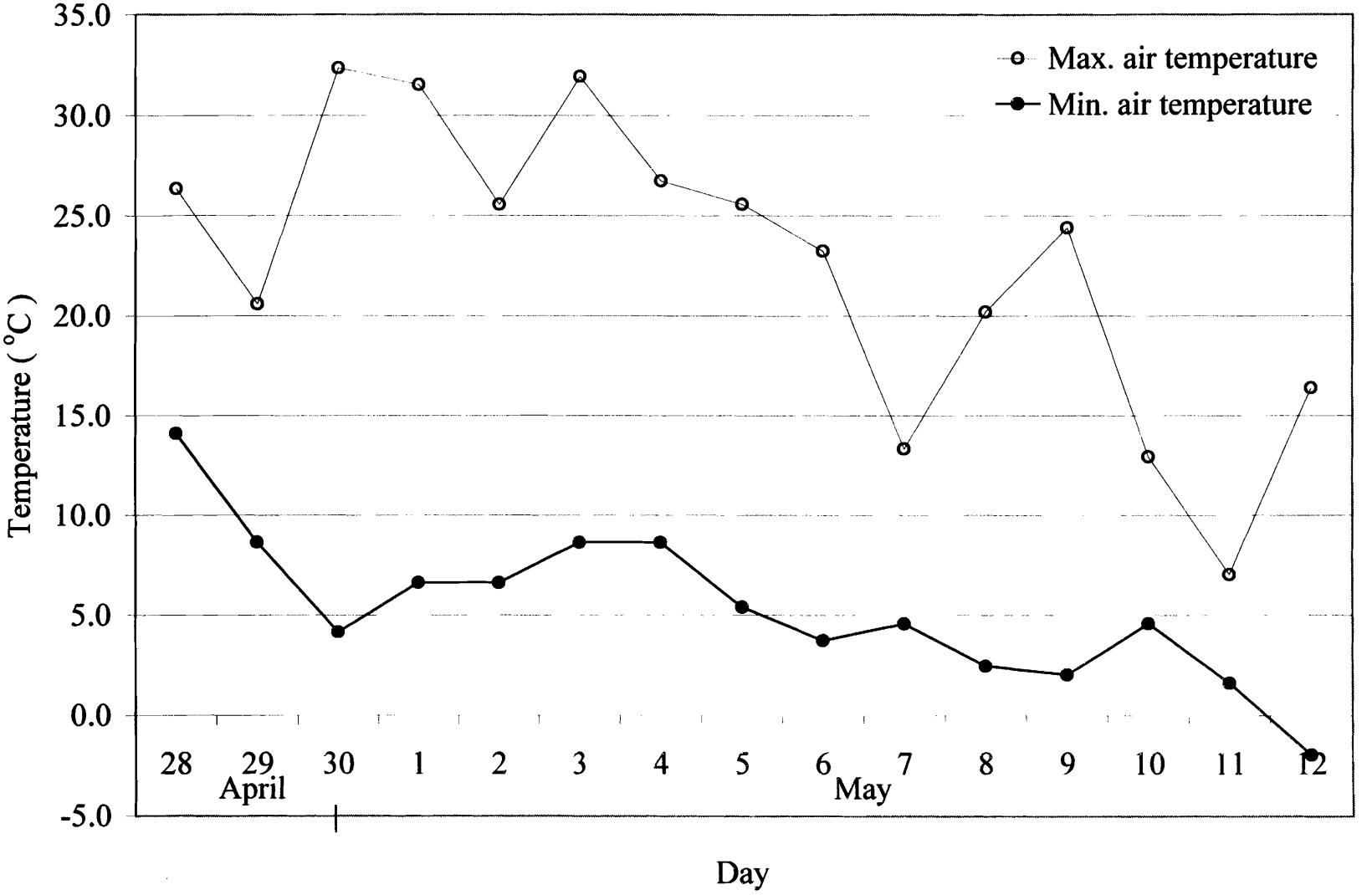

Figure 5.2 Maximum and minimum daily air temperature (°C) at plant height (15 cm) outside the John Mitchell greenhouse during the spring of 2000.

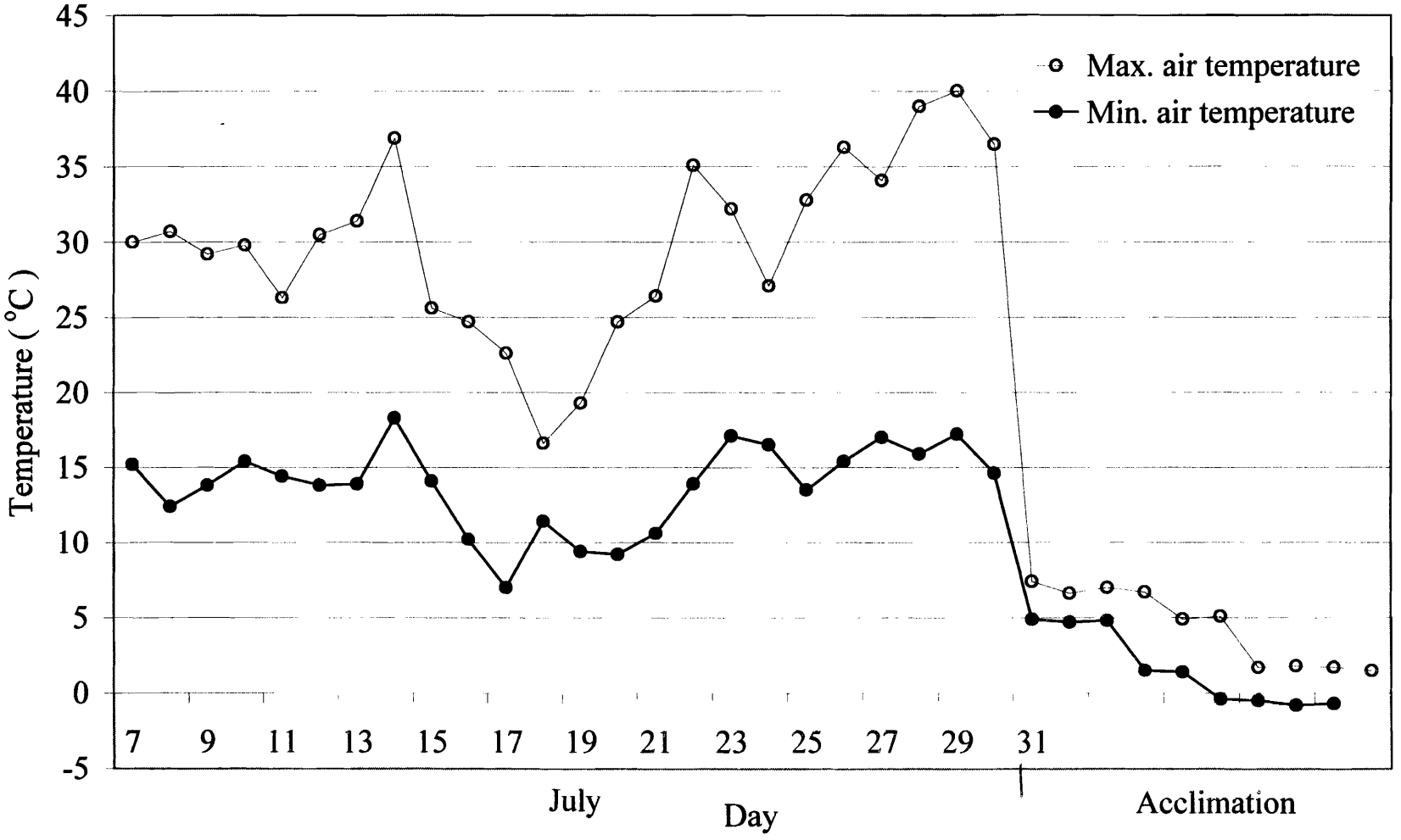

Figure 5.3 Maximum and minimum daily air temperature (°C) at plant height (15 cm) outside the John Mitchell greenhouse during the summer of 2000, and during acclimation in a controlled environment chamber.

Table 5.6 Means for $LT_{50}K$ (kill) (°C) of *Phaseolus* species during acclimation in a natural environment.

Species	Acclimation			Mean
	Control	Seven days	Fourteen days	
	°C			
Phaseolus filiformis	–2.5	–4.0	–3.0	**–3.2**
P. angustissimus	–2.5	–3.5	–3.0	**–3.0**
P. ritensis	–3.5	–4.0	–4.0	**–3.8**
P. acutifolius var. *tenuifolius*	–1.5	–3.0	–2.0	**–2.2**
P. vulgaris var. *mexicanus*	–1.5	–3.0	–1.0	**–1.8**
P. vulgaris var. *aborigineus*	–1.0	–2.0	–2.0	**–1.7**
P. vulgaris cv. CDC Nighthawk	–1.0	–2.0	–1.5	**–1.5**
Mean	**–1.9**	**–3.1**	**–2.4**	
LSD (0.05)		**NS†**		**–0.8‡**

† Least significant difference to compare acclimation treatments.
‡ Least significant difference to compare *Phaseolus* species and subspecies.

Table 5.7 Means for $LT_{50}G$ (regrowth) (°C) of *Phaseolus* species during acclimation in a natural environment.

Species	Acclimation			Mean
	Control	Seven days	Fourteen days	
	°C			
Phaseolus filiformis	–3.5	–4.0	–4.5	**–4.0**
P. angustissimus	–2.0	–3.0	–4.5	**–3.2**
P. ritensis	–3.0	–2.5	–3.0	**–2.8**
P. acutifolius var. *tenuifolius*	–2.0	–2.0	–1.5	**–1.8**
P. vulgaris var. *mexicanus*	–3.0	–2.0	–1.5	**–2.2**
P. vulgaris var. *aborigineus*	–3.0	–2.0	–1.5	**–2.2**
P. vulgaris cv. CDC Nighthawk	–3.0	–2.0	–1.5	**–2.2**
Mean	**–2.8**	**–2.5**	**–2.6**	
LSD (0.05)		**NS†**		**–0.8‡**

† Least significant difference to compare acclimation treatments.
‡ Least significant difference to compare *Phaseolus* species and subspecies.

Table 5.8 Means for $LT_{50}K$ (kill) (°C) of *Phaseolus* species during acclimation in a controlled environment.

Species	Acclimation				
	Control	Three days	Six days	Nine days	**Mean**
	°C				
Phaseolus filiformis	–3.0	–3.0	–3.0	–3.5	**–3.1**
P. angustissimus	–2.0	–3.5	–2.5	–3.5	**–2.9**
P. ritensis	–3.0	–3.0	–4.0	–3.5	**–3.4**
P. acutifolius var. *tenuifolius*	–3.0	–3.0	–1.0	–1.0	**–2.0**
P. vulgaris var. *mexicanus*	–2.0	–2.0	–2.0	–1.0	**–1.8**
P. vulgaris var. *aborigineus*	–2.5	–2.0	–2.0	–1.0	**–1.9**
P. vulgaris cv. CDC Nighthawk	–1.5	–2.0	–2.0	–1.0	**–1.6**
Mean	**–2.4**	**–2.6**	**–2.4**	**–2.1**	
LSD (0.05)			**NS†**		**–0.7‡**

† Least significant difference to compare acclimation treatments.
‡ Least significant difference to compare *Phaseolus* species and subspecies.

Table 5.9 Means for $LT_{50}G$ (regrowth) (°C) of *Phaseolus* species during acclimation in a controlled environment.

Species	Acclimation				
	Control	Three days	Six days	Nine days	**Mean**
	°C				
Phaseolus filiformis	–2.5	–3.0	–2.5	–1.0	**–2.3**
P. angustissimus	–1.0	–3.0	–1.5	–1.0	**–1.6**
P. ritensis	–2.0	–2.0	–1.5	–1.0	**–1.6**
P. acutifolius var. *tenuifolius*	–1.5	–1.0	–1.0	–1.0	**–1.1**
P. vulgaris var. *mexicanus*	–1.0	–1.5	–1.0	–1.0	**–1.1**
P. vulgaris var. *aborigineus*	–1.0	–1.0	–1.0	–1.0	**–1.0**
P. vulgaris cv. CDC Nighthawk	–1.0	–1.5	–1.0	–1.0	**–1.1**
Mean	**–1.4**	**–1.9**	**–1.4**	**–1.0**	
LSD (0.05)			**NS†**		**–0.4‡**

† Least significant difference to compare acclimation treatments.
‡ Least significant difference to compare *Phaseolus* species and subspecies.

