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Yield enhancement genes: seeds for growth

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Yield is a multifactorial trait, integrating various developmental and physiological processes. Despite this complexity, evidence is mounting that yield can be increased by the genetic modification of single genes. Positive results have been obtained by targeting different yield constituents, indicating that there is ample room for further yield improvement by genetic means. Successful targets include photosynthesis, starch biosynthesis, plant architecture and transcriptional networks controlling plant development. Most of the current data have been obtained in a (semi-)controlled environment and relate to yield calculated on a per plant basis. Demonstrating the ability to transfer these effects to field-grown plants and with reference to yield on a per area unit basis will be a crucial step in establishing the agronomic importance of these findings.

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Introduction

For many crops, yield is the primary trait in breeding programs. Yet, until recently, our understanding of yield at the molecular level was very limited. Although the molecular mechanisms of the processes underlying yield — such as photosynthesis, carbon partitioning, flower development and seed production — have been progressively unravelled over the past two decades, very little progress has been made towards the identification of the genetic components that define yield in a quantitative manner. So-called ‘yield enhancement’ genes remained elusive, thus feeding a longstanding debate as to whether such genes actually exist. For many years, opponents in this debate have argued that yield is too complex a trait for specific yield enhancement genes to stand out. Accordingly, the qualification of yield enhancement genes would be futile, at least in the sense that single genes would not have any major effect on yield. Quanti-

tative genetics seems to support this view, as a large number of genetic loci appear to be involved in a complex trait like yield.

Yet, in the past few years significant progress has been made towards the elucidation of plant genes that, as single variables, are able to improve yield. The debate therefore seems to evolve in favour of the proponents of the existence of yield enhancement genes. Whether or not the currently identified genes will also lead to increased performance in the field is, in most cases, still an open question. But, this does not reduce the significance of the finding that single genes can have profound effects on complex, multifactorial traits such as yield.

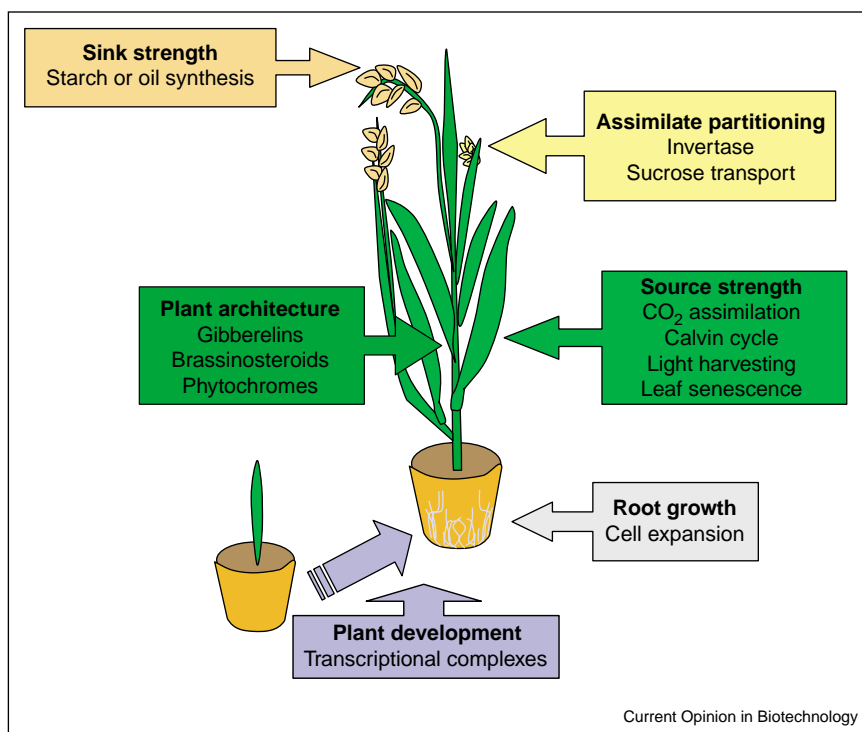
Diverse approaches have been undertaken towards improving yield using single genes (Figure 1). Carbohydrate metabolism has been manipulated both in source tissues, with the aim of increasing the supply of metabolites to heterotrophic sink organs, and in sink tissues to enlarge the sink capacity. Other targets for metabolic changes include the transport functions of metabolites from source to sink. The efficiency of nitrogen use has also been a target for crop improvement, but this subject is not discussed here as it has recently been reviewed elsewhere [1,2].

