The introduction of dwarf varieties of cereals was fundamental to the green revolution, but in the post-genomic era, the manipulation of plant morphology could be more sophisticated. A recent publication by Tahar Ait-ali et al. describes the use of the ethanol-inducible transgene expression system to re-examine plant architecture, and the genes that determine it. Their findings have implications for the manipulation of plant height and yield, and demonstrate the efficacy of regulated transgene expression for functional genomics.

‘Feed the world’ was the LiveAid rallying cry in 1986 to draw attention to the relationship between famine and politics. This remains an important argument of the anti-GM crop lobby: there is enough food in the world — its distribution is just uneven. Will this still be true in the decades to come? It is predicted that the demand for food will double over the next 50 years [1,2], which will be an increasingly difficult challenge for science and politics to resolve. In the 1960s and 1970s, the ‘green revolution’ resulted in substantial increases in the yield of cereal grains, based on new varieties and methods of cultivation [3]. A key element to this was the introduction of shorter plants, from which crop losses were lower. Dwarfism arises from mutations in gibberellin (GA)-mediated plant growth regulation [4]. A recent publication from Nick Harberd’s research group by Tahar Ait-ali and colleagues [5] suggests that a post-genomic green revolution could follow a similar route.

Regulated expression: the transgenic approach to conditional mutations

In the 1960s, mutations were the only approach to understand gene function. A key step in microbial genetics was the use of conditional and conditional-lethal mutations (Box 1). Being able to dissect functional interactions temporally revealed more than phenotypic assays based entirely upon the presence or absence of active gene products. At the time, this was not practical in plants. In the 21st century, the principal method of functional analysis is transgene expression, and yet much of the functional genomics of plants still employs constitutive promoters to drive transgene expression continuously from germination to senescence. However, a variety of inducible gene expression systems offer the equivalent of conditional mutations for plant genomics [6,7]. One of these has the potential for use in the field: the ‘alc switch’ (Figure 1) responds to ethanol, an inducer that is simple and cheap, of low phytotoxicity, biodegradable and of low environmental impact [8,9]. It is effective at low concentrations, in a dose-dependent manner, and effects reversible expression on a series of reporter genes [10]. Not only does the alc switch work in Arabidopsis, but it also functions effectively in several crop species including potato and tomato [11]. The ability to switch a gene of choice on or off at defined developmental or physiological stages allows a dissection of function temporally. Ait-ali et al. [5] have shown its potential for investigating genes that determine plant architecture.

Green revolution and plant height

The determination of plant height involves a complex interplay of several molecular components. Central to this are a family of proteins called DELLA, which are nuclear regulators whose activities are opposed by the growth-promoting hormone GA; these proteins repress growth [12]. In Arabidopsis thaliana, the product of the dominant gai mutant allele lacks a 17 amino acid region identified as characteristic of DELLA proteins (the DELLA domain), which results in a constitutive repression of growth (dwarfism) and a reduced response to GA. Similar mutations (Rht-B1b and Rht-D1b) in wheat underpin the semi-dwarfing varieties. Transgenic expression of the Arabidopsis GAI or gai protein confers dwarfism to

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Box 1. Conditional and conditional-lethal mutations

Microbial geneticists faced a particular problem in attempting to unravel gene function by mutation analysis, namely that in a haploid organism there is only one copy of each gene and therefore a null mutation often results in lethality. So a key step was to recognize conditional mutants, that is, mutant organisms that were normal under one set of conditions but that had a mutant phenotype under other conditions. Those that died in the non-permissive condition were described as conditional-lethals. For example, the most common examples of conditional dependence were temperature-sensitive mutations, which can be viable at 37°C, but inviable at 42°C. Such mutations enabled the discovery of multiple DNA polymerases in E. coli, because temperature-sensitive polA alleles were not lethal at the non-permissive temperature.

Functional genomics faces similar challenges. Accurately assigning functions to many tens of thousands of gene products will require subtle analyses in which functional or mutant homologous, homologous or heterologous genes or their RNAi counterparts are expressed at different stages of development, to varying degrees, and/or reversibly. In this way, the ordered events of, for example, a signalling or developmental pathway can be unravelled, and the particular protein–protein or protein–ligand interactions defined under varying conditions.

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*Corresponding author: Brian Tomsett (tomsett@liv.ac.uk).*
rice [13,14], demonstrating that plant architecture can be controlled transgenically. However, this continuous expression also has detrimental effects: for example, rice expressing GAI constitutively has a reduced ability to extrude the seed-bearing flowers (panicles), reducing the number of seeds. Therefore any increase in yield from shortening the plant to prevent lodging is likely to be counterproductive. What of conditional expression of GAI?

