Wheat (*Triticum spp.* L.) is one of the most important staple food and cereal crop feeding about 36 per cent of world’s population. Wheat is classified into three major groups, Einkorn (Diploid; \(2n=2x=14\)), emmer and durum (Tetraploids; \(2n=4x=28\)), and spelta and bread wheat (Hexaploids; \(2n=6x=42\)). Bread wheat (*Triticum aestivum* L.) is an allohexaploid (\(2n=6x=42\)) and accounts for about 95 per cent of the wheat grown worldwide. Most of the remaining 5% is tetraploid durum wheat (Shewry, 2009). Wheat has a large genome size of 16 Gb (Arunuganathum and Earle, 1991) and 17 Gb (Paux *et al*., 2008; Feuillet *et al*., 2011). It is cultivated extensively in many parts of the world with latitudinal distribution from 30-60°N and 27-40°S, due to its high nutritive value, low water content, ease of transport and processing, and the good storage qualities. Wheat grains are full of essential nutrients, including carbohydrates (60-80%), proteins (8-15%), containing adequate amount of all essential amino acids except lysine, tryptophan and methionine, fats (1.5-2 %), minerals (1.5-2%) and vitamins (Shewry, 2000).

### 1.1 Current status and estimated demand

Wheat is an essential crop for the food security irrespective of climatic variation ranging from temperate to tropical. During the last 50 years, significant improvement in the production and productivity of wheat, leading to Green Revolution has been achieved through exploitation of major genes for important traits like reduced height and photoperiod insensitivity (Reynolds and Borlaug, 2006a, b). Globally, it occupies about 224.70 Mha of area with the production of about 734.80 Mt (Anonymous, 2016a) and supplements almost 19 per cent of our total calories (FAO, 2011). Even though, production of wheat in India has touched 93.501 Mt in the year 2015-16 (Anonymous, 2016b), the demand of wheat is estimated to be about 109 Mt (Nagarajan, 2005) by the year 2020. Hence a sustained increase in grain yield potential is required.
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Most of the high yielding cultivated wheat varieties in India were bred for irrigated condition (http://www.dwr.in). Only a very few varieties (DBW16, HD2888, HI1531, NIAW917 and PBW533) have been bred for drought conditions (http://www.icar.org). Furthermore, the popular rainfed varieties like C306 and K68 were developed before Green Revolution. Therefore, to meet the need for greater wheat demand under increasing water scarcity, it is essential to develop wheat varieties that are high yielding and have acceptable level of tolerance to water stress.

1.2 Drought

Meteorologically, drought is characterized when evapo-transpiration exceeds from precipitation. Although agriculturally, drought is defined as “inadequacy of water availability (including precipitation and soil moisture storage capacity) in quantity and distribution” during the life cycle of a crop plant that resist the expiration of its full genetic yield potential.

1.2.1 Impact of drought on crop plant

World food production is affected primarily by environmental stresses. Among these the drought and heat are considered to be the main stresses for yield reductions (Boyer, 1982). In India, a drought has been reported at least once in every three to five years in the last five decades and is showing increasing frequency. Since the mid-nineties, prolonged and widespread droughts have occurred in consecutive years (Mishra and Singh, 2010). Right now, out of 29 per cent of total cultivable land in India, 10 per cent land is severely affected by drought which causes 20-30 per cent yield losses in wheat (Anonymous, 2012). Moreover, global climate changes suggest a growing increase in aridity and increased frequency of extreme events. Therefore, irrigation and generation of new appropriate varieties to drought is of utmost importance worldwide.

1.2.2 Physiological and biochemical changes in crop plants during drought

Drought is one of the most common environmental stresses which affects growth and development of plants through alterations in metabolism and gene
expression (Leopold, 1990). Physiological and biochemical changes at the cellular level are associated with drought stress. Synthesis of osmo-protectants, osmolytes or compatible solutes are the mechanisms that plants have evolved for adaptation to water deficit. These molecules which act as osmotic balancing agents, are accumulated in plant cells in response to drought and are subsequently degraded after stress relief (Tabaeizadeh et al., 1998). Osmoprotectants include amino acids, polyols, quaternary ammonium and tertiary sulfonium compounds (Rontein, 2002). One of the major signals operating during drought and other abiotic stresses is the plant hormone abscisic acid (ABA), which induces various genes involved in signaling cascades for the regulation of downstream biochemical protective mechanism (Schinozaki and Yamaguchi Schinozaki, 1997). Accumulation of proline which responds to stresses like temperature, drought, and starvation has been recommended to use as a parameter of selection for stress tolerance (Yancy et al., 1982; Sairam et al., 2002).

Another important consequence of drought stress in plants is the excessive production of reactive oxygen species (ROS) such as superoxide anion (O$_2^-$), H$_2$O$_2$ and the hydroxyl radicals particularly in chloroplast and mitochondria (Mittler, 2000; Neill et al., 2002). These ROS cause rapid cell damage by triggering off an electron transport chain reaction (Imlay, 2003). ROS scavenging activities are defended by complex non-enzymatic (Ascorbate, Glutathione, a-Tocopherol) and enzymatic responses such as CAT, APX, GR, SOD, etc., against abiotic stresses (Prochazkova et al., 2001; Vranova et al., 2002).

