CHAPTER 11

PHYSIOLOGICAL INTERVENTIONS IN BREEDING FOR ADAPTATION TO ABIOTIC STRESS

M.P. REYNOLDS[#] AND R.M. TRETHOWAN[##]

[#] *CIMMYT, Int. Apdo. Postal 6-641, 06600 Mexico DF, Mexico.*
[##] *University of Sydney, Plant Breeding Institute, PMB 11, Camden, NSW, Australia.*
E-mail: m.reynolds@cgiar.org

Abstract. The physiological-trait-based breeding approach has merit over breeding for yield *per se* because it increases the probability of crosses resulting in additive gene action. While considerable investment in germplasm characterization is required, conceptual models of crop genotypes can be employed as research tools to quantify likely genetic gains associated with specific trait or in defining traits that may have generic value across different stresses. For example, deeper root growth that permits better access to soil water has obvious benefit under drought, while under hot, irrigated conditions permits leaf canopies to match the high evaporative demand associated with hot, low-relative-humidity environments, resulting in higher leaf gas-exchange rates and heat escape through evaporative cooling. Although improvement in adaptation to abiotic stress may occur as a result of transgressive segregation, exotic parents can be used to increase total allelic diversity for such traits. The bread-wheat-breeding programme at CIMMYT is exploiting new genetic diversity using inter-specific hybridization of the ancestral genomes of bread wheat. Novel genetic diversity is also being accessed more directly by crossing adapted germplasm with landrace accessions originating in abiotically stressed environments that have become isolated from mainstream gene pools. Through studying these genetic resources it has been possible to calculate the theoretical impact of combining their best values of trait expression into the check cultivar to gain some insight into which traits may hold most promise in terms of genetic enhancement. It was apparent that the genetic diversity found for water use efficiency offers the greatest and most consistent opportunity for increasing yield, while increasing stem carbohydrates and access to water at depth also shows some potential. Direct physiological interventions in breeding include (i) characterization of potential parents for more strategic crossing; (ii) early-generation selection; and (iii) evaluation of promising genetic resources in pre-breeding. The early-generation selection trait 'canopy temperature' (measured with an infrared thermometer) has been readily adopted since measurement is quick, easy and inexpensive. Although genetic markers are not currently used in selection for complex traits, as technology advances and combines with gene discovery approaches, more quantitative trait loci (QTLs) associated with adaptation to complex environments will emerge. A multi-staged approach to identifying molecular markers may be the best approach where QTLs for generic traits – i.e., valid across a range of environments – are identified in well controlled field environments and used to optimize germplasm. Subsequently, environment-specific models would be used to factor in additional traits commonly found in a specific region that may not be directly related to moisture stress, factors such as nematodes or microelement deficiency or toxicity that are exacerbated under drought.

INTRODUCTION

Since breeding for adaptation to abiotic stress at a global scale encompasses a range of target environments, an array of adaptive mechanisms must be considered for deployment accordingly. Research into the different physiological adaptations of crops to abiotic stress has been ongoing for decades (e.g., Fischer and Turner 1978; Blum 1988; Boyer 1996; Bruce et al. 2002), and molecular technologies have added a new dimension to the research (Xiong et al. 2002; Chaves et al. 2003; Wang et al. 2003). While a limited amount of research has been applied to crop improvement (Bolaños and Edmeades 1996; Condon et al. 2004; Rebetzke et al. 2002; Richards et al. 2002; Trethowan and Reynolds 2006), much has yet to be applied (Araus et al. 2002). Nonetheless, the physiological-trait-based breeding approach has merit over breeding for yield *per se* because it increases the probability of crosses resulting in additive gene action, although considerable investment in germplasm characterization is prerequisite. This paper discusses some of the ways that physiological intervention can assist in the breeding for abiotically stressed environments.

The use of conceptual models of genotypes is discussed. For example, candidate traits for increasing yield under abiotic stress can be grouped together such that physiological effects among groups are likely to be relatively independent genetically. Grouping traits in this way, while based on an incomplete knowledge of drought adaptation and its genetic basis, does help to establish a broad conceptual framework that can be used not only as a decision support tool in designing crosses between complementary parents but also in more strategic research.

The exploitation of new allelic variation related to abiotic-stress adaptation is discussed using examples from the International Maize and Wheat Improvement Center's (CIMMYT) wheat-breeding programme. The wild D-genome has been exploited for some time for disease resistance genes (Villareal et al. 1995). More recently, wide crossing has been applied to increase yield under drought (Trethowan et al. 2003; 2005) and there is already evidence for impact in drier regions world-wide based on data from recent international drought trials (Trethowan and Reynolds 2006). Collections of genetic resources including landraces originating in abiotically stressed regions are another important source of novel alleles that are relatively easy to work with from a breeding perspective.

The process of incorporating new genetic diversity can be accelerated with suitable early-generation selection tools that enable the best progeny to be identified before costly yield trials are run. For example, canopy temperature (CT) is now used in drought breeding at CIMMYT to select segregating populations with better access to water by roots (Trethowan and Reynolds 2006). Given the current lack of genetic markers for stress adaptation, physiological traits can be viewed as proxy genetic markers at least among locations where the level of genotype × trait interaction is relatively low. As our knowledge of the physiological and genetic basis of stress adaptation increases, it will become more biologically and economically feasible to apply molecular-marker-assisted selection for targeted breeding objectives. The main challenge to their application in breeding will be to determine the right combination of alleles to use, since conditions vary significantly from site to site and

from year to year and significant quantitative trait locus (QTL) × environment interactions exist. A multi-staged approach to identifying molecular markers is discussed.

PHYSIOLOGICAL APPROACHES TO BREEDING

To achieve the maximum impact from using specific traits in breeding strategies, a detailed knowledge of target environments is prerequisite, especially in terms of building a developmental component into genotype models. Some of the most important factors to consider when defining abiotically stressed environments are outlined below. The potential benefits and examples of physiological breeding approaches are also discussed.

