1. INTRODUCTION

Temperature is one of the most important factors determining the global distribution of plants depending upon their respective temperature sensitivities (Repo et al. 2008) and hence affecting their phenology and yield (Hayashi 2001). Plants are exposed to a wide range of temperature fluctuations under natural conditions. The temperature instabilities may be experienced by the plant at micro or macro-environment levels but both are bound to have serious implications on the normal growth and development of a crop. Owing to climate change, it is speculated that plants will be exposed to even more temperature extremes in future (Solomon 2007). A large number of studies have evaluated different plant species for their response to temperature stress such as Arabidopsis thaliana (Deng et al. 2011), tobacco (Nicotiana tabacum; Cui et al. 2013), chickpea (Cicer arietinum; Kaushal et al. 2013) and soybean (Glycine max; Board and Kahlon 2011) but similar information on Lentil (Lens culinaris Medik.) is lacking. Temperature extremes i.e. both high (as heat stress) and low (as cold stress) are injurious to all stages of plant development (Zinn et al. 2010) resulting in severe loss of productivity.

Cold stress has been reported to have very wide implications on various crops grown worldwide (Beck et al. 2007) and many economically important crops are sensitive to temperatures below 10°C (Ouellet 2007). Plants show response to various temperature extremes at molecular, cellular, whole plant and canopy level (Croser et al. 2003). Various abiotic stresses mainly affect the plant by influencing the water relations of the plant at cellular as well as at whole plant level and the effects in response to these stresses may be specific or non-specific i.e. responses may overlap e.g. various drought and cold responses overlap (Beck et al. 2007).
Since the effects of cold stress are multiple and there are various complex mechanisms evoked for injuries and the adaptations exhibited by the stressed plant, the plant’s response may be considered a complex response rather than a single reaction (Beck et al. 2007). Prolonged exposure to chilling temperatures at any stage of development is bound to ultimately affect the yield. Depending upon the intensity and duration of chilling, there can be lower yield or poor quality of the harvest or in severe cases may even lead to complete crop failure (Hudak and Salaj 1999). Chilling injury causes many irreversible changes in the metabolic processes and these changes become evident only during long exposure to chilling periods (Lukatkin 2005). Cold stress can affect the plant at any stage of its life cycle i.e. vegetative as well as reproductive, latter being more susceptible (Nishiyama 1995). Reproductive stage being directly related to the plant’s yield and its products being the major source of food, cold stress during reproductive phase has far reaching socio-economic impacts (Yadav et al. 2004).

Morphological symptoms may be observed in the form of stunted growth, water-soaked (soggy) look, discoloration of shoots and fruits, abnormal curling and crinkling of leaves, cracking and splitting of stems etc. Reproductive stage of angiosperms is quite a complex one involving various sub-stages in it and cold stress has different implications on each one (Staggenborg and Vanderlip 1996; Verheul et al. 1996) but overall responses are negative and expected to reduce the net yield.

Depending upon the intensity and duration of stress experienced, plants may show varying responses to low temperature. Cold-stressed plants adapt by up-regulating the processes leading to the accumulation of osmoprotectants such as proline, glycine betaine, sugars, sugar alcohols, fructans etc. Plants stabilize their membranes
by increasing the ratio of unsaturated fatty acids to saturated ones. Also, the activity of various COR (Cold Responsive Genes; Zhu et al. 2007; Nakashima et al. 2009; Seo et al. 2009; Dong and Liu 2010) and anti-freeze proteins is up regulated (Cutler et al.1989; Moffatt et al. 2006). Cold stress leads to generation of oxidative stress (Prasad et al.1994; Apel and Hirt 2004). The activities of antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase, ascorbic acid peroxidase (APO), glutathione peroxidase, ascorbic acid, glutathione etc. may be thus up-regulated or down-regulated depending upon the intensity of the oxidative stress (Apel and Hirt 2004).

