Challenges in breeding for yield increase for drought

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Crop genetic improvement for environmental stress at the molecular and physiological level is very complex and challenging. Unlike the example of the current major commercial transgenic crops for which biotic stress tolerance is based on chemicals alien to plants, the complex, redundant and homeostatic molecular and physiological systems existing in plants must be altered for drought tolerance improvement. Sophisticated tools must be developed to monitor phenotype expression at the crop level to characterize variation among genotypes across a range of environments. Once stress-tolerant cultivars are developed, regional probability distributions describing yield response across years will be necessary. This information can then aid in identifying environmental conditions for positive and negative responses to genetic modification to guide farmer selection of stress-tolerant cultivars.

Genetic challenge of drought stress

There have been tremendous advances in understanding the physiology, biochemistry and molecular genetics of plant response to drought in the past half-century. Increasing numbers of papers are being published reporting new insights about the factors contributing to increased plant development and growth rates [1]. Yet, this information has had little or no impact to date in improving the intrinsic behavior of plants resulting in increased crop yield [2–5]. For example, the two highly visible, successful transgenic crops – glyphosate tolerance and Bacillus thuringiensis (Bt) insect tolerance – ‘defend’ against pest-mediated yield loss, but do not address fundamental, intrinsic plant performance that would lead to improved crop response to yield-limiting abiotic factors. Indeed, these two commercial transgenes do not rely on acceleration or suppression of fundamental biochemical or physiological pathways altering plant development or growth – they are based on specific chemicals that are alien to plants and reside outside the contributory mechanisms leading to crop yield. These two cases do not offer a model for improving crop performance when subjected to environmental stresses, including drought.

Alteration of an entire synthetic process in plants to get adequate changes at whole plant and field levels is extremely difficult, as proved in nearly a half century of physiological studies [5,6]. Many individual processes, each influenced by a myriad of reactions and molecular controls, contribute and integrate to affect whole plant performance. This is certainly true for drought-adaptive traits [7]. The ‘bottom–up’ approach, starting at the molecular level, is often promoted as a simple and direct means for achieving major yield gains. However, plant modifications via transformation of an existing molecular pathway will likely flounder, given the vast number of interacting biochemical networks and the damping of any simple molecular change when scaling-up the complex physiological hierarchy leading to yield. The route to yield-change expressed by crops is different for each geographical location and new growing season, reflecting new combinations and temporal dynamics in the biotic and abiotic environments [1]. Certainly the DNA code may carry information that influences alternate plant responses to each new environment, but how that information is integrated throughout the growing season at the physiological level and across the community of plants are the critical determinants of crop yield.

An alternative to the bottom–up perspective is a ‘top–down’ approach that starts with a whole-crop perspective to consider how the plant community may perform in the field across a range of environments. What traits can be altered in individual plants to result in an increased yield by a community of such altered plants that is a crop? The advantage of the top–down approach is that the starting point is the performance of intact crop plants that can be studied for expression of a desired behavior, instead of an abstract extrapolation of what a specific molecular-level transformation might contribute at the whole crop level.

The top–down view may sound a bit old fashioned, but it must be remembered that it is the use of field experiments and careful observations that have enabled successful selection and genetic gain for increased crop yield. Indeed, numerous studies have demonstrated yield to be genetically complex and under control of many genes of small impact. The challenge is to integrate the modern tools of molecular genetics and physiology to bear on a practical approach for yield advances in the field [8]. How can these new insights be used to improve plant phenotype, thereby increasing crop yield? Can this new information be used in genotype selection to alter the expression of specific traits and achieve yield increase? The answers to these critical questions are not resolved. There are only a few examples where the top–down approach has been successfully applied to intrinsically alter biochemical or physiological activity to increase crop yield [2,3]. The few examples resulting in cultivar releases with increased yield based on this approach include heat tolerance in cowpea (Vigna unguiculata) [9], improved water use in wheat (Triticum
aestivum) [10], and drought tolerance of nitrogen fixation in soybean (Glycine max) [11] which is the example considered in more detail later in this paper.

