NEW ERA AGRICULTURE MAGAZINE

Genetics and mapping of drought tolerance in wheat

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Introduction:

Drought is one of the most common environmental stresses that affect growth and development of plants. Drought continues to be an important challenge to agricultural researchers and plant breeders. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world's population will live under water-stressed environments. Tolerance to water stress is a complicated parameter in which crops' performance can be influenced by several characteristics (Ingram and Bartels,1996). Tolerance can be divided into two parts including drought avoidance and dehydration tolerance (Kramer and Boyer, 1995). Drought avoidance includes root depth, reasonable use of available water by plants, and changes in plants' lifestyle to use rainfall. Dehydration tolerance consists of plants' capability to partially dehydrate and grow again when rainfall continues (Salekdeh et al. 2002). Adaption of plants to drought stress is a vital issue to develop new improved methods for increasing stress tolerant plants.

Morphological, physiological and biochemical changes during drought stress:

In addition to these confounding environmental factors, a drought research programme should also consider plant phenology. By completing its life cycle before the onset of severe water deficit, plants are often able to escape drought (Chaves *et al.*, 2003). This mechanism of avoidance is deployed by rapid phenological development, developmental plasticity, and remobilization of pre-anthesis assimilates to grain. A short life cycle is particularly advantageous in environments with terminal drought stress or where physical or chemical barriers inhibit root growth. The plant's response to drought can be confounded by the environmental covariates as a result of differing phenology. Plant maturity strongly influences grain yield.

A further confounding factor is plant morphology, particularly plant height and tillering. Small plants with few tillers can show higher Water Use Efficiency (WUE, ratio of the volume of water consumed to the total biomass produced, or ratio of biomass to

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total evapotranspiration) than tall multi-tillered plants. Since the genotypic variation of WUE is mainly driven by variations in water use rather than by variations in plant assimilation, the selection for high WUE may result in smaller plants, instead of high yield under drought. Some QTLs for carbon isotope discrimination (a measurement of WUE) in wheat were actually associated with variation in heading date and plant height. Breeding for a shortened crop life cycle has been a very successful strategy in Mediterranean conditions. However, in well-developed agricultural regions, crop flowering time has already been optimized by breeders so that the plant's phenology matches its environment. Therefore research should now focus on optimizing vegetative development to manage biomass and ensure effective assimilates remobilization to grain when water supply becomes limiting. Some genes are known to be drought influenced and produced different types of drought stress related proteins and enzymes including dehydrins, vacuolar acid invertase, glutathione S-transferase (GST) (Anderson et al. 2004), and late embryo abundant (LEA) protein (Pnueli et al. 2002); expression of *ABA* genes and production of proteins like RAB, rubisco, helicase, proline, and carbohydrates are molecular basis of drought tolerance. Plants respond to stress environments with altering their gene expressions and protein productions. In contrast, available information on drought-responsive genes is still limited as their roles have not been thoroughly determined.

Complexity of drought tolerance:

Drought tolerance is defined as the ability of a plant to live, grow, and reproduce satisfactorily with limited water supply or under periodic conditions of water deficit (Turner, 1979). Crop plants should not only have the ability to survive under drought but also the ability to produce a harvestable yield. Research into the molecular aspects of drought tolerance has tended to focus on plant survival at the expense of yield. However, severe water deficits are rare in viable agriculture, and asking how crops respond to or survive extreme drought is unlikely to have much of a practical impact (Passioura, 2002). The aim is not to ‘convert wheat to a cactus’ but to allow wheat to continue to grow and yield grain under water-limited conditions.

Drought tolerance is a quantitative trait, with complex phenotype and genetic control (McWilliam, 1989). Understanding the genetic basis of drought tolerance in crop plants is a prerequisite for developing superior genotypes through conventional breeding. Given the complexity of the genetic control of drought tolerance (multigenic, low-heritability, and high G×E interactions), marker assisted selection has not contributed significantly to

cultivar improvement for dry environments and breeding has relied on direct phenotypic selection. There are additional problems in investigating the genomics of drought tolerance in species such as wheat: most pathways and candidates can be more effectively studied in model species with smaller and sequenced genomes such as *Arabidopsis* and even amongst the cereals there are more extensive data available for rice and maize when compared with wheat. However, recent technological advances and the imperative to ensure sustainable food production has driven research programmes to improve this crop genetically despite the size and complexity of the genome

Vegetative stage	Yield loss (%)
Early season stress	22
Midseason stress	58
Booting stage	20.74
Tillering stage	46.85
1000-grain weight (vegetative stage)	38.67
Earlier stages	79.7
Spike length (vegetative stage)	16.90
Number of spikelets per spike (vegetative stage)	28.63
Grains number (vegetative stage)	72.51
Grain yield (vegetative stage)	61.38

Plants have adaptive robustness to osmotic stresses such as drought and high salinity. Numerous genes functioning in stress response and tolerance are induced under osmotic conditions in diverse plants. Various signaling proteins, such as transcription factors, protein kinases and phosphatases, play signal transduction roles during plant adaptation to osmotic stress, with involvement ranging from stress signal perception to stress-responsive gene expression.

