

REVIEW PAPER

Genomic approaches for designing durum wheat ready for climate change with a focus on drought

D. Z. Habash^{1,*}, Z. Kehel² and M. Nachit²

¹ Plant Science Department, Centre for Crop Genetic Improvement, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

² Biodiversity and Integrated Gene Management, International Centre for Agricultural Research in the Dry Areas (ICARDA), PO Box 5466, Aleppo, Syria

Received 29 May 2009; Accepted 3 June 2009

Abstract

Climate change is projected to have a significant impact on temperature and precipitation profiles in the Mediterranean basin. The incidence and severity of drought will become commonplace and this will reduce the productivity of rain-fed crops such as durum wheat. Genetic diversity is the material basis for crop improvement and plant breeding has exploited naturally occurring variation to deliver cultivars with improved resistance to abiotic stresses. The coupling of new genomic tools, technologies, and resources with genetic approaches is essential to underpin wheat breeding through marker-assisted selection and hence mitigate climate change. Improvements in crop performance under abiotic stresses have primarily targeted yield-related traits and it is anticipated that the application of genomic technologies will introduce new target traits for consideration in wheat breeding for resistance to drought. Many traits relating to the plant's response and adaptation to drought are complex and multigenic, and quantitative genetics coupled with genomic technologies have the potential to dissect complex genetic traits and to identify regulatory loci, genes and networks. Full realization of our abilities to manipulate metabolism, transduction pathways, and transcription factors for crop improvement ultimately relies on our basic understanding of the regulation of plant networks at all levels of function.

Key words: Breeding, climate change, durum wheat, genetics, genomics, systems biology.

Climate change in the Mediterranean region

It is now generally accepted that human activity has resulted in an increase in average global surface temperatures. The Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report projects the rise in mean global temperatures to be as high as 6.4 °C by 2100 with 1.3 times as much CO₂ entering the atmosphere, compared with just 20 years ago (IPCC, 2007). Even though climate models vary in their detail there is general agreement that climate trends in the Mediterranean are more robust than in many regions and they agree that the Mediterranean basin will become hotter and drier over the next century (IPCC, 2007; Bates *et al.*, 2008). Annual precipitation around the Mediterranean is likely to decrease by 4–27%, and a reduction of 20% appears to be a typical response across models (IPCC, 2007; Fig. 1). Models have also predicted

a 3–5 °C increase in temperature (Fig. 1; IPCC, 2007) and around a 20% loss in soil moisture (Scheiermeier, 2008). The frequency and duration of dry spells and heat waves is also likely to increase especially in the dryland areas. Extremes, variability, and rates of change are all key features in addressing vulnerability and adaptation to climate change. Water scarcity will be a major problem for the region with the added human demands and especially in North Africa where it is estimated that water extraction would exceed renewable levels in most countries by 2025, even assuming the current climatic conditions. The combination of larger population pressure and climate uncertainty will expose millions of people to severe water shortages and this is expected to have a proportionally high impact on the social, economic, and ecological aspects of

* To whom correspondence should be addressed: E-mail: dimah.habash@bbsrc.ac.uk

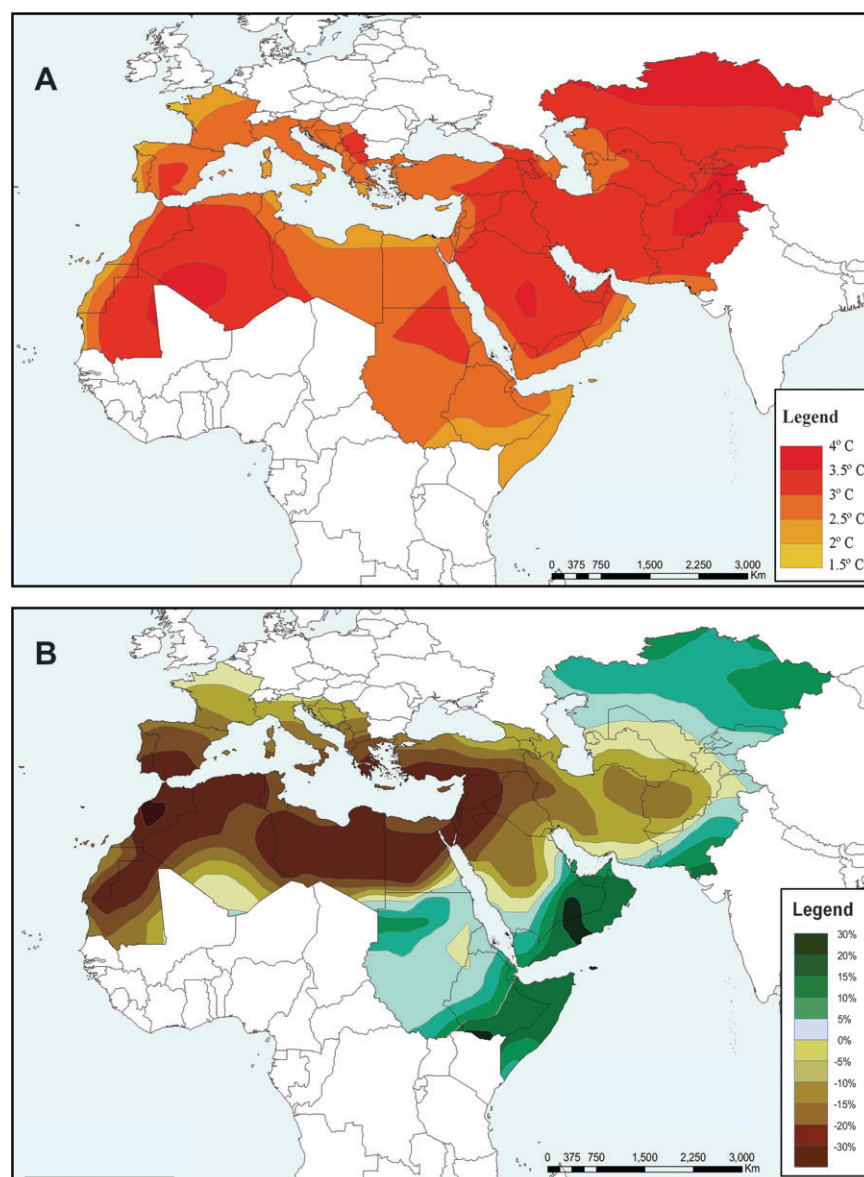


Fig. 1. Multi-model predicted changes in mean annual temperature (A) and annual precipitation (B) from 1980–2099 in parts of Asia, Africa, and Southern Europe. Changes are presented as the average of 21 models for scenario MMD-A1B based on the IPCC fourth assessment report.

this region, compared with other parts of the world (Parry *et al.*, 2005). A variety of adaptation measures are already in place in Mediterranean agricultural systems to adjust to current climate variability and to prepare for future climate change. These include water harvesting and conservation techniques, early warning systems for droughts and floods, improved seasonal forecasting, strengthening and integrating regional management practices, improving conservation strategies, and improved crop cultivars.

