Critical Reviews in Plant Sciences, 22(2):185–219 (2003)

Low-Temperature Stress: Implications for Chickpea (*Cicer arietinum* L.) Improvement

J. S. Croser,[1] H. J. Clarke,[1] K. H. M. Siddique,[1] and T. N. Khan[2]

[1]Centre for Legumes in Mediterranean Agriculture, University of Western Australia; [2]Department of Agriculture, Western Australia

* **Correspondence to:** Professor K.H.M. Siddique, Director, Centre for Legumes in Mediterranean Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia. *ksiddiqu@agric.uwa.edu.au.* +61 8 9380 7012 (Tel.) +61 8 9380 7354 (Fax.)

ABSTRACT: Chickpea is the third major cool season grain legume crop in the world after dry bean and field pea. Chilling and freezing range temperatures in many of its production regions adversely affect chickpea production. This review provides a comprehensive account of the current information regarding the tolerance of chickpea to freezing and chilling range temperatures. The effect of freezing and chilling at the major phenological stages of chickpea growth are discussed, and its ability for acclimation and winter hardiness is reviewed. Response mechanisms to chilling and freezing are considered at the molecular, cellular, whole plant, and canopy levels. The genetics of tolerance to freezing in chickpea are outlined. Sources of resistance to both freezing and chilling from within the cultivated and wild *Cicer* genepools are compared and novel breeding technologies for the improvement of tolerance in chickpea are suggested. We also suggest future research be directed toward understanding the mechanisms involved in cold tolerance of chickpea at the physiological, biochemical, and molecular level. Further screening of both the cultivated and wild *Cicer* species is required in order to identify superior sources of tolerance, especially to chilling at the reproductive stages.

KEY WORDS: abiotic stress, freezing, chilling, flower abortion, wild relatives.

I. GENERAL INTRODUCTION

The production of the cool season grain legume chickpea (*Cicer arietinum* L.) is constrained by low temperatures across much of its geographical range. Botanical, genetic, and archeological evidence points to chickpea originating within the Fertile Crescent, Turkey (Lev-Yadun *et al.*, 2000). From there, it has spread to its present day range, principally concentrated between the latitudes 20° and 40° and including west and central Asia, the Indian subcontinent, southern Europe, Africa (northern parts), Latin America, and more recently North America and Australia (FAO, 2001). Chickpea cultivation has also spread to near equatorial Uganda and Ethiopia, and northern latitudes within Canada and Russia (ca. 50°) (Figure 1). India (6.2×10^6 t) is by far the largest producer of chickpea, accounting for ca. 70% of total world production (8.8×10^6 t) and reflecting the importance of chickpea as a protein source in the diet of people in developing countries. Widespread cultivation has resulted in chickpea being third in terms of world pulse production behind dry bean (*Phaseolus* species) (18.8×10^6 t) and field pea (*Pisum sativum* L.) (10.9×10^6 t) (FAO, 2001).

In most agricultural species, suboptimal temperatures can be divided into chilling range and freezing range. For the purposes of this review we define freezing range temperatures for chickpea as below –1.5°C, which is the typical freezing point of plant tissue (Graham and Patterson, 1982), and chilling range temperatures for chickpea as between –1.5°C and 15°C. Temperatures up to 15°C have been demonstrated to cause flower and pod abortion in parts of the Indian subcontinent

0735-2689/03/$.50

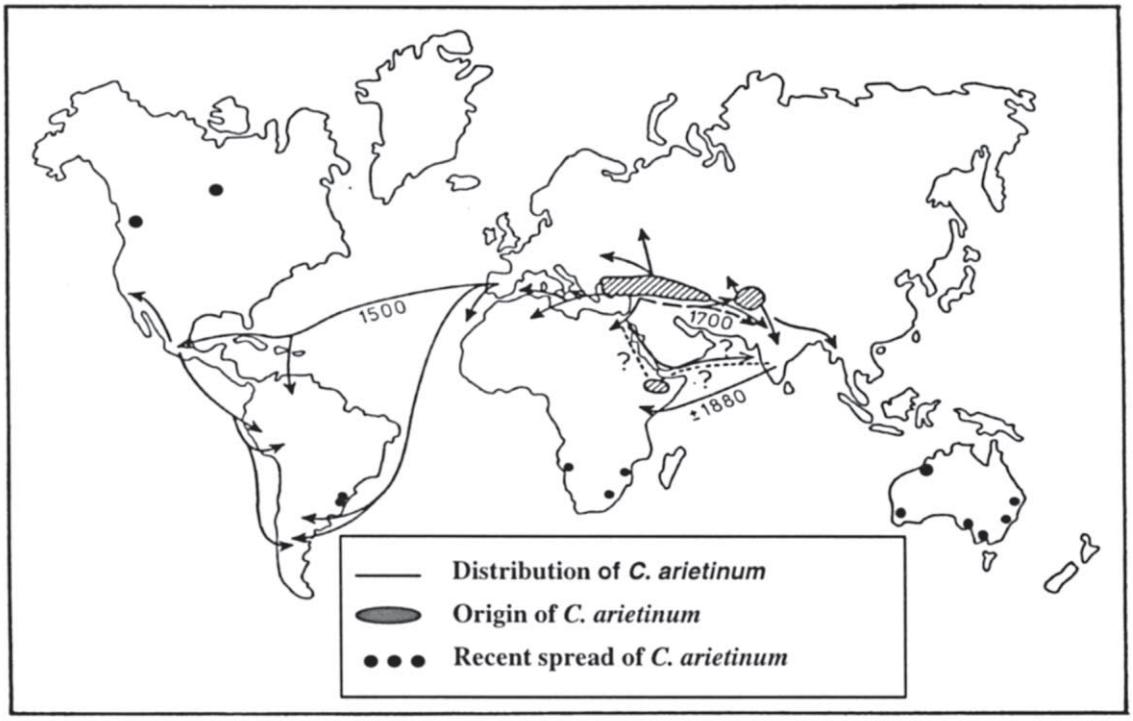

FIGURE 1. Areas of origin of chickpea (*C. arietinum*) and subsequent spread around the world. (Modified from van der Maesen [1972].)

and Australia (Srinivasan *et al*., 1998; Clarke, 2001). Freezing range temperatures are considered an important problem for winter-sown chickpea in the countries surrounding the Mediterranean Sea, the tropical highlands, and temperate growing regions (Singh, 1993). In these regions, freezing stress predominantly occurs during the seedling and early vegetative stages of crop growth. Isolated freezing events (frost) are also a problem across the geographical distribution of the crop, especially when they occur in the late vegetative and reproductive phenological stages. On the other hand, temperatures within the chilling range can limit the growth and vigour of chickpea at all phenological stages, but are considered most damaging to yield at the reproductive stages. Southern Australia and parts of the Indian subcontinent are the most affected by chilling range temperatures at flowering.

This review focuses on the research pertaining to the morphological, physiological, biochemical, and genetic factors involved in the response of chickpea to both freezing and chilling range temperatures. It is important to note that varying levels of tolerance and sensitivity are possible between and even within individual genotypes. This is due to the close linkage of the plants reaction to temperature with the phenological stage of the plant, the preceding temperature regimes, and the prevailing environmental conditions. The effect of chilling range and freezing range temperatures at different phenological stages is considered separately due to this large variation in plant response.

II. IMPACT OF SUBOPTIMAL TEMPERATURES ON CHICKPEA PRODUCTION

A. Impact of Freezing Range Temperatures

Yield instability in chickpea has been chiefly attributed to the diverse geographic distribution of the crop and the subsequent effects of a number of biotic and abiotic stresses (Saxena, 1990; Wery *et al.*, 1994; Singh and Saxena, 1993; Singh *et al.*,

1994; Robertson *et al.*, 1996; Singh *et al.*, 1998; Leport *et al.*, 1999). The term 'stress' when used in this context is defined as any disturbance that adversely influences plant growth (Singh, 1993). Research progress toward ameliorating the effects of abiotic and biotic stresses and stabilizing chickpea yields has been reviewed (Saxena and Singh, 1987; van Rheenen *et al.*, 1990; Singh and Reddy, 1991; Singh and Saxena, 1993). Singh *et al.* (1994) categorized the major abiotic and biotic stresses affecting chickpea production in order of relative impact and compared this to the research effort expended in each area (Figure 2). This figure indicates the rather skewed distribution of research focus in chickpea, with an estimated 80% of the research effort going toward biotic stresses, which account for only an estimated 58% of the impact on production. In contrast, abiotic stresses, which account for an estimated 42% of the impact on production, receive only 20% of the research effort. Of the abiotic stresses, freezing range temperatures were estimated to account for 6% of the impact on production and receive 5% of the research effort. In this context, the authors did not take into account yield reduction from chilling range temperatures at the reproductive stage. It should be noted that impacts are estimations only, and resources are primarily expended in research areas where there is likely to be a favourable outcome.

It is widely acknowledged that freezing range temperatures are detrimental to chickpea yield. Prolonged periods of freezing range temperatures can prevent germination, reduce the vigour and vegetative biomass of the developing plant, and can be fatal to plants, especially those at the late vegetative and reproductive phenological growth stages. Isolated frost events during the reproductive stage commonly results in flower or pod abortion, and this can be detrimental to yield in environments that experience terminal drought.

There is very little quantitative data regarding the effect of freezing range temperatures on seed quality of chickpea. Causal observations have indicated that freezing can reduce seed size, probably due to stress conditions affecting the mobilization of plant resources. In addition, the seed coat can be discolored. More research is required to ascertain if there are biochemical changes to the seed composition following exposure to freezing or chilling range temperatures.

In the last 15 years there has been an increased focus toward temperature tolerance breeding in chickpea (Malhotra and Saxena, 1993). This has resulted in the development of cultivars tolerant to freezing temperatures at the seedling

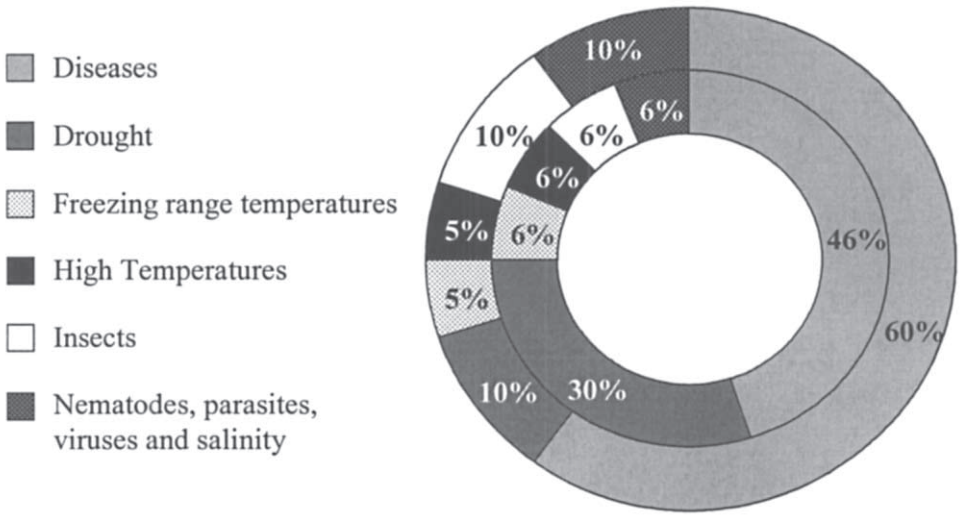

FIGURE 2. Relative importance of abiotic and biotic stresses on chickpea production (inner circle) compared to the research effort expended (outer circle). (Modified from Singh *et al.* [1994].)

and early vegetative stages. This trait, when combined with resistance to ascochyta blight (caused by *Ascochyta rabiei*), has enabled a switch from spring to winter sowing in countries surrounding the Mediterranean Sea. This has increased seed yields by an average of 70% through higher availability of soil water enabling plants to more effectively utilize available water resouces (Singh *et al.*, 1997). If cultivars with increased vegetative growth during the winter months can be developed, they should prove beneficial in many environments. The development of cultivars with a higher degree of cold tolerance would facilitate the spread of chickpea growing regions to both higher altitudes and colder latitudes and therefore are worthy of considerable agronomic and breeding attention.

B. Impact of Chilling Range Temperatures

A prolonged period of chilling range temperatures at any phenological stage of development in chickpea has detrimental effects on final seed yield. During germination, chilling range temperatures result in poor crop establishment, increased susceptibility to soil-borne pathogens, and reduced seedling vigor. At the seedling stage, long periods of chilling range temperatures can retard the growth of the plant and, in severe cases, cause plant death. At the vegetative stage, chilling range temperatures have a pronounced negative effect on plant growth and dry matter production. Less dry matter production reduces the reproductive sink that the plant can support, which, in turn, reduces potential yield. Flower, pod, or seed abortion are further symptoms of chilling range temperatures (Plate 1).

In the Mediterranean type environment of southwestern Australia, chickpea yields are limited by chilling range temperatures during flowering, causing extensive flower and pod abortion (Siddique and Sedgley, 1986). The high yield potential of early sown crops (high biomass) or early flowering genotypes is largely limited by abortion of flowers and pods in late winter and early spring, which in turn leads to low harvest index (Table 1). Although delayed sowing can reduce flower and pod abortion associated with low temperatures, seed yield is often limited by terminal soil moistures, a common feature in this environment (Turner *et al.*, 2001). Early flowering would benefit yield if flowers were fertile, because pod development and seed filling can start earlier and so avoid terminal soil moisture stress (Leport *et al.*, 1999). Greater tolerance to chilling range temperatures at flowering therefore is required in chickpea in order to take advantage of the full benefit of early flowering and high yield potential associated with early sowing in short season Mediterranean-type environments.

In subtropical South Asia, a prevalence of chilling range temperatures during early flowering leading to excessive floral abortion is a major cause of low pod and seed set in chickpea (Saxena, 1980; Srinivasan *et al.*, 1998, 1999). Although such loss is considered an adaptive mechanism that stimulates vegetative growth and provides additional nodes for production of flowers and pods (Saxena, 1984), this in only true in environments that are not limited by soil moisture toward the end of the growing season.

III. FREEZING STRESS

The need for greater tolerance to freezing range temperatures in chickpea has arisen where the distribution of chickpea has been expanded to higher latitudes, such as Canada or Russia, or the growing season has been altered to include colder conditions, such as winter sowing in the Mediterranean basin.

A. Mechanisms of Freezing Injury

Freezing-sensitive plants are damaged or killed by temperatures below –1.5°C. Damage from freezing commonly occurs due to ice forming within the intercellular spaces. The rigid ice lattice structure extends with decreasing temperature and may penetrate cellular walls and membranes to an extent that is irreparable by normal cell processes (Andrews, 1996). Intracellular ice formation is lethal (Guy, 1990), whereas many plant species can tolerate extracellular ice forma-

PLATE 1. Chickpea plant showing abortion of pods following exposure to frost. (Photograph: Couresy of Mr. Ted Knights, New South Wales Agriculture, Tamworth, Australia.)

TABLE 1
Effect of Chilling Range Temperatures at Flowering on Chickpea Productivity at Merredin, Western Australia, 1983 (31°29' latitude; 118°17' longitude).

Date of 50% flowering	Mean daily temperature (°C) at 50% flowering	Aborted flowers (m^{-2})	Biological yield (t ha^{-1})	Seed yield (t ha^{-1})	HI
August 19th	12.5	800	6.76	1.25	0.18
September 1st	13.6	500	5.34	1.13	0.21
September 14th	14.7	200	4.84	1.12	0.23
September 29th	16.8	0	3.98	1.11	0.28
October 6th	17.7	0	3.23	0.94	0.29

Planting dates: May 17th, May 31st, June 14th, June 30th and July 20th.
Source: Modified from Siddique and Sedgley (1986)

tion. It is important to note that during periods of active growth, most crop species do not tolerate freezing. In chickpea, the elongation regions are often the first affected by freezing, and this can show up in a frost-damaged plant by sigmoidal curves around the elongation point, commonly referred to as 'hockey stick' symptom. Depending on the minimum temperature and the duration of the frost, plants may be partially damaged or killed, resulting in lower yield and quality at harvest or even complete crop failure (Hudák and Salaj, 1999).

The freezing tolerance of a plant varies greatly between different tissues, that is, upper and lower leaves of the plant canopy, stems, meristems, or roots (Herzog, 1987; Herzog and Olszewski, 1998). Antifreeze proteins and ice nucleators control the initial formation of ice. Tolerance to freezing is often associated with mechanisms at the cellular level, including increased membrane fluidity and osmotic adjustment (Buddenhagen and Richards, 1988; Wery *et al.*, 1993) as well as supercooling without ice nucleation (Olien and Smith, 1981). A lack of efficient and reliable screening techniques makes it difficult for breeders to routinely screen specifically for these mechanisms (Wery *et al.*, 1994).

B. Acclimation to Freezing Range Temperatures

For many plant species tolerance to the stress imposed by freezing range temperatures is not static, but can change seasonally or when the temperature and other environmental conditions are varied. A large part of the seasonal dynamics of freezing tolerance in plants is related to the process of cold acclimation. Acclimation is a highly active process resulting from both metabolic and physiological alterations in the plant in response to low temperatures. These alterations result in a lowering of the temperature at which a plant is damaged by freezing (Levitt, 1980; Graham and Patterson, 1982; Stepkonus, 1984; Guy, 1990; Prasad, 2001). Acclimation can also be referred to as acclimatization or hardening (Levitt, 1980).