Reproductive stage	Yield loss (%)
Higher grain protein content, fewer days to physiological maturity, smaller kernel weight and diameter, less grain yield	Not applicable
Less grain yield (drought-tolerant variety)	43
Less grain yield (drought-sensitive variety)	26
1000-grain weight	18.29 5
1000-grain weight (anthesis stage)	38.67
Biological yield	10
Maximum grain yield	22
Decreased seed number	64
Grain formation stage	101.23
Grain formation stage	65.5
Number of spikes	19.85
Number of spikes (anthesis stage)	15.79
Spike length (anthesis stage)	16.90
Number of spikelets per spike (anthesis stage)	26.20
Grains number (anthesis stage)	72.51
Grain yield (anthesis stage)	64.46

Recent progress has been made in analyzing the complex cascades of gene expression during osmotic stress response, and especially in identifying specificity and crosstalk in abscisic acid (ABA)-dependent and ABA-independent signaling pathways. In this review, we highlight transcriptional regulation of gene expression governed by two key transcription factors: AREB/ABFs and

DREB2A operating respectively in ABA-dependent and ABA-independent signaling pathways

ABA dependent and independent pathways initiates:

- Activation / regulation of transcription of large number of genes governing tolerance to drought
- Functional protection of proteins by late-embryogenesis abundant proteins (*LEA*) (e.g. dehydrins) and chaperone protein (e.g. heat shock proteins)
- Accumulation of osmolytes (proline, glycine betaine, trehalose, mannitol, *myo*-inositol)
- Induction of chemical antioxidants (ascorbic acid and glutathione) and
- Enzymes reducing the toxicity of ROS (superoxide dismutase)
- Large number of genes activate at different stages of plant growth to express into proteins

Fact is that, each gene is correlated to each other having small cumulative effects. Due to this cumulative behavior of genes, drought tolerance is assigned as complex trait. The potential for improving crop performance under drought stress cannot be achieved until we have identified genes or gene products which are responsible for desired characteristics of drought resistance at different stages of plant growth and development.

QTL mapping:

QTL (quantitative trait loci)- location where quantitative genes are located on chromosomes. Mapping of QTL is a process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits (McCoach and Deorge, 1995)

- Detection of QTLs on chromosome
  - DNA Markers.
  - Software packages.
- QTL mapping – Association between phenotypic data(trait measurements) and genotypic data (molecular markers)
- Describe the effect of the QTL on the trait

QTL Mapping Strategies

1. Select parents that differ for a trait.
2. Screen the two parents for polymorphic marker loci.
3. Generate Mapping population.
4. Obtain marker data/genotypic data for all individuals of the mapping population.
5. Develop a Genetic Linkage map using a computer software program (eg. Mapmaker or Joinmap).
6. Score all individuals for their trait of interest/ phenotypic data.

7. Analyze genotypic and phenotypic data using a computer software program (eg. QTL Cartographer).
8. Determine which QTL positively or negatively affect the trait of interest.

Mapping population:

The prime requirement for the construction of a linkage map is a segregating plant population (i.e. a population derived from sexual reproduction). The parents selected for the mapping population will differ for one or more traits of interest (e.g., highly disease resistant and highly disease susceptible); this is important to enhance the possibility of identifying a large set of polymorphic markers that are well distributed across the genome. Population sizes used in preliminary genetic mapping studies generally range from 50 to 250 individuals [8]. For the analysis of QTLs having small effects on the target trait, large number of individuals (~ 500) is required; however, a mapping population of a size of 200-300 individuals is sufficient for detection of QTLs with major effects

Molecular marker:

The first large scale efforts to produce genetic maps were performed mainly using RFLP markers, the best known genetic markers at the time. Different types of molecular markers used in plant sciences today are Restriction fragment length polymorphisms (RFLPs), microsatellites or simple sequence repeats (SSRs), expressed sequence tags (ESTs), cleaved amplified polymorphic sequence (CAPS), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeat (ISSR), Diversity arrays technology (DArT) and single nucleotide polymorphism (SNP). Each of this marker system has advantages and disadvantages

Analysis of QTL by qtl mapping:

Gahlaut et al. 2017 made a study entitled "QTL mapping for nine drought responsive agronomic traits in bread wheat under irrigated and rain fed environments"

1) Selection of two phenotypically and genotypically diverse parents: Kukri (drought susceptible) and Excalibur (drought tolerant)
2) Development of mapping population: Developed 192 doubled haploid (DH) mapping population using anther culture technique
3) Field experiments and phenotypic evaluation

- Used 192 DH mapping lines
- Four locations under irrigated (IR) and rain-fed (RF) conditions over three crop seasons (2010–11 to 2012–13).
- In IR environments, four irrigations [1st, 21 days after sowing (DAS); 2nd, 40

DAS; 3rd, 60 DAS; 4th, 80 DAS] were given.

