INTRODUCTION

Chickpea (Cicer arietinum L.) is one of the major pulse crops domesticated in the old world ca 7000 years ago. Most probably, it has originated in an area of South eastern Turkey and Syria (Ladizinsky, 1975). The botanical, genetic, and archeological evidences point to chickpea originating with in 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° including west and central Asia, the Indian subcontinent, Southern Europe, Africa (northern parts), Latin America and more recently North America and Australia (FAO, 2001). Three wild annual Cicer species, C. bijugum, C. echinospermum and C. reticulatum, closely related to Chickpea, cohabit with the cultivar in this area. Chickpea is cultivated from the Mediterranean basis to the Indian subcontinent and Southward to Ethiopia and the East African highlands. It has been introduced to the America and gained popularity especially in Mexico. However the crop has greatest significance in the Indian subcontinent.

Chickpea is the only domesticated species under the genus Cicer, which was originally classified in the tribe vicieae of the family Leguminosae and subfamily Papilionoideae. Based on the pollen morphology and vascular anatomy Cicer is now set aside from the members of vicieae and is classified in its own monogeneric tribe, Cicereae Alef. The Cicereae comes closer to the tribe, Trifolieae which
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Chickpea differs from the former in having hypogeal germination, tendrils, stipules free from petiole and nonpapillate unicellular hairs.

Chickpea has one of the highest nutritional compositions of any dry edible legume. On an average chickpea seed has 23% protein, 64% total carbohydrates, 47% starch, 5% fat, 6% crude fiber, 6% soluble sugars and 3% ash.

The mineral components are high in phosphorus (340 mg/100gm), calcium (190mg/100gm), magnesium (140mg/100gm), iron (7mg/100gm), and zinc (3mg/100gm). Chickpea digestibility is the highest among the dry edible legumes. The lipid fraction is high in unsaturated fatty acids, primarily linoleic and oleic acid. In eastern Asia, Chickpeas are sown in October to November. The day length and temperature decrease after planting and only in March, when most of the crop is ripe, does the day length surpass 12 hours. In contrast, the crop in the Western Asia and Mediterranean regions is sown in March to April when the photoperiods are short and temperatures are low initially. In general, long days induce early flowering and short days delay flowering (Eshel, 1967; Vander Maesen, 1972) but differential cultivar response has been noted (Vander Maesen, 1972; Ladizinsky and Adler, 1975).

Cubero (1975) identified two groups of chickpea based on seed size as Macrocarpa and Microcarpa. The Macrocarpa are referred to as Kabuli and the Microcarpa as Desi by plant breeders in the Indian subcontinent and the International crops research institute for the semiarid tropics (ICRISAT). Kabuli chickpea have large seeds that are rounded in shape and of creamy color. The plants are medium to tall with no anthocyanins and have white flowers. They are characteristic of the Mediterranean area and are adapted to spring plantings in Afghanistan, Iran and Countries Westward. In contrast, Desi chickpea have small irregularly shaped seed of rough brown color. The plants are short and contain anthocyanins, the flowers are usually purplish. They
are adapted to winter plantings in Pakistan and countries eastward. Kabuli types are considered relatively more advanced because of their larger seed size and reduced pigmentation achieved through conscious selection. Desi and Kabuli types differ in their dietary fiber components of seed both qualitatively and quantitatively. Kabuli types contain higher amount of dietary fiber, particularly cellulose and hemicellulose. Desi accounts for nearly 90% and Kabuli is grown in around 10% area. Nearly 90% of the crop is cultivated rain-fed mostly on receding soil moisture and on marginal lands. It is adapted to relatively cooler climates. The largest area of adaptation is in the Indian subcontinent. In recent years its cultivation has spread to Australia.

Chickpea (*Cicer arietinum* L.) is a major food legume in many countries. It is cultivated mainly in Algeria, Ethiopia, Iran, India, Mexico, Morocco, Myanmar, Pakistan, Spain, Syria, Tanzania, Tunisia and Turkey. In 1997, it was cultivated worldwide on 11.33 million ha, with 8.80 million tonnes production, of the total world production 91% is produced in Asia, and in Asia, India accounts for 74.8% production of chickpea. The average world productivity of 0.78 t ha\(^{-1}\) is rather low. India (6.2 \times 10^6 t) is by far the largest producer of chickpea accounting for ca 70% of total world production (8.8 \times 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 \times 10^6 t) and field pea (*Pisum sativum* L.) (10.9 \times 10^6 t), (FAO, 2001).