1.3 Challenges in breeding drought tolerant wheat

The pathways involve in plant physiological alterations have a molecular and genetic basis; therefore, genotypes differing in drought tolerance must present qualitative and quantitative difference in gene expression levels. The complex genetic basis (Yamaguchi-Shinozaki and Shinozaki, 1994) and high genotype x environment interaction (Bohnert et al., 2001; Bruce et al., 2002) are the two major problems encountered in understanding the mechanisms and pathways of drought tolerance. Reliable screening for drought tolerance is a continuing challenge for agricultural scientists and plant breeders, because it is time consuming, labour intensive and not so
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effective in identifying the materials, which are actually drought tolerant (O’Toole and Chang, 1979). Traditional methods of plant breeding have made a significant contribution to crop improvement, but they have been slow in targeting complex trait like drought tolerance. In addition, traditional plant breeding has other limitations such as: (i) a large segregating population needs to be screened for yield, its components and other important traits such as quality, biotic and abiotic stress tolerance; (ii) waiting for advanced generations (F$_5$ to F$_7$) to start selection for quantitative traits, as early generation is not effective; (iii) it is often difficult to screen a segregating population for desired traits when they trait are significantly influenced by environment; (iv) contrasting forms are often not distinguishable at seedling stage, making it necessary to grow population up to the adult stage; (v) it is difficult to undertake pyramiding of resistance genes, since selection of additional genes in presence of an existing resistance gene is challenging.

1.4 Application of biotechnological approaches into applied plant breeding

Modern plant research and advance biotechnological techniques are playing a leading role in advancing new and powerful tools to plant breeders (Ronald, 2014). One method receiving growing attention is the mapping of chromosomal regions affecting qualitative or quantitative traits. Polygenic characters, which have been very difficult to analyze using traditional plant breeding methods, can now be easily tagged using molecular markers. Molecular markers allow geneticists and plant breeders to locate and follow the numerous interacting genes that determine a complex trait (Bernardo, 2008). Combining marker-assisted selection methods with conventional breeding schemes has been demonstrated to increase the overall selection gain as well as the efficiency of breeding programmes. In addition, molecular techniques are also helpful to accelerate the introgression of desirable genes between varieties and novel genes from wild species to cultivated varieties.

In 1980s molecular biology entered in a new era when first non-PCR hybridization based molecular marker (RFLPs) came into existence (Botstein et al., 1980) which have been used in the detection of DNA polymorphism and construction
of genetic linkage maps. Later on, invention of polymerase chain reaction (PCR) technology (Mullis and Faloona, 1987) facilitated a large number of approaches and generations of PCR based molecular markers such as RAPDs (Williams et al., 1990), AFLPs (Vos et al., 1995), SSRs and several other markers that have been developed to date, primarily due to its apparent simplicity and high probability of success. Traditionally, the development of such type of markers were costly, iterative process which involved time consuming cloning and primer designing steps that could not easily be parallelized; scoring of these marker panels across target population was also too expensive, laborious and limitations of reproducibility across laboratories. The advent of high throughput SNPs arrays removed this bottleneck from genotyping process, excluding from the discovery process, since the production of a high quality array requires a substantial investment of resources and also markers are specific to the population in which they are developed. Therefore, genotyping of new populations will be biased in the direction of alleles present in the original survey that’s creating a serious problem to studies of highly divergent as well as wild populations.

1.5 Next generation sequencing (NGS) approaches

The next generation sequencing (NGS) has revolutionized genomic approaches; including new tools which are valuable for the discovering, sequencing, genotyping; not hundreds but thousands of markers across almost any genome of interest in a single step (Luikart et al., 2003). They are also useful in validation and assessment of genetic markers in populations, or even in the populations in which little genetic information is available (Davey et al., 2011). The recently developed novel approaches NGS technologies are based on varieties of restriction enzymes (REs) that produce a reduced representation of a genome of interest for genome wide marker discovery. These approaches includes reduced-representation sequencing (RRS) using reduced representation libraries (RRLs) (van Tassell et al., 2008), restriction site associated DNA sequencing (RAD-seq) (Baird et al., 2008), complexity reduction of polymorphic sequences (CRoPS) (van Orsouw et al., 2007) and low coverage genotyping viz. multiplexed shotgun genotyping (MSG) (Andolfatto et al., 2011) and genotyping by sequencing (GBS) (Elshire et al., 2011). In this
context, only the genotyping by sequencing approach has been further discussed since it has been applied in this study.

1.5.1 NGS through genotyping by sequencing (GBS)

The GBS is recently developed NGS platform (Elshire et al., 2011, Poland et al., 2012a). It is simple, robust, rapid, flexible, inexpensive and less laborious sequenced based genotyping approach. The GBS has removed the bottlenecks of traditional genotyping approaches where two steps process; marker discovery followed by assay design and genotyping are involved. The marker discovery and genotyping are completed at same time which facilitates exploration of new sets of germplasm without direct effort of discovering and characterizing DNA polymorphisms. The raw GBS data is dynamic and could be reanalysed which uncovers further information as bioinformatics tool improve, reference genome develop and collection of sequences data increases. Each of above said factor adds additional information into the same raw dataset. GBS can be readily used as potent tool for *de novo* discovery and finding new molecular polymorphism of complex genomes of new sets of germplasm as well as uncharacterized species and also genotyping of diverse panel to association mapping (Myles et al., 2009; Hamblin et al., 2010). Ultimately, the plant breeders continuously make an effort for connecting phenotype with genotypes and *vice versa*; resulting phenotypes will enable to assist genomic assisted breeding for achieving increase global food supplies with the utilization of limited natural resources where anticipated climate change play a greater role (Poland and Rife, 2012).

Keeping in view of the above developments and necessity of breeding for drought tolerance, the present study was aimed at following objectives:

1. Development of RIL population for drought tolerance
2. Molecular mapping for drought tolerance in spring wheat.