Drought-adaptive traits derived from wheat wild relatives and landraces

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Abstract

Exotic parents are being used to increase allelic diversity in bread wheat breeding through (i) interspecific hybridization of the ancestral genomes to produce so-called synthetic derived (SYN-DER) wheat, and (ii) crossing with landrace accessions, originating in abiotically stressed environments, that have become isolated from mainstream gene pools. Evaluation of the inherent genetic diversity encompassed by drought-adapted landraces compared with checks using DNA fingerprinting confirmed that some landraces were not only distant from checks but also showed significant diversity among each other. Improvement in performance of SYN-DER lines compared with recurrent parents was not associated with a larger overall investment in root dry weight, but rather an increased partitioning of root mass to deeper soil profiles (between 60 cm and 120 cm) and increased ability to extract moisture from those depths. The best Mexican landraces showed superior ability in terms of water extraction from soil depth, as well as increased concentration of soluble carbohydrates in the stem shortly after anthesis. Although it can be argued that inferring theoretical yield gains from the over-expression of any of these traits is questionable, since compensatory mechanisms may be at work, the fact remains that mechanistic or genetic linkages among physiological traits remain largely un-established. In the meantime, trait information is being used to make strategic crosses based on the theoretical combination of useful stress-adaptive traits with the possibility of realizing additive gene action in selected progeny. Candidates for crossing with elite check cultivars include landraces identified that showed relatively high biomass under drought combined with favourable expression of physiological traits such as stem carbohydrates, water extraction characteristics, and transpiration efficiency.

Key words: Candidate-trait, genetic diversity, genetic improvement, interspecific hybridization, root:shoot ratio, synthetic-derived wheat, water-use-efficiency.

Introduction

Genetic improvement of crops, whether approached empirically or strategically, depends on different gene expression that will result in more optimal growth and development in a given environment. For example, crosses among conventional lines can achieve new levels of trait expression as a result of transgressive segregation of alleles. On the other hand, exotic parents can be used to increase allelic diversity. The bread wheat breeding programme of The International Maize and Wheat Improvement Centre (CIMMYT) is exploiting new genetic diversity from inter-specific hybridization of the ancestral genomes of bread wheat, more commonly known as synthetic or resynthesized hexaploid wheat (Mujeeb-Kazi et al., 1996). Specifically, these derive from crossing tetraploid durum wheat (AB genome) with the diploid wild species T. tauschii (D genome). Crosses between elite wheat cultivars and synthetic wheat have

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resulted in lines with improved drought adaptation (Trethowan et al., 2005).

A more direct way to exploit novel allelic diversity is to cross elite material with genetic resources of the same genome, for example, landrace accessions originating in abiotically stressed environments that have become isolated from mainstream gene pools. The assumption is that such accessions may provide novel alleles that will complement existing stress-adaptive mechanisms. Since landraces collections frequently exist in the thousands, in order to identify useful physiological traits it is usually necessary to subject them to a pre-screening under the appropriate stress to identify the most promising genotypes. Again, while landraces have been used for some time in breeding barley for adaptation to abiotic stresses (Ceccarelli, 1984), their use in bread wheat breeding is less common and the physiological basis of drought adaptation of cereal landraces has not been extensively studied.

This study describes some of the physiological mechanisms associated with novel genetic resources expressing superior drought adaptation. Firstly, two synthetic derived lines (SYN-DER) and their recurrent parents (REC-PAR) are examined under well-watered and post-anthesis drought scenarios. The second part of the study briefly describes how approximately 2000 Mexican landraces were screened for yield under drought, permitting a handful to be selected for more detailed physiological measurements along with other genetic resources. A DNA fingerprinting study of the selected landraces was also conducted to give an indication of their genetic distance amongst each other and from standard check cultivars. Another objective in the second part of the study was to compare the relative expression of drought-adaptive traits of landraces and other selected genetic resources (GEN-RES) with an elite drought-adapted check cultivar to gain insight into which traits and genotypes may hold promise in terms of genetic enhancement.

