

Crop transformation and the challenge to increase yield potential

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Molecular transformation is commonly offered as a hope to overcome the apparent stagnation in crop yield potential. A basic understanding of the resource limits imposed on crops and the yield hierarchy going from gene expression to harvestable yield leads to a rather negative view that transformations of a few, or even of a complex of genes will result directly in major yield increases. Forty years of biochemical and physiological research illustrate the great difficulty in translating research at the basic level into improvements in crop yield. However, there are a few cases where physiological research has led to improved crop cultivars with increased yield. These successes are instructive in highlighting key elements required to achieve success in developing crop cultivars for increased yield.

Gene transformation and genetic engineering are frequently offered as hopes for increasing crop yields worldwide, particularly in less-developed areas plagued by low crop productivity and malnutrition [1,2]. Crop transformations that allow farmers to deal more effectively with biological pests could contribute to increased crop productivity. Such transformations have been commercialized in countries with high crop yields, although these transformations were not focused on increasing yields. However, once pests are controlled, either using genetically improved plants or various management options, plant transformations are proposed to increase the inherent yielding capability of plants. There are two approaches to increasing yield potential: (i) increase the overall physiological capacity of plants to produce harvestable yield, and (ii) ameliorate the negative consequences of abiotic stresses on plants so as to increase yield. The question considered in this paper is, how can crop transformation be exploited to contribute effectively to these two approaches to increase yield potential?

In spite of the optimistic predictions often made for transformations leading to trait improvement resulting in increased yield potential, a historical perspective indicates that a much more moderate expectation is warranted. Forty years of research on the biochemistry and physiology of plant traits considered crucial for yield increases have resulted in few examples where such research led directly

to a yield increase. Although past research has greatly increased our understanding of the factors associated with crop yields and contributed significantly to the development of molecular genetics, overall there are virtually no examples of such research leading directly to crop yield increase. As Ben Mifflin pointed out [3], an inability in past years to apply discoveries in plant biochemistry and physiology to practical challenges of crop improvement should engender caution concerning the short-term contribution that molecular genetic research might make to increasing crop yields.

The discussion presented here considers past results from research on physiological traits for yield improvement and identifies characteristics of these efforts that might be applied to crop transformation research. First, options are briefly reviewed for substantial improvement in some of the key physiological traits being targeted for improvement by gene transformation. The traits considered include photosynthesis, nitrogen assimilation, seed growth and drought tolerance. Next, three rare cases are presented in which physiological research led to cultivars with increased yielding potential. Each of these cases involved ameliorating the consequences of abiotic stress. Finally, key characteristics of these rare successes are identified as crucial for future success in achieving increased yield potential using crop transformation.

Possible target traits for transformation

Photosynthesis

Increasing leaf photosynthetic rates seems to be a straightforward way of increasing crop yields. Considerable physiological research has been carried out to select and breed for genotypes with superior photosynthetic rates, and was successful in identifying such cultivars in maize [4], wheat [5] and soybean [6]. In soybean, the trait is inherited quantitatively [7]. Nevertheless, no increase in crop yield has been obtained as a result of breeding efforts based on genotypes with high leaf photosynthetic rates [4,8].

Failure to achieve a yield increase appears to be because of the diminished impact that enhanced photosynthetic capacity at the leaf level has when scaled up to the higher levels that lead to grain yield. It is possible to calculate the impact that enhanced photosynthetic activity at one level has on the activity at the next level of complexity going up

the hierarchy leading to grain yield. **Box 1** presents an example of such a calculation for soybean, starting with an assumed capability to increase the production of mRNA for synthesis of RuBP carboxylase, the primary carboxylating enzyme for C3 photosynthesis, by 50%. At each level of the hierarchy, the impact of the assumed 50% increase diminishes until the estimated change in grain yield is variable depending on assumptions about crop nitrogen accumulation. The optimistic yield with additional nitrogen accumulation is an increase of only 6%, but if no additional nitrogen is accumulated by the crop then estimated grain yield is decreased by 6%. The calculations in this example help to explain the historical difficulty of increasing yield by improving leaf photosynthetic capacity.