Acclimation to freezing temperatures is well recorded in many plant species. Extracellular ice is relatively nonharmful and can act as a nucleation site for water drawn out of the cell, promoting cell desiccation. Although ice driven desiccation can continue to the point of irrevocable cellular damage, it is in a stable desiccated state that most cold-stressed tissues overwinter at subzero temperatures (Andrews, 1996). Cellular and metabolic changes that occur during cold acclimation include increased levels of sugars, soluble proteins, prolines, and organic acids as well as the appearance of new isoforms of proteins and altered lipid membrane composition (Hughes and Dunn, 1990, 1996).

The ability to acclimate is essential to freezing tolerance of the cool season grain legumes (Wery *et al.*, 1994). This ability seems to decrease with plant age and is reliant on a slow rate of plant growth as is found in early winter sown crops in colder temperatures (Wery *et al.*, 1993). Winter sown chickpea are exposed to decreasing photoperiods and temperatures that fall gradually as the season progresses from autumn to early winter. Therefore, seedlings of winter planted chickpea have a possibility of acquiring some degree of tolerance to moderate subzero temperatures, as has been shown for faba beans by Herzog (1978).

C. Winter Hardiness of Chickpea

It is important to note that winter survival of plants often requires tolerance to factors other than cold, for example, frost-heaving, water-logging, frost-drought, and diseases (Murray *et al.*, 1988). The primary causes of winterkill are heaving caused by the formation of ice in the soil, either as a solid or as capillary needles or columns, which push the plants upward, breaking and exposing the roots; smothering; physiological drought; and freezing of the plant tissue (Grafius, 1981). Snow cover itself can be a positive environmental factor because the thermal and radiative properties of snow results in a distinct thermal profile, where its surface temperature is at near equilibrium with air temperature (below zero), while temperature gradually increases with snow depth, often to near zero at the soil surface. In this way, snow can act as an effective insulator for underlying plants (Blum, 1988). Cultural prac-

tices such as planting date, fertilizer regime, disease and insect control, and tillage practices that influence snow capture and drainage, combine to influence the overwintering ability of grain legumes (Murray *et al.*, 1988). Thus, winter hardiness of chickpea represents tolerance to a complex of winter rigours. It is important that this complexity be taken into account and reflected in the germplasm screening methodology adopted. Therefore, it is necessary to screen chickpea not only in the field, but also under controlled conditions where the various components of winter hardiness can be separated and selected for individually. To date, the selection of genotypes exhibiting tolerance to freezing in field screening tests have not been verified under controlled conditions (Singh *et al*., 1981, 1989, 1990; Malhotra and Saxena, 1993; Singh *et al*., 1995, 1997).

D. The Importance of Phenological Stage on Response to Freezing Range Temperatures

The chickpea crop germinates, matures, senesces, and dies within 100 to 225 days from sowing, depending on environmental conditions before and after flowering, the magnitude of seed yield, and the rate and synchrony of seed filling (Summerfield and Roberts, 1985). The effect of freezing range temperatures can vary in chickpea according to the phenological stage of the plant when the stress occurs. In light of this, we have dealt separately with the effect of freezing on each of the different phenological growth stages.

1. Pre-Emergent (Heterotrophic) Developmental Stage

The germination of dicotyledonous seeds, such as chickpea, starts the growth process of a quiescent or dormant embryo, and is evidenced by the growth of the embryonic axis (de Rueda *et al.*, 1994). Good germination and seedling emergence are important prerequisites for a successful crop and soil and air temperature is one of the key factors affecting seed germination (Chen *et al.*, 1983). In chickpea, 0°C has been proposed to be the base temperature for germination (Singh and Dahliwal, 1972; Siddique *et al.*, 1983; Ellis *et al.*, 1986; Calcagno and Gallo, 1993). Thus, in freezing soils, chickpea will not germinate.

2. Seedling (Autotrophic) Developmental Stage to Early Vegetative Stage

Plants at the autotrophic stage are more vulnerable to temperature stress than those at the heterotrophic stage prior to germination because their endosperm resources have been exhausted. The degree of vulnerability varies with the age of the plant and in chickpea, tolerance to freezing range temperatures has been shown to decrease as the plant progresses from the seedling stage (most tolerant) to flowering (least tolerant) (Wery 1990; Singh *et al.*, 1995). The main effects of freezing temperatures on the developing seedling are related to membrane injury and include reduced respiration and photosynthesis and loss of turgor, resulting in wilting and temperature-induced drought stress.

The effect of early winter sowing is that chickpea plants in the early vegetative stage encounter gradually decreasing temperatures and photoperiods. During the winter season, night temperatures fall below zero and remain low for a long period. Although some studies report lines that withstand temperatures of –12°C in the post-emergence vegetative stage, the minimum temperature at which chickpea generally seem to survive is –8°C (Wery, 1990). It is difficult to determine an exact threshold temperature for field grown chickpea because snow cover can protect plants, while a wind factor can increase injury.

The effect of freezing range temperatures on the duration of the vegetative stage has been shown to have a substantial role in the final yield of the crop. Yield increases exhibited by winter sown chickpea have been ascribed to the longer vegetative growth periods leading to a larger vegetative structure. This larger vegetative structure intercepts photosynthetically active radiation (PAR) more effectively in spring and supports a proportionally larger reproductive sink with adequate partitioning of dry matter (Singh *et al.*, 1997). Also, the reproductive phase of winter sown

chickpea is longer than spring sown chickpea, contributing to higher seed yield. Better utilization of PAR increases total biomass of winter sown chickpea, while retaining a similar harvest index (HI) to that of spring sown chickpea (Keatinge and Cooper, 1983; Siddique and Sedgley, 1986; Singh *et al.*, 1997).

3. Late Vegetative Stage

Unlike the seedling to early vegetative stage of chickpea, which exhibits a relatively high degree of freezing tolerance, there is a high sensitivity to freezing damage at the more advanced vegetative stage (Calcagno and Gallo, 1993; Singh *et al.*, 1993; Singh *et al.*, 1995). This finding has been mirrored in field pea and faba bean (Scarascia-Mugnozza and Marzi, 1979; Étévé, 1985; Murray *et al.*, 1988). Wery (1990) has suggested that the increased sensitivity to freezing range temperatures is due to the plant being unable to set osmoregulation mechanisms in motion during the active growth phase of the plant. The absence of quantitative data in chickpea makes it difficult to prove this hypothesis.

In many crops, the microclimate has been shown to have a significant effect on the degree of damage from low temperatures. In chickpea there are limited data regarding the relationship between morphological parameters, such as growth habit, flower position, leaf shape, hairs, stomata, and fibrovascular structures, and physiological characteristics such as resistance to water due to increased viscosity, temperature, or light stress in the vegetative growth phase (Calcagno and Gallo, 1993). In chickpea, stem elongation during the vegetative stage means that the shoots are in air strata colder than that nearer to the soil surface. It has been suggested that propensity to branch may be a prerequisite for tolerance to freezing range temperatures in chickpea (Murray *et al.*, 1988) and that rosette rather than upright growth habit may be a means of escaping the worst of the freezing temperatures because the microclimate near the soil surface is less severe than that of the higher air strata (Anderson and Markarian, 1968; Acikgoz, 1982; Lawes *et al.*, 1983).

Frost damage tends to be affected by the microclimate, with great variability occurring within paddocks and even on the same plant. Frost conditions can be amplified by climatic conditions such as clear sky, dry atmosphere, and windless conditions (Blum, 1988). Soil type, soil moisture, position in the landscape, and crop density can also have a bearing on the damage caused by a frost event. In some species, crop nutrition has been shown to mediate the effect of freezing range temperatures on the plant. It is thought that fertilization of the plant, and consequent fast growth rates, can exacerbate the effect of freezing, particularly on the part of the plant undergoing elongation. However, the study of the microclimate involves the understanding of the complex relationships between many variables, and the effect of varying the plant population density requires greater understanding.

In chickpea, the duration of the vegetative phase of growth in either short or long photoperiods is negatively related to the mean diurnal temperature, irrespective of the genotype (Roberts *et al.*, 1985). Freezing temperatures at this point in the crop's phenological development therefore can cause considerable damage and yield losses.

4. Reproductive Stage: Anthesis, Pollination, and Pod Set

The onset and duration of flowering in chickpea are functions of genotype, photoperiod, and temperature (Roberts *et al.*, 1985). Flowering is indeterminate and can extend for up to 60 days with leaf initiation and stem elongation continuing into the reproductive period. Small purple or white flowers are produced singly in auxiliary racemes and are highly self-pollinated (98 to 100%) (Knights, 1991). Self-pollination takes place between 1 and 2 days before the flower opens and anthers commonly dehisce between 9 am and 3 pm (Oraon *et al.*, 1977), although this can vary depending on the temperature regime. During the reproductive stage chickpea is far more likely to encounter temperatures within the chilling range as opposed to temperatures within the freezing range. Singh *et al.* (1993) observed that

plants at the reproductive stage do not tolerate freezing temperatures, such as those encountered via a late frost event. Undoubtedly, this is the case for extended periods of freezing range temperatures; however, due to their indeterminate nature, chickpea may be able to recover to flower and set pods following an isolated frost event provided soil moisture conditions are favorable during the subsequent periods.

The time and duration of flowering affects tolerance and the ability to compensate after the frost has occurred. Early flowers are often aborted in chickpea, but if soil moisture is available long-duration cultivars compensate for the loss. Frosts that occur toward the end of the reproductive period following pod-set are more damaging, resulting in the abortion of pods and large yield reduction.

Srinivasan *et al.* (1999) propose that the sensitivity of chickpea to cold temperatures can be attributed to its evolution as a spring crop in West Asia, where flowering and podding occur in progressively increasing temperatures. Consequently, there has been no selection in chickpea for cold tolerance at the reproductive stage, resulting in a high degree of sensitivity to freezing range temperatures especially when planted in autumn/winter.

IV. CHILLING STRESS

Temperatures within the chilling range for chickpea (–1.5°C to 15°C) are common throughout the growing season across almost all chickpea growing areas. In chickpea, the upper limits of the chilling range are quite acceptable and even optimum for early growth in some genotypes, but the reproductive processes can become susceptible to damage from temperatures of ca. 15°C and lower (Khanna-Chopra and Sinha, 1987; Clarke, 2001). Exposure to prolonged periods of temperatures at the lower end of the chilling range can cause poor germination, slow growth, flower shedding, and pod abortion, and in severe cases cell necrosis and plant death. The resulting yield penalty or reduction in harvest index varies dramatically in the field, but in some cases can be substantial.

The change from summer to winter sowing in the countries around the Mediterranean Sea has increased yield up to 70% (Singh *et al.*, 1994). This shift in planting time means the chickpea crop is exposed to far colder temperatures during the early growing stages of the plant than when it is planted in summer. Increased tolerance to chilling range temperatures at flowering has been identified as a highly desirable trait in Australia and parts of the Indian subcontinent (25° to 30° latitude) due to a smaller window of opportunity for spring or summer sowing (Savithri *et al.*, 1980; Saxena *et al.*, 1988; Siddique *et al.*, 1994; Srinivasan *et al.*, 1998; Srinivasan *et al.*, 1999; Siddique *et al.*, 1999). In these growing regions, flower shed and pod abortion due to chilling range temperatures at flowering is a major cause of poor yield. It should be noted that it is the combination of chilling range temperatures at flowering with terminal drought that is the cause of reduced seed yields in chickpea. Early sowing (winter) is essential in these environments in order to achieve high yield potential and avoid terminal soil moisture stress.

A. Mechanisms of Chilling Injury in Plants

For the purpose of this review, we have defined chilling-sensitive plants as being adversely affected by chilling range temperatures. Chilling sensitivity is a characteristic of plants of tropical or subtropical climates (McWilliam, 1983). Varietal differences in susceptibility to chilling injury have also been reported for a number of species (Lyons *et al.*, 1979). All plants able to survive and grow at temperatures between –1.5°C and 15°C are chilling tolerant. It is important to note that there is no sharp distinction between chilling-intolerant and -tolerant plants (Buddenhagen and Richards, 1988), principally because the phenological stage of the plant at the time of exposure can have such a large effect on the sensitivity of the plant.

There has been very little research regarding the mechanisms of chilling injury in chickpea. In the absence of detailed information for chickpea, we have summarized the effect of chilling at the cellular level on the function of sensitive plants in

general (Table 2). We propose that at the cellular level, the processes by which chilling injures plant cells is likely to have commonalities between species. Therefore, the effects observed in other species may be cautiously extrapolated for chickpea, until further research is undertaken to elucidate the exact mechanisms in chickpea.

1. Pre-Emergent (Heterotrophic) Developmental Stage

Chickpea seed sown early in the season in temperate areas such as parts of Turkey, Russia, and Canada are commonly exposed to chilling (3° to 8°C) or even freezing temperatures during germination, which can result in a reduced stand and low seedling vigor (Chen *et al.*, 1983). A number of interacting factors have been recognized as mediating seed response to low germination temperatures. These are the temperatures to which the seed is exposed, the duration of exposure, the germination period in which the low-temperature exposure takes place, the seed moisture content prior to the start of imbibition (water influx), and the genotype. Generally, the longer a germinating seed of a sensitive species is exposed to a chilling temperature, the greater the injury it will sustain (Wolk and Herner, 1982).

Roberts *et al.* (1980) demonstrated that the rate of germination of chickpea seed is inversely related to daily mean temperature. Ellis *et al.* (1986) showed that chickpea exhibits a linear relationship to the mean value of the temperature fluctuation, and that there is a positive correlation between the numbers of seedlings emerged and the average soil temperature and air temperature. Further to this, Calcagno and Gallo (1993) demonstrated that the number of days between sowing and emergence are negatively correlated with average soil temperature. No correlation was observed between the number of seedlings emerged and the day and night temperature sums. There is considerable variation on the recommended optimum temperatures for chickpea germination (Singh and Dahliwal, 1972; Ellis *et al.*, 1986; Calcagno and Gallo, 1993). For the most part, such differences can be ascribed to the genotypes used and the experimental conditions followed. For example, some authors considered the base and maximum temperatures to be those at which germination became zero, whereas others considered them to be those at which germination was inhibited.

It is common practice to maintain chickpea seed in gene banks at temperatures as low as –20°C. Prior to water imbibition, the seed remains unaffected by even these very low temperatures. It is during the process of water imbibition into the seed that suboptimal temperatures become a potentially damaging factor. Chickpea, along with many other chilling-sensitive species, is very sensitive to 'imbibitional chilling injury' resulting from chilling range temperatures during water influx (Tully *et al.*, 1981). In chickpea, Chen *et al.* (1983) observed that the period of greatest sensitivity to cold corresponds to the first 30 min of imbibition. When dry seeds first begin to imbibe water, a variety of intracellular solutes leak out. These leaked substances include amino acids, sugars, organic acids, gibberellic acid, phenolics, and phosphates (Simon, 1974; Simon and Wiebe, 1975; Simon, 1979), suggesting a general leakage of cellular contents. At optimum temperatures, seed leakage declines rapidly as imbibition proceeds. However, the quantity of material lost during seed hydration is a function of initial seed moisture, temperature, and the condition of the testa (Chen *et al.*, 1983; Christiansen and St. John, 1984). For example, in many species tissue moisture content prior to seeding is inversely related to relative cold tolerance (Wolk and Herner, 1982; Murray *et al.*, 1988). In chickpea, Chen *et al.* (1983) demonstrated prehydrating the seed at 20°C prior to sowing reduced the effect of rapid imbibition and helped to protect the seed from chilling injury.

Solute leakage damages the cells on the cotyledonary surface, resulting in tissue death (Powell, 1989). A further consequence of chilling-induced solute leakage is the establishment of an excellent medium around the seed for the growth of soil pathogens. Thus, under field conditions chilled seed is often subject to extensive infestation by soil organisms, leading to a reduction in seedling survival. Fungicidal treatments can help ameliorate this problem, but the high cost is often prohibitive in developing countries. In Australia, this

TABLE 2
Summary of the Effects of Prolonged Chilling Range Temperatures at the Cellular Level in Susceptible Plant Species

Cause	Effect	Reference/s
Enzymes and Oxidative Stress		
Reduced enzyme activity at low temperatures	Impairment of photosynthesis and respiration	Wolk & Herner, 1982; Van Heerden & Kruger, 2000
Antioxidant enzymes suppressed	Excessive level of free radicals - Reactive Oxygen Species (ROS)	Lyons, 1973; Wise & Naylor, 1987; Hodgson & Raison, 1991; Pastori *et al.*, 2000; Prasad, 2001
Oxidative stress - ROS	Lipid peroxidation by ROS and DNA damage	Shewfelt & Erickson, 1991; Prasad, 1996; Prasad, 2001
	Protein oxidation by ROS. Proteases inactivated, toxic levels of oxidised proteins since no degradation	Fucci *et al.*, 1983; Kenis *et al.*, 1989; Levine *et al.*, 1990; Pacifici & Davies, 1990; Stadtman & Oliver, 1991; Stadtman, 1993
Altered Membrane Function		
Membrane transition from liquid crystalline to solid gel phase As affected by lipid composition As affected by lipid peroxidation As affected by water content of tissue	Membrane permeability changes	Lyons & Raison, 1970; McWilliam, 1983; Minorsky, 1985; Lynch, 1990; Nuotio *et al.*, 2001 Wilson & Crawford, 1974; Stepkonus, 1984; 1990; Roughan, 1985; Kenrick & Bishop, 1986; Hugly & Somerville, 1992; Shewfelt, 1992; Riken *et al.*, 1993 Refer lipid peroxidation above Murray *et al.*, 1988; Chen *et al.*, 1983
	Membrane shrinkage and vacuole disruption	Blum, 1988; Ishikawa, 1996
	Leakage of electrolytes from cell	Wery *et al.*, 1993
	Loss of cell turgour	Levitt, 1980; Wang, 1982; Markhart, 1986; Collins *et al.*, 1995
	Impairment of cytoplasmic streaming	Woods *et al.*, 1984
	Impairment of photosynthesis; particularly in strong light (photoinhibition)	Berry & Bjorkman, 1980; Powles *et al.*, 1983; Nösberger, 1984; Peeler & Naylor, 1988a, b, c; Li *et al.*, 1990; Wery *et al.*, 1993; Van Heerden & Kruger, 2000; Nuotio *et al.*, 2001; Smillie *et al.*, 1988
	Alteration/inhibition of protein synthesis and function	McWilliam, 1983; Cooper & Ort, 1988
	Vacuolisation, disruption of cytoplasmic reticulatum, and organelle disorder in general	Christiansen & St John, 1984; Blum, 1988
Reduced ATP availability		
	Reduced protein synthesis	Prasad *et al.*, 1994
	Inhibition of protein transport (e.g. from cytosol into chloroplast)	Leheny & Theg, 1994
	Reduced respiration in mitochondria Respiration by alternative pathway - heat evolution	Prasad *et al.*, 1994 Moynihan *et al.*, 1995

facet of imbibitional damage has been implicated in the poor establishment of some chickpea genotypes in cold, wet soils (Knights and Mailer, 1989).

