HERBICIDE RESISTANT CROPS AND PASTURES IN AUSTRALIAN AGRICULTURE – PRESENT AND FUTURE

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Summary The potential use of herbicide-resistance genes in crops (e.g. wheat, cotton, lupins, peas) and pasture plants (e.g. subterranean clover) in the Australian context is currently being evaluated against criteria of economic value and environmental impact. Trials currently in progress at sites throughout Australia will help to determine the value of herbicide-resistant crops and pastures as part of weed management practices and provide an assessment of the risks associated with the introduction of this technology. Transgenic cotton, lupins, peas, wheat and subterranean clover carrying genes conferring herbicide tolerance are being evaluated under field conditions, though it is not expected that these trials will all lead to the commercialization of herbicide-resistant crops. In some cases, e.g. wheat, herbicide tolerance has been used to demonstrate that stable transformation of the target species has been achieved. Commercialization will ultimately depend on a thorough assessment of the benefits and risks of the technology and the value of herbicide-resistance as part of an overall strategy for weed management.

INTRODUCTION

The development of herbicide-tolerant plants for use in agriculture has significant agronomic implications and may well play a role in shaping the agricultural practices of the future. Herbicide-tolerant plants have the potential to contribute significantly to an integrated weed management strategy. The careful use of herbicide-resistant crop and pasture plants could provide new options for weed control and promote the more efficient use of the herbicides currently available.

Herbicide-tolerant crops are most likely to be of importance where current weed control systems are inadequate, for example, in cotton, and in certain minor crops where the range of herbicide options is limited or non-existent. Specific herbicide tolerance has been the objective of herbicide chemists and biologists since synthetic organic herbicides came into general use some fifty years ago. Herbicides specific for major crop plants have been developed by the synthesis and testing of numerous compounds until a molecule with an adequate weed control spectrum has been found. However, it is now possible using the techniques of genetic engineering to modify the plant rather than the herbicide to achieve herbicide resistance.

Resistance may be introduced into crop plants in three ways. Firstly, resistance may be identified in natural or mutagenised populations and the resulting germplasm used in breeding programs. For example, the development of brassicas with resistance to triazine herbicides was accomplished by traditional breeding using resistance sourced from a weedy *Brassica* biotype (Beversdorf *et al.* 1988). Secondly, cell/tissue culture techniques may be used to identify genetic material carrying natural resistance to a particular herbicide and incorporating this material into commercial cultivars by conventional breeding. Imidazolinone resistant corn was developed in this way using material selected for resistance in corn cell culture (Newhouse *et al.* 1991). Thirdly, and most importantly, the techniques of genetic engineering may be used to incorporate foreign genes conferring herbicide resistance into established commercial cultivars of crop plants. It is this latter technique which has provided added impetus for the development of herbicide tolerant crops for use in agriculture.

GENETIC ENGINEERING FOR HERBICIDE TOLERANCE

The development of our understanding of the molecular mode of action of specific classes of herbicide and their environmental fate combined with the capacity to move novel genetic material in agriculturally important plants has allowed rational approaches to the design of herbicide tolerant crops using genetic engineering techniques. Three different strategies to engineer herbicide tolerance in crop plants have been developed.

Strategy 1 The introduced foreign genes may code for altered target site enzymes which are less sensitive to the herbicides than the native enzymes.

Organisms selected for tolerance to glyphosate have been reported (Comai *et al.* 1983) in bacteria and shown to have mutant forms of the enzyme target site of glyphosate, 5-enolpyruvyulshikimic acid (EPSP) synthase. The *Aro*A gene encoding the herbicide tolerant form of EPSP synthase has been cloned and transferred into a number of plant species. When correctly engineered for expression in plant chloroplasts, this mutant gene confers significant resistance to glyphosate in a wide range of crop plants (Comai *et al.* 1985).

Resistance to the sulfonylurea herbicides has been engineered similarly by transferring a mutant enzyme target site (acetolactate synthase, ALS) from plant or bacterial sources into crop plants. Tobacco and tomato plants transformed with genes for mutant ALS are resistant to field applications of chlorsulfuron (Lee *et al.* 1988).

Strategy 2 The genetic makeup of the crop plant may be altered to promote the over-production of the herbicide target site thereby diluting out the toxic effect.