Materials and methods

Growing conditions
All experiments were grown in NW Mexico at the CIMMYT experiment station (27°20’ N; 109°54’ W; and 38 m asl). The soil is a heavy clay loam and is described in detail, along with other aspects of environment and the management practices, elsewhere (Sayre et al., 1997). All experiments were sown on raised beds spaced at 80 cm intervals. Yield plots consisted of two beds 5 m long with three rows sown on top of each bed 12 cm apart. Seed rate was 12 g m⁻². Hill plots were sown using 10 seeds clumped at 30 cm intervals on top of the bed. The plots were protected from weeds, pests, and diseases using agrochemical applications as necessary and were well managed in terms of N and P fertilization, although irrigated trials were fertilized with sufficient N to achieve approximately 80–90% of yield potential to avoid the confounding effects on yield of lodging. Trials emerged in early December and reached physiological maturity approximately 110 d later for moisture-stress treatments and 120 d later for irrigated treatments (averaging across genotypes). In the first part of this study, comparing SYN-DER and REC-PAR, experiments were conducted during the 2004, 2005, and 2006 cycles (i.e. sown in December 2003 and 2004 and 2005, respectively, and harvested in April of the following year) under well-watered and moisture-stressed conditions. Total moisture available under stressed conditions was 360 mm in 2004 and 260 mm in 2005, and 190 mm in 2006, while well-watered plots used an estimated 600 mm of water. In the second part of the study, the 2077 landraces were grown as hill plots in 2000 with approximately 175 mm of available water available during the cycle. Of the lines yielding 200 g m⁻² or more, the highest yielding 50 lines were sown in the 2001 spring cycle as small (2 m²) replicated yield plots also with approximately 175 mm of water available during the cycle. Yield plots for the 12 lines comprising the experiment GEN-RES were grown in 2002, 2004, and 2005 cycles with 180 mm, 360 mm, and 250 mm water available, respectively. A well-irrigated (>600 mm available water) yield trial was grown in 2002 only. In all cases, 175 mm water was available in the soil (down to 120 cm) after the seeding irrigation and the rest of the moisture came from intermittent rainfall events during tillering, therefore, for all moisture-stress treatments drought stress intensification from the boot stage onwards (Trethowan et al., 2001). Average monthly minimum/maximum daily temperatures in this environment for the period 2000–2005 were 7.3/26.0, 7.6/25.5, 8.5/25.2, 8.9/27.8, and 11.4/31.5 °C for December, January, February, March, and April, respectively and average daily radiation levels were 14.3, 15.2, 18.1, 23.7, and 25.8 MJ m⁻² d⁻¹, respectively, for the same months. Experiments were set up as randomized alpha-lattice designs and adjusted means calculated using analysis of variance procedures (Barreto et al., 1997).

Germplasm
In total, four lines deriving from synthetic hexaploid wheat were used in this study. Two lines, d67.2/p66.270/Ae.squarrosa (320)/3/Cunningham and Croc_1/Ae.squarrosa (210)/2*Excalibur, were compared directly with their recurrent parents (Cunningham and Excalibur) in the first part of this study. Two others, Croc_1/Ae. squarrosa (224)//Opata and Sokoll were included in the second part of the study comparing different genetic resources. Synthetic derived lines were developed using standard breeding procedures at CIMMYT (Trethowan et al., 2005). In summary, primary hexaploid synthetics were either crossed once or twice to adapted hexaploid bread wheat. These materials were then selected under alternating cycles of drought stress and non-moisture stress during the segregating phase. With the exception of Croc_1/Ae.squarrosa (224)//Opata, the F₂ generation was planted at three times the normal sowing density to exert selection pressure on plant emergence and crop establishment characteristics. The populations were advanced to F₆ using the selected bulk strategy (Singh et al., 1998) and then head-rowed to produce the F₇. The resultant lines were yield tested firstly under irrigated conditions and then under drought. Plots were inoculated with rust at all generations. The recurrent parents, Cunningham and Excalibur, are cultivars released in Australia and recommended for planting in the drier, rainfed areas. Both cultivars have export grade industrial quality. Cunningham is planted in north-eastern Australia whereas Excalibur is adapted to southern Australia. Evidence for their adaptation to the experimental environment in Mexico comes from their comparison with other CIMMYT standards. For example, in the 2006 cycle, growing under rainfed conditions, their yield was not significantly different from that of the drought-adapted check line Pastor. Under irrigated conditions during the same cycle the yield of Cunningham was indistinguishable from Pastor, while Excalibur showed 15% less yield, i.e. within an acceptable range for adapted material.

The 2077 Mexican landraces screened for adaptation to drought stress were collected as described by Skovmand et al. (2002). A subset of eight of these (selected for performance under drought)
were used in DNA fingerprinting as well as for detailed physiological characterization under field-stressed drought conditions. Besides the selected landraces, the physiological characterization was conducted on another genetic resource line, namely MES, which had previously shown drought adaptation, two synthetic derived lines (described above) and a drought-tolerant check cultivar, Woebil 1. The line MES was developed in the 1980s by Ricardo Rodriguez as part of a prebreeding programme to develop lines with enhanced expression of yield components. It is fairly short and shows a very high expression for spike density based on a novel genetic combination of germplasm including one or more of the following sources: *Agrotriticum* (Canada), *Polonicum* (Poland), *Morocco* (Morocco), and semidwarf (*Mexico*) wheats (Rajaram and Reynolds, 2001). The check cultivars, Woebil 1 and W15 91 (the latter was only included in the DNA fingerprinting assay), represent the spectrum in adaptation to drought stress in the Yaqui Valley in northwestern Mexico. Woebil 1 is a non-synthetic wheat with good adaptation to the range of drought stresses generated by CIMMYT in northern Mexico. W15 91 is a cultivar released in Zimbabwe with very high yield potential under well-watered conditions and not well adapted to drought stress in northwestern Mexico.

**Physiological and agronomic measurements**

Water use was determined by measuring gravimetric water content at the following profile depths: 0–30 cm, 30–60 cm, 60–90 cm, and 90–120 cm. This was determined on six random locations per experiment at crop emergence, and on two spots of every plot after harvest. When calculating available water it was assumed that 25% of the available water was lost as surface evaporation. Values of permanent wilting point of 20% and a soil bulk density of 1.3 were assumed, based on previous samplings at the same locality (K. Sayre, personal communication). Water use efficiency was calculated using total water extracted for each genotype and final biomass.