5.4.3 Resistance of Seedlings to Fall Frost

Saskatoon experienced two consecutive fall frosts on September 23 and 24, 2000 when the air temperature at plant height (15 cm) was –4.2°C and –4.9°C, respectively (Figure 5.4). Air temperature remained below the critical temperature of –2°C for three h on September 23 and six h on September 24. On both days, subzero temperatures occurred prior to dawn. The number of plants that survived frost in each species was determined on the second day (i.e., September 24), and the number of surviving plants that showed

regrowth from the aerial shoot was determined on the seventh (i.e., September 30) and 14th day (i.e., October 7) after the first fall frost.

The primary gene pool species *Phaseolus vulgaris* cv. CDC Nighthawk, *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus* were killed by the first fall frost regardless of the growth stage of the plant (Table 5.10). Although the next seven day period was frost free with favourable temperatures (> 20°C) for growth (Figure 5.4), the primary gene pool species showed no sign of recovery or regrowth (Table 5.11). This indicates the extreme susceptibility of the cultivated common bean and its wild relatives in the primary gene pool to frost. The chi-square test of independence of proportions indicated that among the tertiary gene pool species, with the exception of *P. filiformis* and *P. acutifolius* var. *tenuifolius*, plant survival (on the second day after frost) and/or regrowth (on the seventh day after frost) of *P. angustissimus* and *P. ritensis* were dependent on the growth stage of the plants (Tables 5.10 and 5.11). In both *P. angustissimus* and *P. ritensis*, younger plants (transplanted on August 29) had a higher frost survival compared to the relatively older plants (transplanted on August 10).

The chi-square test of independence of proportions also indicated that plant survival and/or regrowth on the second and seventh day after the first fall frost were dependent on the *Phaseolus* species (Tables 5.12 and 5.13). *Phaseolus angustissimus* had the highest percentage survival followed by *P. filiformis* on the second day after the first fall frost (Table 5.12). Absence of frost between September 24 and October 2 coupled with favourable air temperatures (> 20°C) during the same period enabled the surviving plants of *P. angustissimus* and *P. filiformis* to put forth new shoots and/or regrowth from aerial nodes (Table 5.13). Regrowth in *P. filiformis* was from the cotyledonary nodes held at the soil level while the regrowth in *P. angustissimus* was from the axillary buds.

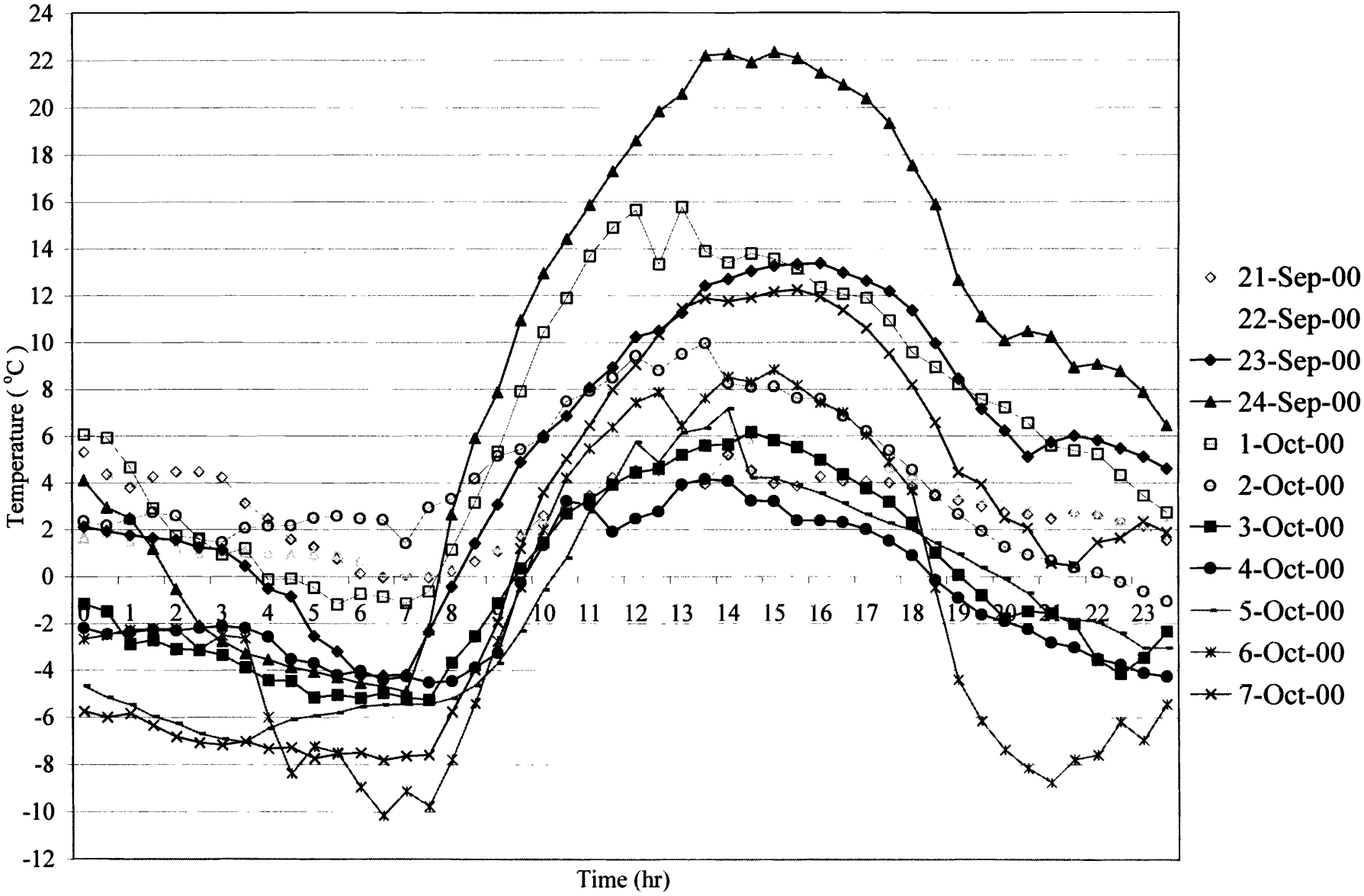

Figure 5.4 Air temperature (°C) at plant height (15 cm) at the Preston field plot in Saskatoon during the fall of 2000.

Table 5.10 Chi-square test of independence for comparing mean number of surviving plants in different growth stages for *Phaseolus* species on the second day after first fall frost in 2000.

Species	Mean number of surviving plants on the second day after first fall frost†		χ^2_{obs}	P value
	Branching stage	Seedling stage		
Phaseolus filiformis	9.0 (98)	7.0 (98.5)	**0.3**	**0.587**
P. angustissimus	61.5 (95.5)	81.0 (97.5)	**8.2**	**0.004**
P. ritensis	1.5 (90.5)	7.0 (28)	**15.9**	**0.000**
P. acutifolius var. *tenuifolius*	3.5 (97.5)	7.0 (97.5)	**0.9**	**0.352**
P. vulgaris var. *mexicanus*	0.0 (100)	0.0 (95)		
P. vulgaris var. *aborigineus*	0.0 (99.5)	1.0 (96.5)		
P. vulgaris cv. CDC Nighthawk	0.0 (99.5)	0.0 (76.5)		

† Numbers in parentheses are the number of plants (out of 100) that were alive prior to the first fall frost on September 23, 2000.

Table 5.11 Chi-square test of independence for comparing mean number of surviving plants in different growth stages for *Phaseolus* species on the seventh day after first fall frost in 2000.

Species	Mean number of surviving plants on the seventh day after first fall frost†		χ^2_{obs}	P value
	Branching stage	Seedling stage		
Phaseolus filiformis	16.5 (98)	15.0 (98.5)	**0.1**	**0.699**
P. angustissimus	60.5 (95.5)	87.0 (97.5)	**12.4**	**0.000**
P. ritensis	1.5 (90.5)	7.0 (28)	**15.9**	**0.000**
P. acutifolius var. *tenuifolius*	2.0 (97.5)	5.5 (97.5)	**2.1**	**0.149**
P. vulgaris var. *mexicanus*	0.0 (100)	0.0 (95)		
P. vulgaris var. *aborigineus*	0.0 (99.5)	0.5 (96.5)		
P. vulgaris cv. CDC Nighthawk	0.0 (99.5)	0.0 (76.5)		

† Numbers in parentheses are the number of plants (out of 100) that were alive prior to the first fall frost on September 23, 2000.

Table 5.12 Chi-square test of independence for comparing mean number of surviving plants of several *Phaseolus* species on the second day after first fall frost in 2000.

Species	Mean number of surviving plants on the second day after first fall frost†			χ^2_{obs}	P value
	Branching stage	Seedling stage	**Total**		
Phaseolus filiformis	9.0 (98)	7.0 (98.5)	**8.0**		
P. angustissimus	61.5 (95.5)	81.0 (97.5)	**71.3**		
P. ritensis	1.5 (90.5)	7.0 (28)	**4.3**		
P. acutifolius var. *tenuifolius*	3.5 (97.5)	7.0 (97.5)	**5.3**	**340.9**	**0.000**
P. vulgaris var. *mexicanus*	0.0 (100)	0.0 (95)	**0.0**		
P. vulgaris var. *aborigineus*	0.0 (99.5)	1.0 (96.5)	**0.5**		
P. vulgaris cv. CDC Nighthawk	0.0 (99.5)	0.0 (76.5)	**0.0**		

† Numbers in parentheses are the number of plants (out of 100) that were alive prior to the first fall frost on September 23, 2000.

Table 5.13 Chi-square test of independence for comparing mean number of surviving plants of several *Phaseolus* species on the seventh day after first fall frost in 2000.

Species	Mean number of surviving plants on the seventh day after first fall frost†			χ^2_{obs}	P value
	Branching stage	Seedling stage	**Total**		
Phaseolus filiformis	16.5 (98)	15.0 (98.5)	**15.8**		
P. angustissimus	60.5 (95.5)	87.0 (97.5)	**73.8**		
P. ritensis	1.5 (90.5)	7.0 (28)	**4.3**		
P. acutifolius var. *tenuifolius*	2.0 (97.5)	5.5 (97.5)	**3.8**	**341.1**	**0.000**
P. vulgaris var. *mexicanus*	0.0 (100)	0.0 (95)	**0.0**		
P. vulgaris var. *aborigineus*	0.0 (99.5)	0.5 (96.5)	**0.3**		
P. vulgaris cv. CDC Nighthawk	0.0 (99.5)	0.0 (76.5)	**0.0**		

† Numbers in parentheses are the number of plants (out of 100) that were alive prior to the first fall frost on September 23, 2000.

Starting October 3, 2000, subzero temperatures were encountered daily until the last day of sampling in this study (Figure 5.4). During this period, the air temperature was as low as −10.2°C (i.e., October 6). Hence by the 14th day after the first fall frost (i.e., October 7), except for the seven (out of 200) plants of *P. angustissimus*, all succumbed to the frost. At about 10 days after the first fall frost, surviving and frost killed plants of *P. angustissimus* from the border rows were dug from the field with soil intact and transferred to a controlled environment chamber maintained at 23/18°C (12 h/12 h) with a

12 h photoperiod. The surviving plants continued to grow normally and in frost killed plants, shoots from the cotyledonary nodes were observed. Plants continued to grow, bloom and set seeds.

The above experiment was repeated in the fall of 2001. Plants were transplanted on the same dates as in the previous growing season. Air temperature below –2°C was recorded on September 12 (minimum air temperature = –2.7°C) at the Preston field plot. However, seedlings showed little or no injury and hence could not be evaluated for their frost resistance. On October 4, a minimum air temperature of –5°C was recorded, however, isolated groups of plants of CDC Nighthawk, *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus* remained alive, indicating non-uniformity of freezing stress in the field. On October 5, an air minimum temperature of –13°C was recorded that resulted in total lethality.

