Integration of phenotyping and genetic platforms for a better understanding of wheat performance under drought

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Abstract

Identifying markers for physiological traits of proven value in breeding, especially ones that are consistent across environments with different patterns of stress, strengthens the toolkit to increase confidence in the value and delivery from physiological breeding. To identify markers relevant to drought adaptation, this review will highlight the importance of development and implementation of robust and repeatable phenotyping that is relevant to the different target drought types, and practical examples of managed environment facilities in Australia and Mexico are given. These facilities can be used as models to: (i) improve reliability and consistency of environments and genetic responses to the environment at a global scale; (ii) improve the capacity to deliver quantitative trait loci (QTLs) as user-friendly markers for enriching populations; and (iii) illustrate the use of populations with a narrow range of variation for phenology allowing the identification of QTLs for drought-adaptive traits. However, the importance of further optimizing phenology and plant height at a global scale is highlighted. Finally, the impact of physiological trait-based crossing is demonstrated and supports the need for urgent development of robust genetic markers.

Introduction

The yield of wheat has doubled over the last 30 years due to a combination of advanced agronomic practices and improved germplasm through selective breeding. Recent evidence suggests overall yield progress has been slowing, and a significant improvement in wheat production will be required if demand from the growing human population is to be met (Odegard and van der Voet, 2014). The challenge of breeding is to increase genetic gains in productivity at a rate that matches growing demand, in combination with good agronomy to minimize the potential for any yield gaps. Analysis of spring wheat lines distributed by the International Wheat and Maize Improvement Center (CIMMYT) between 1995 and 2010 showed average genetic gains in yield of between 0.5% and a little over 1% per year depending on regions, based on analyses of over 1500 spring wheat environments (Lopes et al., 2012; Manès et al., 2012; Sharma et al., 2012).

It is clear that breeding and selection methodologies must become more efficient in order to accelerate genetic progress, especially given the predictions that climate change will bring about more drought and heat stress in the majority of wheat environments (IPCC, 2014). Some progress is already being made in this respect. For example, the Semi-Arid Wheat Yield Trial (SAWYT), one of the nurseries from CIMMYT targeted at maintaining yields under stressed conditions, showed the strongest genetic gains at the warmest temperatures, whereas nurseries targeted to high-yielding conditions did not have an impact on hot environments (Gourdji et al., 2013). The process has been associated with selection of germplasm under environments showing some level of stress at least at some stage of progeny selection, where the environment itself is the ‘natural selection tool’ in combination with breeder’s observations.

Recent impacts have been reported in response to physiological characterization of parents and progeny in the SAWYT (Reynolds et al., 2009). Molecular breeding is also relevant in this context (reviewed by Liu et al., 2012).
Examples of functional markers that may be relevant to selection for drought adaptation are the semi-dwarfing genes Rht-B1b and Rht-D1b (Ellis et al., 2002) and genes regulating phenotype like photoperiod insensitivity (Beales et al., 2007) and vernalization requirement (Yan et al., 2004, 2006; Fu et al., 2005; Chu et al., 2011), among other genes of major effect. Despite the fact that many of the major alleles for appropriate phenology and height have been fixed in commercial breeding programmes (e.g. Richards et al., 2010), we are still far from having a clear understanding of the optimal values of the former traits on a global scale to optimize grain yield. Unfortunately, it has not been possible to further extend these findings to functional markers associated with drought or other abiotic stress adaptation. This has been hampered by the complexity of plant responses to stress and particularly to the difficulties associated with determining all possible genetic responses and interactions or epistasis to specific environmental parameters that happen to occur in random combinations in real environments. This particular bottleneck will restrict the use of markers in selection (from marker-assisted selection to genomic selection) to adaptation to specific environmental conditions (Edae et al., 2014), which may not be desirable for global breeding progress, where wide adaptation has been the pillar of success. Reproducible environments on a global scale representative of different levels of drought and other stresses have to be made available where germplasm can be evaluated under strictly controlled and monitored but realistic conditions, and this will eventually lead to a clearer knowledge of which parts of the genome are responsible for stress adaptation.