Water-soluble stem carbohydrates were estimated on the whole stem of six randomly selected spike-bearing culms 7 d after anthesis and at physiological maturity using the methodology described elsewhere (Yemm and Willis, 1954).

Leaf carbon isotope discrimination (Δ13C) values were measured on leaves (collected approximately 40 d after emergence during tillering stage) on irrigated plots to maximize potential expression of genotypic variation in transpiration efficiency and the methodology is described elsewhere (Condon et al., 2004).

From spectral reflectance measurements taken at the end of tillering (Zadoks 30), the normalized difference vegetation index (NDVI) was calculated as an indicator of biomass differences between treatments (Babar et al., 2006). Canopy temperature was measured with a portable infrared thermometer (Mikron M90 Series, Mikron Infrared Instrument Co., Inc., Oakland, NJ, USA). Two measurements per plot were taken standing close to the two corners of the plot (with the sun behind and avoiding shadows). Data are the means of six measurements taken during the grain-filling stage (Zadoks 70–85) on cloudless days with low wind between 11.00 h and 15.00 h.

Osmotic adjustment was measured in both field and controlled conditions. Three flag leaves were sampled per plot at first light from irrigated and drought-stressed treatments approximately 15 d after anthesis. One centimetre sections from the middle of the leaves were excised with scissors and immediately placed in a tube containing distilled water. Tissue was left to hydrate fully for 3 h at 5 °C before being quickly dried of all surface moisture and placed in a 2 ml propylene tube, sealed with the lid and placed in a deep-freeze to rupture the cells. After this, a drop of cell sap was extracted using a glass rod and placed on the sampling cuvette of a vapour pressure osmometer (Wescor model 5500, Logan, Utah, USA). Values obtained from the leaf sap (in mmol kg−1) were then expressed in Mpa by dividing by the conversion factor 0.02525.

Osmotic adjustment was also measured in controlled conditions where genotypes were grown together in pots in greenhouses. To ensure good control over soil water potential, replicated lattice designs were used where all genotypes from an incomplete block shared a single pot, with three replications. Osmotic potential was measured twice during boot stage, as described above, at average soil water potentials of −0.6 MPa for irrigated treatments and −1.7 MPa for drought treatments.

Root sampling took place between 7 d and 12 d after anthesis. Soil cores of 42 mm width and 120 cm length were extracted (using a hydraulic soil corer, Giddings Corp, Co, USA) from two locations in each plot, one between the beds and one between one of the paired rows on top of the bed. The cores were separated into four profiles, 0–30, 30–60, 60–90, and 90–120 cm and bulked according to profile. The cores were washed carefully in water to separate root tissue from soil and other debris. Root tissue was dried at 75 °C for 24 h and weighed.

Yield and yield components were calculated using standard procedures (Sayre et al., 1997).

**DNA fingerprinting and marker assays**

Genomic DNA was extracted from bulked leaves of young plants according to Saghai-Maroof et al. (1984) and modified according to CIMMYT protocols (http://www.cimmyt.cgiar.org/ABC/Protocols/ manualABC.html). Quality and quantity of the extracted DNA was determined on 1% agarose gels by visually comparing extracted DNA bands to a known concentration of a standard lambda DNA cut with HindIII. Fifty SSR markers were amplified in this study. However, six did not amplify at all and 10 were associated with double bands or a high error rate in general and were removed. The remaining 34 markers represented a total of 169 alleles. PCR reactions for SSR analysis were performed according to Dreisigacker et al. (2004). Briefly, reactions containing a final volume of 20 μl were amplified in a 96-well Peltier thermal cycler (MJ Research, Inc., Watertown, MA) following a standard temperature profile: 29 cycles consisting of 1 min denaturation at 94 °C, 2 min annealing using temperatures between 50 °C and 64 °C (depending on primer combination), and 2 min extension at 72 °C. Primers were labelled with 6-FAM, HEX, or TET fluorescent dyes. PCR products were amplified separately and run on an ABI Prism 377 DNA Sequencer (Perkin Elmer/Applied Biosystems). When possible, multi-loading was applied to increase efficiency, with two to six loci with non-overlapping allele sizes loaded together in one well of the gel. Two control genotypes were loaded on every gel. Fragments were sized using Genescan 3.1 and assigned to allele categories using the software package Genotyper 2.1 (Perkin Elmer/Applied Biosystems Biotechnologies, Foster City, USA).

Frequency of the major allele within the group, number of alleles, gene diversity, heterozygosity, and Polymorphic Information Content (PIC) values were calculated per locus. Genetic distances between cultivars were calculated using the modified Rogers’ distance (MRD; Wright, 1978): 

$$MRD = \sqrt{\frac{1}{2m} \sum_{j=1}^{m} \sum_{l=1}^{m} (p_{ij} - q_{lj})^2}$$

where $p_{ij}$ and $q_{lj}$ are the frequencies of the $i$th allele at the $i$th locus in the two taxonomic units under consideration, $q$ is the number of alleles at the $i$th locus, and $m$ refers to the number of loci. The unweighted pair group method with arithmetic averages (UPGMA) cluster analysis (Sneath and Sokal, 1973) was used to reveal associations and to produce the dendrogram. The clusters formed by the cluster analysis were tested to determine if the genetic distance between the clusters was statistically significant. An analysis of variance was performed on the molecular marker distance data,
assuming that all pairs of distances between the individuals followed a normal distribution. The least significant difference in genetic distances was computed and compared to the distance between clusters, and an F test performed to test if the variances within each cluster were the same, or different.