During 2002-2004 the global chickpea production was 80 million tonnes giving an average productivity of 786 kg ha\(^{-1}\). During the past 20 years the global chickpeas are increased by 7% in yield and by 24% in production. Presently, the most important chickpea producing countries are India (64%), Turkey (8%), Pakistan (7%), Iran (3%) Mexico (3%), Myanmar (3%), Ethiopia (2%), Australia (2%) and Canada (1%).
potential seed yield of chickpea is 5 t ha\(^{-1}\). The realized seed yield of 850 kg ha\(^{-1}\) is a result of lack of widely adapted cultivars and susceptibility to several biotic and abiotic stresses. Generally the crop produces excessive vegetative growth under high input conditions and is unable to convert the biomass into high seed yields.

In India, chickpea is grown as a rain fed, post-rainy season crop on 6-7 million ha across the country. In Punjab, it is an important winter season food legume that is cultivated in about 13 thousand ha with annual production of about 10 thousand tonnes (Singh et al., 1990). Chickpea is raised as a winter legume which can withstand temperatures between 8°C minimum and 22°C maximum during the coldest month. In northern parts of India during the flowering period, the average minimum temperatures are 10 to 14°C and maximum temperatures are 25 to 31°C. These ranges are considered optimum for hybridization and seed set in most cultivars.

The most important factors affecting chickpea development are generally temperature, photoperiod and moisture. Temperature (Singh and Dhaliwal, 1972; Siddique and Sedgley, 1986), moisture (Saxena, 1990) and depth of sowing (Saxena, 1987) mainly control the duration from sowing to emergence. After emergence, temperature and photoperiod (Sandhu and Hodges, 1971; Summerfield et al., 1980), coupled with the availability of soil moisture (Khanna-Chopra and Sinha et al., 1987; Singh, 1993) control the rate of progress towards any phenological stage. In chickpeas flowering is considered the critical stage, because environmental conditions that prevail at flowering and the duration of reproductive phase determine to a large extent the percentage of fruit set and the final yield (Savithri et al., 1980; Saxena, 1984).

Even though a cool season food legume chickpea evolved as a spring crop in West Asia, with flowering and podding occurring in
progressively increasing temperature and thus without selection to confer cold tolerance to these processes (Saxena and Johansen, 1990). The production of cool season chickpea is constrained by low temperature across much of its geographical range. Temperatures below 15°C have been demonstrated to cause flower and pod abortion in parts of Indian sub-continents 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). Temperatures with in chilling range can limit the growth and vigour of chickpea at all the phenological stages (Nayyar et al., 2005a). The phenological stages of chickpea growth may be broadly classified as emergence, flowering, pod set, and physiological maturity. Being indeterminate the last three stages occur simultaneously in different parts of plant along with vegetative growth (Summerfield and Wien, 1980; Saxena, 1984).

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; Nayyar et al., 2004, 2005a). Exposure to prolonged period of temperatures at 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 (Croser et al., 2003; Nayyar et al., 2005a).

Yield instability in chickpea has been chiefly attributed to the diverse geographic distribution of the crop and subsequent effects of a number of biotic and abiotic stresses (Saxena, 1990; Wery et al., 1994; Leport et al., 1999). In the Mediterranean type of 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 genotype is largely limited by abortion of flowers and pods in late winter and early spring, which in turn leads to low harvest index. Although delayed sowing can reduce flower and pod abortion associated with low temperatures, seed yield is often limited by terminal soil moistures (Turner et al., 2001).

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 the pollen to reach or germinate on stigma or the failure of the pollen tube to penetrate the stigma and grow in style (Singh et al., 1997).

Chilling temperatures at flowering cause floral abortion in most chickpea cultivars (Nayyar et al., 2005a). Recent evaluations of germplasm showed distinct genotypic differences in pod and seed set at low temperature but the morpho-physiological basis for such variation is unclear (Nayyar et al., 2005c). Observations in the field during cold spells of December and January, and at 15/5°C and 15/0°C (day/night) regimes showed distinct variation in flower morphology, gamete development (viability and size of pollen and ovules) and function (pollen germination and tube growth, ovule viability and fertilization etc.) (Srinivasan et al., 1998, 1999; Saxena and Johansen, 1990; Nayyar et al., 2004, 2005a).

Chilling injury may occur at temperature below 15°C but by definition, it occurs in the absence of ice-nucleation in plant cells i.e. between 15°C and 0°C. Cold injury is the physical and/or physiological changes that are induced by exposure to low temperature. 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 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 membrane and proteins and indirect participation of many metabolites called cryo-protectants in regulating the 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).

