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Physiological breeding☆

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Physiological breeding crosses parents with different complex but complementary traits to achieve cumulative gene action for yield, while selecting progeny using remote sensing, possibly in combination with genomic selection. Physiological approaches have already demonstrated significant genetic gains in Australia and several developing countries of the International Wheat Improvement Network. The techniques involved (see Graphical Abstract) also provide platforms for research and refinement of breeding methodologies. Recent examples of these include screening genetic resources for novel expression of Calvin cycle enzymes, identification of common genetic bases for heat and drought adaptation, and genetic dissection of trade-offs among yield components. Such information, combined with results from physiological crosses designed to test novel trait combinations, lead to more precise breeding strategies, and feed models of genotype-by-environment interaction to help build new plant types and experimental environments for future climates.

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Introduction

Global cereal demand is predicted to outstrip genetic gains by 2050 [2], while climate change threatens to reduce their impact [3•]. To accelerate yield improvement, physiological traits at all levels of integration (Figure 1) need to be considered in breeding [4–6]. Annual genetic yield gains in cereals are currently in the region of 0.5–1% [7], due almost entirely to conventional approaches. These have come about as a result of two main factors: unspecified recombination of genes of minor effect among elite germplasm, and the introduction of new genetic diversity often associated with disease resistance and grain quality [1,8]. Physiological breeding complements this approach by adding two main elements: knowledge of well-characterized genetic resources to design crossing strategies, and the ability to enrich for favorable alleles through phenomic (and genomic) screening of progeny. This increases the probability of achieving cumulative gene action for yield compared to crossing physiologically uncharacterized lines. In practice it differs from conventional breeding by considering a larger range of traits — including genetically complex physiological characteristics [9–12] — and differs from molecular breeding by encompassing both phenomic and genomic information. The key steps are presented below:

- Designing a plant with theoretically improved adaptation;
- Identifying genetic resources likely to encompass new and/or complementary allelic variation (for crossing);
- Developing and implementing phenotyping protocols and experimental treatments to maximize resolution of physiological trait expression (to select parents);
- Genetic dissection of traits, and development of gene-based selection approaches;
- Strategic hybridization to achieve cumulative gene action for yield, combined with application of high throughput phenotyping and genotyping to select progeny;
- Analysis of the trait/allele combinations that achieve environmentally robust genetic gains based on multi-location trial data (to design new crosses);
- Informatics services underpinning the iterative refinement of breeding strategies across all steps.

Since a comprehensive genetic basis explaining cultivar level differences in performance does not yet exist for any crop, physiological breeding currently relies heavily on phenomics. However, it can also make use of markers for alleles associated with genes of major effect — such as *Ppd*, *Vrn*, and *Rht* in wheat [8] — and will increasingly make use of both genomic selection (GS) [13,14] and marker assisted selection (MAS) associated with genes of minor effect [15]. In that sense, phenomics and genomics go hand in hand in the physiological breeding approach. Other examples include: selecting parents with complementary traits and alleles [8]; progeny selection using a