Results

Synthetic derived wheat

The interaction of genotypes with the three growth cycles (years) was not statistically significant for agronomic traits. The effects of SYN-DER germplasm and drought treatment (DRT), averaged across both genetic backgrounds, for above- and below-ground growth parameters are summarized in Table 1. There was no significant interaction of SYN-DER with DRT for yield or biomass. While DRT reduced overall yields and biomass by 58% and 35% respectively, the main effect of SYN-DER was to increase yield and biomass by approximately 18% and 37%, respectively, in both environments compared with REC-PAR. For harvest index and kernel weight there was no interaction and SYN-DER was associated with a reduction in harvest index of 14% and an increase in kernel weight of 18% averaged across environments (Table 1), neither of which are unexpected for synthetic derived germplasm. However, there were clear interactions for root growth parameters that were measured in 2004 and 2005 cycles. While total root biomass increased under DRT the effect was larger for REC-PAR (52%) than SYN-DER lines (21%) and this was reflected in root:shoot ratios (Table 1). However, when considering only the roots in the top 30 cm of the soil, the SYN-DER lines showed a small decrease in root mass of 17% in response to DRT while REC-PAR showed a sizeable increase of 85% (Table 1).

The SYN-DER derived material showed a greater capacity for water uptake especially at intermediate root depths (Fig. 1). By the end of the cycle, REC-PAR had approximately 50 mm of water remaining in the soil profile (down to 120 cm) while SYN derived lines had less than half that amount, representing an increase in total water absorption of 11%. The increased capacity of SYN-DER lines to take up water was noticeable at anthesis as well as after physiological maturity (Fig. 1). On average (data for 2004 and 2005 cycles) SYN-DER lines used 210 mm of water throughout the cycle down to 120 cm while REC-PAR used 185 mm; using total biomass data (above-ground+root biomass), water-use efficiency values were calculated as 6.8 g m⁻² mm⁻¹ and 5.2 g m⁻² mm⁻¹ for SYN-DER and REC-PAR, respectively.

Carbon isotope discrimination data of leaf tissue showed values of 20.0% for SYN-DER lines and 19.0% for REC-PAR. These differences are highly significant, however, they suggest that the SYN-DER had a slightly lower transpiration efficiency than REC-PAR (Condon et al., 2004), and therefore do not explain the apparent increase in WUE of SYN-DER.

Landrace selection and genetic diversity

Based on preliminary screening of the 2077 landraces for yield under drought (Fig. 2) the top 50 yielding lines were yield tested under drought in 2001 and showed an average yield of 280 g m⁻². The top eight lines yielded 315 to 415 g m⁻² and were therefore similar to the drought-adapted check Baviacora with a yield of 400 g m⁻² (LSD=95 g m⁻²); these were included in the experiment GEN-RES (Table 2). Seven of the eight landraces were subjected to DNA fingerprinting along with drought-adapted check lines, Weebil-1 and Sokoll also from the experiment GEN-RES, and an additional drought-susceptible but high yield potential line W15.91. The DNA fingerprinting of the landraces showed a range of genetic diversity among the lines selected. The dendrogram generated from similarity coefficients indicated two main groups (Fig. 3) that were tested for statistical significance and confirmed at the 0.001 level of probability. One group was relatively homogeneous consisting of three landraces (2 from the state of Mexico and one from the neighbouring state of Puebla), and a more heterogenous group including the landraces from Mexico and Oaxaca as well as the check lines. The closer association of the synthetic derivative, Sokoll, with the adapted wheats W15 91 and Weebil 1 to a large extent

<table>
<thead>
<tr>
<th>Moisture level</th>
<th>Genotype</th>
<th>Grain yield (g m⁻²)</th>
<th>Biomass above-ground (g m⁻²)</th>
<th>Harvest index</th>
<th>Kernel weight (g)</th>
<th>DW roots⁡0–30 cm (g m⁻²)</th>
<th>DW roots⁡0–120 cm (g m⁻²)</th>
<th>Root:shoot ratio⁡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigated</td>
<td>SYN-DER</td>
<td>579</td>
<td>1400</td>
<td>0.416</td>
<td>39.5</td>
<td>52.6</td>
<td>96.5</td>
<td>0.071</td>
</tr>
<tr>
<td>Irrigated</td>
<td>REC-PAR</td>
<td>503</td>
<td>1080</td>
<td>0.464</td>
<td>33.8</td>
<td>40.7</td>
<td>85.0</td>
<td>0.082</td>
</tr>
<tr>
<td>Drought</td>
<td>SYN-DER</td>
<td>267</td>
<td>970</td>
<td>0.255</td>
<td>28.2</td>
<td>43.6</td>
<td>117.2</td>
<td>0.092</td>
</tr>
<tr>
<td>Drought</td>
<td>REC-PAR</td>
<td>225</td>
<td>645</td>
<td>0.318</td>
<td>23.2</td>
<td>75.1</td>
<td>128.9</td>
<td>0.171</td>
</tr>
<tr>
<td>P (SYN v REC)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P (moisture)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P (interaction)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>31.8</td>
<td>117</td>
<td>0.024</td>
<td>1.47</td>
<td>11.9</td>
<td>16.4</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

* Based on data for 2004 and 2005 cycles only.
reflects selection for genomic regions present in the adapted parents used to produce Sokoll. These regions likely confer improved agronomic type and quality, whereas the drought tolerance from the original primary synthetic is probably linked to a relatively smaller area of the genome.