One current target for molecular modification of photosynthesis is to introduce the precursor pathway for organic acid fixation of CO₂ (C4 pathway) into C3 species. Transgenic lines of rice have been developed that have higher levels of phosphoenolpyruvate carboxylase activity [9], but their leaf photosynthetic rates were comparable to untransformed lines [10]. The failure to increase the leaf photosynthetic rate is not unlike past studies using species

that naturally have intermediates in C3 and C4 photosynthesis [11]. For example, no advantage was found in physiological studies using a partial C4 enzymatic pathway without accompanying C4 morphological changes or vice versa [11]. Synchronized changes in the biochemistry and morphology of the photosynthetic apparatus are required to obtain an effective C4 pathway in C3 plants [12]. Even if the putative advantage of increasing the leaf photosynthetic rate was achieved by completely converting a C3 plant to C4 photosynthesis, there is no guarantee that grain yield would increase (**Box 1**).

Nitrogen assimilation

Increased nitrogen accumulation by crops has been a crucial feature of past yield increases [13]. Increased nitrogen accumulation has usually resulted from applications of nitrogen to the soil and genetic improvement of plants to accumulate and store greater quantities of nitrogen in the plant. One important consequence of genetic alterations has been that the fraction of accumulated nitrogen ultimately stored in the grain (i.e. nitrogen harvest index) has been increased to values commonly in

Box 1. Carbon assimilation and the yield hierarchy

The impact on yield of hypothetical improvements in the molecular capacity of photosynthesis is calculated at each scale of organization from the molecular level to grain yield. The calculations begin by assuming that soybean leaves can be transformed to produce 50% more mRNA than currently for synthesis of the small and large subunits of Ribulose biphosphate carboxylase (Rubisco), the primary carboxylating enzyme of C3 photosynthesis (Figure 1). Based on a linear extrapolation of the results in Ref. [31], this assumed increase in mRNA production should result in the synthesis of 37% more Rubisco. This estimated increase in Rubisco is, in turn, calculated to result in a 33% increase in light-saturated leaf photosynthetic rate [31].

The next step in the hierarchy is to calculate the impact of changes in leaf photosynthesis on total plant carbon assimilation. By assuming that an isolated plant has as much as 60% of its leaf area exposed to photosynthetically saturating light levels, photosynthesis is calculated to increase by 30% because of the increase in mRNA. A further decrease in yield advantage occurs in moving to assimilation by a community of plants where there is competition for light. The original assumption of a 50% increase in mRNA diminishes to an increase of canopy carbon assimilation of only 18% [32].

Finally, the benefit of mass accumulation on seed production is estimated. The accumulated mass must be combined with nitrogen to form the essential components of grain. Consequently, the estimate of grain yield is dependent on assumptions about nitrogen uptake by transformed plants. Rubisco is a primary storage site for nitrogen during plant vegetative development. When grain development begins, Rubisco is broken down and most of the released nitrogen is transferred to the grain. In calculations of yield hierarchy, nitrogen is required to synthesize photosynthetic enzymes as well as all the other nitrogen components of the larger plant. If nitrogen is readily available in the soil to meet this increased requirement for nitrogen, then sufficient nitrogen will have been stored in Rubisco to allow a 6% increase in soybean yield. To achieve yields any greater than 6%, the transformed plant will also have to be modified to enhance other nitrogen storage mechanisms.

The calculations of yield increase can become negative if nitrogen accumulation by the transformed plants does not also increase. The larger vegetative plants that form as a result of stimulated photosynthesis mean that more nitrogen needs to be incorporated into structural components of the vegetative tissue and therefore this

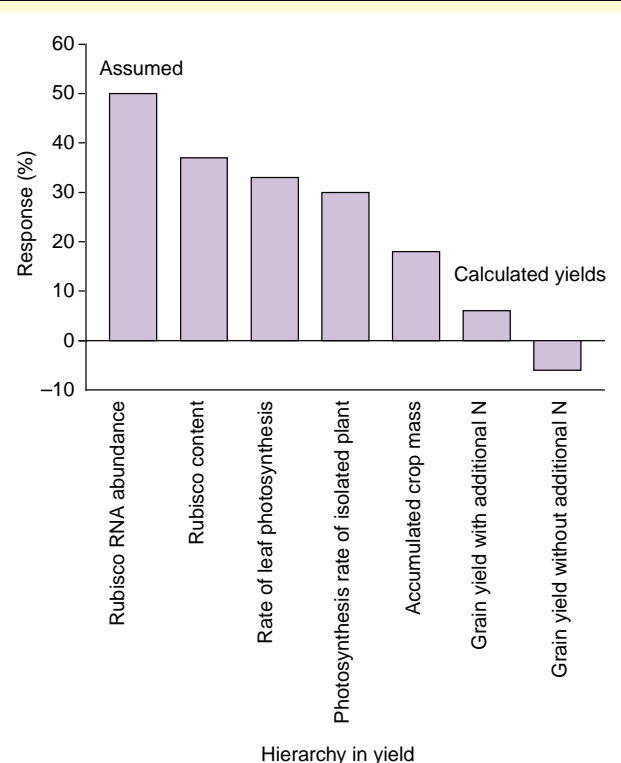


Figure 1. Carbon assimilation capacity and grain yield calculated at each increasing scale of crop hierarchy beginning with an assumed 50% increase in mRNA concentration.