Durum wheat production in the Mediterranean basin

Durum wheat, *Triticum turgidum* L. var. durum $2n=4x=28$ genome AABB, originated in the Eastern Mediterranean

and has been farmed in this region for the last 12 thousand years (Key, 2005). Whilst farming has spread globally, a premium is set on durum wheat quality cultivated in the Mediterranean basin and this can account for up to 75% of the world total production (Nachit, 1998a). The largest durum producers in this region are Syria, Turkey, and Italy followed by Morocco, Algeria, Spain, France, and Tunisia. Durum wheat grain is used in the production of pasta, bread, couscous, frekeh, and bulgur (Elias and Manthey, 2005). The major environmental constraints limiting the production of durum wheat in this region are drought and temperature extremes with productivity ranging from 0–6 t ha⁻¹ (Nachit and Elouafi, 2004). Changes in total seasonal precipitation and its pattern of variability are both important, and the occurrence of moisture stress during flowering, pollination, and grain-filling is harmful to wheat. Drought,

combined with terminal heat at the grain-filling period, is a characteristic of farming wheat in this Mediterranean environment and is ultimately detrimental to grain yield (Nachit, 1998a). The frequency of drought, with injury occurring during the middle to the end of winter and coinciding with the tillering–booting developmental stages, has increased during the last three decades in North Africa and has had a negative impact on durum wheat production (Belaid *et al.*, 2005). Furthermore, crop duration has been shortened by almost one month since 1970 in some areas of southern Morocco. Warmer and drier winters have also exacerbated the effect of some diseases and insects which target wheat and are major biotic constraints on production (A Yahyaoui, personal communication). For the leaf, *Puccinia striiformis* (yellow rust) and *Pyrenophora tritici-repentis* (tan spot) are problematic in the continental areas and *Septoria tritici* (leaf rust) is prevalent during milder winters. Root diseases and insects are increasing in importance as limiting factors to durum production in the Mediterranean region with major problems of dryland root rot caused by *Fusarium* species, particularly *culmorum* and *graminearum* and by *Helminthosporium sativum*. The effect of global warming has also extended the area of damage caused by several diseases and insects of durum and bread wheat. *Septoria tritici* remains a major constraint to farming wheat in Morocco and Tunisia and this has now spread to Spain, France, and Northern Europe (Great Britain, Holland, Germany, and Denmark). Similarly, *Mayetiola destructor* (hessian fly) has been a major constraint in durum wheat production in North Africa and this has recently expanded to Spain and France (Nsarellah *et al.*, 2000).

Key traits for wheat yield stability under drought stress

Drought is a normal feature of climate. In agricultural terms, drought occurs when there is insufficient soil moisture to meet the needs of a particular crop at a particular time, and is measured in relation to an average balance between precipitation and evapotranspiration for any given environment. A deficit of rainfall over cropped areas during critical periods of the growth cycle can result in destroyed or underdeveloped crops with greatly depleted yields. It is also defined in terms of the timing of its occurrence in relation to key physiological stages of crop development, in terms of its intensity, and in terms of the presence of additional abiotic stresses such as extremes of temperature. Any factors that originate either from physical factors such as seasonal temperature changes or from societal factors such as increased use of water resources, due to population increase, will exaggerate the level and impact of drought. Plants sense, respond, and adapt to water stress at the molecular, biochemical, physiological, and crop levels. To understand how breeders have been able to screen and develop cultivars capable of resisting drought, it is important to examine the concepts defining plant resistance to drought. Plants respond and adapt to water

deficit using various strategies that have evolved at several levels of function. These form components of the conceptual framework developed by Levitt (1972), which defines drought resistance in terms of dehydration escape, tolerance or avoidance. In dehydration avoidance, plants retain cellular moisture by adopting strategies such as increased soil moisture capture (via modification of root traits), reduced water use or increased water use efficiency (changes in plant size, leaf area, and leaf area index, leaf waxiness, plant density) and osmotic adjustment (production of osmolytes). This strategy is argued to be one of the major mechanisms that plants have evolved and breeders have selected for in targeted traits for drought resistance (Blum, 2005). Another strategy of maintaining cellular metabolism and plant function during water stress is dehydration tolerance with examples such as stem reserve mobilization (Plaut *et al.*, 2004), functional stay green phenotype (Sanchez *et al.*, 2002), and mechanisms observed in resurrection plants. Dehydration escape is the third major strategy, and has been successfully exploited by introducing early flowering alleles in wheat germplasm to enable biomass accumulation and flowering before the period of major drought during grain fill, which is typical of a Mediterranean-type drought (Richards *et al.*, 2002; Slafer *et al.*, 2005). Adoption of any combination of these drought-resistance strategies requires a balance between reduced water use and maximizing yield potential—defined as the maximum yield obtained in cultivation under non-stress environmental conditions.

Genetic improvement under drought can be achieved through direct or indirect selection for yield in the target environment (Ceccarelli and Grando, 1996; Araus *et al.*, 2008). Whilst selecting for yield remains the simplest to implement, it is characterized by low heritability and high genome×environment ($G\times E$) interactions, hence making it one of the most difficult to breed for genetically under drought environments. A complementary strategy is also adopted which is to target traits closely correlated with yield and yield potential and this has been coined indirect selection, analytical or physiological breeding. Crop physiological studies on cereals under drought environments have identified several indirect traits that can be considered for physiological breeding: radiation and water use efficiency, green leaf duration, harvest index, rate of senescence, grain fill duration, leaf area index, deep roots, vigorous crop establishment, stem-reserve utilization, and maintaining cellular hydration (Araus *et al.*, 2008; Reynolds and Tuberosa, 2008). Other ‘constitutive’ type traits have also been selected and have proven very useful in escaping drought such as time to flowering. The challenge and difficulty that breeders face is in the manner of how these traits should be combined to design cultivars ready for drought stress that can potentially hit it at any development stage. Crop simulation models combined with high resolution climate change scenarios may identify key high level traits important under drought and high temperature stress in wheat (Semenov and Halford, 2009). However, modelling is further compounded by the projected climate change

scenario of increasing fluctuations in temperature and the projected increase in atmospheric CO₂. The climate-induced warming in the Mediterranean basin is projected to result in an accelerated growth period for crops which will reduce potential yield (Rozenzweig and Parry, 1994). Applying crop models has also identified primary causes of the global decrease in yield: the shortening of the growing period especially during grain fill, decreases in water availability due to increased evapotranspiration and poor vernalization due to higher average temperatures (Parry *et al.*, 2005). However, care has to be taken in projections for changes in evapotranspiration since some studies have highlighted that global values of pan evaporation have been declining (Roderick *et al.*, 2009). Another factor has to be considered in the modelling of climate change impact on durum wheat production, that of the projected increase in CO₂ which has a direct impact on lowering stomatal conductance and transpiration rate, both potentially beneficial under drought. This result has indeed been established in free-air CO₂ experiments (FACE) on wheat only if the temperature is held constant (Wall *et al.*, 2006). The importance of the rise in temperature in preventing a full realization of the benefits of increased CO₂ on photosynthesis and yield for lower latitudes was also highlighted by Parry *et al.* (2005). Recently, modelling has drawn a complex picture where the beneficial effect of CO₂ on durum yield in Mediterranean climates, in ameliorating the effects of higher temperature and lowered precipitation, is projected for the next 30 years, after which no benefit is forecast (Ferrise *et al.*, 2009). The results of such modelling necessitate further studies, both *in silico* and in the field, to enable us to understand the influence of these interacting climatic factors upon the target traits in durum wheat.

