



REVIEW PAPER

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Utilization of synthetics for drought tolerance in bread wheat (*Triticum aestivum* L.)

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Abstract

Wheat (*Triticum aestivum* L.) is the staple food of 36% of world population. One of the wheat ancestors, D genome donor *Aegilops tauschii* is supposed to impart resistance against the biotic and abiotic stresses. At the time of wheat evolution, very few accessions of *Aegilops* were available so little tolerance was found in wheat varieties prevailing in that era. In hexaploid wheat, there is only 25% polymorphism so *Triticum turgidum* (4x) was crossed with different accessions of *Aegilops tauschii*, and genetic diversity was enhanced artificially. Because of synthetics production, it became easy to study wheat genome for improving yield under stress condition to ensure food security.

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Introduction

Wheat (*Triticum aestivum* L.) is used mainly for human consumption and supports nearly 35% of the world population (USDA, 2008). To meet the needs of the growing world population, the forecast demand for the year 2020 is projected to 1 billion tons. Due to land limitations, the enhancement of wheat production must come from higher absolute yields by increasing efforts in plant breeding along with biotechnological tools and expanded genetic diversity (Rajaram, 2001). It is the need of time to enhance wheat (*Triticum aestivum* L.) production by synthesizing the artificial wheat by introducing stress tolerant alleles in wheat genome. In wheat, D genome is responsible for donating stress tolerance characteristics so synthetic wheat became the source of enhanced wheat yield in drought prone areas.

Wheat

Wheat (*Triticum aestivum* L.) is third most prominent grain crop in the world. Though rice and corn are also important crops, there are more acres of wheat field globally. Wheat belongs to genus *Triticum* having genome of AABBDD ($2n=6x=42$). It is an allohexaploid, which evolved from the hybridization of a tetraploid wheat (AABB) with *Aegilops tauschii* ($2n=2x=14$, DD). The contributing genomes A, B and D are from three wild grasses belonging to the family Poaceae. Its originate about 10,000 year ago in the 'Fertile Crescent' a region with rich soil in the upper areas of the Tigris-Euphrates drainage basin (Briggle and Curtis, 1987).

Cultivated wheat belongs to the tribe Triticeae of the grass family (Gramineae) with basic chromosome number 7. Wide crosses and polyploidy have played a major role in evolution of Triticeae and thus interspecific and intergeneric crosses around Triticeae progenitor sources are being exploited and proving successful (Mujeeb-Kazi *et al.*, 2006).

Wheat is used mainly for human consumption and supports nearly 35% of the world population. The demand for wheat is expected to grow faster than any other major agricultural crop. To meet the needs of the growing world population, the forecast demand

for the year 2020 varies between 840 (Rosegrant *et al.*, 1995) and 1050 million tons (Kronstad, 1998). Due to land limitations, the enhancement of wheat production must come from higher absolute yield, which can only be met by the concerted action of scientists involved in diverse agricultural disciplines and in particular by increased efforts in plant breeding (Braun *et al.*, 1998).

D-Genome

About 8000 years ago, the wheat plant, which human beings selected for the domesticated purpose, was actually the result of natural cross between two distantly related wild grasses *Aegilops speltoides* (BB or SS) and *Triticum uratu* (AA). The spontaneous F1 hybrid ($2n=2x=14$, AB) naturally doubled to give rise to the wild emmer or durum wheat with $2n=4x=AABB$ composition. Around 6000 BC one more hybridization took place in natural condition between wild emmer and the progenitor or donor of D genome i.e. *Ae. tauschii* which resulted in modern hexaploid bread wheat i.e. *Triticum aestivum* with $2n=6x=42$, AABBDD (Jiang *et al.*, 1994). The D genome is more drought and salt tolerant as compared to other genomes like A genome and B genome. So the hexaploid wheat is genomically AABBDD exhibits more salt and drought tolerance as compared to other synthetic hexaploid like AAAABB and AABBBB (Xu *et al.*, 2002).