This provides an alternative strategy to protect crop plants from glyphosate by reintroducing the sensitive gene coding for the target enzyme, but under the control of a strong gene promoter to elevate levels of EPSP synthase protein to tritate out the herbicide. For example, when the petunia EPSP synthase gene was linked to the cauliflower mosaic virus 35S promoter and expressed in tobacco, the transgenic plants exhibited enhanced glyphosate tolerance and were unaffected by commercial use rates of this compound (Shah *et al.* 1986).

Phosphinothricin (PPT) is an irreversible inhibitor of the enzyme glutamine synthetase of bacteria and plants. Selection of a *Medicago sativa* cell line on increasing levels of PPT resulted in genomic amplification of glutamine synthetase and increased tolerance to the herbicide. Over-expression of the glutamine synthetase under the control of the 35S promoter conferred an increased level of PPT tolerance to transgenic tobacco (Eckes 1987).

Strategy 3 An exogenous detoxification system may be introduced to metabolize the herbicide before it has exerted its phytotoxic effect.

Many crops are already tolerant to certain herbicides and this forms the basis for their selective use for weed control. Quite often the basis for selectivity arises from differences in the metabolism of the herbicide by the tolerant plants although, in most cases, the multi-enzyme nature of such differences makes them difficult targets for direct genetic manipulation. Soil micro-organisms, on the other hand, are involved in herbicide breakdown in the environment and are more easily characterized biochemically and genetically. Microbial herbicide degradation genes have proved to be a useful source of detoxification genes for transgenic plants and have been used to confer tolerance to PPT, 2,4-dichlorophenoxyacetic acid (2,4-D), glyphosate, and the benzonitrile herbicides, bromoxynil and ioxynil.

A bacterial resistance gene coding for a PPT acetyl transferase (*bar* gene) has been used to detoxify the herbicide PPT. When expressed in transgenic tobacco, tomato and potato plants under control of the 35S promoter the *bar* gene has conferred high levels of resistance to PPT in both glasshouse and field studies (De Block *et al.* 1987). Similarly, a soil isolate of *Alcaligenes eutrophus* has been characterized and shown to degrade 2,4-D. The pathway for catabolism is plasmid encoded and many of the genes have been cloned and sequenced. The first gene in the pathway, the *tfd*A gene encoding a 2,4-D monooxygenase has been expressed in transgenic tobacco and cotton (Lyon *et al.* 1986, 1993). Cotton plants expressing the *tfd*A gene with a 35S promoter are significantly (3–5 times) less sensitive to 2,4-D than non transgenic plants. The broad-leaf herbicide, bromoxynil, a potent photosystem II inhibitor, is deactivated by a nitrilase enzyme found in a soil bacterium, *Klebsiella ozaenae.* The gene coding for this enzyme, when introduced into tobacco and cotton plants conferred resistance to 10–20 times the normal field rates of bromoxynil (Stalker *et al.* 1988). Likewise, a glyphosate oxidoreductase (*gox*) gene has been isolated from an *Achromobacter* species and been utilized in transgenic plants to detoxify glyphosate.

Each of these strategies has been used to successfully introduce herbicide tolerance into crop plants. The former two strategies rely on a detailed knowledge of the gene coding for the particular herbicide target enzyme. The last strategy has particular utility because it does not depend on the detailed knowledge of the mode of action of the herbicide and can be used if the herbicide has multiple sites of action.

DISCUSSION

Herbicide-resistant plants developed through genetic engineering are currently subject to controlled release under GMAC guidelines in several trial sites around Australia. Generally, these trials are a result of an industry interest in increasing the options for weed management. However, in certain instances genes conferring herbicide resistance are included for use as selectable marker genes as part of the mechanism for inclusion of other desirable traits into the transgenic plant. The *bar* gene conferring resistance to the herbicide PPT is particularly useful in transformation systems for various plant species including wheat, subterranean clover, peas, white clover, lucerne and lupins.

The CSIRO Division of Plant Industry has addressed the issue of benefits and risk of this area of research in consultation with other interested groups. In the Australian context, the development of herbicide-resistant crops should only be pursued for clear economic or environmental reasons. The risks of herbicide-resistant plants becoming major weeds or having the potential to outcross to weedy relatives needs to be recognised and carefully assessed on a case by case basis.