The breeding strategies

Two complementary strategies have been employed to develop wheat cultivars for farming in dry environments. In the International Maize and Wheat Improvement Centre (CIMMYT), efforts have focused on coupling semi-dwarf material with disease resistance so that yield potential will be realized under wet conditions and also with some reasonable productivity during dry conditions subjected to biotic and abiotic stresses (Reynolds and Borlaug, 2006). In the International Centre for Agricultural Research in the Dry Areas (ICARDA) scientists invested in a strategy that targeted drought-adaptive plant mechanisms, enabling cereal production in a very diverse environment often characterized by multiple biotic and abiotic stresses (Ceccarelli, 1994). The Mediterranean climate is characterized by low and variable annual rainfall which can fluctuate from 200–800 mm and irrigation is not an available option for durum commercial production (Nachit and Elouafi, 2004). Because of this high year-to-year variability, breeding cultivars that combine drought resistance, yield potential, and yield stability are a prerequisite for stable productivity in this region. A key approach has been to introgress resistance

genes from landraces and wild relatives to durum advanced genotypes and to test germplasm in a matrix of stress environments. Landraces and wheat wild relatives are the main sources of drought resistance and Mediterranean durum landraces were found to possess desirable traits for resistance to drought and their use in the hybridization programme has shown substantial progress in yield and yield stability (Valkoun, 2001). Further, *Triticum* wild relatives are used to widen the genetic base of durum and to improve its resistance to abiotic and biotic stresses. Genotypes generated from hybridization with *Triticum* wild relatives have been found to produce high grain yield under favourable and dry conditions. Under drought, the best crosses were those with *Triticum carthlicum*, *T. dicoccoides*, *Aegilops* species, *T. monococcum*, *T. polonicum*, and *T. dicoccum*. At ICARDA, durum wheat segregating populations and advanced lines are subjected to the stresses encountered in the representative selection environments of the Mediterranean drylands. Hence a strategy was developed which allows the identification of germplasm that combines drought resistance, productivity, stability, and resistance to biotic and other abiotic stresses (e.g. cold and heat). To achieve this, a double gradient selection technique (DGST) for temperature extremes varying from cold to hot and for water regimes varying from severe drought to irrigated conditions is used (Nachit and Elouafi, 2004). This strategy was coupled with the application of physiological screening tools and the results showed that significant genetic gain, in terms of grain yield, was associated with physiological traits such as chlorophyll content and water use efficiency using the surrogate carbon isotope discrimination and osmotic adjustment (Nachit *et al.*, 1993, 1998a, b). Drought resistance was also found to be associated with some molecular markers and the combination of the physiological and molecular research tools have generated promising genetic material for the Mediterranean drylands (Nachit and Elouafi, 2004); this germplasm is now used as a benchmark for further genetic improvement. Nevertheless, breeding for yield under drought remains a difficult task and it is anticipated that the application of genomic tools will help in identifying the number and type, as well as the nature of dominance and epistatic interactions, of genes underlying a given quantitative genetic trait (Kearsey, 2002).

Molecular markers an essential cornerstone for genomic and genetic research

Molecular markers allow discrimination in DNA sequence amongst cultivars and breeding lines and thus offer the scientific community singularly powerful tools to monitor, track, and exploit sequence variation in germplasm. Many types of markers have been developed and are now an essential part of functional, structural genomics and molecular breeding (Varshney *et al.*, 2007). Microsatellite markers or single sequence repeats (SSR) have proved useful in wheat research since they offer reproducibility, multiallelic nature, codominant inheritance, genome specificity, relative

abundance, and good genome coverage (Varshney *et al.*, 2005; Ganal and Roder, 2007). They have been used in the localization of genes to chromosomes (Roder *et al.*, 2004), identification of quantitative trait loci (QTLs) for yield and quality traits (Ganal and Roder, 2007), characterization of wheat varieties and germplasm (Donini *et al.*, 2000; Roder *et al.*, 2004), marker assisted selection (MAS), and back-crossing. Another promising type is single-nucleotide polymorphism (SNP) or biallelic markers because they form the basis for most genetic variation between individuals, they are widely distributed and amenable to high multiplex detection systems (Ganal and Roder, 2007). The identification of SNP markers depends on comparative sequencing of lines or analysis of expression sequence tags (ESTs). This was delayed in wheat due to a lack of adequate technologies that can handle the problems associated with polyploidy and sequencing large genomes. Recent advances in new DNA sequencing technologies (454, Solexa, and SOLiD) (Pettersson *et al.*, 2009) will enable low-cost SNP discovery over larger genomic regions in species including wheat. Molecular markers can be derived either from within or from outside the gene sequence and the selection of the most suitable marker system depends on the objective and cost. For wheat molecular studies on drought stress, the development of functional genic markers are essential in linking traits to sequence polymorphism.

QTL discovery

The advances in functional genomics in delivering bacterial artificial chromosomes (BACs), ESTs, partial gene sequences, full-length cDNA clones, genes and markers have enabled the establishment of molecular maps based on genic markers. The creation of suitable mapping populations and the development of molecular markers have enabled linkage studies in wheat and many QTLs have been identified for yield under drought environments (Varshney *et al.*, 2006). Linkage studies have shown that QTLs for grain yield reside in several chromosomal regions, and measurements of yield components allow us to dissect complex traits to smaller genetic components more amenable for building our knowledge of trait architecture which will inform our future strategies for exploitation. Mapping populations have also been developed in durum wheat for the study of biotic and abiotic stresses (Blanco *et al.*, 1998; Nachit *et al.*, 2001; Nachit and Elouafi, 2004). QTLs have been established for traits relating to durum wheat yield under rain-fed and irrigated conditions in field trials typically accounting for 10–21% of the phenotypic variation. Studies on a cross Omrabi 5/*Triticum dicoccoides* 600545//Omrabi 5 in 18 environments identified QTLs for test weight on chromosomes 7AS and 6BS, explaining 30% of the total variation. Thousand kernel weight showed a significant transgressive inheritance and five QTLs were identified, explaining 32% of the total variation, out of which 25% was of a genetic nature, and showing QTL $\times$ E interaction (Elouafi and Nachit, 2004). Loci for yield, yield components, heading date, plant height, and physiological