nitrogen will not be available for subsequent transfer to the grain. Hence, without additional nitrogen accumulation there will be a net decrease in nitrogen available for the grain and this results in a calculated grain yield decrease of 6%.

Box 2. Improved water-use efficiency of wheat

Water deficits can cause serious losses of yield in wheat production in Australia. Graham Farquhar, Richard Richards, Anthony Condon and colleagues initiated a research effort seeking to increase the transpirational water-use efficiency of wheat so that more plant mass and yield would be obtained with the same amount of limited water. A major limitation in the initiation of this research was an inability to characterize plants for such a complex trait as transpirational water-use efficiency. Their first study published in 1984 examined the possibility of using a carbon isotope discrimination methodology as a surrogate of a direct measure of transpiration water-use efficiency [33]. The expected negative correlation between carbon isotope discrimination and water-use efficiency was found in an initial study of four wheat cultivars grown in a greenhouse and confirmed in additional cultivars [34]. Field studies were undertaken to examine variation in carbon isotope discrimination among cultivars and the correlation between isotope discrimination and yield was often positive rather than negative [35,36]. It was concluded that

the anticipated association was hidden because correlations between carbon isotope discrimination and grain yield can be influenced by several physiological differences among genotypes [37]. To overcome this problem, a breeding program was initiated in an attempt to introduce improved transpirational water-use efficiency into germplasm within similar genetic backgrounds [37]. Initially, two hundred $F_{2:3}$ families were measured for carbon isotope discrimination under field conditions, and six families at each extreme of isotope discrimination were used in the backcross-breeding program. Carbon isotope measurements were again made on $BC_2F_{2.4}$ families to select genotypes for testing in nine environments. Those lines selected for increased water-use efficiency using carbon isotope discrimination produced significantly greater yields in eight of the nine field environments – the greatest relative yield increase being nearly 11% ($\sim 120 \text{ kg ha}^{-1}$) in the driest environment [37]. Several wheat varieties are to be released from this program to growers and the first, 'Drysedale', is already available.

the range of 0.70 to 0.80. That is, nearly all the accumulated nitrogen ends up in the grain except that which is locked into structural components of vegetative tissue. Consequently, once crops have accumulated nitrogen, the plants are highly efficient in using the nitrogen to produce grain.

The goal of molecular genetic researchers is to increase 'nitrogen use efficiency' [14], although it is not clear what this might mean in terms of crop yield. The activities of specific enzymes involved in nitrogen metabolism within the plant have been targeted for transformation. For example, plants have been transformed to overexpress a glutamine synthetase gene, which is crucial in the assimilation of NH_3 , but this either had no influence on nitrogen accumulation or decreased mass accumulation [15]. With regards to altered nitrogen metabolism, David Lawlor [16] concluded that 'it is likely (and now frequently demonstrated) that changes to the genome will not result in major alterations to basic metabolism'. Some gain in growth has been reported for isolated plants in a greenhouse [17] that had been transformed, but considerable caution is needed in extrapolating results to increased grain yield in field-grown crops. The crucial nitrogen limitation on yield for field-grown crops is the ability to increase total nitrogen accumulation from the soil, and also the soil might not have additional soluble nitrogen available for plant uptake.

Seed growth

Increasing the growth rate of individual seeds seems to be an obvious approach to increasing crop yield. Consequently, a large amount of past research has focused on avoiding ovule and embryo abortion, and increasing individual seed growth rates. This research has shown that there is a great deal of compensation among seed mass, seed number and seed growth rate [18]. Further, increases in individual seed growth rate in a community of plants are compensated by altered duration of seed growth so that little or no increase in yield results [19]. Consequently, considerable flexibility in seed growth traits exists in modern crop plants resulting in high, conservative ratios of harvested grain to total accumulated crop mass, i.e. harvest index [20]. Improvements in seed growth

traits that result in substantially increased harvest indices do not seem likely [21].