The spring frost resistance of *Phaseolus* species was evaluated in 2001. *Phaseolus* species were grown in a controlled environment chamber and ten seedlings of each species, at the V3 growth stage, were transplanted to the Preston field plot on April 23, 2001. In the case of *P. vulgaris* var. *aborigineus*, only seven seedlings were transplanted to the field. Two replications were used. Seedlings were watered daily and protected from subzero temperatures by a cloth tunnel during the first week of transplanting. Upon establishment in the field, seedlings that survived transplanting shock were counted and the cloth tunnel removed. The number of surviving seedlings on the second, seventh and 14th day after a spring frost was determined. A subzero temperature of –6.6°C (Figure 5.5) was recorded on May 3. Seedlings of most species, with the exception of *P. angustissimus*, succumbed to freezing injury (Table 5.14). *Phaseolus angustissimus* had 55% seedling survival while CDC Nighthawk and its closely related species *P. vulgaris* var. *mexicanus* had no surviving seedlings. *Phaseolus vulgaris* var. *aborigineus* had 20% seedling survival. The surviving seedlings of *P. angustissimus* withstood a subsequent frost of –1.9°C on May 8 and continued to grow, flower and produce seeds as the season progressed while all but one seedling of *P. vulgaris* var. *aborigineus* was killed.

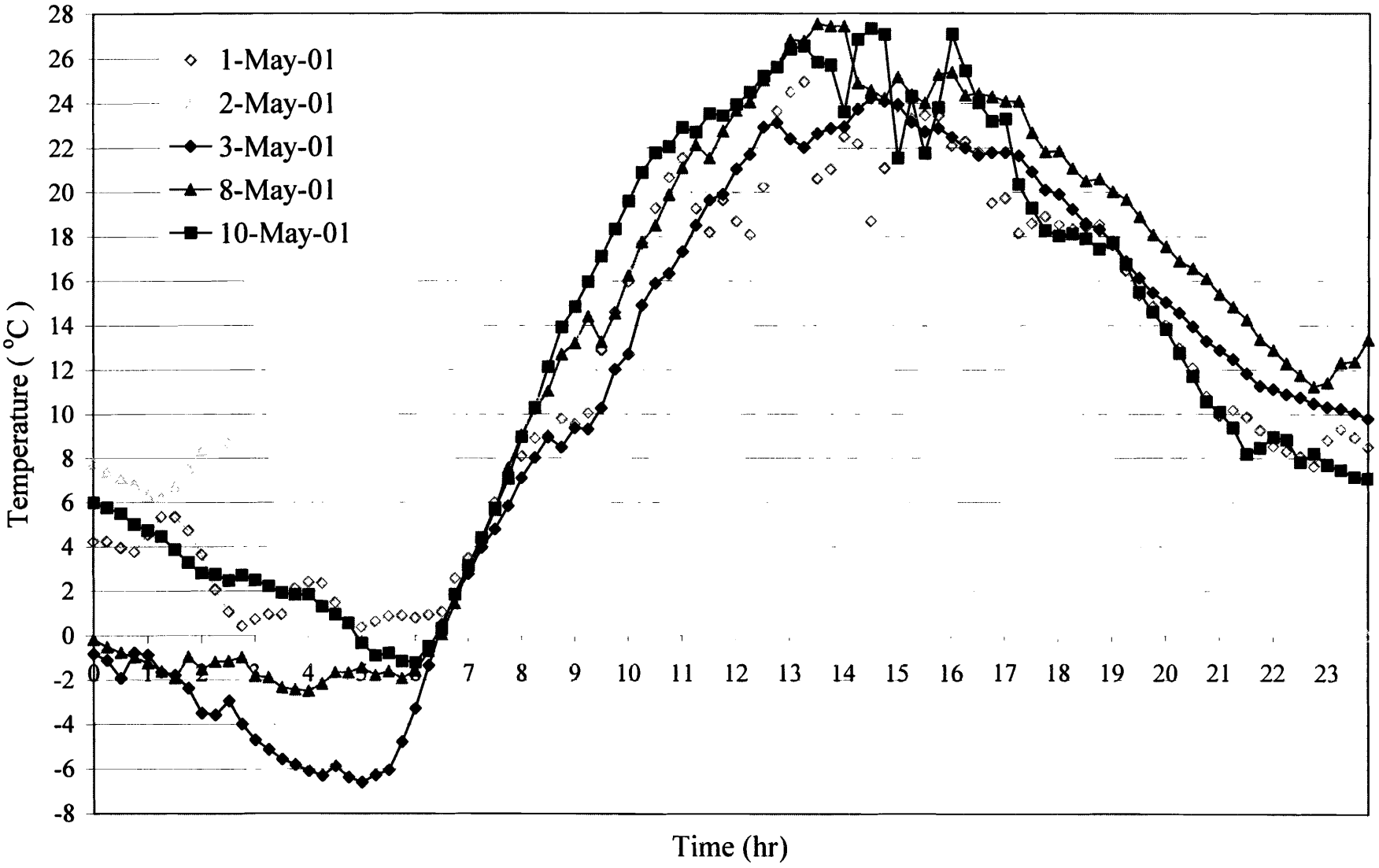

Figure 5.5 Air temperature (°C) at plant height (15 cm) at the Preston field plot in Saskatoon during the spring of 2001.

Table 5.14 Means of surviving seedlings of *Phaseolus* species after a spring frost in 2001.

Species	Mean number of surviving plants after a spring frost†		
	Second day	Seventh day	Fourteenth day
Phaseolus filiformis	1.0 (9)	1.0 (9)	1.0 (9)
P. angustissimus	5.5 (10)	5.5 (10)	5.5 (10)
P. ritensis‡	-	-	-
P. acutifolius var. tenuifolius	1.0 (8)	1.0 (8)	1.0 (8)
P. vulgaris var. *mexicanus*	0.0 (8.5)	0.0 (8.5)	0.0 (8.5)
P. vulgaris var. *aborigineus*	1.5 (6.5)	1.0 (6.5)	0.5 (6.5)
P. vulgaris cv. CDC Nighthawk	0.0 (10)	0.0 (10)	0.0 (10)

† Numbers in parentheses are the number of seedlings (out of ten, except for *P. vulgaris* var. *aborigineus,* which had seven seedlings) that were alive prior to the spring frost on May 3, 2001.

‡ Seedlings did not establish well in the field.

5.5 Discussion

At higher latitudes, low temperatures are of such magnitude that only taxa with special adaptive features grow naturally (Stushnoff et al., 1984). Cultivated common bean is a tropical species and suffers from both chilling and freezing injury. Ashworth et al. (1985) reported that common bean seedlings either in the controlled or in the natural environment were killed at a mean temperature of –1.3°C. Freeze tolerance evaluation using leaf tissue (Table 5.3) and seedlings (control treatment in Tables 5.6 and 5.8) confirm the extreme sensitivity of dry bean to subzero temperatures. The LT_{50} mean for the dry bean cultivar CDC Nighthawk was –2°C or higher.

In the absence of acclimation, subzero temperatures (frost) of –1 to –3°C will kill many tropical plants (Levitt, 1980). After acclimation, however, the survival temperature of plants can be lowered considerably below 0°C depending on the species and tissue. Acclimation decreased the LT_{50} of winter pea from –3°C to –9°C (Swensen and Murray, 1983). Acclimation, however, had little or no effect in increasing the freezing tolerance of the primary gene pool species, which were consistent in the lethal subzero temperature at which they were killed (between –1.5 and –2°C), and the lethal subzero temperature above which they showed regrowth (between –1 and –2°C) (Tables 5.6, 5.7, 5.8 and 5.9). Although, accessions of *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus* of the primary gene pool come from the high altitudes of the Sierra Madre and the Andes, respectively, their freezing response was very similar to cultivated dry bean. During

acclimation in a controlled environment chamber, at the end of the 2/0°C temperature regime, leaves of the primary gene pool species wilted, indicating chilling injury. Subsequent frost tolerance evaluation on wilted plants showed no change in their $LT_{50}K$ mean. Squeo et al. (1996) reported that at 3750 m elevation of the Chilean Andes, the mean annual temperature was 4.3°C with July being the coldest (−1.8°C) and January and February being the warmest (9.9°C) months. Altitudes at which *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus* grow in the wild, in Mexico and in Peru, respectively, probably do not encounter subzero temperatures during the growing season, possibly explaining the absence of frost resistance in the primary gene pool of common bean. It is interesting to note that wilting of plants was not observed in the tertiary gene pool species. Accessions of the tertiary gene pool species were likely collected from altitudes of 1500 to 2000 m in the South western United States and North western Mexico (Buhrow, 1983).

With the exception of *P. acutifolius* var. *tenuifolius*, tertiary gene pool species had similar levels of freezing tolerance (Tables 5.3, 5.6 and 5.8). However, the lowest subzero temperature at which regrowth was observed was significantly lower for *P. filiformis* compared to *P. angustissimus* and *P. ritensis* (Tables 5.7 and 5.9). In *P. filiformis*, cotyledonary nodes are positioned at the soil level and regrowth was primarily from these nodes. In *P. angustissimus* and *P. ritensis*, germination is hypogeal. Regrowth from cotyledonary nodes was observed only in *P. angustissimus* but was not considered for the $LT_{50}G$ determination. Hypogeal germination, if successfully bred into a crop species lacking constitutive freezing tolerance, will lead to delayed maturity. On the prairies with a short growing season, the "set back" in plant growth would delay maturity and expose a bean crop to a fall frost, resulting in poor seed yield and quality.

The tertiary gene pool species, *P. angustissimus* and *P. filiformis* had thin, pale green leaves when grown at a low light intensity (< 400 μmol m^{-2} s^{-1}) (personal observation). However, when grown outside in direct sunlight or at light intensities above 1000 μmol m^{-2} s^{-1}, leaves become thick with a greyish hue. When grown under sunlight, some species develop thicker leaves with a thicker cuticle and more layers of palisade mesophyll consisting of longer cells (Salisbury and Ross, 1992). A thick cuticle, if continuous, may be an effective barrier to nucleation by external ice (Burke et al., 1976).

Increased layers of palisade mesophyll prevents or reduces leaf injury in frost resistant wild relatives of potato (Estrada, 1982). Hence, the decrease in $LT_{50}K$ mean from –1.9°C for the control plants to –3.1°C for plants acclimated under a natural environment for seven days (Table 5.6), can partly be attributed to structural changes in leaves when plants were transferred from low light intensity of a controlled environment chamber to sunlight. The decrease in $LT_{50}K$ mean was not statistically significant likely due to fewer degrees of freedom for replication x acclimation interaction (d.f. = 2), which is the appropriate error to test the significance of the acclimation treatment. In acclimation under a controlled environment condition, little or no change in $LT_{50}K$ was observed between acclimation treatments, for plants initially grown outdoors (Table 5.8).

Infrared video thermography enables direct observation of i) ice nucleation and propagation, ii) effect of plant structure on the freezing process, and iii) relationship between specific pattern of freezing and visual pattern of injury (Wisniewski and Fuller, 1999). Plants grown in small pots, such as those used in the acclimation study, were subjected to a freeze test and observed under infrared thermography. The Redi-earth® froze before the seedlings when the chamber temperature was decreased to approximately –4°C (personal observation). Hence, seedling death in *P. filiformis*, *P. angustissimus*, and *P. ritensis*, all of which had a low $LT_{50}K$ mean (–3°C or lower) (Tables 5.6 and 5.8), and lack of regrowth from the cotyledonary nodes in species with a hypogeal germination, may have been partly influenced by earlier freezing of the Redi-earth® which in turn nucleated the seedlings. This is uncommon in the field due to a higher buffering capacity of the soil during light frosts.

