

## Invited Expert Review

# An Integrated Approach to Crop Genetic Improvement<sup>□</sup>

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## Abstract

The balance between the supply and demand of the major food crops is fragile, fueling concerns for long-term global food security. The rising population, increasing wealth and a proliferation of non-food uses (e.g. bioenergy) has led to growing demands on agriculture, while increased production is limited by greater urbanization, and the degradation of land. Furthermore, global climate change with increasing temperatures and lower, more erratic rainfall is projected to decrease agricultural yields. There is a predicted need to increase food production by at least 70% by 2050 and therefore an urgent need to develop novel and integrated approaches, incorporating high-throughput phenotyping that will both increase

production per unit area and simultaneously improve the resource use efficiency of crops. Yield potential, yield stability, nutrient and water use are all complex multigenic traits and while there is genetic variability, their complexity makes such traits difficult to breed for directly. Nevertheless molecular plant breeding has the potential to deliver substantial improvements, once the component traits and the genes underlying these traits have been identified. In addition, interactions between the individual traits must also be taken into account, a demand that is difficult to fulfill with traditional screening approaches. Identified traits will be incorporated into new cultivars using conventional or biotechnological tools. In order to better understand the relationship between genotype, component traits, and environment over time, a multidisciplinary approach must be adopted to both understand the underlying processes and identify candidate genes, QTLs and traits that can be used to develop improved crops.

**Keywords:** Germplasm variation; nitrogen; TILLING; transformation; trait; water; wheat; yield.

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## Introduction

The concern for global food security results from an impending imbalance between the supply and demand of the major food crops (wheat, rice and maize). The increased demand results not only from the growing population and wealth but also from non-food uses for crops and land, notably for bioenergy feedstock. Ensuring food security is a major challenge given

the projected need to increase world food production by 40% in the next 20 years and 70% by 2050 (FAO forecasts). Although the recent global average increases in yields for wheat, rice or maize (FAO data) have been sufficient to meet this projected demand to date, future production is further threatened by urbanization, degradation of arable land and global climate change. The predicted increase in temperatures as well as decreased and more erratic rainfall as a result of global climate

change, are projected to decrease global yields of crops. A need for a substantial acceleration in crop improvements is required.

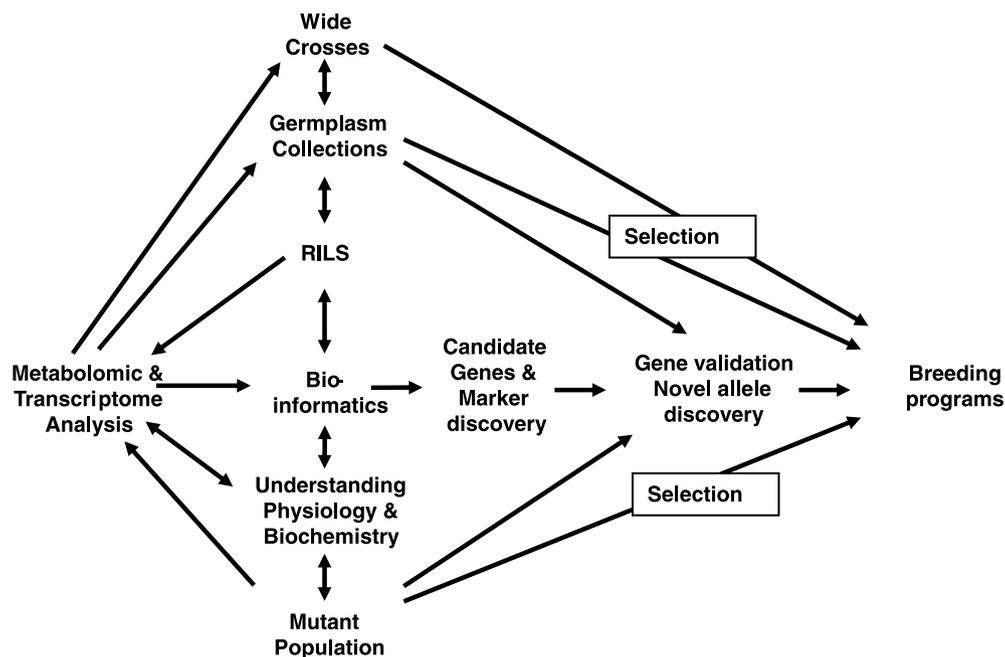
To supplement conventional breeding programs, there is an urgent need to develop novel integrated approaches that will both increase crop production per unit area and are sustainable in terms of the resource use efficiency. Increased productivity can be achieved both through plant breeding and improvements in agronomy to increase both the yield potential and the actual yields achieved by farmers, minimizing losses due to biotic and abiotic stress. Improvements in agronomic practice alone are unlikely to allow us to meet projected world food demands and genetic gains will be required in addition to agronomic improvements (Leegood et al. 2010).