This review attempts to summarize the advances and procedures available in breeding to accelerate genetic gains for wheat under drought conditions. It also attempts to describe up-to-date phenotypic and genetic tools available and how these two must integrate effectively to return more efficient identification of parental lines for crossing, together with defining the genetic variation available for relevant traits for targeted introgression and physiological breeding (Reynolds et al., 2009).

Development and implementation of robust and repeatable phenotyping relevant to the target drought types

Understanding environment types and their frequency

There are few greater challenges confronting breeders than selecting for improved performance under drought. Reduced genetic variance and non-systematic genotype×environment interaction contribute to changes in line ranking across environments to reduce breeder confidence (Calhoun et al., 1994; Ceccarelli 1994). Variation in the timing and amount of rainfall commonly confounds selection, while breeders cannot reliably sample the potential range in on-farm environments in any given testing season. There is value in describing the nature of environments over the long term, and considering how well these are sampled and represented in a given testing season. Furthermore, analysis of historic datasets should identify which rainfall patterns and subsequent environment types are most common, therefore allowing targeting of environment types relevant to a breeding programme (Chenu et al., 2013, Lopes et al., 2014).

It has been argued that there is significant value in broadly defining rain-fed environment types around timing and average rainfall amount (Chenu et al., 2013). Chapman (2008) applied this understanding in analysing a historic dataset containing 116 years of weather data across multiple sites in the northern Australian wheatbelt. Changes in the timing and amount of rainfall on grain yield in these rain-fed environments were simulated for a target genotype (cv. Hartog). The simulations encompassed several management regimes and planting dates for each part of the region, and a strong focus was given to the effects of water stress immediately before and at flowering owing to the sensitivity of this stage on floret fertility and final grain number. Analysis of yield response summarized into repeatable and systematic environmental groups ranging from seasons of mild or no stress (less than 25% of environments) to the more frequent terminal stress (over 50%) environment types. While such summaries are very useful in a priori planning to sample potential target environments, their use has been extended to classifying genotype response from large multi-environment experiments to different environment types (Chapman, 2008; Chenu et al., 2013). Without classification of the environment type, genotype×environment interaction variance is substantially larger than the genotypic variance to reduce heritability for yield and slow potential genetic advance. Inclusion of environment type in the statistical model partitions into repeatable components of the genotype×environment interaction to increase the relative magnitude of the genotypic variance for the different environmental types.

Managed environments

The simulation-based approaches above illustrate the critical need for breeders to interpret multi-environment trials in such a way as to adequately sample representative droughts in their target regions. However, their sampling and use of existing locations and seasons (years) is stochastic and thereby subject to the vagaries of weather for a given region. Increasing effort towards physiological and genetic understanding of traits has challenged pre-breeders in defining and delivering relevant trait information for immediate use by commercial breeders (Rebetzke et al., 2013a). Organizations such as CIMMYT (Trethewan et al., 2005) and others (Campos et al., 2004; Kirigwi et al., 2004; Rebetzke et al., 2013a) have strongly advocated the use of field-based screening in environments rigorously controlled for the timing and amount of water availability. These managed environments should allow capacity to assess many hundreds and potentially thousands of research-based lines and populations common to many research programmes, and include meaningful comparisons with the best available commercial varieties. When located in regions of low rainfall, they preclude the need for large and expensive glasshouses and provide excellent control of water availability via use of accurate overhead sprinklers.
CIMMYT have long and successfully employed the use of managed environments for assessing wheat genotypes at their reliably dry Obregon site in northwest Mexico (Trethowan et al., 2005).