Leaflets of all *Phaseolus* species supercooled to a certain degree prior to freezing by intrinsic nucleators (Table 5.3). As expected, leaflets from plants grown in a controlled environment chamber supercooled extensively compared to leaflets from the field likely due to the presence of fewer extrinsic ice nucleators such as bacteria and fungi in controlled environment conditions compared to the field. Leaflets of the primary gene pool species from a controlled environment chamber froze at –7.0°C, indicating the presence of intrinsic ice nucleators that prevented further supercooling (Table 5.3). Marcellos and Single (1979) reported that the mean freezing temperature of greenhouse grown snap bean stems was –7.1°C. However, ice nucleators were effective in the range

of –4 to –8°C. Among the tertiary gene pool species, the LT_{50} for leaflets of *P. filiformis* and *P. ritensis* from a controlled environment chamber was not achieved at –10°C in five and three out of six replications, respectively. This indicates their ability to supercool to low temperatures and also shows that the nucleators are either inactive or of low abundance at the temperatures tested. In the presence of effective extrinsic nucleators (e.g., ice), leaflets of *Phaseolus* sp. exhibit little or no supercooling (Table 5.3).

During the growing season, radiation frosts on clear, windless nights are the most common type of freezing stress (Chen et al. 1995). Plants lose heat rapidly to the open sky through black body radiation and can cool to temperatures substantially below ambient. Advective cooling can also occur during the growing season due to movement of cold air, and the leaf and air temperatures drop at similar rates. Under these circumstances, constitutive freezing tolerance or supercooling may enable plant survival. Field results from the fall of the 2000 growing season indicated that *P. angustissimus* of the tertiary gene pool is the most promising species for radiation frost survival. Depending on the growth stage of the plants, *P. angustissimus* had between 60 to 80% survival with little or no damage to leaves (Tables 5.10 and 5.11). For *P. angustissimus*, the increased number of surviving plants on the seventh day compared to the second day after the first fall frost was due to i) recovery of plants which suffered little damage to leaves, ii) growth of axillary shoots in plants with damaged terminal bud, or iii) growth of axillary shoots in plants in which the stem alone survived the frost. The above result was confirmed in the spring of 2001 when *P. angustissimus* had 55% survival after a spring frost. The relatively lower seedling survival after spring frosts of 2001 compared to fall frosts of 2000 may be due to a lower air temperature (–6.6°C) (Figure 5.5) during the spring frost compared to the fall frosts (–4.2 and –4.9°C) (Figure 5.4). The relatively poor freezing resistance of *P. angustissimus* when evaluated in controlled environment conditions compared to the field may be related to i) the technique, where individual leaflets were used instead of whole plants, and ii) freezing of Redi-earth® in 102 mm pots at –4°C when whole plants were used. Spatial variability in the fall frost, with subsequent very low freezing temperatures rendered the fall frost study of 2001 inconclusive.

5.6 Conclusions

Both freezing tolerance and avoidance were observed in the *Phaseolus* species, although to varying proportions in the primary and the tertiary gene pools. Freezing studies in the field indicate that common bean and its primary gene pool species are extremely sensitive to frost while the tertiary gene pool species showed large variation in freezing response. Controlled environment chamber studies with detached leaflets indicated that avoidance may be the primary mechanism of survival of subzero temperatures in the tertiary gene pool species. Leaflets of both *P. filiformis* and *P. angustissimus* plants grown in the field supercooled extensively when evaluated in a controlled environment chamber. In the field, however, in response to the first fall frost in 2000 and spring frost in 2001, *P. filiformis* had less than 20% survival while *P. angustissimus* had 60 to 80% survival. This indicates that either *P. filiformis* could not supercool to the extent of *P. angustissimus* in the field or *P. angustissimus*, in addition to supercooling (avoidance) has other frost survival strategies that require further investigation. It is possible that *P. angustissimus* has low abundance of intrinsic nucleators and efficient barriers to nucleation from the environment. Freezing resistance of *P. angustissimus* in the field evaluations, so far, is promising. Introgression of freezing resistance into common bean genotypes may eventually enable early to mid May planting of dry bean cultivars on the Canadian prairies. This could also expand the geographic distribution of bean crop, possibly to higher altitudes in the tropics.

6. Interspecific Hybridization of Common Bean with *Phaseolus angustissimus* A. Gray and *P. filiformis* Bentham

6.1 Abstract

Frost resistant cultivars may enable expansion of dry bean production into areas with a short growing season. In this study, frost sensitive common bean and wild relatives in its primary gene pool were crossed with frost resistant *P. angustissimus* and *P. filiformis* of the tertiary gene pool to develop F_1 interspecific hybrids. Interspecific hybrids were obtained by embryo rescue only when the primary gene pool species were used as the female parent. In reciprocal crosses, flowers aborted about three days after pollination. Black bean cv. ICA Pijao and *P. vulgaris* var. *aborigineus* accession PI266910 were the only female parents that produced embryos which developed into plants. Hybrid embryos from black bean cv. CDC Nighthawk, breeding line 5-593 and *P. vulgaris* var. *mexicanus* accession G11024 failed to grow on three-quarter strength MS medium. The F_1 interspecific hybrid plants grew to produce flowers, but failed to set seed due to pollen sterility.

6.2 Introduction

Common bean (*Phaseolus vulgaris* L.) is the largest of the food legume crops both in acreage and production (Singh, 1999). Although the bean crop is widely adapted, the growth of common bean is favoured by a mean growing temperature of 18 to 22°C and a well distributed precipitation of 500 to 800 mm during the growing season (Singh, 1992). Diseases, drought, and low soil fertility are among the most widespread and endemic production problems of cultivated common bean in the tropics and subtropics. Common bean is also sensitive to both chilling (< 15°C) and sub-zero temperatures at all stages of plant growth. Hence at the upper limits of agriculture (i.e., high latitudes and high

altitudes), periods of low (< 15°C), but above zero temperatures and frosts during the growing season are the major abiotic constraints for bean production.

Common bean improvement thus far has relied on the primary gene pool, which includes dry and snap bean cultivars, and the wild relatives, *P. vulgaris* var. *mexicanus* from Central America and *P. vulgaris* var. *aborigineus* from South America (Singh, 2001). The secondary gene pool of common bean includes *P. coccineus*, *P. polyanthus* and *P. purpurascens* (Delgado-Salinas, 1985) and has been a source of several disease resistance traits (Singh, 2001). The tertiary gene pool comprises about 51 wild species including *P. acutifolius*, *P. angustissimus*, *P. filiformis*, and *P. ritensis* (Delgado-Salinas, 1985; Debouck, 1991). With the exception of species in the secondary gene pool and *P. acutifolius* in the tertiary gene pool, the remaining wild species have not been used in common bean improvement primarily due to hybridization barriers and lack of appropriate embryo, ovule or pod culture protocols.

Buhrow (1980, 1983) reported frost resistance in several tertiary gene pool species of common bean. *Phaseolus acutifolius* var. *tenuifolius*, *P. angustissimus*, *P. filiformis* (syn. *P. wrightii*), and *P. ritensis* (syn. *P. metcalfei*) survived several successive nights of radiation frost when grown in a germplasm nursery in Tucson, AZ. The above species, with the exception of *P. ritensis*, are also drought tolerant (Buhrow, 1981). Furthermore, rooted stem cuttings of *Phaseolus vulgaris* x *P. filiformis* hybrids had higher nitrogen fixation rates and accumulated more total nitrogen than either parent (Lülsdorf and Holl, 1992).

An earlier study (chapter 5) confirmed *P. angustissimus* and *P. filiformis* as frost resistant. Frost resistance, if successfully introgressed into common bean germplasm, may enable early spring planting of dry bean on the Canadian prairies and expand the geographic distribution of bean crop, possibly to higher altitudes in the tropics. The objective of this study was to obtain F_1 interspecific hybrids of *P. vulgaris* with *P. angustissimus* and *P. filiformis* that would aid breeders in transferring frost resistance from the tertiary, into the primary gene pool.

6.3 Materials and Methods

Phaseolus vulgaris cultivars CDC Nighthawk and ICA Pijao, a breeding line 5-593, *P. vulgaris* var. *mexicanus* (G11024), *P. vulgaris* var. aborigineus (PI266910), *P. filiformis* (Unknown) and *P. angustissimus* (PI535272) were used in this study. Seeds of wild accessions were scarified by nicking the seed coat.

Plants were grown in controlled environment chambers at 23/18°C (12 h/12 h) with a 12 h photoperiod. Relative humidity was set at 40% and increased to 60% at the time of anthesis and pollination. With the exception of ICA Pijao, which was relatively late flowering, the other genotypes flowered at about the same time under the conditions used in this study. CDC Nighthawk, ICA Pijao, 5-593, G11024 and PI266910 were crossed as females with *P. filiformis* (unknown) and *P. angustissimus* (PI535272), and flowers were tagged. Cross combinations with *P. filiformis* and *P. angustissimus* as female parents were not successful. Developing pods used for embryo rescue were removed from the female parent at approximately 20 days after pollination or when yellowing of pod was observed, whichever was earlier. Pods were sterilized and F_1 hybrid embryos rescued from the aborting ovules under a binocular dissecting microscope.

Excised embryos were cultured in test tube slants containing three-quarter strength Murashige and Skoog medium supplemented with 0.125 μM benzyladenine, 0.7 μM glutamine, 0.8% (w/v) agar, and 3% (w/v) sucrose. Embryos were incubated in the dark at 25°C until hypocotyl elongation took place. Developing embryos were then transferred to a controlled environment chamber set at 23/18°C (12 h/12 h) with a 12 h photoperiod. Ten to 15 days after the embryo rescue, vigorously growing plants were transferred to magenta boxes containing full-strength MS medium and grown for another 10 to 15 days. Plants were then transferred to pots containing Redi-earth® (W.R. Grace and Co. of Canada Ltd., Ajax, ON), fertilized with Osmocote (14-14-14), a slow release fertilizer, and covered with transparent domes to maintain high relative humidity. By adjusting the domes, plants were gradually acclimated to ambient growing conditions over a period of seven days.

Number of pollinations, number of pods obtained, number of pods with at least one culturable embryo, number of embryos cultured and number of plants acclimated were determined. Data were subjected to the chi-square test of independence of proportions.

6.4 Results

In crosses with *P. filiformis* as the male parent, the chi-square test of independence of proportions indicated that number of pods obtained, number of pods with at least one culturable embryo and number of plants acclimated were dependent on the female parent used in the cross combinations (Table 6.1). Pollinations with CDC Nighthawk as the female parent resulted in the highest number of pods and pods with at least one culturable embryo. However, none of the rescued embryos developed into plants. In contrast, with ICA Pijao, although the number of pods obtained and pods with at least one culturable embryo were among the lowest, 75% of the rescued embryos developed into plants. *Phaseolus vulgaris* var. *aborigineus* accession PI266910 was also promising, with 25% of the rescued embryos developing into plants. In crosses with *P. angustissimus* as the male parent, the chi-square test of independence indicated that number of pods obtained and number of plants acclimated were dependent on the female parent used in the cross combinations (Table 6.2). ICA Pijao and PI266910, when crossed with *P. angustissimus,* were the only two parents that resulted in hybrid embryos developing into plants.

The acclimated plants grew to produce flowers, but failed to set any seed. Aceto-carmine (2 g carmine in a mixture of 45 ml glacial acetic acid and 55 ml distilled water) staining showed poorly or unstained pollen grains of the F_1 interspecific hybrid plants indicating pollen sterility. Interspecific hybrids were intermediate between the parents for leaf size and shape. Flower size of interspecific hybrids was more similar to that of the female parent whereas, the growth habit was indeterminate prostrate and similar to that of the male parent.

Table 6.1 Chi-square test of independence of proportion for mean number of pods obtained, pods with at least one culturable embryo and plants acclimated for crosses with *Phaseolus filiformis* (Unknown) as the male parent.