Here I discuss four topics concerning the application of molecular and physiological knowledge that may help to resolve these questions for improving adaptation to drought: (i) assessing potential yield benefit and variation within a germplasm population for a proposed drought trait, (ii) developing suitable means of phenotyping the specific trait of interest, (iii) developing techniques to track expression of a desired trait through the breeding process, and (iv) creating new criteria for cultivar selection and marketing. While the focus is specially on developing crop drought tolerance, it is anticipated that many of the conclusions of this discussion apply to other abiotic stresses.

Assessment of benefit
It is necessary to move beyond intuitive anticipation of a significant benefit from a putative trait before launching a major research program to develop an improved cultivar. Intuition is not a reliable guide in the face of the complexity of response to soil drying, and it gives no insight about the amount of yield change that might be expected. An example illustrating the failure of intuition in developing drought tolerance is the substantial investment in transgenic lines that display tolerance under severe water deficit. Experiments are reported that impose drought stresses resulting in the death or near death of wild-type and survival of transgenic sister lines. The problem is that simply enhancing survival of grain crops is irrelevant in commercial production. Any situation where water availability is so low to threaten survival means that even if the plants survive, crop yield will necessarily be very low and the farmer will be economically devastated. Rather than survival, realistic yield improvements likely need to address one of the following traits: (i) increase plant access to water, most likely through deeper rooting, to support greater crop growth; (ii) conserve soil water for use during late-season water deficits; or (iii) overcome special sensitivities in the plant that limit yield formation under water deficit.

The initiation point to genetically enhance crop yield when subjected to environmental stress should be the collection of evidence giving a solid basis for anticipating a relevant benefit under production conditions and for quantifying the expected yield gain. Unfortunately, many current studies started with expensive and sophisticated molecular studies without any direct evidence that modification would have practical benefit. Important sources of such evidence are experimental results in which trait response is induced experimentally in high-yield commercial cultivars to mimic anticipated genetic modification. How large is the yield-increase observed in such experiments? In studies on increasing drought tolerance of nitrogen fixation, such results were obtained by applying high amounts of nitrogen fertilizer to soybean plots subjected to drought stress [12,13]. In these experiments, removing plant dependence on symbiotically-fixed nitrogen during water deficits resulted in yield gains of 15–20%.

A very useful approach to fully understand possible yield changes as a result of trait modification is to use mechanistic crop models to simulate yield response over a range of growing seasons and geographical locations [8,14]. We have used a relatively simple model of soybean development, growth, and yield to simulate soybean yield changes that could be expected across the USA with modified drought traits ([15], Box 1).

Trait phenotyping
The expression of any particular physiological trait or transformation event is highly dependent on the environment in which the crop is grown. The existence of desired genetic code in a genotype offers no assurance about the expression of plant phenotype in a given environment. The challenge to make progress in developing higher-yielding crops is that physiological phenotype other than yield must be documented at some point in the breeding process [14,16].

Ben Miflin [17] argued that “Undue or sole emphasis on genomics will lead to an ever increasing gap between the genetic information acquired and an understanding of the phenotype, a ‘phenotype gap’”. Indeed, the lack of ability to phenotype plants for specific trait performance has been a critical limitation in applying physiological information for more than half a century [6]. The challenge is that it is very difficult to accurately phenotype plants for anything but the most obvious trait. Most of the physiological traits that impact on response to environmental stress require detailed, sophisticated and usually expensive techniques to phenotype plants, and can be applied only to a very limited number of genotypes. Not surprisingly, many physiological studies searching for genetic variation have frequently involved no more than 20 genotypes, and almost never more than 50 genotypes. Conversely, there have been a few attempts to search a large number of genotypes for genetic variation, and these attempts have failed due to the lack of sophistication in the screen.

A solution to the dilemma of phenotypic screens either being too difficult and sophisticated, or too crude and with poor resolution is a multi-tiered screening sequence involving both types of screens [7]. A simple but less accurate screen that allows a large number of genotypes to be examined is a first-tier screen followed by tiers of more sophisticated screens of decreasing numbers of genotypes. Such multi-tiered screens for crop improvement are not new in classical breeding efforts. ‘Tandem’ selection breeding is used regularly in some crops. We developed a three-tiered sequence of physiological screens (Box 2) in our efforts to identify candidate genotypes for use as parents in breeding efforts to sustain nitrogen fixation activity during soil water deficit [18].