Transformation of ADP-glucose pyrophosphorylase in seed, resulting in less sensitivity to phosphorus inhibition, has been studied as an approach to sustain and increase seed growth rate. Although increased seed growth for isolated plants of transformed wheat [22] and rice [23] has been reported, a striking feature of the results was that overall plant growth was stimulated and that seed growth was associated directly with overall increases in the growth of individual plants. Therefore, no change in the plant harvest index was achieved in rice and only a small increase in wheat as a result of the transformation.

Drought stress

Inadequate water availability is a crucial limitation to crop yield in most environments, and has been the focus for genetic improvement of crops for many years. The ratio between plant mass accumulation and transpiration (i.e. crop water-use efficiency) has limited flexibility owing to the physics and physiology of leaf gas exchange [24] and this relationship directly influences yield potential [25]. However, there has been some success in improving crop water use efficiency in wheat (Box 2), but major increases in yield will require increased water uptake by the crop, which means the crop must access more soil water under water-deficit conditions.

Much biochemical and physiological research has focused on drought tolerance to allow plants to survive periods of extended drought. However, for most annual grain crops, a drought that is sufficiently severe to threaten plant survival will result in crop yields so low that survival is often a moot point [26]. Therefore, there might be little or no point in transforming plants using exotic genes from, for example, resurrection plants to increase drought survival mechanisms for sustaining food production [27].

One approach that has received considerable attention is the possibility that solute accumulation in plants, or osmoprotection, might confer drought tolerance [28]. Transformations are being attempted for osmolyte accumulation and increased production of compatible solutes [29] under water-deficit conditions. Considerable

Box 3. Cowpea heat-stress tolerance

In the early 1980s, Anthony E. Hall and colleagues identified failure in seed set as a potential problem in cowpeas. Initial findings showed that the reproductive development of cowpea was much more sensitive to heat than was photosynthesis, and that high night temperatures were more damaging to reproductive development than were high day temperatures [38]. This observation was further supported in field tests in Imperial Valley, California, a location with high night temperatures that were consistently at levels that caused yield loss in production areas. There was a substantial decrease in pod set and in grain yield in the field experiments [39]. It was found that high night temperature resulted specifically in indehiscence of anthers and low pollen viability [40] as well as premature degeneration of the tapetal layer and lack of endothelial development in the pollen [41].

The solution to the yield loss from this stress was to identify tolerance among diverse germplasm sources. A field screen of 58 cultivars of cowpea was undertaken in 1981 in a location of consistently high night temperatures [40]. Of the three lines that were originally identified as having high levels of pod set, two were considered useful in breeding, 'Prima' and TVu 4452, but they were not well adapted to California. Subsequent tests in growth chambers showed that both cultivars had dehiscent anthers and had highly viable pollen under a 30°C night temperature treatment. A pedigree breeding program was initiated using backcrosses to California cultivars to develop heat-tolerant lines that had desirable agronomic traits [42]. Segregating progeny were screened for high pod-set levels under hot field and glasshouse conditions. In 1999, 'California Blackeye No. 27' was released as a heat-tolerant commercial cultivar [43].

Box 4. Soybean nitrogen fixation tolerance to water deficit

Nitrogen fixation rates in soybean have been reported to decrease with soil drying in advance of other processes, including leaf gas exchange [44]. After showing the sensitivity of the nitrogen fixation activity in soybean grown under field conditions [45], our colleagues and we initiated a research effort to ameliorate this stress. The extent of this problem was documented in a field test under rainfed conditions where applying large quantities of nitrogen fertilizer to supplant the dependency on nitrogen fixation during water deficit resulted in an 18% increase in soybean yield [46]. It was discovered that increased accumulation of ureides, the products of nitrogen fixation transported to the plant shoot, was associated with the sensitivity of nitrogen fixation to soil drying [47,48]. A multi-stage, physiological screen of soybean germplasm was undertaken and eight plant introduction lines were identified that had a greatly enhanced tolerance of nitrogen fixation to soil drying [49].