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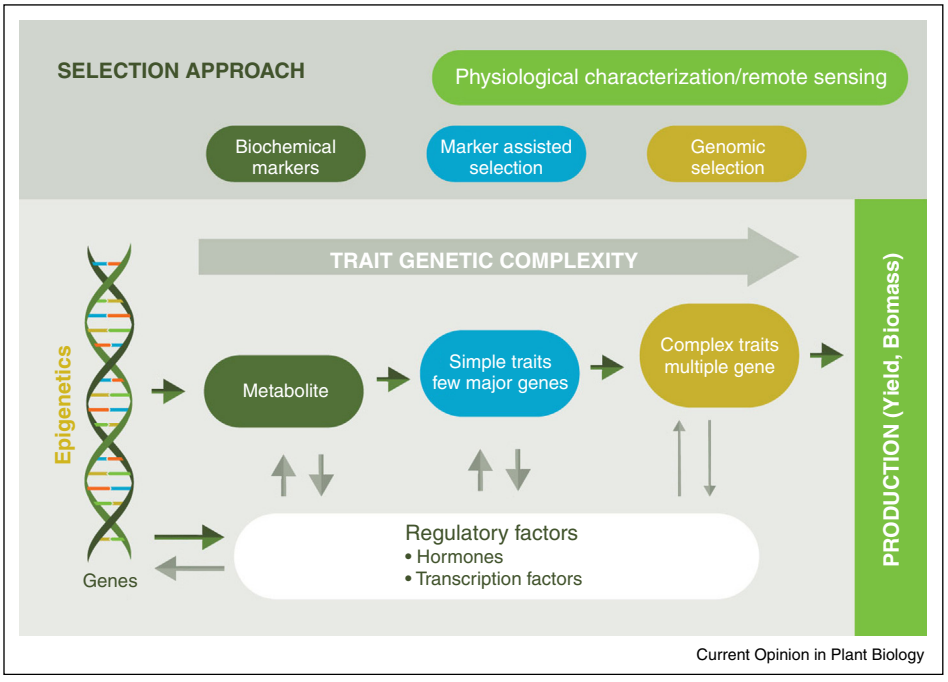
Figure 1



Selection approaches for traits at different levels of integration and genetic complexity. The figure represents plant selection approaches that may be used at different intervention points across the spectrum of traits, starting with simple metabolites and culminating in polygenic productivity traits such as yield and biomass. Regulatory factors that may interact with the expression of traits at any level are indicated. Environmentally mediated epigenetic factors may directly influence gene expression and therefore increase expression of genotype by environment interaction.

combination of remote sensing of integrative traits, MAS [16•], and GS [14]; and genetic dissection to demonstrate successful combinations of traits and the trade-offs among them, for which QTL analysis adds considerable weight [16•]. The main objective of this review is to present the rationale for the main activities of the physiological breeding pipeline, as outlined in the Graphical Abstract.

Crop design

Designing improved plant types is a unique aspect of physiological breeding, in the sense that through implementing new strategic crosses, novel trait combinations can be rigorously tested across a range of target environments in terms of their impact on yield. With the exception of yield *per se*, selection for genetically simple traits has traditionally dominated plant breeding. However, developments in phenomics and genomics are increasing access to more complex traits [17••,18,19,20•], resulting in renewed interest in designing improved plant types [9,12,18,21–23,24•,25••,26•], including under climate change [4,10,27]. Designing new plant types is not trivial since genotype–environment–crop management interactions (G × E × M) are unpredictable, being determined largely by future weather (E), farmer choices (M), and specific location (E × M). Another barrier to effective crop design is significant gaps in knowledge of the physiological and genetic bases of adaptation (G), further exacerbated by interactions with E [28].

These hurdles led to a belief that stochastic approaches, supported by modern tools, would achieve breakthroughs more readily; for example '-omics' tools combined with bioinformatics to cut through the biological complexity and deliver empirical solutions [29]. On the other hand, detailed studies in controlled conditions with rapid life-cycle model species (of relatively small genome size) were expected to extrapolate to crop species and environments [30]. Unfortunately, neither of these approaches has yet revolutionized practical plant breeding [15]. This is partly because they are still confounded by the G × E × M paradigm (e.g. [31••]), in addition to being retrospective, in the sense that they focus on extant germplasm rather than extrapolating to the requirements of improved genotypes. For example, genomic selection models are predictive within a pre-determined genepool, but they breakdown when new genetic diversity is introduced into the breeding genepool [32].

However, physiological and genetic dissection of complex traits [11–13,15,16•,19,20•,33••,34,35,36•,37–43], and the development of high throughput phenotyping approaches translated to the field environment [17••,44•,45•], have provided valuable insights for the

design of new plant types; for example by establishing the genetic bases for trait synergies and tradeoffs (e.g. [16•,35,36•]), and thereby improved crossing strategies [11–13,16•]. Analysis of the performance of new lines developed by physiological breeding helps inform crop design by demonstrating which combinations of traits/alleles improve yield and in which environments [16•,40]. Using such outputs, and with the help of simulation models, hypotheses regarding the value of new levels of trait expression and trait combinations can also be tested theoretically across a range of environments [24•,28,33••].