**Genetic resources**

The interaction of genotypes with the three drought environments (years) was statistically significant. However, trait expression across years was generally highly correlated among genotypes indicating that there was relatively little crossover interaction. Therefore, growth (Table 2) and water relations parameters (Table 3) are presented as main effects across the three cycles. Evaluation of performance under well-watered conditions was not a prime objective in this experiment, most of the material not being adapted for high yield environments. Nonetheless, irrigated yields are presented in Table 2 as a point of reference. The highest yielding line was the check variety Weebil-1 while the landraces showed a considerable range. The effect of drought on this material was to reduce yield by over 50% on average, with the exception of one landrace with very low yield potential. The check was among the highest yielding lines under drought along with the synthetic derived line Sokoll, and the high spike density line MES, which all yielded just over 300 g m$^{-2}$. The lines that were high yielding under drought, Sokoll, and MES both showed significantly higher biomass than the check by 17% and 25% respectively, indicative of superior drought adaptive mechanisms. Kernel weights and harvest indices were within fairly normal ranges for these conditions while some of the landraces were up to 10 d later in maturity than the check (not shown). Landraces also tended to be taller than the other materials, although differences were no more than 10–20 cm under moisture stress.

**Traits relating to water uptake and water use efficiency (WUE)** also showed significant variation (Table 3). Landraces generally showed better water uptake characteristics than the check at soil depths below 60 cm, but not between 30–60 cm. On the other hand WUE was largest for the two lines showing the highest biomass while most landraces showed values of WUE lower than the check. Canopy temperature measured in the driest of the three cycles (2002) showed a very good relationship with water uptake between 30–120 cm (Fig. 4). Osmotic adjustment (OA) measured in the field and in controlled conditions showed significant variation between genotypes, but not with each other, presumably because of the very different ways in which they were measured, field measurements being confounded by differences in the ability of genotypes to extract water from the soil profile; OA measured

![Figure 1](https://academic.oup.com/jxb/article-abstract/58/2/177/537881)

**Fig. 1.** Average difference between two synthetic derived (SYN) wheat lines and their recurrent parents (REC) for residual moisture in different soil profiles, shortly after anthesis (ant) and after physiological maturity (mat), averaged across 2004 and 2005 spring wheat cycles. (Standard error of differences between means within depth profiles increased sequentially with depth as follows: 0.30, 0.61, 0.79, 2.1 mm.)

![Figure 2](https://academic.oup.com/jxb/article-abstract/58/2/177/537881)

**Fig. 2.** Distribution within yield categories for 2077 Mexican landraces grown as hill plots with 150 mm of available water (applied at sowing), NW Mexico, 2000.
in the field was positively associated with residual soil water (Table 2) supporting this conclusion (Table 3). Values of OA measured in controlled conditions, however, showed no association with performance, WUE or water uptake parameters. Carbon isotope discrimination was associated negatively with residual water as might be expected when the regulation of stomatal conductance is the main source of changes in transpiration efficiency (Condon et al., 2002). The CID trait showed weak negative association with WUE \( (r = -0.45) \) and final biomass \( (r = -0.42) \).

### Discussion

#### Synthetic derived wheat

The use of inter-specific hybridization to widen the hexaploid wheat gene pool is a revolutionary step in terms of overcoming the genetic bottle-neck that occurred when the genomes (AB+D) fist combined, at least 10 000 years ago (Mujeeb-Kazi et al., 1996). While the diploid D genome has been exploited for disease resistance genes for some time (Villareal et al., 1995), it is only more recently that the potential for increasing drought adaptation has been realized (Trethowan et al., 2003, 2005), although there is already evidence for impact in drier regions worldwide based on data from recent international drought trials (Trethowan and Reynolds, 2007). The data presented in this study indicate a response of SYN-DER to moisture stress in terms of changes in partitioning of assimilates to roots and an increased ability to take up water from a greater depth.

Increase in root:shoot ratio and absolute root mass in response to moisture stress have been shown previously in wheat (Blum et al., 1983) and were observed in this study (Table 1). Perhaps counter intuitively, despite superior yield and biomass SYN-DER displayed a relatively modest increase in total root mass and root:shoot ratio.

