CHAPTER I
INTRODUCTION

Wheat is one of the first domesticated food crops of the world and has been the basic staple food of the major civilizations of Europe, West Asia and North Africa. It is grown from temperate and irrigated to dry and high-rainfall areas and from warm-humid to dry-cold environments. Undoubtedly, this wide adaptation has been possible due to the complex nature of the plant’s genome, which provides great plasticity to the wheat crop. Wheat is a C$_3$ plant and as such it thrives in cool environments. Today, wheat is grown on more land area than any other commercial crop and continues to be the most important food grain source for humans. Its production leads all crops, including rice, maize and potatoes.

Wheat which is classified within the genus *Triticum* belongs to the poaceae family. The genus *Triticum* is further subdivided into a number of species which are classified according to the number of chromosome pairs they contain; (a) Diploid, have 7 pairs of chromosomes (2n=2x=14) e.g. einkorn wheat, (b) Tetraploid, have 14 pairs of chromosomes (2n=4x=28) e.g. durum wheat and (c) Hexaploid, have 21 pairs of chromosomes, (2n=6x=42) e.g. common bread wheat (Curtis *et al.*, 2002). The diploid donor species diverged 2.5 – 4.5 million years ago (Ma) and are termed AA, BB and DD. They are understood to have combined to produce *Triticum aestivum* in two distinct hybridization events. First, *Triticum urartu* (AA) and an unknown relative of *Aegilops speltoides* (BB) are believed to have produced the tetraploid *Triticum turgidum*, followed by hybridization with *Aegilops tauschii* (DD) to produce the hexaploid (Chantret *et al.*, 2005).

The worldwide cultivated area of wheat is 270 million hectares with production of 830 million tonnes and productivity 3093 tonnes per hectare. While in India, area of cultivation is 30.72 thousand hectares with production 97.44 million tonnes at 3172 kg/ha productivity. In Gujarat, cultivation area is 996 thousand hectares, 2938 thousand tonnes production with 2950 kg/ha productivity (Anon., 2017).

Although useful as a livestock feed, wheat is used mainly as a human food. It is nutritious, concentrated, easily stored and transported, and easily processed into
Introduction

various types of food. Unlike any other plant derived food, wheat contains gluten protein, which enables leavened dough to rise by forming minute gas cells that hold carbon dioxide during fermentation. This process produces light textured bread.

Wheat supplies about 20 percent of the food calories for the world's people and is a national staple in many countries. In Eastern Europe and Russia, over 30 percent of the calories consumed come from wheat. The per capita consumption of wheat in the United States exceeds that of any other single food staple. Besides being a high carbohydrate food, wheat contains valuable protein, minerals, and vitamins. Wheat protein, when balanced by other foods that supply certain amino acids such as lysine, is an efficient source of protein.

Wheat grain contains high energy value (1,368 kJ energy) and high nutritive value carbohydrate (71.18g), sugar (0.41 g), dietary fiber (12.2g), protein (12.61g) and fat (1.54g) par 100g of wheat grain. Wheat grain also contain trace amount of vitamins (Thiamine, Riboflavin, Niacin, Pantothentic acid, Vitamin B6, Vitamin E, Vitamin K, Folate, Choline) and some minerals (Ca, Fe, Mg, Mn, Zn, Na, K). This ratio might have variation as per form of the wheat product. For example, wheat germ contains highest protein (26.7 %) content followed by wheat bran (14.1%), wheat whole meal flour (12.7%), and white flour (8.9%). On the other hand, white flour contains highest carbohydrate content (77.7%) while wheat bran contains lowest carbohydrate content (26.8%) (Kumar et al., 2011).

The time-span of development phases of wheat essentially depends on genotypes, temperature, day-length and sowing date etc. Various environmental stresses, particularly heat but also water and salinity, may shorten the wheat growth phases. High temperature, which is often combined with drought, reduces the grain yield and quality of wheat (Blumenthal et al., 1995; Maestri et al., 2002; Wardlaw et al., 2002). Heat stress during grain filling leads to significant reduction in yield, biomass, grain number, harvest index and thousand-kernel weight (Ball et al., 2009). Different traits like early ground cover, leaf rolling, plant height, earliness, grain filling duration, and stay green are associated with tolerance to heat stress (Fokar et al., 1998; Reynolds et al., 2001). Farooq et al. (2011) noted that grain filling rate is accelerated by heat stress, which results in the reduction of grain filling duration (Dias and Lidon, 2009). According to Yin et al. (2009), 5°C increase over 20°C reduced the grain filling duration by 12 days in wheat even though it increased the grain filling
rate. In another study, Streck (2005) reported that for every 1°C increase above a temperature of 15–20°C, grain-filling duration was reduced by 2.8 days.