and developmental traits under drought have also been established in mapping populations (Maccaferri *et al.*, 2008; DZ Habash *et al.*, unpublished data). An alternative to linkage analysis is association mapping which has enabled the establishment of a suite of QTLs for yield-related traits in panels of diverse germplasm (Maccaferri *et al.*, 2005, 2006). Despite the available maps, populations, and marker technology, advances in transferring knowledge from QTL studies on yield under drought to breeding remains slow. This is due to three major factors: first, yield is a quantitative trait with poorly defined genetic architecture of multiple interacting loci, the second is the fact that these loci have a high $G\times E$ interaction component, and the third is the accurate phenotyping of the traits under study (often an underestimated problem). QTLs established in one environment often disappear in another and thus QTLs have been defined as constitutive versus adaptive. However, this categorization can be artificial since certain genes underlying 'constitutive' traits can also respond to the environmental conditions such as flowering genes (Collins *et al.*, 2008). To enable a study of the interaction of $G\times E$, phenotyping trials can be carried out on the same population in multiple field sites where environmental covariates are considered in the analysis (Nachit *et al.*, 1992; DZ Habash *et al.*, unpublished data). This enables the modelling and interpretation of QTL by environment interaction, or the differential expression of QTLs in relation to changing environmental conditions, as demonstrated in maize (Vargas *et al.*, 2006) and bread wheat (Kuchel *et al.*, 2007). Reymond *et al.* (2003) argued for an alternative approach combining genetic and ecophysiological models for understanding and dissecting plant responses to water deficit so that the response of one genotype can be genetically dissected and mapped as a function of varying degrees of stress. An alternative and potentially powerful mathematical approach based on quantitative genetics has been developed, linking gene models with quantitative genetics (Podlich and Cooper, 1998). This allows the simulation of various gene actions or models in the context of selection and breeding strategies. Despite the complexity of studying yield and its genetic architecture under drought, data are now available from multiple studies and from various genetic material to enable us to start identifying important loci for further work (Varshney *et al.*, 2006).

Exploring and fine mapping a QTL

Loci correlated to yield traits under water-limited field trials in durum wheat germplasm contrasting for drought tolerance have recently been explored (Diab *et al.* 2008). Twelve genes and 103 differentially expressed sequence tags (dESTs) were selected from previous studies on drought in barley (Talame *et al.*, 2007) and polymorphism was sought in the durum wheat Jennah Khetifa $\times$ Chaml mapping population (Diab *et al.*, 2008). Results show that several dESTs and candidate genes were statistically correlated with QTLs for traits relating to tolerance under drought. Figure 2 shows an example of such an approach and

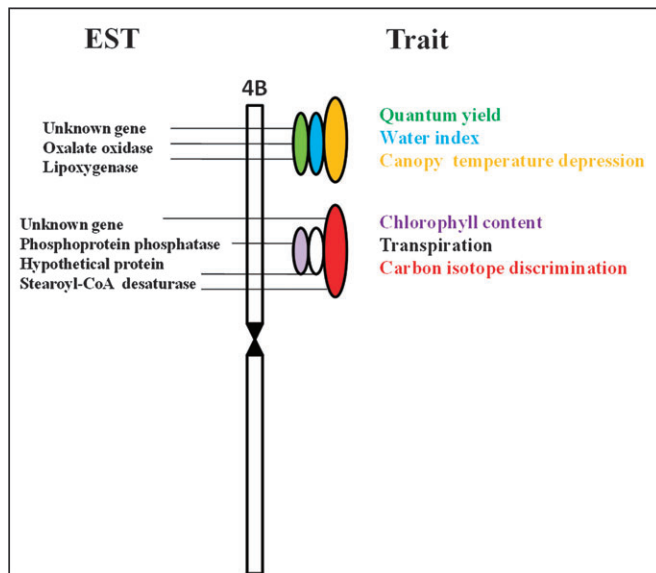


Fig. 2. Co-localizations between mapped dESTs and QTLs for various traits on chromosome 4B relating to plant performance under drought redrawn with permission from *Scientific Research and Essays* (Diab *et al.*, 2008).

identified some candidate genes involved in fatty acid/lipid metabolism (lipoxygenase, stearoyl-CoA desaturase), regulation (phosphoprotein phosphatase), and defence (oxalate oxidase) that were statistically correlated with traits on chromosome 4B (quantum yield, chlorophyll content, transpiration, water index, canopy temperature depression, and carbon isotope discrimination). Therefore, this gives us the first link between candidate genes and trait chromosomal location and future work is necessary to fine map the QTL and establish how these candidate genes are involved in the response to drought. Primary QTL mapping, will identify a QTL to within 10–30 cM resolution. To fine map a locus to a higher resolution, near isogenic lines (NILS) specific for the particular QTL under study are required to mendelize the locus (Alonso-Blanco and Koornneef, 2000). Only a handful of studies have achieved the cloning of a wheat QTL via positional cloning, most notably the cloning of *VRN1* (Yan *et al.*, 2003). Several putative loci relating to abiotic stress tolerance have been identified in studies using allelic variation and are reviewed by Collins *et al.* (2008). In durum wheat, a recent study has identified robust QTLs in yield traits across 16 environments on chromosome groups 2B and 3B (Maccaferri *et al.*, 2008) which are now under fine mapping (R Tuberosa, personal communication). When a QTL region is resolved to a few cM genetic distance, markers closest to the QTL are then used to anchor the genetic map to a physical map and BAC libraries are then used to identify potential candidate sequences. The use of model species, synteny, and bioinformatics tools is critical at this stage to enable candidate genes to be identified in wheat. Whilst QTL cloning represents a huge undertaking in terms of the technology, time, and resources required, the advantages derived from its success are directly applicable to MAS and to improving our

knowledge of genome function and trait dissection (Salvi and Tuberosa, 2007). This will even become more accessible in the near future with the recent breakthroughs in DNA sequencing technologies (Pettersson *et al.*, 2009). A promising new approach, coupling linkage analysis with high throughput gene expression, coined genetical genomics (Jansen and Nap, 2001) has enabled the establishment of expression QTLs (eQTLs) which can identify potential candidate genes residing in a QTL. This has recently been applied to wheat studies identifying *cis* and *trans*-acting regulatory regions controlling seed development (Jordan *et al.*, 2007).

International efforts, genomic technologies, and resources

Advances in cereal genomic research have produced major milestones in the efforts to understand the structure and function of the wheat genome. A proposal developed to establish a public database of ESTs from Triticeae species, Triticeae International Triticeae EST Cooperative (ITEC; <http://wheat.pw.usda.gov/genome/>) has delivered over 1.5 million ESTs for Triticeae of which ~1 million are for wheat (<http://www.ncbi.nlm.nih.gov/>). These have been essential for the development of molecular markers, genetic and physical mapping, comparative genomics, annotation of sequences, and analysis of gene expression under biotic and abiotic stresses (Matthews *et al.*, 2004). An international effort is underway to sequence the genome of wheat because of the necessity to provide knowledge specifically for polyploidy where model species cannot always deliver insight into the functioning of traits in wheat. The international wheat genome sequencing consortium (IWGSC; <http://www.wheatgenome.org/>) was therefore established by scientists and other stakeholders to sequence the wheat genome chromosome by chromosome. Paux *et al.* (2008) have recently published the first physical map for chromosome 3B in wheat using chromosome-sorted large-insert BAC libraries and genetic mapping strategies to anchor the physical map. This critical achievement demonstrated that this strategy works and has allowed the identification of genes and QTL, of agronomic importance, in contigs which can then be sequenced and studied in depth (Paux *et al.*, 2008). Furthermore, they have also demonstrated inversions and non-colinear regions in wheat chromosome 3B and its colinear rice chromosome 1 thus stressing that caution is required in predicting gene order from the rice genome. This lends further weight to the need to sequence each specific crop genome. Sequencing the full chromosome of 3B will now become tangible, both in terms of cost, time, and effort, with the arrival of high throughput next-generation sequencing technologies (Pettersson *et al.*, 2009). The technical barriers in DNA sequencing technologies are breaking down and it is envisaged that soon it should be possible to sequence one whole genome for \$1000 in one day (Service, 2006; Eid *et al.*, 2009). The need to exploit functional genomics for breeding in crops has also led to the establishment of various high throughput technologies for