### Table 2. Yield components, early NDVI indices and stem carbohydrate values for wheat genetic resources selected for drought adaptation, average values from three growth cycles under moisture stress, (except for yield potential) NW Mexico

<table>
<thead>
<tr>
<th>Cross</th>
<th>Yield potential ( (\text{g m}^{-2}) )</th>
<th>Yield ( (\text{g m}^{-2}) )</th>
<th>Biomass above-ground ( (\text{g m}^{-2}) )</th>
<th>Kernel weight (g)</th>
<th>NDVI at zadoks 30 (relative units)</th>
<th>Stem CHO at anthesis+7 d (%)</th>
<th>Stem CHO maturity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEEDIL1 (Check)</td>
<td>793</td>
<td>309</td>
<td>757</td>
<td>38.6</td>
<td>0.707</td>
<td>19.1</td>
<td>2.2</td>
</tr>
<tr>
<td>MES (genetic resource)</td>
<td>752</td>
<td>303</td>
<td>945</td>
<td>25.9</td>
<td>0.754</td>
<td>4.9</td>
<td>1.6</td>
</tr>
<tr>
<td>SOKOLL synthetic derived (SYN)</td>
<td>715</td>
<td>316</td>
<td>884</td>
<td>39.3</td>
<td>0.757</td>
<td>15.9</td>
<td>1.8</td>
</tr>
<tr>
<td>MEX94.2.19 (landrace)</td>
<td>658</td>
<td>248</td>
<td>656</td>
<td>32.6</td>
<td>0.778</td>
<td>15.7</td>
<td>2.4</td>
</tr>
<tr>
<td>CROC_1/ AE.SQUARROSA (224)/OPATA (SYN)</td>
<td>647</td>
<td>227</td>
<td>609</td>
<td>34.4</td>
<td>0.731</td>
<td>18.9</td>
<td>2.0</td>
</tr>
<tr>
<td>MEX94.15.34 (landrace)</td>
<td>584</td>
<td>164</td>
<td>378</td>
<td>37.4</td>
<td>0.798</td>
<td>19.9</td>
<td>2.3</td>
</tr>
<tr>
<td>MEX94.2.18 (landrace)</td>
<td>563</td>
<td>202</td>
<td>640</td>
<td>38.0</td>
<td>0.768</td>
<td>15.2</td>
<td>1.8</td>
</tr>
<tr>
<td>MEX94.12.2.39 (landrace)</td>
<td>516</td>
<td>218</td>
<td>519</td>
<td>38.8</td>
<td>0.749</td>
<td>24.1</td>
<td>3.1</td>
</tr>
<tr>
<td>PUB94.16.24 (landrace)</td>
<td>486</td>
<td>212</td>
<td>614</td>
<td>39.7</td>
<td>0.790</td>
<td>23.1</td>
<td>4.1</td>
</tr>
<tr>
<td>PUB94.15.1.12. (landrace)</td>
<td>427</td>
<td>224</td>
<td>569</td>
<td>41.0</td>
<td>0.756</td>
<td>25.8</td>
<td>3.6</td>
</tr>
<tr>
<td>MEX94.27.1.20 (landrace)</td>
<td>418</td>
<td>213</td>
<td>477</td>
<td>39.3</td>
<td>0.698</td>
<td>26.1</td>
<td>3.6</td>
</tr>
<tr>
<td>OAX93.24.35 (landrace)</td>
<td>200</td>
<td>182</td>
<td>501</td>
<td>34.2</td>
<td>0.769</td>
<td>20.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Mean</td>
<td>563</td>
<td>235</td>
<td>629</td>
<td>36.6</td>
<td>0.755</td>
<td>19.1</td>
<td>2.55</td>
</tr>
<tr>
<td>LSD(5%)</td>
<td>51.8</td>
<td>38.0</td>
<td>133</td>
<td>2.34</td>
<td>0.072</td>
<td>7.36</td>
<td>1.91</td>
</tr>
</tbody>
</table>

\( ^a \) Yield potential based on a single cycle with irrigation.
### Table 3. Water use efficiency, water uptake parameters, for wheat genetic resources selected for drought adaptation, average values from three growth cycles under moisture stress, NW Mexico, osmotic adjustment also estimated in controlled environment

