Comprehensive overview for developing drought tolerant transgenic wheat (*Triticum aestivum* L.)

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Abstract

Wheat is the most vital staple food crop in the world and is adversely affected by drought. Drought stress is a major restriction in the development of higher and more stable yielding bread wheat genotypes for arid and semi-arid regions throughout the world. Previously efforts have been made to introduce genes for drought tolerance through conventional breeding methodologies. The genomes of wheat and other harvested crops have been sequenced by a number of researchers, and numerous genes have been identified that can be exploited for developing drought stress tolerance. Drought tolerance enhancing genes have been discovered by using molecular applications, molecular markers, gene mapping and QTLs. In addition to using these approaches, microarray technology provides new ways to study gene expression and its utilization in the development of drought tolerant varieties. It is especially useful to consider the time when a specified gene associated with water deficit was identified. The full length cDNAs with required genes are being cloned by using Agrobacterium-mediated transformation. Thus the use of non-conventional breeding might be helpful in enhancing the production of wheat under drought stress conditions.

Key words: drought; microarray; cDNA; QTL; molecular marker; *Agrobacterium*

INTRODUCTION

Water shortage is a severe threat for world wheat production. Recent advancements in genomics have made possible the molecular transformation of wheat as a valuable technique for the regulation and manipulation of genetic material for drought tolerance. Higher yielding
and more drought tolerant wheat varieties may be developed through molecular transformation of novel genes. It is a time saving technique replacing conventional plant breeding in the hope of coping with future food shortages. This review summarizes all the novel aspects of genetic transformation in wheat.

Wheat (Triticum aestivum L.) is one of the most extensively consumed cereal crops worldwide (Farooq et al. 2011). Conventional and non-conventional breeding have significantly increased wheat production. But such improvements are insufficient to cope with the increasing food demands of a rapidly growing human population. It is not possible through conventional breeding methods that are limited because of gene pool accessibility and very slow procedures. With the advent of recombinant DNA technology and various advanced molecular techniques wheat production is increasing. The evolution of wheat led the civilizations that spread out from the Middle East to new regions, resulting in huge wheat production for a growing world population. The tribe Triticeae consists of over 300 species including wheat, rye and barley and is considered as a germplasm source for bread wheat improvement by conventional and non-conventional breeding programs.

Wheat produces its supreme yield potential only in stress free environments while environmental stress causes a decrease in this potential. Abiotic stresses are the major danger for wheat crop production and cause up to 71% reduction in yield. Drought is the most challenging threat with global distribution that directly reduces the growth and development of crop plants by disturbing normal biochemical processes and gene expression (Jones et al. 2003, Akram et al. 2004). Drought stress limits plant growth, development and ultimately productivity in almost all the cereals, thus it is one of the most serious threats to world agriculture (Aazami et al. 2010, Hamayun et al. 2010, Subhani et al. 2011). Drought negatively affects the chlorophyll content in plants. Water stress increases the grain filling rate, while grain filling duration is substantially decreased. Cultivars staying green, better grain filling and duration at the reproductive stages under water deficit are better able to survive drought stress (Nawaz et al. 2013).

Relative water content and stomatal conductance are reliable characteristics that regulate the rate of transpiration from stomata. They work as indicators for drought tolerance in wheat and can reliably be used in further breeding programs for drought tolerance (Baloch et al. 2013). Drought severely affects yield production in cereals. The foliar application of growth-enhancers may be useful in reducing the effects of drought by increasing the antioxidant levels and secondary metabolites of the plant (Yasmeen et al. 2013). This situation demands crop breeding for drought stressed areas utilizing modern molecular techniques instead of using traditional time consuming breeding methodologies (Razzaq et al. 2004).

The genes for drought tolerance are regulated at once under drought stress and produce the respective products that respond to signal transduction, and stress response and help the plant to withstand drought conditions (Zhou et al. 2010). Many stress-inducible genes produce: osmo-protectants, chaperones, and detoxification enzymes that directly protect against environmental stresses. The gene expression and signal transduction are also regulated by transcription factors and protein kinases under water scarce conditions (Seki et al. 2003). This indicates that the sensible stress-responsive genes expression in time is very harmful for the plants' capacity to survive and tolerate the various abiotic stresses (Chinnusamy et al. 2007, Shinozaki and Yamaguchi-Shinozaki 2007). Marker-assisted selection is a useful tool in accelerating the wheat breeding against different abiotic stresses (Wei et al. 2009). Marker assisted breeding has provided an opportunity to check the utility of various genomic regions of a crop germplasm under water scarce conditions, which were not possible in the past (Ashraf 2010). The use of various molecular markers including random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP) have made it easy and simple. As wheat has a large genome size and is allohexaploid in nature so the cloning of genes creates problems. Therefore, markers are usually developed through polymorphisms within transcribed regions of functional genes which are entirely associated with the function of the gene (Andersen and Lübberstedt 2003, Wei et al. 2009).