At the other extreme, rising global temperature worldwide have potential implications on overall production of various crops (Hall 2001), as evident from the studies on corn (Zea mays) and soybean (Lobell and Asner 2003) in U.S. where 17% reduction in the yield was recorded whereas in Phillipines, 15% reduction in the yield of rice (Oryza sativa) was observed for every 1°C increase in temperature during growing season (Peng et al. 2004). The effects of heat stress are expected to intensify over a larger area of crop production to adversely affect their performance. High temperature affects the number, morphology and even wall patterns of pollen grains, anther dehiscence as well as pollen metabolism and composition (Sato et al.2002, 2006; Koti et al. 2005). Heat injury is a major limiting factor for the tropical and sub-tropical plants ultimately resulting in decreased plant yield (Wahid 2007). High temperature leads to the breakdown of photosynthetic machinery by disrupting the organization of thylakoids (Gounaris et al. 1983), oxygen Evolving Complex (OEC) of PSII (Strasser 1997) and also reduced RUBISCO (ribulose-1,5-bisphosphate carboxylase) activity (Law and Crafts-Brandner 1999). Morphologically, the symptoms may be visualized as sunburn and scorching of aerial parts of the plant, leaf senescence and abscission,
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retarded shoot and root growth, fruit discoloration and damage and hence reduced yield (Guilioni et al. 1997; Ismail and Hall 1999; Vollenweider and Gunthardt-Goerg 2005).

Plants show varying response to heat stress depending on the intensity, duration, and rate of temperature change (Wahid et al. 2007). It induces several physiological responses in the plant by up-regulating the production of osmotins, dehydrins and production of various heat-shock proteins, the production of various compatible solutes for stabilizing proteins and biomembranes (Lee et al. 2000; Sung et al. 2001), altering the levels and compartmentalization of various biomolecules, especially the hormones (Maestri et al. 2002). Heat stress induces production of reactive oxygen species (ROS), which at very high levels may lead to severe cell injury and even cell death (Schoffl et al.1999; Apel and Hirt 2004) and in response to it the concentrations of various enzymatic and non-enzymatic anti-oxidants may increase (Larkindale and Knight 2002; Apel and Hirt 2004).

Legumes cater to about 33% of our protein dietary requirements, are socio-economically beneficial, ecologically desirable and their demand has increased many folds over past few decades resulting in legume cultivation under new areas thereby exposing the crops to new climatic conditions(Popelka et al. 2004; Vadez et al. 2012). The plants are thus subjected to various environmental stresses especially temperature stress. Effects of both high as well as low temperatures on various developmental stages of legumes have been studied by workers worldwide e.g. low temperature was found to reduce the seedling growth and survival in chickpea (Croser et al. 2003; Heidervand et al. 2011), early vegetative stage in pea (Pisum sativum; Badaruddin and Meyer 2001) and soybean (Kurosaki et al. 2004; Posmyk et al. 2005). Further, when reproductive stages approach, they are also affected by low temperatures as reported in chickpea,
lentil (*Lens culinaris*), pea and faba (*Vicia faba*) beans (Maqbool et al. 2010). Low temperatures have been found to disrupt pollen viability, pollen germination, impair stigma receptivity, pollen load, ovule viability and result in fertilization arrest, abscised flowers, reduced grain filling and pod set (Kumar et al. 2010; Shafiq et al. 2012). Sub-optimum temperatures have been proposed to be affecting normal male and female gametophytic development due to energy deprivation (Nayyar et al. 2005b; Oliver et al. 2005), whereas Clarke and Siddique (2004) credited the abortive pollen tube growth to failed fertilization as confirmed by Kumar et al. (2010) in cold-stressed chickpea. Stressful low temperatures further disrupt source-sink relationship thereby impairing grain filling stage leading to heavy losses in the yields and related attributes e.g. pea (Guilioni et al. 1997), chickpea (Nayyar et al. 2005 b; Berger et al. 2006), soybean (Ohnishi et al. 2010; Board and Kahlon 2012) and pigeon pea (*Cajanus cajan*; Sandhu et al. 2007). At metabolic levels, chilling affects the photosynthesis negatively as described in chickpea (Nayyar et al. 2005 a,b,c,d), beans (*Phaseolus vulgaris*; Tsonev et al. 2003), faba beans (Hamada et al. 2001; Goltsev et al. 2010). Respiratory rate may at first increase in response to chilling (Kaur et al. 2008) but on continued exposure, respiration decreases (Munro et al. 2004) or plants may resort to some alternative respiratory pathway as found in case of mungbean (*Vigna radiata*) and pea leaves (Gonzalez-Meler et al. 1988, 1999). Besides these implications, other harmful effects of low temperature reported are loss of membrane fluidity and rigidification (Vigh et al. 2007; Jewell et al. 2010), generation of ROS (Wang et al. 2009; Turan and Ekmekci 2011), impaired nodulation and nitrogen fixation (Lia et al. 2005; Duzan et al. 2006). Likewise, the effects of heat stress on legumes have also been reported on the various developmental stages i.e. ranging from vegetative (Piramila et al. 2012; Chakraborthy and Pradhan 2013) to reproductive stages (Barghi et al. 2013;
Devasirvatham et al. 2013). High temperatures impaired pollen viability and germination in groundnut (*Arachis hypogea*; Kakani et al. 2005) and chickpea (Kumar et al. 2013), lentil (Barghi et al. 2013). If the unfavorable high temperatures continue to prevail, it restrains the stigma receptivity, pollen load and ovule viability (Kumar et al. 2013) resulting in abscised flowers and fertilization arrest as reported in mungbean (Suzuki et al. 2003), soybean (Board and Kahlon 2011), chickpea (Kumar et al. 2013) leading to reduced embryogenesis, poor grain filling and seed set (Board and Kahlon 2011; Kumar et al. 2013). Other high temperature induced responses may be summarized as impaired photosynthesis (Board and Kahlon 2011; Kumar et al. 2013) reduced respiration (Hasanuzzam et al. 2013; Kumar et al. 2013), membrane instability (Kumar et al. 2013; Mansoor and Naqvi 2013) and oxidative stress (Gomez et al. 2012; Chakraborty and Pradhan 2013).

