DEVELOPMENT OF TRANSGENIC OILSEED RAPE RESISTANT TO OXYNIL HERBICIDES

M. FREYSSINET[1], P. CREANGE[1], M. RENARD[2], P. McVETTY[3], R. DEROSE[1] and G. FREYSSINET[4]

[1]Rhône-Poulenc Secteur Agro, BP 9163, 69263 LYON Cedex 09, France; [2]INRA, Domaine de la Motte, BP 29, 35650 LE RHEU, France; [3]University of Manitoba, WINNIPEG, Manitoba, Canada R3T 2N2; [4]Rhône-Poulenc, 25 Quai Paul Doumer, 92408 COURBEVOIE, France.

ABSTRACT

A gene coding for a nitrilase specific for the transformation of hydroxybenzonitrile (oxynil) herbicides into the non-phytotoxic benzoic form has been introduced into oilseed rape (Westar) through *Agrobacterium*-mediated transformation. This gene is under the control of either the CaMV 35S promoter or a sunflower RuBisCO small subunit promoter. The agronomic performance of the homozygous progeny from several transformation events was evaluated under field conditions in France and Canada. Lines showing the best level of tolerance to oxynil treatment was kept for further analyses. Several analyses were performed on the selected lines: i.e. agronomic performance, quality of the harvest and the processing of the seeds. We have observed no yield penalty due to the presence of the "oxy" gene nor the herbicide treatment. All the biochemical analyses show identity between the non-transgenic Westar treated by normal agronomic practices and the transgenic lines treated with oxynils. The sequence at the position of the gene insertion done for a line containing a gene controlled by the CaMV promoter, confirms the fidelity of gene transfer. The selected line is currently used for breeding the herbicide tolerance trait into winter and spring elite lines.

INTRODUCTION

Hydroxybenzonitriles (oxynils) are a family of herbicides (bromoxynil and ioxynil) which have been used for several years to control broadleaf weeds in cereal, onion and garlic crops. They act by interfering with photosynthetic electron transport. Cereals and a few other crops are resistant to oxynils because they have a nitrilase which degrades them into a non-phytotoxic benzoic acid form (Buckland *et al.*, 1973). Oxynils have a two-week half life in the soil due to the presence of micro-organisms able to degrade them into benzoic acid and other derivatives. A gene coding for a nitrilase has been cloned from *Klebsiella ozaenae* isolated from bromoxynil treated soil (Stalker and Mc Bride, 1987). This gene has been transferred to various dicot crops such as tobacco (Delon *et al.*, 1993) and cotton (Fillatti and Mc Call, 1989).

The control of weeds in oilseed rape is not adequately solved with the available herbicide treatments, and there is need for a system which allows a control of cruciferous weeds, such as wild mustard. To this end, we have introduced the nitrilase gene into oilseed rape, and we present here the results obtained.

EXPERIMENTAL

Gene construction

The coding region of the nitrilase was put under the control of either a constitutive (CaMV 35S) or a light-inducible (RuBisCO small subunit) promoter, the terminator being from the nopaline synthase gene. These chimeric genes (construct 235 for the CaMV promoter; construct 237 for the RuBisCO promoter) were transferred between the borders of the T-DNA.

Plant transformation

An *Agrobacterium*-mediated transformation system applied to the terminal internodes of Westar, a spring cultivar, was used for gene transfer. *In vitro* selection of transformants was made with bromoxynil (5-10 mg/l, Delon *et al.*, 1993). The regenerated plants were selfed and homozygous progeny identified.

Plant analysis

Transformation success was evaluated at several stages, phenotypically during leaf disc assays, spraying in greenhouse with bromoxynil, and at the molecular level by DNA, RNA and protein analyses using conventional techniques. In addition, analyses of the seed composition (fatty acids, protein, glucosinolate) was performed on field grown seeds.

Herbicide treatments

Under field conditions, we have evaluated the herbicide tolerance of the transgenic oilseed rape, and the weed control of oxynil herbicides in fields containing transgenic oilseed rape.

RESULTS

Herbicide treatments

Herbicide tolerance: The level of tolerance was checked by spraying the regenerated plants with 600-1,200 g a.i./ha. Plants with no sign of phytotoxicity were selfed and the progeny was evaluated under field conditions. Field trials have been carried out in France and Canada following the necessary regulations for such experiments. To select the best lines, progeny from each transformation event was treated with increasing concentrations of bromoxynil from 280 g a.i./ha (the agronomic dose applied for control of broadleaf weeds) to 4,800 g a.i./ha. Lines showing no sign of phytotoxicity at the highest dose were kept for further analyses. The transgenic lines are tolerant to oxynil at all stages of their development.

Weed control: When bromoxynil or ioxynil or a mixture of both was used in the field on transgenic oilseed rape, we observed a very good control of broadleaf weeds, especially wild mustards which were destroyed by a treatment with 280 g a.i./ha.

Plant analyses

Molecular analyses: We have performed Southern, northern and western analyses. Only plants with one transgene were kept for further experimentation. Expression of the gene (mRNA and protein) was as expected, in all tissues for the plant containing the 235 construct (CaMV promoter), and in all green tissue for the plants containing the 237 construct (RuBisCO SSU promoter).

In addition, we have determined the DNA sequence at the site of insertion for the selected 235-type plant. This sequence shows that four bases 5' of the right border, and 22 bases from the 3' end of the left border, were integrated into the plant DNA.

Agronomic parameters: Transgenic Westar was compared to its non-transgenic counterpart for parameters such as plant height, days to flowering, lodging and yield. No significant differences were observed between these two types of plants.

Biochemical analyses: These analyses were made on non-transgenic Westar treated with conventional herbicides and on transgenic Westar treated with bromoxynil (4,800 g a.i./ha). The oil, protein and erucic acid contents are not significantly different between the two types of seeds. Interestingly, we observed a lower amount of glucosinolates in the transgenic seeds versus non-transgenic controls, probably due to the isolation of a plant with a low amount during the regeneration process.

In addition we have determined the composition of fatty acids, sterols, tocopherols and observed no significant differences between the two types of seeds.

CONCLUSIONS

We have introduced a gene coding for a specific nitrilase into Westar. This nitrilase allows the plant to resist a treatment with bromoxynil or ioxynil up to at least 16 times the agronomic dose applied for broadleaf weed control. A line has been selected for the two types of constructs. These lines have been used for breeding the herbicide resistance trait into elite lines of winter and spring oilseed rapes.

Results from field experiments show that there is no yield penalty and no difference for several morphological and biochemical parameters critical for the commercialisation of oilseed rape.

ACKNOWLEDGEMENTS

This work was part of the Bio Avenir programme supported by Rhône-Poulenc and the French Ministries of Research and of Industry.

REFERENCES

Buckland, J.L., Collins, R.E. and Pullin E.M. (1973). Metabolism of bromoxynil octanoate in growing wheat. *Pesticide Science*, **4**, 149-162.

Delon, R., Pelissier, B., San, L.H., Borrod, G. et Freyssinet, G. (1993). Des tabacs transgéniques résistants aux herbicides de la famille des oxynils : cinq années d'expérimentation. *Annales du Tabac*, **25**, 793-798.

Fillatti, J.J. and Mc Call C. (1989). Herbicide resistant cotton plants. *In vitro Cellular and Developmental Biology*, **25**, 57A.

Stalker, D.M. and Mc Bride K.E. (1987). Cloning and expression in *Escherichia coli* of a *Klebsiella ozaenae* plasmid-borne gene encoding a nitrilase specific for the herbicide bromoxynil. *Journal of Bacteriology*, **169**, 955-960.