Many of the traits (yield, yield stability, nutrient and water use) important in crop productivity and sustainability are complex and multigenic traits. While there is genetic variability in these traits, they are often difficult to breed for. Nevertheless molecular plant breeding has the potential to deliver improvements, once the component traits and the genes underlying these traits have been identified. These can then be incorporated into new cultivars using conventional or biotechnological tools. In order to better understand the relationship between genotype, component traits, and environment over time we are adopting a multidisciplinary approach to both understand the underlying processes and identify candidate genes, QTLs and traits that

can be used to develop improved crops. In this review we present 'an integrated approach to crop genetic improvement' and focus primarily on traits related to yield potential. In addition to classical visual and performance-related phenotyping and selection, the integrated approach encompasses detailed omics technologies, specific expertise in biochemistry and physiology with all of the tools of modern molecular breeding, including, increasingly, genome sequence information (see **Figure 1**). We fully recognize the importance of both abiotic and stress tolerance but they are outside the scope of this review.

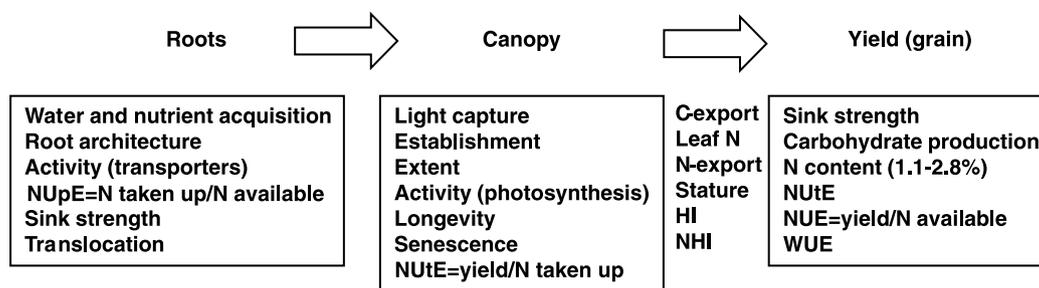
## Traits

The complex traits relating to resource use efficiency (RUE), whether in reference to light, water or nutrients may be subdivided into tractable processes; this may be at the level of crop performance, plant anatomy and physiology or cellular biochemistry including pathway functions, or individual proteins and enzymes (**Figure 2**). Ultimately the goal is the identification of the alleles or genes that encode these traits or components. An apparently clear divide exists between capture and use efficiency but in fact both are complex and often interacting components. Pathways involved in, for example, photosynthetic efficiency will depend on nutrient acquisition efficiency (e.g. to provide the N for canopy formation), and conversely nutrient use efficiency depends upon photosynthetic efficiency



**Figure 1. Trait analysis, gene function and outputs.**

A flow diagram indicating the linkages between multidisciplinary basic research, crop improvement and delivery to breeding programs.



**Figure 2. Yield depends upon efficient capture and use of resources.**

Complex agronomic traits can be broken down into subtraits, still complex but based on existing physiological and biochemical knowledge, facilitating the identification of molecular targets for breeding.

for yield production as well as the breakdown of the physical components of this trait to enable nutrient recycling.

### Carbon assimilation

The cumulative rate of photosynthesis over the growing season is the primary determinant of crop biomass.  $CO_2$  enrichment experiments have clearly demonstrated that increasing photosynthesis will increase crop yields provided that other constraints do not become limiting (Ainsworth and Long 2005). Crop photosynthesis, and biomass, is dependent on (i) the ability of the canopy to intercept and capture light; (ii) the duration of light capture; and (iii) the photosynthetic capacity and efficiency of the canopy (Parry et al. 2011). Each of these is determined by a range of component traits and all of these are valid targets for crop improvement. However, under conventional high-input systems, canopy architecture in the major crops has already been optimized for light capture and there are few obvious opportunities for further improvements (Horton 2000). In some environments, provided water is not limited, there is the potential to extend the duration of photosynthesis by improving the rate of early leaf area growth or introducing 'functional stay-green' phenotypes (Dohleman et al. 2009; Dohleman and Long 2009). In  $C_3$  crops, the largest potential benefits would be achieved through increasing the photosynthetic rate per unit leaf area (Raines et al. 2006; Long et al. 2006; Parry et al. 2007, 2011). Most often the concentration of  $CO_2$  inside the leaf is much lower than the outside concentration. The assimilation rate versus internal  $CO_2$  concentration (Figure 3) illustrated in (A) the potential benefit to photosynthetic rate of any selection or modification that increases the internal  $CO_2$  concentration and in (B) the benefit of enhanced RuBP regeneration and the replacement of the wheat Rubisco with that from *Limonium gibertii* (Parry et al. 2011). Zhu et al. (2008) calculated that the theoretical photosynthetic energy conversion efficiency of  $C_3$  plants is about 4.6%, but in the field the energy conversion efficiency is only 30% of this value. Therefore there is substantial room to increase photosynthetic