Apart from metabolism, transgenic strategies aimed at modifying development have also been investigated. Desired developmental alterations include the optimization of plant architecture towards high density cultivation or the increase in harvestable organ size and number. In this review, some of the most significant advances in these different areas are highlighted.

Increasing source strength

Photosynthesis is the main source of carbon assimilation in crop plants. Carbohydrates produced in the leaves during photosynthesis, so-called photoassimilates, are mobilized to heterotrophic parts of the plants, such as the growth zones and the storage organs. Tissues and organs that are net importers of carbohydrates are referred to as sinks, and the partitioning of photoassimilates from source organs to various sinks is under strict developmental control. The relative strength of different source and sink organs will determine the flow of carbohydrates and other nutrients within the plant and will largely delimitate the growth potential of different plant organs. An obvious target for increasing the source strength is the production of photoassimilates during photosynthesis. The first enzyme in photosynthetic CO₂ assimilation is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Oxygen

Figure 1



Transgenic strategies for yield enhancement. A major target is carbohydrate metabolism, from CO₂ assimilation in leaves to carbohydrate transportation and conversion into starch (or oil) in storage organs. Other approaches are aimed at modifying root growth, leaf and stem architecture, and at plant development. For each strategy, the main molecular, biochemical or cellular targets are specified.

can compete with CO₂ at the active site of Rubisco, thus initiating a process called photorespiration. Photorespiration reduces the efficiency of carbon fixation during photosynthesis, with a loss of up to 50% of the carbon fixed [3]. C₃ plants, including most of the important crops such as wheat, rice, soybean, potato and tomato, undergo photorespiration. However, some plants, including a few important crops such as maize, sugar cane and sorghum, have developed mechanisms to concentrate CO₂ near the active site of Rubisco, thus avoiding Rubisco oxygenation and photorespiration [4,5]. These mechanisms consist of both metabolic and anatomical adaptations. In these plants, the C₄ cycle is used for CO₂ assimilation, which releases CO₂ in the vicinity of Rubisco by enzymatic decarboxylation.

Many attempts have been undertaken to introduce a more efficient, C₄-like photosynthesis in C₃ plants, mostly in rice, potato and tobacco [4,6]. The introduction of single C₄ enzymes in these plants has so far not resulted in an improvement of photoassimilate production, most likely because of perturbations in the fluxes of C₄ intermediates for metabolic pathways other than the C₄ cycle. Interestingly, the combined expression of two C₄ cycle enzymes in rice was reported to increase photosynthetic capacity by 35% and grain yield by 22% [7]. In this study, the maize genes were transferred to rice together with

their respective promoters, which might have resulted in a better spatial and temporal expression of the C₄ cycle enzymes than the more conventional expression of transgenes by constitutive promoters. More analysis is needed to understand the importance of the promoters for targeting the desired metabolic alterations in these transgenic plants. Also, the performance of these plants in more variable and often less optimal field conditions remains to be evaluated.