Abiotic-stress environments

Drought and temperature are the two most important abiotic stress factors influenced directly by climate; the former is discussed in more detail elsewhere (Reynolds et al. in press). One important characteristic of the drought environment includes the distinct types of water distribution profile in relation to evaporative demand, creating different combinations of pre- or post-anthesis stress and determining the relative value of drought-adaptive traits. Another is the presence of soil factors such as microelement deficiency or parasitic nematodes, whose effects on productivity are severely exacerbated under moisture deficit, confounding potential genetic gains associated with drought adaptation *per se*.
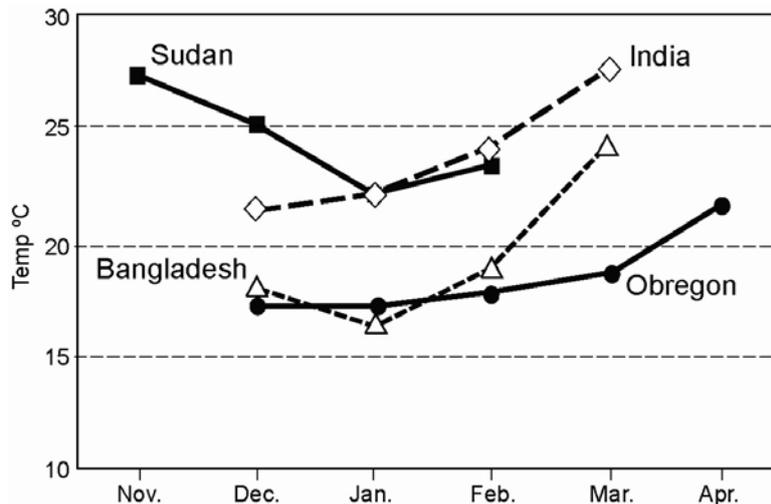

Figure 1. Mean daily temperature for wheat crop cycle at four locations (Wad Medani, Sudan; Dharwad, India; Dinajpur, Bangladesh; Obregon, Mexico) of the International Heat Stress Genotype Experiment -IHSGE- (Reynolds et al. 1994)

High temperature is detrimental to temperate cereals, defined (for wheat) as a mean average temperature of the coolest month higher than 17.5 °C (Fischer and Byerlee 1991). The heat-prone environment encompasses a range of temperature profiles when comparing different locations across a range of countries (Figure 1).

For breeding purposes CIMMYT defined two distinct sub-environments, one with high relative humidity and another with low relative humidity. This distinction is made primarily because of the fact that diseases are a severe problem in the humid environment, especially *Helminthosporium sativum*. Nonetheless, results of international yield trials (Reynolds et al. 1998) grown at over 40 environments (where disease incidence was controlled with fungicide application) confirmed that the main factor determining genotype × environment interaction (G×E) in hot climates was relative humidity (Figure 2). More recent and comprehensive analysis
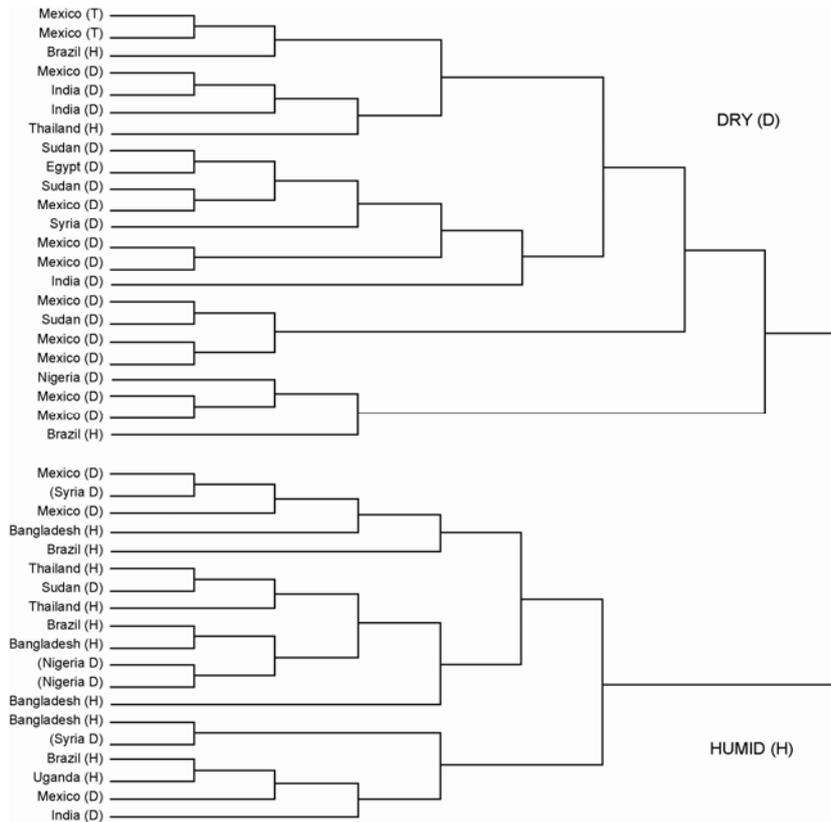

Figure 2. *Cluster analysis of hot wheat-growing environments internationally (Reynolds et al. 1998). Cluster analysis was based on cross-over interaction of genotypes as described by Vargas et al. (1998). The analysis indicates two main groups in which either hot, low-relative-humidity environments (upper group) or hot, high-relative-humidity (lower group) environments predominate. Temperate environments (T) are thus indicated; environments in parentheses indicate extreme low yield*

using CIMMYT international nursery yield data indicated that main genotype clusters correspond to three main types of environment, viz., temperate, continuous heat stress and terminal heat stress, and confirmed relative humidity as an important factor determining G×E within some of these clusters (Lillemo et al. 2005).

The other main abiotic stress factors associated with soils are discussed in detail elsewhere. For example, saline soils cover over 900 Mha of land area, and at least a third of the area under irrigation is affected by salinity (Ghassemi et al. 1995). While soil salinity is a global problem, it is especially so in arid or semi-arid climates where average rainfall is less than evapotranspiration. Other important soil stresses are associated with acid soils (see Hede et al. 2001) and micronutrient deficiency and toxicity (see Ascher-Ellis et al. (2001) for description of environments).

The merits of the physiological approaches to breeding

When breeding for defined target environments, whether broad or specific, the physiological approach has an advantage over empirical breeding for yield *per se* because it increases the probability of crosses resulting in additive gene action for stress adaptation. However, the corollary is that germplasm must be much more thoroughly characterized than for yield and agronomic type alone. Before they can even be characterized, individual traits must be conceptualized and defined in terms of (i) the stage of crop development at which they are pertinent; (ii) the specific attributes of the target environment for which they are adaptive; (iii) their potential contribution to yield over a range of crop cycles.

The physiological approach also has merit over the genetic approach for the very simple reason that there is a lack of in-depth understanding of the genetic basis of stress adaptation in crops in general. As a result, very few genes or gene markers exist that can be implemented in breeding for adaptation to abiotic stresses such as drought or heat (Snape 2004). On the other hand, physiological traits can be used to dissect stress adaptation into some of its components. Such physiological traits represent the closest approximation available to genetic markers, assuming they are applied to a restricted range of environments within which the traits show acceptable levels of heritability.