Desi types generally suffer less damage from low temperatures at germination than kabuli types. The rapidity of imbibition is a factor controlled principally by the thickness of the testa (Tully *et al.*, 1981; Christiansen and St John, 1984). Kabuli types generally have a thinner testa than desi types, resulting in more rapid imbibition of water and consequently greater levels of imbibitional damage. Another factor affecting germination success at cold temperatures is the phenolic content of the seed (Auld *et al.*, 1983; Wery, 1990), which presumably confers fungistatic properties (Wery *et al.*, 1994). When white and brown seeded near isolines of kabuli chickpeas were sown into cold and wet soils, the establishment of white-seeded lines was inferior (Knights and Mailer, 1989). Thus, the poor germination of kabuli types is partly due to their thin white testa and resulting higher level of susceptibility to soil pathogens.

Calcagno and Gallo (1993) suggested that chickpea genotypes suited to autumn and winter sowing should germinate and emerge rapidly in cold soils with suboptimal moisture. Ellis *et al.* (1986) found genotypic differences in the rate of germination with temperature. In view of this existing genetic variability, it should be possible to select genotypes that are resistant to temperature stress during germination. Because there is no association between days to emergence and tolerance to freezing range temperatures, days to emergence cannot be used as a criterion for preliminary selection of lines for cold tolerance (Singh *et al.*, 1995). We suggest that selection techniques for genotypes that exhibit tolerance to freezing range temperatures should initially be conducted under controlled environment conditions to separate the effect of soil moisture stress.

2. Post-Emergent Seedling (Autotrophic) Developmental Stage

In chickpea, sensitivity to freezing and chilling range temperatures increases as the plant progresses from germination to flowering (Wery 1990; Singh *et al.*, 1995). The visual symptoms of chilling injury at the seedling stage can include the inhibition of seedling growth, accumulation of anthocyanin pigments, waterlogged appearance with browning of mesocotyls, and the browning and desiccation of coleoptiles and undeveloped leaves (Prasad, 2001). Nonvisible symptoms include alterations in gene expression, membrane properties, proteins, lipids, carbohydrate composition, and solute leakage. The main effects of chilling range temperatures on the developing seedling are related to membrane injury and include reduced respiration and photosynthesis and loss of turgor, resulting in wilting and cold-induced water stress. It is also evident that the damage caused by rapid imbibition of water into cold-affected seeds carries over to the developing seedling, resulting in reduced vigor.

Exposure to chilling range temperatures during early growth of established seedlings can exert macroscopic formative effects on leaf shape and size, plant height, root development, and floral initiation (Christiansen and St. John, 1984). For example, the growth of kidney bean (*Phaseolus vulgaris* L.) is reduced by 10°C chilling at the 3–leaf stage and intermittent chilling during the lifecycle was shown to reduce yield by as much as 34% (Wierenga and Hagan, 1966). Other effects of chilling on seedling growth are summarized (Figure 3). These include water loss due to increased membrane permeability; slow closure of stomatal openings and reduced root hydraulic conductivity and water uptake through both roots and shoots (Wolk and Herner, 1982; McWilliam *et al.*, 1982). In sensitive species chilling reduces the conductivity of the plasmalemma and tonoplast of the guard cells, rendering the stomata less responsive to changes in leaf water potential. The combination of reduced water uptake and slow closure of stomata under conditions of continued evaporative demand in the light causes a reduction in water potential leading to wilting and, ultimately, to severe tissue dehydration (McWilliam *et al.*, 1982). In a controlled environment, treatments can be applied to ameliorate wilting in cold-affected plants. These include affecting stomatal closure by spraying leaves with ABA or holding plants in the dark prior to chilling to reduce transpiration and retain a more favorable plant water balance. Maintaining a

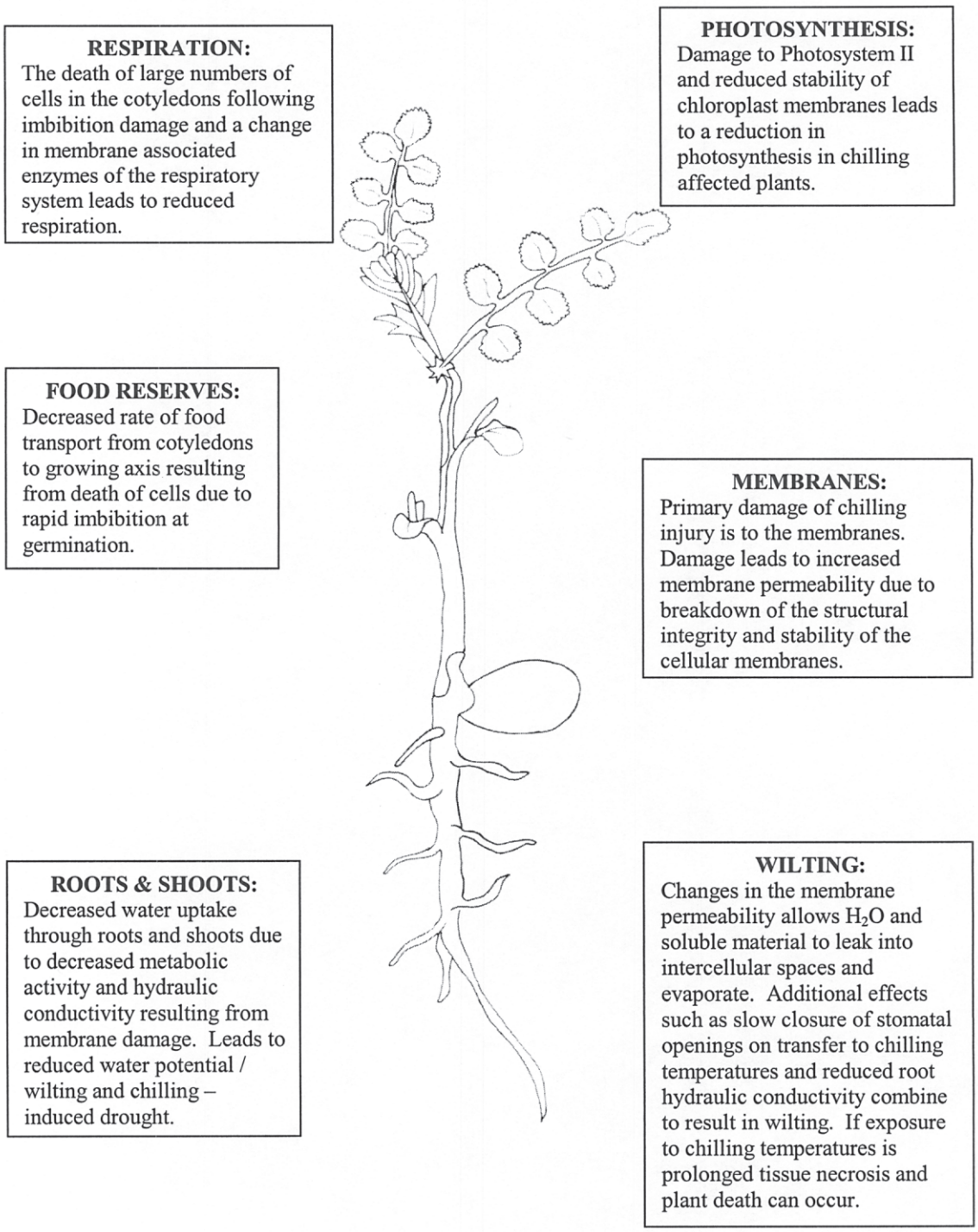

FIGURE 3. Effect of chilling injury on chickpea at the seedling to vegetative phenologcial stage. (Illustration of chickpea seedling adapted from Cubero [1987].)

water-saturated atmosphere around the shoots of chilling-sensitive seedlings at low temperature is another means of restricting loss of water through transpiration to prevent wilting (Wilson, 1976; McWilliam *et al*., 1982). The reduction in the growth of seedlings from damaged seeds is also associated with a slower rate of food reserve transfer from the cotyledons to the growing axis. Mobilization of food reserves is reduced in tissues in which dead cells occur, because enzyme activity is reduced (Powell and Matthews, 1978).

a. Acclimation of Seedlings

Seedlings may be conditioned or hardened by exposure to temperatures slightly above the chilling range (Wolk and Herner, 1982). Plants conditioned in this way are more resistant to subsequent chilling than seedlings transferred directly to chilling temperatures. In readily hardened, chilling-sensitive plants, the degree of fatty acid unsaturation associated with the phospholipid fraction increased during low-temperature conditioning. Hardening does not cause an increase in total leaf fatty acids or in the degree of unsaturation of the glycolipids. The use of low temperature to harden plants has also been shown to prevent the loss of ATP, which normally occurs during chilling (Wilson, 1978). In addition, acclimated maize seedlings were found to have an increased antioxidant defense system that scavenged reactive oxygen species (ROS) during acclimation followed by chilling stress (Prasad, 1996). It appears likely that ROS-induced lipid peroxidation is, at least in part, responsible for increased chilling sensitivity in maize seedlings and, perhaps, all of the chilling-sensitive crop plants (Prasad, 2001).

3. Mild to Late Vegetative Stage

Chilling range temperatures at the mid to late vegetative stage retard growth rate and reduce plant vigor. Siddique *et al.* (1983) describe a close linear correlation between air temperature and the appearance of expanded leaves in chickpea. Khanna-Chopra and Sinha (1987) report the optimum temperature for achieving maximum leaf growth as 10°C to 25°C. These effects are due to the same mechanisms that affect post-emergent seedling growth, that is, reduced respiration and photosynthesis, and in severe cases a loss of turgor and subsequent water stress. Wilson and Crawford (1974) demonstrated the degree of fatty-acid unsaturation and the weight of phospholipids decreased with age in leaves of plants grown at 25°C. They proposed the decrease in unsaturation and weight of the phospholipids may be related to an increase in sensitivity of older leaves to chilling injury.

4. Reproductive Stage: Anthesis, Pollination, and Pod Set

Air temperature and photoperiod have a major influence on the timing of reproductive events in chickpea, with the rate of progress to flowering being a linear function of mean temperature (Summerfield *et al.*, 1980; Roberts *et al.*, 1985). Roberts *et al.* (1985) demonstrated that longer photoperiods at any temperature result in faster accumulation of the thermal sum required for flowering. The earliest flowering genotypes are the least responsive to photoperiod, and there is no apparent correlation between relative sensitivity to temperature and relative sensitivity to photoperiod. This fact, together with the lack of interaction between these environmental parameters, suggests that while the response to temperature and photoperiod both affect time of flowering, they are under separate genetic control.

Nonoptimum temperatures constrain reproductive development in higher plants at various stages, including pollen development, transfer of viable pollen to the stigma, pollen germination and tube growth, and ovule fertilization and seed development. Recent research by Clarke (2001) indicated that pollen germination and vigour is affected by chilling range temperatures. Sensitive and tolerant cultivars can be ascertained under chilling range temperatures from the relative germination of pollen and growth of pollen tubes *in vivo* (Figure 4). Additional demonstrated and hypothesized effects of chilling range temperatures on chickpea reproduction are summarized (Table 3). It is important to note that the sensitiv-

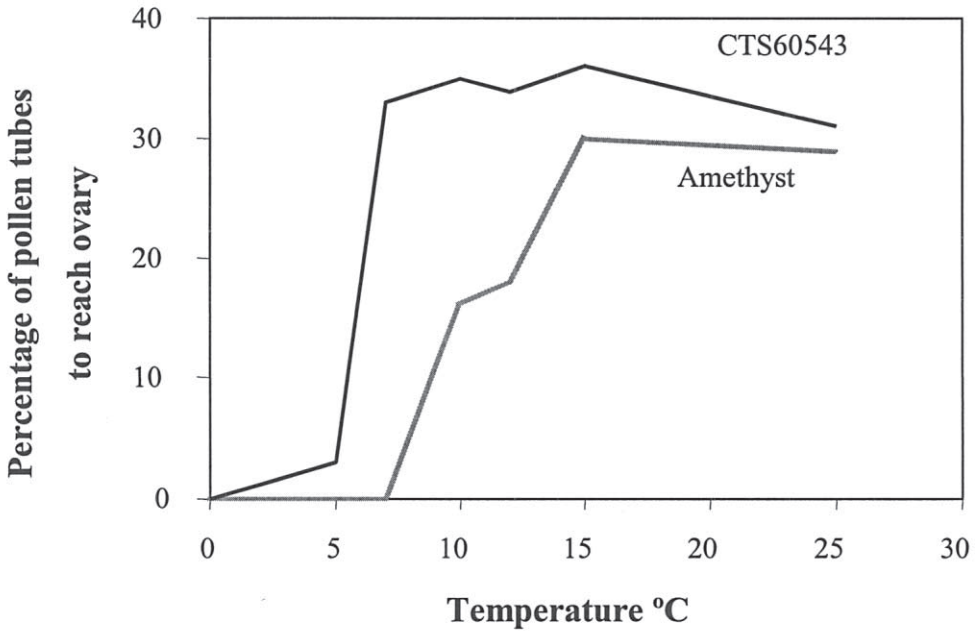

FIGURE 4. Difference in pollen tube growth between chilling sensitive (cv. Amethyst) and chilling tolerant (CTS 60543) chickpea genotypes. (Source: modified from Clarke [2001].)

ity of each of these stages may vary with the severity of stress and the genotype.

Srinivasan *et al.* (1999) propose that the sensitivity of chickpea to cold temperatures can be attributed to the evolution of the species as a spring/summer crop in West Asia, where flowering and podding occur in progressively increasing temperatures. Consequently, there has been no selection in chickpea for cold tolerance at the reproductive stage, resulting in a high degree of sensitivity to both freezing range and chilling range temperatures.

It should be noted that factors other than cold can result in abortion of reproductive structures; however, there is likely an interaction with temperature stress effects. It is suggested that abortion of reproductive structures in chickpeas and other grain legumes can also be caused by water stress (both inadequate or excess) (Keatinge and Cooper, 1983; Siddique and Sedgley, 1986; Leport *et al.*, 1999; Davies *et al.*, 2000). In chickpea, cloudy weather, which can reduce incident solar radiation (Hay and Walker, 1989) and is often associated with rainfall and high humidity, has also been implicated in high abortion rates (Aziz *et al.*, 1960; Varma and Kumari, 1978; Dahiya *et al.*, 1987; Khanna-Chopra and Sinha, 1987), and reduced seed yield (Verghis *et al.*, 1999). In chickpea, experimental work by a number of authors have shown that reduced light intensity on its own can increase abortion of reproductive structures due to the premature bursting of the anther sacs or the adverse effect of the weather on the pollinated stigma and pollen (Chandrasekharan and Parthasarathy, 1963; Khanna-Chopra and Sinha, 1987).

V. BREEDING FOR TOLERANCE TO FREEZING RANGE TEMPERATURES IN CHICKPEA

In 1974/75, the Arid Lands Agricultural Development Program (now International Centre for Agricultural Research in the Dry Areas — ICARDA) undertook investigations into the feasibility of growing chickpea over winter in the Mediterranean region. All 192 genotypes sown in the early winter in the Bekaa Valley of Lebanon survived the winter, despite the occurrence of subzero temperatures on several occasions (Hawtin and Singh, 1984). These initial investigations

TABLE 3
Effect of Chilling Range Temperatures on Chickpea Reproduction

Effect	Description	Authors/s
Flower shedding / floral abortion **Pod shedding / drop**	Sudden low temperatures (0° to10°C) during flowering induces flower shedding, which causes partitioning of assimilates to vegetative growth, resulting in lowered Harvest Index. Major cause of low pod and seed set in subtropical South Asia and Australia.	Sinha, 1977 Varma & Kumari, 1978 Saxena, 1980 Savithri *et al.*, 1980 Siddique & Sedgley, 1986 Saxena *et al.*, 1988 Saxena & Johansen, 1990 Srinivasan *et al.*, 1998
Interrupted pollen tube growth	Temperatures up to 25°C shown to interrupt pollen tube growth. Failure of fertilisation results from poor germination and slow growth of pollen tubes in susceptible genotypes at low temperatures (Figure 4).	Savithri *et al.*, 1980 Calcagno and Gallo, 1993 Clarke, 2001
Lowered pollen viability	Pollen in tolerant genotypes more viable (90%) compared to susceptible genotypes (60%). Two stages of pollen sensitivity at 5 and 9 days before anthesis have been identified.	Srinivasan *et al.*, 1999 Clarke, 2001
Reduced ovule size	Ovules in flowers opening on cool days were 9-45% smaller than warm day ovules – more pronounced in chilling susceptible than tolerant genotypes.	Srinivasan *et al.*, 1999
Reduced pistil size	Heterostyly – the distance between the anther and stigma at the time of flower opening is greater in sensitive than in tolerant genotypes.	
Reduced stigmatic esterase activity	Reduced esterase activity was identified in susceptible genotypes suggesting the stigmas were less receptive to pollen tube growth.	
Delayed anther dehiscence	Anther dehiscence is delayed by chilling temperatures, reducing fertilisation events.	
Reduced pollen germinability	Possibly due to smaller amount of storage material in pollen from sensitive genotypes.	
Reduced pollen turgor	Turgidity is an absolute requirement for germination. Pollen cells with leaking membranes cannot become turgid and germinate.	

spawned more intensive screening tests of chickpea germplasm, and subsequent incorporation of both tolerance to freezing range temperatures at the seedling to early vegetative stage and ascochyta blight resistance has led to the development of cultivars suitable for early winter planting in the Mediterranean areas. Since these early investigations, attention has been focused on the development of effective field screening tests for freezing tolerance, identification, and widespread evaluation of sources of freezing tolerance, incorporation of freezing tolerance into a range of genetic backgrounds and studies on the inheritance of freezing tolerance.