In Australia, the Grains Research and Development Corporation has supported a national network of managed environment facilities (MEFs) located to broadly represent the potential range of rain-fed wheat environments (Fig. 1). These MEFs are geographically widespread and so sample quite contrasting rainfall and photothermal conditions to which commercial crops are adapted. Four environment types (ET1–4) reflecting different patterns of post-anthesis water use are encountered with varying frequency and influence on final yield across the different MEFs (Chenu et al., 2013). Each site is managed to minimize soil constraints (e.g. root disease and other chemical-based subsoil toxicities), thereby allowing maximum root growth and potential water use. Soil conductivity is monitored across all MEF sites using EM38 (e.g. Fig. 2) to locate experiments in areas within each MEF of greatest soil uniformity. A reliable crop rotation is used to control root disease and soil nutrients, and the soil profile is managed so that starting water at sowing is consistent with the expectation for the region and the season (e.g. Chenu et al., 2013). As much as 4 ha accommodating up to 2500 large 10 m² plots can be sown in each MEF. Weather data is coupled with gypsum blocks and Arducrop® sensors (O’Shaughnessy et al., 2011) permitting constant monitoring of soil water and canopy temperatures, respectively, for a selected group of indicator or ‘probe’ genotypes (Rebetzke et al., 2013a) (Fig. 3). These probe genotypes are commercial wheat varieties and are representative of the performance and potential water use of commonly grown, adapted varieties. Their inclusion provides real-time information of the environments being sampled and so ‘inform’ the managers of each MEF of the need for scheduling of irrigation for a given season scenario. Commonly, up to 10 current commercial varieties represent the probe genotypes. Importantly, other genotypes of interest (e.g. high-transpiration-efficiency genotypes) independent of the probe genotypes are also quantified for water use. The probe genotypes also inform researchers of the development of the crop and timing of sampling for key measures of water use or growth (e.g. leaf water potential). Notably, traits and germplasm are assessed in seasons representative of the regions in which each MEF is located (i.e. decile 5 for rainfall). At each site, an additional rainfall scenario is included requiring extra irrigation aimed at target yields of 25–50% above the rain-fed treatments. By contrasting, average and above-average rainfall scenarios, the value of the trait, and germplasm can be assessed when water is limiting and in more favourable environments where growers commonly make considerable income. By having both environment types (average and above-average rainfall scenarios), breeders can address the potential for trade-offs commonly observed in selection for improved performance in stress environments (e.g. Rosielle and Hamblin, 1981). A final development critical to the assessment of germplasm across

Fig. 1. Climate characteristics of the MEF sites of Merredin, Narrabri, and Yanco in the Australian wheatbelt (shaded). Mean monthly rainfall and temperature are given for data collected for 1889–2011 (http://www.longpaddock.qld.gov.au/silo; Jeffrey et al., 2001). The sowing window for wheat is commonly from late April to mid-June. The water-stress pattern for four environment types (ETs) has been modelled for the three MEF locations across the Australian wheatbelt: ET1 (no or short-term stress; blue, plain line), ET2 (mild stress commonly easing through grain-fill; purple, dash), ET3 (intermediate stress relieved through grain-fill; orange dash-dot), and ET4 (severe stress through grain-fill; brown dot) (see text for explanation). The stress index [here as the supply:demand ratio (sdr)] is represented as a function of thermal time relative to flowering, and ranges from 1 (no water stress) to 0 (full stress, no water available to the crop) (the insert pie-chart contains the frequency of the four environment types at each location) (after Rebetzke et al., 2013a Functional Plant Biology http://www.publish.csiro.au/nid/102/paper/FP12180.htm.). Monthly rainfall is given as bars and temperature as a solid line.
multiple MEFs is the need for a standardized set of protocols (e.g. http://prometheuswiki.publish.csiro.au/tiki-index.php?page=Field+Experiments+in+Crop+Physiology) (Pask et al., 2012; Reynolds et al., 2012; Rebetzke et al., 2013a). This permits the assessment of performance across the different MEFs for data standardized and is therefore consistent for all germplasm being assessed.

In Turkey, CIMMYT is mapping soils using the EM38 of national and regional agricultural stations where most breeding programmes are located, to detect major problematic areas and design strategies for soil improvement (e.g. soil levelling and adequate crop rotation). One major difficulty of an international institution like CIMMYT is the variable-quality data received by some of the cooperators when CIMMYT lines are distributed worldwide. Improving field uniformity, rotation programmes, and soil and climate monitoring of key stations in the world will give major support to the global wheat programme in the future, and the example
given in Australia above will be useful and relevant for further global application provided funds are available.

