Chapter 1

INTRODUCTION

Chickpea (Cicer arietinum L.) is one of the earliest grain crops cultivated by man and has been found in Middle Eastern archaeological sites dated at 7500-6800 BC (Zohary and Hopf, 2000). Today, chickpea continues to play an important role in agricultural systems, ranking third behind dry bean (Phaseolus vulgaris L.) and field pea (Pisum sativum L.) in terms of world grain legume production. It is an annual species that originated in southeastern Turkey (Ladizinsky, 1975). It exists as two groups based on seed size, 'macrocarpa' and 'microcarpa'. The 'macrocarpa' are also known as 'Kabuli' (large, rams-head shaped and light brown seeds) and 'microcarpa' as 'Desi' (small, angular and dark-brown colored seeds) by plant breeders in Indian subcontinent.

Cicer, which was classified under tribe Vicieae Alef, was later reported to the monogeneric tribe Cicereae (Kupicha, 1981), which differs from related genera in Vicieae by its glabrous style, inflated pods and glandular pubescence. The genus includes 9 annuals and 34 perennial herbs (Van der Maesen, 1972; Muehlbauer, 1993). Chickpea is a self-pollinated crop and cross-pollination is rare; only 0-1% is reported (Smithson et al., 1985). Although spoken as day neutral, chickpea is a quantitative long day plant and produces flowers in every photoperiod (Smithson et al., 1985). Chromosome number in Cicer species can be generalized as 2n = 2x = 16, although varying numbers both for chickpea (2n = 2x = 14, 16, 24, 32, 33) and other wild species (2n = 2x = 14, 16, 24) have been reported but could not be confirmed by other workers.
Chickpea is grown in tropical, subtropical and temperate regions. Kabuli type is grown in temperate region while Desi type chickpea is grown in semi-arid tropics (Muehlbauer and Singh, 1987; Malhotra et al., 1987). India is the largest producer of chickpea (6.2 × 10^6 t), accounting for 74% of the 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. In India, it is grown as rainfed, post rainy season crop especially in northern part of the country. It is an important winter season food legume of Punjab and is cultivated in about 13 thousand hectares with an annual production of about 10 thousand tons (Singh et al., 1990; Singh and Ocampo, 1993.). It is valued for its nutritive seeds with high protein content (25.3-28.9%) as well as 38-59% carbohydrates, 3% fiber, 4.8-5.5% oil, 3% ash, 0.2% calcium and 0.3% phosphorus (Hulse, 1991).

Chickpeas are propagated from seeds by broad-casting method or (more often) drilled in rows 25-60 cm apart, spaced at 10 cm between seeds, at a depth of 2-12 cm. Seed is sown in mid September to November, rarely later in India and Pakistan, spring (late March-mid April) in Turkey, February-April around the Mediterranean, September-January or April, Ethiopia, depending upon the area and seed type (Smithson et al., 1985).

Chickpea may be grown as a sole crop, or mixed with barley, linseed, mustard, peas, corn, safflower, potato, sweet-potato, sorghum or wheat. In rotation, it often follows wheat, barley or rice (Van der Maesen, 1972). In India, chickpeas are grown as a catch crop in sugarcane fields and often as a second crop after rice. Because, usually it is considered as a dry-land crop, so it develops well on rice lands. Even though a cool season crop, chickpea evolved as a spring crop in West Asia, with flowering and podding occurring in progressively increasing temperatures and thus without selection to confer cold tolerance to these processes (Saxena and Johansen, 1990).

As a winter legume, chickpea can withstand temperatures of 8°C minimum and 22°C maximum during the coldest month. The
optimum temperature range for its normal flowering, hybridization and seed set is 10 to 14°C (as the average minimum temperature) and 25 to 31°C (as the average maximum temperature). In northern parts of the country, where it is grown as a cool/winter season crop, the temperature remains low throughout the season. The minimum temperature may fall below 8°C, some times approach freezing levels. Temperature within the chilling range can limit the growth and vigour of chickpea at all phenological stages but considered most damaging to yield at reproductive stage. Parts of Indian subcontinent and southern Australia are the most affected regions by chilling range temperatures at flowering phase. It does not affect much seedling as well as vegetative growth except for slow growth rate. But during its reproductive phase, it is lethal for normal flowering and pod development, which cause prolonged reproductive phase, floral abortion, poor pollen germination, impaired ovule development, failure in pod set and reduction in seed filling that drastically affects the crop productivity (Singh et al., 1993, 1997). Early maturing lines suffer severe cold damage and do not produce seeds (Singh et al., 1993).