A. Genetics of Tolerance to Freezing at the Seedling Stage

In chickpea, Murray *et al.* (1988) has reported the smallest range of tolerance to freezing temperatures at the seedling and early vegetative stage compared with the other three major grain legumes, faba bean, field pea, and lentil. The inheritance of freezing tolerance in chickpea has been the subject of only two studies (Malhotra and Singh, 1990, 1991). Malhotra (1998) summarized the results of these studies, suggesting that both additive and dominance gene effects, additive being the more important, govern the inheritance of tolerance to freezing at the seedling to early vegetative stage. These genetic studies indicated the presence of additive × additive, additive × dominance, and dominance × dominance interactions. However, additive and dominance parameters alone were not adequate to explain the tolerance to freezing exhibited in these crosses, and it is suggested that genetic interactions may be responsible for the expression of tolerance to freezing in chickpea. Tolerance to freezing was found to be dominant over susceptibility to freezing in the material examined. Further to this, heritability of freezing tolerance was high and visual selection possible under field conditions. Malhotra and Singh (1991) suggest that selection in early generations should be effective due to the high heritability of the trait and the limited number of genes governing the inheritance of tolerance to freezing range temperatures. However, Singh *et al.* (1994) suggest that selection should be delayed until the F_3 or later generations when dominance effects are reduced. Although additive and additive × additive gene effects, which can be fixed, are present in almost all crosses, the presence of dominance and duplicate epistasis would tend to retard the pace of progress through selection in early generations. Thus, selection for tolerance to freezing would be more effective if the dominance and epistatic effects were reduced after a few generations of selfing.

It is important to note that all studies pertaining to genetics of 'cold tolerance' in chickpea have focused on tolerance to freezing at the seedling and early vegetative stages. There is a clear lack of information regarding the genetics of tolerance to chilling range temperatures at other phenological stages in chickpea, for example, germination, flowering, and podding.

B. Screening Techniques to Identify Genotypes with Tolerance to Freezing Range Temperatures

The lack of rapid and accurate screening methods is often a limiting factor in breeding programs aimed at increasing freezing tolerance and available methods are often subjective and impractical (van Heerden and Krüger, 2000). A screening method is effective if it can show distinct differences in injury to a tissue or process (Srinivasan *et al.*, 1996). The efficiency of a screening technique depends on its ability to reproduce the most probable conditions of development of the stress in the target environment. It requires characterization of the most probable stress in the actual position in the plant cycle (Wery *et al.*, 1993) and its reproduction in conditions where screening a large number of genotypes can be made. These two steps are essential for the representativeness and the reproducibility of screening techniques (Wery *et al.*, 1994). It is also essential to identify the stage of development at which freezing stress occurs in the target environment of the breeder. To date in chickpea, field screening has been primarily confined to freezing tolerance at the seedling and early vegetative stages.

Measurement of freezing tolerance is further complicated by the various factors that contribute to winter hardiness and by the transient nature and the variable magnitude of freezing tolerance within and between species. Laboratory- and controlled condition-based methods are particularly beneficial in understanding the physiology of plant responses to freezing stress. They are absolutely necessary to enable reproducibility of experimental conditions. The main drawback of controlled condition screening is that they cannot assess the ability to survive the combined stresses of winter (winter hardiness). Laboratory assessments of freezing tolerance are based on artificial hardening and freezing conditions that may not reflect those experienced by the plants in the field. In addition to this, it is not possible to assess large numbers of germplasm under laboratory conditions. On the other hand, field methods assess overall winter hardiness but often fail to separate the various stresses causing winter kill (Murray *et al.*, 1988) and are not reproducible. However, a large number of germplasm lines can be screened at a given time. For the purposes of this review, the benefits and disadvantages of field and laboratory screening are considered separately.

1. Screening for Freezing Tolerance in the Field

Field trials for freezing tolerance require no specific equipment and allow for screening of thousands of genotypes. The inclusion of an appropriate sensitive check cultivar acts as a control to verify freezing stress conditions. ICARDA field screening tests for freezing tolerance at the early phenological stages have demonstrated it is usually sufficient to sow the same sensitive check after every nine test genotypes (Singh *et al.*, 1989). The rating of the cultivars is made only after the sensitive check suffers 100% mortality. The presentation of the results must be made with due consideration of daily minimum air temperatures, the amount and importance of snow cover, and the ratings of known lines covering the scale of sensitivity (Wery, 1990). The screening test must be repeated successfully in another place or during a second year.

Singh *et al.* (1989) developed a field screening technique for freezing tolerance in chickpea grown in countries surrounding the Mediterranean Sea (Table 4). The authors noted that screening for freezing tolerance was most productive at low elevations in the West Asia North Africa (WANA) region. Data indicated that freezing tolerance in different genotypes of chickpea varied by planting date and that susceptibility was increased with early planting. The effect of freezing was gradually reduced with later planting dates and disappeared in the material planted after mid-December. The advancement of sowing date to mid-autumn, with irrigation for rapid emergence, allows the crop to reach an advanced stage of growth when it is susceptible to freezing injury. Based on these parameters, the following screening technique was proposed for the ICARDA screening trials:

- Sow in October and irrigate to ensure plants enter the winter season in the late vegetative growth stage.
- Sow a susceptible check at frequent intervals (commonly ILC 533).
- Evaluate test lines only if environmental conditions are severe enough to kill the susceptible check.

Using these criteria, extensive screening of chickpea for freezing tolerance during the early vegetative stage has been undertaken. The main constraint to this process is to ensure the target range of low temperature each year. In an effort to attain the required temperatures, screening at high elevations has been used extensively in chickpea. At high elevations, snow cover can protect the plants or the temperature is lower than that of normal growing conditions, thus making it difficult to extrapolate results to the conventional growing regions. An alternative solution suggested by Wery *et al.* (1994) is to screen at lower altitudes in regions with days of -10°C without snow cover. One major drawback of this technique is that the very early sowing date can alter the phenological development pattern of chickpea and hence the susceptibility to freezing range temperatures.

TABLE 4
Field Screening Freezing Tolerance Ratings Developed for Chickpea (Singh *et al.*, 1989)

Rating	Description
1	no visible symptoms of damage
2	highly tolerant, up to 10% leaflets show damage
3	tolerant, 11-20% leaflets show damage
4	moderately tolerant, 21-30% leaflets and up to 20% branches show withering and drying, but no killing
5	intermediate, 41-60% of leaflets and 21-40% branches show withering and drying, up to 5% plant killing
6	moderately susceptible, 61-80% leaflets and from 41-60% branches show withering and drying, 6-25% plant killing
7	susceptible, 81-99% leaflets and 41-80% branches show withering and drying, 26-50% plant killing
8	highly susceptible, 100% leaflets and 81-99% branches showing withering and drying, 51-99% plant killing
9	100% plant killing

The most susceptible stage of the plant should be matched with the period of frost and while allowing the plants enough time to emerge and harden before the first frost (Wery, 1990). Field observations with chickpea have confirmed the observations with other crops that seedlings are not as sensitive to freezing injury as plants at the late vegetative stage of growth (Sutcliffe and Pate, 1977). Thus, a sudden frost event at the latter phenological stage can cause considerable damage to plants and permit better discrimination among genotypes for their freezing tolerance level. Screening germplasm and breeding lines at the late vegetative stage thus has been recommended (Cousin *et al.*, 1993; Singh *et al.*, 1995). This can be obtained with very early fall sowing in the Mediterranean regions. It should be noted that no correlation between frost tolerance at the late vegetative stage and frost tolerance in the earlier seedling stages has been established. Therefore, screening in the late vegetative stage may not be relevant to the majority of production areas where at that stage the crop is unlikely to encounter very low temperatures. As a further precaution it is generally recommended to sow at two dates spaced 1 month apart to more likely meet the correct conditions each year of testing (Wery, 1990; Cousin *et al.*, 1993). Altering the sowing depth of chickpea to either above or below the normal depth of 10 cm did not affect the freezing susceptibility of the crop (Malhotra *et al.*, 1990).

On the other hand, a high level of soil nitrogen is known to decrease relative tolerance to freezing range temperatures in field pea (Kephart and Murray, 1989). Malhotra *et al.* (1995) investigated whether increased soil nitrogen enhanced discrimination between chickpea cultivars for tolerance to freezing range temperatures. Nitrogen application at the rate of 100 kg/ha was effective in enhancing the discrimination between the genotypes. This technique was subsequently recommended as a way to ensure good preliminary field screening of susceptibility to freezing range temperatures in chickpea. Such a high nitrogen application induces excessive vegetative growth and branching. Although it does enhance discrimination between the genotypes,

caution should be exercised in adopting this technique, because in some species the balance of nutrients affects the plants relative freezing tolerance response. The nitrogen interacts with the growth pattern, and hence true genotypic response to freezing range temperatures is difficult to determine.

2. Screening Under Controlled Conditions

In addition to field screening, there are a number of controlled condition- and laboratory-based tests available for the identification of genotypes with tolerance to either freezing range or chilling range temperatures. A number of the more common techniques utilized in other species are summarized (Table 5). While these techniques enable separation of germplasm with tolerance to specific temperature regimes, they do not take into account the other stresses imposed by overwintering, for example, snow cover or ice heaving, and results therefore will need to be confirmed by field screening. Laboratory-based methods may find a broader application in determining genotypes that have tolerance to chilling at the reproductive stages, because field conditions for this stress are easy to replicate. Laboratory-based methods can also be useful in screening a limited number of parental genotypes for a given trait, such as pollen vigor at chilling range temperatures. Appropriate genotypes identified from this screening can then be used in a hybridization program to generate progenies with variable tolerance to either freezing or chilling stress. Only recently have laboratory-based screening for tolerance to low temperatures, either freezing or chilling, been undertaken in chickpea. Clarke (2001) has developed a pollen tube growth screening technique in order to identify germplasm with chilling tolerance at the reproductive stages. This technique compares pollen tube growth of different genotypes at varying temperatures and has been used to select putative chilling-tolerant lines as parents in the breeding program at the Western Australian Department of Agriculture.

VI. SOURCES OF TOLERANCE TO SUBOPTIMAL TEMPERATURES

A. Sources of Tolerance to Freezing Range Temperatures at the Early Vegetative Stage

1. Cultivated Species

Some 20,000 lines of *C. arietinum* are held within the gene backs of ICARDA and the International Centre for Research in the Semi-Arid Tropics — ICRISAT (Singh *et al.*, 1995). To date, breeders and researchers have screened *ca*. 10,000 germplasm and breeding lines and some mutants (derived from the cultigen ILC 482) for tolerance to freezing at the seedling to early vegetative growth stage. Initial work by Singh *et al.* (1981) reported 100% plant survival in four of 3158 kabuli lines sown in October near Ankara, Turkey (Altitude 1055 m). These lines survived snow cover for 47 days during December to March, when the air temperature reached –26.8°C. Three of the landrace lines came from India (ILC 2636, 2479, and 2491) and one from Iran (ILC 410). Under these conditions, nearly 90% of the accessions did not survive. Subsequent screening identified further lines of the cultivated species with some tolerance to freezing (rating of 4); however, all these lines were susceptible to ascochyta blight, which explains why winter sowing had not been adopted previously in the Mediterranean areas.

Since this early research, field screening of chickpea freezing tolerance at the early vegetative stages using the criteria developed by Singh *et al*. (1989) has been extended by ICARDA to 9095 accessions, landraces, and breeding lines (R.S. Malhotra, personal communication). To date, no *C. arietinum* genotype has been identified with a freezing tolerance rating of less than three, and the bulk of the genotypes (ca. 86%) are rated as moderately to completely susceptible (Figure 5). In the cultivated species, the best sources of tolerance to freezing range temperatures at the seedling to vegetative stage come from 13 kabuli lines. There are three tolerant lines with a field rating of three (ILC 1464, 3287, 3465) and 10 moderately tolerant lines with a field rating of four (ILC 3470, 5638, 5663, 5667, 5947, 5951, 5953, 8262,

TABLE 5
A Summary of Controlled Environment and Laboratory-Based Screening Techniques for the Identification of Tolerance to Chilling and/or Freezing Range Temperatures

Technique	Methodology	Example Reference/s
Controlled environment frost screening	Plants are subjected to gradually decreasing temperature over a period of 3 weeks, which is increased when 50% of plants show frost damage.	Cousin *et al.*, 1993
Controlled environment chilling screening	Plants are subjected to chilling temperatures during flowering period and assessment is based on pod and seed set	Srinivasan *et al.*, 1998 Lawlor *et al.*, 1998
Chlorophyll Fluorescence	Based on the fact that fluorescence emission is strong when leaves are irradiated after a dark period but is reduced if stress has damaged the cells.	Klossen & Krause, 1981 Smillie & Hetherington, 1983 Greaves & Wilson, 1987 Calkins & Swanson, 1990 Ratinam *et al.*, 1994
Ion Efflux	Measurement of ion efflux in leaves	Herzog & Olszewski, 1998
Controlled freezing tests	Freezing whole plants/parts under a specific regime and then assessing for visible injury.	Murray *et al.*, 1988
Triphenyl tetrazolium chloride test (TTC)	A cell viability test based on the reducing capacity of living cells. Healthy, non-injured cells can reduce TTC better than injured cells.	Stepkonus & Lanphear, 1967
Leachate test	Based on amount of naturally-occurring compounds that diffuse from cells following cold exposure. Larger amounts of leachate are indicated by greater electrical conductivity.	Dexter *et al.*, 1932 Sukumaran and Weiser, 1972 Palta *et al.*, 1977
Plasmolysis test	Based on the fact healthy cells plasmolyse in a hypertonic solution such as calcium chloride, whereas injured cells do not.	Siminovitch *et al.*, 1964 Olien, 1967
Pollen tube growth*	Cold sensitive genotypes yield pollen with reduced tube growth and fewer pollen tubes reaching the ovule.	Clarke, 2001

* Used in chickpea

8617, and a mutant line 482 M 17033). Singh *et al*. (1992) selected from ILC 3470 to develop the germplasm line ILC 8262. Singh *et al.* (1995) suggest that absence of freezing tolerance in the desi type could be because traditionally they have been grown and selected in a relatively warmer climate, for example, the Indian subcontinent and Ethiopia, than the kabuli type.

For chickpea, Singh and Jana (1993) identified a strong association between freezing tolerance at the early vegetative stage and growth habit, seed size, and plant height. The majority of the freezing tolerant kabuli lines had medium-small size leaves and seeds, medium height, and were late maturing. Unlike the parental germplasm, some of the tolerant breeding lines were medium flowering, large seeded, or both. The pedigree of the breeding lines did not reveal any specific trend for the contribution of freezing tolerance genes. However, diversity for responses to freezing in kabuli accessions were low (Singh and Jana, 1993). This suggests that a greater emphasis should be placed on collecting chickpea germplasm from regions with high frequencies of genotypes resistant to individual stresses, followed by a breeding strategy to increase multiple stress tolerance.

Elsewhere, Wery (1990) found genetic variation for tolerance to freezing temperatures (minimum temperatures between –10° and –18°C) at the seedling to early vegetative stage in southeastern France in 27 chickpea lines, which included several freezing tolerant lines from ICARDA. Saccardo and Calcagno (1990) evaluated 835 chickpea lines in Italy and found that 27 were freezing tolerant. Unlike Singh and Jana (1993), they found no association between plant growth habit (prostrate vs. erect) and freezing tolerance.

Singh *et al.* (1990) suggests that winter sowing in colder temperate regions calls for a still higher level of tolerance to freezing range temperatures in chickpea such as that achieved for field pea (*Pisum sativum*) and faba bean (*Vicia faba*) (Cousin *et al.*, 1985; Picard *et al.*, 1985). Thus, researchers have turned to the wild *Cicer* genepool, in particular the annual species, in an effort to identify further and possibly better sources of freezing tolerance.