Chronic high temperature is defined as mean temperature during the growth cycle ranging from 18 to 24°C, with maximum day time temperature reaching up to 32°C. Heat shock occurs if the maximum day time temperature exceeds 32°C after the mid-reproductive stage 2 (Wardlaw and Wrigley, 1994; Cossani and Reynolds, 2012). Only five to six days of 28-32°C average temperature can cause 20% yield reduction in wheat (Stone and Nicolas, 1994). According to Acevedo et al. (1991), every 1°C increase over 17 to 24°C average temperatures during grain filling can causes 4% yield reduction. Almost all the temperate, arid, semi-arid, tropical, and sub-tropical wheat production is affected by heat stress (Paliwal et al., 2012; Fischer, 1986).

With industrialization, natural environment deterioration and climate change, heat stress has become an increasingly important factor affecting crop growth, and it is thought that crop production may be severely affected by an increase in mean global temperature (Han et al., 2009). For example, models indicate that a mean temperature rise of 1°C could reduce wheat yields by 10% in some regions (Anon., 2013a,b).

Heat stress-induced decrease of the duration of developmental phases leading to fewer and smaller organs, lower light perception due to a reduced life cycle and altered carbon assimilation is of major importance for cereal yield losses (Han et al., 2009, Stone, 2001). High temperature may slow down or prevent germination, depending on plant species and stress intensity, and, at later stages, may adversely affect photosynthesis, respiration, water relations and membrane stability, as well as modulate levels of hormones and primary and secondary metabolites. Furthermore, throughout plant ontogeny, enhanced expression of a variety of heat shock and other stress-related proteins, and enhanced production of reactive oxygen species (ROS) constitute major plant responses to heat stress (Wahid et al., 2007).

High temperatures reduce photosynthesis by changing the structural organization of thylakoids (Karamanos and Papatheohari, 1999, Wahid et al., 2007). In general, it is evident that high temperature considerably affects anatomical structures not only at the tissue and cellular levels, but also at the sub-cellular level. The cumulative effects of all these changes may result in poor plant growth and productivity (Wahid et al., 2007).
Numerous biochemical reactions are involved in plant growth and development that are sensitive to high temperature (HT). Plant response depends on the plant type, degree and duration of high temperature. Catastrophic collapse of cellular organization occurred at high temperature leads to cell damage and cell death. High temperature affects productivity and yield as well as germination, stability of various proteins, RNA species, membranes, nuclear membrane and enzymatic efficiency in the cell which retard the growth. HT reduces the concentration of CO$_2$ which promotes the Reactive Oxygen Species (ROS). These species use the excess O$_2$ due to shortage of CO$_2$ and leads to oxidative stress. Increase in antioxidant capacity increases the plant ability against high temperature (Hasanuzzaman et al., 2013) (Fig. 1.1).

**Fig. 1.1: Effect of high temperature on plant growth**

Plants can adapt to changing environmental conditions by a series of strategies aimed at the maintaining of cellular metabolism, molecular activities, growth and development, through a variety of molecular networks that allow a rapid and efficient sensing of the stress, resulting in the triggering of response activation (Rampino et al. 2006). Heat stress highly affects on plant metabolism as well as other systems i.e. ROS production, CO$_2$ solubility, activity of various enzyme activity which ultimately lead to the reduction of the photosynthesis and cause plant lower production or plant death (Fig. 1.2).

Earlier studies have revealed the nature of heat stress effects on mature, well-developed green leaves (Al-Khatib and Paulsen, 1999; Dash and Mohanty, 2001;
The impact of heat stress on seedling growth and leaf development has also been established based upon pigmentation sensitivity (Crafts-Brannder and Salvucci, 2000; Kariola et al., 2006; Mathews et al., 2008) and Photosystem II (PS II) function in wheat (Dash and Mohanty, 2001; Mohanty and Mohanty, 1988; Mohanty et al., 1987). Genotypes of wheat exhibited considerable variation in their sensitivity to heat stress (Ali et al., 1994; Al-Khatib and Paulsen, 1999; Dash and Mohanty, 2001).

**Fig. 1.2: Effect of heat stress on photosynthesis**

Differences in the sensitivity of chloroplast photoreactions to heat stress, however, could not be established detected between wild and cultivated wheat species (Dash and Mohanty, 2001; Rekika et al., 1997) or between temperate and tropical cereals cultivars, including wheat (Dash and Mohanty, 2001; Havaux et al., 1988; Sayed et al., 1989).

**Transcriptome and gene expression:**

New advances in “omics” technologies have provided new opportunities and hopes for the identification of transcriptional, translational and post-translational mechanisms and signalling pathways that regulate the plant response(s) to abiotic stress including HT. Such omic approaches help to systematic analysis and correlation between the changes in the genome, transcriptome, microme, proteome and metabolome to the variability in plant’s response to temperature extremes and their application to increase the chances of developing stress tolerant plants (Hasanuzzaman et al., 2013) (Fig. 1.3).
The genome is made up of DNA a long, winding molecule that contains the instructions needed to build and maintain cells. For these instructions to be carried out, DNA must be transcribed into corresponding molecules of RNA referred to as transcripts. A transcriptome is a collection of all the transcripts present in a given cell. The transcriptome is the set of all RNA molecules, including mRNA, tRNA, rRNA and other non-coding RNA, as hnRNA, snoRNA, exRNA, long ncRNA, produced in one or a population of cells and transcriptome provides information on gene expression, gene regulation and amino acid content of proteins.