The genes that produce late embryogenetic proteins are stimulated under the stress that assembles in seedlings to avoid desiccation, within vegetative tissues and cells where there is loss of water from the plant body which damages the cell; the proteins help the plant cells to resist against water stress (Hu et al. 2003, Khanna and Daggard 2003). Transgenic
strategy has been used for effectively presenting and over expressing of barley HVA1 gene development for late embryogenesis proteins by using the particle bombardment procedure (Sivamani et al. 2000, Cattivelli et al. 2008). The majority of the transgenic genotypes examined showed enhancement in essential agronomic characteristics, such as water use efficiency, fresh shoot and dry weight, total dry matter and fresh root and dry weight when plants were sown in water deficit conditions. Generally, this particular analysis demonstrated how the transgenic genotypes indicating the HVA1 gene expression to enhance development features such as a good improved biomass per plant under water limited conditions. The actual breakthrough is associated with the discovery of genes, gene expression under water stress conditions and induction of resistance against abiotic stress may be successful by using functional genomics methods and effective genetic engineering strategies leading to better stress tolerance (Hiei et al. 1994, Ishida et al. 1996, Cushman and Bohnert 2000, Hu et al. 2003, Vishnudasen et al. 2005, Bhatla et al. 2006, Jauhar 2006, Khurana et al. 2008).

Wheat genetic transformation procedures
Genetic engineering for the improvement of wheat requires the separation, recombination and expression of distinct foreign genes into appropriate re-generable explants. In the past attempts were made to induce genes in wheat through protoplast fusion as explants due to lack of a cell wall. It was found that a marker gene introduced into protoplasts gave very important evidence regarding the gene expression and tissue specific variety of promoters and gene regulatory elements in the transformed explant tissue (Bajaj 1990, Chibbar et al. 1991, Maheshwari et al. 1995, Vasil and Vasil 1999, Cooper et al. 2004, Akiyama and Jin 2007, Shinozaki and Yamaguchi-Shinozaki 2007). Firstly, the required gene is identified and introduced into a specific vector to check the expression of that gene before the transfer into the desired plant in genetic transformation. Previous research on transformation in wheat explained that the cDNA can be transferred into the plant cell by using the Escherichia coli gene for the enzyme chloramphenicol acetyl transferase (CAT) (Hauptmann et al. 1988, Bajaj 1990, Chibbar et al. 1991, Maheshwari et al. 1995). The identification of the transgene by enzymatic and immune-chemical approaches for competency of CAT activity and quantity of CAT protein was a tedious job and also the existence of inhibitors of CAT activity and the endogenous CAT activity limited its use as a reporter gene in wheat (Chibbar et al. 1991, Vasil et al. 1992, Maheshwari et al. 1995). The accessibility of a procedure for the fast analysis of the b-glucuronidase gene from E. coli, appeared as the most widely used scorable marker in wheat transformation (Chibbar et al. 1991, Vasil et al. 1992, 1993, Weeks et al. 1993, Becker et al. 1994, Maheshwari et al. 1995, Akiyama and Jin 2007). The b-glucuronidase enzyme hydrolyzes b-glucuronide compounds and its products which are quantified by spectrophotometric techniques (Jefferson et al. 1987, Vasil et al. 1992, 1993). The b-glucuronidase reporter gene system is really suitable for optimization of traits for the transformation of genes by using a simple histochemical exposure method. The destructive nature of its assay is one of the major restrictions of using the b-glucuronidase reporter gene system. The anthocyanin biosynthesis, firefly luciferase and green fluorescent protein have been used effectively to evaluate the outcome by using the reporter genes to introduce transgenes in living cells. R genes of Zea mays stimulate the synthesis and accumulation of endogenous anthocyanin in the vacuoles which is a valuable scorable marker in differentiated and mature plant cells. The R genes have been used in wheat for transformation (Weeks et al. 1993, Dhir et al. 1994, Maheshwari et al. 1995, McCormac et al. 1998, Chawla et al. 1999a, b, Jordan 2000, Bortolotti et al. 2004). A synthetic and spectrally modified shape of green fluorescent protein extracted from jellyfish and Aequorea victoria has been used as an essential marker gene in the transformation of wheat (Maheshwari et al. 1995, Pang et al. 1996, McCormac et al. 1998, Jordan 2000, Akiyama and Jin 2007). It was reported that the use of a modified green fluorescent protein as a detectible marker gene for the recognition of transgenic wheat plants was very reasonable. The luciferase gene (fire fly) and Photinus pyranus, have been used effectively in secure transformation of the required gene in wheat which provides a non-destructive type of transgene analysis and provides an easy way to evaluate the differences in the use of various marker efficiency in wheat (Lonsdale et al. 1998, Harvey et al. 1999, Jordan 2000).