Cold stress induced abnormal reproductive growth in chickpea may occur due to failure of either or both male and female parts (Srinivasan et al., 1998, 1999; Nayyar et al., 2005a). Before anthesis, poor pod set may be due to low pollen viability or high ovule sterility and during anthesis and fertilization, it may be due to failure of pollen to reach or germinate on the stigma, or the failure of the pollen tube to penetrate the stigma and grow in style (Singh et al., 1997). Chilling injury may occur at temperatures below 15°C, but by definition, it occurs in the absence of ice-nucleation in plant cells, i.e. between 15 and 0°C. Cold injury is the physical and/or physiological changes that are induced by exposure to low temperatures. The physiological changes may be considered primary or secondary. The primary injury is the initial rapid response that causes a dysfunction in the plant, but is readily reversible if the temperature is raised to non-chilling conditions. Secondary injuries
are dysfunction that occur, because of the primary injury and may not be reversible. The characteristic visual symptoms are the consequence of secondary cold injury. The cell, thus, perceives the stress by a primary, temperature-induced event, which is readily reversible that causes several metabolic alterations, which are secondary in nature. The resultant imbalance in metabolism initiates autolysis that is irreversible and culminates in cell death (Nilsen and Orcutt, 1996). Cold stress is known to cause injury at various levels of plant organization (Thomashow, 1998). A perusal of literature reveals a direct role of membranes and proteins and indirect participation of many metabolites, called cryo-protectants in regulating the cold tolerance at cellular and sub-cellular levels. The enzymes affecting the metabolism of carbon, and nitrogen have been strongly implicated in altering the thermo-tolerance (Nilsen and Orcutt, 1996).

Plant species can vary visually and temporally for onset of symptom expression due to cold stress. Depending upon the severity of stress and sensitivity of plant, the expression may take few hours to months. Symptoms of cold injury include cellular changes (changes in membrane structure composition and function, decreased protoplasmic streaming, electrolyte leakage and plasmolysis), altered metabolism (increased or reduced respiration, depending upon the severity of the stress, production of abnormal metabolites due to anaerobic conditions) and reduced plant growth (Saltveit and Morris, 1990). The cessation of protoplasmic streaming is an early symptom and one of the earliest observations of cold injury at the cellular level that was made by Sachs (1864) (cited by Saltveit and Morris, 1990). Since protoplasmic streaming requires energy in the form of ATP, cold may limit energy metabolism in the mitochondria. Electron microscopy has shown the mitochondria of sensitive species to be swollen and distorted after cold. The two cellular sites of injury common to all forms of cold injury are metabolism and membrane integrity, representing protein and lipid changes, respectively. The existence of a metabolic imbalance caused
by the primary injury implies that an enzyme or metabolic pathway has been disproportionately inhibited by low temperatures, leading to the accumulation of a toxic intermediate metabolite (nature unknown). This toxin would disrupt membrane integrity and contribute to the expression of other cellular and visual symptoms. An alternative viewpoint is that the membrane itself perceives the low temperature (Thomashow, 1998).

Crop plants experience the lowest temperatures during the night and although cold injury in the dark is not as severe as in the light, it is significant. Though cold at night can disrupt whole chain of electron transport, the capacity of the thylakoid electron transport system remains in excess of metabolic requirements for reducing equivalents (Hallgren and Oquest, 1990). Alternatively, damage may occur to alternative sites including (a) enzymes involved in CO$_2$ fixation (Sassenrath et al., 1990), (b) translocation of sugars from source leaves to sinks leading to feedback inhibition of photosynthesis (Bagnall et al., 1988), or (c) altered water relations, due to slow stomatal responsiveness or reduced hydraulic conductivity of roots (McWilliam et al., 1982). The activity of enzymes that scavenge activated oxygen decreases at low temperature reducing these protective systems (Richter et al., 1990). For example, catalase is photo-inactivated at low temperatures in cold sensitive cucumber and maize (Feierabend et al., 1992). This allows hydrogen peroxide to accumulate and activated oxygen to "escape" to other sites in the chloroplast or cytosol where it initiates degradative reactions. Cold injury is mediated, in part, by oxygen free radicals as agents causing the secondary injuries to membranes and photosystems. The activation of oxygen by the photosystems in the presence of excessive light is probably the major site of free radical production in leaves, but other electron transport systems, including those on the mitochondria or plasmalemma, may contribute, especially in non-photosynthetic tissue. There is experimental evidence to indicate that mitochondria are a major source of superoxide in cold-sensitive plant tissues at low temperatures (Purvis...
et al., 1995). According to Raison and Lyons (1986), oxidative stress must be considered as a secondary response to a primary lesion in a redox enzyme system. The development of cold injury symptoms is frequently coincident with peroxidation of fatty acids (Parkin et al., 1989; Nayyar and Kaushal, 2002; Nayyar and Chander, 2004). Shewfelt and Erickson (1991) proposed that lipid peroxidation would alter the physical properties of membrane lipids and thereby inhibit the function of membrane-bound proteins contributing to the development of visual symptoms of cold injury.