Glasshouse phenotyping

Earlier, we indicated that the nature of populations assessed for understanding genetic control can be varied, diverse in genotypic composition, and commonly large (e.g. genome-wide association studies). Glasshouses are invariably too small, and plant growth is constrained to small pots and the limited soil volumes in which plants are typically grown. Furthermore, conditions in glasshouses commonly do not reflect well the outdoor environments to which growth and genotype performance is being inferred (e.g. Rebetzke et al., 2004, 2014). Large, portable rainout shelters are now being developed and deployed with capacity for phenotyping large populations in the field (Fig. 4). These portable shelters permit experiments to be grown in the field under conditions of adequate light intensity and quality, and allow access to all available soil water in the profile. Shelters can be moved over experiments at key times to permit the development of a slow but representative drought stress and then removed after the implementation of the stress if necessary. Figure 5 shows how use of a rainout shelter can allow differentiation between wheat genotypes for performance under a controlled drought in small, bordered plots.

Quality high-throughput phenotyping

Drought ideotypes in wheat

The so-called drought environment is highly variable and therefore ideotypes are at best generic and must be refined for specific target environments (Reynolds and Tuberosa, 2008). A simple framework has been developed where grain yield (GY) under water limitation is a linear function of water use or evapotranspiration (WU), water use efficiency (WUE), and harvest index (HI) (Passioura, 1977) as:

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GY = WU \times WUE \times HI
\]

The broader benefit in this partitioning is that selection can be focused towards the individual drivers of water-limited yield potential, and then genetic variation for these components assessed separately before combining to deliver elite germplasm. Traits that are currently applied in CIMMYT’s breeding and pre-breeding for drought are listed under three main drivers to yield: (i) traits to increase WU, including deeper roots to tap subsoil water, and early ground cover to reduce losses of water at the soil surface back to the atmosphere; phenotyping protocols such as infrared thermometry and spectral radiometry, respectively, are discussed later; (ii) traits to increase WUE, including transpiration efficiency (estimated through carbon isotope discrimination of unstressed leaves), epicuticular wax expressed on stressed plants (and easily observed visually), and biomass; and (iii) the main trait selected to increase partitioning of carbon to grain (apart from HI itself)—accumulation and remobilization of water-soluble carbohydrates (mainly from the stem) to grains. There are many other candidate traits in the generic conceptual model; spike photosynthesis is a prime candidate under drought conditions, where leaves may dry but the spike remains photosynthetic (Tambussi et al., 2007; Maydup et al., 2012). However, consistent phenotyping protocols are still being established.

Fig. 4. Large, portable rainout shelter deployed at the Yanco MEF. The wheels illustrate the portability, while the large, grey cinder blocks are used to anchor the shelters. The sides are made of curtains, which are rolled up to permit air flow.
Measuring complex traits associated with the drought ideotype

Improved understanding of genotype×environment interaction and subsequent characterization of environment types (above) coupled with improved statistical methodology (e.g. Smith et al., 2001) has contributed to reduced error variances to increase heritabilities and response to selection for yield. Similarly, improved experimental techniques are being employed to reduce experimental error in measuring yield. For example, removal of border rows at maturity will reduce the effects of inter-genotypic competition between adjacent plots (Rebetzke et al., 2014) while breeders are careful in selecting uniform sites and/or mapping field variability for known soil constraints (e.g. micronutrients or salinity) (Fig. 2).

Yet, in spite of the improvements in yield-based selection, empirical selection for yield in dry environments has largely delivered improved adaptation through earlier flowering and reduced height (Richards et al., 2010). With many of the major alleles for appropriate phenology and height now fixed in commercial breeding programmes, there is reliance on the identification and deployment of new alleles underpinning traits for improved productivity under drought. Increases in biomass or yield per unit of water used should permit a broader consideration of component traits that account for the nature, timing, and variability of water limitation in the target environment.