the study of gene expression such as differential display, DNA microarray, SAGE, oligoarrays, EST sequencing, and RNA sequencing. The application of EST analysis and transcript profiling has uncovered hundreds of genes in plants whose level of expression is altered by abiotic stresses. This has enabled us to move away from the single gene to phenotype approach and towards cataloguing the genome's global response to stress. These studies have uncovered multiple strategies that plants employ in response to stress and have enabled us to sketch regulatory networks altered by stress (Sreenivasulu *et al.*, 2004; Bohnert *et al.*, 2006; Valliyodan and Nguyen, 2006). Studies have also highlighted the importance of cell-specific gene expression in *Arabidopsis* (Birnbaum *et al.*, 2003) and organ-specific responses in rice (Zhou *et al.*, 2007), and the method of stress imposition on transferability of results to crops in field conditions (Talame *et al.*, 2007). Similar considerations will apply to the use of high throughput studies of the proteome and metabolome in the future.

Candidate gene and genetic engineering approach

High throughput transcriptome studies and functional genomic approaches have been used to identify genes correlated with a plant's response to water stress. A large number of genes have been identified and the diversity of these responsive genes and pathways reflects the complexity of the mechanisms involved in sensing and responding to water stress. These genes have been categorized by Yamaguchi-Shinozaki and Shinozaki (2006) into two major groups: the first codes for functional proteins involved in protecting cellular function (water channels, transporters, detoxification, proteases, protection factors of macromolecules, chaperones, and osmolyte biosynthesis); the other group codes for regulatory proteins involved in signal transduction such as transcription factors, protein kinases, protein phosphatases, and enzymes of lipid metabolism (Shinozaki *et al.*, 2003). Genetic modification of the expression of members belonging to these two major groups of genes, further categorized as abscisic acid (ABA)-dependent or ABA-independent, has resulted in some improvement in traits relating to tolerance under water stress in a variety of plant species (Zhang *et al.*, 2004; Vinocur and Altman, 2005; Yamaguchi-Shinozaki and Shinozaki, 2006). However, only a few genes have been successfully engineered in crops to enhance resistance to drought under field conditions. To date, modulating the NFYB2 transcription factor in maize has resulted in enhanced performance under water-limited field trials (Nelson *et al.*, 2007) and field trials are underway in CIMMYT to test wheat genetically engineered to modulate the expression of the dehydration responsive element binding (DBF/DREB1) transcription factor (M Reynolds, personal communication). An important aspect that is worth highlighting here is that fine-tuning the modulation of the target gene(s) expression to specific cell types may be essential in a successful strategy for modulating the system. This is supported by recent global

analysis of the proteome and metabolome of *Arabidopsis* root tissue showing cellular specificity in response to abiotic stress (Dinnyen *et al.*, 2008). However, this will rely on our ability to identify and utilize cell/tissue-specific promoters to fine-tune and target genetic modification. This, coupled with an improved understanding of the complex regulatory networks involved in sensing and responding to water stress, will improve our genetic engineering outcome.

Exploiting and mining information

A considerable body of information is now available from the application of new genomic and genetic technologies in cereal research. However, this information is often dispersed among different databases with varying formats and this presents major problems of accessibility and utility for scientists. Bioinformatics, through computational biology, has therefore emerged as a new discipline focused on the collection, manipulation, storage, retrieval, and use of biological information and the development of programmes and databases to enable this. The release of the genome sequence for *Oryza sativa* and *Arabidopsis thaliana* has had an enormous impact on genomic research in cereals in transferring gene and locus-specific sequence information to non-model cereals such as wheat. This has been aided by comparative genomics which involves the projection of structure and function between model species and others, and this is critical for research in wheat because of the lack of a sequenced genome. Furthermore, the discovery of colinearity in molecular markers between grasses (Devos and Gale, 2000; Feuillet and Keller, 2002) has enabled the transfer of some genome information from rice. Comparative studies in cereals have enabled the development of genetic maps, molecular markers, and map-based cloning of agronomically important genes (Salse and Feuillet, 2007). This knowledge is now stored in databases such as GrainGenes (<http://wheat.pw.usda.gov/GG2/germplasm.shtml>) which specifically targets genetic data for the Poaceae (Gramineae) family. This includes several types of information such as genetic and cytogenetic maps, genomic probes, genes, alleles, and gene products, nucleotide sequences, associated phenotypes, quantitative traits and QTLs, genotypes and pedigrees of cultivars, genetic stocks, and other germplasms. Another database, Gramene (<http://www.gramene.org/>) holds data for comparative genome analysis in the grasses and is currently integrating QTL data from open sources. These databases also enable useful comparison of QTL data between related species and will facilitate the exploitation of genetic information research and breeding in wheat. In our genomic research on durum wheat responses to drought, a programme called ONDEX has been used (<http://ondex.sourceforge.net/index.php>) which links, integrates, analyses, and visualizes data from diverse biological sources (Fig. 3). This programme was used to import durum wheat gene expression results in order to compare sequences against rice and *Arabidopsis*, to identify orthologous proteins, to map them to metabolic pathways using AraCyc (<http://www.arabidopsis.org/biocyc/index.jsp>),