2. Wild Cicer

There are many examples of crops improved by means of wide hybridization (Prescott-Allen and Prescott-Allen, 1983). Wild relatives of chickpea are a promising source of genes for tolerance to the major biotic and abiotic stresses affecting yield stability in chickpea (Singh *et al.*, 1989; Singh and Reddy, 1993; Kaiser *et al.*, 1994; Singh and Weigand, 1994; Di Vito *et al.*, 1996). The *Cicer* genus comprises 43 species divided into four sections on the basis of growth habit and morphology, *Monocicer*, *Chamaecicer, Polycicer,* and *Acanthocicer* (Popov, 1928 to 29; van der Maesen, 1972; Muehlbauer, 1993). Eight of these wild species are of particular interest to breeders because they share an annual growth habit and a chromosome number of 2n = 16 with the cultivated species. Two, *C. reticulatum* (the progenitor of *C. arietinum*) and *C. echinospermum* can be routinely hybridized with chickpea and have been utilized for genetic improvement in breeding programs. There have been isolated reports of hybridization between the cultigen and the species of the secondary and tertiary genepools of *Cicer* using conventional techniques (Singh *et al.*, 1994; Verma *et al.*, 1995; Archana-Singh *et al*., 1999; Singh *et al*., 1999); however, the success rate of such crosses is very low. Recently, there has been renewed interest in *in vitro* embryo rescue techniques to facilitate interspecific hybridization in chickpea (Badami *et al.*, 1997; van Dorrestein *et al.*, 1998; Mallikajuna, 1999).

Singh *et al.* (1990) evaluated the reaction to freezing at the seedling to vegetative stage of 137 accessions of the eight wild annual *Cicer* species. The level of tolerance in *C. bijugum* K.H. Rech., *C. echinospermum* P.H. Davis, *C. reticulatum* Ladiz., and *C. pinnatifidum* Jaub. & Spach was significantly superior to the cultivated species; in *C. cuneatum* Hochst ex Rich, *C. yamashitae* Kitamura, and *C. judaicum* Boissier it was inferior, and in *C. chorassanicum* (Bunge) M. G. Popov it was approximately equal to the cultivated species. Among the tolerant accessions, five *C. bijugum* accessions and four *C. reticulatum* accessions had a rating of two (Figure 5) (Singh *et al*., 1995).

Singh *et al*. (1997) hybridized *C. reticulatum* and *C. arietinum*, resulting in improved tolerance

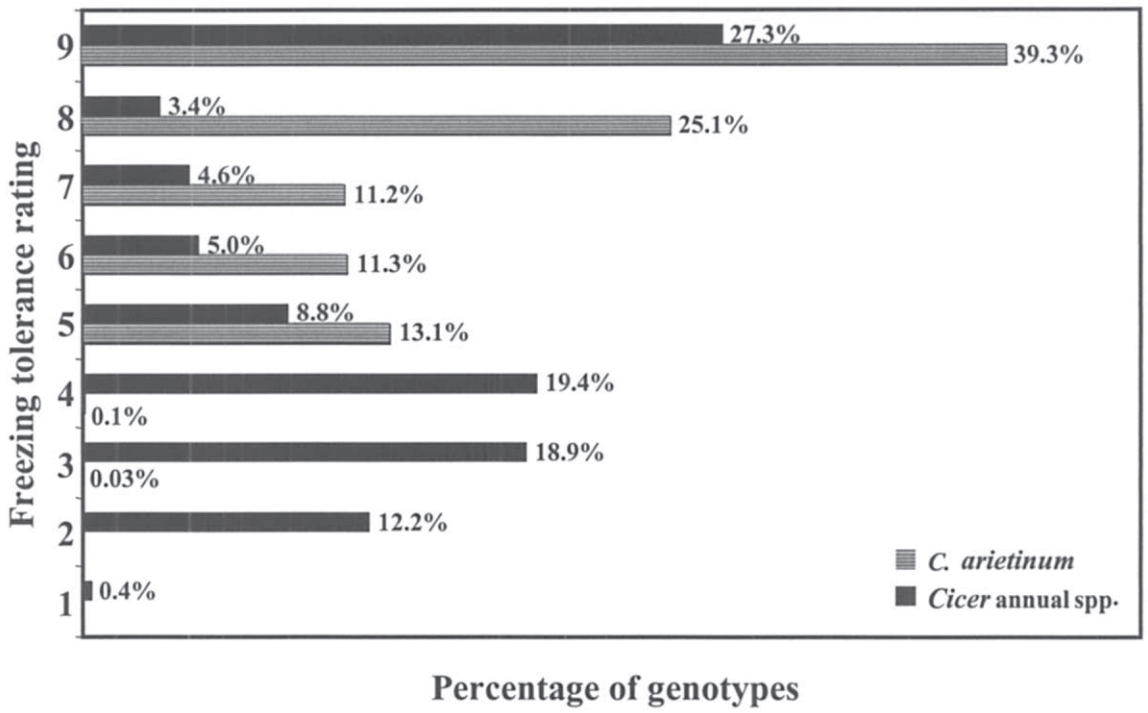

FIGURE 5. Comparison of freezing tolerance ratings at the seedling to early vegetative stage within the cultivated and the wild annual *Cicer* germplasm. (Source: Robertson *et al.* (1995), Dr. R.S. Malhotra, personal communication.)

to freezing range temperatures at the seedling to early vegetative stage. One such derived entry (from a cross between cultigen ILC 482 and *C. reticulatum* ILWC 36) when planted in autumn, gave as much as 5700 kg seed ha^{-1} with a biomass of about 13 t ha^{-1} when compared with about 2000 kg ha^{-1} for the best yielding winter sown line. This and other promising lines have been utilized as parental genotypes in ICARDA's breeding program along with wild *Cicer* species to further enhance tolerance to freezing range temperatures and to encourage onset of flowering and pod setting at lower temperatures (Singh *et al.*, 1997; Malhotra, 1998).

More recent field screening for freezing tolerance at the seedling to early vegetative stage undertaken within the ICARDA breeding program has revealed a *C. bijugum* line (ILWC 66) with a freezing tolerance rating of one. Of the accessions and lines tested, 72% of the wild *Cicer* germplasm accessions exhibited freezing tolerance compared to 0.2% of lines from the cultivated species (R.S. Malhotra, personal communication). It is important to note that, as for the cultivated species, there is considerable duplication in the gene bank collections of wild species that may confound these results (Abbo *et al.*, 2003). It is fair to assume, however, that there is a higher level of tolerance in the wild *Cicer* germplasm. This is to be expected given the occurrence of some of these species at high altitudes and cold climates and the absence of continued selection under a Mediterranean-type climate by early farmers. The response of *C. bijugum* and *C. pinnatifidum* is particularly promising because accessions from these two species are also resistant to important chickpea diseases such as ascochyta blight (Singh *et al.*, 1990). It is clear that tolerance to freezing range temperatures in the cultivated species can be improved via the introgression of genes from the wild species (Singh *et al.*, 1995).

B. Sources of Tolerance to Chilling Range Temperatures during the Reproductive Stage

1. Cultivated Species

ICRISAT has released three cultivars that set pods at chilling range temperatures (ICCV 88503, ICCV 88506, ICCV 88510) (ICRISAT Plant

Material Description No. 53). All of these cultivars are derived from a cross involving a common parent, ICC 8923, originating from Russia (Identity: K 1189) (Sethi, S.C. personal communicatin). This parent appears to be contributing the chilling tolerance at pod set in these cultivars. Material derived from this parent is also being utilized in controlled environment studies in Western Australia. These studies have identified two stages of sensitivity to chilling range temperatures in chickpea (Clarke *et al.*, 1998). The first occurs during pollen development in the flower bud, resulting in infertile pollen even in open flowers. The second stage of sensitivity occurs at pollen tube growth. At chilling range temperatures the pollen tubes grow slowly, fertilization is less likely to occur, and the flower often aborts (Clarke, 2001). The rate of pollen tube growth at low temperature is closely related to the chilling tolerance of the whole plant (Plate 2a and 2b). Therefore, this trait can be used to select more tolerant varieties.

Experiments have shown that, except in the case of isolated frost events, it is the average of the day/night temperature that is more important for flowering and pod set rather than any specific effects of either the maximum or minimum temperatures. The critical average daily temperature for abortion of flowers in most varieties currently grown in Australia is about 15°C (Lawlor et al., 1998). New hybrids that set pods at about 13°C are being developed. In the field, chilling tolerant breeding lines set pods 1 to 2 weeks earlier than most current varieties. As well as conventional methods for plant improvement, DNA-based techniques such as amplified fragment length polymorphisms (AFLPs) are also being investigated (Clarke, 2001).

2. Wild Cicer

To our knowledge, there has been very little screening of wild *Cicer* species for tolerance to chilling range temperatures at the reproductive stage. Preliminary screening undertaken under controlled conditions at CLIMA, The University of Western Australia, has indicated that *C. reticulatum*, the wild progenitor of cultivated chickpea, may avoid injury from chilling range temperatures at this stage by delaying flowering until the temperatures have reached a critical level (H. Clarke, unpublished data). Further screening of the wild annual genepool is required before it is known if there is a source/s of chilling tolerance from these species.

The annual *Cicer* species have received the most attention from plant geneticists and breeders. However, it is likely that genes for tolerance to both chilling and freezing range temperatures are present in the wild perennial species. Van der Maesen and Pundir (1984) have indicated that the perennial species *C. microphyllum* is tolerant to freezing range temperatures and may be an important source of tolerance. To date, perennial species have not been utilized in *Cicer* breeding programs, and there is a paucity of information on the availability or cultivation of wild perennial *Cicer* species (Kaiser *et al.*, 1997). Factors contributing to this lack of knowledge include difficulty in seed collection and growth. Many perennial species grow in poorly accessible mountainous areas, and travel to some countries where these species are indigenous has been very difficult or impossible due to political reasons (Kaiser *et al.*, 1997). It is also often difficult to grow and maintain perennial *Cicer* species outside their naturally adapted areas (van der Maesen and Pundir, 1984).

As pointed out by Ocampo *et al.* (1998), the evaluation of wild genetic resources of *Cicer* is only a preliminary step in the exploitation of wild relatives for the genetic improvement of crops. Comprehensive estimation of the breeding value of wild accessions is possible only after their introgression into cultivated genotypes. Genetic reshuffling of diverse taxa, along with breeding procedures that enable breakage of undesirable linkages, may produce agronomically suitable genotypes not expected from parental performances.

VII. NEW TECHNOLOGIES FOR IDENTIFICATION AND INTRODUCTION OF TOLERANCE TO FREEZING AND CHILLING RANGE TEMPERATURES

A. Molecular Markers, Genome Mapping

Genome mapping in chickpea has progressed slowly over the last decade from linkage maps based

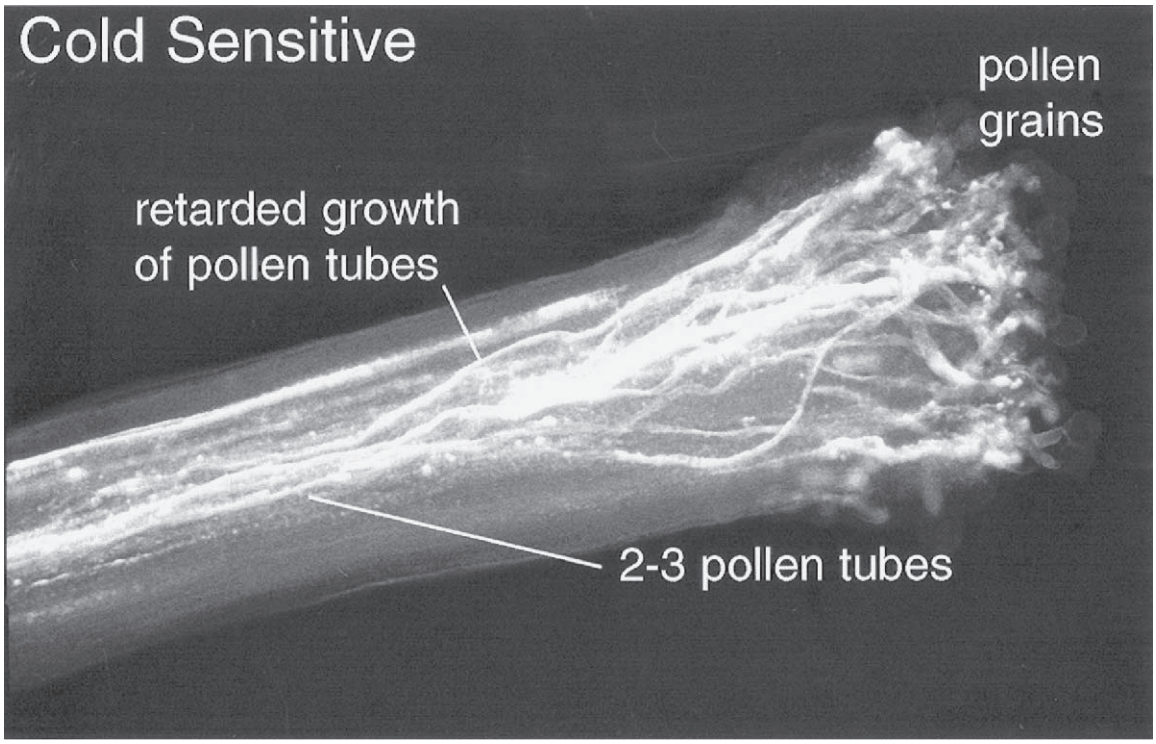

PLATE 2a. Pollen tube growth in cold sensitive cultivar Amethyst following exposure to chilling range temperatures. (Photograph courtesy of Dr Heather Clarke, University of Western Australia.)

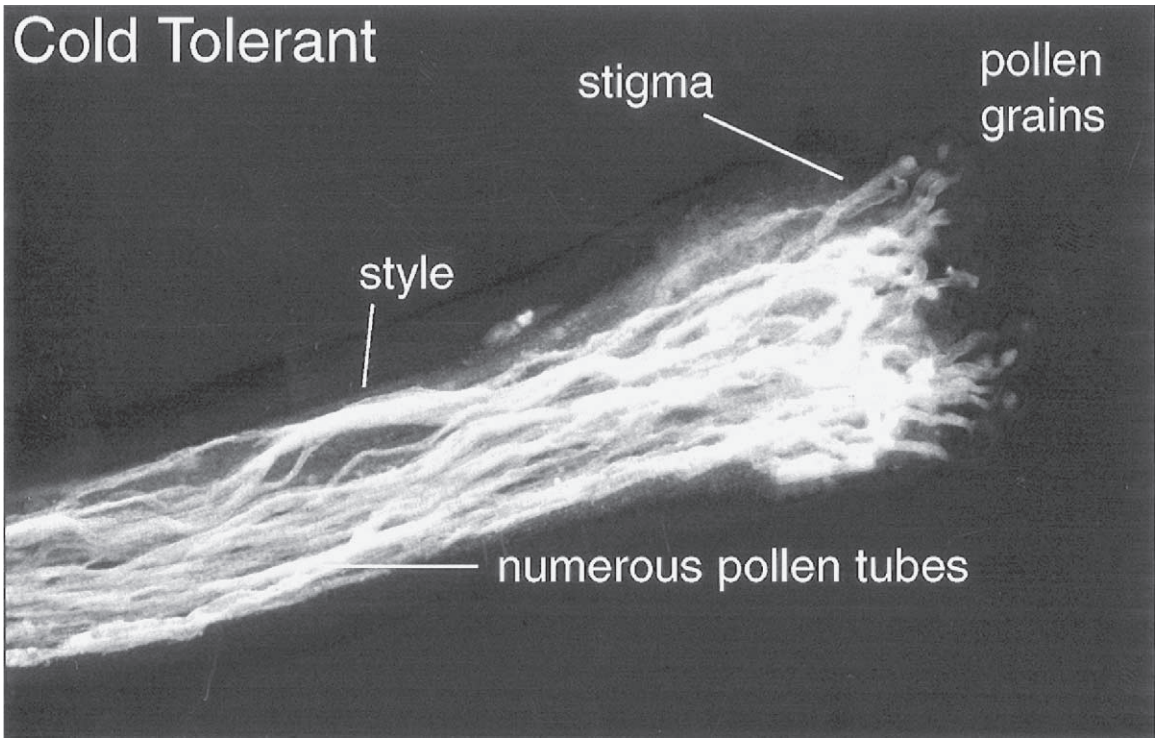

PLATE 2b. Pollen tube growth in cold-tolerant cultivar CTS 60543 following exposure to chilling range temperatures. (Photograph courtesy of Dr Heather Clarke, University of Western Australia.)

on isozymes (Gaur and Slinkard, 1990; Muehlbauer *et al.*, 1990; Kazan *et al.*, 1993), to RFLPs, RAPDs (Udupa *et al.*, 1998; Simon and Muehlbauer, 1997), and to a number of microsatellite-based markers. Weising *et al.* (1992) were the first to demonstrate the presence of simple repetitive sequences (SSRs), or microsatellites, in chickpea. This DNA fingerprinting technique is highly polymorphic, enabling intraspecific mapping in chickpea (Sharma *et al.*, 1995a, 1995b).