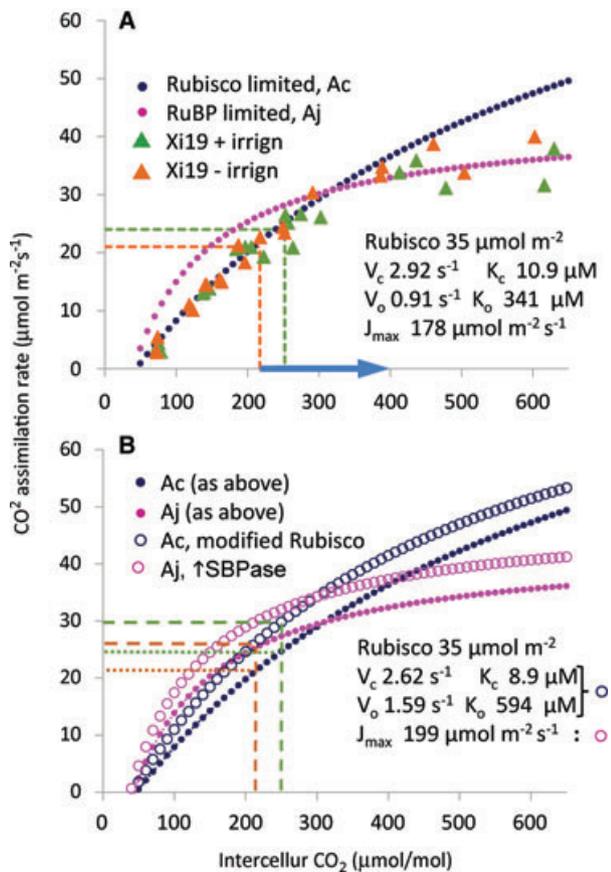
energy conversion efficiency in the field (Reynolds 2000; Zhu et al. 2008).

### Water

Over much of the land surface of the globe the availability of water is the major limitation to crop productivity. Where water supply is inadequate it may be provided by irrigation; up to 70% of available water is used in agriculture. However, this is not sustainable because fossil water reserves are being exploited more quickly than they are being replenished and climate change is projected to not only decrease the amount of rainfall but also the crops' demand by increasing transpiration. While numerous plant species are adapted to cope with limited water availability, they are generally not that productive. High productivity and water use efficiency requires the assimilation of  $CO_2$  without losing too much water through transpiration. Crop productivity is dependent on (i) the ability to extract water from the soil; (ii) the duration of water extraction and (iii) the efficiency with which the water is used (Parry et al. 2005). These traits are determined by several component traits, each of which is a valid target for crop improvement. Molecular breeding should best focus on constitutive traits that give good performance under drought stress. Traits related to root architecture (root angle, root distribution and density) and function (the ability to penetrate drying soils and extract water) are often difficult to assess under field conditions but are obvious targets for both selection and manipulation. Canopy traits that relate to sustaining high photosynthetic rate at low stomatal conductance are also important.

### Nutrition

Nitrogen is a major determinant of crop yield and an essential contributor to quality where protein is a desired trait. Nitrogen is, however, expensive to produce, distribute and apply, resulting in a large environmental (carbon) footprint, exacerbated by the potential of damaging greenhouse gas nitrogen emissions from



**Figure 3. Dependence of measured and modeled photosynthetic rates on intercellular  $\text{CO}_2$  concentrations ( $C_i$ ).**

The Rubisco-limited ( $A_c$ ) and electron-transport limited ( $A_j$ ) rates of  $\text{CO}_2$  assimilation for wheat are represented as blue and red (solid) circles, respectively, derived from the kinetic constants of Carmo-Silva et al. (2010) and the biochemical model of Farquhar et al. (1980). The maximal electron transport rate ( $178 \mu\text{mol}/\text{m}^2$  per s) predicted assimilation rates that resembled the data points above  $300 \mu\text{mol CO}_2/\text{mol}$  air. Other assumptions were: Rubisco content ( $35 \mu\text{mol}/\text{m}^2$ ), dark respiration rate ( $1.2 \mu\text{mol}/\text{m}^2$  per s), saturating light ( $2000 \mu\text{mol PAR}/\text{m}^2$  per s for measurements),  $210 \text{ mbar O}_2$  and intracellular  $\text{CO}_2$  conductance non-limiting. The actual rate of assimilation is the lower of the two values ( $A_c$  or  $A_j$ ) at any  $C_i$ . (A) Data for wheat cultivar Xi19 grown with (+) and without (-) irrigation under field conditions. The potential benefit to photosynthetic rate of any selection or modification that increases the intercellular  $\text{CO}_2$  concentration (see blue arrow) towards the ambient (about  $400 \mu\text{mol CO}_2/\text{mol}$ ) is apparent. (B) Predicted effect of replacing wheat Rubisco with that from *Limonium gibertii* in combination with a 12% increase in the electron transport rate, resulting from overexpression of sedoheptulose 1,7-bisphosphatase (SBPase).

ill-managed inputs. Efficient use of nitrogen is an essential trait, but one that cannot be considered in isolation. Nitrogen use efficiency (NUE) may be defined as the yield as a function of available nitrogen, and therefore improvements in yield will implicitly deliver enhanced NUE; this may, however, be at the expense of quality if the increased yield (starch) dilutes the protein, or indeed any other nutritional component; clearly an 'integrated' approach is required when considering this trait.