The Division is researching the introduction of genes for resistance to 2,4-D, glyphosate and bromoxynil into

Australian cotton cultivars. Transgenic cotton plants expressing the *tfd*A gene can survive field application rates of 2,4-D but do show some reduction in growth soon after spraying. It is anticipated therefore that overhead spraying of cotton crops with 2,4-D would not be commercially viable and the presence of the *tfd*A gene would only be useful for protection against damaging spray drift from other sources. The glyphosate resistance gene is currently being incorporated into Australian material by cotton breeders both in CSIRO and in commercial companies. The bromoxynil resistance gene is also being introduced into elite cotton cultivars by direct transformation to provide an alternative weed control option for this crop. A recent analysis by Charles *et al.* (1995) of the introduction of transgenic cotton carrying herbicide resistance showed that both glyphosate and bromoxynil resistance had the potential to reduce weed control costs and the overall quantity of herbicide used in Australian cotton. Their data suggested that herbicide resistant cotton plants would play a significant role in weed control, but emphasized the need for integrated weed management because of the risks that may be associated with herbicide resistance and the possible development of a new weed spectrum with continuing use of a single broad spectrum herbicide.

A collaborative project between CSIRO Plant Industry and NSW Agriculture has led to the trialling of a transgenic subterranean clover with increased tolerance to bromoxynil. Subterranean clover was transformed with the gene which detoxifies bromoxynil leading to a significant enhancement of subterranean clover's natural tolerance to this herbicide. In this case the introduction of the bromoxynil resistance gene was used to enhance a pre-existing characteristic of sub-clover and will enable more effective weed control to improve the productivity of clover-based pastures. The economic and environmental benefits of bromoxynil-resistant subterranean clover are outlined by Dear *et al.* (1995).

In Western Australia, the introduction of the *bar* gene for phosphinothricin resistance has enabled CSIRO in collaboration with The University of Western Australia to field test lupin plants resistant to this herbicide. In the Western Australia environment this trait represents a useful option in weed control and it may well significantly reduce the production costs for lupins in this area. A similar evaluation of transgenic peas resistant to phosphinothricin is being undertaken in eastern Australia in collaboration with NSW Agriculture. In both cases, a thorough analysis of the impact of the transgenic crops on management options for weeds will be undertaken prior to any commercial release.

CONCLUSION

Herbicide resistant crops are already an option in some farming systems. However, further trials in Australia are necessary prior to the commercial release of such crops. The extent to which herbicide resistant crops are used in agricultural systems will ultimately depend on commercial viability and a clear assessment of the risk involved in their use. Judicious use of herbicide resistant crops has the potential to reduce the overall application of herbicide. For example, a genetically engineered crop having resistance to a broad spectrum post-emergent herbicide could eliminate the use of pre-emergent herbicides in certain cropping systems. Furthermore the use of herbicide resistant crops should shift the pattern of herbicide use away from older relatively high dose compounds towards more modern low dose herbicides with negligible residue problems.

A number of risks associated with the use of herbicide resistant crops have been recognised. The emergence of herbicide resistance in weeds has been an increasing problem and it is feared that the use of herbicide resistant crops could exacerbate the spread of this resistance. Other potential risks include the possibility of the herbicide resistant biotype itself becoming a weed and the chance of gene transfer between the herbicide resistant crop and weedy relatives. Another issue which may arise with the use of the broad spectrum herbicides under these conditions could be the emergence of a new weed spectrum as weeds more tolerant to the herbicide invade to replace the native group.

A further significant limitation to the widespread introduction of herbicide resistant crops is the lack of total herbicides with target sites well defined at the molecular level. It is noteworthy in this regard that in over 80% of all field trials of genetically engineered herbicide resistant crop plants, the cultivars generally carry resistance to one of only two herbicides, glyphosate or phosphinothricin. Thus the range of herbicides available for introduction of resistance into a cropping or pasture system is necessarily limited.

Herbicide resistant crops will provide another option for inclusion in integrated weed management systems. The transgenic crops and pastures currently under trial in Australia will provide further information about the viability and safety of herbicide resistant plants in the Australian environment. It is, however, likely that the development of a wide range of herbicide resistant crops will be constrained by management and regulatory issues as well as by economic considerations.

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