Molecular markers
The assorted rate of recurrence associated with DNA shipping within cell or tissue related with various explants necessitated the actual
improvement associated with effective selection of cell or tissue which has transformed gene sequences. Selection of the required transformed cell is based on the resistance to some cytotoxic material, frequently a good antibiotic or even a good herbicide. Molecular guns which label DNA sequence variability for such types of transformation bacterial cells are used as target DNA (Jordan 2000, Jansen and Nap 2001, Johnson 2004, Shinozaki and Yamaguchi-Shinozaki 2007), particularly for traits defined by responses to developmental, environmental or physiological changes. Mostly, a bar gene (a bialaphos resistance gene) is used as a selection marker in wheat transformation for the encoding of phosphine-thrincin acetyl transferase isolated from various Streptomyces species. The aminoglycoside antibiotic is commonly used for transformation in wheat amongst the entire antibiotic resistance neomycin phosphotransferase II marker gene. The herbicide resistance genes provide a substitute for the antibiotic-resistant marker gene. The gene coding for 5-enolpyruvyl-shikimate-3-phosphate synthase (critical enzyme for aromatic amino acid biosynthesis) and phosphinothricin acetyl transferase respectively provide tolerance to glyphosate and glufosinate ammonium herbicides. The enolpyruvyl-shikimate-phosphate synthase gene has been isolated from the Agrobacterium strain CP4 and the glyphosate oxidoreductase also used for transformation in wheat that provides resistance against glyphosate by degrading glyphosate into amino methyl phosphoric acid (Zhou et al. 1995, Cooper et al. 2004, Shinozaki and Yamaguchi-Shinozaki 2007). The hygromycin phosphotransferase gene (widely used in rice transformation) is an effective marker for achieving useful wheat genetic transformation (Ortiz et al. 1996, Cooper et al. 2004, Eathington et al. 2007, Shinozaki and Yamaguchi-Shinozaki 2007). The effectiveness of hygromycin phosphotransferase gene as a marker gene is comparable to the other extensively used marker genes encoding antibiotics and herbicides resistance. Most recently, mannose-6-phosphate isomerase encoded by manA from E. coli, has been selected as a useful marker for wheat (McCormac et al. 1998, Chawla et al. 1999a, b, Jordan 2000, Bortolotti et al. 2004). The mannose-6-phosphate isomerase marker allows transformants to grow on mannose as an exclusive carbon source and has shown a positive way of action which relatively boosted the development of the transformed tissues more than immediately allowing it (Chawla et al. 1999a, b, Hansen and Wright 1999, Weeks 2000, Cooper et al. 2004, Eathington et al. 2007, Shinozaki and Yamaguchi-Shinozaki 2007).