The NUE trait comprises a component relating to capture efficiency, and a component relating to conversion into useable biomass (Figure 2). In addition, harvest index and nitrogen harvest index are important parameters for ensuring optimal resource, especially nitrogen use efficiency. Grain total protein content and composition of protein has a fundamental influence on quality parameters of wheat flour. Finally, while there is generally an inverse relationship between grain yield and grain protein due to the dilution phenomenon described above, on the other hand, some varieties show exceptional combinations of yield and protein, a highly desirable trait that has been referred to as grain protein deviation. Genetic variation exists in all of these traits and component traits and improvement strategies need to clearly define the targeted components and identify specific genetic variation in each, as well as environmental interactions. Effective evaluation of nutritional traits requires field experimentation for the full expression of the relevant phenotypes. Furthermore, year to year seasonal variation can have the strongest influence on trait expression, which is both a useful and valuable experimental parameter, as well as a hindrance in terms of the need for replication. Stability of traits is a highly desirable attribute with huge economic implications.

In addition to nitrogen, crops require between 13 and 15 other nutrients in variable proportions (Hawkesford and Barraclough 2011). Natural occurrence varies considerably, and while the major macronutrients (N, P, K and S) are common constituents of fertilizers, other essential minerals may also be present in suboptimal amounts, may be biologically unavailable, or may be present in excess, which may also have negative consequences. Of particular concern is the consumption of non-renewable reserves of key fertilizers such as phosphorus (Cordell et al. 2009). Germplasm selection for more efficient varieties can go some way to alleviate the impending problem but management and resource recycling will also be required. The molecular and physiological basis of efficient nutrient use in crops has been summarized in a volume containing reviews of specific aspects of nitrogen nutrition as well as a systematic analysis of other nutrient efficiencies (Hawkesford and Barraclough 2011).

## Germplasm Availability and Selection (G)

Underpinning all crop improvement is the availability of the appropriate germplasm. This is self-evident for traditional crop

breeding approaches that seek to combine together the best genes/alleles and express them in elite varieties. However, in addition, knowledge gained from wide germplasm screening will inform on requirements for novel genes/alleles to be introduced through non-classical means, such as gene transformation (see below). Whatever the means of implementation, the basic requirement is the access to suitable genetic material.

Improvements in genotyping have encouraged a resurgence in QTL (quantitative trait loci) approaches. In the UK, a single population (Avalon × Cadenza) has been widely used to assess multiple traits; the germplasm and associated map have been made publically available by the John Innes Centre and many research projects have focused on a diversity of traits. The encouragement of early release of the phenotyping data allows rapid comparison and identification of overlapping loci. Furthermore, as it is feasible and preferable to produce multiple mapping populations to target traits, and for example, the Avalon × Cadenza population along with others has been in 'meta' analyses, which provides greater resolution and confidence in identified loci (Griffiths et al. 2009, 2012).

Mutagenesis has been widely used to generate new variation in genomes. Mutations may be generated by irradiation or chemical treatment; the changes can result in large scale deletions of DNA, or may only involve point mutations. While most often the performance of mutants is generally inferior to the wild type, occasionally lines with improved performance with respect to any trait can be selected. Given the simplicity of the approach and ease of mutating and screening large numbers of individuals, many new crop varieties derived from mutagenesis programs have been released (Parry et al. 2010). Although in polyploid species genetic redundancy may make it difficult to identify useful variation on the basis of phenotype, a number of reverse genetic approaches have been developed to identify point mutations and small indels in target genes (see e.g. high-resolution melt analysis (Ririe et al. 1997), endonuclease cleavage for detection of mismatches in heteroduplexes (Oleykowski et al. 1998), and most recently by next-generation sequencing (Tsai et al. 2011)). Mutations in individual homoecologs can then be combined by crossing to investigate the effect on the trait, although some backcrossing to remove excessive extraneous mutations is usually required. Such reverse genetics techniques facilitate the investigation of gene function and the development of novel alleles of genes that have effects on important traits. Mutagenesis also has the advantage over RNAi and similar methods of being able to target individual members of gene families and therefore potentially target gene function in specific tissues and at specific times.

Gene function, and the effect of novel genes for crop improvement can be assessed by gene transformation. Transformation protocols have been developed for wheat, (Vasil

et al. 1993), rice (Christou et al. 1991), maize (Gordon-Kamm et al. 1990) and most other crop species. However, there is still substantial scope to improve their efficiency. In addition, the availability of promoters to target gene expression to specific times, organs, cell types or in response to environmental cues will further refine and expand capabilities. Transgenic crops with resistance to herbicides, pests or quality traits are widely commercialized. However, despite the optimistic predictions, examples of transgenic plants with improved traits for resource use efficiency leading to increased yield are not yet available (Sinclair et al. 2004). The identification of the key genes for the agriculturally important traits remains the major limitation. Importantly it is not usually sufficient to simply identify a single gene associated with a trait, but it is also necessary to understand how the gene is regulated in concert with the rest of the pathway and what pleiotropic effects may affect performance.