Extension from empirical selection for GY to selection for components underpinning water productivity discussed earlier should facilitate targeted selection for heritable traits relevant to the nature of the drought being selected for. In spite of this potential, factors contributing to improved WU, WUE, and HI are commonly genetically and physiologically complex traits (e.g. water-soluble carbohydrates) and/or traits expensive to measure (e.g. carbon isotope discrimination). These will limit their adoption and subsequent selection in breeding programmes despite their value in improving performance. New tools are becoming increasingly available for phenotyping and selection of complex traits by breeders in early and later stages of family evaluation. Spectral information obtained through near-infrared spectroscopy is inexpensive and reliable with potential as a surrogate for leaf N, carbon isotope discrimination, and water soluble carbohydrates content (e.g. Ruuska et al., 2006; Rebietzke et al., 2008; Dreccer et al., 2014). Similarly, remote sensing of multispectral reflectance enables rapid assessment of biomass and green leaf area, canopy architecture (as photosynthetically active radiation absorption), plant nitrogen, and water status of large breeding populations assessed as plots (Babar et al., 2006). Indications are that spectral reflectance indices are repeatable with high heritability and are correlated with GY across contrasting irrigation regimes (Babar et al., 2006). Indeed, application of reflectance in high-throughput phenotyping of large mapping populations was successfully undertaken for canopy stay-green with a commercially available Greenseeker®, which measures the spectral index Normalized Difference Vegetation Index and estimates ground cover, biomass, or stay-green, depending at what crop stage it is measured (Lopes and Reynolds, 2012).

Other seemingly less-complex tools have application for high-throughput phenotyping in breeding programmes. Use of digital cameras and availability of free or inexpensive digitizing software (e.g. ImageJ: http://imagej.nih.gov/ij/) can be used for rapid assessment of vegetation indices, ground cover, or plant establishment counts for large numbers of lines (e.g. Mullan and Reynolds, 2010). Plant water status can readily be assessed from canopy temperature using hand-held infrared thermometers (Olivares-Villegas et al., 2007; Rebietzke...
Marker discovery relevant to drought adaptation

Until relatively recently, the major bottlenecks to identifying drought-related quantitative trait loci (QTLs) and markers, especially for physiological traits explaining a significant variation in yield were: (i) the availability of a mapping population with controlled height and phenology to avoid confounding major gene effects; and (ii) the ability to phenotype large experimental populations in a time frame that would avoid confounding diurnal or weather-related effects (Reynolds and Tuberosa, 2008). In an analysis of a large mapping population where phenology was not controlled, apparently only genes of major effect were associated with drought adaptation. However, it was demonstrated that, by performing QTL analysis on early and late subpopulations individually, a set of QTLs that had previously been masked were then identified, and they were not necessarily the same for early and late subgroups (Reynolds et al., 2009). This study highlighted the inherent problem of trying to identify genes of minor effect when genes of major effect confounding expression or response are not controlled.

At the International Maize and Wheat Improvement Center (CIMMYT), important advances have been made in population development with a narrow range of variation for phenology in both biparental populations and diverse panels for association mapping [e.g. Wheat Association Mapping Initiative (WAMI)]. The Seri/Babax biparental population was developed for genetic analysis since it has a relatively narrow range of flowering time of approximately 10 days (Olivares-Villegas et al., 2007). In the WAMI and Seri/Babax phenology-controlled populations, co-location of *Vrn* genes and GY associations were rare although observable, showing that the effects of days to heading to GY in these populations were reduced, and other potential markers may be discovered (Lopes et al., 2013; Edae et al., 2014). However, co-locations of minor plant height-associated markers with GY have been observed, even in these controlled populations (Lopes et al., 2013). Most of these new populations have been selected mainly on the phenotypic expression of days to heading and plant height. In the future, it might be useful to develop large populations (around 1000 individuals), screen for major genes (vernalization, photoperiod sensitivity, and semi-dwarf stature), and only characterize and compare individuals monomorphic for those major genes and with a controlled phenotypic expression of heading and height.