The design and testing of improved plant types can be founded on two main hybridization tactics: either by synergistic re-combination of traits/alleles already present in extant genepools, or via the introduction of new levels of trait expression/alleles from exotic sources [46,47]. One of the functions of crop design is to estimate likely cost–benefits of using different classes of genetic resources.

Genetic resources

Physiological profiling among genetic resources can broaden the crop genepool in a highly targeted way. However, most breeders show a pragmatic skepticism towards crossing with exotic germplasm and precedents generally relate to imperatives such as avoiding disease epidemics (e.g. [47]). Three main classes of genetic resources can be utilized: crop wild relatives, isolated genepools of the same genome (e.g. landraces), and modern breeding lines.

Interspecific hybridization is the most difficult to achieve as it normally results in sterility. However, it has resulted in some impressive yield gains [47] and — as a century old technology accelerated by marker technology — is relatively uncontroversial in terms of moving genes between related species [48]. Nonetheless, less than 10% of the wild relatives collected have been used in inter-specific crossing, and fewer still have been surveyed for genetic diversity of traits with potential to boost yield or adaptation. One recent exception was a study of diversity in Calvin cycle enzymes and Rubisco in the Triticeae. Rubisco from species related to wheat showed promising catalytic properties and modeling of photosynthesis at 25 °C and 35 °C demonstrated the potential benefit of replacing Rubisco of *T. aestivum* with Rubisco from *Ae. cylindrica* or *H. vulgare*, in terms of higher assimilation rates [49].

Polyploid crops like wheat can withstand the introgression of alien chromatin due to the buffering presence of homoeologous genomes. The D genome of hexaploid wheat (ABD) exists in abundance as a wild grass (*Triticum tauschii*) and will cross with durum wheat (AB) to generate a synthetic hexaploid with relatively little linkage drag of detrimental alleles [50]. While typically difficult to thresh, such primary synthetics can express yields equal to modern cultivars and with substantially higher biomass (Figure 2). One or more backcrosses to cultivated wheat can result in lines with significantly higher yield, including under heat and drought stress [37], although precise genetic bases still need elaboration. Screening of both AB and D genomes for stress adaptive traits presents the opportunity to combine both sources into a single synthetic hexaploid genome with unique alleles not represented in the conventional genepool.

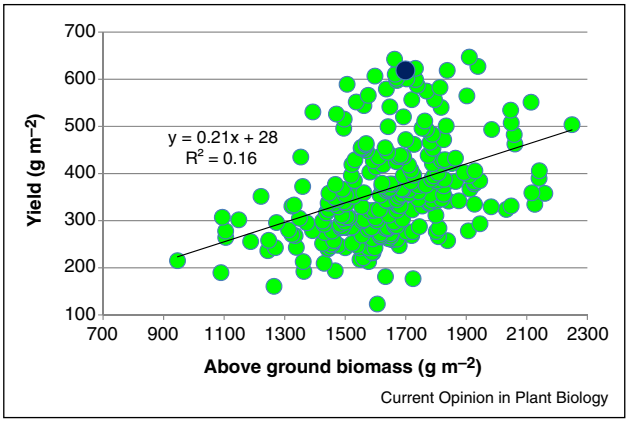
Compared to crop wild relatives, landraces are much easier to cross with while still representing novel pools of allelic diversity; most countries have extensive and overlapping collections (e.g. [51]). Techniques such as the Focused Germplasm Identification Strategy (FIGS) help identify accessions originating in conditions of relevance to breeding targets (http://www.figs.icarda.net/). A good illustration of their value came from recent field phenotyping of FIGS wheat panels selected under heat and drought stress, revealing dozens of lines with final biomass significantly larger than adapted checks [52] (final biomass being an indicator of agronomic potential, especially under stress).