## The Environmental Component (E)

Screening germplasm with appropriate tools involves not only phenotyping and genotyping technology but also the appropriate environmental conditions. While some simple traits like coleoptile length (Wang et al. 2009) can be meaningfully assessed in controlled environment facilities or glasshouses, screening of others requires field conditions. Unfortunately fully replicating the field environment in controlled conditions is not yet possible. The greatest difficulty lies in the provision of a substrate for growth; most crop species produce roots that extend >1 m below ground and have complex responses to soil structure and composition. Furthermore, both water and nutrient availability vary during the growth cycle due to natural seasonal variation and may have variable spatial distributions. In some cases mature traits may be inferred from proxy measurements, but this is never ideal; specific exceptions for simple traits may exist, for example arsenic tolerance (Lee et al. 2003), where the screening for toxic mineral sensitivity or salinity tolerance of seedlings may be achieved in pot-based systems, but generally most screening is, and should be, field based. Field conditions provide an integrated environment relevant to whole crop development to maturity. Most breeding programs are for specific climatic regions, although there is a strong case for using overlapping varieties in separate programs in trials to aid in comparisons, although the problem of trialing non-locally adapted material is not facile. Such an approach is used by the CIMMYT and ICARDA wheat programs, which shuttle breeding lines between diverse and stressful environments, which has the additional value in facilitating multiple generations per year. However in most climatic regions there can be huge year to year variation, necessitating multiple year trials at any one site (e.g. Barraclough et al. 2010).

For individual trials the most important elements, apart from suitable replication and randomization, are plots of adequate size for destructive sampling and accurate final yield determination. Crop performance comprises multiple complex traits including yield and resource use efficiency, and these may be broken down into subtraits at many levels. Even a basic resolution of traits indicates wide variation and independent rankings of performance: for example performance in terms of yield, NUE and its component sub-traits for trials undertaken from 2003 to 2006 have been published recently as part of

the WGIN (Wheat Genetic Improvement Network) program funded by Defra (UK Department of Environment, Food and Rural Affairs) (Barraclough et al. 2010). The WGIN project at Rothamsted was initiated in 2003 and evaluates a number of traits including nitrogen use efficiency for a range of commercial wheat germplasm (>40 varieties to date, with a common core subset grown every year). Independent significant variation was seen for NUE component sub-traits and varieties ranked in terms of performance (Figure 4). It is clear that while some varieties perform in the uppermost quartile for some traits, no

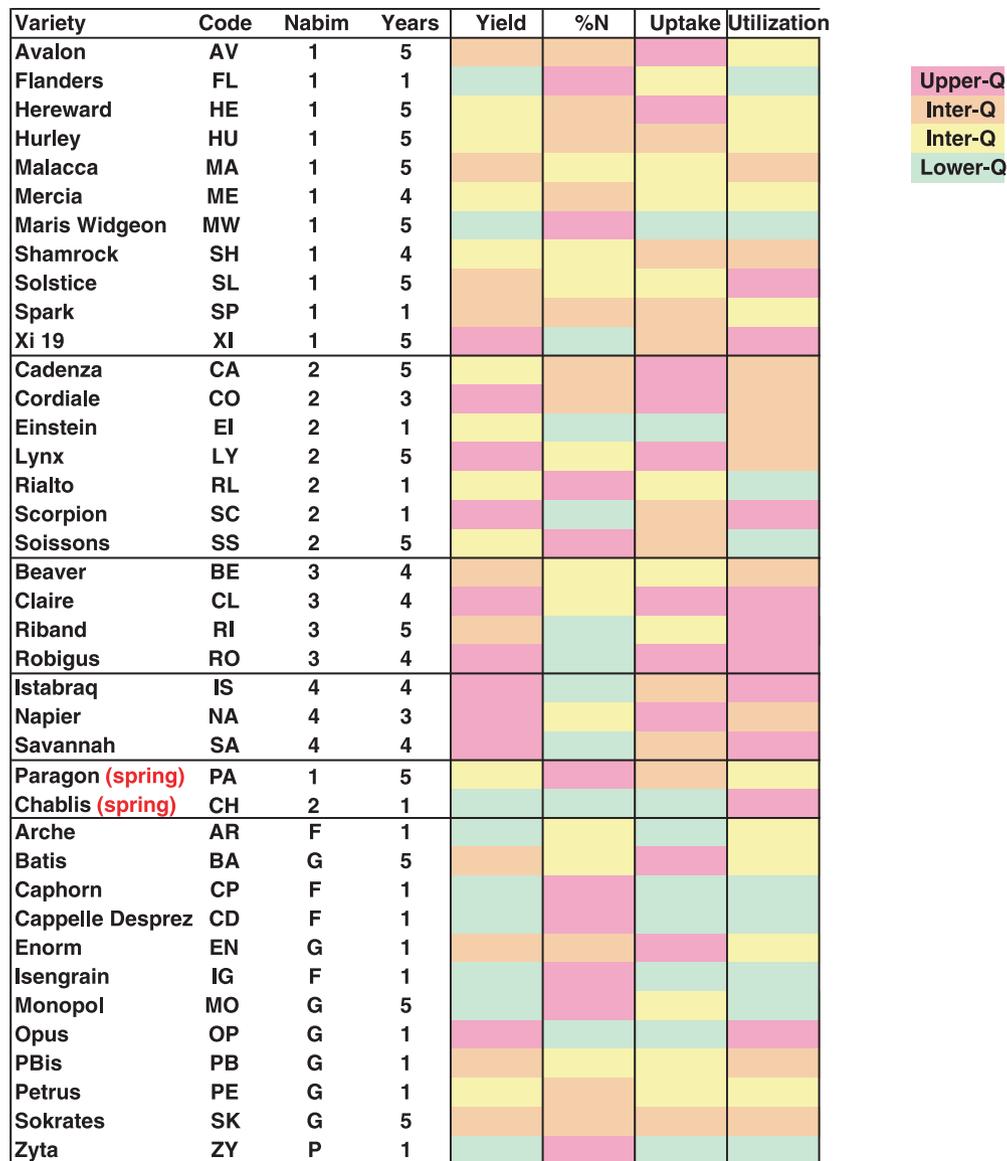


Figure 4. Ranking of agronomic trait performance amongst 39 elite wheat cultivars.