Application of a general conceptual model

A general model for drought adaptation of wheat was developed by physiologists and breeders at CIMMYT encompassing most of the traits for which evidence had been presented of a potential role in dry environments (Reynolds et al. 2000; 2005). The model is explained in detail elsewhere (Reynolds et al. 2005) and is summarized here. The many candidate traits for increasing yield under drought stress are grouped together such that physiological effects among groups are likely to be relatively discrete genetically. Grouping traits in this way, while based on an incomplete knowledge of drought adaptation and its genetic basis, helps to establish a broad conceptual framework. The model describes four main groups of traits relating to: (i) Pre-anthesis growth; rapid ground cover to shade the soil from evaporation

(Richards et al. 2002), and strong assimilation capacity between jointing and lag-phase to permit accumulation of stem carbohydrates (Blum 1998). (ii) Access to water as a result of rooting depth or intensity that would be expressed by a relatively cool canopy (Reynolds et al. 2005) or favourable expression of water-relations traits (Blum et al. 1989). (iii) Water use efficiency (WUE) as indicated by relatively higher biomass per mm of water extracted from the soil, transpiration efficiency of growth (TE = biomass per mm water transpired) indicated by C-isotope discrimination ($\Delta^{13}C$) of leaves (Condon et al. 2002), and WUE of spike photosynthesis associated with refixation of respiratory CO_2 (Bort et al. 1996). (iv) Photoprotection including energy dissipation (Niyogi 1999; Havaux and Tardy 1999), anti-oxidant systems (Mittler and Zilinskas 1994) and anatomical traits such as leaf wax (Richards et al. 2002).

The model is used to assist with breeding decisions permitting a strategic approach whereby drought-adaptive genes, for example, are more likely to be accumulated when parents with contrasting drought-adaptive mechanisms are crossed. In a subsequent section germplasm will be described that was generated using this approach. The conceptual platform can also be used as a decision support
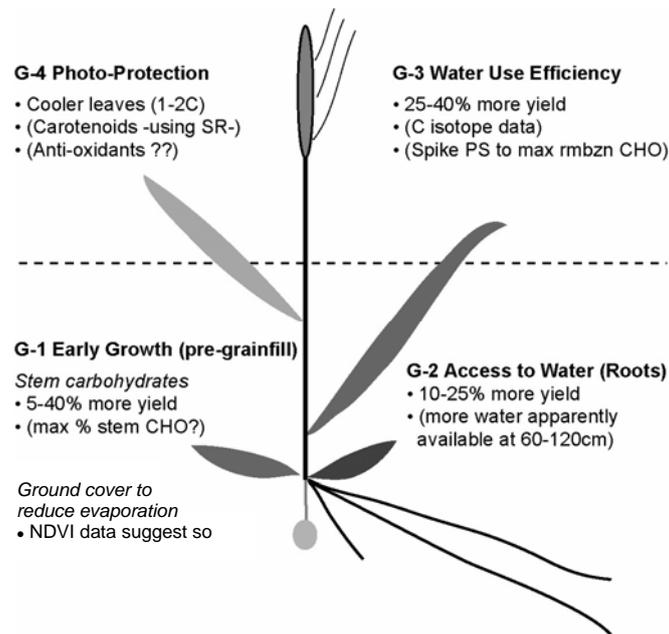

Figure 3. *Potential genetic gains associated with over-expression of drought-adaptive traits theoretically expressed in an elite check background, based on empirical data from controlled field studies involving 11 elite genetic-resource genotypes and the check cultivar. Potential genetic gains are expressed for the range of differences in trait expression (i.e. comparing the check with the best expression among all genotypes) across three drought cycles, NW Mexico (2002, 2004, 2005). Traits are grouped according to a conceptual model for drought-adaptive traits (Reynolds et al. 2005)*

tool for activities such as (i) defining suitably contrasting parents in development of molecular-mapping populations and subsequent gene discovery; (ii) quantifying the potential benefits of enhanced trait expression (Figure 3), and therefore indicating targets for exploration of genetic resources; and (iii) identifying common physiological bases between drought and other abiotic stresses such as high temperature.

For example, Figure 4 presents a generic conceptual model of a core-set of traits for adaptation to dry as well as hot, irrigated environments in wheat. It is clear when considering the groups of traits that a number of physiological mechanisms are likely to be of benefit in both situations. For example, rapid ground cover is a useful trait for avoiding the wasteful evaporation of soil water under pre-anthesis drought stress (Loss and Siddique 1994). The trait may also be of value under hot, irrigated conditions where rapid early ground cover could increase light capture and partially compensate for reduced tiller number associated with accelerated development rate at higher temperatures (Rawson 1986). Accumulation of stem carbohydrates and their subsequent remobilization in the post-anthesis period provide an extra source of assimilates for grain growth when either of these stress factors is experienced during the grain-filling stage (Blum 1998). Similarly, root growth that permits better
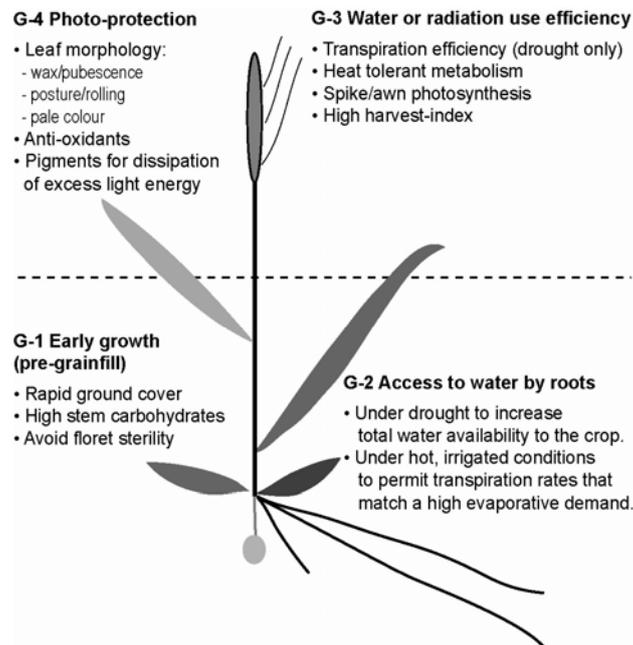

Figure 4. *Conceptual model for generic traits associated with adaptation to moisture-stressed and/or hot, irrigated environments (adapted from Reynolds et al. 2005; Reynolds and Borlaug 2006)*

access to soil water has obvious benefit under drought, while enabling heat-stressed canopies to match the high evaporative demand associated with hot, low-relative-humidity environments, resulting in higher leaf gas-exchange rates and heat escape through cooler canopies (Reynolds et al. 2000). There are also traits that will impact either WUE or radiation use efficiency (RUE) depending on the environmental conditions. For example, heat-sensitive metabolic processes such as starch synthesis (Keeling et al. 1994), photo-respiration and photo-inhibition (Osmond and Grace 1995) will impair performance when tissues reach supra-optimal temperatures, whether the cause is reduced evaporative cooling due to soil water deficit, the inability of the vascular system to match evaporative demand, or simply an ambient temperature or radiation load that precludes heat escape. Similarly, photo-protective mechanisms that either dissipate excess light energy (Niyogi 1999) or deactivate reactive oxygen species resulting from excess light (Mittler and Zilinskas 1994) are likely to be important under drought because there is insufficient water to permit full utilization of light energy, while under heat stress the metabolism may become impaired, leading to the same problem.