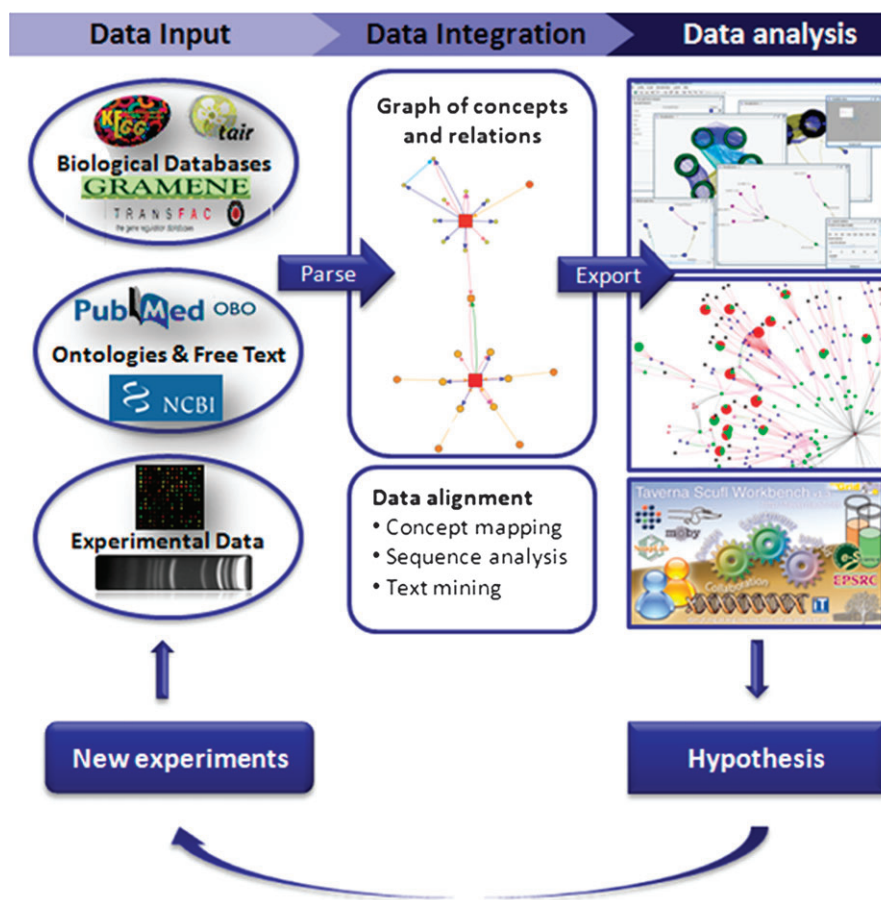


Fig. 3. A typical usage workflow for Oindex. Data from disparate and heterogeneous biological databases and public sources is combined with experimental observations and converted into a graph of concepts and relations. Data alignment and integration techniques are applied to merge and infer new relations within the data. This integrated data store is used as a resource to enrich further statistical, bioinformatics, and graph analysis. Oindex visualization tools can overlay further quantitative measures such as gene expression, confidence scores, and graph topology (nodes, connectivity) statistics. This facilitates the reduction and dissection of experimental data. Hypotheses generated from this workflow lead to further experiments which then feed into further iterations of this workflow.

and to visualize the results in various graphical formats (DZ Habash *et al.*, unpublished data). Wheat target sequences have also been analysed for transcription factor motifs using databases such as TRANSFAC (<http://www.biobase-international.com/pages/index.php?id=transfac>) and the WhETS programme (Wheat Estimated Transcript Server V1.3; Mitchell *et al.*, 2007) utilized to design specific primers for qPCR analysis in wheat using rice sequence information.

Scientists have documented and collated an unprecedented amount of biological information arising from the application of new high throughput post-genomic technologies to study drought stress. Whilst this accumulation of knowledge is important, there is a growing view that this approach is not enough to enable us to capture the totality of plant function, especially in the study of quantitative traits (Oltvai and Barabasi, 2002).

Integrating genomics and genetics towards a system

The preceding sections have outlined the approaches and advances made in crop and wheat genetics and our current

status and understanding with regard to drought research in durum wheat. What is still lacking is a comprehensive conceptual framework linking genotype to phenotype—the intractable problem. Since wheat responses to drought involve quantitative traits then our framework should include the study of their genetic architecture which is essential for our ability to manipulate them for breeding purposes. The genetic architecture of a quantitative trait is complex and is influenced by the number of genes, their effect on a trait, number of alleles, allele haplotypes, linkage, epistatic interactions, and pleiotropy. The genomic advances are adding further detail to this architecture in the form of gene networks, hubs, transcription factors, regulators, and epigenetic information. However, there is growing evidence that information storage, processing, and execution of various cellular programmes reside at different levels of organization at the genome, transcriptome, proteome, and metabolome and are not necessarily unidirectional (Oltvai and Barabasi, 2002). Therefore, these levels of control and organization should not be perceived as distinct but that they exist as interacting and dynamic networks (Kitano, 2002). The emergence and development of the global omics concept

which covers the genome (genomics), protein (proteomics), metabolite (metabolomics), the molecular fluxes through metabolic networks (the fluxome), and the plant phenotype (phenomics) will enable us to capture the global dynamic features of plant function. Identifying these networks and their interactions forms one of the major challenges in biology in the effort to link genotype to phenotype (Benfey and Mitchell-Olds, 2008). A shift in thinking and new experimental designs are required to re-synthesize knowledge and to uncover global relationships at and between different levels of function (gene, protein, metabolome, and phenotype) and the application of systems thinking to this problem offers one way mathematically to integrate the vast amount of knowledge at different biological organizational levels. To enable this view, it is necessary to integrate this omic information into quantitative genetics, one of the most widely used frameworks linking the genotype with the phenotype. One approach has developed mathematical tools to enable the theoretical quantification of various gene action models (epistasis for example) and explore their contribution to phenotype under various environments using simulation (Chapman *et al.*, 2002; Cooper *et al.*, 2002). Another emerging powerful approach is that of genetical genomics introduced by Jansen and Nap (2001) which identifies gene expression QTLs by integrating high throughput gene expression with quantitative genetics using segregating germplasm. This has the potential to identify genes statistically correlated with an established QTL, thus linking gene expression with studied traits and also to uncover *cis*- and *trans*-acting regulatory elements. This approach could also uncover loci governing regulatory networks across the levels of function by integrating the metabolome, proteome, and phenome (Jansen *et al.*, 2009).

In conclusion, the advances in genome sequencing, development of high throughput omic technologies, quantitative genetics, and bioinformatics offer us an unprecedented opportunity to dissect and re-synthesize the molecular regulation of quantitative traits in crops. It is only a matter of time for this to be exploited fully in durum wheat research.

Acknowledgements

This work was funded by an EU-FPVI project 'TRITIMED' INCO-CT-509136. DZH is supported by Rothamsted Research which is grant aided by the Biotechnology and Biological Sciences Research Council of the UK. We acknowledge the contribution of Matthew Hindle and Chris Rawlings, Rothamsted Centre for Mathematical and Computational Biology, in the construction of Fig. 3.