Rankings are quite distinct for each trait: individual varieties perform best for some traits but not others. Deconvoluting traits even at this broad level indicates opportunities to combine best performance. Performance is indicated by quartiles (pink, orange, yellow and green colors represent highest to lowest quartiles, respectively). Used with permission from Barraclough et al. 2010.

varieties perform ideally for all traits: independent selection and then the combination of the different traits is required. An aerial view of this trial in 2011 is shown in **Figure 5**.

### Base phenotyping

The key integrative base measurement of crop performance is yield, for wheat, specifically grain yield. For selection in breeding programs it is typical to break this down into the key yield components: grain size, number, tiller number, grain per ear, etc. Another critical component is harvest index (HI), i.e. the proportion of total yield, which is the usefully harvested product, e.g. grain yield as a proportion of total biomass. HI also has implications with regard to all mineral nutrient use efficiency including nitrogen, and consequences for key nutritional quality traits (high Fe, Zn, Se etc.). All of these key measurements may be made at final harvest, together with measurements of protein (often measured as total N), minerals and other phytochemicals. Such phenotyping is slow and expensive and can only be accomplished after final harvest. A key goal is to have proxies for these key measurements,

facilitating early indications of performance and accelerating selection timescales. Examples may be measurements of canopy development by NDVI (normalized difference vegetation index) or similar indexes, quantification of early anatomical indicators such as tiller number, or early diagnostic/prognostic chemical measures of crop performance, for example S content (Blake-Kalff et al. 2000) or stem carbohydrate content (Ruuska et al. 2008). Spectral reflectance has been used to evaluate nitrogen nutritional indexes (Mistele and Schmidhalter 2008), and potentially, when combined with hyper spectral imaging (including NIR spectroscopy) and chemiometric calibrations, could be extended to measurement of many other parameters (Batten 1998). Such proxies would have direct positive benefits in terms of cost and timescale of breeding programs.

### Deep phenotyping

Behind all yield components is the underpinning biochemistry determining resource (light, water, nutrients) capture and use. Traditional approaches focus on single target aspects of physiology and biochemistry, and although definitive, are



**Figure 5. An aerial photo of germplasm trials at Rothamsted in 2011.**

Two trials are shown: in the foreground is the Defra sponsored WGIN (Wheat Genetic Improvement Network) Diversity Trial, comprising 25 modern varieties grown at four nitrogen levels in a randomized block design. In the top left is the Biotechnology and Biological Sciences Research Council (BBSRC) sponsored Wheat Prebreeding Long and Large Project (LOLA) trial of 240 diverse elite and land race wheats, grown at two nitrogen levels in triplicate. In both cases basic and advanced phenotyping is applied to dissect yield traits.

highly labor intensive, and perhaps even more critically, seldom reflect the integrated picture; while it may be possible to see a biochemical/physiological response on a single plant in the greenhouse, this may not be apparent in a field crop stand.

Variation in key characteristics may have developmental components and therefore measurements are ideally recorded continuously; however, screening may usefully and more practically be performed at specific developmental stages. While meaningful screening at very immature stages (prior to canopy closure) may be desirable, such analyses may give misleading indications of enhanced performance, not achievable in a typical mature crop stand for most traits. An exception is clearly seedling-specific traits such as seedling cold-hardiness. Similarly, measurement of individual plants, as discussed above, also may not reflect performance.

An optimal alternative approach is the deployment of high-throughput phenotyping systems, either static setups in controlled environments or preferably mobile units in the field (Furbank and Tester 2011). The latter especially facilitates deep phenotyping in a relevant situation. Such analyses will typically include a visual imaging and detailed image analysis to extract key yield component information, spectral measurements for NVDI and chemical analysis as detailed above, and fluorescence measurements for high-throughput estimations of photosynthetic capability (Scholes and Rolfe 2009; Doughty et al. 2011).

### Omics

In addition to detailed morphological, physiological and targeted biochemical approaches, non-biased omics approaches are an essential component of a fully integrated analysis. Such approaches including transcriptomics and metabolomics have been successfully applied to crop systems, including at the field trial scale (Lu et al. 2005; Howarth et al. 2008; Wan et al. 2009). Snapshots of expression or metabolite profiles are extremely informative and can provide new and unexpected leads for crop improvement; however interpretation must be in the context of wider phenotyping. Again, while such approaches are time consuming and costly, they serve to provide essential underpinning mechanistic knowledge of traits and will potentially provide new diagnostic and prognostic markers for crop performance.