Using such an approach CIMMYT, in collaboration with CSIRO-Brisbane, has designed experiments using the Seri/Babax mapping population (Olivares-Villegas et al. in review; McIntyre et al. 2006) where QTLs for (i) stem carbohydrate accumulation and (ii) canopy temperature are being evaluated under both drought and hot, irrigated environments to establish whether there is a common genetic basis for the expression of either trait across environments.

INTRODUCING NEW ALLELIC VARIATION

While crosses among elite breeding lines may result in increased levels of trait expression due to transgressive segregation of alleles, exotic parents can be used to increase allelic diversity. The bread-wheat-breeding programme at CIMMYT is exploiting new genetic diversity using inter-specific hybridization of the ancestral genomes of bread wheat. Specifically tetraploid durum wheat is crossed with *Aegilops tauschii*, the ancestral donor of the D-genome to recreate hexaploid bread wheat (Mujeeb-Kazi et al. 1996). When elite wheat cultivars are crossed to these so called 'synthetic wheats', some of the progeny show considerable improvement in drought adaptation (Trethowan et al. 2005), though the physiological and genetic basis is not established. Novel allelic diversity can also be accessed more directly, for example, by crossing adapted germplasm with landrace accessions originating in abiotically stressed environments that have become isolated from mainstream gene pools (Reynolds et al. submitted). While landraces have been used for some time in breeding barley for adaptation to abiotic stresses (Ceccarelli et al. 2001), their use in bread-wheat breeding is less common.

Synthetic derived wheat

Although synthetic wheat possesses significant new variation for adaptation to moisture-limited environments (Trethowan et al. 2000; 2003; Villareal et al. 2003a;

2003b), the primary synthetics do not necessarily show better adaptation to drought compared with adapted modern cultivars. However, they sometimes carry complementary genes for drought adaptation that segregate transgressively in combination with modern materials. Table 1 shows yield advantages of up to 23% over the high-yielding adapted bread-wheat cultivar, Bacanora. This cultivar was crossed to drought-tolerant but low-yielding primary synthetic wheat and the resultant progeny were tested for yield under drought stress in northern Mexico. In association with their improved yield the synthetics maintain seed size under stress, an important quality characteristic in many cultures (Trethowan et al. 2003).

Table 1. *Mean yield (absolute and relative to that of Bacanora) of grain-derived lines based on crosses between Bacanora and primary synthetic wheat grown under drought stress in northwestern Mexico during 2000 (adapted from Trethowan et al. 2000)*

Pedigree	Yield (t ha^{-1})	Yield as % of Bacanora
Bacanora//Sora/*Ae. tauschii* (323) [b] CASS94600121S-1Y-2B-1PR-0B-0HTY	3. 838[a]	123
Bacanora//Sora/*Ae. tauschii* (323) CASS94Y00121S-1Y-2B-2PR-0B-0HTY	3.697[a]	118
Bacanora/Rabi//Gs/Cra/3/*Ae. tauschii* (895) CASS94Y00160S-40Y-7B-1PR-0B-0HTY	3.660[a]	117
Bacanora//Sora/*Ae. tauschii* (323) CASS94Y00121S-1Y-2B-3PR-0B-0HTY	3.536[a]	113

[a] Significantly different from Bacanora at $P < 0.05$.
[b] This line was evaluated in a separate trial with a different randomization.

Recent work compared two synthetic derived (SYN-DER) lines with their recurrent parents under moisture-stressed conditions for which a full description of methods and results can be found elsewhere (Reynolds et al. submitted). In summary, SYN-DER lines showed significant increases in yield (17 and 33%) and substantial increases in total biomass (45 and 66%) (Table 2). When considering water uptake characteristics, SYN-DER lines were more effective at removing water from the soil at all depth profiles, resulting in on average 11% increase in water use (Table 2). Despite this, SYN-DER lines showed a lower root:shoot ratio associated with less investment in root mass in the top 30 cm of the soil (Reynolds et al. submitted). Nonetheless, increased water extraction was not of sufficient magnitude to explain the increase in biomass of SYN-DER relative to recurrent parents. Using the SYN-DER value for WUE of 5.5 g m^{-2} mm^{-1}, an extra 26 mm of water would account for approximately 150 g m^{-2} of additional biomass. Mechanisms that may explain a larger WUE are increased transpiration efficiency associated with intrinsically low stomatal conductance and carbon-isotope discrimination (Condon et al. 2002; 2004; Rebetzke et al. 2002), and decreased losses of soil water early in crop establishment due to improved early ground cover (Richards et al. 2002). Although neither trait was estimated in this experiment, SYN-DER lines have been reported to display considerable early vigour and increased early ground cover

(Trethowan et al. 2005), which, along with higher values of above-ground biomass during tillering stage, suggests that this trait does play a role.