Early in the research, cultivar 'Jackson' was selected from field and greenhouse screens as having substantial tolerance of nitrogen fixation to soil drying [50]. A breeding program was initiated by crossing Jackson with high-yielding lines. Progeny lines have now been field tested under rainfed conditions at six locations in the southern USA for yield performance under non-irrigated conditions. The average yield of several of these lines was 5% or greater than the commercial lines, with one line having a 19% greater yield than the commercial lines. The superior lines are now available to soybean breeders for development of locally improved varieties and such a breeding program is underway at the University of Arkansas.

physiological research has already identified genetic differences in solute accumulation under water deficit conditions in several crop species, but the overall results of these studies have provided no consistent evidence that solute accumulation improves yield [26].

Research characteristics to achieve yield increase

Physiological and biochemical research has led to considerable insight about the influence of various traits on plant growth and crop yield. However, nearly all this research has failed to lead to cultivar improvement by aiding in problem identification, germplasm selection and cultivar development. Such failures do not bode well for molecular genetics research, which is even further removed in the yield hierarchy from grain yield. Molecular genetic research is likely to confront many of the same obstacles previously limiting the application of biochemical and physiological approaches.

However, there are a few, rare examples where physiological research, together with genotype selection, has been a necessary and integral part of cultivar development, producing cultivars that give increased crop yields. We have identified three successful cases that applied a physiological approach to develop crop cultivars with decreased sensitivity to abiotic stress: water use by wheat (Box 2), heat stress in cowpea seed set (Box 3), and symbiotic nitrogen fixation sensitivity to water deficit in soybean (Box 4). The crucial steps that appeared to lead to success in each of these research programs are described in Boxes 2–4.

We identified four common features among these cases that were essential in achieving success: early assessment of the putatively beneficial trait, effective phenotyping of

genetic modifications, multi-disciplinary effort and long-term commitment.

Beneficial trait assessment

The successful examples discussed above were initiated using traits near the top of the yield hierarchy and included field studies early in the project to assess potential impact on yield. By contrast, molecular approaches select genes (or pathways) for modification at the bottom end of the hierarchy and the ultimate influence on yield of a transformation is unknown. As seen in Box 1, the impact can be greatly diminished as the influence is tracked up through the yield hierarchy. Integration of genomics, mapping and physiology might enable scientists to develop molecular hypotheses starting near the top of the trait hierarchy. An early assessment of the benefits resulting from the hypothesized modifications requires the input of whole-plant physiologists and agronomists at an early stage to obtain a realistic perspective on the potential benefit of a transformation in the yield hierarchy. Further, an additional component for evaluation of transgenic cultivars is likely to be an economic assessment of whether additional costs and liabilities incurred by growers can be justified.

Phenotyping of genetic modification

A crucial challenge for a transgenic approach to yield improvement is forecasting consequences in the expression of a transformed trait. As pointed out by Mifflin [3] 'farmers cultivate phenotypes' so it is crucial to adequately document variations in phenotype that result from gene modification. Both the genetic background into which a gene is inserted and the physical and biological

environments in which the transformed crops are grown will almost certainly have large influences on gene expression and value. Consequently, extensive testing of phenotypic expression is required, including evaluation of plant performance and yield in a cropping situation. The need for rapid and inexpensive methods for phenotyping plants, particularly for traits that do not have a readily visible expression, is likely to increase substantially with the advent of molecular technologies. Mifflin [3] argues powerfully about the problem of developing a 'phenotype gap' between genetic information and our understanding of phenotypic expression.

Multi-disciplinary effort

The three successful physiological efforts leading to improved crop yield each involved scientists from several disciplines, including crop physiologists, agronomists and breeders, throughout the research effort. A molecular transformation approach adds another layer to the team and the team approach will require input from all participants at each phase of the research. Consequently, use of a molecular genetics approach to increase yield requires an even larger number of participating disciplines represented by scientists who must be committed to interactive research throughout the effort. Full coordination and integration of such teams from inception to cultivar release is a challenge that does not appear to be easily met within the framework of many public research organizations. This challenge might be increasingly difficult to meet in view of the declining commitment to research in whole-plant physiology and crop breeding [30].

Long-term commitment

The successful physiological programs required 15 or more years from identification of the specific problem to cultivar release. Although the molecular genetics approach might offer a few shortcuts, it still seems likely that a successful program to generate improved cultivars will take at least ten years. Consequently, a sustained commitment is required of scientists to apply molecular understanding to the task of crop yield improvement. Thus, funding organizations need to put aside short-term funding perspectives in favor of long-term funding horizons for such research efforts. The challenge for funding organizations is to recognize the benefits of long-term commitments and to implement a funding framework for long-term support of research targeted to achieving crop yield increases.

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