GUEST EDITORIAL

Georges Freyssinet, born 1946. Scientific Advisor, Bayer CropScience-BioScience (since 2002) and President of the Management Board of RhoBio (since 2001). Doctor es-Sciences, U. of Lyon, France (1977). 1970-72, Research Assistant, Brandeis U., Mass., USA and Montreal University, Canada; 1967-79, Teaching Assistant, U. of Lyon, France; 1977-79, Visiting Prof., U. of Champaign, Illinois, and U. of Brandeis, USA; 1979-84, Prof., U. of Lyon and U. of Limoges, France; 1984-92, Head of Molecular and Cellular Biology, Rhône-Poulenc Agrochimie, Lyon; 1992-98, Scientific Advisor, Rhône-Poulenc, Paris; 1998-99, CEO of RhoBio, Lyon; 2000-01, Head of Genomics, Aventis CropScience, Lyon. *Main fields of interest:* Plant genomics; crop protection and crop improvement; use of plants as factories.

Herbicide-Resistant Transgenic Crops - A Benefit for Agriculture

Herbicide resistance was one of the first traits engineered into plants. The main reasons are: the knowledge of herbicide resistance mechanisms; the availability of genes; the possibility to use the gene and the herbicide as a marker set to select transformed tissues; and the commercial interest in such a trait for farmers and agrochemical companies.

Two approaches have been used successfully:

a. Amplification of the target In this case the enzyme that serves as the target for the herbicide was reintroduced into the crop using strong constitutive promoters to increase the target level, allowing the plant better to resist the herbicide treatment. Since in most of the cases this does not give a sufficient level of resistance, a resistant target is introduced into the crop. This resistant target is obtained either from organisms which are naturally resistant to the herbicide or by introducing mutations into the target to make it resistant. Using this approach, crops resistant to glyphosate (Roundup), a total herbicide, or to the herbicides of the sulfonylurea and imidazolinone families, have reached the market. In the case of glyphosate, the target is 5-*enol*-pyruvylshikimate-3-phosphate synthase (EPSPS, EC 2.5.1.19), which is present in both plants and micro-organisms (23). Genes have been isolated from several bacteria and plant species. The genes have been mutated to obtain enzymes resistant to higher doses of glyphosate and introduced into several crops. The first mutant, produced in *Salmonella typhimurium*, allowed the first production of a glyphosate-resistant tobacco plant (5). Another gene was isolated from maize in which two mutations, known as T-102 and P-106, were introduced to obtain a resistant enzyme (12). Since the enzyme is located inside the chloroplast, an optimized transit peptide (11) was linked to the coding region of the enzyme. This gene, controlled by a constitutive promoter such as that for rice actin, has been introduced into maize, resulting in the development of glyphosate-resistant maize (20) that is on the market today. In the case of soybean and cotton, the CP4 gene isolated from an *Agrobacterium* strain has been used to confer glyphosate resistance (15). The target of the herbicides from the sulfonylurea and imidazolinone families is acetohydroxyacid synthase (EC 4.1.3.18). Plant cells and bacteria resistant to these herbicides have been obtained (3). A mutated gene is at the origin of this resistance. Several plants have been made either by transgenesis or through the direct regeneration of resistant plant tissues (17,19). Their commercial application has, however, been limited to maize and canola. For a few other herbicides, resistant enzymes have been obtained and the corresponding genes introduced into plants: this is the situation for diflufenican acting on phytoene desaturase (14) and for asulam acting on dihydropteroate synthase (EC 2.5.1.15). In this case, the gene had been introduced into potato to control parasitic weeds (24). More recently, our team has been working on the introduction of resistance to the new family of isoxazole herbicides. These herbicides act by blocking hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27) (16). Several genes from bacteria or plants, natural or mutated, have been used to introduce resistance into numerous crops which are under development (1).

