Chapter 1

Introduction

Wheat is the world's second most widely grown staple food crop and accounts for more than 35% of the human population (FAO 2016). It is grown on about 220 million hectares land area worldwide. It fulfills 21% of the total calorie and 20% of the protein requirements of more than 4.5 billion population in developing countries (Braun et al. 2010). It belongs to Triticeae tribe having different species with a different type of genome. Wheat genetics is more complicated than that of most other domesticated cereal crops. Wheat had been originated in the Middle East (Zohary et al. 2000) and Einkorn was earliest domesticated species of wheat. It became the main crop in the region of Turkey for several thousand years (Feuillet et al. 2007). Due to hardy and low yielding in nature, this species is not cultivated these days; however, it is available as a wild species in the Fertile Crescent (Heun et al. 1997). Besides this species, other species of wheat had originated in this area and played a significant role in improving the genomic constitution of wheat. Initially, natural hybridization of diploid wheat Triticum urartu (AA genome) (Chapman et al. 1976) with Aegilops speltoides (BB genome) generated sterile hybrid of wheat containing both the genome. Someway, doubling of chromosomes in nature leads to the generation of fertile wild tetraploid, T. dicoccoides (AABB) (Ozkan et al. 2011). Domesticated form of this species is widely known as Emmer wheat (Ozkan et al. 2011). Meanwhile, another event of interspecies hybridization had occurred between cultivated Emmer wheat and Ae. tauschii (DD genome) followed by polyploidization produced the hexaploid bread wheat T. aestivum (AABBDD genome) (Charmet 2011).

Some wheat species are diploid, with two sets of chromosomes, but many are stable polyploids, with four sets of chromosomes (tetraploid) or six (hexaploid). Einkorn wheat (T. monococcum) is diploid (AA, two complements of seven chromosomes, 2n=14) species with wild and cultivated variants. Durum (T. durum) and Emmer (T. dicoccum) are tetraploid species. Durum wheat is widely used today for making pasta & burgher and it is the second most widely cultivated wheat. Common wheat (T. aestivum L.), also known as bread wheat, is most widely cultivated in the world. Bread wheat is allohexaploid consisting of three closely-related sub genomes (A, B, D) (Hancock 2004) and it independently maintains genomes that are the result of a series of naturally occurring hybridization events. Each sub-genome (each having 7 chromosomes, i.e. 21 chromosome pairs) has almost 5500 million bases which are twice than the human genome.
Several individual groups around the world are working on sequencing of the wheat genome. The international wheat genome sequencing consortium (IWGSC) presented the draft sequence of 17 giga-base pairs (gbp) hexaploid bread wheat (T. aestivum cv. ‘Chinese spring’) genome on 18th July 2014. IWGSC annotated 1,24,201 gene loci well distributed evenly across the homologous chromosomes of sub-genomes.

An estimated 1.2 billion poor people depend on wheat, a crop that is particularly vulnerable to climate change. In South Asia, wheat yields are projected to decline 20-30 percent by 2050. According to the most recent report by the United Nation, currently, there are 7.3 billion people and may reach 9.7 billion by 2050. Food demand is expected to increase anywhere between 59-98% by 2050. Particularly, the demand for wheat in the developing world is projected to increase 60% by 2050. At the same time, climate-change-induced temperature increases are likely to reduce wheat production in developing countries by 20-30%. It is estimated that by 2030, India needs to produce 130 million tonnes of wheat to feed constantly growing population. In India, during 2016-17 crop season, wheat occupied about 30.72 million hectare of total available 181.95 million hectare agriculture land (Land use statistics, Directorate of Economics and Statistics, Government of India) and is the second highest contributor to the total food grain production of the country after rice and established a new record with 97.44 million tonnes production in 2016-17 in its history (Third Advance Estimates 2016-17, Directorate of Economics and Statistics, Government of India). India is a major contributor to the world wheat production after China has witnessed a tremendous increase in production during the last four decades by way of having the green revolution through the development and utilization of input-responsive, high yielding disease resistant and adoption of environment-friendly cultivation practices such as conservation tillage/agriculture.