<table>
<thead>
<tr>
<th>Cross</th>
<th>Water use efficiency (g m⁻² mm⁻¹)</th>
<th>Residual H₂O 30–120 cm (mm)</th>
<th>Residual H₂O 30–60 cm (mm)</th>
<th>Residual H₂O 60–90 cm (mm)</th>
<th>Residual H₂O 90–120 cm (mm)</th>
<th>Δ¹³C (%o)</th>
<th>Osmotic Adj field (MPa)</th>
<th>Osmotic Adj controlled (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEEBILL1 (Check)</td>
<td>4.25</td>
<td>29.2</td>
<td>5.2</td>
<td>10.7</td>
<td>13.4</td>
<td>22.10</td>
<td>-0.462</td>
<td>-0.284</td>
</tr>
<tr>
<td>MES (genetic resource)</td>
<td>5.74</td>
<td>47.6</td>
<td>18.0</td>
<td>15.1</td>
<td>14.6</td>
<td>22.15</td>
<td>-0.238</td>
<td>-0.166</td>
</tr>
<tr>
<td>SOKOLL synthetic</td>
<td>4.87</td>
<td>25.1</td>
<td>6.7</td>
<td>7.5</td>
<td>10.9</td>
<td>22.26</td>
<td>-0.553</td>
<td>-0.134</td>
</tr>
<tr>
<td>MEX94.2.19 (landrace)</td>
<td>3.68</td>
<td>29.0</td>
<td>6.5</td>
<td>10.8</td>
<td>11.8</td>
<td>21.94</td>
<td>-0.491</td>
<td>-0.029</td>
</tr>
<tr>
<td>CROC_1/Opata (SYN)</td>
<td>3.47</td>
<td>33.3</td>
<td>9.2</td>
<td>11.0</td>
<td>13.1</td>
<td>22.36</td>
<td>-0.556</td>
<td>-0.189</td>
</tr>
<tr>
<td>MEX94.15.34 (landrace)</td>
<td>2.24</td>
<td>42.2</td>
<td>10.9</td>
<td>15.7</td>
<td>15.6</td>
<td>22.05</td>
<td>-0.470</td>
<td>-0.089</td>
</tr>
<tr>
<td>MEX94.2.18 (landrace)</td>
<td>3.63</td>
<td>32.3</td>
<td>6.4</td>
<td>11.5</td>
<td>14.4</td>
<td>22.10</td>
<td>-0.424</td>
<td>-0.083</td>
</tr>
<tr>
<td>MEX94.12.2.39 (landrace)</td>
<td>2.84</td>
<td>23.6</td>
<td>7.4</td>
<td>6.7</td>
<td>9.5</td>
<td>23.00</td>
<td>-0.525</td>
<td>-0.192</td>
</tr>
<tr>
<td>PUB94.16.24 (landrace)</td>
<td>3.39</td>
<td>25.7</td>
<td>9.2</td>
<td>7.8</td>
<td>8.7</td>
<td>23.10</td>
<td>-0.479</td>
<td>-0.183</td>
</tr>
<tr>
<td>PUB94.15.1.12.i (landrace)</td>
<td>3.13</td>
<td>24.2</td>
<td>8.7</td>
<td>7.9</td>
<td>7.7</td>
<td>22.79</td>
<td>-0.451</td>
<td>-0.192</td>
</tr>
<tr>
<td>MEX94.27.1.20 (landrace)</td>
<td>2.60</td>
<td>22.6</td>
<td>4.6</td>
<td>7.0</td>
<td>11.0</td>
<td>23.21</td>
<td>-0.422</td>
<td>-0.055</td>
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<tr>
<td>OA93.24.35 (landrace)</td>
<td>2.80</td>
<td>28.0</td>
<td>8.9</td>
<td>8.3</td>
<td>10.7</td>
<td>22.68</td>
<td>-0.514</td>
<td>-0.048</td>
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<tr>
<td>Mean</td>
<td>3.15</td>
<td>30.2</td>
<td>8.5</td>
<td>10.0</td>
<td>11.8</td>
<td>22.48</td>
<td>-0.5</td>
<td>-0.15</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>0.67</td>
<td>6.7</td>
<td>2.78</td>
<td>2.87</td>
<td>2.61</td>
<td>0.384</td>
<td>-0.236</td>
<td>-0.196</td>
</tr>
</tbody>
</table>

Fig. 4. Association between canopy temperature measured at high vapour pressure deficit in the field during grain-filling and residual soil moisture measured on genotypes after physiological maturity, NW Mexico, 2002. (NB. The check Weebil can be identified as showing the largest value for canopy temperature.)

(21% and 29%, respectively) compared with REC-PAR (52% and 110%, respectively) in response to drought (Table 1). However, SYN-DER showed a marked change in root distribution under drought with 63% of their root biomass between 30–120 cm compared with 42% for REC-PAR, and approximately 50% for either under irrigated conditions (Table 1). This would appear to be a significant drought adaptation which probably explains their ability to extract more water from deeper in the soil profile (Fig. 1) and more water in total (26 mm) compared with REC-PAR. Large investment of roots in the top 30 cm of soil makes biological sense for well-watered conditions, but not with a receding moisture profile. Nonetheless, neither the increased water extraction nor the small reduction in total root mass (8 g m⁻²) were of sufficient magnitude to explain the increase in biomass of SYN-DER relative to REC-PAR. Using the SYN-DER value for WUE of 6.8 g m⁻² mm⁻¹, an extra 26 mm of water would account for 178 g m⁻² of additional biomass. Possible mechanisms that may explain an apparently larger WUE are increased transpiration efficiency associated with intrinsically low stomatal conductance and, therefore, a reduction in discrimination against fixation of the heavier isotope of carbon (Condon et al., 2002, 2004; Rebetzke et al., 2002), and decreased losses of soil water early in crop establishment due improved early ground cover (Richards et al., 2002). Although ground cover was not measured in this trial SYN-DER lines have been reported to display considerable early vigour and increased ground cover (Trethewan et al., 2005). Carbon isotope discrimination was measured, but did not account for differences between SYN-DER and REC-PAR.

### Genetic diversity of landraces

The origin of the wheat lines introduced by Spanish colonists to Mexico is unknown and their potential value as a genetic resource has gone largely unrecognized, although records of introduction date back to the early 16th Century (Skovmand et al., 2002). Given the dry climate in Mexico and the genetically diverse nature of landrace populations, there is good reason to believe that natural and human selection would have favoured drought-adaptive mechanisms. The screening of over 2000 Mexican landraces for yield under drought suggested considerable phenotypic diversity (Fig. 2) and DNA fingerprinting confirmed genetic difference between landraces and checks as well as among landraces themselves (Fig. 3). Given the generally acceptable agronomic and favourable drought-adaptive
characteristics of the selected lines, this is a positive outcome, suggesting that such approaches can be employed to broaden the genetic base of modern wheat in terms of abiotic stress adaptation.