b. Metabolic detoxification of the herbicide In this case an enzyme which detoxifies the herbicide is introduced into the crops. Resistance to the total herbicide phosphinotricine (Basta, also known as glufosinate), and bialaphos, has been obtained by the introduction into plant cells of the bacterial enzyme phosphinothricin *N*-acetyltransferase (PAT or BAR), isolated from a *Streptomyces* species and which detoxifies the herbicide (25). Approximately 40 plant species resistant to these herbicides have been obtained; 36 are listed in ref. (4). This success is principally because the marker system is one of the best for the selection of transgenic plant cells in the laboratory. Among those plant species, canola was the first crop to reach the market in Canada (6). Other herbicide resistance has been obtained using a detoxification approach: bromoxynil and ioxynil, which are two herbicides acting on photosystem II. A specific nitrilase was isolated from *Klebsiella ozaenae* (21) and introduced into crops such as cotton, canola and tobacco (7,8). Resistance to glyphosate has also been obtained by a detoxification mechanism. A glyphosate oxidoreductase (GOX) has been isolated from *Achromobacter* sp. strain LBAA (9). When this enzyme is introduced into plants, it confers resistance to the herbicide. It has been associated with the CP4 gene described above, for commercial applications in canola and maize (18). More recently, a new detoxification system has been described for glyphosate; it uses a glyphosate acetyltransferase and has been evaluated with success in tobacco (2). Detoxification systems have been isolated and evaluated for other herbicides such as 2,4-D in cotton using a 2,4-D dioxygenase (13).

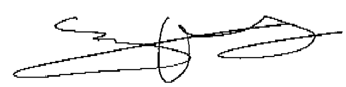
These two approaches have been used with success up to the commercial level. When a detoxification system is readily available, it is an easy system to put in place; the level of expression of the enzyme needed is generally low since it degrades the herbicide. This has positive aspects when the degradation is complete but may be problematic if new metabolites are produced. In most cases it is more difficult to obtain a fully resistant crop plant using the mutated target strategy, probably because of the competition between the two types of targets present in the plant. Also, it is sometimes difficult to produce a mutated target which has a good resistance to the herbicide and yet retains full enzymatic activity.

After almost 20 years of experience with this agronomic trait, which has been exploited under commercial growing conditions for over 5 years, the technology has proven its robustness. The stability of the introduced genes has been shown under laboratory and field conditions over several generations. No significant deleterious effects such as yield reduction or phytotoxicity have been observed when a fully resistant plant is used. The trait also has no effect on plant development (flowering time, seed set). One of the issues raised by environmentalists is the risk of spreading these herbicide-resistant genes in the environment. Reports have confirmed the transfer of genes through the pollen (22). However, so far, there has been no report of agronomic problems due to the escape of these herbicide genes, suggesting that either the rate of spreading is low or it has no agronomic impact. Another concern is whether the development of such practices will increase the occurrence of resistant weeds. It seems too early to answer this question. However, it is possible that changes in the weed flora due to repetitive treatments with the same herbicide will be more of a problem than development of resistant weeds.

As indicated above, work on herbicide resistance began with the setting up of plant transformation technologies in 1983. Thus, it is not surprising that herbicide-resistant crops have been the first to reach the market. In 1994, about 10 years after the first plant transformation, the first crops resistant to herbicides were authorized for commercialization in the USA, Canada and Europe. The first commercialization was in 1995 and since that time the area grown has increased annually. In 2002, herbicide resistance alone represented 75% of the 58.7 million hectares of transgenic crops; another 8% was covered with maize and cotton resistant to both herbicide and insects (10). This clearly represents a commercial success.

The reasons for this success are numerous. The use of herbicide-resistant crops simplifies farming practices; the crops are resistant to the herbicide throughout their life, enabling flexibility in treatment periods, which can be tailored to good weather conditions and weed infestation. It also allows the development of no-till practices, thus limiting soil erosion. In addition to these benefits for the farmer, a significant decrease in the overall treatment costs has been achieved. These crops also benefit the environment, since, in addition to the development of no tillage, the total amount of herbicide sprayed in the environment has decreased, limiting the risk for residues in the crops and in the environment. Moreover, herbicide-resistance traits will allow the use of more environment-friendly herbicides, since one of the constraints in the discovery and development of new herbicides – resistance to the target crops – has been removed.

In the future, the current herbicide-resistance traits already on the market will continue to develop, more through their expansion into new countries than by an increase of areas in countries where they are already well used. In parallel, the herbicide-resistant genes will be introduced into new crops, several of which will soon be commercialized. Moreover, new herbicide-resistance traits will reach the market as new broad-spectrum herbicides are discovered.