Table 2. *Growth and water-use parameters for two synthetic derived wheat lines and the recurrent parents under moisture stress (average two cycles) for two genetic backgrounds, NW Mexico, 2003-2005*

GENOTYPE	Grain yield (g m^{-2})	Total biomass (g m^{-2})	Root: shoot ratio	Water used (mm)	WUE (biomass) (g m^{-2} mm^{-1})
CROC_1/AE.SQUARROSA (210)//2×EXCALIBUR	320	1125	0.09	252	4.46
EXCALIBUR (recurrent parent 1)	240	777	0.20	221	3.51
D67.2/P66.270//AE. SQUARROSA (320)/3/ CUNNINGHAM	350	1659	0.09	253	6.57
CUNNINGHAM (recurrent parent 2)	300	1000	0.14	233	4.29
Average effect	24%	57%	–46%	11%	41%
Least significant difference (LSD) (P ≤ 0.05)	27	302.4	0.013	11.9	1.35

Quantifying the potential value of land races and other genetic resources

One of the benefits of interdisciplinary collaboration between physiology, breeding and genetic resources is the continuum provided by ready access to a vast range of genetic resources, the ability to evaluate germplasm in realistic field environments in comparison to up-to-date cultivars, and the opportunity to use and evaluate promising genetic resources in pre-breeding work. Through such collaboration, CIMMYT's wheat physiology programme has been assembling elite genetic resources and comparing them in controlled field environments with the best check cultivars in terms of expression for a number of stress-adaptive traits including early ground cover, the accumulation and remobilization of soluble stem carbohydrates, the ability to access water at different soil depths down to 120 cm, and apparent water use efficiency (calculated as the ratio of above-ground biomass and water used). Some of this work has already been reported and indicates that some of the best landraces collected in Mexico's driest regions have a significantly greater ability to extract water at depth compared to the elite checks, while other genetic resources including synthetic derived lines excel in WUE or the percentage of stem weight found as soluble carbohydrate at anthesis (Reynolds and Condon submitted;

Reynolds et al. submitted). A principal objective in assembling and comparing such materials was to calculate the theoretical impact of combining their best values of expression into the check cultivar to gain some insight into which traits may hold most promise in terms of genetic enhancement.

A rough quantitative assessment was made by identifying the highest expression of any trait amongst all genetic resources and comparing the value of its expression with that of the check cultivar, and estimating what the theoretical yield would be if the trait were expressed at the same level in the check cultivar. The calculations were performed for each of three years when water availability ranged from 175 to 300 mm; the range of potential benefits in terms of yield gain are presented in Figure 3 using the conceptual model developed previously as a frame of reference (Reynolds et al. 2005). It was apparent that the genetic diversity found for WUE offers the greatest and most consistent opportunity for increasing yield, while increasing stem carbohydrates and access to water at depth also show some potential. However the increased expression of a number of other traits studied could not be extrapolated directly to yield gains. For example, differences in early ground cover, estimated using spectral reflectance (Gutiérrez-Rodríguez et al. 2004; Babar et al. 2006), for which most genotypes were superior to the check, indicate a greater potential for early ground cover and thus reduced evaporation of soil moisture. Previous analysis using estimates of transpiration efficiency based on ^{13}C-isotope discrimination analysis and final biomass with a larger selection of genotypes estimated that genetic effects on water losses because of evaporation from the soil could vary from 20 to 40% (Reynolds and Condon submitted), indicating a substantial potential advantage associated with increased ground cover. Canopy temperature showed genetic effects associated with soil moisture extraction (Reynolds et al. 2005; submitted) and cooler leaves, and may also be associated with increased photo-protection.

DIRECT PHYSIOLOGICAL INTERVENTIONS IN BREEDING

Relatively few crop-breeding programmes have actively selected for genetically complex physiological traits due to expense and the time-consuming nature of their measurement. Exceptions would include selection for anthesis-silking interval (ASI) in breeding maize for drought adaptation (Bolaños and Edmeades 1996) and carbon isotope discrimination (Δ^{13}C), which provides an indirect measure of WUE (Condon et al. 2002), used to develop the Australian spring-wheat cultivar, Drysdale (Descriptions: *Triticum aestivum* 2002). The wheat-breeding programme at CIMMYT uses physiological interventions at three stages of the breeding process: (i) parental characterization; (ii) early generation selection; and (iii) pre-breeding. The first and second of these will be discussed subsequently, while the latter encompasses a combination of both, i.e., selection tools are used to screen large collections of genetic resources, and elite genetic resources are characterized and used as new sources of physiological traits for introgression into improved backgrounds.

Physiological characterization of parents

Potential parents are characterized for a range of physiological traits, thereby
allowing plant breeders to combine these traits in a strategic manner in crosses
(Reynolds et al. 2005). Table 3 shows an example of the range in physiological traits
among key parental materials grown under drought stress in NW Mexico. These
data were collected on lines using a managed drought-stress regime described in
Trethowan et al. (2001). Quantitative analysis of physiological traits in a broad
range of genetic backgrounds (including materials derived from inter-specific
hybridization and selected landraces) suggest that traits like WUE, stem
carbohydrates and access to water at depth in the soil, if combined into modern
varieties, could increase yields under drought by at least 20–30% over current elite
checks (Reynolds and Condon submitted; Reynolds et al. submitted). Many of the
same traits have potential to improve yields under hot, irrigated environments
(Figure 4), although quantitative data have yet to be analysed for this environment.
This kind of information has been used for several years in breeding and pre-
breeding at CIMMYT to design crosses (Trethowan and Reynolds 2006). These
results show that it is possible to combine extreme expression of several
physiological traits in one genetic background. However, the challenge remains to
compile expression of all relevant stress-adaptive physiological traits into one
genotype.

Table 3. *Physiological traits measured on parental materials at Ciudad Obregon 2003-2004
(from Trethowan and Reynolds 2006)*

Pedigree	Yield	Biomass (Anthesis)	CT[a] (Vegetative)	CT (Gr. fill)	Carbon isotope discrim.	Stem CHO[b] at anthesis	Water extraction by roots
	g m^{-2}	g m^{-2}	°C	°C		% stem dry weight	% available water
Jun/Gen	338	424	19.2	21.8	−23.1	13.3	84
Weebill 1	348	513	19.3	21.7	−22.5	17.5	83
Synthetic	278	510	19.8	22.6	−22.5	19.1	79
Frame	213	503	20.5	23.2	−21.7	6.8	79
Klein Cacique	247	638	20.1	23.3	−22.6	3.4	82
Prointa Federal	223	572	20.0	22.9	−22.4	11.2	79

[a] Canopy temperature; [b] carbohydrate.

