Plant breeding

Engineering herbicide resistance

from Robert Shields

ONE of the much-vaunted targets for genetic engineering in agriculture has been the production of herbicide-resistant plants. To be useful, herbicides must be selective, that is they must kill plants but not animals, and weeds but not the crop. There are many problems in achieving the second objective, so it is not surprising that several agrochemical companies are looking to genetic engineering to produce herbicide-resistant crop plants. On page 741 of this issue[1], Comai *et al.* show that this prospect is not just an idle dream.

It is relatively easy to produce herbicides selective for plants. Many in current use affect functions unique to plants: atrazine and diuron interfere with photosynthesis, and glyphosate, the sulphonylureas and imidazolinones block the synthesis of essential amino acids. But crop plants share these processes with weeds, so crop plants must be protected in other ways, for example through differential uptake and metabolism of the herbicide by the crop and weed or by careful choice of the time and site of herbicide application. The differential sensitivity of plants to herbicides can cause problems. For instance, atrazine is an effective herbicide for use with maize, which can detoxify the compound, but residues remaining in the soil cause problems when maize is rotated with the more sensitive soybean. If soybean could be made atrazine resistant, the problem would be avoided. Other broader spectrum herbicides such as glyphosate are rapidly broken down by soil microorganisms, so soil residues are less of a problem, but glyphosate is indiscriminate in the plant attacked. If selected plants could be made glyphosate resistant then this herbicide could have wider applications — and higher sales.

Comai and colleagues started from the observation that the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase), an enzyme involved in aromatic amino acid synthesis, is the *in vivo* target for glyphosate in bacteria, and then selected glyphosate-resistant strains of *Salmonella typhimurium*. Some of these bacteria contained the gene coding for a resistant version of EPSP synthase. The mutant form of the enzyme differs from the wild type by a single amino acid. The coding region of the resistant bacterial EPSP synthase was hooked up to eukaryotic promoter and poly (A) addition sequences derived from the T regions of agrobacteria and the resulting chimaeric constructs recombined into the T_i region of *Agrobacterium rhizogenes*, which is the causative agent of 'hairy root' disease. Like the better studied tumour-forming species *A. tumefaciens*, to which it is related, *A. rhizogenes* contains a large plasmid. The transferred (T) region of this root-inducing (Ri) plasmid contains genes with eukaryotic transcription signals and is transferred to the plant, where it is stably incorporated in nuclear DNA. The strain of *A. rhizogenes* used in these experiments has two T DNA regions in its Ri plasmid (termed T_L and T_R), which are independently transferred to the plant. Genes in both the T_L and T_R region promote root formation in transformed plant tissue; genes in the T_L region are responsible for the extreme hairy phenotype. The advantage of *A. rhizogenes* over *A. tumefaciens* is that it is relatively easy to regenerate plants from 'hairy roots', whereas plants can only be regenerated from *A. tumefaciens*-transformed tissues if the tumour genes have previously been mutated or deleted.

In the experiments reported by Comai *et al.*, a strain of *A. rhizogenes* carrying the chimaeric EPSP synthase in their Ri plasmids was used to infect tobacco leaf disks and the resulting roots were regenerated into plants. The resistant EPSP gene inserted into the plant genome was correctly transcribed and translated, so that the regenerated plants were resistant to glyphosate, with the degree of tolerance depending on the level of EPSP synthase expression. So these experiments show that it is indeed possible to transfer herbicide resistance into a previously sensitive plant.

One intriguing aspect of these results is that plant amino-acid biosynthesis is conducted by enzymes encoded by nuclear genes that function in the chloroplast, whereas the transferred resistant EPSP synthase works in the cytoplasm. Presumably the chloroplast is permeable to the precursors and products of the synthase. It would be interesting to know if higher levels of herbicide tolerance could be achieved if the resistant enzyme were directed into the chloroplast where the sensitive plant enzyme resides.

Recent experiments have shown that it is indeed possible to transfer foreign proteins encoded in the nucleus to chloroplasts if the proteins are linked to chloroplast-specific transit peptides[2,3]. In these experiments the bacterial enzyme, neomycin phosphotransferase (npt-II), was transferred to chloroplasts of plants transformed by *Agrobacterium* vectors carrying the *nptII* gene in a chimaeric construct with the transit sequence from the gene for the small subunit of ribulose bisphosphate carboxylase. These experiments are of great practical importance for the engineering of herbicide resistant crops. Several herbicides besides glyphosate block chloroplast-located amino acid biosynthesis; for instance, the very broad spectrum sulphonylurea herbicides interfere with acetolactate synthase (ALS), the first enzyme in the biosynthetic pathway of branched-chain amino acids. Genes coding for a resistant version of ALS have been located in yeasts and it should now be a relatively straightforward matter to transfer them to plants.

A bigger challenge for the genetic engineers is provided by the herbicides such as atrazine and the chemically unrelated diuron that interfere with the binding of plastoquinone to the Q_B protein, part of the photosystem II complex. The Q_B protein is encoded by chloroplast DNA and is firmly anchored in the thylakoid membrane of that organelle. Plants and a number of microorganisms have been found that are resistant to atrazine, and a recent report describes the cloning of a gene coding for diuron-resistant Q_B protein from a cyanobacterium[4]. The atrazine- and diuron-resistant Q_B proteins from a number of sources differ from the wild type by a single amino-acid substitution at the same place in the protein chain. Would it be possible to engineer atrazine and diuron resistance in plants by expressing chimaeric genes encoded in the nucleus that code for a mutant Q_B protein linked to a chloroplast transit peptide? Problems could arise with such a strategy because the extremely hydrophobic nature of the Q_B protein might prevent transport across the chloroplast membrane, even if a suitable transit peptide were provided.

A more exciting prospect comes from the observation that *Agrobacterium*-based vectors are capable of transferring and integrating DNA into the chloroplast as well as the nuclear genome[5]. If such genes were linked to chloroplast-specific promoters and possibly regions of homology with chloroplast DNA, it might prove possible to direct genes into specific places in the chloroplast genome. It might even be possible, by designing a suitable vector, to replace the genes that encode normal Q_B proteins by cloned genes for herbicide-resistant Q_B proteins.

A more down to earth problem is that gene transfer systems based on agrobacteria are available for a narrow range of dicotyledenous plants. As yet no transformation system exists for major monocot crop plants such as the cereals (but see ref. 6). So the challenge for the future is to find ways of genetically engineering these crops or to persuade the public and animals to forego the delights of cereals and eat tobacco and petunia instead. □

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5. de Block, M. *et al. EMBO J.* **4**, 1367 (1985).
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Robert Shields is at Unilever Research, Sharnbrook, Bedford MK44 1LQ, UK.