The sensitivity of the plant towards cold stress depends upon its stage of growth (Thomashow, 1998). Reproductive growth being most crucial stage deserves particular attention. Interest in cold tolerance at flowering is largely associated with either the abnormal development of flowers or the failure to set seed or fruit at chilling temperatures. Pollen sterility is a common cause of low fruit set under cold temperatures at flowering in rice, sorghum, strawberry and tomato (Lin and Peterson, 1975; Toriyama and Hinata, 1984). Although reduced viability under low temperature was associated with anther morphology or proline content of pollen (Lin and Peterson, 1975), the exact cause of injury remains unresolved. In rice, meiotic anthers were observed to be more injured than mature anthers (Toriyama and Hinata, 1984). The reduced amount of pollen produced under low temperatures may also cause a reduction in fruit set (Rylski, 1979). Low temperatures may cause deformation of flowers or floral parts (Rylski, 1979) leading to functional sterility or the formation of deformed fruit of low marketing quality. Cold can detrimentally affect flower induction, pollen production and germination, and in some sensitive species will cause male sterility. An example of this injury occurs in soybean where the lowest temperature for fertilization and pod formation varies among cultivars between 9 and 18°C (Hume and Jackson, 1981), a difference that has been traced to pollen abnormalities at low temperature (Lawn and Hume, 1985). This cold sensitivity was related more to low night temperature than to low temperature during the day. Even one cold
night at 8°C was sufficient to inhibit pod formation in field grown plants. The underlying causes for these disturbances are not clear. It was observed that pod set failure and deformation in fruit could be associated with abnormal ovary development (Rylski, 1979).

**Fertilization process, subsequent embryo development and seed filling are potential targets of cold stress.** Cold stress at flowering, as expressed in fertility, is largely a function of floral structure and function under stress. The mechanisms by which pollen viability is maintained under stress are unclear. While the physiological link between low temperatures and formation of nonviable pollen is missing, in sorghum, reduction in proline content was related to pollen sterility (Brooking, 1976). The involvement of proline in cold tolerance in other reproductive organs is worth examining.

Yield losses up to 50% have been reported in many cases (Saxena and Johansen, 1990) depending upon severity of the cold stress and sensitivity of the genotype. Hence, incorporation of cold tolerance in chickpea cultivars is an important prerequisite for its winter sowing (Singh et al., 1990) and it thus becomes imperative to investigate the mechanisms of cold injury to maximize chickpea’s potential yield than its actual yield.

The reproductive failure due to cold injury may have its origin at cellular and sub-cellular levels that are least known and understood in chickpea. The knowledge about these mechanisms may lead to the identification of some reliable indicators of cold tolerance useful for early screening of chickpea genotypes against low temperature stress as well as development of cold tolerant cultivars. Some chickpea genotypes showing differential sensitivity to cold stress at reproductive phase and some tolerant genotypes have been identified by Punjab Agricultural University (Ludhiana), and ICRISAT (Hyderabad) that were employed in the present study.

The important criteria for development of cold tolerant genotypes require characterization of stress, identification of genetic variation and availability of simple screening methods. The present
research work was thus planned to study these aspects in differentially sensitive chickpea genotypes with following objectives:

1.1. OBJECTIVES

• Evaluation of the existing chickpea germplasm for cold tolerance to identify tolerant and susceptible genotypes on the basis of reproductive growth and yield traits
• Characterization of cold injury to reproductive growth at various levels of organization in contrasting genotypes
• Comparative evaluation of certain genotypes under contrasting temperature regimes.