Tracking of phenotypic improvement
Unfortunately, the approach of using multi-tiered screens is likely only suited for identifying parental lines with desired physiological traits. This approach is too laborious for tracking a trait through each stage of a breeding effort. How can the status of a physiological trait in a breeding effort be resolved?

Of course, one very appealing approach is to identify a genetic marker or maybe a specific nucleotide sequence completely linked with the desired trait. The challenge, as
discussed above, is that response to water-deficit is not likely to be under the control of a single or even a few genes. One possibility is that a collection of gene markers might be identified that contribute to physiological phenotype. Nevertheless, genetic markers only offer information about the genetic potential related to expression of physiological trait(s) for drought tolerance. While markers offer important information, they do not provide insight about the level of expression of a trait over a range of field environments. Trait expression is likely to be very much dependent on the biotic and abiotic environment in which the plant is grown. Within the plant the level of redundancy, location of the gene within the genome, and regulation by other genes may also impact trait expression. Therefore, the genetic tag could be useful in ensuring the possibility of phenotypic expression, but unfortunately, such tags do not offer definitive information about phenotypic expression being sought in superior lines [19].

The ultimate solution is to have ‘tools’ available to document phenotypic expression at each stage of breeding and in each test environment. However, such comprehensive tools seem improbable, if not impossible, and unrealistic for practical breeding programs. What can be the alternative to tracking phenotypic expression at each stage in the breeding process? One possibility may be to focus efforts on phenotyping for physiological performance only at a few critical stages in the breeding cycle. Certainly, one critical stage would be the initial identification of parent lines, either existing genotypes or transgenic lines that readily express the desired trait under a range of conditions. This can be accomplished by the multi-tiered approach described above.

Box 1. Model assessment of enhanced nitrogen fixation drought tolerance

A mechanistic soybean growth and yield model was used to simulate over growing seasons the soil water budget and its impact on crop development, growth and yield [15]. A Geographical Information System data base of at least 50 years of weather data for individual 30 km × 30 km grid locations across the U.S. were the input for the model simulations. To simulate yield for all grid locations and all years across the U.S., the model was run for >130,000 environments. The model was used to first simulate a ‘standard’ soybean and then re-run after modifying the model to simulate the behavior of a specific drought trait hypothesized to influence crop yield in response to drought. The probability of yield gain is color coded for all locations in Figure Ia as a result of the trait of nitrogen fixation tolerance to drought. Yield gain was achieved in >85% of the growing seasons, and in many regions it was >95%. The absolute amount of the yield change is color coded in Figure Ib, c and d for the 75 percentile, median and 25 percentile years for each grid. The greatest absolute yield gains were in the drier years (25 percentile). But the median and 75 percentile years also showed positive gains in yield, indicating that even in these growing seasons there was sufficient soil water deficit to impair nitrogen fixation activity. Almost no penalty in yield was simulated from the nitrogen fixation drought tolerant trait.

Figure I. Simulation results for incorporation of the trait for drought tolerance of nitrogen fixation in soybean. (a) Probability of yield gain and (b-d) absolute yield change for yield percentiles of 75% (wet), median and 25% (dry). Reproduced with permission from [15].
Box 2. Multi-tier selection scheme for nitrogen fixation drought tolerance

A three-tier selection scheme (outlined in Figure 1) was developed to identify candidate parental soybean lines with high tolerance of nitrogen fixation under drought conditions [18]. The broadest, and least accurate, screen was based on the concentration of ureides in the petioles of well-watered soybean genotypes. High leaf ureide concentrations had been shown to be associated with sensitivity to nitrogen fixation to drought. From a screen of about 3500 plant introduction (PI) lines, about 250 lines or slightly less than 10% with low petiole ureide concentrations were selected for a field test of nitrogen accumulation under dry conditions. The lines in the second tier screen were grown on a sandy soil, which had both a low nitrogen content and low water holding capacity. The lines were lightly irrigated approximately every other day to maintain them for about three weeks at or slightly above a soil water content resulting in slight leaf wilting. Again about 10% of these field-tested lines (24 lines) were selected for the third tier of screening for intensive measurement of nitrogen fixation response to soil drying in the greenhouse. Ultimately, 11 PI lines were identified that had substantial superiority in nitrogen fixation tolerance to soil drying.