Lentil (*Lens culinaris* Medik.) is an important food legume of South Asia, West Asia and North Africa (Ferguson and Robertson 1999). It is supposed to have coevolved with wheat, barley and other cool season crops in Near East arc about 8000 years ago (Cubero 1981; Ladizinsky 1979). It is presumed to be the most ancient legume to be domesticated (Bahl et al. 1993). Lentil is a bushy annual herb, stem may be erect or semi-erect, branched and with soft hair. Plant may reach a height of 15-75 cm (Muehlbauer et al. 1985, 2006). It is a very good source of proteins, vitamins, dietary fibers, iron and amino acids. In fact, out of all cool-season legumes, Lentil is the richest in the important amino acids viz., lysine, arginine, leucine, and sulphur containing amino acids (Williams et al. 1994). Besides being an important pulse crop, its husks, dried leaves, stems, fruit walls and bran serve as very good fodder. Also, the green plants may be used as a valuable green manure. Seeds besides being consumed can be used
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commercially as a source of starch for textile and printing industries (Kay 1979).

Amongst various abiotic stresses experienced worldwide by Lentil; cold, drought and heat were listed to be the most important ones by Singh and Saxena (1993). Susceptibility of lentil to hot-growing regions and cold highlands has been supported by studies carried out by number of workers worldwide (Erskine and El Ashkar 1993; Oktem et al. 2008). Temperature variation also affects legume-rhizobium association as evident from delays in the appearance of nodules, and decrease in nodule number, nodule size and nodule growth rate in case of lentil (Junior et al. 2005). Due to increasing population and ever increasing demand for various food legumes, lentil is now being grown in marginal lands thereby exposing it to various biotic and abiotic stresses (Muehlbauer 1993).

With a recent trend in climate change and shift in the cropping pattern in various regions at national and international level, there is need to examine the sensitivity of each crop species for its response to a range of temperatures. It has also been observed that while the chilling periods are getting relatively shorter, the heat periods are becoming longer that exposes the cool season crops to heat stress at the time of reproductive stage. Moreover, Lentil is also being grown in relatively warmer regions of the central and southern parts of the country that exposes the crop to supra-optimal temperatures causing reduction in its yield potential.

Relatively, no information exists about the response of Lentil to cold and heat stress, especially at its reproductive stage; studies are required to be conducted to probe these responses. Keeping in view this, the proposed objectives of the study are as follows:
OBJECTIVES:

- Evaluation of response of Lentil genotypes to cold and heat stress at reproductive stage.

- Assessment of the stress injury at various organizational levels to find out the traits related to stress sensitivity.

- Investigation of reproductive and metabolic dysfunction and adaptive responses of Lentil genotypes.