The Synthetic Hexaploid (SH)

Among different methods of wheat improvement wide hybridization has received much attention over last few decades (Mujeeb-Kazi *et al.*, 2008). The different accessions of *Aegilops* species (*Ae. cylindrica* Host, *Ae. geniculata* Roth and *Ae. neglecta* Req. ex Bertol.) have the potential to be used for wide hybridization programmed of wheat for abiotic stresses like drought resistance and high temperature (Zaharieva *et al.*, 2001; Zaharieva *et al.* 2003; Baalbaki *et al.*, 2006). Introduction of valuable genes from exotic donors via wide crosses has been proposed to broaden the genetic base of many crop plants with known and closely related wild relatives, such as the diploid and tetraploid progenitors of bread wheat

(BW) (*Triticum*; $2n=6x=42$, AABBDD). Among these wild relatives *Aegilops tauschii* the donor of D genome has attention of the breeder, as much potential is available in this wild relative for wheat improvement against different biotic and abiotic stresses (Zohary *et al.*, 1969).

The synthetic hexaploid wheat were produced artificially crossing tetraploid forms such as modern durum wheat (*Triticum turgidum*, $2n=4x=28$ AABB) with *Aegilops tauschii* ($2n=2x=14$, DD) (Mujeeb-Kazi *et al.*, 1996). In this regard, a core collection of *Aegilops tauschii* comprised 450 accessions (Kawahara *et al.*, 2003) were used in CIMMYT to produce more 1000 synthetic hexaploid wheat (Mujeeb-Kazi, 2003). Over the past decade CIMMYT has produced numerous synthetic and their derivatives with exceptional expression for traits relating to agronomic features, resistance/tolerances to abiotic and biotic stresses and quality (Trethowan and Mujeeb-Kazi, 2008). The synthetic hexaploid wheat is a good source for incorporating genes in common bread wheat that confer resistance to biotic and abiotic stresses like drought and salinity and can be used to induce new genetic diversity in to wheat gene pool (Lage *et al.*, 2003).

The bread wheat offers an enormous potential for the incorporation of genetic resources, because its allopolyploid nature enables for the incorporation of genetic resources, because its allopolyploid nature enables the introgression of genes from wild species by recombination of the homologous chromosomes (Valkoun, 2001). Many novel alleles for biotic and abiotic stresses in many synthetic hexaploids, which were stably, inherited in the backcross derived lines. This indicated that the synthetic hexaploids and its backcross derivatives could also be used for broadening the genetic base of elite breeding programmed (Zhang *et al.*, 2005).

Trethowan and Mujeeb-Kazi (2008) also reported on the value of exotic germplasm that comprised of D genome diversity and land races in addressing environmental stress constrains which limits yield

output and elucidate their alleviation through this unique genetic resource. Due to this reason, the 50 percent advanced lines developed in CIMMYT for drought tolerance have synthetic hexaploid (SH) in their pedigrees. Molecular evaluation of these SH helps breeders all over the world to target their crossing programmed around this diversity (Mujeeb-Kazi *et al.*, 2004). Synthetic hexaploid wheat and their derivatives were evaluated in Mexico under managed drought stress condition the results showed that the yield of these synthetic were 23 percent and 33 percent higher as compared to their adapted recurrent parents and the best local check respectively (Trethowan *et al.*, 2000; Reynolds and Trethowan, 2007). The genetic diversity in synthetic hexaploid wheat derived from *Aegilops tauschii* enabled them to show resistance to major wheat diseases and tolerance to abiotic stresses. At molecular level these new synthetic derivatives had been shown to boost genetic diversity (Villareal and Mujeeb-Kazi, 1999; Van Ginkel and Ogbonnaya, 2007).

Drought

Drought is a worldwide problem, seriously influencing crop productivity. Moreover about 60% of land area on the globe belongs to arid or semiarid zone (Zong, 2006). The worldwide water shortage and uneven distribution of rainfall makes the improvement of drought resistance especially important. Fulfillment of this goal would be enhanced by understanding of the genetic and molecular basis of drought resistance (Bring *et al.*, 2006). The drought is one of devastating environmental stresses. Currently about 25 percent of world's agricultural land is effected by drought (Jajarmi, 2009; Quarrie *et al.*, 2003).