More recently, sequence tagged microsatellites (STMS) have been developed for chickpea (Huttel *et al.*, 1999). Subsequent research has shown that some of the repeat sequences are distributed nonuniformly across the chickpea genome, while others are better candidates for genetic mapping (Gortner *et al.*, 1998). Taking this into account, Winter *et al.* (1999) is currently exploiting the immense potential of such STMS markers for genome mapping. In particular, this marker system could play an important role in the construction of an anchor map for chickpea since STMS are transferable across populations segregating for different traits. In the future they could be used to bring together existing linkage maps based on isozymes, RFLPs, and microsatellites. In addition, the occurrence of retrotransposan-like sequences has been demonstrated in the chickpea genome (Staginnus *et al.*, 1999). This family of repetitive DNA sequences is currently being exploited as a marker system in field pea (Ellis *et al.*, 1998; Pearse *et al.*, 2000). Comparative mapping between chickpea and other legumes demonstrates extensive homology between chickpea and lentil (Kazan *et al.*, 1993). These results, along with previous studies, suggest that pea, lentil, and chickpea have several common linkage groups consisting of homologous genes and between chickpea and field pea (Simon and Muehlbauer, 1997; Pandian *et al.*, 2000; *http://jic-bioinfo.bbsrc.ac.uk/bioinformatics-research/comparative/index.html;* T.H.N. Ellis, personal communication). This suggests future researchers could take advantage of the more extensively mapped field pea genome, which can be viewed as the 'model' system for legumes. The use of model plants such as barrel medic (*Medicago trunculata)* should also be explored.

Chickpea possesses eight distinct chromosomes containing approximately 1×10^9 base pairs DNA (Bennett and Smith, 1976) and has the potential to be an excellent system for genome mapping and map-based analysis. DNA markers could play an important role in tagging genes for tolerance to freezing and chilling range temperatures (Eujayl *et al.*, 1999), but to our knowledge there has been no publication of molecular markers linked to this trait in *Cicer*. In other species, the use of molecular markers to enhance the breeding for tolerance to abiotic stresses, including chilling tolerance, has been reported in tomato and maize (Vallejos and Tanksley, 1983; Guse *et al.*, 1988), where isozyme markers were found associated with the trait. Linkage between DNA markers and genes conferring frost tolerance has also been observed. Byrne *et al.* (1997), using a DNA marker-based genetic map of a large population, reported quantitative trait loci influencing frost tolerance in *Eucalyptus*. Eujayl *et al.* (1999) developed a population of recombinant inbred lines (RILs) in lentil from a cross between a tolerant and a susceptible parent. Their purpose was to identify DNA markers linked to a gene conferring tolerance to radiation frost and to determine the mode of inheritance of radiation-frost tolerance in lentil. The results of the *Chi*-square test suggested a single major gene controlling tolerance to radiation-frost injury. In contrast to these results, winter hardiness in cereals appears to be the final expression of a number of interacting component traits and freezing tolerance genes have been assigned to many different chromosomes in wheat, barley, and maize (reviewed by Hayes *et al.*, 1996).

The development of doubled haploid lines of chickpea, which are currently unavailable, would significantly accelerate the development of marker systems for traits such as freezing and chilling tolerance. Researchers at The University of Sasktachewan, Canada, and The University of Western Australia have reported the development of chickpea haploid embryos to the heart-shaped stage from isolated microspore culture, and research is continuing in order to overcome barriers to embryo maturation and germination (Lülsdorf *et al.*, 2001). A detailed genetic map of chickpea would benefit gene cloning, functional genomics,

and the development of transgenic plants. The rapid development of the science of functional genomics offers the opportunity to gain a further understanding of the genetic basis of tolerance to freezing range and chilling range temperatures and the identification of specific genes associated with this stress.

B. Transgenic Approach

The transformation approach, which first became available in chickpea in the early 1990s (Fontana *et al*., 1993), offers an alternative system for the introduction of genes from distant relatives or novel sources for tolerance to freezing range or chilling range temperatures. Researchers at CLIMA have successfully transformed all the major winter grown grain legumes and have developed a routine, variety independent protocol for chickpea (Hamblin *et al.*, 1998). However, the lack of information regarding the actual genes involved in conferring tolerance (chilling or freezing) in chickpea has limited the application of transformation technology in combating this problem.

C. Crop Simulation Models

Soltani *et al*. (1999) developed a simple mechanistic model for the simulation of chickpea phenology, development of leaves as a function of temperature, accumulation of biomass as a function of intercepted radiation, dry matter accumulation of grains as a function of time and temperature, and soil water balance. The use of such physiologically based crop simulation models in management of temperature stress in chickpea deserves greater attention. Our ability to accurately assess the interaction of the numerous processes over the crop life cycle is limited, and the development and use of crop simulation models can help in selecting relevant physiological traits for breeding. Agronomic practices such as time of sowing, sowing density, and irrigation can complement freezing- or chilling-tolerant varieties to ensure the increased productivity of chickpea in regions subject to stress. Crop simulation models can assist in identifying the optimum agronomic practices such as time of sowing to avoid damage to chickpea from suboptimal temperatures at critical stages. In addition, breeders could utilize simulations to choose the best field locations and sowing times for precision screening for either freezing range or chilling range temperatures.

VIII. SUMMARY AND FUTURE DIRECTIONS

The deleterious effect of low-temperature stress in chickpea on plant survival, growth, and biological yield has been described by a number of authors. Despite this, there has been little elucidation of the basic physiological and biochemical changes that occur at the cell and organ level of chickpea as a result of either freezing or chilling range temperatures. A limited amount of research has been undertaken into the effects of low temperature on the reproductive processes of chickpea; however, mechanisms and genetic control have yet to be identified.

Screening of chickpea germplasm, landraces, and breeding lines have confirmed that there is a low level of tolerance to freezing range temperatures available within the cultivated germplasm, consistent with the general lack of genetic diversity within this species. In contrast to the domesticated chickpea, the wild *Cicer* species experienced limited selection pressure and are hence more genetically diverse. Screening of the wild annual *Cicer* species has identified valuable sources of tolerance to freezing stress at the seedling and early vegetative stage, most probably due to their evolution in regions experiencing cold winters.

Further screening of wild *Cicer* species, both annual and perennial, is now required to identify the best source of genes for freezing and chilling tolerance. It appears that the major sources of tolerance have come from specific geographical regions. Greater emphasis therefore should be given to collecting wild accessions from these regions and evaluating current collections on the basis of eco-geographic data. Promising acces-

sions can be targeted as parents in wide hybridization crossing programs; however, greater emphasis is required on the development of *in vitro* techniques to enable crossing of the cultivated species with *Cicer* species outside the primary genepool. Such techniques would also enable the transfer of genes from accessions that exhibit superior levels of resistance compared with the cultivated species for other biotic and abiotic stresses.

In the Indian subcontinent and Australia, where chickpea is primarily a winter crop, production is far more likely to be constrained by chilling range temperatures during the reproductive stages rather than freezing injury during the early vegetative stages. Increased adoption of winter sowing technology and expansion of production regions to higher altitudes in the West Asia–North Africa region means producers in this area will also require cultivars with tolerance to chilling stress at the reproductive stages. There has been far less effort in screening of either cultivated chickpea or the wild *Cicer* species for tolerance to this stress. Further screening of wild *Cicer* accessions and more targeted collection is required for this trait.

Rapid and reliable screening techniques such as molecular markers or pollen selection would be of considerable value to plant breeders. A detailed map of chickpea will greatly facilitate marker-assisted selection. The exploitation of the more extensive maps already available for other legumes such as field pea, lentil, or barrel medic could also indicate potential sites for important genes.

It is clear that suboptimal temperatures in both the freezing and chilling range can be detrimental to chickpea production worldwide. Further effort in screening germplasm, including *Cicer* wild relatives, for tolerance to chilling and freezing range temperatures is now required, as is a better understanding of the mechanisms involved in conferring tolerance to this stress. This information will lead to the development of widely adapted cultivars that will be instrumental in the spread of chickpea cultivation to new production regions.

ACKNOWLEDGMENTS

This review is the outcome of a number of projects on grain legume research funded by Grains Research and Development Corporation, Australia. The authors acknowledge Dr. S.C. Sethi, a visiting fellow from ICRISAT, for his initial discussions about the review, and the Centre for Legumes in Mediterranean Agriculture for financial support in the writing of this manuscript. The authors thank Mr. Ted Knights, Dr. Neil Turner, and Professor Lars Christiansen for their helpful comments, discussions, and suggestions during the preparation of this manuscript.

REFERENCES

Abbo, S., Berger, J., and Turner, N.C. 2003. Evolution to cultivated chickpea: four genetic bottlenecks limit diversity and constrain crop adaptation. *Genome*. (submitted).

Acikgoz, E. 1982. Cold tolerance and its association with seedling morphology and chemical composition in annual forage legumes. II. Vetch (*Vicia*) species. *Z. Pflanzenzuchtung*. **88**(4), 278–286.

Anderson, R.L. and Markarian, D. 1968. The inheritance of winter-hardiness in *Pisum* III. Stem branching in autumn growth. *Euphytica* **17**, 473–478.

Andrews, C.J. 1996. How do plants survive ice? *Ann. Bot*. **78**, 529–536.

Archana Singh, Singh, N.P., and Asthana, A.N. 1999. Genetic potential of wide crosses in chickpea. *Legume Res*. **22**(1), 19–25.

Auld, D.L., Adams, K.J., Swensen, J.B., and Murray, G.A. 1983. Diallel analysis of winterhardiness in peas. *Crop Sci*. **23**, 763–766.

Aziz, M.A., Khan, M.A., Shah, W. 1960. Causes of low setting of seed in gram (*C. arietinum*). *Agric. Pak*. **11**(1), 37–48.

Badami, P.S., Mallikajuna, N., and Moss, J.P. 1997. Interspecific hybridization between *C. arietinum* and *C. pinnatifidum*. *Plant Breed*. **116**, 393–395.

Bennett, M. D. and J. B. Smith. 1976. Nuclear DNA amounts in angiosperms. *Phil. Trans. Royal Soc. Lon*. **274**(Ser 13), 227–274.

Berry, J. and Björkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol*. **31**, 491–543.

Blum, A. 1988. *Plant Breeding for Stress Environments*. Boca Raton, Florida, CRC Press.

Buddenhagen, I.W. and Richards, R.A. 1988. Breeding cool season food legumes for improved performance in stress environments. In: *World Crops: Cool Season Food Legumes*, pp. 81–95. (Summerfield, R.J., Ed.) Dordrecht, Kluwer Academic.

Byrne, M., Murrell, J.C., Owen, J.V., Williams, E.K., and Moran, G.F. 1997. Mapping of quantitative trait loci influences frost tolerance in *Eucalyptus nitens*. *Theor. Appl. Genet.* 95**,** 975–979.

Calcagno, F and Gallo, G. 1993. Physiological and morphological basis of abiotic stress resistance in chickpea. In: *Breeding for Stress Tolerance in Cool-Season Food Legumes*, pp. 293–309. (Singh, K.B. and Saxena, M.C., Eds,) West Sussex, UK., John Wiley & Sons.

Calkins, J.B. and Swanson, B.T. 1990. The distinction between living and dead plant tissue – viability tests in cold hardiness research. *Cryobiology* **27**, 194–211.

Chandrasekharan, S.N. and Parthasarathy, N. 1963. Botany of useful plants — Indian gram. *Madras Agric. J*. **16**, 5–11.

Chen, T.H.H., Yamamoto, S.D.K., Gusta, L.V., and Slinkard, A.E. 1983. Imbibitional chilling injury during chickpea germination. *J. Am. Soc. Horti. Sci*. **108**(6), 944–948.

Christiansen, M.N. and St. John, J.B. 1984. Chemical modification of plant response to temperature extremes. In: *Bioregulators: Chemistry and Uses. Amer. Chem. Soc., Washington, D.C, USA*, pp. 235–243. Washington, USA, USDA.

Clarke, H.J. 2001. Improving tolerance to low temperature in chickpea. In: *4th European Conference on Grain Legumes. Towards the Sustainable Production of Healthy Food, Feed and Novel Products*. Cracow, Poland, July 8–12, 2001, pp. 34–35. European Association for Grain Legume Research, Paris.

Cooper, P. and Ort, D.R. 1988. Changes in protein synthesis induced in tomato by chilling. *Plant Physiol*. **88**, 454–461.

Cousin, R., Burghoffer, A., Marget, P., Vingere, A., and Étévé, G. 1993. Morphological, physiological and genetic bases of resistance in pea to cold and drought. In: *Breeding for Stress Tolerance in Cool-Season Food Legumes*, pp. 311–320. (Singh, K.B. and Saxena, M.C., Eds.) Chichester, UK, John Wiley & Sons.

Cousin, R., Messager, A., and Vingére, A. 1985. Breeding for yield in combining peas. *The Pea Crop: A Basis for Improvement*, pp. 115–129. (Heblethwaite, P.D., Heath, M.C., and Dawkins, T.C.K., Eds.) London, Butterworths.

Cubero, J.I. 1987. Morphology of chickpea. In: *The Chickpea*, pp. 40. (Saxena, M.C. and Singh, K.B., Eds) Oxon, UK., CAB International.

Dahiya, B.S., Ram Kumar, and Waldia, R.S. 1987. Thermoinsensitivity in chickpea. *Int. Chickpea Newsl*. **17**, 37.

Davies S.L., Turner, N.C., Palta, J.A., Siddique, K.H.M., and Plummer, J.A.. 2000. Remobilisation of carbon and nitrogen supports seed filling in chickpea subjected to water deficit. *Aust. J. Agric. Res*. **51**, 855–866.

De Rueda, P.M., Matilla, A.J., Sanchez-Calle, I.M., Bueno, M., and Gallardo, M. 1994. Thermoinhibition alters the polyamine levels in cotyledons and embryonic axes during germination of stratified chickpea seeds. *Plant Sci*. **101**, 143–150.

Dexter, S.T., Tottingham, W.E., and Graber, L.E. 1932. Investigations of the hardiness of plants by measurement of electrical conductivity. *Plant Physiol*. **7**, 63–78.

Di Vito, M., Singh, K.B., Greco, N., and Saxena, M.C. 1996. Sources of resistance to cyst nematode in cultivated and wild *Cicer* species. *Genet. Resour. Crop Evol*. **43**, 103–107.

Ellis, R.H., Covell, S., Roberts E.H., and Summerfield, R.J. 1986. The influence of temperature on seed germination rate in grain legumes. XI. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *J. Exp. Bot*. **37**(183), 1503–1515.

Ellis, T.H.N., Poyser, S.J., Knox, M.R., Vershinin, A.V., and Ambrose, M.J. 1998. Polymorphism of insertion sites of *Ty1–Copia* class retrotransposans and its use for linkage and diversity analysis in pea. *Mol. Gen. Genet*. **260**, 9–19.

Étévé, G. 1985. Breeding for cold tolerance and winter hardiness in pea. In: *The Pea Crop: A Basis for Improvement*, pp. 131–136. (Heblethwaite, P.D., Heath, M.C., and Dawkins, T.C.K., Eds.) London, Butterworths.

Eujayl, I., Erskine, W., Baum, M., and Pehu, E. 1999. Inheritance and linkage analysis of frost injury in lentil. *Crop Sci*. **39**, 639–642.

FAO. "FAOSTAT DATABASE". Food and Agriculture Organisation of the United Nations. 1998. <http://apps.fao.org/lim500/nphapp.pl?Production.Crops.Primary&Domain=SUA&servlet=1. (May 2001).

Fontana, G.S., Santini, L., Caretto, S., Frugis, G., and Mariotti, D. 1993. Genetic transformation in the grain legume *Cicer arietinum* L. (chickpea). *Plant Cell Rep*. **12**, 194–198.

Fucci, L., Oliver, C., Coon, M., and Stadtman, E. 1983. Inactivation of key metabolic enzymes by mixed-function oxidation reactions: possible implication in protein turnover and aging. *Proc. Nat. Acad. Sci. USA* **80**, 1521–1525.

Gaur, P.M. and Slinkard, A.E. 1990. Genetic control and linkage relationships of additional isozyme markers in chickpea. *Theor. Appl. Genet*. **80**, 648–656.

Gortner, G., Nenno, M., Weising, K., Zink, D., Nagl, W., and Kahl, G. 1998. Chromosomal localization and distribution of simple sequence repeats and the *Arabidopsis*-type telomere sequence in the genome of *Cicer arietinum* L. *Chromosome Res*. **6**, 97–104.

Grafius, J.E. 1981. Breeding for winter hardiness. In: *Analysis and Improvement of Plant Cold Hardiness*, pp. 161–174. (Olien, C.R. and Smith, M.N., Eds.) Boca Raton, Florida, CRC Press.

Graham, D. and Patterson, B.D. 1982. Responses of plants to low, nonfreezing temperatures: proteins, metabolism, and acclimation. *Annu. Rev. Plant Physiol*. **33**, 347–372.

Greaves, J.A. and Wilson, J.M. 1987. Assessment of the frost sensitivity of wild and cultivated potato species by chlorophyll fluorescence analysis. *Potato Res*. **30**, 381–395.

Guse, R.A., Coors, J.G., Drolsom, P.N., and Tracy, W.F. 1988. Isozyme marker loci associated with cold tolerance and maturity in maize. *Theor. Appl. Genet*. **76**, 398–404.

Guy, C.L. 1990. Molecular mechanisms of cold acclimation. In: *Environmental Injury to Plants*, pp. 35–61. (Katterman, F., Ed.) London, Academic Press Inc.

Hamblin, J., Barton, J., Atkins, C., Jones, M., Smith, P., Wylie, S., Schroeder, H., Molvig, L., Tabe, L., and Higgins, T.J. 1998. The development and status of transgenic pulses in Australia. In: *3rd European Conference on Grain Legumes. Opportunities for High Quality, Healthy and Value-Added Crops to Meet European Demands*. Valladolid, Spain, November 14–19, 1988, pp. 70–71. European Association for Grain Legume Research, Paris.

Hawtin, G.C. and Singh, K.B. 1984. Prospects and potential of winter sowing of chickpeas in the Mediterranean region. In: *Ascochyta Blight and Winter Sowing of Chickpeas*, pp. 7–16. (Saxena, M.C. and Singh, K.B., Eds.) The Hague, Martinus Nijhoff/ Dr. W. Junk Publishers.