Crosses	No. of pollinations	Number of pods obtained		Pods with at least one culturable embryo		No. of embryos cultured	No. of Plants transplanted†	
		No.	%	No.	%		No.	%
Female parents								
Phaseolus vulgaris cv. CDC Nighthawk	29	17	58.6	10	34.5	17	0	0
P. vulgaris cv. 5-593	19	3	15.8	3	15.8	3	0	0
P. vulgaris cv. ICA Pijao	42	5	11.9	4	9.5	8	6	75
P. vulgaris var. mexicanus (G 11024)	46	9	19.6	2	4.3	2	0	0
P. vulgaris var. *aborigineus* (PI 266910)	42	9	21.4	6	14.3	20	5	25
χ^2_{obs}		**23.7**		**14.4**			**11.1**	
P value		**0.005**		**0.006**			**0.001**	

† Chi-square test was performed on the means of *Phaseolus vulgaris* var. *aborigineus* (PI266910) and *P. vulgaris* cv. ICA Pijao versus *P. vulgaris* cv. 5-593, *P. vulgaris* cv. CDC Nighthawk and *P vulgaris* var. mexicanus (G11024).

Table 6.2 Chi-square test of independence of proportion for mean number of pods obtained, pods with at least one culturable embryo and plants acclimated for crosses with *Phaseolus angustissimus* (PI 535272) as the male parent.

Crosses	No. of pollinations	Number of pods obtained†		Pods with at least one culturable embryo†		No. of embryos cultured	No. of Plants transplanted†	
		No.	%	No.	%		No.	%
Female parents								
Phaseolus vulgaris cv. CDC Nighthawk	9	5	55.6	5	55.6	7	0	0
P. vulgaris cv. 5-593	11	9	81.8	2	18.2	4	0	0
P. vulgaris cv. ICA Pijao	20	6	30.0	5	25.0	13	11	84.6
P. vulgaris var. mexicanus (G 11024)	5	2	40.0	2	40.0	3	0	0
P. vulgaris var. *aborigineus* (PI 266910)	12	4	33.3	4	33.3	10	9	90.0
χ^2_{obs}		**6.1**		**0.4**			**26.5**	
P value		**0.014**		**0.526**			**0.000**	

† Chi-square test was performed on the means of *Phaseolus vulgaris* var. *aborigineus* (PI266910) and *P. vulgaris* cv. ICA Pijao versus *P. vulgaris* cv. 5-593, *P. vulgaris* cv. CDC Nighthawk and *P. vulgaris* var. mexicanus (G11024).

6.5 Discussion

Plant breeders have relied on wild relatives as sources of desirable traits in crop improvement. Based on the ease with which a progeny could be obtained by crossing a

cultivated with a wild plant, crop plants and their relatives are classified into primary, secondary or tertiary gene pools in the order of decreasing ease of hybridization (Harlan and de Wet, 1971). Both *P. angustissimus* and *P. filiformis* belong to the tertiary gene pool of common bean (Debouck, 1991). As a result, interspecific crosses with *P vulgaris* required embryo rescue to obtain F_1 hybrids. Embryos were rescued from all five female parents, indicating the absence of pre-fertilization barriers when *P. vulgaris* is the female parent. Reciprocal crosses however, were unsuccessful as observed by earlier researchers with other *Phaseolus* species (Al-Yasiri and Coyne, 1966). In the reciprocal cross, flowers aborted at approximately three days after pollination which may indicate pre-fertilization rather than post-fertilization barriers.

In general, abortion of pods was observed between 15 and 20 days after pollination in crosses with *P. vulgaris* as female parents. Belivanis and Doré (1986) reported 19 days after pollination as the optimal time for embryo rescue in crosses between *P. vulgaris* and *P. angustissimus*. Female parents differed significantly in their ability to produce pods, pods with at least one culturable hybrid embryo and hybrid plants (Tables 6.1 and 6.2). A three gene model for incompatibility in interspecific hybridization has been proposed for common bean. They include blocked cotyledon lethal (*BCL*), crinkle leaf dwarf (*CLD*) and dwarf lethal (*DL*) (Ferwerda and Bassett, 2000). The lack of development of hybrid embryos in crosses with CDC Nighthawk, 5-593 or G11024 as female parent may be due to the above genetic factors or alternately, the embryo culture medium composition and culture conditions did not support hybrid embryo growth. The black bean cultivar ICA Pijao and the breeding line 5-593 are recessive for the above three incompatibility barriers (Ferwerda and Bassett, 2000). Ferwerda and Bassett (2000) also observed that the breeding line 5-593 could serve as a bridging parent to transfer desirable traits from the secondary gene pool species *P. coccineus* into dry bean. In our study, however, 5-593 failed to yield hybrid plants when crossed with the two tertiary gene pool species, *P. filiformis* and *P. angustissimus*. The black bean cultivar ICA Pijao has been the most successfully used cultivar in interspecific hybridization (Lülsdorf and Holl, 1991; Mejía-Jiménez et al., 1994). Furthermore, in the cross between *P. vulgaris* and *P. acutifolius*, Mejía-Jiménez et al. (1994) observed that ICA Pijao greatly facilitated

the production of vigorous F_1 plants, which could be backcrossed efficiently with other common bean cultivars.

6.6 CONCLUSIONS

Barriers to interspecific hybridization in legumes are well documented in the literature. In this study, the F_1 interspecific hybrids *P. vulgaris* x *P. angustissimus* and *P.* vulgaris x *P. filiformis* were obtained using embryo rescue with relative ease. Backcrossing the F_1 hybrids to either the same or different accession/cultivar of their parental species will be challenging, due to imbalance in the genetic constitution of parental species in the backcrossed (BC_1) hybrid. Transferring frost resistance from the tertiary gene pool species, *P. angustissimus* into dry bean cultivars is a long term objective, one that will require concerted efforts by plant breeders, physiologists, cytogeneticists and tissue culture specialists. Frost resistant bean cultivars, when developed, could have a profound effect on the production of this crop, particularly at high latitudes and altitudes.

7. Summary

7.1 General Discussion and Conclusions

In Saskatchewan, dry bean production is concentrated in the Dark Brown and Black soil zones. In 2001, dry bean accounted for 0.1% (2,000 ha) of the total pulse acreage in the province (Saskatchewan Agriculture and Food, 2001). The low production compared to other pulse crops can partly be attributed to the abiotic constraints associated with the production of this crop in Saskatchewan. Poor emergence in cool wet soils of early spring, seedling death from late spring frosts, and poor seed yield and quality due to mid September frost are major deterrents to dry bean production. The mean daily soil temperature for the Dark Brown and Black soil zones indicates that above suboptimal temperatures (> 20°C) for dry bean germination and emergence are usually achieved around May 20 (Environment Canada, 1977-84). The long term temperature records for Saskatoon (Dark Brown soil zone) indicates that a killing spring frost can be expected on average until May 23 (Environment Canada, 1966-2000). The above data also indicate that the earliest killing fall frost (–2.8°C) was on September 4, 1972. In most years a killing frost can be expected by mid September (Sept. 15). In any given growing season, the conventional practice of planting dry bean in late May will effectively overcome poor emergence and avoid spring frost. However, the risk of an early fall frost is always present. Dry bean cultivars with the ability to emerge under suboptimal temperatures and survive light frost (–3°C) will enable early spring plantings and therefore early maturity of the crop.

Earlier studies demonstrated variation for common bean germination and emergence under suboptimal temperature in controlled environments (Kooistra, 1971; Dickson and Boettger, 1984; Hucl, 1993; Zaiter et al., 1994). Kooistra (1971) and Dickson (1971) also showed that suboptimal temperature emergence in common bean is heritable and could be easily selected for in breeding programs. Common bean emergence under

suboptimal temperatures in the field has not been widely studied. Furthermore, dry bean is chilling sensitive, and hence the cumulative effect of suboptimal temperatures during germination and emergence on subsequent growth and development in the field needs to be determined prior to breeding elite bean cultivars with the ability to emerge under low seedbed temperatures of early spring. The cumulative effect, if any, of suboptimal temperatures during germination on subsequent growth and development of dry bean cultivars will offset the advantages of early spring seeding. The first objective of this thesis addresses the above concern.

Results of this study (chapter 3) confirmed earlier observations under controlled conditions that planting dry bean under suboptimal temperatures (mid May) results in reduced seedling stand. Percent emergence, however, increased with warming of the seedbed but was still lower compared to that of late May planting. Bean growth and development as determined by cumulative thermal units to anthesis and to maturity did not differ significantly between planting dates. This indicates that suboptimal temperatures of early spring only influenced seedling emergence and not subsequent plant development. Crop maturity, however, differed significantly between planting dates in 1999. Two indeterminate cultivars, CDC Whistler and UI 906, failed to mature prior to the first fall frost on September 13. As a result, the mid May planting had the highest seed yield with little or no percent frost damaged seed. In 2000, when the first fall frost was on September 23, the planting dates did not differ significantly for seed yield. Thus, a mid May planting of dry bean cultivars may result in higher seed yields and good quality compared to late May plantings in growing seasons with a mid September frost. Even in years with a delayed fall frost, early spring plantings may decrease the risk of late season drought stress. Although a limited number of cultivars was used for each of the determinate bush and indeterminate bush growth types, cultivar responses indicate that indeterminate cultivars would benefit from a mid May planting due to their late maturity.

The above study (chapter 3) demonstrated the chilling sensitivity of dry bean emergence when planted in mid May. The second objective of this thesis was to evaluate dry bean accessions for emergence under suboptimal temperatures (chapter 4). Results of this study indicated that accessions can be broadly divided into two groups based on their

emergence response with suboptimal temperatures. The first group consisted of accessions with early emergence, usually occurring between 20 and 30 days after seeding (i.e., in late May, for example G8823, G9345, CDC Nighthawk). The second group consisted of accessions with a substantial increase in emergence only after 30 days from planting (i.e., in mid June, for example UI 906). At 50 days from planting (June 22), most accessions in both groups had more than 80% emergence, indicating that both chilling tolerant (first group) and chilling sensitive (second group) accessions retained seed viability. Accession G8823 from the Netherlands consistently had a higher percent emergence than the check cultivar CDC Nighthawk. The accession G8823 would serve as an excellent parent to improve the suboptimal temperature emergence in common bean. Emergence in UI 906 was delayed in both years until the seedbed temperature exceeded 15°C. Further, more than 70% of emergence in UI 906 occurred after May 23, when the risk of late spring frosts is low. In the absence of subzero temperature resistance, UI 906 and accessions with a similar emergence response could be used to develop dry bean cultivars that would enable producers to seed in early May but still minimize the risk of seedling death.

Frost resistance in common bean has not been widely investigated, partly because the bean crop has traditionally been grown in regions with a low risk of spring or fall frosts. Limited work on common bean indicates that bean seedlings are extremely sensitive to light frost (–2°C). Ashworth et al. (1985) reported that bean seedlings froze at a mean temperature of –1.3°C in a controlled environment chamber and in response to natural frost. The median temperature at which the seedlings froze was –4°C in the controlled environment chamber and –3°C in the natural environment. Bean plants can be expected to freeze or be severely damaged in response to a radiation frost of –2°C depending on the duration of exposure. Buhrow (1980) observed regrowth from wild species of the tertiary gene pool of common bean in response to fall frosts. Regrowth in *P. filiformis* (syn. *P. wrightii*) was observed even after plants were exposed to –7°C. Wild species in general are of the bush type with a prostrate growth habit, which may protect the stem and axillary buds that show regrowth. A long term genetic improvement strategy for incorporation of frost resistance from the wild species into common bean cultivars should involve investigation of the mechanism and the extent of frost resistance in wild species.

The third objective of this thesis was to investigate the freezing resistance of *Phaseolus* species (chapter 5). Results indicated that avoidance is the primary mechanism of frost survival. Leaflets of the tertiary gene pool species, with the exception of *P. acutifolius* var. *tenuifolius*, showed extensive supercooling when evaluated under controlled conditions. In the field, however, *P. angustissimus* was the only species with more than 60% seedling survival in response to both spring and fall frosts. It is speculated that supercooling, coupled with structural modification in aerial shoots, may have ensured the survival of this species. Based on field evaluations it is safe to conclude that frost resistance of *P. angustissimus* if introgressed "as is" into common bean cultivars, will ensure survival when plants are exposed to unseasonal killing frosts.