### Data Integration and Management

Integrated approaches generate huge datasets often comprising quite different formats and types of information. It is therefore absolutely essential that these are combined in a useful way and can be exploited to understand the interactions between the component traits underlying plant performance in the field. Bioinformatic approaches struggle to integrate multiple omic datasets, although there are numerous examples

of combining transcriptomics and metabolomics (for example, Ghassemian et al. 2006). Tools such as Ondex combine the datasets with a wider knowledge base (Köhler et al. 2006) facilitating interpretation. The challenge for plant breeding is to adopt these tools and handle the added dimension of large numbers of genotypes. A recent summary of tools for bioinformatics and omics datasets relating to crops including Poaceae, Solanaceae, Fabaceae highlights the multitude of developing tools (Mochida and Shinozaki 2011).

### Prospects

The complex traits underlying yield, both in terms of production and crop protection, require multi-disciplinary approaches to facilitate de-convolution and targeted breeding. The combination of advanced phenotyping, genomics and employment of multiple environments (geographic or year) for selection provide the required tools to identify novel targets for crop improvement. Sourcing germplasm from diverse regions and historic collections will provide the novel genes and alleles required for future crops.

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### References

- Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. *New Phytol.* **165**, 351–372.
- Barracough PB, Howarth JR, Jones J, Lopez-Bellido R, Parmar S, Shepherd CE, Hawkesford MJ (2010) Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. *Eur. J. Agron.* **33**, 1–11.
- Batten GD (1998) Plant analysis using near infrared reflectance spectroscopy: The potential and limitation. *Aust. J. Exp. Agric.* **38**, 697–706.
- Blake-Kalff MMA, Hawkesford MJ, Zhao FJ, McGrath SP (2000) Diagnosing sulphur deficiency in field-grown oilseed rape (*Brassica*

- napus* L) and wheat (*Triticum aestivum* L.). *Plant Soil* **225**, 95–107.
- Carmo-Silva AE, Keys AJ, Andralojc PJ, Powers SJ, Arrabaca MC, Parry MAJ** (2010) Rubisco activities: Properties and regulation in three different C<sub>4</sub> grasses under drought. *J. Exp. Bot.* **61**, 2355–2366.
- Christou P, Ford TL, Kofron M** (1991) Production of transgenic rice (*Oryza sativa* L.) plants from agronomically important indica and japonica varieties via electric discharge particle-acceleration of exogenous DNA into immature zygotic embryos. *Nat. Biotechnol.* **9**, 957–962.
- Cordell D, Drangert J-O, White S** (2009) The story of phosphorus: Global food security and food for thought. *Global Environ. Change* **19**, 292–305.
- Dohleman FG, Heaton EA, Leakey DB, Long SP** (2009) Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of Miscanthus relative to switchgrass. *Plant Cell Environ.* **31**, 1525–1537.
- Dohleman FG, Long SP** (2009) More productive than maize in the Midwest: How does Miscanthus do it? *Plant Physiol.* **150**, 2104–2115.
- Doughty CE, Asner GP, Martin RE** (2011) Predicting tropical plant physiology from leaf and canopy spectroscopy. *Oecologia* **165**, 289–299.
- Farquhar GD, von Caemmerer S, Berry JA** (1980) A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta* **149**, 78–90.
- Furbank RT, Tester M** (2011) Phenomics – technologies to relieve the phenotyping bottleneck. *Trends Plant Sci.* **16**, 635–644.
- Ghassemian M, Lutes J, Tepperman JM, Chang HS, Zhu T, Wang X, Quail PH, Lange BM** (2006) Integrative analysis of transcript and metabolite profiling data sets to evaluate the regulation of biochemical pathways during photomorphogenesis. *Arch. Biochem. Biophys.* **448**, 45–59.
- Gordon-Kamm WJ, Spencer TM, Mangano ML, Adams TR, Daines RJ, Start WG, O'Brien JV, Chambers SA, Adams WR Jr., Willetts NG, Rice TB, Mackey CJ, Krueger RW, Kausch AP, Lemaux PG** (1990) Transformation of maize cells and regeneration of fertile transgenic plants. *Plant Cell* **2**, 603–61.
- Griffiths S, Simmonds J, Leverington M, Wang Y, Fish L, Sayers L, Alibert L, Orford S, Wingen L, Herry L, Faure S, Laurie D, Bilham L, Snape J** (2009) Meta-QTL analysis of ear emergence in elite European winter wheat germplasm. *Theor. Appl. Genet.* **119**, 383–395.
- Griffiths S, Simmonds J, Leverington M, Wang Y, Fish L, Sayers L, Alibert L, Orford S, Wingen L, Snape J** (2012) Meta-QTL analysis of the genetic control of crop height in elite European winter wheat germplasm. *Mol. Breed.* **29**, 159–171.
- Hawkesford MJ, Barraclough PB** (2011) *The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops*. Wiley-Blackwell, Chichester, UK.
- Horton P** (2000) Prospects for crop improvement through the genetic manipulation of photosynthesis: Morphological and biochemical aspects of light capture. *J. Exp. Bot.* **51**, 475–485.
- Howarth JR, Parmar S, Jones J, Shepherd C, Carol C, Galster A, Hawkins N, Miller S, Baker J, Ward J, Beale M, Verrier P, Barraclough P, Hawkesford MJ** (2008) Co-ordinated expression of amino acid metabolism in response to N and S deficiency during wheat grain filling. *J. Exp. Bot.* **59**, 3675–3689.
- Köhler J, Baumbach J, Taubert J, Specht M, Skusa A, Rüegg A, Rawlings C, Verrier P, Philippi S** (2006) Graph-based analysis and visualization of experimental results with ONDEX. *Bioinformatics* **22**, 1383–1390.
- Lee DA, Chen A, Schroeder JI** (2003) *Ars1*, an Arabidopsis mutant exhibiting increased tolerance to arsenate and increased phosphate uptake. *Plant J.* **35**, 637–646.
- Leegood RC, Evans JR, Furbank RT** (2010) Food security requires genetic advances to increase farm yields. *Nature* **464**, 831.
- Long SP, Zhu XG, Naidu SL, Ort DR** (2006) Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* **29**, 315–330.
- Lu C, Hawkesford MJ, Barraclough PB, Poulton PR, Wilson ID, Barker GL, Edwards KJ** (2005) Markedly different gene expression in wheat grown with organic or inorganic fertilizer. *Proc. Roy. Soc. Ser. B* **272**, 1901–1908.
- Oleykowski CA, Mullins CRB, Godwin AK, Yeung AT** (1998) Mutation detection using a novel plant endonuclease. *Nucl. Acids Res.* **26**, 4597–4602.
- Mistele B, Schmidhalter U** (2008) Estimating the nitrogen nutritional index using spectral canopy reflectance measurements. *Eur. J. Agron.* **29**, 184–190.
- Mochida K, Shinozaki K** (2011) Advances in omics and bioinformatics tools for systems analyses of plant functions. *Plant Cell Physiol.* **52**, 2017–2038.
- Parry MAJ, Flexas J, Medrano H** (2005) Prospects for crop production under drought: Research priorities and future directions. *Ann. Appl. Biol.* **147**, 211–226.
- Parry MAJ, Madgwick PJ, Carvahlo JFC, Andralojc PJ** (2007) Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. *J. Agric. Sci.* **145**, 31–43.
- Parry MAJ, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabou H, Labhili M, Phillips AL** (2009) Mutation discovery for crop improvement. *J. Exp. Bot.* **60**, 2817–2825.
- Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu X-G, Price GD, Condon AG, Furbank R** (2011) Raising yield potential of wheat: (II) Increasing photosynthetic capacity and efficiency. *J. Exp. Bot.* **62**, 453–468.
- Raines CA** (2006) Transgenic approaches to manipulate the environmental responses of the C<sub>3</sub> carbon fixation cycle. *Plant Cell Environ.* **29**, 331–339.
- Reynolds MP, Maarten van Ginkel M, Ribaut J-M** (2000) Avenues for genetic modification of radiation use efficiency in wheat. *J. Exp. Bot.* **51**, 459–473.