To get around the second bottleneck, physiological phenotyping of the mapping population focused on canopy temperature since it can be measured quickly and easily (in about 10 s) with an inexpensive infrared thermometer (Pinto et al., 2010). Combining these two approaches led to identification for 15–20 cores per day (A. Wasson, personal communication). Other methods for indirect assessment of rooting depth include the use of canopy temperature where access to water with deeper roots has been linked to cooler canopies (e.g. Lopes and Reynolds, 2010; Wasson et al., 2014).

Fig. 6. Thermal image of a large experimental site (~2 ha) taken from a manned aircraft using a high-resolution thermal camera at an altitude of 300 m. Lighter colours represent warmer canopies. (Image courtesy of David Deery, CSIRO Plant Industry, Canberra, ACT, Australia; with permission).
of the first QTLs that were not collocated with phenology traits and explained a substantial proportion of variation in canopy temperature as well as GY and other agronomic traits. An added bonus, since the same population was phenotyped under hot-irrigated conditions, was the identification of common genetic bases for heat and drought adaptation (Pinto et al., 2010), which have subsequently been shown to be associated with optimal root distribution across the soil profile (R. Pinto and M. Reynolds, unpublished data).

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**Finding markers for complex traits of proven value in breeding consistent across realistic environments with different patterns of stress**

It is widely accepted that drought commonly accelerates plant development, i.e. hastens heading and flowering date, while plant height is considerably decreased independently from specific genotype responses (Tardieu, 2012). These two traits are said to be simple genetically in nature, presenting small genotype×environment interactions, and genes or markers are readily available to breeders. Genotype×environment interaction in complex traits with proven value in breeding such as GY has been explained by these simple traits with major effects. For example, Yan and Hunt (2001) showed this when correlating the principal components from site regression with several winter wheat traits, concluding that genotype×environment interactions were explained by different traits according to year: some years related to winter survival scores, while other years could relate to heading dates, plant height, and lodging. Yan and Hunt (2001) suggested that an increase or decrease in the levels of expression of these traits would therefore improve the specific adaptation of the genotypes to certain environments but was unlikely to lead to improved overall cultivar performance. The concept suggested by Yan and Hunt (2001) was to target trait optimization rather than maximizing or minimizing a specific trait, which will lead only to specific adaptation. Optimization has to be targeted towards groups of environmental types like those defined for Australia and described above.

When it comes to complex traits like GY, different types of genotype×environment interaction may occur (Crossa et al., 2013) due to the different alleles that respond to drought and other stresses occurring at random stages of crop development according to the environment. However, in the context of climate change, realistic environments are subjected to a wide range of conditions (particularly on a year-to-year basis) and resilience is one of the top priorities for breeding, whereas specific adaptation will not be desirable (Braun et al. 2010). As a result, it becomes difficult to find consistent markers across environments. While a few methodologies are available to define QTLs and markers associated with complex traits in a wide range of environments, often results are unstable and not representative. This problem is particularly observed when comparing the QTLs identified for each environment and the QTLs identified with the average of all environments, where new regions (not previously identified in individual environments) appear. In Fig. 7, we used phenotypic data collected in several trials (Lopes et al., 2012, Shivakumar et al., 2013) and used it for genome-wide association studies. It was possible to structure the data according to the genotype×environment components (using scores from a biplot) and subsequently use them in genome-wide association. This procedure allowed us to determine common genomic regions associated with yield in a wide range of environments. Figure 7 shows clusters of markers associated with GY in several regions of chromosomes 1A and 6A, and this procedure seems promising for structuring common patterns of markers associated with yield. The possibility of dealing with genotype×environment interactions for GY as a complex trait itself would be very useful to avoid expensive multi-location trials, and the results in Fig. 7 look promising.