Besides the above strategies based on C₄ photosynthesis, other approaches have been taken to improve the efficiency of photosynthetic carbon assimilation. One of these directs the enzyme Rubisco activase, a key regulator of Rubisco activity [8]. Rubisco activase is sensitive to heat [9], a feature that was strongly alleviated in a mutant version of the enzyme generated by the company Verdia (now part of DuPont-Pioneer; <http://www.pioneer.com>) through gene shuffling technologies. Transgenic *Arabidopsis* plants expressing this heat-tolerant version of Rubisco activase showed a clear improvement in photosynthesis and leaf growth when exposed to heat stress [10]. Following another strategy, a cyanobacterial inorganic carbon transporter was expressed in plants with the aim to increase the carboxylation reaction of Rubisco [11]. Beneficial effects were most pronounced under low

humidity conditions, suggesting that photosynthesis in such environments may be limited by inorganic carbon accumulation. Steps in the Calvin cycle downstream of Rubisco have also been subject to genetic engineering. Overexpression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco chloroplasts enhanced photosynthesis and growth in hydroponic culture [12].

Other studies have targeted the light reactions of photosynthesis. A possible pitfall of altering the light-harvesting efficiency of plants is that the effects can be either beneficial or detrimental, depending on the light conditions in which the plants are grown. This was shown to be the case when a light-harvesting complex protein subunit was overexpressed in tobacco [13].

Photoassimilate production may not only be enhanced by improving photosynthetic efficiency, but it can also be promoted by increasing the total photosynthetic capacity of a plant. The latter can be achieved by delaying leaf senescence, thereby prolonging the time span over which a leaf contributes to the photoassimilation of a plant. Senescence is a process of controlled tissue degeneration, during which metabolites are mobilized from the deteriorating cells to other parts of the plants. Unexpectedly, a transgenic strategy for modifying fatty acid biosynthesis in tobacco also had effects on leaf longevity and seed production [14^{*}]. Chloroplast transformation with the plastidic subunit of acetyl-CoA carboxylase resulted in increased fatty acid levels in the leaves, but not in the seeds, of tobacco. The change in leaf fatty acid content was accompanied by extended leaf longevity and a two-fold increase of seed yield. Plants that flower later often have higher seed production as a result of the extended vegetative growth phase and a concomitant increase in source strength; however, delayed maturation is generally an undesired trait in agriculture. Whether or not the increase in seed yield accompanied a prolonged life cycle was not mentioned in this study. The authors instead suggested that the improved yield performance possibly resulted from the increased source strength in fatty acids or, although not directly measured, from increased photosynthetic capacity owing to delayed senescence.

Enhancing sink strength

In many cases, sink strength has been altered by modifying the biochemistry of the sink organs. Favoured biochemical targets are enzymes directly or indirectly involved in the conversion of sucrose into starch. One approach has been to alter the adenylate pools in potato, as adenylate levels have been shown to be important for starch content in potato tubers [15]. Downregulation of the plastidial isoform of adenylate kinase resulted in a 60% increase of starch in potato and, interestingly, in a 39% increase in tuber yield [16^{*}]. Notably, this increase was achieved by modifying the levels of a cofactor that is

involved in many more reactions than just starch synthesis.

Other approaches for yield improvement are more specifically directed towards starch synthesis. Many of these attempts showed only limited success [17,18]; however, one positive example is particularly compelling as it has proven to work in different plant species. ADP-glucose pyrophosphorylase (AGP) produces the substrate for starch synthesis and is thought to catalyse a rate-limiting step in the starch biosynthetic pathway. AGP is inhibited by the allosteric factor orthophosphate. The introduction of a bacterial or mutant form of the large subunit of AGP that is less sensitive to allosteric inhibition has previously been shown to increase starch biosynthesis and yield in potato [19] and maize [20]. Positive data have now also been obtained for wheat [21^{*}] and rice [22]. In both cases, seed yield was increased by more than 20%, primarily as the result of an increase in the total number of seeds. The transgenic AGP large subunit was driven by an endosperm-specific promoter. To explain the fact that the primary effect of the transgene was on seed number, it was hypothesized that during the early stages of development, a sink may require a threshold rate of supply of resources to continue development. Consistent with such a model is the fact that in wheat and rice not all of the flowers that are initiated develop into mature seeds. In addition, the increase in sink strength may reduce the feedback inhibition of sugars on photosynthesis in leaves. The latter could also explain why, in addition to the increase in seed yield, more plant biomass was also observed in the transgenic wheat and rice plants [21^{*},22^{*}]. In maize, AGP is possibly the most heat-labile enzyme of starch biosynthesis, and a single amino acid substitution in the large subunit of AGP has been described that stabilizes the AGP complex [23]. Combining the mutations for reduced allosteric inhibition and heat sensitivity in a single transgene could thus result both in increased yield and in improved yield stability under heat stress.