Early generation selection

The difficulty of selecting for improved adaptation to abiotic stresses makes the use
of indirect measures attractive to plant breeders. A good example is canopy
temperature, for which measurement is quick (10 seconds), easy (aim and pull the
trigger) and inexpensive. Previous studies have shown strong association between
yield and canopy temperature (CT) in random-inbred lines (RILs) under drought,

indicating the potential of the trait as an indirect selection criterion for achieving genetic gains in drought adaptation (Reynolds et al. 2000; Olivares-Villegas et al. in review). At CIMMYT, canopy temperature is evaluated in breeders' F4 populations to change gene frequency in favour of cooler lines (presumably better able to explore deep soil water profiles) and in combination with visual selection. The trait is measured during the late vegetative stage and again during grain filling, and bulks that are consistently cooler are selected (Figure 5) assuming they meet the visual criteria. The visually selected lines showing consistently warmer canopies are not thrown out; however, a greater number of plants are selected from the cooler bulks, thereby skewing gene frequency in the early generations in favour of these cooler materials (Figure 5). The trait could conceivably be measured as early as $F_{2:3}$ populations. Since CT has been shown to be well associated with ability to extract water from depth (Reynolds et al. 2005) selection for CT is most probably increasing gene frequencies for root-related traits.
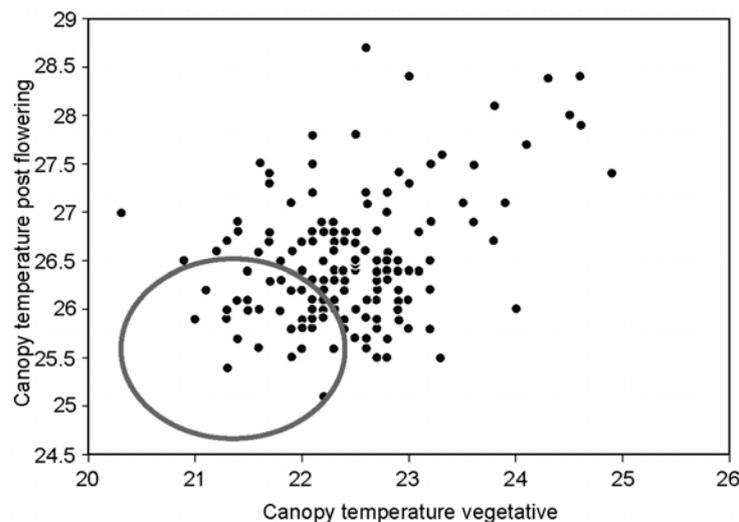

Figure 5. *Canopy temperature measured pre- and post-anthesis on visually selected F4 bulks grown under drought stress at Ciudad Obregon in 2004*

CT has also been shown to be associated with genetic gains in RILs under hot, irrigated conditions (Reynolds et al. 1998). As well as having application in early generation selection, CT was shown to be a powerful tool for selecting advanced lines within the breeding environment for performance at a number of international heat-stressed target environments (Reynolds et al. 1994; 1998; 2001). For example, when comparing the association of yield in wheat environments in target countries (Sudan, India, Bangladesh) with yield and CT measured in the selection environment (NW Mexico), it was found that both traits explained approximately equal amounts of variation in yield of 60 advanced lines, about 40% (Reynolds et al. 2001). However, CT was measured on plots of 2 m^2 instead of yield plots of 10 m^2,

in about 10 seconds compared with several minutes to harvest and weigh a yield plot, and with an instrument that costs less than $200 compared with a small plot harvester. Although we are not suggesting that CT should replace yield estimates in a breeding programme, this example illustrates the point that indirect selection criteria like CT have a role to play in improving the efficiency of selection.

Another attraction of integrative traits like CT is that they may combine several important physiological mechanisms (Araus et al. 2002). When CT is measured on genetically diverse material during early generation selection, for example, under hot, irrigated conditions, cooler canopies would be found in lines that combine a number of the traits considered important (see Figure 4). These might include (i) a root system that can match evaporative demand at high vapour-pressure deficit; (ii) high intrinsic RUE; and (iii) photo-protective mechanisms that maintain RUE throughout the cycle. By measuring CT strategically, for example, at different phenological stages and times of the day, genotypes that are deficient in any of those three areas could be detected and eliminated while genotypes showing consistently cool CT would be advanced to the next generation. Similarly, under drought, a cool CT may – in certain environments – be related directly to genetic potential for root depth. In other environments, however, cooler genotypes would be found only for those lines that combine a number of relevant genes. This could be the case, for example, in environments where micro-element deficiency or soil-borne diseases are affecting root growth. Clearly the value of integrative selection tools such as CT can be augmented with a strategic use of selection environments, and conceptual models such as those described can play a role in developing such strategies.

Marker-assisted selection

As our understanding of the physiological basis of yield and how cultivars interact with environment accumulates, methods for manipulation of DNA such as marker-assisted selection (MAS) will become increasingly powerful (Snape 2004). For example, MAS is already applied in international wheat breeding to screen for a number of genetically simple traits (Trethowan and Reynolds 2006). Good examples are *Cre1* and *Cre3* (Lagudah et al. 1997) for cereal cyst nematode, genetic resistance being a key element of root health (Trethowan et al. 2005). A marker for tolerance to high boron (*Bo1*) is also routinely used; boron toxicity is frequently associated with soils in marginal environments world-wide, which exacerbates yield loss when water is limited (Ascher-Ellis et al. 2001). Diagnostic markers are available for the gibberellic-acid-insensitive Rht1 and Rht2 genes, and markers are either available (Rht8) or under validation for alternative gibberellic-acid-sensitive dwarfing genes such as Rht 12 and Rht 13 (Ellis et al. 2005). These gibberellic-acid-sensitive dwarfing genes can improve emergence characteristics due to longer coleoptiles when seed is sown in hot and dry environments (Rebetzke et al. 1999; Trethowan et al. 2005). The wheat-breeding programme at CSIRO, Canberra in Australia also make routine use of molecular markers for a large array of traits (Bonnett et al. 2005). Despite heavy investment, there has been significantly less success identifying markers for QTLs such as drought adaptation (Snape 2004). Given that

QTL analysis is essentially a statistical rather than a deterministic analysis of genes, the likelihood of identifying all of the genes associated with a complex trait decreases with the number of loci involved and the size of the individual effects. However, as marker technology advances and combines with gene-discovery approaches, more QTLs associated with adaptation to complex environments will emerge. Nonetheless, the main challenge to their application in breeding will be to determine the right combination of alleles to use, since conditions vary significantly from site to site and from year to year and significant QTL × environment interactions exist. A multi-staged approach to identifying molecular markers may be the best approach. For example, conceptual models for generic drought-adaptive traits (e.g., Figure 4) can be used to identify suitable crosses and populations that would be grown in well-controlled field environments so as to develop QTLs associated with performance under purely water-limited conditions. Generic traits for drought adaptation might include capacity of roots to access water deep in the soil, high intrinsic WUE, antioxidant systems for photo-protection, etc. (Reynolds et al. 2005). Once germplasm has been optimized for adaptation to moisture stress, environment-specific models would be used to include other factors commonly found in farmers' fields in a region that are not directly related to moisture stress, such as micro-element deficiencies/toxicities and soil-borne diseases. Environment-specific models could also be used to fine-tune QTLs related to rainfall distribution, temperature profiles and photoperiod etc.