With the exception of *P. acutifolius*, species of the tertiary gene pool have not been used in common bean improvement due to barriers to interspecific hybridization (Singh 2001). In crosses involving these species with common bean, the F_1 is sterile and backcrosses have proven futile. In chapter 6, F_1 interspecific hybrids were developed to investigate the feasibility of using the congruity backcrossing technique to introgress freezing resistance.

7.2 Future Research

Breeding for frost resistance in dry bean is a long term objective. Black bean genotypes like UI 906 and accessions with a similar emergence pattern may enable early spring seeding on the prairies and still allow avoidance of late spring frosts. Due to limited seed availability, dry bean check cultivars and diverse accessions were not evaluated for emergence, time to anthesis, time to maturity and yield by planting them at an optimum seedbed temperature (i.e., late May). This would have enabled us to determine the effects of chilling injury during germination and emergence on anthesis, maturity and yield. UI 906 was relatively late to flower and mature compared to CDC Nighthawk. This may be attributed either to its inability to emerge early at suboptimal temperatures and hence establish early plant stand, or to its inherent tendency to flower and mature later even when seeded at optimum seedbed temperatures. If the latter were the case, then bean lines with emergence similar to UI 906 but with early anthesis, early

maturity and high seed yield can be selected. Such cultivars may enable early spring seeding in the absence of frost resistance. Thus, the interaction of planting date, cultivar maturity and growth habit on agronomic traits needs to be further evaluated prior to deciding on an appropriate breeding strategy for suboptimal temperature emergence. In combination with frost resistance, however, dry bean cultivars with an emergence response similar to G8823 would be appropriate.

The mechanism of chilling resistance in the dry bean accession G8823 is not known. Cold tolerant maize and soybean lines are able to initiate germination and seedling establishment by utilizing an alternative respiratory (CN-insensitive) pathway not present in cold sensitive lines (McDonald, 1994). In tomato, the genotype with a higher germination rate at 9°C had a higher respiration rate compared to a cold sensitive genotype (Leviatov et al., 1994). The cold resistant genotype also had a higher activity of endomannanase, an enzyme that mobilizes galactomannan in the endosperm cell walls at both 12 and 25°C, compared to the cold sensitive genotype (Leviatov et al., 1995). Zaiter et al. (1994) observed changes in polypeptides in common bean genotypes with superior emergence at a constant temperature of 8°C or an alternating temperature regime of 10/8°C. Polypeptide changes were not detected in common bean genotypes with poor emergence at the above temperatures. Physiology and molecular studies on cold tolerant and cold sensitive common bean genotypes will shed more light on the genes and biochemical pathways involved in germination and emergence under suboptimal temperatures.

Leaflets of *Phaseolus angustissimus* showed extensive supercooling. It is speculated that distinct changes in leaf colour and cuticle thickness occur in response to increased light intensity or sunlight, which plays an important role in restricting external ice nucleators. Estrada (1982) observed double or triple layers of palisade parenchyma in frost resistant wild relatives of potato. Burke et al. (1976) suggested that in the absence of intrinsic nucleators, a thick continuous cuticle may serve as an effective barrier to nucleation by external ice. Further studies on the mechanisms of frost survival in *P. angustissimus* may provide breeders and physiologists with additional tools for developing frost resistant dry bean.

Barriers to interspecific hybridization between common bean and its tertiary gene pool species are well documented. Delgado-Salinas et al. (1999) in their revised classification of *Phaseolus* species, identified some new species (*P. albescens* and *P. costaricensis*) that are more closely related to common bean than *P. angustissimus* or *P. filiformis*. If freezing resistance is present in these new species, this trait could be introgressed into common bean with less effort compared to that required to hybridize from *P. angustissimus*.

Congruity backcrossing (Haghighi and Ascher, 1988) has been used by bean breeders to increase pollen fertility and introgress desirable traits by forced recombination between common bean and *P. acutifolius*, a tertiary gene pool species (Mejía-Jiménez et al., 1994; Anderson et al., 1996). It remains to be determined if the congruity backcrossing technique can be used to introgress frost resistance from *P. angustissimus* into common bean.

Future research suggested above would provide much needed insight into chilling and freezing resistance in common bean. The study on the interaction of planting date, cultivar maturity and growth habit will enable breeders to develop elite dry bean cultivars that could be planted in early spring even in the absence of frost resistance. The above study is particularly important given the barriers to interspecific hybridization in common bean. Simple backcrossing of the F_1 interspecific hybrids (*P. vulgaris* x *P. angustissimus* and *P. vulgaris* x *P. filiformis*) to either parent has failed. Currently, the nature of the hybridization barrier (pre or postfertilization) is being investigated. It is speculated that high percent survival of *P. angustissimus* seedlings in the field is primarily due to a thick waxy layer on the aerial shoot, which prevents extrinsic nucleators during subzero air temperatures. The above trait, if identified and introgressed from a more closely related species into common bean germplasm, may result in frost resistant bean cultivars.

8. References

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9. Appendices

Appendix 1. Means for percent emergence at 20, 30, 40 and 50 days after planting of common bean cultivars, accessions and wild relatives grown at Saskatoon, SK, during 2000.

Plot no.†	Accession no.	Origin	Altitude (m)	Bean Type	Seed size‡	Growth habit§	Emergence at 20 days	30 days	40 days	50 days
							%			
Check	CDC Whistler	Canada		Dry (D)	S	2	1.3	24.2	47.7	49.7
Check	CDC Nighthawk	Canada		D	S	2	1.7	70.7	89.3	91.0
Check	UI 906	USA		D	S	2	7.5	16.7	87.3	93.7
B2-17	G9345	USA		D	S	1	49.8	84.5	91.5	93.1
B3-13	G8823	Netherlands		D	S	1	30.2	75.5	83.8	89.4
B4-12	G9256	Netherlands		D	M	1	16.8	50.5	84.8	86.1
B5-2	G8800	Netherlands		D	S	1	16.8	49.5	69.8	69.8
B5-24	G11285	Sweden		D	M	1	14.8	59.5	81.8	81.8
B5-13	G11197	Netherlands		D	M	1	12.8	49.5	57.8	57.8
B6-17	G4498	USA		D	S	1	10.8	43.2	50.8	50.8
B4-1	G9430	Netherlands		D	M	4	8.8	50.5	74.8	77.1
B4-4	G5673	Japan		D	S	4	8.8	46.5	64.8	66.1
B4-13	G7493	Netherlands		Snap	S	1	8.8	43.5	46.1	46.1
B2-6	G7504	Netherlands		Snap	S	1	8.8	31.5	66.1	66.1
B2-23	G7551	Netherlands		D	S	1	7.8	49.5	67.5	75.1
B4-15	G1460	Sweden		D	S	1	6.8	45.5	76.8	80.1
B1-2	G1541	Poland		Dry	S	1	6.5	30.9	73.1	74.8
B4-26	G744	Netherlands		D	S	4	5.8	41.5	73.8	76.1
B3-5	G8090	Netherlands		D	S	1	5.2	32.5	62.8	64.4
B3-12	G7544	Germany		Snap	S	1	5.2	27.5	65.8	67.4
B2-13	G2902	Sweden		D	M	1	4.8	41.5	62.5	86.1
B4-27	G6352	Netherlands		D	S	1	3.8	15.5	49.8	56.1
B6-33	G14632	Denmark		Snap	M	1	2.8	30.2	88.1	89.8
B4-2	G2974	Guatemala		D	S	2	2.8	17.5	75.8	83.1
B6-14	G7556	Netherlands		D	S	1	2.8	16.2	59.8	59.8

B1-1	G7498	Netherlands		Snap	S	1	2.5	16.9	67.1	80.8
B1-4	G7577	Netherlands		Snap	S	1	2.5	15.9	70.1	71.8
B6-21	G9509	Belgium		D	S	1	1.8	50.2	81.1	81.8
B5-7	G7519	Netherlands		Snap	S	1	1.8	28.5	69.8	69.8
B5-29	G1090	Chile		D	S	4	1.8	22.5	75.8	80.8
B3-28	G20324	Sweden		D	M	1	1.2	50.5	76.8	82.4
B4-17	G16805	Tanzania		D	M	1	0	54.5	86.1	86.1
B6-2	G8811	Sweden		Snap	S	1	0	50.2	81.8	81.8
B4-18	G9042	Netherlands		D	M	1	0	46.5	96.1	96.1
B4-16	G22208	Bulgaria		D	S	2	0	46.5	58.8	78.1
B2-18	G1540	Sweden		D	M	1	0	43.5	98.5	100.0
B6-26	G5478	USA		D	M	4	0	42.2	87.8	87.8
B6-25	G8752	Sweden		D	S	1	0	42.2	77.8	77.8
B5-8	G5024	Brazil		D	M	1	0	38.5	89.8	89.8
B1-9	G741	Netherlands		D	M	1	0	35.9	93.1	93.8
B2-31	G9499	Netherlands		D	L	1	0	35.5	80.5	84.1
B4-30	G9290	Netherlands		Snap	S	1	0	34.5	68.1	68.1
B2-14	G7576	Netherlands		D	S	1	0	34.5	54.5	55.1
B2-26	G8756	Netherlands		Snap	M	1	0	34.5	41.5	59.1
B4-21	G6668	Sweden		D	M	1	0	28.5	88.8	90.1
B2-4	G8760	Netherlands		Snap	S	1	0	27.5	56.5	62.1
B2-8	G14739	Bulgaria		D	L	4	0	23.5	92.5	94.1
B3-27	G9605	Japan		D	M	4	0	23.5	78.8	84.4
B6-30	G8654	Netherlands		D	M	2	0	21.2	77.8	77.8
B4-25	G8835	Netherlands		D	M	1	0	20.5	78.8	80.1
B2-15	G7500	Netherlands		Snap	S	1	0	19.5	61.5	71.1
B6-23	G2511	Colombia		D	S	4	0	19.2	74.1	77.8
B5-19	G7511	Netherlands		Snap	S	1	0	18.5	72.8	72.8
B5-12	G8886	Netherlands		Snap	S	1	0	18.5	65.8	70.8
B2-5	G1683	Sweden		Snap	S	1	0	17.5	72.5	81.1
B1-11	G23554A	Mexico	1950	Weedy	S	3	0	16.9	67.1	68.8

B2-28	G8807	Netherlands		D	M	1	0	16.5	100.0	100.0
B3-10	G8960	Denmark		D	M	1	0	16.5	74.8	76.4
B2-19	G7562	Netherlands		Snap	M	1	0	15.5	66.5	74.1
B2-30	G9519	Netherlands		Snap	M	1	0	15.5	34.5	37.1
B2-20	G23450	Peru	2840	Weedy	S	4	0	14.5	52.1	52.1
B2-24	G8839	Norway		D	S	1	0	14.5	46.5	68.1
B6-16	G7581	Netherlands		D	S	1	0	14.2	61.8	61.8
B1-5	G7499	Netherlands		D	S	1	0	13.9	73.1	76.8
B2-16	G7503	Netherlands		D	S	1	0	13.5	30.5	42.1
B1-3	G7528	Netherlands		Snap	S	1	0	12.9	38.1	39.8
B4-7	G1283	Sweden		Snap	S	1	0	12.5	74.8	84.1
B3-9	G13182	Japan		D	M	1	0	12.5	44.8	54.4
B6-10	G3353	Mexico		D	M	4	0	12.2	85.8	85.8
B6-12	G6405	Ecuador		D	M	2	0	12.2	81.1	81.8
B4-10	G8985	Netherlands		Snap	M	1	0	9.5	62.8	69.1
B5-1	G7515	Netherlands		D	S	1	0	9.5	57.8	60.8
B5-14	G5711	Guatemala		D	S	4	0	8.5	73.8	77.8
B6-4	G8873	Denmark		Snap	M	1	0	8.2	75.8	75.8
B1-31	G9356	Netherlands		D	S	1	0	7.9	68.1	80.8
B1-24	G9248	Unknown		D	M	1	0	6.9	26.1	64.8
B6-9	G8969	Netherlands		Snap	M	1	0	6.2	60.1	60.8
B1-12	G15955	Netherlands		D	M	4	0	5.9	30.1	54.8
B5-27	G8795	Netherlands		D	S	1	0	5.5	52.8	56.8
B5-22	G15967	Netherlands		D	M	1	0	5.5	48.8	60.8
B1-30	G8976	Netherlands		D	S	1	0	4.9	48.1	54.8
B5-17	G7506	Netherlands		Snap	S	1	0	4.5	19.8	19.8
B1-28	G746	Netherlands		D	M	4	0	3.9	69.1	80.8
B1-32	G9033	Netherlands		D	M	1	0	3.9	61.1	68.8
B1-6	G8851	Denmark		D	M	1	0	3.9	53.1	67.8
B4-32	G7574	Netherlands		Snap	M	1	0	2.5	57.1	57.1
B3-2	G8952	Netherlands		D	S	1	0	2.5	52.8	67.4