The above strategies can obviously only work in cereals, potato and other crops that accumulate starch as a major carbon reserve in sink organs. Altering sink strength in oilseed crops in an analogous manner would require modifications of the biochemical pathways that convert sugars into triacylglycerols [24]. Interesting in this regard is the recent identification of a novel pathway involving Rubisco and the photosystems for more efficient conversion of carbohydrates into oils in green seeds [25]. This pathway opens up new opportunities for the genetic engineering of yield traits in certain oilseed crops.

Modifying assimilate partitioning

Directly linked to sink strength is the ability to unload assimilate in the sink organ. Apoplastic invertase is a prime candidate for driving this process, as it cleaves

sucrose into the monosaccharides glucose and fructose [26,27]. Expression of apoplastic invertase under the control of a meristem-specific promoter increased seed yield by more than 20% in *Arabidopsis* [28*]. This increase in total seed yield resulted from an increased number of seeds produced in the transgenic lines, which in turn was due to the formation of additional axillary inflorescences. Apoplastic targeting of the enzyme was essential, as cytoplasmic expression of the invertase had a negative effect on yield. The positive effect of apoplastic invertase overexpression on seed yield was only discernible under long-day conditions, concurrent with a shortening of the vegetative phase of the plants and a reduction in the number of rosette leaves. A model was therefore proposed in which, compared to wild-type, assimilate supply in invertase overexpressors was channelled earlier into flowering and inflorescence formation. Recent work indicates that inhibition of invertase activity may play an important role in ovary abortion of maize under conditions of water deficit [29]. It would be interesting to test if these effects can be alleviated by invertase overexpression.

Other molecular candidates for increasing sink strength through intervention with assimilate unloading are sucrose transporters. Constitutive overexpression of a sucrose transporter in potato resulted in a reduction of sugars in the leaves and an increase of sugars in tubers; however, there was little change in either tuber starch content or tuber yield [30]. Quite surprisingly, tobacco plants overexpressing a maize pathogenesis-related (PR) protein of the PR-1 group showed improved growth and increased seed yield [31]. Sucrose levels in these plants were threefold to fourfold higher than those of wild-type plants. The maize PR protein was localized at the plasmodesmata of tobacco cells and transgenic leaves showed up to 16-fold higher export rates of sucrose compared with control plants. It was therefore proposed that a symplastic pathway of sucrose transport [32], operating parallel to the apoplastic pathway mediated by sucrose transporters, would be facilitated by the maize PR protein in tobacco. The increase in symplastic sucrose transport could thus account for the enhanced growth.

Modifying plant architecture

In general, field crops are grown at high density and with high nitrogen input, both factors promoting stem elongation. Stem elongation is often an undesired effect, as tall plants more easily fall over — a process commonly referred to as lodging. Moreover, resources diverted to stem growth are not available for seed production. High density cultivation also requires optimization of lateral branching. Another target for yield improvement has therefore been the adaptation of plant architecture to current agricultural practices. The great potential of this approach is illustrated by the green revolution in the 1960s, which was based on the improvement of plant architecture; the introduction of semi-dwarf varieties

doubled the crop yield in wheat and rice [33]. The mutations that are responsible for the short stature in wheat and rice have now been identified. Both relate to the plant hormone gibberelin. The role of gibberelin in plant dwarfing and the nature of the green revolution genes has recently been reviewed and will not be discussed here in further detail [34]. The same review also deals with the recent identification of two genes, *MONOCULMI* [35] and the rice ortholog of *TEOSINTE BRANCHED1* [36], which control the formation of tillers (i.e. branches carrying inflorescences) in rice.