However, the most accessible source of genetic variation — in terms of its use in strategic crossing — is that within current breeding material. Interestingly, detailed physiological [53•] and genetic dissection [20•,38,39] is not yet a routine procedure for selecting parents among advanced breeding lines, but developments in field phenotyping — in combination with high throughput genotyping — will identify more candidate lines for use in trait-based crossing.

Phenotyping

Recent advances in high throughput field phenotyping have boosted the power of physiological breeding

Figure 2



Yield and total above ground biomass (AGB) of a panel of 250 primary synthetic hexaploid wheat lines (green dots) compared to the best elite check (Tacupeto blue dot) under yield potential conditions (as described in [82]), Obregon, 2014; LSD for yield and AGB = 135 and 390 g m^{-2}, respectively.

[17••,45•]. Almost by definition, high throughput implies use of non-invasive approaches like proximal/remote sensing of spectral reflectance from plant tissue. The traits measured relate either to thermal/hydration properties of plant tissue assessed in the infrared region of the electromagnetic spectrum, or pigment profiles estimated in visible bands [45•]. These spectral indices cover an important range of traits including plant temperature, water relations, photosynthesis, nutrient status, and agronomic traits. Water index in particular has been shown to be predictive of cultivar level differences in leaf and soil water potential, as well as yield and biomass in both stressed and favorable environments [54].

Remotely sensed traits express good resolution for large scale screening, and in some cases remote sensing is sufficient for detailed genetic or physiological dissection, as required for mapping quantitative trait loci (QTL), for example. Canopy temperature (CT) has been used to identify QTL for drought and heat stress tolerance [35,40], and QTL common to both stresses were linked to adaptive root response [36•]. Dedicated spectral sensors, such as the GreenSeeker that measures the normalized difference vegetation index (NDVI), are also used for simple growth analysis [17••,45•].

Aerial imaging (either by manned or unmanned low-flying vehicles) is revolutionizing field phenotyping [44•] by offering two main advantages that increase throughput and precision compared to ground-based systems. Firstly, the ability to include hundreds of field plots in a single image avoids confounding effects of environmental drift associated with lengthy plot-to-plot measurement. Secondly, image analysis permits data curation by removing outlying pixels in each plot. Both of these factors improve associations between remote-sensed traits and yield in comparison to ground based readings [55]. As sensor and image analysis technologies develop, the scope and precision of remote sensing is expected to increase. Work is underway (at the International Maize and Wheat Improvement Center, CIMMYT, for example) to develop algorithms associated with spike characteristics — including size, density, and phenological stages — based on a combination of visible and infrared bands. Elsewhere, indices have been reported that may eventually substitute for gas exchange measurements (e.g. [56]), perhaps in combination with chlorophyll fluorescence [57]. However, there are still many important phenotypic traits that do not lend themselves to remote sensing, such as detailed growth analysis where the required level of precision necessitates destructive harvests, or measurements of traits that are partially or fully obscured from view such as stem or root characteristics.

At the other end of the phenotyping spectrum (Figure 1), some metabolites have been associated with performance traits; for example, fructans with stress tolerance and quality, and major loci controlling fructan biosynthesis, have been mapped [58]. It is now practical to investigate interactions between metabolome and phenotype at a large scale [59•], and new loci controlling metabolites have been associated with agronomic traits [60]. A study in drought-stressed wheat detected QTL for 238 metabolites with significant genetic associations ranging from one QTL for 125 metabolites to nine QTL just for malate [60]. Consequently, the translation of metabolite information to practical breeding remains elusive. An alternative application has been the direct modification of metabolite levels through genetic engineering, focusing mainly on compounds associated with osmotic adjustment and amino acid metabolism including trehalose, proline, mannitol, and ornithine (reviewed in [61]).