- In the RF environments, single irrigation was given at 21 DAS to allow the crop to establish and to avoid complete crop failure

Phenotypic evaluation was carried out for nine characters:

1. Germination percentage
2. Days to anthesis
3. Days to maturity
4. Grain filling duration
5. Plant height
6. Grain weight / ear
7. Productive tillers
8. 1000 grain weight
9. Grain yield / plot

4) Polymorphism and Construction of linkage maps

- 392 polymorphic genetic markers
- Including 222 DArT (Diversity Arrays Technology) markers, 169 SSR (simple sequence repeats) markers.
- The markers were placed in linkage groups using the program MAPMAKER/EXP v3.0b
- A LOD score of 3.0 was set as the minimum threshold to indicate linkage between markers.
- Kosambi mapping function was used to convert recombination frequencies in cM values.
- The final map was drawn using the MapChart program, v.2.1

5) QTL analysis

- A total of 66 QTL were detected for nine different agronomic traits using CIM, they were located on 19 different chromosomes (except 4D and 5D).
- The A sub-genome had the highest coverage, with 168 marker loci, while the D sub-genome had the lowest coverage with 70 markers; the B sub-genome had 154 markers
- It is apparent that A and B sub-genomes had each more than double the number of markers mapped on the D sub-genome
- The smallest linkage group belonged to chromosome 4D (4 markers; 20.2 cM) and the longest linkage group belonged to 7D (13 markers; 107.9 cM).
- QTL were identified both in IR (34 QTL) and in RF (23 QTL); only 9 QTL were identified in both;
- LOD scores for individual QTL ranged from 1.80 to 10.50 and PVE ranged from 3.85% to 20.43%. Of the above 66 QTL, 12 QTL were major because each had >10% PVE

6) Construction of linkage maps using QTLCartographer or MAPMAKER softwares.

Association mapping (AM):

An alternative to traditional QTL mapping. Uses the recombination events from many lineages o Discovers linked markers associated (=linked) to gene controlling the trait. Major goal is to discover the causative SNP in a gene. It exploits the natural variation found in a species, landraces, cultivars from multiple programs. Discovers associations of broad application is variation from regional breeding programs can also be utilized.

Analysis of QTL using association mapping:

Association mapping studies by Erena et al. 2013. entitled "Genome‐wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes"

1) Mapping population: 287 diverse lines of Wheat association mapping II (WAMII) panel was originally developed by the International Maize and Wheat Improvement Center (CIMMYT) with the intention of identifying QTL/genes for drought and heat tolerance
2) Experimental design and phenotypic trait evaluation
   - Association mapping panel included two local check cultivars, Reeder and Butte 86
   - The irrigated treatment was supplemented three times with drip irrigation, (twice before flowering and once during the grain filling stage), while the rain-fed treatment was irrigated only once at flowering to avoid complete failure of the experiment.
   - Phenotypic evaluation:
     1. Plant height
     2. Days to heading
     3. Days to maturity
     4. Grain filling duration
     5. Leaf senescence
     6. Flag leaf length
     7. Flag leaf area (cm^2)
     8. Single kernel weight
     9. Spike length
     10. Thousand kernel weight
3) Genotypic evaluation: Linkage disequilibrium and population structure
   - A total of 1,863 DArT markers
   - Seventy-eight markers (3–4 markers spaced >10 cM per chromosome) were selected from all chromosomes (except for chromosome 4D and 5D) from a total of 1,863 markers for analysis of population structure. To determine population structure, an admixture model with correlated allele frequency in STRUCTURE software was applied
   - LD among markers was calculated using observed versus expected allele

frequencies of the markers in TASSEL v.3.0

4) QTL analysis:

- Chromosomes 4A (62 %) and 1B (55 %) showed a higher percentage of significant ($P < 0.01$) marker pairs in LD whereas chromosomes 5A (20 %), 2B (23 %) and 7A (23 %) had the least number of significant ($P < 0.01$) marker pairs
- Kernel size-related traits, single-kernel weight, single-kernel diameter and thousand-kernel weight had QTL in common on chromosomes 1BL, 4AL and 7DL

➢ Test weight also shared the same regions with one or more kernel size-related traits on chromosomes 1B, 2DL, 4BL, 7BL and 7DL.

➢ Similarly, clusters of QTL for flag leaf characters were found on chromosomes 3BL and 5BL.

Utilization of drought tolerance in crop improvement

➢ Many wild species also retain superior genetic resources that have not yet been investigated. One such species is *Aegilops tauschii*, the diploid D-genome progenitor of hexaploid wheat (*T. aestivum*). *Ae. tauschii* is more drought resistant than *T. aestivum* and wild emmer wheat (*T. dicoccoides*) and harbors drought-resistance traits that were lost during the breeding processes (Ashraf et al. 2009).

➢ Several *Agropyron* species and wheat X *Ae. elongatum* lines have been reported to enhance tolerance to abiotic stresses, including drought waterlogging and salinity

➢ Genetic diversity may be introduced into common wheat by the 'bridge' of synthetic hexaploid wheat (SHW) derived from artificial synthesis of hexaploid wheat (*T. durum*× *Ae. tauschii*) in a manner analogous to the evolution of hexaploid wheat.

➢ Through marker assisted selection QTLs present could be identified.

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