Hay, R.K.M., Walker, A.J. 1989. *An Introduction to the Physiology of Crop Yield*. Essex, U.K, Longman Scientific and Technical.

Hayes, P.M., Chen, F.Q., Kleinhofs, A., Kilian, A., and Mather, E., Eds. 1996. *Barley Genome Mapping and Its Application. Methods of Genome Analysis in Plants*. Boca Raton, Florida, CRC Press.

Herzog, H. 1978. Growth and cold tolerance of *Vicia faba* under various test conditions. 2. CO_2 assimilation and its change after a freezing stress. *Z. Acker Pflanzenbau*. **147**, 111–120.

Herzog, H. 1987. Freezing resistance and development of faba beans as affected by ambient temperature, soil moisture and variety. *J. Agron. Crop Sci*. **159**, 90–100.

Herzog, H. and Olszewski, A. 1998. A rapid method for measuring freezing resistance in crop plants. *J. Agron. Crop Sci*. **181**(2), 71–79.

Hodgson, R.A.J. and Raison, J.K. 1991. Superoxide production by thylakoids during chilling and its implication in the susceptibility of plants to chilling-induced photoinhibition. *Planta*. **183**, 222–228.

Hudák, J. and Salaj, J. 1999. Effect of low temperatures on the structure of plant cells. In: *Handbook of Plant and Crop Stress*, pp. 441–464. (Pessarakli, M., Ed.) Marcel Dekker Inc.

Hughes, M.A. and Dunn, M.A. 1990. The effect of temperature on plant growth and development. *Biotech. Genet. Engin. Rev*. **8**, 161–188.

Hughes, M.A. and Dunn, M.A. 1996. The molecular biology of plant acclimation to low temperature. *J. Exp. Bot*. **47**, 291–305.

Hugly, S. and Somerville, C. 1992. A role for membrane lipid polyunsaturation in chloroplast biogenesis at low temperature. *Plant Physiol*. **99**, 197–202.

Huttel, B., Winter, P., Weising, K., Choumane, W.K., Weigand, F., and Kahl, G. 1999. Sequence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). *Genome* **42**, 210–217.

Ishikawa, H.A. 1996. Ultrastructural features of chilling injury: injured cells and the early events during chilling of suspension-cultured mung bean cells. *Am. J. Bot*. **83**, 825–835.

Kaiser, W.J., Alcalá-Jiménez, A.R., Hervás-Vargas, A., Trapero-Casas, J.L., and Jiménez-Diaz, R.M. 1994. Screening of wild *Cicer* species for resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*. *Plant Dis*. **78**, 962–967.

Kaiser, W.J., Hellier, B.C., Hannan, R.M., and Muehlbauer, F.J. 1997. Growing techniques and conservation of wild perennial *Cicer* species in the U.S. Pacific Northwest. *Int. Chickpea Pigeonpea Newsl*. **4**, 7–8.

Kazan, K., Muehlbauer, F.J., Weeden, N.F., and Ladizinsky, G. 1993. Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). *Theor. Appl. Genet*. **86**, 417–426.

Keatinge, J.D.H. and Cooper, P.J.M. 1983. Kabuli chickpeas as a winter-sown crop in northern Syria: moisture relations and crop productivity. *J. Agric. Sci*. **111**, 233–254.

Kenis, J.D., Morlans, J.D., and Trippi, V.S. 1989. Effect of hydrogen peroxide on nitrate reductase activity in detached oat leaves in darkness. *Physiol. Planta* **76**, 216–220.

Kenrick, J.R. and Bishop, D.G. 1986. The fatty acid composition of phosphatidylglycerol and sulfoquinovosyldiacylglycerol of higher plants in relation to chilling sensitivity. *Plant Physiol*. **81**, 946–949.

Kephart, K.D. and Murray, G.A. 1989. Nitrogen, phosphorus and moisture effects on acclimation of winter peas. *Can. J. Plant Sci*. **69**(4), 1119–1128.

Khanna-Chopra, R. and Sinha, S.K. 1987. Chickpea: physiological aspects of growth and yield. In: *The Chickpea*, pp. 163–189. (Saxena, M.C. and Singh, K.B., Eds.) Wallingford, U.K., CAB International.

Klossen, R.J. and Krause, G.H. 1981. Freezing injury in cold-acclimated and unhardened spinach leaves. XI. Effects of freezing on chlorophyll fluorescence and light scattering reactions. *Planta* **151**, 347–352.

Knights, E.J. 1991. Chickpea. In: *New Crops. Agronomy and Potential of Alternative Crop Species*, pp. 27–38. (Jessop, R.S. and Wright, R.L., Eds.) Melbourne, Inkata Press.

Knights, E.J. and Mailer, R.J. 1989. Association of seed type and colour with establishment, yield and seed quality in chickpea (*Cicer arietinum*). *J. Agric. Sci*. **113**, 325–330.

Lawes, D.A., Bond, D.A., and Poulson, M.H. 1983. In: *The Faba Bean*, pp. 23–26. (Hebblethwaite, P.D., Ed.) Cambridge, UK, Cambridge University Press.

Lawlor, H.J., Siddique, K.H.M, Sedgley, R.H., and Thurling, N. 1998. Improving cold tolerance and insect resistance in chickpea and the use of AFLPs for the identification of molecular markers for these traits. *Acta Hortic*. **461**, 185–192.

Leheny, E.A. and Theg, S.M. 1994. Apparent inhibition of chloroplast protein import by cold temperatures is due

to energetic considerations not membrane fluidity. *Plant Cell*. **6**, 427–437.

Leport, L., Turner, N.C., French, R.J., Barr, M.D., Duda, R., Davies S.L., Tennant, D., and Siddique, K.H.M. 1999. Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *Eur. J. Agron*. **11**, 279–291.

Levine, R.I., Garland, D., Oliver, C., Amici, A., Climent, I., Lenz, A., Ahn, B., Shaltiel, S., and Stadtman, E.R. 1990. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol*. **186**, 464–478.

Levitt, J. 1980. *Response of Plants to Environmental Stresses; Chilling, Freezing and High Temperature Stresses 1*, Academic Press, pp. 497. New York.

Lev-Yadun, S., Gopher, A., and Abbo, S. 2000. The cradle of agriculture. *Science* **288**, 1602–1603.

Li, G., Knowles, P.F., Murphy, D.J., and Marsh, D. 1990. Lipid-protein interactions in thylakoid membranes of chilling-resistant and –sensitive plants studied by spin label electron spin resonance spectroscopy. *J. Biol. Chem.* **265**, 16867–16872.

Lülsdorf, M., Dament, T., Croser, J., Warkentin, T., and Vandenberg, A. 2001. Development of microspore culture technology for the production of doubled-haploid lines of chickpea (*Cicer arietinum* L.). *Proceedings of the North American Pulse Improvement Association*, Fargo, USA, Oct 28th to 30th, pp. 17.

Lynch, D.V. 1990. Chilling injury in plants: the relevance of membrane lipids. In: *Environmental Injury to Plants*, pp. 17–34. (Katterman, F., Ed.) Academic Press, London.

Lyons, J.M. 1973. Chilling injury in plants. *Annu. Rev. Plant Physiol.* **24**, 445–466.

Lyons, J.M. and Raison, J.K. 1970. Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. *Plant Physiol*. **45**, 386–389.

Lyons, J.M., Raison, J.K., and Stepkonus, P.L. 1979. The plant membrane in response to low temperature: an overview. In: *Low-Temperature Stress in Crop Plants: The Role of the Membrane*, pp. 1–24. (Lyons, J.M., Graham, D., and Raison, J.K., Eds.) Academic Press, New York.

Malhotra, R.S. 1998. Breeding chickpea for cold tolerance. In: *3rd European Conference on Grain Legumes. Opportunities for High Quality, Healthy and Value-Added Crops to Meet European Demands*. Valladolid, Spain, November 14–19, 1988, pp. 152. European Association for Grain Legume Research, Paris.

Malhotra, R.S. and Saxena, M.C. 1993. Screening for cold and heat tolerance in cool-season food legumes. In: *Breeding for Stress Tolerance in Cool-Season Food Legumes*, pp. 227–245. (Singh, K.B. and Saxena, M.C., Eds.) Chichester, John Wiley & Sons.

Malhotra, R.S. and Singh, K.B. 1990. The inheritance of cold tolerance in chickpea. *J. Genet. Breed*. **44**, 227–230.

Malhotra, R.S. and Singh, K.B. 1991. Gene action for cold tolerance in chickpea. *Theor. Appl. Genet*. **82**, 598–601.

Malhotra, R.S., Singh, K.B., and Saxena, M.C. 1990. Effect of depth of sowing on cold tolerance in chickpea. *Int. Chickpea Newsl*. **22**, 19–21.

Malhotra, R.S., Singh, K.B., and Saxena, M.C. 1995. Effect of nitrogen fertilizer application on cold tolerance in chickpea. *Int. Chickpea Pigeonpea Newsl*. **2**, 24–25.

Mallikajuna, N. 1999. Ovule and embryo culture to obtain hybrids from interspecific incompatible pollinations in chickpea. *Euphytica* **110**, 1–6.

Markhart, A.H. III. 1986. Chilling injury: a review of possible causes. *HortSci*. **21**, 1329, 1333.

McWilliam, J.R. 1983. Physiological basis for chilling stress and the consequences for crop production. In: *Crop Reactions to Water and Temperature Stresses in Humid, Temperate Climates*, pp. 113–132. (Raper, C.D., Jr. and Kramer, P.J., Eds.) Westview Press, Boulder, Colorado.

McWilliam, J.R., Kramer, P.J., and Musser, R.L. 1982. Temperature-induced water stress in chilling-sensitive plants. *Aust. J. Plant Physiol*. **9**, 343–352.

Minorsky, P.V. 1985. An heuristic hypothesis of chilling injury in plants: a role for calcium as the primary physiological transducer of injury. *Plant, Cell Environ*. **8**, 75–94.

Moynihan, M.R., Ordentlich, A., and Raskin, I. 1995. Chilling-induced heat evolution in plants. *Plant Physiol*. **108**, 995–999.

Muehlbauer, F.J. 1993. Use of wild species as a source of resistance in cool-season food legume crops. In: *Breeding for Stress Tolerance in Cool-Season Food Legumes*, pp. 359–372. (Singh, K.B. and Saxena, M.C., Eds.) John Wiley & Sons, Chichester, UK.

Muehlbauer, F.J., Simon, C.J., Spaeth, S.C. and Haddad, N.I. 1990. Genetic improvement of chickpea: key factors to be considered for a breakthrough in productivity. In: *Chickpea in the Nineties: Proceedings of the Second International Workshop on Chickpea Improvement*, pp. 209–216. (van Rheenen H.A. and Saxena M.C., Eds.) ICRISAT, India**.**

Murray, G.A., Eser, D., Gusta, L.V., and Eteve, G. 1988. Winterhardiness in pea, lentil, faba bean and chickpea. In: *World Crops: Cool-Season Food Legumes*, pp. 831–845. (Summerfield, R.J., Ed.) Kluwer Academic, Dordrecht.

Nösberger, J. 1984. Effects of low temperature on photosynthesis and growth characteristics. In: *Efficiency in Plant Breeding*, pp. 163. (Lange, W., Zeven, A.C., Hogenboom, N.G., van Hasselt, P.R., and Woltjes, J., Eds.) Wageningen, Centre for Agricultural Publishing and Documentation.

Nuotio, S., Heino, P., and Tapio Palva, E. 2001. Signal transduction under low-temperature stress. In: *Crop Responses and Adaptations to Temperature Stress*, pp. 151–175. (Prasad, A.S., Ed.) The Haworth Press, Inc., New York.

Ocampo, B., Robertson, L.D., and Singh, K.B. 1998. Variation in seed protein content in the annual wild *Cicer* species. *J. Sci. Food Agric*. **78**, 220–224.

Olien, C.R. 1967. Freezing stresses and survival. *Annu. Rev. Plant Physiol*. **18**, 387–408.

Olien, C.R. and Smith, M.N (Eds.). 1981. In: *Analysis and Improvement of Plant Cold Hardiness*, pp. 215. CRC Press, Boca Raton, Florida.

Oraon, P., Prakash, R., and Haque, Md.F. 1977. Floral biology of chickpea (*Cicer arietinum* L.). *Tropic. Grain. Legume Bull*. **7**, 19–20.

Pacifici, R.E. and Davies, K.J.A. 1990. Protein degradation as an index of oxidative stress. *Methods Enzymol*. **186**, 485–502.

Palta, J.P., Levitt, J., and Stadelmann, E.J. 1977. Freezing injury in onion bulb cells. I. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. *Plant Physiol*. **60**, 393–397.

Pastori, G., Foyer, C.H., and Mullineaux, P. 2000. Low-temperature-induced changes in the distribution of H_2O2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. *J. Exp. Bot*. **51**(342), 107–113.

Pearce, S.R., Knox, M., Ellis, T.H.N., Flavell, A.J., and Kumar, A. 2000. Pea *Ty1–copia* group retrotransposans: transpositional activity and use as markers to study genetic diversity in *Pisum*. *Mol. Gen. Genet*. **263**, 898–907.

Peeler, T.C. and Naylor, A. W. 1988a. The influence of dark adaptation temperature on the reappearance of variable fluorescence following illumination. *Plant Physiol*. **86**, 152–154.

Peeler, T.C. and Naylor, A.W. 1988b. A comparison of the effects of chilling on thylakoid electron transfer in pea (*Pisum sativum* L.) and cucumber (*Cucumis sativus* L.). *Plant Physiol*. **86**, 147–151.

Peeler, T.C. and Naylor, A.W. 1988c. A comparison of the effects of chilling on leaf gas exchange in pea (*Pisum sativum* L.) and cucumber (*Cucumis sativus* L.). *Plant Physiol*. **86**, 143–146.

Picard, J., Duc, G., and Pelletier, R. 1985. Cote d'Or, a highly frost resistant population of *Vicia faba*. *FABIS Newsl*. **13**, 1–2.

Popov, M.G. 1928–29. The genus *Cicer* and its species. *Trudy Prikl. Bot. Genet. Sel*. 21(1), 1–254. Also translated by INSDOC, New Delhi, 1976.

Powell, A.A. 1989. The importance of genetically determined seed coat characteristics to seed quality in grain legumes. *Ann. Bot*. **63**, 169–175.

Powell, A.A. and Matthews, S. 1978. The damaging effect of water on dry pea embryos during imbibition. *J. Exp. Bot*. **29**(112), 1215–1229.

Powles, S.B., Berry, J.A., and Björkman, O. 1983. Interaction between light and chilling temperature on the inhibition of photosynthesis in chilling-sensitive plants. *Plant Cell Environ*. **6**, 117–123.

Prasad, T.K. 1996. Mechanisms of chilling-induced oxidative stress injury and tolerance: changes in antioxidant system, oxidation in proteins and lipids and protease activities. *Plant J*. **10**, 1017–1036.

Prasad, T.K. 2001. Mechanisms of chilling injury and tolerance. In: *Crop Responses and Adaptations to Temperature Stress*, pp. 1–52. (Basra, A.S., Ed.) Hawthorne Press, New York.

Prasad, T.K., Anderson, M.D., Martin, B.A., and Stewart, C.R. 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell*. **6**, 65–74.

Prescott-Allen, R. and Prescott-Allen, C. 1983. *Genes From the Wild*. International Institute for Environment and Development, London, United Kingdom.

Ratinam, M., Abd-El-Moneim, A.M., and Saxena, M.C. 1994. Assessment of sensitivity to frost in ochrus chickling (*Lathyrus ocrus* (L) D.C.) by chlorophyll fluorescence analyses. *J. Agron. Crop Sci*. **173**, 338–344.

Riken, A., Dillwith, J.W., and Bergman, D.K. 1993. Correlation between the circadian rhythm of resistance to extreme temperatures and changes in fatty acid composition in cotton seedlings. *Plant Physiol*. **101**, 31–36.

Roberts, E.H., Hadley, P., and Summerfield, R.J. 1985. Effects of temperature and photoperiod on flowering in chickpeas (*Cicer arietinum* L.). *Ann. Bot.* **55**(6), 881–892.

Roberts, E.H., Summerfield, R.J., Minchin, F.R., and Hadley, P. 1980. Phenology of chickpeas (*Cicer arietinum*) in contrasting aerial environments. *Exp. Agric*. **16**, 343–360.

Robertson, L.D., Singh, K.B., Willie Erskine, and Ali M. Abd El Moneim. 1996. Useful genetic diversity in germplasm collections of food and forage legumes from West Asia and North Africa. *Genet. Resour. Crop Evol*. **43**, 447–460.

Roughan, P.G. 1985. Phosphatidylglycerol and chilling sensitivity in plants. *Plant Physiol*. **77**, 740–746.

Saccardo, F. and Calcagno, F. 1990. Considerations of chickpea plant ideotypes for spring and winter sowing. In: *Present Status and Future Prospects of Chickpea Crop Production and Improvement in Mediterranean Countries*. Options Méditerranéennes. Serie A: Séminaires Mediterranéens: No. 9. Zaragoza, Spain: CIHEAM.

Savithri, K.S., Ganapathy, P.S., and Sinha, S.K. 1980. Sensitivity to low temperature in pollen germination and fruit-set in *Cicer arietinum* L. *J. Exp. Bot*. **31**(121), 475–481.

Saxena, M.C. 1990. Problems and potential of chickpea production in the nineties. In: *Chickpea in the Nineties, Proceedings 2nd International Workshop Chickpea Improvement*, pp. 13–25. (Van Rheenen, H.A. and Saxena, M.C., Eds,) Patancheru, India, ICRISAT.