While phenotyping protocols exist for simple through to highly integrative traits (Figure 1), the genetic improvement of more integrative traits (canopy temperature, biomass, fruiting efficiency, harvest index, etc.) are among the 'lowest hanging fruits' in terms of increasing genetic gains, since they show considerable genetic variation within modern cultivars and have not been systematically considered in conventional breeding. However, as these more integrative traits are optimized and fixed in elite lines (accelerated by high throughput phenotyping), subsequent genetic gains will come from understanding their component traits, physiological mechanisms, metabolic pathways, etc. (Figure 1), in combination with genetic analysis.

Genetic analysis

Genetics and physiology are inextricable in the context of crop improvement. Genetic dissection of complex traits, boosted by the tools of modern biotechnology, permit models of improved plant functions to be rigorously tested [16•,20•,24•]. Genome-wide association studies readily identify parents with contrasting inheritance of key traits [39] for genetic dissection using bi-parental crosses/nested association mapping (as well as for designing new crosses). When phenotyping genetically complex traits in experimental populations, it is crucial to control genes of major effect to avoid masking detection of novel QTL [11,39,40]. The ideal genetic marker is a diagnostic one that allows direct identification of specific alleles, as seen in the selection for phenology and plant height genes in wheat [8]. Details on how DNA sequence information can be used to develop molecular markers for screening a range of agronomic traits is provided elsewhere (http://maswheat.ucdavis.edu/).

Nonetheless, even in crops where the complete genome sequence is available (reviewed in [62]), trait-based markers are still not in mainstream use for complex trait selection in major breeding programs [15]. This goes back to the G × E × M paradigm (or QTL × E × M), since most QTL are not robust across environments [41]. The

Table 1

Summary of genetic progress achieved through physiological trait (PT) based breeding/crossing at CIMMYT for different target environments as indicated by multi-location yield trials (in Asia, North Africa, and Mexico), conducted within the International Wheat Improvement Network (IWIN)

Breeding target environment	Trial name and # PT lines included	# International sites	Year of harvest	Indication of genetic progress
Water deficit	17th SAWYT PT advanced[a] lines	64	2010	Best PT line higher yielding (avg 16%) than local check[c] at 50/64 sites; best PT lines expressed cooler canopies and larger biomass than checks (http://apps.cimmyt.org/wpgd/index.htm; [68]).
Yield potential	2nd WYCYT 35 rapid-cycle[b] PT lines	26	2014	Best PT line higher yielding (avg 10%) than best check[d] at 23/26 sites; best PT lines expressed larger biomass than checks at all sites where measured [67].
Heat stress	4th SATYN 25 rapid-cycle PT lines	24	2015	Best three PT lines higher yielding (avg 8%) than best check at 23/24 sites; best PT lines expressed larger biomass than checks at all sites where measured [69].
Yield potential	Bacanora/Weebil experimental population with 105 double haploid PT lines	9	2007–2010	Best PT lines expressed higher yield than best parent in all 9 environments, most spectacularly in S. Chile where 34% of PT lines were higher yielding, and the best PT line showed 22% higher yield than the best parent (avg 2 seasons) [16•,42].
Heat/drought	Seri/Babax experimental population with 167 recombinant-inbred PT lines	12 & 9	2002–2013	Considering yield averaged across all 12 international sites, 15 PT lines were higher yielding than the best parent by as much as 13%; Considering 9 environments in Mexico, the best 3 PT lines were higher yielding (avg 24%) than the best parent at all 9 sites [40].

Abbreviations: SAWYT, semi-arid wheat yield trial; WYCYT, wheat yield collaboration yield trial; SATYN, stress adaptive trait yield nursery; DH, doubled haploid (lines); RIL, recombinant inbred line.
[a] Advanced lines having <1% of gene loci heterozygous.
[b] Rapid cycle lines having <10% of gene loci heterozygous (i.e. products of pre-breeding).
[c] Local check = best adapted local cultivar.
[d] Best check = best performing conventional CIMMYT elite line (where superior to local check).

challenge will be to focus on markers closely linked to functional genes (exome capture for example in barley [63•]), and to define the environmental factors causing GxE. In this context, research into epigenetics suggests that it may be necessary to consider the epi-genome if GxE is to become more predictable [64].