Georges Freyssinet
Bayer CropScience
55 Avenue René Cassin,BP 106
69266 Lyon Cedex 09, France
e-mail: Georges.Freyssinet@bayercropscience.com

REFERENCES

1. Boudec, P., Bourdon, H., Dumas, F., Rodgers, M.W. and Sailland, A. (1999) Mutated hydroxyphenylpyruvate dioxygenase, DNA sequence and isolation of plants which contain such gene and which are resistant to herbicides. Patent application WO 99/24585.
2. Castle, L., Siehl, D., Giver, L.J., Minshull, J., Ivy, C., Chen, Y.Y. *et al.* (2002) Novel glyphosate N-acetyl transferase. Patent application WO 02/36782 A2.
3. Cole, D.J. (1994) Molecular mechanisms to confer herbicide resistance. *in:* Marshall, G. and Walters, D. [Eds.] Molecular Biology in Crop Protection. Chapman and Hall, London, UK. pp. 146-176.
4. Cole, D.J. and Rodgers, M.W. (2000) Plant molecular biology for herbicide-tolerant crops and discovery of new herbicide targets. *in:* Cobb, A.H. and Kirkwood, R.C. [Eds.] Herbicides and Their Mechanisms of Action. Sheffield Academic Press, Sheffield, UK. pp. 239-278.
5. Comai, L., Facciotti, D., Biatt, W.R., Thompson, G., Rose, R. and Stalker, D. (1985) *Nature* 317:741-744.
6. Copping, L.G. (1998) Genetically Modified Crops II? Genetic Engineering for Herbicide Tolerance. Agrow Report DS 162, PJB Publications, Richmond, Surrey, UK.
7. Freyssinet, G., Leroux, B., Lebrun, M., Pelissier, B., Sailland, A. and Pallett, K. (1989) *Proc. Brighton Crop Protection Conf.* (Farnham, UK), pp. 1223-1234.
8. Freyssinet, G., Pelissier, B., Freyssinet, M. and Delon, R. (1996) *Field Crops Res.* 45:125-133.
9. Hallas, L.E., Hahn, E.M. and Korndorfer, C. (1988) *J. Ind. Microbiol.* 3:377-385.
10. James, C. (2002) Global Status of Commercialized Transgenic Crops: 2002. ISAAA Brief vol. 27.
11. Lebrun, M., Leroux, B. and Sailland, A. (1996) Chimeric gene for the transformation of plants. U.S. patent no. 5,510,471.
12. Lebrun, M., Sailland, A., Freyssinet, G. and Degryse, E. (1997) Mutated EPSPS gene coding for said protein and transformed plants containing said gene. Patent application WO 97/04103.
13. Lyon, B.R., Cousins, Y.L., Llewellyn, D.J. and Dennis, E.S. (1993) *Transgenic Res.* 2:162-169.
14. Misawa, N., Masamoto, K., Hori, T., Ohtani, T., Böger, P. and Sandmann, G. (1994) *Plant J.* 6:481-489.
15. Padgette, S.R., Kolacz, K.H., Delanny, X., Re, D.B., LaVallee, B.J., Tinius, C.N. *et al.* (1995) *Crop Sci.* 35:1451-1461.
16. Pallett, K.E., Cramp, S.M., Little, J.P., Veerasekaran, P., Crudace, A.J. and Slater, A.E. (2001) *Pestic. Manage. Sci.* 57:133-142.
17. Saari, L.L. and Marvin, C.J. (1996) Sulfonylurea herbicide-tolerant crops. *in:* Duke, S.O. [Ed.] Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory and Technical Aspects. CRC Press, Boca Raton, FL, USA. pp. 127-142.
18. Saroha, J.V., Sridhar, P. and Malik, V.S. (1998) *J. Plant Biochem. Biotechnol.* 7:65-72.
19. Shaner, D.L., Bascombe, N.F. and Smith, W. (1996) Imidazolinone-resistant crops: selection, characterisation and management. *in:* Duke, S.O. [Ed.] Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory and Technical Aspects. CRC Press, Boca Raton, FL, USA. pp. 127-142.
20. Spencer, M., Mumm, R. and Gwyn, J. (2000) Glyphosate tolerant maize line. U.S. patent no. 6,040,497.
21. Stalker, D.M. and McBride, K.E. (1987) *J. Bacteriol.* 169:955-960.
22. Staniland, B.K, McVetty, P.B.E., Friesen, L.F., Yarrow, S., Freyssinet, G. and Freyssinet, M. (2000) *Can. J. Plant Sci.* 80:521-528.
23. Steinrücken, H.C. and Amrheim, N. (1980) *Biochem. Biophys. Res. Commun.* 94:1207-1212.
24. Surov, T., Aviv, D., Aly, R., Joel, D.M., Goldman-Guez, T. and Gressel, J. (1998) *Theor. Appl. Genet.* 96:132-137.
25. Wehrmann, A., Van Vliet, A., Opsomer, C., Botterman, J. and Schulz, A. (1996) *Nature Biotechnol.* 14:1274-1278.