Drought is actually a meteorological event which implies the absence of rainfall for a period of time, long enough to cause moisture depletion in soil and consequently in water potential of plant tissues. But from agriculture point of view, it's working definition would be the inadequacy of water availability, including precipitation and soil-moisture storage

capacity, in quantity and distribution during the life cycle of a crop plant, which restricts the expression of full genetic potential of the plant. It acts as serious limiting factor in agricultural production by preventing a crop from reaching the genetically determined theoretical maximum yield (Jiban, 2001). Water stress condition and high temperature are the key factors which affects yield in cereals (Barnabas *et al.*, 2008; Das *et al.*, 2007).

Among cereals, wheat is grown in both irrigated and rain fed areas around the globe and the major limiting factors which affects its productivity and stability are drought and salinity (Morris *et al.*, 1992). Productivity with high yield is dependent upon resistance or tolerance in the crop varieties that face various biotic and abiotic stress constraints. The major constraints in Pakistan for biotic stresses are the three rusts, karnal bunt, barley, yellow dwarf virus, some spot blotch, powdery mildew, aphids, smuts being of lesser concern that cumulatively are important for food security. The major abiotic stresses are drought, salinity and heat. These stresses form the backbone of national wheat breeding programs and are separated into two classes; biotic being the “dynamic” group because of active pathogen presence and abiotic considered “static” due to the absence of pathogen (Mujeeb-Kazi and Kimber, 1985).

Drought stress is a major limitation to bread wheat productivity and its yield stability in arid and semiarid region of the world including parts of Pakistan. It has been estimated that about one third of the world’s potentially viable land suffer from an insufficient supply of water, and crop yields are periodically reduced by drought (Khan *et al.*, 2007). In Pakistan drought and high temperature can occur during seedling emergence of wheat as well as grain filling stage. These stresses can badly affect the seedling emergence, coleoptile length, seedling growth and photosynthesis in wheat (Muhammad, 2005). Drought stress is one of the most extensive environmental stresses when the available water in the soil is reduced and atmospheric conditions cause

continuous loss of water by transpiration and evaporation. Numerous regions of earth are often or permanently exposed of drought. Up to 26 percent from the usable areas of the earth is exposed to drought. Drought is the most severe stress and the major cause of considerable losses in growth, productivity of crop plans, and finally their yields (Demirevska, 2008).

Water deficits affect every aspect of plant growth and yield modifying the anatomy, morphology, physiology, bio-chemistry and finally the productivity of crop (Akram *et al.*, 2004). Different parameters like physiological, morphological and molecular used to characterize wheat germplasm for drought tolerance (Deneie *et al.*, 2000; Bhutta, 2006). It is most important need of time to improve crop varieties for drought resistance but there are many problems in understanding, designing, and acting upon crop plant improvement programs for drought conditions. Plants bring a lot of changes in its physiological and morphological characteristics to cope with drought (Blum, 2005).

Characterization of D Genome

In vitro characterization

For in vivo studies drought was induced under laboratory conditions mainly by using PEG (polyethylene glycol) and on the basis of different parameters, drought tolerant varieties were identified. In 2000, the effect of drought and salt stresses on the water soluble carbohydrate contents were studied in wheat seedling and for this purpose four varieties of wheat were grown hydroponically under (polyethylene glycol PEG) and salt (NaCl) stresses and then the total water soluble carbohydrate (WSC), glucose, fructose, sucrose and fructose content of stem were determined. Results showed that tolerant genotype accumulates more soluble carbohydrates as compare to other once, thus indicated that that WSC as useful marker for selecting tolerant genotype for drought and salinity (Kerepesi and Galiba, 2000).

Afterwards response of various wheat cultivars,

stabilized wheat-wheatgrass lines and intergeneric wheat amphiploids were studied to in vitro cultivation using 5 and 10 percent polyethylene glycol 6000 MW. Results showed that wheat-wheatgrass line No. 31 and amphidiploid *Triticum durum* were found to be relatively tolerant to drought thus they can be efficiently utilized in various wheat breeding programs to improve drought tolerance in wheat (Tyankova *et al.*, 2004). Similarly in vitro effects of drought using PEG in 13 genotypes of winter wheat, one genotype of winter wheat, one genotype of spring wheat, and three Triticale genotypes of different geographic origin were also studied. Results revealed statistically significant differences among the genotypes in their response to induce stress and thus different genotypes were identified showing tolerance to drought (Galovic *et al.*, 2005).