**Gene transformation**

Different methodologies have been developed for the transfer of genes into intact plant tissues by particle bombardment which has revolutionized the technology of plant transformation. The concepts of accelerating DNA-coated particles into cells and tissues are an innovative established tool in plant molecular biology (Klein et al. 1987, Oard et al. 1990, Klein and Jones 1999). The particle bombardment technique of introducing DNA into cells was evolved to decrease the biological restrictions of Agrobacterium and the problems associated with plant regeneration from protoplasts. The early research on particle bombardment as a gene delivery technique attained transient expression of b-glucuronidase gene subsequent bombardment of cell suspensions (Wang et al. 1988, Chawla et al. 1999a, b, Weeks 2000, Cooper et al. 2004), leaf bases and apical tissues (Oard et al. 1990, Weeks 2000) and immature embryos (Chibbar et al. 1991). Vasil et al. (1991) gained fully transformed callus lines that expressed all the marker genes tested (b-glucuronidase, neomycin phosphotransferase II and enolpyruvyl-shikimate-phosphate synthase-gene). Vasil et al. (1992) reported the first successfully transformed wheat plant through the particle bombardment method by using plasmid vector BARGUS into cells of type-C (long-term regenerable embryogenic callus) which was isolated from immature embryos. The succeeding improvement in techniques condensed the time needed for the creation of transgenic wheat plants through particle bombardment of immature embryos and embryogenic cali in 5–7 months (Oard et al. 1990, Vasil et al. 1993) by about 4 months by immature embryos (Weeks et al. 1993, Weeks 2000) and about 3 months by using an enhanced regeneration system using isolated scutella as the starting explant (Nehra et al. 1994) and more recently to 56–66 days through particle bombardment of cultured immature embryos (Altpeter et al. 1996, Weeks 2000). Mostly, subsequent gene transfer researches employed immature embryos, isolated scutellum, and cali initiated from immature embryos work as the target tissue for bombardment. Bommineni et al. (1997) reported the first successful gene transformation in durum wheat by particle bombardment of isolated scutella. The particle bombardment of explants like pollen embryos and microspore-derived
embryos (Shimada et al. 1991, Loeb and Reynolds 1994, Altpeter et al. 1996, Weeks 2000) resulted in the successful introduction of transgenes as shown by transient expression of b-glucuronidase gene, but regeneration of plantlets has not been reported. Iglesias et al. (1994) investigated the direct transformation of foreign DNA to the meristem cells of immature embryos by microtargeting, and transgenic sectors were achieved in both coleoptiles and leaf primordial and were affected by spermidine concentration, the amount of plasmid DNA, the osmotic pretreatment of target tissues and vacuum pressure on gene transformation into wheat tissues (Srivastava et al. 1996, Rasco-Gaunt et al. 1999, Jauhar 2006, James 2007). Mostly investigations have not found any clear correlation between transient expression and stable transformation but the biolistic technique displays a significant degree of stability in assimilation and expression in succeeding generations (Altpeter et al. 1996, Srivastava et al. 1996, Baenziger et al. 2006, Jauhar 2006, Varshney et al. 2006, James 2007). It was reported that the multiple copies of insertions of the transgenes is very common in transgenics acquired through the particle bombardment procedure (Srivastava et al. 1999, Stoger et al. 1999, Bieri et al. 2000, Jauhar 2006). The particle bombardment technique has appeared as a reproducible procedure for the transformation of several marker genes in wheat. It may be used to increase the yield and quality of the wheat plant, reduce genetic male sterility, drought resistance, fungal resistance and resistance against other diseases and insect/pests through engineering and transposons labeling (Leckband and Lorz 1998, Bieri et al. 1999, Chen et al. 1999, Stoger et al. 1999, Bieri et al. 2000, Sivamani et al. 2000, Baenziger et al. 2006, Jauhar 2006, Varshney et al. 2006, James 2007).