Marker technology is used stochastically in genomic selection in animal breeding and is being evaluated for crops [65]. Some results are promising, for example, in wheat [34], and maize [66], while incorporating phenotypic data from training populations into models significantly improves prediction [13,14].

Crossing and selection

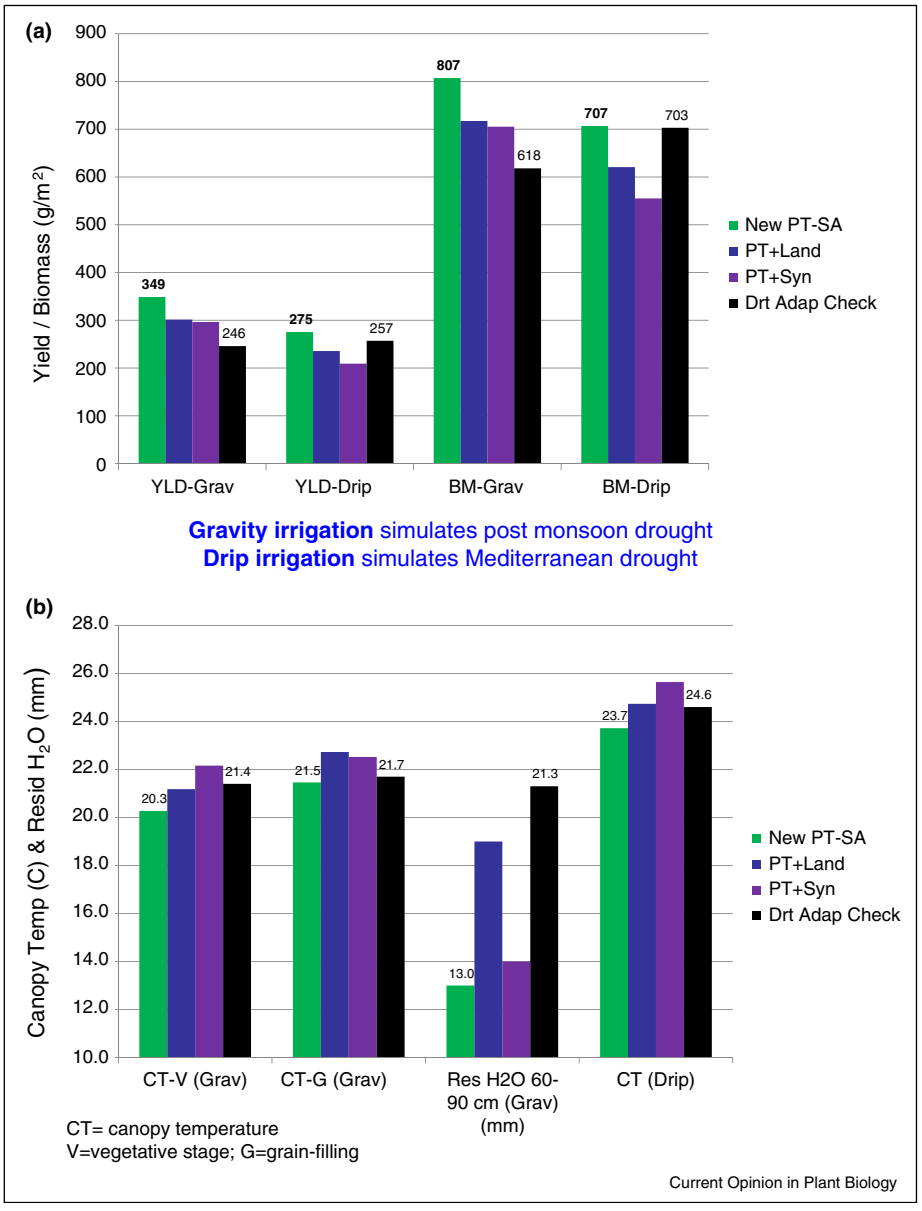
With a modest investment in some phenotyping equipment,[3] any breeding program can implement physiological trait (PT) based crossing strategies (see Graph Abst). Based on traits identified in conceptual models (e.g. [9,10,12]) and the use of simple quantitative models to estimate potentially complementary traits [11,52], systematic screening of genetic resources has been employed at CIMMYT to identify complementary parental sources for adaptive traits. For example, parents with cool canopies associated with more extensive root systems are crossed with lines expressing ability to store and remobilize stem water soluble carbohydrates under heat and drought stress (see [52] and references therein). To improve yield potential, parents with good spike fertility characteristics, for example, [26•] are crossed with sources of high radiation use efficiency [67]. Progeny selection is facilitated by remote sensing.