Saxena, M.C. 1993. The challenge of developing biotic and abiotic stress resistance in cool-season food legumes. In: *Breeding for Stress Tolerance in Cool-Season Food Legumes*, pp. 3–14. (Singh, K.B. and Saxena, M.C., Eds.) John Wiley & Sons, Chichester.

Saxena, M.C. and Singh, K.B. 1987. In: *The Chickpea*. (Saxena, M.C. and Singh, K.B., Eds,) Oxon, UK, CAB International.

Saxena, N.P. 1980. Pod setting in relation to temperature in Hissar. *Int. Chickpea Newsl*. **2**, 11.

Saxena, N.P. 1984. Chickpea. In: *The Physiology of Tropical Field Crops*, pp. 419–452. (Goldsworthy, P.R. and Fisher, N.M., Eds,) Wiley, New York.

Saxena, N.P., Johansen, C. 1990. Realised yield potential in chickpea and physiological considerations for further genetic improvment. In: *Proc. Int. Congress Plant Physiol., Vol, 1, 15–20 February, 1988, New Delhi, India*, pp. 279–288. (Sinha, S.K., Sane, P.V., Bhargava, S.C., Agarwal, P.K., Eds.) India, Indian Soc. Plant Physiol. and Biochem.

Saxena, N.P., Johansen, C., Sethi, S.C., Talwar, H.S., and Krishnamurthy, L. 1988. Improving harvest index in chickpea through incorporation of cold tolerance. *Int. Chickpea Newsl*. **19**, 17–19.

Scarascia-Mugnozza, G.T. and Marzi, V. 1979. *Retrospective and prospective views for the Vicia faba crop in southern Italy*, pp. 7–22. Publication, Commission of the European Communities.

Sharma, P.C., Huttel, B., Winter, P., Kahl, G., Gardner, R.C., and Weising, K. 1995a. The potential of microsatellites for hybridisation and polymerase chain reaction-based DNA fingerprinting of chickpea (*Cicer arietinum* L.) and related species. *Electrophoresis* **16**, 1755–1761.

Sharma, P.C., Winter, P., Bunger, T., Huttel, B., Weigand, F., Weising, K., and Kahl, G. 1995b. Abundance and polymorphism of di-, tri-, and tetra-nucleotide tandem repeats in chickpea (*Cicer arietinum* L.). *Theor. Appl. Genet*. **90**, 90–96.

Shewfelt, R.L. 1992. Response of plant membranes to chilling and freezing. In: *Plant Membranes*, pp. 192–219. (Leshem, Y.Y., Ed.) Dordrecht, The Netherlands, Kluwer.

Shewfelt, R.L. and Erickson, M.C. 1991. Role of lipid peroxidation in the mechanism of membrane-associated disorders in edible plant tissue. *Trends Food Sci. Technol*. **2**, 152–154.

Siddique, K.H.M. and Sedgley, R.H. 1986. Chickpea *Cicer arietinum* L., a potential grain legume for southwestern Australia: seasonal growth and yield. *Aust. J. Agric. Res*. **37**, 245–261.

Siddique, K.H.M., Loss, S.P., Reagan, K.L., and Jettner, R.L. 1999. Adaptation and seed yield of cool season grain legumes in Mediterranean environments of southwestern Australia. *Aust. J. Agric. Res.* **50**, 375–387.

Siddique, K.H.M., Marshall, C., and Sedgley, R.H. 1983. Temperature and leaf appearance in chickpea. *Int. Chickpea Newsl*. **8**, 14–15.

Siddique, K.H.M., Sedgley, R.H., Davies, C.L., and Punyavirocha, S. 1994. Genotypic differences in chickpea for pod set at low temperature during flowering. In: *International Symposium on Pulses Research*, April 2–6, pp. 85. New Delhi, India.

Siminovitch, D., Therrien, H., Gfeller, F., and Rheaume, B. 1964. The quantitative estimation of frost injury and resistance in black locust, alfalfa, and wheat tissues by determination of amino acids and other ninhydrin-reacting substances released after thawing. *Can. J. Bot*. **42**, 637–649.

Simon, C.J. and Muehlbauer, F.J. 1997. Construction of a chickpea linkage map and its comparison with maps of pea and lentil. *J. Hered*. **88**, 115–119.

Simon, E.W. 1974. Phospholipids and plant membrane permeability. *N. Phytol*. **73**, 377–420.

Simon, E.W. 1979. Seed germination at low temperatures. In: *Low-Temperature Stress in Crop Plants: The Role of the Membrane*, pp. 37–45. (Lyons, J.M., Graham, D., and Raison, J.K., Eds.) Academic Press, New York.

Simon, E.W. and Wiebe, H.H. 1975. Leakage during imbibition, resistance to damage at low temperature and the water content of peas. *N. Phytol*. **74**, 407–411.

Singh, K.B. 1993. Problems and prospects of stress resistance breeding in chickpea. In: *Breeding for Stress Tolerance in Cool-Season Food Legumes*, pp. 17–37. (Singh, K.B. and Saxena, M.C., Eds.) John Wiley & Sons, Chichester.

Singh, K.B. and Reddy, M.V. 1991. Advances in disease-resistance breeding in chickpea. *Adv. Agron*. **45**, 191–222.

Singh, K.B. and Jana, S. 1993. Diversity for responses to some biotic and abiotic stresses and multivariate associations in kabuli chickpea (*Cicer arietinum* L.). *Euphytica* **68**, 1–10.

Singh, K.B. and Reddy, M.V. 1993. Sources of resistance to ascochyta blight in wild *Cicer* species. *Neth. J. Plant Path*. 99, 163–167.

Singh, K.B. and Saxena, M.C (Eds.). 1993. *Breeding for Stress Tolerance in Cool-Season Food Legumes*. John Wiley & Sons, Chichester, UK.

Singh, K.B. and Weigand, S. 1994. Identification of resistant sources in *Cicer* species to *Liriomyza cicerina*. *Genet. Resour. Crop Evol*. **41**, 75–79.

Singh, K.B., Malhotra, R.S., and Muehlbauer, F.J. 1984. *An Annotated Bibliography of Chickpea Genetics and Breeding 1915–1983*. ICARDA and ICRISAT.

Singh, K.B., Malhotra, R.S., and Saxena, M.C. 1989. Chickpea evaluation for cold tolerance under field conditions. *Crop Sci*. **29**, 282–285.

Singh, K.B., Malhotra, R.S., and Saxena, M.C. 1990. Sources for tolerance to cold in *Cicer* species. *Crop Sci*. **30**, 1136–1138.

Singh, K.B., Malhotra, R.S., and Saxena, M.C. 1992. Registration of ILC 8262, a cold-tolerant germplasm line of chickpea. *Crop Sci*. **32**, 508.

Singh, K.B., Malhotra, R.S., and Saxena, M.C. 1993. Relationship between cold severity and yield loss in chickpea (*Cicer arietinum* L.). *J. Agron. Crop Sci*. **170**(2), 121–127.

Singh, K.B., Malhotra, R.S., and Saxena, M.C. 1995. Additional sources of tolerance to cold in cultivated and wild *Cicer* species. *Crop Sci.* **35**(5), 1491–1497.

Singh, K.B., Malhotra, R.S., Halila, M.H., Knights, E.J., and Verma, M.M. 1994. Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica* **73**, 137–149.

Singh, K.B., Malhotra, R.S., Saxena, M.C., and Bejiga, G. 1997. Superiority of winter sowing over traditional spring sowing of chickpea in the Mediterranean region. *Agron. J.* **89**(1), 112–118.

Singh, K.B., Ocampo, B., and Robertson, L.D. 1998. Diversity for abiotic and biotic stress resistance in the wild annual *Cicer* species. *Genet. Resour. Crop Evol*. **45**, 9–17.

Singh, K.B., Saxena, M.C., and Gridley, H.E. 1981. Screening chickpeas for cold tolerance and frost resistance. In: *Proceedings of the Workshop on Ascochyta Blight and Winter Sowing of Chickpeas*, 4–7 May, ICARDA, Syria, pp. 167–177.

Singh, N.H. and Dhaliwal, G.S. 1972. Effect of soil temperature on seedling emergence in different crops. *Plant Soil*. 37, 441–444.

Singh, N.P., Archana Singh, and Asthana, A.N. 1999. Studies on inter-specific crossability barriers in chickpea. *Indian J. Pulses Res.* 12, 13–19.

Sinha, S.K. 1977. *Food Legumes: Distribution, Adaptability and Biology of Yield.* Rome, FAO.

Smillie, R.M. and Hetherington, S.E. 1983. Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence *in vivo*. Chilling, freezing, ice cover, heat and high light. *Plant Physiol*. **72**, 1043–1050.

Smillie, R.M., Hetherington, S.E., He, J., and Nott, R. 1988. Photoinhibition at chilling temperatures. *Aust. J. Plant Physiol*. **15**, 207–222.

Soltani, A., Ghassemi-Golezani, K., Khooie, F.R., and Moghaddam, M. 1999. A simple model for chickpea growth and yield. *Field Crops Res*. **62**, 213–224.

Srinivasan, A., Johansen, C., and Saxena, N.P. 1998. Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): characterization of stress and genetic variation in pod set. *Field Crops Res.* **57**(2), 179–191.

Srinivasan, A., Takeda, H., and Senboku, T. 1996. Heat tolerance in food legumes as evaluated by cell membrane thermostability and cholorophyll fluorescence techniques. *Euphytica* **88**, 35–45.

Srinivasan, A., Saxena, N.P., and Johansen, C. 1999. Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): genetic variation in gamete development and function. *Field Crops Res.* **60**(3), 209–222.

Stadtman, E.R. 1993. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. *Annu. Rev. Biochem*. **62**, 797–821.

Stadtman, E.R. and Oliver, C.N. 1991. Metal-catalyzed oxidation of proteins. Physiological consequences. *J. Biol. Chem*. **266**, 2005–2008.

Staginnus, C., Winter, P., Desel, C., Schmidt, T., and Kahl, G. 1999. Molecular structure and chromosomal localization of major repetitive DNA families in the chickpea (*Cicer arietinum* L.) genome. *Plant Mol. Biol*. **39**, 1037–1050.

Stepkonus, P.L. 1984. Role of plasma membrane in freezing injury and cold acclimation. *Annu. Rev. Plant Physiol*. **35**, 543–584.

Stepkonus, P.L. 1990. Cold acclimation and freezing injury from a perspective of the plasma membrane. In: *Environmental Injury to Plants*, pp. 1–16. (Katterman, F., Ed.) Academic Press, London.

Stepkonus, P.L. and Lanphear, F.O. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. *Plant Physiol.* **42**, 1423–1426.

Sukumaran, N.P. and Weiser, C.J. 1972. Freezing injury in potato leaves. *Plant Physiol*. **50**, 564–567.

Summerfield, R.J. and Roberts, E.H. 1985. *Cicer arietinum*. In: *Handbook of Flowering, Vol 1*, pp. 92–99. (Halevy, A.H., Ed.) CRC Press, Boca Raton, Florida.

Summerfield, R.J., Minchin, F.R., Roberts, E.H., and Hadley, P. 1980. The effects of photoperiod and air temperature on growth and yield of chickpea. In: *Proceedings of the International Workshop Chickpea Improvement*, pp. 121. (Green, J.M., Nene, Y.L., and Smithson, J.B., Eds.) India, ICRISAT.

Sutcliffe, J.F. and Pate, J.S. 1977. *The Physiology of the Garden Pea*. Academic Press, New York.

Tully, R.E., Musgrave, M.E., and Leopold, A.C. 1981. The seed coat as a control of imbibitional chilling injury. *Crop Sci*. **21**, 312–317.

Turner, N.C., Wright, G.C., and Siddique, K.H.M. 2001. Adaptation of grain legumes (pulses) to water-limited environments. *Adv. Agron.* **71**, 193–231.

Udupa, S.M., Weigand, F., Saxena, M.C., and Kahl, G. 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in ascochyta blight pathogen of chickpea." *Theor. Appl. Genet.* **97**, 299–307.

Vallejos, C.E. and Tanksley, S.D. 1983. Segregation of isozyme markers and cold balance in an interspecific backcross of tomato. *Theor. Appl. Genet.* **66**, 241–247.

Van der Maesen, L.F.G. 1972. *Cicer L. a monograph of the genus with special reference to chickpea (Cicer arietinum L.), its ecology and cultivation.* Veenman and Zonen, Wageningen, The Netherlands.

Van der Maesen, L.J.G. and Pundir, R.P.S. 1984. Availability and use of wild *Cicer* germplasm. *FAO/IBPGR Plant Genet. Resour. Newsl*. **57**, 19–24.

Van Dorrestein, B., Baum, M., and Malhotra, R.S. 1998. Interspecific hybridisation between cultivated chickpea (*Cicer arietinum* L.) and the wild annual species *C. judaicum* Boiss., *C. pinnatifidum Jaub*. & Sp and *C. bijugum* K.H. Rech. In: *3rd European Conference on Grain Legumes. Opportunities for High Quality, Healthy and Value-Added Crops to Meet European Demands*. Valladolid, Spain, November 14–19, 1988,

pp. 362–363. European Association for Grain Legume Research, Paris.

Van Heerden, P.D.R. and Krüger, G.H.J. 2000. Photosynthetic limitation in soybean during cold stress. *South Afr. J. Sci.* 96, 201–206.

Van Rheenen, H.A., Saxena, N.P., Singh, K.B., Sethi, S.C., and Acosta-Gallegos, J.A. 1990. Breeding chickpea for resistance to abiotic stress: what are the problems and how can we solve them? In: *Chickpea in the Nineties: Proceedings of the Second International Workshop on Chickpea Improvement, 4–9th December, 1989, ICRISAT Centre, India*, pp. 239–244. (Van Rheenen, H.A., and Saxena, M.C., Eds.) India, ICRISAT.

Varma, S.K. and Kumari, P. 1978. Nature, extent, periodicity and intensity of flower and pod shedding in gram (*Cicer arietinum* L.). *Legume Res.* **1**(2), 108–114.

Verghis, T.I., McKenzie, B.A., and Hill, G.D. 1999. Effect of light and soil moisture on yield, yield components, and abortion of reproductive structures of chickpea (*Cicer arietinum* L.) in Canterbury, New Zealand. *NZ J. Crop Hortic. Sci.* **27**, 153–161.

Verma, M.M., Ravi, and Sandhu, J.S. 1995. Characterization of the interspecific cross *C. arietinum* L. x *C. judaicum* (Boiss). *Plant Breed*. **114**, 549–551.

Wang, C.Y. 1982. Physiological and biochemical responses of plants to chilling stress. *HortScience* **17**(2), 173–186.

Weising, K., Kaemmer, D., Weigand, F., Epplen, J.T., and Kahl, G. 1992. Oligonucleotide fingerprinting reveals various probe-dependant levels of informativeness in chickpea (*Cicer arietinum)*. *Genome* **35**, 436–442.

Wery, J. 1990. Adaptation to frost and drought stress in chickpea and implication in plant breeding. In: *Present Status and Future Prospect of Chickpea Crop Production and Improvement in the Mediterranean Countries*, pp. 77–85. (Saxena, M.C., Cubero, J.I., and Wery, J. Eds.) Options Méditerranéennes. Serie A: Séminaires Méditerranéens: No. 9, Zaragoza, Spain. (Saxena, M.C., Cubero, J.I., and Wery, J., Eds.) Spain, CIHEAM.

Wery, J., Silim, S.N., Knights, E.J., Malhotra, R.S., and Cousin, R. 1994. Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes. *Euphytica* **73**, 73–83.

Wery, J., Turc, O., and Lecoeur, J. 1993. Mechanisms of resistance to cold, heat and drought in cool-season legumes, with special reference to chickpea and pea. In: *Breeding for stress tolerance in cool-season food legumes*, pp. 271–291. (Singh, K.B. and Saxena, M.C., Eds.) John Wiley & Sons, Chichester.

Wierenga, P.J. and Hagan, R.M. 1966. Effects of cold irrigation water on soil temperature and crop growth. *Calif. Agric.* **20**, 14.

Wilson, J.M. 1976. The mechanism of chill and drought hardening of *Phaseolus vulgaris* leaves. *N. Phytol*. **76**, 257–270.

Wilson, J.M. 1978. Changes in the fatty acids and water status of *Phaseolus vulgaris* leaves at temperatures inducing chill-hardiness. *Opportunities for Chemical Plant Growth Regulation.* British Crop Protection Council, Croydon, UK: Monograph No. 21, 69–74.

Wilson, J.M. and Crawford, R.M.M. 1974. Leaf fatty-acid content in relation to hardening and chilling injury. *J. Exp. Bot.* 25, 121–131.

Winter, P., Pfaff, T., Udupa, S.M., Huttel, B., Sharma, P.C., Sahi, S., Arreguin-Espinoza, R., Weigand, F., Muehlbauer, F.J., and Kahl, G. 1999. Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Mol. Gen. Genet*. **262**, 90–101.

Wise, R.R. and Naylor, A.W. 1987. Chilling-enhanced photoxidation. Evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants. *Plant Physiol*. **83**, 278–282.

Wolk, W.D. and Herner, R.C. 1982. Chilling injury of germinating seeds and seedlings. *HortScience* **17**(2), 169–173.

Woods, C.M., Reid, M.S., and Patterson, B.D. 1984. Response to chilling stress in plant cells. I. Changes in cyclosis and cytoplasmic structure. *Protoplasma* **121**, 8–16.