Specific drought-adaptive characteristics of the selected landraces were determined in these experiments (Tables 2, 3) and included the ability to extract water from the deepest part of the soil profile. The best Mexican landrace (Pub94.15.1.12) in terms of water extraction from depth, extracted 8.5 mm more water between 60–120 cm than the check line, which at a WUE of the check cultivar (4.25 g m⁻² mm⁻¹) is equivalent to an extra 36 g m⁻² of biomass (a 15% increase over the mean trial yield). A number of the selected landraces also showed high values of soluble stem carbohydrates up to 26% of stem dry mass shortly after anthesis compared with 19% for the check. Other landraces showed increased early ground cover, a trait that has been identified as useful component of drought adaptation (Richards et al., 2002). Selected landraces have already been employed in CIMMYT’s drought crossing programme and are providing initially promising results (Trethowan and Reynolds, 2007).

Genetic resources

Research into the physiological basis of drought adaptation in crops is well established (Fischer and Tumer, 1978; Blum, 1998; Loss and Siddique, 1994; Richards et al., 2002) and molecular technologies have added a new dimension to the research (Chaves et al., 2003). While some of the research has been applied to wheat improvement (Condon et al., 2002; Rebetzk et al., 2002; Richards et al., 2002), much has yet to be applied (Araus et al., 2002). While new molecular technologies offer powerful ways to identify and manipulate drought-adaptive genes, applications are ultimately limited by the availability of suitable genetic resources in which novel allelic diversity can be utilized. Notwithstanding transgenes from alien taxa, novel trait expression must be identified within germplasm collections. The 12 lines represented some of the broadest and, at the same time, most promising genetic resources available to CIMMYT’s drought-breeding programme. However, a large number of drought-adaptive responses exist and it can be overwhelming for researchers to know which traits to study first given a lack of quantitative information. The main objective of comparing the lines in GEN-RES was to assess their theoretical potential for yield improvement under drought. Passioura (1977) developed an identity that considers the fundamental drivers of yield under drought:

\[
\text{Grain yield} = \text{Crop water use} \times \text{Water use efficiency} \times \text{Harvest index}
\]

The potential of some GEN-RES lines to extract water from depth (discussed in the previous section) could be of potential benefit in improving the first element of this equation, crop water use. Another way to increase water use is to reduce direct evaporation from the soil surface through increased early ground cover (Richards et al., 2002), and a number of GEN-RES showed superior values of NDVI than the check (Table 2) indicating potential for improving this trait. There was also variation for osmotic adjustment among GEN-RES which, in previous work, has been shown to be associated with increased water uptake from the soil (Morgan and Condon, 1986), however, in the case of this trait it was also very well expressed in the check.

In terms of the second element of the equation, the best value for WUE among genetic resources was 5.74 g m⁻² mm⁻¹, expressed by the line MES, compared with 4.25 g m⁻² mm⁻¹ for the check Weebil. The former had a considerably higher biomass than Weebil although both had the same yield. Although CID, indicative of TE, was only weakly associated with WUE, it also showed genetic variation and, as expected, low values of CID were found among the most productive lines.

In terms of the third element of Passioura’s (1977) identity, harvest index is a function of constitutive factors relating to Rht genes, but it can also be influenced by the proportion of stem mass in the form of soluble stem carbohydrates that are remobilized to grains as well as interacting with the other drivers post-anthesis when grain-filling rate will be influenced by water availability and WUE, respectively (Richards et al., 2002). Among GEN-RES one of the traits with potential to contribute is, therefore, genetic variation in soluble stem carbohydrates for which the best genotypes showed a value of 26% compared with 19% for the check.

It can be argued that inferring theoretical yield gains from the over-expression of any one or a combination of these traits is unfounded, since compensatory mechanisms may be at work. For example landraces that extract water at depth also showed low WUE, and those with high stem CHO tend to have low biomass. The important questions is whether there is genetic linkage between high water extraction and low WUE or between high stem CHO and low biomass at anthesis, and if so whether such linkages are mechanistic or can be broken at the genetic level. These questions cannot be answered given our current level of understanding of the genetic basis of the physiological processes that determine differences in productivity under drought at the cultivar level (Cooper et al., 2005). In the meantime, trait information is being used to make strategic crosses based on combining useful stress-adaptive traits with the possibility of realizing additive gene action in selected progeny (Reynolds et al., 2005; Reynolds and Trethowan, 2007). Obvious candidates for crossing with elite check cultivars include landraces that already show relatively high biomass under drought combined with favourable expression of physiological traits such as stem carbohydrates (PUB94.16.24) good water extraction characteristics (also PUB94.16.24), and TE (MEX94.2.19).
Conclusions

Exotic germplasm such those described appear to have considerable potential to improve drought-adaptive mechanisms in wheat. In this experimental environment at least, novel genetic sources could potentially contribute to increased water extraction from depth in the soil, accumulation of soluble stem carbohydrates, and water use efficiency. Furthermore, in addition to their use in strategic crossing, some of these traits are amenable to early generation screening or large-scale screening of genetic resources since they can be measured rapidly using remote sensing techniques. Differences in early ground cover and biomass can be rapidly estimated using spectral reflectance (Babar et al., 2006) while canopy temperature, a very easy trait to measure, shows strong genetic effects associated with soil moisture extraction (Fig. 4).

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References