Another strategy to alleviate density effects is to reduce photomorphogenic responses [37]. Responses to low-red to far-red light ratios are potentially adaptive and increase the competitive ability of plants; yet, they enhance interference amongst plants and are therefore usually undesired in field crops. The overexpression of phytochromes has previously been shown to improve photosynthetic performance and harvest index in tobacco [38] and potato [39]. The positive effect of phytochrome overexpression on photosynthesis and tuber yield in potato has now also been confirmed in the field [40*]. Leaf morphology could possibly also be adapted towards optimal light capture under high-density conditions. Genes that control the leaf index (i.e. the ratio of leaf length to leaf width) have now been identified. Examples include *ANGUSTIFOLIA*, *ROTUNDIFOLIA3* and *DRL1* [41–44], but applications of these genes for modifying plant yield have so far not been reported.

Altering plant development

Cell division is one of the prime determinants of plant growth rate. The potential of cell division control for yield enhancement is part of a separate article in this themed issue (see the review by Beemster, Mironov and Inzé). An interesting novel upstream regulator of cell division is ARGOS. Overexpression of *ARGOS* was shown to increase lateral organ size in *Arabidopsis* [45]. Besides cell division, cell elongation is the second major mechanism that contributes to growth. Whether stimulating cell elongation is generally desirable can be questioned, as cellular expansion may often simply increase the water content and thus the fresh weight of plant organs, without having any impact on the dry biomass production. Expansins are cell-wall proteins characterized by their ability to stimulate wall loosening during cell expansion. Overexpression of an expansin in soybean stimulated root elongation of agar-grown seedlings [46], but it is currently unclear whether this approach holds promise for increasing root growth in soil. Also, expansin overexpression affected cellular organization in leaves, which could indicate that the transgene expression might need to be confined to the root. Moreover, earlier work has shown a negative impact on growth when another expansin isoform was overexpressed [47]. It is likely therefore that the stimulating effect on cell elongation is either specific

to certain members of the expansin family or, alternatively, is rather tightly linked to the level of expression.

The identification of transgenic plants with improved growth or yield can also reveal new developmental pathways, the existence of which or their importance for plant development was previously unknown. Transgenic suppression of deoxyhypusine synthase (DHS) in *Arabidopsis* delayed natural and drought-induced senescence and led to a concomitant increase in leaf, root and seed biomass [48]. DHS catalyzes the first of two reactions resulting in the post-translational activation of the eukaryotic initiation factor 5A and is thought to play a role in selective messenger RNA translocation for translation during senescence and possibly also in cell proliferation. Delaying senescence may at the same time extend the time to maturity of the plant, a feature that is often unwanted in agriculture. One strongly suppressed DHS line was also severely delayed in seed production. The question as to whether all DHS suppressed plants exhibited some delay in maturity was not addressed in the article.

The adoption of high-throughput tools for testing the effects of transgenes on plant development is also yielding novel information on genes and pathways involved in growth regulation. Most high-throughput testing has been performed by private companies. Mendel Biotechnology (<http://www.mendelbio.com/>) has constitutively overexpressed over 1700 transcription factors in *Arabidopsis*. In this way, an NF-YB transcription factor that confers a stay-green phenotype and an AT-hook transcription factor that promotes biomass production and seed yield have been identified (N Gutterson, Abstract W137, *Plant and Animal Genomes XIII Conference*, 15–19 January 2005, San Diego) [49]. Through a partnership with Monsanto (<http://www.monsanto.com/>), these genes are now being tested in various crops.