Such approaches have achieved impacts over conventional breeding in water stressed Australian environments [12]. CIMMYT and collaborators have achieved consistent genetic gains in heat and drought stressed environments, as well as raising yield potential [11,41,42,52,68,69] (Table 1), resulting in uptake of new lines and phenotyping methods by national programs [68]. The best new PT lines expressed the highest average yield across all sites, indicating yield stability (Table 1).

Experimental PT crosses between judiciously selected parents have also demonstrated substantial genetic gains. Double haploid progeny of the wheat cross Bacanora/Weebil — where both parents express high yield via contrasting expression of yield components — generated progeny with exceptional yield potential [42] (Table 1). Similarly, random inbred lines of the Seri/Babax wheat

[3] Infra-red thermometer for canopy temperature, Greenseeker for NDVI, lab facility to estimate aboveground dry biomass and assimilate partitioning at key growth stages [68].

Figure 3



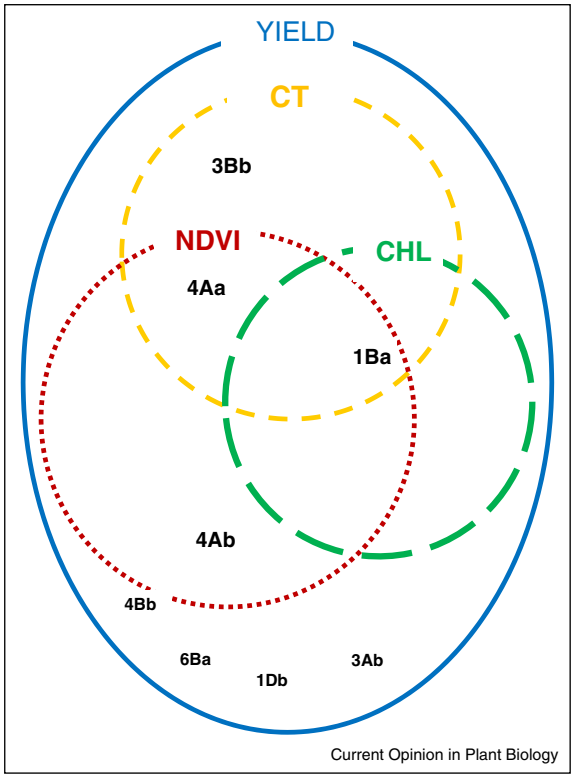
New PT line SOKOLL/3/PASTOR//HXL7573/2*BAU/4/WBLL4//OAX93.24.35/WBLL1 under two distinct drought environments: **(a)** yield and biomass under gravity and drip irrigation simulating post-monsoon stored soil moisture, and Mediterranean drought environments, respectively. **(b)** Improved water relations in new PT line showing canopy temperature in season, and residual soil moisture at harvest. Traits are compared with the drought adapted check Vorobey (from [52]). LSD values ($P < 0.05$) of genotypic differences for yield, biomass, CT, and residual soil moisture ~45 g m^2, 100 g m^2, 0.9 °C and 4 mm, respectively.

cross — chosen to combine a widely adapted parent (Seri) with a source of drought adaptive traits (Babax) — expressed superior yield to either parent across multiple sites [40] (Table 1). Results such as these lend themselves to dissection of genetic gains to refine PT-based crossing strategies.