Under PEG studies different parameters were evaluated like praline content, protein content, sugar analysis, SOD (Superoxide dismutase) and chlorophyll content to identify drought tolerant varieties. The role of antioxidant system in the drought tolerance of wheat cultivars were studied under in vivo and in vitro conditions. In field, drought tolerance was evaluated by the capacity to maintain the grain yield under water stress but it did not show a clear correlation between water-stress tolerance and antioxidant system so under in vitro conditions leaves of tolerant and sensitive cultivars were subjected to osmotic stress. Results indicated that tolerant varieties show an increase in antioxidant contents and less oxidative damage, thus suggesting that this behavior of antioxidant system can be used as an early selection tool for drought tolerant varieties (Lascono *et al.*, 2001).

Molecular Characterization

Although the morphological traits can be used to assess genetic diversity but molecular markers are more reliable and receiving much attention (Manfiesto *et al.*, 2001; Huang *et al.*, 2002 and Ahmed, 2002). Molecular biotechnologies helped plants breeders and physiologists to select the genotypes with improved yield under water

conditions (Zhao *et al.*, 2008). Among different molecular technologies, molecular markers provide a powerful tool to access diversity within and among germplasm and to monitor the flux of diversity over time (Heckenberg *et al.*, 2002).

The genetic diversity based on molecular markers has been studied in plants for about three decades. Although these markers allowed large number of samples to be analyzed, only a limited number of loci could be stored and the comparison of samples from different species and laboratories were problematic. During the past decade the focus shifted to surveys at the level of DNA. Using DNA markers, diversity are measured as the average allelic divergence between any two individuals for given loci. Sufficiently large numbers of samples allow robust analysis of open questions in population genetics (Hamrick and Godt 1990). Detailed characterization of genetic resources can be done by the help of molecular markers. These markers have a great potential to identified genetic diversity within and among accessions which can help breeders to optimize the collections, the planning of seed regeneration and successful implementation of pre-breeding approaches (Borner *et al.*, 2000).

Among the molecular markers microsatellite or simple sequence repeats (SSRs) detects high level of polymorphism. In hexaploid wheat there are some difficulties to use molecular markers due to presence of high portion of repetitive DNAs, large genome, continuous inbreeding caused by self-pollination and narrow genetic base (Joshi and Nguyen, 1993). Simple sequence repeats (SSRs) are valuable molecular markers in many plant species. In common wheat (*Triticum aestivum* L.), which is characteristics of its large genomes and allopolyploidy, SSRs are one of the most useful markers (Torado *et al.*, 2006). The high level of polymorphisms detection, ability to be analyzed by automated systems, high accuracy and repeatability make SSR suitable for large scale DNA fingerprinting in 1998 (Roder *et al.*, 1998). The micro satellites, or simple sequence repeats SSR, are PCR-based DNA markers that are highly polymorphic, show co-dominant inheritance, are evenly distributed

throughout the genome and are locus specific (Powell *et al.*, 1996). Ninety five synthetic hexaploid of wheat by using 45 SSR primers for genetic diversity were analyzed. The results showed that the D genome exhibits greatest genetic diversity among A, B and D genome of synthetic hexaploids. They suggested that the variations of D genome from *Aegilops tauschii* present in synthetic hexaploid wheat are an ideal way to improve the wheat for various biotic and abiotic conditions (Guo-Yue and Li-Hu 2007).

Conclusions

In short, it can be said that drought is the major problem and *Triticum aestivum* L. is the staple food of more than 36% of the world population, which is vulnerable to drought stress. To enhance its production, synthetic wheats were synthesized to ensure the food security. D genome donor *Aegilops tauschii* played a key role in enhancing stress tolerance in bread wheat.

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