**Agrobacterium-mediated transformation method**

The *Agrobacterium*-mediated transformation procedure is a very simple, cheap and most proficient substitute to direct gene transformation procedures. The benefits of the *Agrobacterium* method involved the distinct insertion of a discrete segment of DNA into the recipient genome. The early experiments on *Agrobacterium* and wheat were supported by the agro-infection phenomenon which initially showed that the soil bacterium can interact in a restricted manner with the cells of a monocot plant and transfer its DNA into the plant cell (Woolston et al. 1988, Dale et al. 1989, Bieri et al. 2000, Hu et al. 2003, Khanna and Daggard 2003, Chauhan and Khurana 2011). The agro-infection is actually the transfer of viral DNA located between the T-DNA border sequences of *Agrobacterium* into plant cells and delivers an appropriate system to monitor transformation of DNA into target explants by recognition of disease symptoms in the plant. The wheat dwarf virus (WDV) worked as a sensitive marker for observing the wheat-*Agrobacterium* relations. Symptoms and detection of the disease due to amplification of the viral DNA generate is confirmed by the ELISA test. In wheat, the effective agro-infection of dwarf virus paved the way for natural advancement of procedures for the introduction of non-viral builds with scorable and selectable markers. It is also necessary for a good understanding of the parameters requisite for the introduction of foreign DNA into wheat. Most of the studies on *Agrobacterium*-mediated transformation worked incubation of suitable explants with the *Agrobacterium* inoculum. Mooney and Goodwin (1991) initially showed that wounding is not essential for the introduction of bacteria into explants of wheat and described an increase in the adherence of bacteria at the wound site produced by mechanical and enzymatic actions. Mahalakshmi and Khurana (1995) studied the fitness of various explants for *Agrobacterium*-mediated gene introduction and described an increase in the transient expression of b-glucuronidase, neomycin phosphor transferase II and enolpyruvyl shikimate-phosphate synthase gene in mature seeds exposed to mechanical injury by scratch as compared to intact seeds. The introduction of viral genes is essential as transfer of the gene has been attained by injury and also by using chemical inducers like aceto-syringone (Mahalakshmi and Khurana 1997, Hu et al. 2003, Fleury et al. 2010, Nevo and Chen 2010). Chen and Dale (1992) maintained that a higher frequency of infection is observed in exposed apical meristems of dry wheat seeds as compared to intact seeds. Significant advancement is achieved in rice, maize and wheat in the region of *Agrobacterium*-mediated transformation (Chen et al. 1993, Hiei et al. 1994, Ishida et al. 1996, Hu et al. 2003) which has contributed enormously to increase understanding of the various factors required for the effective generation of transgenic cereals (Fleury et al. 2010, Nevo and Chen 2010). Cheng et al. (1997) first reported the stable transformation of wheat by *Agrobacterium*-mediated co-cultivation and demonstrated the successful transmission.
of the transgene to the next generation. The transgenic plants were developed within a total time of 2.5 to 3 months by co-cultivating freshly isolated pre-cultured immature embryos and embryogenic calli. The factors which influenced the generation of transgenic plants included the inducers in the inoculation, explant tissue, co-cultivation media and the surfactants present in the inoculation medium. It was reported that nearly 35% of the transgenic plants received a single copy of the transgene and one to five copies of the transgene were integrated into the wheat genome without rearrangement of the genome (Cheng et al. 1997, Hu et al. 2003, Fleury et al. 2010, Nevo and Chen 2010). McCormac et al. (1998) used cell autonomous reporter genes (b-glucuronidase, neomycin phosphor transferase II and enolpyruvyl shikimate-phosphate synthase genes) for transformation purposes. The localization of the transformed cells revealed a non-random distribution throughout each embryo and callus piece. It has been found recently that, Agrobacterium-mediated transformation of immature inflorescence tissue may be successfully used for gene transformation in wheat (Amoah et al. 2001, Khanna and Daggard 2003, Fleury et al. 2010, Nevo and Chen 2010). This study achieved optimal T-DNA transformation (measured by b-glucuronidase gene activity) in explants pre-cultured for 21 days and sonicated (subjected to brief periods of ultrasound in the presence Agrobacterium). In the near future, it can be expected that Agrobacterium will be employed as an efficient, reliable and economical vector for the introduction of exogenous genes into wheat to produce abiotic stress resistant genotypes, by various laboratories throughout the world.

**Marker assisted selection in wheat breeding**

The latest information on the advancement in gene targeting technology and the production of meiotically transmissible plant mini-chromosomes pave the way for transferring more characters with high complexity (Ow 2007, Yu et al. 2007, Nevo and Chen 2010). Currently, Biotechnology is controlled to accumulate beneficial genetic diversity from necessary sources. This constructs the concentrate favorable gene action and exploits heritability for a greatly expanded set of traits; molecular plant breeding increases favorable gene action. It is very necessary to highlight the QTL studies, which, when conducted on a suitable scale and with the precision to recognize causal genes, showed itself to be a strong functional genomics method (Fleury et al. 2010, Nevo and Chen 2010). The molecular cloning of QTLs has produced novel insights about the science of quantitative traits that could not have come from the analysis of gene knockouts or over expression approaches; especially the influences of regulatory variation on phenotypic variation and evolution (Cong et al. 2002, Yan et al. 2004, Clark et al. 2006, Salvi et al. 2007). Genomics, molecular markers and biotechnology are now applied in an interactive network to express genetic diversity for crop development. The genomic information permits the finding of favorable alleles via QTL mapping and cloning, followed by the use of information learned from the molecular characterization of QTLs to design optimal transgenic strategies for crop improvement. Marker assisted selection refers to selection based on DNA markers linked to QTLs. The DNA markers are very powerful tools which are identified by their mapping carried out on the chromosome in relation to QTLs. The presence of QTLs for drought tolerance can then be tracked by cautious monitoring of these DNA markers (Salvi et al. 2007, Gosal et al. 2009). The list of QTLs associated with drought tolerance is given in Table 1. Various DNA markers like amplified fragment length polymorphism, restriction fragment length polymorphism and simple sequence repeats have been used to tag QTLs for drought stress in wheat (Quarrie et al. 2005, Salvi et al. 2007). The application of microsatellite markers in wheat for tagging QTLs for grain protein contents, disease resistance and yield have also been documented by a number of scientists (Huang et al. 2000, Del Blanco et al. 2003, Huang et al. 2003, Prasad et al. 2003, Salvi et al. 2007). Kirigiwi et al. (2007) used a simple sequence repeat/expressed sequence tag marker for mapping QTL on chromosome 4A for grain yield and yield attributes in wheat. The markers associated with the QTL were Xwmc89, XBE637912 and Xwmc420. The DNA markers closely linked with QTLs conferring drought tolerance would greatly enhance the selection efficiency and selection response (Cattivelli et al. 2008, Gosal et al. 2009).