Evaluation of genetic gains

Strategic crossing for PTs, in addition to incorporating genetic diversity for complex traits into the genepool, provides an 'acid test' for validating hypotheses about trait interactions, genetic background effects, and the value of plant ideotypes across environments. For example, among the material developed for drought adaptation, expression of the key physiological traits identified in parents — such as deep water extraction, cooler canopies, and storage of water soluble carbohydrates — were also expressed in the best progeny [36•,52]. Figure 3 shows an example of progeny from parents crossed for

Figure 4



Genetic dissection of yield by physiological traits. The Venn diagram shows how QTL for yield are shared by a number of different physiological traits contributing to its expression, including canopy temperature (CT); NDVI, and chlorophyll (CHL); weaker QTL effects for water soluble carbohydrates were common to all four [40]. Bold fonts denote QTL for yield that are stable across environments, while non-bold fonts denote environment dependant QTL for yield (adapted from [40]).

good expression (under drought) of deep water extraction in one case (purple bar), and cooler canopies (blue bar) in the other. The progeny (green bar) shows superior yield and biomass to either parent under two different drought environments, suggesting that combining both traits resulted in cumulative gene action. Another PT line, resulting from a cross between a genetic resource with good expression of stem water soluble carbohydrate (WSC), and a line adapted in terms of yield to the Mediterranean type of drought (where WSC is expected to be of most value), showed superior expression of both traits compared to either parent in the target environment [52]. It is important to keep in mind that the expression and value of any PT will be a function of the growing environment [33••] as well as its genetic background.

Determining the genetic bases of successful trait combinations helps validate cumulative gene action. Genetic dissection of the wheat cross Bacanora/Weebil described above revealed two loci influencing grain yield on chromosomes 1B and 7B, increasing grain number and grain weight, respectively [16•]. These two yield components typically show a negative correlation, however, the lack of a trade-off between them in some progeny of this cross led to extremely high yields in these lines [42], making these loci good targets for MAS [16•]. Genetic dissection of the Seri/Babax wheat population [40] showed that yield QTL under heat and drought stress were collocated with QTL for the following physiological traits: CT (three QTL), NDVI (three QTL), and chlorophyll (one QTL), demonstrating the genetic contribution of each trait to yield (Figure 4). In summary, while phenotyping can indicate the potential for cumulative gene action in contrasting genotypes, genetic dissection of physiological traits can indicate potentially favorable allelic combinations between genotypes expressing similar phenotypes. Furthermore, genetic dissection is the only definitive way to show, for example, that two apparently different traits may share a common genetic basis, and vice versa, or that two apparently valuable traits may be mutually exclusive when combined in a common background.

Conclusions

An increasingly challenging crop environment and the rapid advances in genetic technologies both call for better understanding of the physiological processes involved in achieving crop productivity, and their interaction with environment. Three new factors can help achieve this, while at the same time contributing directly to crop improvement: first, new models of improved plant processes and crop ideotypes capitalizing on more than half a century of physiological research; second, high throughput phenotyping technologies that permit evaluation of complex trait expression on a breeding scale in realistic field environments; third, renewed focus on preserving and utilizing plant genetic resources and a growing awareness that climate change will make it increasingly difficult to achieve needed genetic gains unless new allelic diversity is brought into existing genepools. Along with hypothesis driven physiological breeding and multi-location testing, these factors contribute to better genetic understanding, which itself drives the design and selection of improved cultivars. Physiological breeding as described herein is a central pillar of the newly formed International Wheat Yield Partnership (http://iwyp.org/) that aims to raise wheat yield potential closer to its biological limit, and will be important in similar initiatives of the CGIAR (e.g. the Heat and Drought Wheat Improvement Consortium) that aim to adapt crops to climate change and underpin the need for global food security.

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- •• of outstanding interest

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