DNA fingerprinting to identify key genomic regions associated with adaptation to abiotic stress

The CIMMYT wheat programme has generated an extensive data set of yield and disease performance collected from yield and screening nurseries over the past 3 decades. There is scope to use these data and fingerprints of the key germplasm representing this 30-year period to identify genomic regions linked to performance under defined sets of environmental conditions. It may be possible in the near future to link, for example, drought performance with specific genomic regions always present in materials performing well under drought stress. Wheat breeders could then ensure that these regions are present in their parental materials and could actively select for them in segregating populations. It would also be valuable to establish the traits associated with these regions to determine if they have been apparently optimized or indeed if they are associated with abiotic or biotic factors or a combination of both.

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REFERENCES

Araus, J.L., Slafer, G.A., Reynolds, M.P., et al., 2002. Plant breeding and drought in C$_3$ cereals: what should we breed for? *Annals of Botany,* 89 (Special Issue), 925-940.

Ascher-Ellis, J.S., Graham, R.D., Hollamby, G.J., et al., 2001. Micronutrients. *In:* Reynolds, M.P., Ortiz-Monasterio, J.I. and McNab, A. eds. *Application of physiology in wheat breeding.* CIMMYT, Mexico, 219-240. [http://www.cimmyt.org/research/wheat/map/research_results/wphysio/ WPhysio_Nutritional.pdf]

Babar, M.A., Reynolds, M.P., Van Ginkel, M., et al., 2006. Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. *Crop Science,* 46 (3), 1046-1057.

Blum, A., 1988. *Plant breeding for stress environments.* CRC Press, Boca Raton.

Blum, A., 1998. Improving wheat grain filling under stress by stem reserve mobilisation. *Euphytica,* 100 (1/3), 77-83.

Blum, A., Shpiler, L., Golan, G., et al., 1989. Yield stability and canopy temperature of wheat genotypes under drought-stress. *Field Crops Research,* 22 (4), 289-296.

Bolaños, J. and Edmeades, G.O., 1996. The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. *Field Crops Research,* 48 (1), 65-80.

Bonnett, D.G., Rebetzke, G.J. and Spielmeyer, W., 2005. Strategies for efficient implementation of molecular markers in wheat breeding. *Molecular Breeding,* 15 (1), 75-85.

Bort, J., Brown, R.H. and Araus, J.L., 1996. Refixation of respiratory CO$_2$ in the ears of C$_3$ cereals. *Journal of Experimental Botany,* 47 (303), 1567-1575.

Boyer, J.S., 1996. Advances in drought tolerance in plants. *Advances in Agronomy,* 56, 187-218.

Bruce, W.B., Edmeades, G.O. and Barker, T.C., 2002. Molecular and physiological approaches to maize improvement for drought tolerance. *Journal of Experimental Botany,* 53 (366), 13-25.

Ceccarelli, S., Grando, S., Bailey, E., et al., 2001. Farmer participation in barley breeding in Syria, Morocco and Tunisia. *Euphytica,* 122 (3), 521-536.

Chaves, M.M., Maroco, J.P. and Pereira, J.S., 2003. Understanding plant responses to drought: from genes to the whole plant. *Functional Plant Biology,* 30 (3), 239-264.

Condon, A.G., Richards, R.A., Rebetzke, G.J., et al., 2002. Improving intrinsic water-use efficiency and crop yield. *Crop Science,* 42 (1), 122-131.

Condon, A.G., Richards, R.A., Rebetzke, G.J., et al., 2004. Breeding for high water-use efficiency. *Journal of Experimental Botany,* 55 (407), 2447-2460.

Descriptions: *Triticum aestivum,* 2002. *Plant Varieties Journal,* 15 (2), 80-81.

Ellis, M.H., Rebetzke, G.J., Azanza, F., et al., 2005. Molecular mapping of gibberellin-responsive dwarfing genes in bread wheat. *Theoretical and Applied Genetics,* 111 (3), 423-430.

Fischer, R.A. and Turner, N.C., 1978. Plant productivity in the arid and semiarid zones. *Annual Review of Plant Physiology,* 29, 277-317.

Fischer, R.A. and Byerlee, D.R., 1991. Trends of wheat production in the warmer areas: major issues and economic considerations. *In:* Saunders, D.A. ed. *Wheat for the nontraditional warm areas: a proceedings of the international conference, July 29-August-3, 1990, Foz do Iguacu, Brazil.* CIMMYT, Mexico, 3-27.

Ghassemi, F., Jakeman, A.J. and Nix, H.A., 1995. *Salinisation of land and water resources: human causes, extent, management and case studies.* CAB International, Wallingford.

Gutiérrez-Rodríguez, M., Reynolds, M.P., Escalante-Estrada, J.A., et al., 2004. Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. *Australian Journal of Agricultural Research,* 55 (11), 1139-1147.

Havaux, M. and Tardy, F., 1999. Loss of chlorophyll with limited reduction of photosynthesis as an adaptive response of Syrian barley landraces to high-light and heat stress. *Australian Journal of Plant Physiology,* 26 (6), 569-578.

Hede, A.R., Skovmand, B. and López-Cesati, J., 2001. Acid soils and aluminum toxicity. *In:* Reynolds, M.P., Ortiz-Monasterio, J.I. and McNab, A. eds. *Application of physiology in wheat breeding.* CIMMYT, Mexico, 172-182. [http://www.cimmyt.org/research/wheat/map/research_results/wphysio/ WPhysio_Nutritional.pdf]

Keeling, P.L., Banisadr, R., Barone, L., et al., 1994. Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. *Australian Journal of Plant Physiology,* 21 (6), 807-827.

Lagudah, E.S., Moullet, O. and Appels, R., 1997. Map-based cloning of a gene sequence encoding a nucleotide-binding domain and a leucine-rich region at the Cre3 nematode resistance locus of wheat. *Genome,* 40 (5), 659-665.

Lillemo, M., Van Ginkel, M., Trethowan, R.M., et al., 2005. Differential adaptation of CIMMYT bread wheat to global high temperature environments. *Crop Science,* 45 (6), 2443-2453.

Loss, S.P. and Siddique, K.H.M., 1994. Morphological and physiological traits associated with wheat yield increases in mediterranean environments. *Advances in Agronomy,* 52, 229-276.

McIntyre, C.L., Chapman, S.C., Mathews, K.L., et al., 2006. Identification of germplasm and genomic regions for traits relevant to wheat production in drought environments. *In: Proceedings of the 13th Australasian Plant Breeding Conference: breeding for success: diversity in action, 18-21 April 2006, Christchurch, New Zealand.*

Mittler, R. and Zilinskas, B.A., 1994. Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *Plant Journal,* 5 (3), 397-405.