B6-13	G1461	Sweden		Snap	S	1	0	2.2	68.1	75.8
B6-24	G7476	Netherlands		Snap	M	1	0	2.2	51.8	51.8
B1-22	G8773	Netherlands		D	S	1	0	1.9	76.1	77.8
B1-25	G8737	Netherlands		D	M	1	0	1.9	35.1	55.8
B5-6	G8954	Netherlands		D	S	1	0	1.5	58.8	67.8
B4-11	G9037	Netherlands		D	S	1	0	1.5	53.8	69.1
B4-14	G23554E	Mexico	1950	Weedy	S	3	0	0.5	53.8	71.1
B4-20	G8759	Netherlands		Snap	M	1	0	0	92.8	94.1
B5-20	G8231	Japan		D	M	4	0	0	83.8	84.8
B2-33	G13184	Japan		D	L	1	0	0	82.5	84.1
B3-15	G19504	Peru	2140	Weedy	S	4	0	0	80.8	87.4
B1-19	G1293	Colombia		D	L	1	0	0	79.1	83.8
B5-23	G9025	Netherlands		D	M	1	0	0	77.8	91.8
B4-5	G9000	Netherlands		Snap	M	1	0	0	75.8	82.1
B3-32	G15821	Mexico		D	M	3	0	0	74.8	80.4
B4-19	G21200	Argentina	2340	Wild	S	3	0	0	73.8	78.1
B3-21	G9249	France		D	M	1	0	0	72.8	78.4
B6-1	G6962	Netherlands		D	M	4 (seg)	0	0	72.1	74.8
B6-20	G8999	Netherlands		Snap	M	1	0	0	72.1	97.8
B4-6	G23500A	Peru	2650	Weedy	S	4	0	0	71.1	71.1
B3-18	G19899	Argentina	2100	Wild	S	3	0	0	69.8	81.4
B3-16	G8813	Netherlands		D	S	1	0	0	67.8	73.4
B5-28	G4509	UK		D	M	1	0	0	66.8	75.8
B2-9	G1544	Poland		Snap	M	1	0	0	66.5	94.1
B3-14	G2338	Mexico		D	M	4	0	0	65.8	77.4
B3-7	G9012	Netherlands		Snap	S	1	0	0	64.8	76.4
B6-7	G23500	Peru	2650	Wild	S	4	0	0	62.1	65.8
B1-16	G8981	Netherlands		D	M	1	0	0	61.1	81.8
B1-33	G1545	Poland		Snap	M	1	0	0	61.1	99.8
B6-19	G991	Soviet Union		D	S	4	0	0	61.1	88.8

B2-32	G23445	Bolivia	2100	Wild	S	3	0	0	59.5	93.1
B2-21	G11034	Mexico	1960	Wild	S	3	0	0	55.5	68.1
B6-18	G23506	Mexico	2000	Wild	S	3	0	0	55.1	62.8
B3-20	G7524	Netherlands		Snap	S	1	0	0	53.8	65.4
B5-3	G23452	Peru	2650	Weedy	S	4	0	0	52.8	53.8
B6-22	G2551	Ecuador		D	M	1	0	0	52.1	79.8
B1-7	G4541	Colombia		D	L	1	0	0	51.1	67.8
B1-10	G11283	Poland		Snap	M	1	0	0	50.1	76.8
B2-11	G9348	Netherlands		Snap	M	1	0	0	48.5	90.1
B6-11	G9198	Netherlands		Snap	S	1	0	0	43.1	70.8
B2-10	G8838	Netherlands		Snap	S	1	0	0	42.5	58.1
B3-33	G23443	Bolivia	2380	Wild	S	3	0	0	41.8	72.4
B3-4	G23501	Peru	2650	Escape	S	3	0	0	38.8	40.4
B1-18	G23448	Peru	2850	Weedy	S	4	0	0	38.1	67.8
B1-26	G23502F	Peru	2650	Escape	S	3	0	0	36.1	36.8
B3-31	G21201	Argentina	2600	Wild	S	3	0	0	35.8	65.4
B2-22	G23456A	Peru	2300	Wild	S	3	0	0	32.5	43.1
B5-4	G23444	Bolivia	2040	Wild	S	3	0	0	28.8	49.8
B3-8	G11223	Poland		D	L	1	0	0	27.8	54.4
B4-3	G23423	Peru	2050	Wild	S	3	0	0	27.8	73.1
B2-1	G23425F	Peru	2760	Weedy	S	3	0	0	27.5	32.1
B5-21	G23460	Peru	2560	Wild	S	4	0	0	26.8	42.8
B3-19	G23493	Peru	2760	Weedy	S	4	0	0	25.8	45.4
B4-23	G23491	Peru	2740	Weedy	S	3	0	0	21.8	35.1
B2-3	G9454	Netherlands		D	M	1	0	0	21.5	25.1
B1-17	G23428	Peru	2780	Weedy	S	4	0	0	19.1	66.8
B5-16	G23495	Peru	2880	Weedy	S	4	0	0	17.8	18.8
B6-3	G23459A	Peru	2370	Wild	S	3	0	0	17.8	17.8
B3-30	G8861	Netherlands		Snap	S	1	0	0	16.8	18.4
B2-2	G23454	Peru	2460	Weedy	S	3	0	0	16.5	35.1
B5-9	G23422	Peru	2560	Wild	S	3	0	0	14.8	18.8

B6-27	G23427A	Peru	2580	Weedy	S	4	0	0	14.1	27.8
B5-30	G7225	Peru	2300	Weedy	S	3	0	0	12.8	60.8
B5-5	G23454E	Peru	2460	Weedy	S	4	0	0	11.8	37.8
B4-24	G23425	Peru	2760	Wild	S	3	0	0	10.8	16.1
B6-6	G23541	Mexico	1950	Wild	S	3	0	0	10.1	24.8
B3-29	G23426A	Peru	2440	Weedy	S	3	0	0	9.8	44.4
B2-27	G23427	Peru	2580	Wild	S	3	0	0	9.5	29.1
B3-26	G23426	Peru	2440	Wild	S	3	0	0	7.8	17.4
B2-12	G11027	Mexico	1950	Wild	S	3	0	0	7.5	17.1
B3-24	G23442	Bolivia	2270	Wild	S	3	0	0	6.8	41.4
B4-28	G23589	Peru	2300	Wild	S	3	0	0	6.8	27.1
B4-31	G23460A	Peru	2560	Weedy	S	4	0	0	6.8	12.1
B6-5	G23559	Peru	2900	Weedy	S	4	0	0	5.1	6.8
B1-23	G11028	Mexico	2020	Wild	S	3	0	0	4.1	21.8
B3-1	G23427C	Peru	2580	Weedy	S	3	0	0	3.8	27.4
B5-10	G23553	Mexico	1950	Wild	S	3	0	0	3.8	26.8
B5-15	G11030	Mexico	2050	Wild	S	3	0	0	1.8	40.8
B5-25	G12851	Guatemala	2000	Wild	S	3	0	0	1.8	18.8
B1-14	G23453	Peru	2600	Weedy	S	4	0	0	1.1	1.8
B6-8	G23424	Peru	2340	Weedy	S	3	0	0	1.1	29.8
B6-29	G12959	Mexico	2100	Wild	S	3	0	0	1.1	2.8
B1-13	G23545	Mexico	1990	Wild	S	3	0	0	0.1	30.8
B1-20	G23555	Mexico	1950	Wild	S	3	0	0	0	71.8
B1-21	G23546	Mexico	1950	Wild	S	3	0	0	0	49.8
B1-27	G23554	Mexico	1950	Wild	S	3	0	0	0	34.8
B3-3	G12873	Mexico	1981	Wild	S	3	0	0	0	4.4
B3-11	G23526A	Mexico	1950	Wild	S	3	0	0	0	15.4
B3-17	G11050	Mexico	2040	Wild	S	3	0	0	0	66.4
B3-25	G23456	Peru	2300	Wild	S	3	0	0	0	4.4
B4-8	G23518	Mexico	1950	Wild	S	3	0	0	0	6.1
B4-33	G11024	Mexico	1970	Wild	S	3	0	0	0	4.1

B5-31	G23678	Mexico	2100	Wild	S	3	0	0	0	1.8
B5-32	G11031	Mexico	2270	Wild?	S	3	0	0	0	34.8
B5-33	G11032	Mexico	2030	Wild	S	3	0	0	0	24.8
B6-15	G8855	Netherlands		D	S		0	0	0	0
L.S.D. (0.05) to compare check cultivars							NS	15.1	8.3	9.0
L.S.D. (0.05) to compare two accessions in the same block							20.2	37.0	20.4	22.0
L.S.D. (0.05) to compare two accessions in different block							23.3	42.7	23.5	25.4
L.S.D. (0.05) to compare an accession with a check cultivar							17.8	32.6	18.0	19.4

† B2-17 should be read as Plot no. 17 in Block no. 2.

‡ Seed size: S = small, M = medium and L = large.

§ Growth habit: 1 = determinate bush; 2 = indeterminate bush; 3 = indeterminate prostrate and 4 = indeterminate climbing.

Appendix 2. Means for cumulative thermal units to 50% anthesis (°C d) of common bean cultivars, accessions and wild relatives grown at Saskatoon, SK, during 2000.

Plot no.†	Accession No.	Origin	Bean Type	Seed size‡	Cumulative thermal units to 50% anthesis	Plot no.†	Accession no.	Origin	Bean Type	Seed size‡	Cumulative thermal units to 50% anthesis
					— °C d —						— °C d —
Check	CDC Whistler	Canada	Dry (D)	S	548.8						
Check	CDC Nighthawk	Canada	D	S	527.3						
Check	UI 906	USA	D	S	544.6						
B6-21	G9509	Belgium	D	S	374.0	B1-25	G8737	Netherlands	D	M	486.5
B5-24	G11285	Sweden	D	M	379.3	B6-2	G8811	Sweden	Snap	S	487.3
B2-14	G7576	Netherlands	D	S	380.6	B3-5	G8090	Netherlands	D	S	491.6
B2-23	G7551	Netherlands	D	S	380.6	B3-21	G9249	France	D	M	491.6
B4-12	G9256	Netherlands	D	M	380.6	B6-13	G1461	Sweden	Snap	S	497.8
B6-25	G8752	Sweden	D	S	383.5	B6-17	G4498	USA	D	S	497.8
B3-10	G8960	Denmark	D	M	387.8	B2-28	G8807	Netherlands	D	M	504.3
B3-28	G20324	Sweden	D	M	387.8	B4-25	G8835	Netherlands	D	M	504.3
B5-2	G8800	Netherlands	D	S	389.7	B1-10	G11283	Poland	Snap	M	505.0
B5-13	G11197	Netherlands	D	M	389.7	B1-22	G8773	Netherlands	D	S	505.0
B2-15	G7500	Netherlands	Snap	S	391.0	B6-4	G8873	Denmark	Snap	M	505.8
B2-26	G8756	Netherlands	Snap	M	391.0	B6-24	G7476	Netherlands	Snap	M	505.8
B4-13	G7493	Netherlands	Snap	S	391.0	B3-2	G8952	Netherlands	D	S	510.1
B1-5	G7499	Netherlands	D	S	391.7	B3-8	G11223	Poland	D	L	510.1
B2-5	G1683	Sweden	Snap	S	400.5	B2-30	G9519	Netherlands	Snap	M	514.8
B2-13	G2902	Sweden	D	M	400.5	B2-33	G13184	Japan	D	L	514.8
B2-18	G1540	Sweden	D	M	400.5	B3-9	G13182	Japan	D	M	514.9
B4-18	G9042	Netherlands	D	M	400.5	B1-30	G8976	Netherlands	D	S	515.5