- Ririe KM, Rasmussen RP, Wittwer CT** (1997) Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal. Biochem.* **245**, 154–160.
- Ruuska SA, Lewis DC, Kennedy G, Furbank RT, Jenkins CLD, Tabe LM** (2008) Large scale transcriptome analysis of the effects of nitrogen nutrition on accumulation of stem carbohydrate reserves in reproductive stage wheat. *Plant Mol. Biol.* **66**, 15–32.
- Scholes JD, Rolfe SA** (2009) Chlorophyll fluorescence imaging as tool for understanding the impact of fungal diseases on plant performance: A phenomics perspective. *Funct. Plant Biol.* **36**, 880–892.
- Sinclair TR, Purcell LC, Sneller CH** (2004) Crop transformation and the challenge to increase yield potential. *Trends Plant Sci.* **9**, 70–75.
- Tsai H, Howell T, Nitcher R, Missirian V, Watson B, Ngo KJ, Lieberman M, Fass J, Uauy C, Tran RK, Khan AA, Filkov V, Tai TH, Dubcovsky J, Comai L** (2011) Discovery of rare mutations in populations: TILLING by sequencing. *Plant Physiol.* **156**, 1257–68.
- Vasil V, Srivastava V, Castillo AM, Fromm ME, Vasil IK** (1993) Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos. *Bio-Technol.* **11**, 1553–1558.
- Wan Y, Underwood C, Toole G, Skeggs P, Zhu T, Leverington M, Griffiths S, Wheeler T, Gooding M, Poole R, Edwards KG, Gezan S, Welham S, Snape J, Mills ENC, Mitchell RAC, Shewry PR.** (2009) A novel transcriptomic approach to identify candidate genes for grain quality traits in wheat. *Plant Biotechnol. J.* **7**, 401–410.
- Wang J, Chapman SC, Bonnett DG, Rebetzke, GJ** (2009) Simultaneous selection of major and minor genes: Use of QTL to increase selection efficiency of coleoptile length of wheat (*Triticum aestivum* L.). *Theoret. Appl. Genet.* **119**, 65–74.
- Zhu X-G, Long SP, Ort DR** (2008) What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Curr. Opin. Biotechnol.* **19**, 153–159.

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