Mujeeb-Kazi, A., Rosas, V. and Roldan, S., 1996. Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s.lat. x *T. tauschii*; 2n = 6x = 42, AABBDD) and its potential utilization for wheat improvement. *Genetic Resources and Crop Evolution,* 43 (2), 129-134.

Niyogi, K.K., 1999. Photoprotection revisited: genetic and molecular approaches. *Annual Review of Plant Physiology and Plant Molecular Biology,* 50, 333-359.

Olivares-Villegas, J.J., Reynolds, M.P. and McDonald, G.K., in review. Drought resistance characteristics in a population of random inbred lines from Seri/Babax. *Functional Plant Biology.*

Osmond, C.B. and Grace, S.C., 1995. Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? *Journal of Experimental Botany,* 46, 1351-1362.

Rawson, H.M., 1986. High-temperature-tolerant wheat: a description of variation and a search for some limitations to productivity. *Field Crops Research,* 14 (3), 197-212.

Rebetzke, G.J., Richards, R.A., Fischer, V.M., et al., 1999. Breeding long coleoptile, reduced height wheats. *Euphytica,* 106 (2), 159-168.

Rebetzke, G.J., Condon, A.G., Richards, R.A., et al., 2002. Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. *Crop Science,* 42 (3), 739-745.

Reynolds, M.P. and Borlaug, N.E., 2006. Applying innovations and new technologies for international collaborative wheat improvement. *Journal of Agricultural Science,* 144 (2), 95-110.

Reynolds, M.P. and Condon, A.G., submitted. Quantifying potential genetic gains in wheat using a conceptual model of drought adapatation. *Euphytica.*

Reynolds, M.P., Balota, M., Delgado, M.I.B., et al., 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Australian Journal of Plant Physiology,* 21 (6), 717-730.

Reynolds, M.P., Singh, R.P., Ibrahim, A., et al., 1998. Evaluating physiological traits to complement empirical selection for wheat in warm environments. *Euphytica,* 100 (1/3), 85-94.

Reynolds, M., Skovmand, B., Trethowan, R., et al., 2000. Evaluating a conceptual model for drought tolerance. *In:* Ribaut, J.-M. and Poland, D. eds. *Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments: a strategic planning workshop held at CIMMYT, El Batan, Mexico, 21-25 June 1999.* CIMMYT, Mexico, 49-53.

Reynolds, M.P., Nagarajan, S., Razzaque, M.A., et al., 2001. Heat tolerance. *In:* Reynolds, M.P., Ortiz-Monasterio, J.I. and McNab, A. eds. *Application of physiology in wheat breeding.* CIMMYT, Mexico, 124-135. [http://www.cimmyt.org/research/wheat/map/research_results/wphysio/WPhysio_adaptation.pdf]

Reynolds, M.P., Mujeeb-Kazi, A. and Sawkins, M., 2005. Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought- and salinity-prone environments. *Annals of Applied Biology,* 146 (2), 239-259.

Reynolds, M.P., Rebetzke, G., Pellegrinesci, A., et al., 2006. Drought adaptation in wheat. In: Ribaut, J.-M. ed. *Drought adaptation in cereals.* Haworth's Food Products Press, New York, 401-446.

Reynolds, M.P., Dreccer, F. and Trethowan, R., accepted. Drought adaptive traits derived from wheat wild relatives and landraces. *Journal of Experimental Botany.*

Richards, R.A., Rebetzke, G.J., Condon, A.G., et al., 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. *Crop Science,* 42 (1), 111-121.

Snape, J.W., 2004. Challenges of integrating conventional breeding and biotechnology: a personal view! *In: Proceedings of the 4th international crop science congress, Brisbane, Australia, 26 September-1 October, 2004.* [http://www.cropscience.org.au/icsc2004/plenary/3/1394_snapejw.htm]

Trethowan, R.M. and Reynolds, M.P., 2006. Drought resistance: genetic approaches for improving productivity under stress. *In: Proceedings of the 7th International Wheat Conference, Mar del Plata, Argentina, November 27-December 2, 2005.*

Trethowan, R.M., Van Ginkel, M. and Mujeeb-Kazi, A., 2000. Performance of advanced bread wheat × synthetic hexaploid derivatives under reduced irrigation. *Annual Wheat Newsletter,* 46, 87-88. [http://wheat.pw.usda.gov/ggpages/awn/46/Textfiles/MEXICO.html]

Trethowan, R.M., Crossa, J., Van Ginkel, M., et al., 2001. Relationships among bread wheat international yield testing locations in dry areas. *Crop Science,* 41 (5), 1461-1469.

Trethowan, R.M., Borja, J. and Mujeeb-Kazi, A., 2003. The impact of synthetic wheat on breeding for stress tolerance at CIMMYT. *Annual Wheat Newsletter,* 49, 67-69. [http://wheat.pw.usda.gov/ggpages/awn/49/Textfiles/MEXICO.html]

Trethowan, R.M., Reynolds, M., Sayre, K., et al., 2005. Adapting wheat cultivars to resource conserving farming practices and human nutritional needs. *Annals of Applied Biology,* 146 (4), 405-413.

Vargas, M., Crossa, J., Sayre, K., et al., 1998. Interpreting genotype by environment interaction in wheat by partial least squares regression. *Crop Science,* 38 (3), 679-689.

Villareal, R.L., Fuentes-Dávila, G. and Mujeeb-Kazi, A., 1995. Synthetic hexaploids x *Triticum aestivum* advanced derivatives resistant to Karnal bunt (*Tilletia indica* Mitra). *Cereal Research Communications,* 23 (1/2), 127-132.

Villareal, R.L., Bañuelos, O.T., Rajaram, S., et al., 2003a. Backcross-derived, synthetic bread wheats under drought stress. *Annual Wheat Newsletter,* 49, 65-66. [http://wheat.pw.usda.gov/ggpages/awn/49/Textfiles/MEXICO.html]

Villareal, R.L., Bañuelos, O.T., Rajaram, S., et al., 2003b. Elite, synthetic bread wheats (*Triticum turgidum/Aegilops tauschii*) under one irrigation. *Annual Wheat Newsletter,* 49, 66. [http://wheat.pw.usda.gov/ggpages/awn/49/Textfiles/MEXICO.html]

Wang, W., Vinocur, B. and Altman, A., 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta,* 218 (1), 1-14.

Xiong, L., Schumaker, K.S. and Zhu, J.K., 2002. Cell signaling during cold, drought, and salt stress. *Plant Cell,* 14 (Suppl.), s165-s183.