References

- Alonso-Blanco C, Koornneef M. 2000. Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. *Trends in Plant Science* **5**, 22–29.
- Araus J-L, Slafer G, Royo C, Serret MD. 2008. Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Sciences* **27**, 377–412.
- Bates BC, Kundzewicz ZW, Wu S, Palutikof JP, eds. 2008. *Climate change and water*. Technical Paper of the Intergovernmental Panel on Climate Change. Geneva: IPCC Secretariat.
- Belaid A, Nsarellah N, Laamari A, Nachit M, Amri A. 2005. *Assessing the economic impact of durum wheat research in Morocco*. Aleppo, Syria: International Centre for Agricultural Research in the Dry Areas (ICARDA).
- Benfey PN, Mitchell-Olds T. 2008. From genotype to phenotype: systems biology meets natural variation. *Science* **320**, 495–497.
- Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, Benfey PN. 2003. A gene expression map of the *Arabidopsis* root. *Science* **302**, 1956–1960.
- Blanco A, Bellomo MP, Cenci A, *et al.* 1998. A genetic linkage map of durum wheat. *Theoretical and Applied Genetics* **97**, 721–728.
- Blum A. 2005. Drought resistance, water-use efficiency, and yield potential- are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* **56**, 1159–1168.
- Bohnert HJ, Gong Q, Li P, Ma S. 2006. Unravelling abiotic stress tolerance mechanisms- getting genomics going. *Current Opinion in Plant Biology* **9**, 180–188.
- Ceccarelli S. 1994. Specific adaptation and breeding for marginal conditions. *Euphytica* **77**, 205–219.
- Ceccarelli S, Grando S. 1996. Drought as a challenge for the plant breeder. *Plant Growth Regulation* **20**, 149–155.
- Chapman SC, Hammer GL, Podlich DW, Cooper M. 2002. Linking biophysical and genetic models to integrate physiology, molecular biology and plant breeding. In: Kang MS, ed. *Quantitative genetics, genomics and plant breeding*. Wallingford, UK: CABI Publishing, 167–187.
- Collins NC, Tardieu F, Tuberosa R. 2008. Quantitative trait loci and crop performance under abiotic stress: where do we stand? *Plant Physiology* **147**, 469–486.
- Cooper M, Podlich DW, Micaleff KP, Smith OS, Jensen NM, Chapman SC, Kruger NL. 2002. Complexity, quantitative traits and plant breeding: a role for simulation modelling in the genetic improvements of crops. In: Kang MS, ed. *Quantitative genetics, genomics and plant breeding*. Wallingford, UK: CABI Publishing, 143–166.
- Devos KM, Gale MD. 2000. Genome relationships: the grass model in current research. *The Plant Cell* **12**, 637–646.
- Diab AA, Kantety RV, Ozturk NZ, Benschel D, Nachit MM, Sorrells ME. 2008. Drought-inducible genes and differentially expressed sequence tags associated with components of drought tolerance in durum wheat. *Scientific Research and Essay* **3**, 9–26.
- Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN. 2008. Cell identity mediates the response of *Arabidopsis* roots to abiotic stress. *Science* **320**, 942–945.
- Donini P, Law JR, Koebner RMD, Reeves JC, Cooke RJ. 2000. Temporal trends in the diversity of UK wheat. *Theoretical and Applied Genetics* **100**, 912–917.

- Eid J, Fehr A, Gray J, et al.** 2009. Real-time DNA sequencing from single polymerase molecules. *Science* **323**, 133–138.
- Elias EM, Manthey FA.** 2005. End products: present and future uses. In: Royo C, Nachit MM, Di Fonzo N, Araus JL, Pfeiffer WH, Slafer GA, eds. *Durum wheat breeding: current approaches and future strategies*, Vol. 1. New York, London, Oxford: Food Products Press, an Imprint of The Haworth Press Inc, 63–85.
- Elouafi I, Nachit MM.** 2004. A genetic linkage map of the durum×*Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. *Theoretical and Applied Genetics* **108**, 401–413.
- Ferrise R, Moriondo M, Bindi M.** 2009. Climate change and projected impacts in agriculture: an example on Mediterranean crops. *Geophysical Research Abstracts* **11**, EGU2009–7229.
- Feuillet C, Keller B.** 2002. Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution. *Annals of Botany* **89**, 3–10.
- Ganal MW, Roder MS.** 2007. Microsatellite and SNP markers in wheat breeding, Vol. 2. In: Varshney RK, Tuberosa R, eds. *Genomic assisted crop improvement: genomics applications in crops*. The Netherlands: Springer, 1–24.
- IPCC.** 2007. Climate change 2007: the physical science basis. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK, New York, USA: Cambridge University Press.
- Jansen RC, Nap JP.** 2001. Genetical genomics: the added value from segregation. *Trends in Genetics* **17**, 388–391.
- Jansen RC, Tesson BM, Fu J, Yang Y, McIntyre M.** 2009. Defining gene and QTL networks. *Current Opinion in Plant Biology* **12**, 241–246.
- Jordan MC, Somers DJ, Banks TW.** 2007. Identifying regions of the wheat genome controlling seed development by mapping expression quantitative trait loci. *Plant Biotechnology Journal* **5**, 442–453.
- Kearsey MJ.** 2002. QTL analysis: problems and (possible) solutions. In: Kang MS, ed. *Quantitative genetics, genomics and plant breeding*. Wallingford, UK: CABI Publishing, 45–58.
- Key JM.** 2005. Wheat: its concept, evolution, and taxonomy. In: Royo C, Nachit MM, Di Fonzo N, Araus JL, Pfeiffer WH, Slafer GA, eds. *Durum wheat breeding: current approaches and future strategies*, Vol. 1. New York, London, Oxford: Food Products Press, an imprint of the Haworth Press Inc, 3–61.
- Kitano H.** 2002. Systems biology: a brief overview. *Science* **295**, 1662–1664.
- Kuchel H, Williams K, Langridge P, Eagles HA, JeVeries SP.** 2007. Genetic dissection of grain yield in bread wheat. II. QTL-by-environment interaction. *Theoretical and Applied Genetics* **115**, 1015–1027.
- Levitt J.** 1972. *Responses of plants to environmental stresses*. New York: Academic Press.
- Maccaferri M, Sanguineti MC, Corneti S, et al.** 2008. Quantitative Trait Loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. *Genetics* **178**, 489–511.
- Maccaferri M, Sanguineti MC, Natoli V, et al.** 2006. A panel of elite accessions of durum wheat (*Triticum durum* Desf.) suitable for association mapping studies. *Plant Genetic Resources* **4**, 79–85.
- Maccaferri M, Sanguineti MC, Noli E, Tuberosa R.** 2005. Population structure and long-range linkage disequilibrium in a durum wheat elite collection. *Molecular Breeding* **15**, 271–289.
- Matthews DE, Lazo GR, Carollo VL, Anderson OD.** 2004. Bioinformatics and triticeae genomics: resources and future developments. In: Gupta PK, Varshney RK, eds. *Cereal genomics*. The Netherlands: Kluwer Academic Publishers, 425–446.
- Mitchell RAC, Castells-Brooke N, Taubert J, Verrier PJ, Leader DJ, Rawlings CJ.** 2007. Wheat estimated transcript server (WhETS): a tool to provide best estimate of hexaploid wheat transcript sequence. *Nucleic Acids Research* **35**, W148–W151.
- Nachit MM.** 1998b. Durum breeding research to improve dry-land productivity in the Mediterranean region. In: Rao SC, Ryan J, et al. eds. *SEWANA durum research network*. ICARDA editions, 1–15.
- Nachit MM.** 1998b. Association of grain yield in dryland and carbon isotope discrimination with molecular markers in durum (*Triticum turgidum* L. var. durum). *Proceedings of the 9th international wheat genetics symposium*. Saskatoon, Saskatchewan, Canada, 218–223.
- Nachit MM, Baum M, Autrique E, Sorrells ME, Ali Dib T, Monneveux P.** 1993. Association of morphophysiological traits with RFLP markers in durum wheat. In: Monneveux P, Salem M Ben, eds. *Tolerance a la secheresse des cereales en zone mediterranneenne. Diversite genetique et amelioration varietale*. Montpellier, France, 15–17 December 1992, 159–171.
- Nachit MM, Elouafi I.** 2004. Durum adaptation in the Mediterranean dryland: breeding, stress physiology, and molecular markers. In: Rao SC, Ryan J, eds. *Challenges and strategies for dryland agriculture*. CSSA Special Publication 32. Madison, Wisconsin, USA: Crop Science Society of America Inc., American Society of Agronomy Inc, 203–218.
- Nachit MM, Elouafi I, Pagnotta MA, et al.** 2001. Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. durum). *Theoretical and Applied Genetics* **102**, 177–186.
- Nachit MM, Nachit G, Ketata H, Gauch HG, Zobel RW.** 1992. Use of AMMI and linear regression models to analyze genotype-by-environment interaction in durum wheat. *Theoretical and Applied Genetics* **83**, 597–601.
- Nelson DE, Repetti PP, Adams TR, et al.** 2007. Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proceedings of the National Academy of Sciences, USA* **104**, 16450–16455.
- Nsarellah N, Nachit M, Lhaloui S.** 2000. Breeding durum wheat for biotic stresses in the Mediterranean region. *CIHEAM publications - Options Meditteraneennes*.
- Oltvai ZN, Barabasi A-L.** 2002. Life's complexity pyramid. *Science* **298**, 763–764.
- Parry M, Rozenzweig C, Livermore M.** 2005. Climate change, global food supply and risk of hunger. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 2125–2138.
- Paux E, Sourdille P, Salse J, et al.** 2008. A physical map of the 1-gigabase bread wheat chromosome 3B. *Science* **322**, 101–104.

