INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a major pulse crop of India, Middle-East and North-Africa and is an important source of dietary protein in the Third World countries. Globally, chickpea is cultivated on about 10.4 million ha area adding 8.57 million tonnes of grains to the global food basket, with an average productivity of 826 kg/ha. India grows chickpea on about 7.29 million ha, producing 5.77 million tonnes seed, which represents 30% and 38% of the national pulse acreage and production respectively (Ali and Kumar, 2005). It is an important self-pollinated pulse crop in the Indian subcontinent, and ranks third in production among pulses in the world (Plate I). It provides high quality protein, particularly for the vegetarian population and also plays a significant role in farming system as a substitute for fallow in cereal crop rotations, where it contributes towards the soil nitrogen thereby reducing the need for nitrogenous fertilizers through symbiotic fixation of atmospheric nitrogen (Singh *et al.*, 2002). However, the chickpea production has more or less stagnated for the past two decades. One major limiting factor has been the susceptibility of cultivars to various biotic and abiotic stresses that adversely affect the yield.

Stress is an integral component of the forces that drive the course of evolution. Changes in the physical environment generate stress, which in turn affects homeostasis. It is usually defined as an external factor that exerts a disadvantageous influence on plants. Abiotic stresses such as drought, high temperature, salinity etc are location specific, exhibiting internal variations in occurrence, intensity and duration and generally cause reduced crop productivity. There is a serious concern for food security in developing countries like India where the population is increasing exponentially with each passing day. Therefore, there is an urgent need to increase agricultural productivity and to expand productive areas of the world. This can be achieved only by making a conscious effort to improve the production by extending the cultivation of stress tolerant crops to areas commonly exposed to abiotic stresses such as high temperature, salinity, drought, chilling stress etc.

Plants are exposed to various environmental stresses both during the changes in season and more rapidly over the course of the individual days. The most typical kind of stress plants experience from the surrounding is the "temperature stress" and

Plate I: Cultivation of chickpea (*Cicer arietinum* L.) varieties in the field the severity of this situation is increasing due to global warming. The temperature of an individual plant cell can change much more rapidly than other factors that cause stresses. Temperature stress exists in many forms both within and between the different climates of the world. In particular, plants that exist in regions of higher latitudes must endure dramatic seasonal temperature change, which can span from -40°C to + 40°C. At one extreme, the effects of freezing can induce dehydration, ice nucleation, protein inactivation and in extreme cases, cell death. Alternately, plants exposed to excessive heat can also succumb to dehydration, protein degradation and cell death. High temperature stress is detrimental to plant growth. It is also known to severely reduce seed germination, pollen viability and pollen germination in several crop species.

Levitt (1980) and Ho (1987) have suggested that there are three direct effect of heat stress on cells which could be the site(s) of perception of temperature: cell membrane, metabolic reaction and protein structure. Elevated temperatures will immediately alter the membrane fluidity and affect iron transport in the cell, at the same time, enzyme reaction rates will increase as a function of temperature and alter the concentration of metabolites in the cell, concomitantly, thermo-sensitive proteins will assume non-native configurations, again as a consequence of the heat stress. Adverse effects of high temperature stress leads to various metabolic changes deleterious to plant health and often leads to oxidative stress due to accumulation of toxic oxygen species (Inze and Van Montagu,1995; Dat *et al*.,1998a,1998b and Jiang and Huang, 2001) These toxic radicals can be removed by the scavenging enzyme systems comprising of peroxidase, ascorbate peroxidase, catalase, superoxide dismutase, glutathione reductase etc. However, the function of these antioxidative enzyme systems can be interrupted by heat stress, which results in increase in lipid peroxidation and consequent membrane damage (Jagtap and Bhargava,1995; Dat *et al*.,1998a,b and Jiang and Huang, 2001).

Plants have evolved various mechanisms to cope with stresses in a particular niche. Plants can resist high temperature stress either by avoidance or by tolerance mechanisms. Examples of heat avoidance mechanisms are insulation, decreased respiration, decreased absorption of radiant energy through reflectance or decreased chromophore content or transpirational cooling. Obviously, expression of some of these types of avoidance mechanisms require the coordination of many cell and tissue types. Potential mechanisms of heat tolerance are the synthesis of protectants, increased thermostability of enzymes and increased saturation of fatty acids.