Transcriptome analysis is essential to interpret the functional elements of the genome and reveal the molecular constituents of cells and tissues. Transcriptome or EST sequencing is an efficient way to generate functional genomics level data for non-model organisms. Whole transcriptome analysis is very important in understanding how altered expression of genetic variants contributes to complex plant phenotypes. Plant researchers utilize a variety of approaches to understand gene expression. In some cases, whole transcriptome profiling is preferred to target RNA sequencing, which generally involves panels. In order to study plant functional genomics, researchers also utilize epigenetic tools as well as plant miRNA, small RNA and RNAi studies to decipher the root cause behind phenotypes of interest.

![Integrated circuit of different “omics” approaches](image)

**Fig. 1.3: Expression of gene: Integrated circuit of different “omics” approaches**

The transcriptome represents a comprehensive set of transcribed regions throughout the genome. Studying transcriptome dynamics provides important insights into the functional elements of the genome, their expression patterns and the regulation of transcribed regions in different tissues and under different conditions.
Various technologies have traditionally been applied to deduce and quantify the transcriptome, such as cDNA or expressed sequence tag (EST) library sequencing, microarray hybridizations and serial analysis of gene expression (SAGE), next-generation sequencing (NGS) and array solutions that provide high-quality gene expression and transcriptome analysis data for a broad range of sample types. NGS-based RNA sequencing (RNA-Seq) methods can detect and quantify any active gene or transcript, including novel transcripts. Expression microarray technology measures the relative activity of known, predefined genes and transcripts. However, these techniques have different drawbacks such as low throughput, high cost, low sensitivity, and cloning bias.

The most frequently applied technique for gene expression profiling, i.e. microarray hybridization suffers from high background signals (Miller et al., 2002 and Bengtsson et al., 2004) and relies on the need for pre-determined probes designed against known target sequences, making it unable to identify novel transcribed regions. In addition, probes cover only a portion of the annotated gene and hence the complete gene structure is not represented. The development of next-generation high-throughput RNA sequencing technologies, called (m)RNA-Seq, provides a novel method for identifying, mapping, and quantifying transcriptome. It has already been demonstrated that deep RNA sequencing is a powerful tool for comparing gene expression and discovering the full extent of 5’- and 3’-untranslated regions, novel splice junctions, novel transcripts, alternative transcription start sites, and rare transcripts. The results of RNA-Seq also show high levels of reproducibility, for both technical and biological replicates (Nagalakshmi et al., 2008; Zhang et al., 2010).

Gene expression profiling constitutes an exciting tool to unveil mechanisms involved in the response of plants to environmental stress. It has allowed the identification of hundreds of genes induced when plants are exposed to stress. Several methods are currently being employed to analyze the profiles of gene expression in plants. Most commonly used techniques are cDNA-AFLP, SAGE (Serial Analysis of Gene Expression), MPSS (Massively Parallel Signature Sequencing), RT-PCR and Microarrays (Torres et al., 2009). Analyzing gene expression patterns requires tools enabling sensitive, precise, and reproducible quantification of specific mRNAs. Quantitative real-time polymerase chain reaction (qRT PCR) is currently the technique of choice for this purpose. Reverse transcription (RT) followed by a polymerase chain reaction (PCR) represents the most powerful technology to quantify
gene expression, to amplify and detect trace amounts of mRNA (Heid et al., 1996 and Livak and Schmittgen, 2001). Gene expression analysis has provided insight into complex biological processes, increasing our understanding of signaling and metabolic pathways that underlie environmental responses and development. Real-time reverse transcription PCR is currently the standard method for accurate expression profiling of a moderate number of selected genes, its main advantages being a higher sensitivity and specificity and a broader quantification range than previous molecular techniques (Van Guilder et al., 2008).

**Objectives:**

1. Transcriptome sequencing and assembly in wheat under heat stress condition at booting stage.
2. To identify a set of candidate transcripts (genes), whose expression dynamics indicate their involvement in heat stress.
3. To convert the massive transcriptome data into Gene Ontology categories to facilitate a better knowledge of the stress responsive metabolic pathways at booting stage.
4. Expression analysis of genes possibly involved in heat response in wheat.
5. To examine protein profiling at booting stage of wheat genotypes under heat stress.

**Significance of Research Work:**

The development of molecular techniques for genetic analysis will led to increase in our knowledge about wheat genetics and understanding of the structure as well as behaviour of genome. Transcriptome analysis will helpful to understand the gene function and transcript level of wheat genotype at various stages under stress condition. While biochemical analysis will help to analyze up regulation and down regulation of various protein under stress condition.

Due to high complex nature of wheat genome, it requires more efficient method for annotation of expression of various genes. Transcriptome sequencing of wheat will be valuable for identification of putative gene, to perform gene annotation and compared with genomic SSR markers. Expression study will help in revealing up-regulation and down-regulation of gene identified and involved in abiotic stress.

Protein profiling is also useful to understand heat tolerant mechanism in control and treated plants at seedling stage.