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 (Salveit and Morris, 1990). The cessation of protoplasmic streaming is an early symptom and one of the earliest observation of cold injury at the cellular level that was made by Sachs (1864) (cited by Salveit 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 temperature leading to the accumulation of a toxic intermediate metabolite (nature unknown). This toxin intermediate disrupts membrane integrity and contributes 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 temperature 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 at alternative sites including (a) enzymes involved in CO₂ fixation (Sassenrath et al., 1990), (b) translocation of sugars from source leaves to sinks leading to feedback inhibition of photosynthesis (Bagna 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 temperature 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 photo systems. The activation of oxygen by the photo system 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 tissues. 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 a frequently coincident with peroxidation of fatty acids (Parkin et al., 1989; Nayyar and Kaushal, 2002; Nayyar and Chander, 2004).

Interest in chilling tolerance at flowering is largely associated with either the abnormal development of flowers or the failure to set seed or fruit at chilling temperature. Pollen sterility is a common cause for low fruit or seed set under chilling temperature at flowering in plants such as rice, sorghum strawberry and tomato although reduced pollen viability under low temperatures was associated with anther morphology or proline content of pollen. The exact cause of injury is unresolved (Lin and Peterson, 1975; Toriyama and Hinata, 1984). In rice meiotic anthers were observed to be more injured than mature anthers (Toriyama and Hinata, 1984). The time of highest sensitivity to cold coincides with the time of peak tapetal activity; the transition of the tetrad to early uninucleate stage (young microspore). Low temperature at this stage of pollen development results in an accumulation of sucrose in the anthers, accompanied by decreased activity of cell wall bound acid invertase and depletion of starch in mature pollen grains. Expression analysis of two cell wall (OSINV4) and one vacuolar (OSINV2) acid invertase genes showed that OSINV4 is anther specific and down regulated by cold treatment. OSINV4 is transiently expressed in the tapetum cell layer at the young microspore stage and later from the early binucleate stage in the maturing microspores. The down regulation of OSINV4 expression in the tapetum at young microspore may cause a disruption in hexose production and starch formation in the pollen grains. In cold tolerant cultivars, OSINV4 expression is not reduced by cold; sucrose did not accumulate in the anthers and starch formation in the pollen grains was
not affected (Oliver et al., 2005). The reduced amount of pollen produced under low temperature 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. 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 stage 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 temperature 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.

Abscisic acid (ABA), polyamines and osmolytes have been the focus of research in stress biology (Rajaskaran and Blake, 1999). These have been found to be either acting in stress signalling (Sanders et al., 1999; Leing and Giraudat, 1998) or in protection of cellular metabolism (Compos and Pham, 1997). A central role in low temperature signal transduction has been attributed to plant hormone ABA, although much evidence exists for ABA independent cold signalling (Hughes and Dunn, 1996; Xin and Browse, 2000). ABA has been implicated as a cryoprotectant in certain cases and its exogenous application has led to the induction of cold tolerance (Gong et al., 1998). Similarly, polyamines like putrescine (PUT) spermine (SPM) and spermidine (SPD) have a key role in governing cold response (Shen et al., 2000). Proline and glycine betaine are the two major compatible solutes that get accumulated in
response to low temperature (Grey et al., 1997). It has been assumed that the accumulation of the molecules in response during cold acclimation plays an important role in winter survival (Hurry et al., 1994, 1995).

Exogenous application of certain growth regulating chemicals has been found to elevate cold tolerance (Nayyar et al., 2004). The functional involvement of these molecules as putative cryoprotectants is not known yet and required to be explored in chilling sensitive species.

Due to yield instability in chickpea because of cold injury, 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 subcellular 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.

The relative sensitivity of ‘Desi’ and ‘Kabuli’ genotypes to chilling stress is not known at various stages of development that is imperative to induce cold tolerance. The proposed study would examine these aspects including some markers/traits associated with tolerance/susceptibility that can be explored in breeding for cold tolerance.

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 Macrosperma (Kabuli) and Microsperma (Desi) genotypes with following objectives:-
1.1. OBJECTIVES

- Comparative evaluation of Desi and Kabuli genotypes for their relative sensitivity at different developmental phases towards cold stress.
- Assessment of genetic variation for this response.
- Probing the involvement of cryoprotectants like polyamines, proline, glycine betaine and ABA in cold response.