The use of molecular markers in wheat depends on the hybridization based restriction fragment length polymorphism method. Restriction fragment length polymorphism maps provided a direct process for choosing desired genes via their linkage to easily visible markers thereby expediting the movement of desirable genes among different varieties (Tanksley et al. 1989). The factors that had been contributory for the use
of RFLPs in wheat were the limited number of polymorphisms detected among different wheat genotypes and more significantly the availability of aneuploid stocks for the detection of chromosomal location of genes. In wheat RFLP have been used to map seed storage protein loci (Dubcovsky et al. 1997), mapping of genes for dwarfing (Rht12) cultivar identification (Vaccino et al. 1993), loci associated with flour colour (Parker et al. 1998), vernalization (Vrn1) and a frost resistance gene on chromosome 5A (Galiba et al. 1995), intra-chromosomal and vernalization (Korzun et al. 1997), quantitative trait loci (QTL’s) controlling tissue culture response (Ben Amer et al. 1997), resistance to pre-harvest sprouting (Anderson et al. 1993), milling yield (Parker et al., 1999), resistance to chlorosis induction by Pyrenophora tritici-repentis (Faris et al. 1997) and nematode resistance (Eastwood et al. 1994, Schachermayr et al. 1995, Williams et al. 1996, Lagudah et al. 1997). Restriction fragment length polymorphism markers are also beneficial for the selection programs of insect/pests resistance and pathogens resistance. Otherwise, it is very laborious and time consuming to identify homozygous individuals but has been used for resistance to wheat spindle streak mosaic virus (Khan et al. 2000), resistance to barley yellow dwarf virus (Crasta et al. 2000), resistance against leaf rust (Schachermayr et al. 1994, Autrique et al. 1995, Feuillet et al. 1995, Schachermayr et al. 1995, Dedryver et al. 1996), resistance against powdery mildew (Hartl et al. 1993, Ma et al. 1994, Hartl et al. 1995), and resistance against cereal cyst nematode (Eastwood et al. 1994, Schachermayr et al. 1995, Lagudah et al. 1997). The use of Restriction fragment length polymorphism analysis in wheat has been restricted use in the inter-varietal analysis due to the low level of polymorphism and the high cost of screening in breeding situations. The development of the polymerase chain reaction (PCR) methodologies produced RAPD to appear as an appropriate and convenient method for tracing alien chromosome segments in translocation lines (Devos and Gale 1992, Lagudah et al. 1997, Cong et al. 2002, Clark et al. 2006, Salvi et al. 2007). The RAPD markers provide a beneficial substitute to restriction fragment length polymorphism markers as screening markers linked to a single trait within near isogenic lines and bulked segregants. He et al. (1992) described the development of a DNA polymorphism detection procedure by combining RAPD with denaturing gradient gel electrophoresis for pedigree analysis and fingerprinting of wheat cultivars. RAPD markers can be altered to more user-friendly sequence characterized amplified area markers that display a less complex banding pattern. Sequence characterized amplified region markers linked to resistance genes against fungal pathogens have been characterized in combination with RAPD markers and AFLP markers (Lagudah et al. 1997, Procionier et al. 1997, Myburg et al. 1998, Liu et al. 1999, Clark et al. 2006, Salvi et al. 2007). RAPD markers and other PCR based markers like Sequence Characterized Amplified Regions, Sequence Tagged Sites and Differential Display Reverse Transcriptase PCR are increasingly being used for identification of desirable traits in wheat and related genera. These markers have been used specifically for disease resistance