CropDesign (<http://www.cropdesign.com/>) has built a high-throughput platform for rice and has so far tested 1400 transgenic constructs. Although slower and more costly as a testing system than *Arabidopsis*, rice has the advantage that it is a crop and serves as a better model for cereal crops than *Arabidopsis*. Evidence is emerging that the effects of certain genes involved in yield-related developmental processes might be quite different in rice and *Arabidopsis*. For example, constitutive overexpression of DWARF4, a gene involved in brassinosteroid signalling, enhances vegetative growth and seed yield in *Arabidopsis* [50]. Yet, a similar approach leads to a reduction in growth and seed production in rice (W Van Camp, unpublished). These results indicate that DWARF4, or possibly brassinosteroid signalling altogether, has a different effect on plant development in monocots and dicots.

The reverse situation, namely a gene that when overexpressed confers a deleterious effect in *Arabidopsis* but a

positive effect in rice, has also been observed. Constitutive overexpression of SYT1, a protein involved in chromatin remodelling [51], leads to enhanced vegetative growth in *Arabidopsis*, but this effect is accompanied by sterility (http://www.checkbiotech.org/root/index.cfm?fuseaction=searchandsearch=leaf%20growthanddoc_id=9317andstart=1andfullsearch=0). By contrast, SYT1 overexpression in rice leads to increased seed size and seed yield (W Van Camp, unpublished). Similarly, overexpression of STZ, a protein involved in stress responses, causes growth retardation and dwarfism in *Arabidopsis* [34], while it enhances biomass production and seed yield in rice. Both SYT1 and STZ proteins are transcriptional co-activators and thus form part of complex regulatory systems. It is tempting to speculate therefore that the use of *Arabidopsis* as a general model system for other species, including distant species such as rice, may fall short when applied to complex traits such as plant development and yield, in particular if multicomponent regulatory mechanisms such as the control of transcriptional networks are involved.

Conclusions

Yield is a very difficult trait to study at the molecular level. Experimental methods that have advanced our molecular understanding of plants in many different ways, have been of little aid in the study of yield. For example, loss-of-function mutants are very informative for establishing the involvement of a gene in a specific biological process; however, in the case of yield, loss-of-function would be manifested as a deficiency in growth or a reduction in fruit and/or seed production, phenotypes that are obviously shared by many essential genes and not diagnostic of a specific role in yield. Gain-of-function could be manifested by a yield improvement. Yet, the relative yield increase in such a case will often not surpass the resolution limit of standard greenhouse or growth chamber setups. Also, plant growth and the contribution of plant growth to harvestable yield are continuous processes, features that make the dissection of these processes (e.g. through time-course based transcriptome and proteome analyses) quite difficult.

Considering these limitations, the successes that have been achieved in recent years, many of which have been discussed in this review, are particularly encouraging. It is striking that the many and diverse approaches have all been successful in enhancing yield. The genes deployed in these strategies are prime candidates for the development of new commercial biotech traits, but the hurdles that remain are still significant. First, the ability to transfer yield enhancement technologies from one species to another is, in most cases, still to be established. The modification of AGP is a good example of a technology that appears to be quite broadly applicable, but the above-mentioned examples of transcriptional regulators indicate that, at least with regard to yield, *Arabidopsis* may not

always be a trustworthy predictor for distantly related plant species such as cereal crops. Possibly, functional conservation between plant species will generally turn out to be higher for enzymes than for proteins that operate as part of a regulatory complex. Second, genetic modifications that appear beneficial when analyzed at the plant level might not necessarily translate into higher yields in the field. Such genetic modifications might increase the maximum yield potential, while in the field greatest improvements may come from overcoming numerous yield limitations, thus realizing a larger part of the maximum yield potential. Moreover, breeders will generally measure plant yield in the field on a per unit area basis, and not on a per plant basis. In this context, it is noteworthy that most of the yield increase achieved in maize over the past decades has come from the development of new varieties that show improved performance under high planting density. When planted under low density, these varieties did not outperform the older varieties in yield. For agricultural biotechnology, however, the potential benefits of yield enhancement genes are too large to be restrained by these considerations. With the introduction and testing of these yield enhancement genes in field-grown crops, plant biotechnology has exciting years ahead.

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