- Pettersson E, Lundeberg J, Ahmadian A.** 2009. Generations of sequencing technologies. *Genomics* **93**, 105–111.
- Plaut Z, Butow BJ, Blumenthal CS, Wrigley CW.** 2004. Transport of dry matter into developing wheat kernels and its contribution to grain yield under post-anthesis water deficit and elevated temperature. *Field Crops Research* **86**, 185–298.
- Podlich DW, Cooper M.** 1998. QU-GENE: a simulation platform for quantitative analysis of genetic models. *Bioinformatics* **14**, 632–653.
- Reymond M, Muller B, Leonardi A, Charcosset A, Tardieu F.** 2003. Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiology* **131**, 664–675.
- Reynolds MP, Borlaug NE.** 2006. Impacts of breeding on international collaborative. Wheat improvement. *Journal of Agricultural Science* **144**, 3–17.
- Reynolds MP, Tuberosa R.** 2008. Translational research impacting on crop productivity in drought-prone environments. *Current Opinion in Plant Biology* **11**, 171–179.
- Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF.** 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. *Crop Science* **42**, 111–121.
- Roder MS, Huang XQ, Ganai MW.** 2004. Wheat microsatellites in plant breeding: potential and implications. In: Loerz H, Wenzel G, eds. *Molecular markers in plant breeding*. Heidelberg: Springer-Verlag, 255–266.
- Roderick ML, Hobbins MT, Farquhar GD.** 2009. Pan evaporation trends and the terrestrial water balance. I. Principles and observations. *Geography Compass* **3/2**, 746–760.
- Rozenzweig C, Parry M.** 1994. Potential impact of climate change on world food supply. *Nature* **367**, 133–138.
- Salse J, Feuillet C.** 2007. Comparative genomics of cereals. In: Varshney RK, Tuberosa R, eds. *Genomic assisted crop improvement: genomics approaches and platforms*, Vol. 1. The Netherlands: Springer, 177–205.
- Salvi S, Tuberosa R.** 2007. Cloning QTLs in plants. In: Varshney RK, Tuberosa R, eds. *Genomic assisted crop improvement: genomics approaches and platforms*, Vol. 1. The Netherlands: Springer, 207–225.
- Sanchez AC, Subudhi PK, Rosenow DT, Nguyen HT.** 2002. Mapping of QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). *Plant Molecular Biology* **48**, 713–726.
- Scheiermeier Q.** 2008. A long dry summer. *Nature* **452**, 270–273.
- Semenov MA, Halford NG.** 2009. Identifying target traits and molecular mechanisms for wheat breeding under a changing climate. *Journal of Experimental Botany* **60**, 2791–2804.
- Service RF.** 2006. Gene sequencing. The race for the \$1000 genome. *Science* **311**, 1544–1546.
- Shinozaki K, Yamaguchi-Shinozaki K, Seki M.** 2003. Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology* **6**, 410–417.
- Slafer GA, Araus J-L, Royo C, Del Moral LFG.** 2005. Promising ecophysiological traits for genetic improvement of cereals in Mediterranean environments. *Annals of Applied Biology* **146**, 61–70.
- Sreenivasulu N, Varshney RK, Kishor K, Weschke W.** 2004. Functional genomics for tolerance to abiotic stress in cereals. In: Gupta PK, Varshney RK, eds. *Cereal genomics*. The Netherlands: Kluwer Academic Publishers, 483–514.
- Talame V, Ozturk NZ, Bohnert HJ, Tuberosa R.** 2007. Barley transcript profiles under dehydration shock and drought stress treatments: a comparative analysis. *Journal of Experimental Botany* **58**, 229–240.
- Valkoun J.** 2001. Wheat pre-breeding using wild progenitors. *Euphytica* **119**, 17–23.
- Valliyodan B, Nguyen HT.** 2006. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Current Opinion in Plant Biology* **9**, 189–195.
- Vargas M, Eeuwijk FA, Crossa J, Ribaut J-M.** 2006. Mapping QTLs and QTL×environment interaction for CIMMYT maize drought stress program using factorial regression and partial least squares methods. *Theoretical and Applied Genetics* **112**, 1009–1023.
- Varshney RK, Balyan HS, Langridge P.** 2006. Wheat. In: Kole C, ed. *Genome mapping and molecular breeding in plants*, Vol. 1. *Cereals and millet*. Berlin: Springer, 79–134.
- Varshney RK, Graner A, Sorrell AE.** 2005. Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology* **23**, 48–55.
- Varshney RK, Mahender T, Aggarwal RK, Börner A.** 2007. Genetic molecular markers in plants: development and applications. In: Varshney RK, Tuberosa R, eds. *Genomic assisted crop improvement: genomics approaches and platforms*, Vol. 1. The Netherlands: Springer, 13–30.
- Vinocur B, Altman A.** 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotechnology* **16**, 123–132.
- Wall GW, Garcia RL, Kimball BA, et al.** 2006. Interactive effects of elevated carbon dioxide and drought on wheat. *Agronomy Journal* **98**, 354–381.
- Yamaguchi-Shinozaki K, Shinozaki K.** 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annual Review of Plant Biology* **57**, 781–803.
- Yan L, Loukoinov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J.** 2003. Positional cloning of the wheat vernalization gene *VRN1*. *Proceedings of the National Academy of Sciences, USA* **100**, 6263–6268.
- Zhang JZ, Creelman RA, Zhu J-K.** 2004. From the laboratory to field. Using information from *Arabidopsis* to engineer salt, cold, and drought tolerance in crops. *Plant Physiology* **135**, 615–621.
- Zhou J, Wang X, Jiao Y, et al.** 2007. Global genome expression analysis of rice in response to drought and high-salinity stresses in shoot, flag leaf, and panicle. *Plant Molecular Biology* **63**, 591–608.