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**Trait-based crossing in breeding**

Trait selection has made systematic progress in wheat breeding through improving agronomic traits such as height and flowering time, resistance to a spectrum of common diseases, end-use quality parameters, and yield expressed at multiple...
locations (Braun et al., 2010). However, traits associated specifically with drought adaptation have rarely been used as breeding criteria. The main goal of trait-based crossing is to accumulate traits that will be complementary for a given target environment. Under moisture-limited situations, traits that improve WU, WUE, and partitioning to yield could work synergistically to maximize productivity (Richards, 2006; Reynolds and Tuberosa, 2008). Achieving this in a wheat improvement pipeline involves the following interventions:

1. Target traits are identified based on conceptual models.
2. Genetic resources are screened to provide potential sources of target traits.
3. Potential parents are characterized for traits that may contribute to genetic gains in target environments.
4. Hybridization schemes are designed such that traits expressed by respective parents encompass as many of the target traits as possible. Top crosses may be used to facilitate the accumulation of traits.
5. Early-generation progeny are screened as bulks for integrative traits such as canopy temperature in relevant environments; families with warm canopies, compared with checks, are mainly discarded.

The emphasis on past physiological research has been to identify traits for early-generation selection that can be used to screen progeny for favourable trait expression. There is consensus that the more feasible traits are either genetically relatively simple or integrative traits that explain a reasonable degree of the variation in yield and are relatively quick and easy to measure (Richards, 2006). In fact very few traits meet these criteria and have found application. A successful application in wheat was the use of carbon isotope discrimination to select progeny with a high transpiration efficiency conferring a conservative rate of water use that is associated with increased yield when the soil water profile is restricted (Rebetzke et al., 2002). Another trait that has found application in early-generation selection under a range of environments is canopy temperature as discussed earlier. The trait is used routinely by CIMMYT’s wheat breeding programme for rain-fed environments to enrich for alleles associated with dehydration avoidance. For example, F\textsubscript{3} and F\textsubscript{4} bulks are screened for canopy temperature under drought; a larger number of plants—also expressing favourable agronomic traits—are selected from cooler canopy temperature families, while bulks with warmer canopy temperature (compared with checks) are discarded. The development of relatively easy-to-use spectral radiometers offers another high-throughput screening approach for comparing spectral reflectance indices of genotypes.

Investment in physiological trait-based crossing has generated advanced lines distributed by CIMMYT starting with the 23rd Semi-Arid Wheat Screening Nursery (SAWSN) and the 17th Semi-Arid Wheat Yield Trial (17th SAWYT). For example, of the 205 candidate genotypes for SAWSN in 2009, 48 (23%) were derived from physiological trait crossing and a similar proportion were represented in the SAWYT. The physiological trait lines performed well in 2010, with the average yield of the physiological trait lines beating the group of conventionally bred lines at 75% of the international sites. The most recent products of physiological breeding have shown spectacular results, with 70% of the newest physiological trait lines beating the drought-adapted check Vorobey under drought in Mexico in 2012, some by as much as 30%. The underlying assumption for the physiological trait strategy is that crosses between parents with different but potentially complementary physiological trait expression will realize cumulative gene action in selected progeny. This has been borne out in previous analyses (Reynolds et al., 2009) and was shown again in the newest physiological trait lines. The impact has been demonstrated recently with the release of physiological trait lines by the Pakistan national wheat programme.

Conclusions

A better understanding of the frequency and nature of water limitation in the target population of environments will guide trait identification and selection methods to enrich populations with a greater likelihood of improved performance. Complex traits underpinning drought adaptation include increased WU, WUE, and carbon partitioning to grain, and all are genetically complex and somewhat challenging and/or costly to phenotype. New high-throughput phenotyping tools coupled with the use of managed environments will allow better trait understanding and their relative value in improving performance, while new genetic platforms are providing greater insight into gene\texttimes{}environment and potential for gene\texttimes{}gene interactions and pleiotropy. Improved genetic understanding under controlled drought and testing populations with controlled phenology and plant height is allowing the dissection of genetically complex traits and the opportunity to assess more broadly other alleles common to or rare in commercial breeding programmes targeting water-limited environments. This strategy must be coupled with selection for wide adaptation (within water-limited environments) to avoid losing alleles empirically brought together by selection under different sampled environments representative of the target regions. Finally, through the inclusion of trait-based crossing in breeding, several lines have been released and adopted by national programmes where droughts are frequent, showing the value of this approach.

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