CHAPTER 1

INTRODUCTION

Wheat is the dominant cereal crop grown in temperate countries, and globally, one of the three most important crops for human and livestock feed (**Reaping the benefits: Science and the sustainable intensification of global agriculture, 2009),** with over 600 million tones being produced annually. For example, in 2007, the total world harvest was about 690 m tones as compared to 652 m tones of rice and 785 m tones of maize (**Shewry, 2009**). With the world's population forecasted to reach nine billion by 2050, food security has become a critical global challenge for the 21st century.

It has been estimated that cereal production needs to increase by 50% by 2030 **(Foresight: The Future of Food and Farming, 2011)**. Consequently, increasing wheat yields is now one of the top priorities for agricultural research (**Reaping the benefits: Science and the sustainable intensification of global agriculture, 2009**). When compared with other crops, the increase in wheat yields has slowed since the 'green revolution' of the 20th century **(Alston *et al*., 2010)**.

Wheat (*Triticum aestivum* L.) has been the main cereal grain grown in Egypt for thousands of years, serving as the principal source of calories in Egyptian diet. The local production is about 8 million tons however; it covers less than 60% of local consumption. So, Egypt remains the world's largest wheat importer. Accordingly, cereal import requirements in the current marketing year 2013/14 (July/June) are put at about 15.4 million tonnes, about 16 percent higher than last year but some 4 percent lower than the five-year average **(FAO 2013).**

Climate change and abiotic stress affect agriculture and crop production adversely. Among the various climatic factors affecting agriculture, temperature is one of the most important because higher temperatures adversely affect plant growth and yield (**Zou *et al.*, 2011; Madan *et al.*, 2012**). In the 21st century, average surface temperatures of the earth are likely to increase by 2–4.5 °C, with an increase in the magnitude and frequency of extreme temperature events **(Stevenson *et al.*, 2005).** Global warming as a result of climate change affect negatively at wheat grain yields potentially increasing food insecurity and poverty **(Tubiello *et al*., 2000)**. The **CIMMYT-ICARDA (2011)** reported that world wheat production will decrease due to global warming and developing countries will be highly affected by the negative effects on wheat production. Climate change studies predict a reduction in the productivity of two major crops in Egypt: wheat and maize by 15% and 19% respectively by 2050 **(NEEDS, 2010)**.

Heat stress affects the metabolism and structure of plants, especially cell membranes and many basic physiological processes such as photosynthesis **(Wahid *et al*. 2007)**.

The cell membranes are thought to be the primary site of direct high temperature injury **(Blum, 1981)**. Leakage of solutes through the membrane after heat stress has been measured by electrical conductivity (EC) and used in many crops (eg: wheat, soybean, vegetables) as an index of membrane stability to identify heat tolerant genotypes **(Saadalla *et al*, 1990)**. Electrolyte leakage is a measure of cell membrane thermo stability **(Sullivan and Ross, 1979)**.

Increased heat stress leads to the overproduction and accumulation of various organic and inorganic osmolytes. These osmolytes protect the plants from stresses by cellular osmotic adjustment, detoxification of ROS, protection of biological membranes and stabilisation of enzymes/proteins **(Verbruggen and Hermans 2008)**.

Although heat sensitive plants apparently lack this ability, heat tolerance in such plants can be improved by exogenous application of such osmoprotectants and nutrients **(Rasheed *et al*. 2010).**

Heat stress reduces photosynthesis through disruptions in the structure and function of chloroplasts, and reductions in chlorophyll content. Photosynthesis is the most sensitive physiological process to elevated temperature Photosystem II (PSII) complex is thermally labile and is considered as the most heat sensitive component of the electron transport chain **(Almeselmani *et al*. 2006)**.

High temperature decreases leaf chlorophyll **(Ristic *et al*., 2007)** and suggested that Chlorophyll is harbored in the thylakoid membranes, and loss of chlorophyll may be due to high temperature-induced electrolytic leakage of thylakoid membrane and/or lipid peroxidation of chloroplast membranes **(Djanaguiraman *et al*., 2010)**. These measurements indicate that many physiological processes are dependent on the function of this single chaperone protein and that the production of this specific HSP is required for thermotolerance.

At the biochemical and molecular levels, Supra-optimal temperatures induce the synthesis and accumulation of many new proteins including heat shock proteins **(Law and Brandner, 2001)**. Heat shock proteins (HSPs) are a class of proteins whose expression is increase when cells are exposed to elevated temperatures or other stress. HSPs are involved in the folding of denatured proteins. High temperatures and other stresses, such as altered pH and oxygen deprivation, make it more difficult for proteins to form their proper structures and cause some already structured proteins to unfold. Increased expression of HSPs is mediated at multiple levels: mRNA synthesis, mRNA stability, and translation efficiency. Plants synthesize HSPs proportionally with the severity of heat shock until the greatest level to tackle the shock. HSP synthesis is completely induced for survival with maximum activation of other protection mechanisms at near deadly temperatures. However, plants probably synthesize middle level HSPs at mild heat stress conditions at first, but if the heat stress continues, then more synthesis of HSPs is carried out **(Ahn *et al*., 2004)**. HSP100 family is up-regulated by environmental stress. Proteins of this family generally function to protect protein denaturation and protein aggregation **(Wang *et al*., 2004).** In many plant species (i.e. Arabidopsis thaliana, soybean, rice, maize, wheat, and beans), many cDNAs and genomic clones, coding for different forms of HSP101 which is a member of a small gene family strongly induced by heat **(Agarwal, *et al* 2001 and 2002)**.

Single nucleotide polymorphisms (SNPs) and small insertions deletions (indels) are the most abundant forms of DNA sequence variations. SNPs detection technologies are used to scan for new polymorphisms and to determine the allele(s) of a known polymorphism in target sequences. They can thus provide a huge number of useful markers for ultra-dense genetic mapping, population genetics, evolutionary and genotype phenotype association studies.

Several authors have underlined the interest of developing SNP markers in plants **(Rafalaski 2002a, 2002b)**. In bread wheat, **Guillaumie *et al.* 2004 and Boisson *et al.* 2005** showed that, SNP studies have been limited to single genes or DNA fragments allowing association studies or genetic mapping. To the best of our knowledge, there was no previous report in Egyptian wheat cultivars about SNPs in HSP101 gene associated with thermotolerance.

Therefore, the present work was undertaken to:

1. Evaluate heat tolerance of six Egyptian bread wheat varities at seedling stages via physiological measurements including cell membrane thermostability, proline cotent and chlorophyll content,
2. Isolation and characterization of cDNAs coding for HSP101c in Egyptian wheat and examined gene expression after short durations of heat stress by using Quantitative *Real-time PCR* technique,
3. Developing a SNP marker in the *HSP101c* gene of Egyptian bread wheat and quantifying the average deleterious impact of substituting one amino acid with another to identify heat tolerant and heat susceptible cultivars using gene sequencing method.