Ability of organisms to acquire thermotolerance to normally lethal temperatures is an ancient and conserved adaptive response (Hong *et al*, 2003). Thermotolerance represents a property of all living cells and refers to the capacity of the cells to survive or recover from normally lethal exposures to abrupt, severe heat shock if, before the lethal stress, the cells are exposed to milder or short period of heat stress condition. The various chemical pre-treatments and heat- acclimation treatments that induce the accumulation of heat shock proteins (HSPs) often result in thermotolerance of plants like *Arabidopsis*, Kentucky Bluegrass, *Zea maize* etc. The degree of thermotolerance of different plant species have been found to be directly related with the over and under expression of HSPs. Thermotolerance is characterized by halted plant growth in high temperature conditions. It is suspected that insensitivity to hormones such as auxin and cytokinin is responsible for the onset of thermotolerance, as these hormones normally induce growth responses (Michels, 2004).

One of the promising areas in increasing thermal stress resistance is the induction of thermotolerance by exposure to sub-lethal temperatures prior to sowing in high temperature soils. A preliminary treatment with a moderately elevated, non-lethal temperature can temporarily render plants more resistant to a subsequent potentially lethal heat shock- this phenomenon is known as heat-acclimation. Besides, in several studies, certain chemical treatments including salicylic acid (SA), calcium chloride ($CaCl_2$) and abscisic acid (ABA) have also been shown to induce thermotolerance to a certain degree (Jiang and Huang, 2001; Jiang and Zhang, 2001; Burke, 2001 Larkindale and Knight, 2002; and He *et al.*, 2005;). Acquisition of thermotolerance is likely to be of particular importance to plants that experience daily temperature fluctuations and are unable to escape to more favorable environments.

In an interesting study, Kloepper *et al.* (2004) have reported the role of Plant Growth Promoting Rhizobacteria (PGPR) in heat tolerance. PGPRs are the bacteria that inhabit the rhizosphere-the soil immediately surrounding the roots and exert beneficial effects on plants. Rhizospheric microbe-plant interactions have a great influence on plant health and soil quality since these root assisted microorganisms are able to help the host plant to deal with drought, nutritional and soil-borne pathogen stress conditions. PGPRs are widely studied for their growth promoting nature and also for their role as resistance inducers in many crop species (Burdman *et al.*, 2000; Ramamoorthy *et al.*, 2001; Kloepper *et al.*, 2004).Though the research on PGPR- mediated resistance originated several decades ago, the putative mechanisms underlying such resistance mechanisms especially with regard to thermoprotection still remains to be fully understood. In this context, the present research will throw light on the possible mechanism of PGPR induced heat tolerance mechanism and will reinforce the existing knowledge in this area.

Although heat stress has become a subject of much interest owing to global warming and global climate change, not much research work on cool season legume, like chickpea have been carried out. The methods of screening the suitable genotypes for cultivation still eludes the growers because of the lack of information on the range in genetic diversity for heat tolerance and screening techniques. Hence, the present study was designed with the objectives to study the biochemical response of chickpea to heat stress and to induce thermotolerance by heat acclimation, treatment with salicylic acid (SA), abscisic acid (ABA), $CaCl_2$ and Plant Growth Promoting Rhizobacterial (PGPR) strain -*Bacillus megaterium*. The main objectives of the present study are:

(i) Screening of different genotypes of chickpea (*Cicer arietinum* L.) for heat tolerance; (ii) characterization of biochemical responses of different genotypes to elevated temperatures in terms of changes in the cellular constituents including proteins, carbohydrates, proline, phenols, chlorophylls, carotenoids etc; (iii) determination of the effect of elevated temperatures on enzyme activities-specially antioxidative enzymes like peroxidase, ascorbate peroxidase, catalase, superoxide dismutase and glutathione redutase; (iv) determination of the effect of heat stress on cell membrane stability and lipid peroxidation of membranes; (v) induction of thermotolerance by heat acclimation and various chemical pre-treatments before exposure to lethal temperature; (vi) determination of the specific expression of new protein(s) or enzymes during heat shock and to determine their involvement in thermotolerance; (vii) analysis of the effect of elevated temperatures on *in vitro* callus formation and that of pre-treatments on thermoprotection and (viii) development of biochemical markers related to acquired thermotolerance in *Cicer arietinum* L.

In order to achieve the above-mentioned objectives, standard methods have been used which are described in the following pages. Besides, a brief review of literature in the line of work has also been presented.