Despite this generally increasing trend, wheat production in India is being constantly challenged by many threats as reported worldwide. They include climate changes leading to temperature extremes, weather changes, erratic precipitation and changes in pest dynamics (Mamrutha et al. 2014). Overall changes in the micro-environments are expected to be more severe in the coming years, coupled with challenges due to a decrease in the arable land caused by increasing urbanization, land degradation and limited water for irrigation (Anonymous 2011a). The biotic and abiotic stresses are the major factors which affect the crop in different ways like plant growth, height, strength, and microbe’s infection, etc., ultimately led to the variation in yield by making the interference in normal metabolic
activities of plants. Climate change will increase existing problems of biotic and abiotic stresses. Abiotic stress alone causes more than 50% yield loss worldwide in major crops (Acquaah 2007). India is a country which possesses different climatic and agronomic zones affected by different abiotic stresses. Till date, various abiotic stresses, drought, salinity, heat, waterlogging, cold, aluminum toxicity, micronutrient deficiency etc., are reported to hamper wheat yield. Among the abiotic stresses, temperature extremes, drought, and salinity are the major ones and affects the maximum area in wheat in India (Lobell et al. 2008; Battisti and Naylor 2009; Butler and Huybers 2013; Makhloifi et al. 2014). Hence, there is a need to develop wheat cultivars which efficiently combat major abiotic stresses like drought, heat, and salinity.

Currently, the genetic improvement of wheat in terms of yield potential and its genetic stability through conventional breeding are being confronted slow pace. Although conventional breeding programs have led to continuous improvements in some of the economically important traits, the emergence of new threats and climatic challenges calls for a faster action towards the development of varieties resistant to emerging pests and diseases, and abiotic stresses. In conventional breeding methods, progeny receives a complete set of genes carrying traits along with unwanted traits, through which it takes longer time to produce a cultivar/genotype that contains desired traits. This problem can be resolved with the application of biotechnology to increase the productivity as well as the quality of wheat. We need to exploit the existing sources, the conventional wheat germplasm, landraces, cultivars or its relative species distributed across the Triticeae gene pools, of allelic diversity to produce climate resilient wheat to meet the growing demand. The elucidation of genomic regions associated with the expression of traits involved in abiotic stress adaptation, the novel genes discovery or the determination of their expression patterns in response to abiotic stress will provide the basis for effective engineering strategies leading to enhanced wheat germplasm for specific agro-ecological niches. The identification of novel abiotic stress responsive genes can be done by transcriptome analysis, differential expression analysis, RNA sequencing, etc.

The transgenesis/cisgenesis techniques facilitate the transfer of novel genes from one plant to another plant. The transgenic approach allows transfer of genes from one species to another non-compatible species (Holme et al. 2013). Cisgenics provides the transfer of genes in its intact form within a species (Schouten et al. 2006). Cisgene includes its own promoter, coding region, and terminator region. In intragenesis approach, allows in vitro
recombinations of genetic elements from sexually compatible DNA pool (Rommens 2004). There is a need of the hour to develop climate resilient wheat varieties that can bear a wider range of changing climate variability with an increase in yield. For this, it is necessitated to discover various abiotic and biotic stress responsive genes and to transfer them to existing superior wheat genotypes through biotechnological approaches. As Indian wheat genotypes are exposed to diverse environmental conditions, we believed that they do have highly climate resilient genes compared to other earlier reported ones and hence, there is a need to mine these novel genes.

With the advent of recombinant DNA (rDNA) technique and transformation methods, a new way of incorporating defined genetic changes in plants is quite possible. Although, all currently available techniques are not applicable to all plant species. Wheat is one of them, which requires a more precise technique to place the desired gene in its genome. It has remained a challenge for biotechnologists due to its recalcitrant nature for in vitro propagation which makes the process inefficient and costly. However, many researchers have been reported to establish reproducible regeneration systems for wheat, but these reports have either genotypic, explants, culture media, growth regulator dependency, poor or no regeneration, or prolonged and tedious culture conditions (Ganeshan et al. 2006; Khurana et al. 2007; Raziuddin et al. 2010). To keep pace with functional genomics analysis involving transgenic approaches, it is imperative to have an efficient and robust regeneration protocol. Hence, there is a need to develop reproducible and efficient regeneration protocols suitable for different type of explants and genotype towards an effective wheat functional genomics program. With this background, the present research work has been taken up with the following objectives:

**Objectives:**

1. Identification, cloning and characterization of abiotic stress responsive genes in Indian wheat.
2. Standardization of regeneration protocol in Indian wheat genotypes.
3. Standardization of transformation protocol in Indian wheat genotypes using marker gene.