The second critical stage for physiological phenotyping might be after the breeding population has been decreased to a more limited, yet substantial number of candidate lines by using standard agronomic selection criteria. That is, initially remove lines from the population that do not contain the marker for the desired trait, show disease or insect susceptibility, vulnerability to lodging, or have inappropriate agronomic characteristics. At this stage, the number of candidate lines can be narrowed further using second or third tier screens to a manageable number of lines for yield test.

The final critical stage is to document that those high-yielding lines identified in field tests also readily express the desired physiological trait. This information provides assurance that the yield increase might be a result of the putative physiological trait. (Of course, if the high-yielding lines are shown not to have the desired traits then the breeder is left with a high yielding line, and additional studies will be needed to resolve the reason for the yield improvement). We [18] employed such multi-tiered pheno-

typing at critical stages with success in efforts to develop genotypes with nitrogen fixation tolerance to soil drying (Box 3).

Box 3. Cultivar selection for enhanced nitrogen fixation drought tolerance

In the effort to develop cultivars expressing nitrogen fixation tolerance to water-deficit conditions and high yield, plants were phenotyped at critical stages in the breeding program. Initial effort was given to identifying and characterizing the expression of nitrogen fixation drought tolerance in the soybean cultivar Jackson [21,22]. Jackson was then crossed with the high-yielding cultivar KS4895 and the derived population was advanced by single seed descent to the F2 stage. Dr. Pengyin Chen (University of Arkansas) eliminated genotypes from the population, based on agronomic traits for robustness and uniformity in plant maturity in several environments. Then, 100 selected lines from this population were tested by Drs. C.A. King and L.C. Purcell (University of Arkansas) in the greenhouse for nitrogen accumulation by potted plants subjected to a constant, mild drought stress for two weeks. This relatively simple physiological screen resulted in the identification of 17 candidate lines for field yield test in a number of locations and years. From the field yield tests, two lines were identified as having higher yield than the commercial checks under moderate drought conditions. The final step was to test these two lines with controlled soil drying in the greenhouse to determine directly that both lines expressed nitrogen fixation drought tolerance [18].

Cultivar selection and marketing

Due to complex interactions between physiological processes and the abiotic environment in the field, it is unlikely that small changes in a physiological trait will result in a uniform increase in yield across all environments. Physiological trait improvement for drought conditions, unlike disease or insect tolerance for which the presence of the desired trait itself has virtually no negative consequences on yield, can have quite variable impact on yield across environments [15]. Instead of evaluating candidate cultivars based on grand mean yields over environments, which is a common approach, the more useful information is a probability response for yield change in the targeted environments. What are the environmental circumstances and probability for yield gain and by how much, and conversely what are the environmental circumstances and probability for yield loss and by how much?

Results from field tests with soybean lines identified as having drought tolerance of nitrogen fixation provided information on yield response across environments [20]. Yield tests were made at a number of locations in the southeast USA and the yield in each case was compared with the average yield of commercial cultivars. The results of these tests showed a yield advantage for one genotype (R01-518F) in those environments where average yield of commercial cultivars was 250–360 g m⁻² (Figure 1). Outside this commercially important range of yield, there was no consistent yield advantage or disadvantage for R01-518F. Therefore, if there is a reasonable fraction of growing seasons in the 250–360 g m⁻² range, the nitrogen fixation drought tolerance trait would be advantageous for farmers.

Farmers seem willing to digest probability data on yield response if such information will improve their economic wellbeing. Farmers need to know the fraction of growing seasons a yield gain might be anticipated, the fraction of...
years when there is no yield benefit, and the fraction of years when the stress-tolerant cultivar will result in a yield loss. Cultivars with stress-tolerant traits might be sold like insurance in that the improved cultivar gives increased yields in low-yield, low-income years but the insurance premium is 'paid' in the very good years when a yield decrease would result in a small income loss in years of high revenue.

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