**gai Expression**

Ait-ali et al. [5] have tested the flexibility with which gai can be used for crop improvement, and in the process established important findings for functional genomics. By using an ethanol-inducible gai construct (alc-gai), they have shown that there is no gai protein in the absence of ethanol, but it is detectable within 6 h of treatment with the inducer. Thus, plants grown in the absence of ethanol are similar in stature to wild-type plants, but those grown in its presence exhibit dwarfism similar to a transgenic line expressing gai constitutively, or a gai mutant line. The degree of dwarfing can be controlled in a dose-dependent manner, and, in the greenhouse, a reduction in mutant plant height to 83% of the wild-type plant height results in no net loss in seed yield. The increase in yield in the field attributed to the reduction of plant height results from an increase in plants reaching maturity, and/or being harvestable, not from increased seeds per plant. Because ethanol has been tested and shown to work under field conditions (A. Martinez, pers. commun.), this work thus opens the possibility of a further manipulation of plant height to fuel yield increases, but through a transgenic mechanism in which defined aspects of plant architecture are altered rather than through constitutive expression of a ‘dwarfing’ gene, and its associated side-effects.

This work also has some important lessons for functional genomics. The alc switch allows experimenters to determine the functional effects of gene activity in a quick and controlled way. In this study, it was possible to show that because GA cannot stimulate growth in alc-gai plants in the presence of ethanol, but its effect is restored when ethanol is withdrawn, that therefore gai must inhibit the normal response to GA. Furthermore, by applying ethanol at different stages of growth, it was clear that gai has its effect on actively growing parts of the plant. This means that plant architecture might be changed in different ways at different developmental stages, a conclusion that had previously not been possible to draw.

**Future perspectives**

If food production needs to double in the next 50 years, then our understanding of how plants grow will need to increase dramatically. Plants will need to be precision engineered to grow optimally in a range of environments with their particular suite of biotic and abiotic stresses. We will need to know how to optimize agronomic traits and direct the energy of the plant into yield. Plant functional genomics will be the key and this excellent demonstration of the alteration of plant architecture [5] is just the beginning.

The ability to regulate gene expression can be made more sophisticated by expressing the transcription factor using tissue-specific promoters, thus providing spatial and temporal control [15]. The expression of the functional gene, a dominant-negative mutation, or an antisense construct will allow the researcher and perhaps the
farmer, to switch genes on or off [16]. These developments thus open up plant genomes to rigorous functional analysis and the potential for precise modification of important agronomic traits.

References
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SH2 domains in plants imply new signalling scenarios

Jeffrey G. Williams1 and Marketa Zvelebil2

1School of Life Sciences, University of Dundee, MSI/WTB Complex, Dow Street, Dundee, UK DD1 5EH
2Ludwig Institute for Cancer Research, The Cruciform Building, University College London, Gower Street, London, UK WC1E 6BT

The SH2 (src homology 2) domain is a regulaatable protein–protein interaction domain that is integral to many animal cell signalling pathways. In such pathways, a monospecific tyrosine kinase phosphorylates a target protein and the resultant phospho-tyrosine moiety is bound by an SH2 domain [1]. The social amoeba Dictyostelium also uses SH2 domain-phosphotyrosine signalling [2,3], but it has as yet not been described elsewhere. However, when we used the SH2 domain of a Dictyostelium STAT (signal transducer and activator of transcription) protein as the query sequence in a BLAST search, we identified two highly related Arabidopsis SH2 domain-containing proteins and a close homologue in Oryza. This finding suggests the existence of a mode of cellular signalling in plants that is of central importance in animal cell and developmental biology.

Identification of three plant SH2 domain-containing proteins

A BLAST search, performed using the SH2 domain of Dd-STATa (residues 588 to 657), yielded three matches in the plant databases. The proposed names and GenBank accession numbers for the three predicted SH2 domain-containing proteins are: AtSHA (Arabidopsis thaliana SH2 domain protein A), Accession no. B86306; AtSHB, Accession no. NP 177795.1 and OsSHA (Oryza sativa SH2 domain protein A), Accession no. AAL79683. Figure 1a is an alignment of the three plant SH2 domains with several prototypic SH2 domains. There is a region of homology towards the N-terminus of the plant SH2 domains but the C-terminal halves of all the SH2 domains shown are much less well conserved. However, this is the most divergent region among SH2 domains, so even with the help of the STAT1 and v-src crystal structures, it was only possible to align two C-terminus-proximal residues in the two proteins [4]. Crucially, the highly conserved core of the canonical SH2 domain, the sequence GTF**RF (where * represents L, I or V), is present in all three plant proteins. The arginine residue within this motif is of particular significance because it is the residue primarily responsible for interacting with the phosphate group of phospho-tyrosine.

Other bio-informatic analyses strongly support the conclusion that these are SH2 domains (see supplementary material, http://archive.bmn.com/supp/plants/Williams_Supp.pdf) and Figure 1b is a structural model for the OsSHA SH2 domain. This model shows that although the plant sequence is shorter than the animal SH2 domains, its