<table>
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<tr>
<th>Cross</th>
<th>Trait</th>
<th>QTL Mapping</th>
<th>Number of QTL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese Spring x Ciano 67</td>
<td>ABA concentration</td>
<td>DHL*</td>
<td>1</td>
<td>Quarrie et al. (1994)</td>
</tr>
<tr>
<td>Songlen × Cobodor 4/3Ag14</td>
<td>Osmoregulation under Drought</td>
<td>RIL*</td>
<td>1</td>
<td>Morgan and Tan (1996)</td>
</tr>
<tr>
<td>Trident × Molineux</td>
<td>Yield interaction with water supply and hot conditions</td>
<td>DHL</td>
<td>1</td>
<td>Kuchel et al. (2007)</td>
</tr>
<tr>
<td>Durum × Wild emmer</td>
<td>Various morpho-physiological Traits</td>
<td>RIL</td>
<td>Many</td>
<td>Peleg et al. (2009)</td>
</tr>
<tr>
<td>Seri M82 × Babax</td>
<td>Various productivity and physiological traits</td>
<td>RIL</td>
<td>Many</td>
<td>McIntyre et al. (2010)</td>
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* DHL = doubled haploid; RIL = recombinant inbred lines

Table 1. List of QTLs associated with drought tolerance in wheat
against viral and fungal pathogens and also for insect, pest, nematode, and have the potential of pyramiding of resistance genes for effective breeding programs. The PCR based markers have been widely characterized for genes of resistance against leaf rust, *Puccinia recondita* (Feuillet et al. 1995, Dedryver et al. 1996, Seyfarth et al. 1999), common bunt, *Tilletia tritici* (Demeke et al. 1996); powdery mildew, *Erysiphe graminis* (Hartl et al. 1995, Qi et al. 1996, Korzun et al. 1997, Roy et al. 1999), Russian wheat aphid, *Diuraphis noxia* (Korzun et al. 1997, Myburg et al. 1998, Venter and Botha 2000) and resistance against Hessian fly, *Mayetiola destructor* (Dweikat et al. 1994). The microsatellites or simple sequence repeats are more promising molecular markers for the identification and differentiation of genotypes within a species. The higher level of polymorphism and easy handling has constructed microsatellites tremendously beneficial for different techniques in wheat breeding (Devos et al. 1995, Roder et al. 1995, Bryan et al. 1997, Korzun et al. 1997, Roy et al. 1999, Stachel et al. 2000). Simple sequence repeats or microsatellites have also been used to identify resistance genes like Yr15 from bread wheat (Chague et al. 1999) and Pm6 from *Triticum timopheevi* (Tao et al. 1999). The molecular markers can deliver immediate and consecutive selection of agronomical significant genes in wheat breeding programs. It permits the screening of numerous agronomically significant characters at initial stages and efficiently substitutes time consuming bioassays in initial generation screens.

Drought stress is the major restriction to crop production. Wheat being an important food crop has always been of curiosity to plant breeders. Several efforts have been made simply to enhance its production in general, as well as under drought stress. To develop improved drought tolerant cultivars, plant breeders have explored several useful traits including stomatal conductance, cell membrane stability and osmotic adjustment etc. and released better performing varieties. Applications of conventional selection based breeding are limited due to the complex nature of drought stress and drought tolerance. Advancements in genomics have paced molecular transformation of wheat, as the regulation and manipulation of the genome for drought tolerance is possible. Genetic transformation has an advantage over conventional and marker-assisted breeding as the introduction of the new gene is only at the favorite targeted locus. Several genes have been discovered from wheat and indicated association in vital biological pathways that confers drought tolerance in wheat. However, there is still a lot to be explored about these discovered genes as most of them have only been studied in model plants (e.g. *Arabidopsis* etc.). The molecular based tools would ultimately help us to identify potential candidate genes and valuable QTLs for drought tolerance and their effective utility in marker assisted breeding. The next phase must be the incorporation of drought tolerant genes either through genetic transformation or gene breeding coupled with marker assisted selection.

It is concluded that the higher yielding and drought tolerant wheat varieties must be developed through genetic transformation of novel genes found after screening of wheat germplasms which cope up the future food requirement of world population.

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