B4-30	G9290	Netherlands	Snap	S	400.5	B1-31	G9356	Netherlands	D	S	515.5
B1-1	G7498	Netherlands	Snap	S	401.2	B3-30	G8861	Netherlands	Snap	S	520.5
B3-12	G7544	Germany	Snap	S	410.0	B5-19	G7511	Netherlands	Snap	S	521.5
B5-27	G8795	Netherlands	D	S	410.2	B2-3	G9454	Netherlands	D	M	522.8
B4-16	G22208	Bulgaria	D	S	411.5	B2-10	G8838	Netherlands	Snap	S	522.8
B2-31	G9499	Netherlands	D	L	422.7	B2-17	G9345	USA	D	S	522.8
B4-21	G6668	Sweden	D	M	422.7	B1-3	G7528	Netherlands	Snap	S	523.5
B4-27	G6352	Netherlands	D	S	422.7	B5-17	G7506	Netherlands	Snap	S	526.3
B4-5	G9000	Netherlands	Snap	M	434.3	B2-8	G14739	Bulgaria	D	L	527.6
B3-13	G8823	Netherlands	D	S	435.1	B1-4	G7577	Netherlands	Snap	S	527.9
B3-20	G7524	Netherlands	Snap	S	435.1	B1-2	G1541	Poland	D	S	528.3
B5-23	G9025	Netherlands	D	M	446.5	B1-19	G1293	Colombia	D	L	528.3
B3-7	G9012	Netherlands	Snap	S	447.0	B5-12	G8886	Netherlands	Snap	S	531.9
B2-6	G7504	Netherlands	Snap	S	447.8	B4-15	G1460	Sweden	D	S	539.0
B4-7	G1283	Sweden	Snap	S	447.8	B4-1	G9430	Netherlands	D	M	546.5
B4-20	G8759	Netherlands	Snap	M	447.8	B4-11	G9037	Netherlands	D	S	546.5
B1-16	G8981	Netherlands	D	M	448.5	B1-9	G741	Netherlands	D	M	547.2
B6-9	G8969	Netherlands	Snap	M	455.1	B6-30	G8654	Netherlands	D	M	553.9
B6-11	G9198	Netherlands	Snap	S	455.1	B3-16	G8813	Netherlands	D	S	573.6
B6-14	G7556	Netherlands	D	S	455.1	B6-1	G6962	Netherlands	D	M	581.0
B6-16	G7581	Netherlands	D	S	455.1	B5-1	G7515	Netherlands	D	S	585.0
B6-33	G14632	Denmark	Snap	M	455.1	B4-2	G2974	Guatemala	D	S	586.3
B5-6	G8954	Netherlands	D	S	458.4	B1-12	G15955	Netherlands	D	M	587.0
B5-28	G4509	UK	D	M	458.4	B1-24	G9248	Unknown	D	M	587.0
B2-9	G1544	Poland	Snap	M	459.7	B6-10	G3353	Mexico	D	M	593.6
B4-17	G16805	Tanzania	D	M	459.7	B6-23	G2511	Colombia	D	S	593.6
B4-32	G7574	Netherlands	Snap	M	459.7	B5-8	G5024	Brazil	D	M	609.3
B6-20	G8999	Netherlands	Snap	M	468.8	B4-4	G5673	Japan	D	S	626.4
B5-7	G7519	Netherlands	Snap	S	470.8	B1-7	G4541	Colombia	D	L	627.1
B5-22	G15967	Netherlands	D	M	470.8	B6-22	G2551	Ecuador	D	M	644.1
B2-19	G7562	Netherlands	Snap	M	472.1	B1-32	G9033	Netherlands	D	M	677.4

B4-10	G8985	Netherlands	Snap	M	472.1	B5-20	G8231	Japan	D	M	690.1
B1-33	G1545	Poland	Snap	M	472.8	B5-14	G5711	Guatemala	D	S	703.0
B2-4	G8760	Netherlands	Snap	S	485.8	B3-27	G9605	Japan	D	M	703.9
B2-11	G9348	Netherlands	Snap	M	485.8	B6-19	G991	Soviet Un.	D	S	713.5
B2-16	G7503	Netherlands	D	S	485.8	B5-29	G1090	Chile	D	S	715.3
B2-24	G8839	Norway	D	S	485.8	B3-15	G19504§	Peru	Weedy	S	730.4
B1-6	G8851	Denmark	D	M	486.5	B6-26	G5478	USA	D	M	797.1

L.S.D. (0.05) to compare check cultivars	NS
L.S.D. (0.05) to compare two accessions in the same block	58.1
L.S.D. (0.05) to compare two accessions in different block	67.1
L.S.D. (0.05) to compare an accession with a check cultivar	51.2

† B6-21 should be read as Plot no. 21 in Block 6.

‡ Seed size: S = small, M = medium and L = large.

§ Accession G19504 is from an altitude of 2140 m (Peru).

Appendix 3. Means for cumulative thermal units to 50% maturity (°C d), yield m^{-2} (g), number of plants m^{-2}, 100-seed weight (g) and percent frost damaged seed of common bean cultivars, accessions and wild relatives grown at Saskatoon, SK, during 2000.

Plot no.†	Accession no.	Origin	Bean type	Seed size‡	Cumulative thermal units to 50% maturity	Yield m^{-2}	No. of plants m^{-2}	100-seed weight	Percent frost damaged seed
					°C d	g		g	%
Check	CDC Whistler	Canada	Dry (D)	S	1070.0	187.0	21.4	15.3	7.8
Check	CDC Nighthawk	Canada	D	S	958.5	449.8	65.0	16.6	0
Check	UI 906	USA	D	S	1004.2	358.7	66.1	14.4	0.7
B2-23	G7551	Netherlands	D	S	1011.3	547.8	67.0	25.5	13.2
B2-17	G9345	USA	D	S	1012.4	505.8	73.6	16.3	1.2
B6-9	G8969	Netherlands	Snap	M	1112.3	496.2	44.1	25.9	19.2
B6-14	G7556	Netherlands	D	S	988.7	492.7	42.5	26.6	7.2
B5-23	G9025	Netherlands	D	M	1050.4	490.3	59.2	36.8	5.9
B2-19	G7562	Netherlands	Snap	M	1012.4	481.7	65.3	25.9	1.2
B1-22	G8773	Netherlands	D	S	1029.5	479.9	58.0	26.9	4.2
B6-21	G9509	Belgium	D	S	985.2	459.2	64.1	27.4	0.2
B5-6	G8954	Netherlands	D	S	1115.2	453.0	37.5	23.9	13.9
B1-5	G7499	Netherlands	D	S	987.6	451.4	58.0	25.4	2.2
B5-19	G7511	Netherlands	Snap	S	1034.8	440.0	52.5	22.4	6.9
B2-28	G8807	Netherlands	D	M	1140.5	439.0	73.6	26.5	16.2
B2-15	G7500	Netherlands	Snap	S	984.0	437.3	52.0	26.9	1.2
B1-4	G7577	Netherlands	Snap	S	1029.5	434.4	51.3	21.9	0
B2-5	G1683	Sweden	Snap	S	1033.0	430.0	55.3	21.9	1.2
B1-31	G9356	Netherlands	D	S	1070.1	420.7	51.3	21.9	0
B4-5	G9000	Netherlands	Snap	M	958.8	411.5	60.8	26.8	0
B5-27	G8795	Netherlands	D	S	1091.4	410.3	42.5	26.3	9.9
B2-24	G8839	Norway	D	S	1012.4	407.7	73.6	23.6	1.2
B6-17	G4498	USA	D	S	988.7	406.0	25.8	17.1	7.2
B5-8	G5024	Brazil	D	M	1078.4	403.2	60.8	24.2	0
B3-8	G11223	Poland	D	L	1019.9	396.3	34.1	30.4	8.9

B2-10	G8838	Netherlands	Snap	S	1071.3	395.3	47.0	28.7	10.2
B1-6	G8851	Denmark	D	M	1094.5	388.7	48.0	28.0	0
B2-26	G8756	Netherlands	Snap	M	1012.4	387.8	45.3	29.4	1.2
B3-7	G9012	Netherlands	Snap	S	1018.8	378.3	49.1	24.4	1.9
B1-16	G8981	Netherlands	D	M	959.5	373.7	68.0	26.5	0
B4-2	G2974	Guatemala	D	S	986.1	359.1	59.2	20.4	0
B1-1	G7498	Netherlands	Snap	S	1025.0	343.2	49.7	29.3	2.2
B1-3	G7528	Netherlands	Snap	S	1070.1	331.4	29.7	22.1	6.2
B5-22	G15967	Netherlands	D	M	1095.9	330.5	39.2	24.5	16.9
B5-7	G7519	Netherlands	Snap	S	1034.8	327.7	35.8	21.1	3.9
B5-20	G8231	Japan	D	M	1137.9	325.8	50.8	25.9	22.9
B1-10	G11283	Poland	Snap	M	974.9	316.7	58.0	25.3	0
B6-30	G8654	Netherlands	D	M	1063.5	316.7	54.1	30.5	8.2
B2-3	G9454	Netherlands	D	M	1138.4	310.3	28.6	29.6	22.2
B5-12	G8886	Netherlands	Snap	S	1014.5	289.3	40.8	20.1	7.9
B3-16	G8813	Netherlands	D	S	1088.5	284.9	44.1	26.3	6.9
B1-30	G8976	Netherlands	D	S	1070.1	284.1	28.0	23.6	16.2
B2-14	G7576	Netherlands	D	S	957.8	283.7	45.3	24.4	2.2
B1-2	G1541	Poland	D	S	1029.5	261.6	43.0	25.8	2.2
B3-13	G8823	Netherlands	D	S	978.0	260.9	74.1	17.5	1.9
B6-11	G9198	Netherlands	Snap	S	981.8	257.2	59.1	27.6	14.2
B3-2	G8952	Netherlands	D	S	1106.0	255.1	45.8	20.2	34.9
B4-1	G9430	Netherlands	D	M	1046.1	249.1	55.8	18.8	7.9
B5-1	G7515	Netherlands	D	S	1135.8	248.2	45.8	26.0	11.9
B3-5	G8090	Netherlands	D	S	1015.4	231.3	37.5	16.7	5.9
B4-32	G7574	Netherlands	Snap	M	958.8	230.5	27.5	27.7	0
B4-27	G6352	Netherlands	D	S	1052.2	230.1	22.5	23.4	10.9
B4-10	G8985	Netherlands	Snap	M	989.6	215.8	35.8	21.0	2.9
B4-13	G7493	Netherlands	Snap	S	987.2	214.5	22.5	30.6	0
B4-15	G1460	Sweden	D	S	1052.2	212.3	44.2	16.8	13.9
B1-12	G15955	Netherlands	D	M	1115.6	209.6	36.3	22.6	3.2

B6-23	G2511	Colombia	D	S	1114.4	197.8	54.1	18.7	2.2
B3-20	G7524	Netherlands	Snap	S	949.9	179.6	44.1	27.9	6.9
B2-30	G9519	Netherlands	Snap	M	1089.6	133.0	18.6	26.3	37.2
B3-30	G8861	Netherlands	Snap	S	1091.2	-5.7§	4.1	27.8	12.9
L.S.D. (0.05) to compare check cultivars					28.1	92.5	11.0	1.0	2.9
L.S.D. (0.05) to compare two accessions in the same block					68.7	226.6	26.9	2.8	7.2
L.S.D. (0.05) to compare two accessions in different block					79.4	261.7	31.1	2.9	8.3
L.S.D. (0.05) to compare an accession with a check cultivar					60.6	199.9	23.8	2.2	6.3

† B2-23 should be read as Plot no. 23 in Block 2.

‡ Seed size: S = small, M = medium and L = large.

§ Negative estimate due to block adjustment.