

## Applications for molecular biology in weed management

N.G. Paltridge, CRC for Weed Management Systems and the Department of Plant Science, The University of Adelaide, Waite Campus, Glen Osmond, South Australia 5064, Australia. Email: crcweeds@waite.adelaide.edu.au

### Summary

The purpose of this review is to outline the potential of molecular approaches and techniques to make new contributions in the general field of weed management. Firstly, different approaches through which improved weed control might be attained are identified. These encompass methods for improved chemical, ecological and biological control, and the development of a better understanding of problems in weed management. The technology developed by molecular biologists and relevant to weed management is then identified. Techniques described include those for molecular genetic analysis, for the discovery and isolation of genes of interest, for genetic transformation and for introducing 'suicide' genes into plants of interest. Finally, areas where molecular biology could contribute further to weed management in future are identified. Strategies explored include the development of improved herbicides and herbicide resistant crops, more competitive crops, and more effective biocontrol agents. It is concluded that herbicide-based approaches are of continuing importance, but that future research efforts should also focus on preventing the escape of herbicide resistance genes and on the development of more competitive crop plants.

### Introduction

Molecular biology is widely accepted to have diverse and important roles in plant breeding and agricultural biotechnology. In the general field of weed management, the major contributions of the science have been the development of herbicide resistant crops and, to a lesser extent, in the field of weed population genetics. However, the potential of molecular biology to make additional contributions to weed management has not been explored in the literature, and the purpose of this review is to investigate the subject.

The paper begins by asking what weed scientists, agriculturalists and the custodians of natural ecosystems would really like to be able to do in weed management. What are the major problems they see and how could they be overcome? In the next section, the technology developed by molecular biologists and relevant to weed management is described, and the unique

opportunities the technology presents are highlighted. Finally, specific areas are identified where molecular biology might make new contributions to weed management in future.

The review is based on one prepared for a workshop held by the CRC for Weed Management Systems in February 1999. Though every effort was made to consult as widely as possible during its preparation, it should only be regarded as a starting point for further discussion.

### General objectives in weed science

In broad terms, weed managers (farmers, conservationists and weed scientists) aim to reduce the impact of weeds on crop yield and/or quality, and on the integrity of natural ecosystems. A long term objective is to cause weed seed banks to decline, to effect weed population control rather than 'symptom' control.

Following discussions with more than 20 different Australian crop and weed scientists, a number of objectives in weed management were identified. These are necessarily general and speculative but, if met, could represent major advances in weed management.

### Improved herbicide efficiency

The herbicide-based approach to weed management dominated weed science from the late 1940s to the late 1980s, and particularly during this latter period as soil conservation issues rose to prominence (Lovett and Knights 1996). There are, however, problems associated with a heavy reliance on herbicides; namely, the evolution of herbicide resistant weeds and the adverse effects the chemicals may have on human health and the environment in general. The development of herbicide resistant crops in recent years has only served to increase our reliance on herbicides, and has raised new concerns over the possible escape of herbicide resistance genes into related weed species and in volunteer crop plants.

Specific objectives as regards herbicides and weed management are as follows. Though meeting these objectives would further increase our reliance on herbicides, they would, at least in the short term, offer management advantages.

i. New herbicides that operate through biochemical mechanisms different

from those targeted already, offering greater flexibility in the chemical management of herbicide resistant weeds. These novel herbicides could be 'natural' toxins, derived from any organism with biocidal activity; for example, from plant pathogens.

- ii. New selective herbicides, offering the potential to control particular species in diverse plant populations.
- iii. Herbicides that do not affect non-target organisms, especially humans.
- iv. Herbicides that do not persist in the soil any longer than is desired for herbicide action.
- v. Herbicide resistant crops, enabling otherwise broad-spectrum herbicides to be used selectively. In theory, herbicide resistance genes could be sourced from any organism, including herbicide resistant weeds, and could lead to the development of improved herbicides with new modes of action. It is important that herbicide resistant crop plants do not create a weed problem themselves as volunteer weeds.
- vi. Weeds that are *not* resistant to herbicides. It is of the utmost importance that herbicide resistance genes incorporated into crops should not escape to related weed species through out-crossing, thereby rendering the herbicide concerned ineffective. If related weeds exist, the transformed crop plant should be incapable of out-crossing. Alternatively, the herbicide resistance should be designed to be ineffective in genotypes other than the genotype of the herbicide resistant cultivar.

### Increased competitiveness

In general terms, farmers would like their crops and pastures to compete more efficiently with weeds to reduce herbicide usage. Genetic strategies that enhance the ability of crop and pasture species to compete against weeds have not been applied to any great extent; however, this may need to change if herbicide-based approaches become less effective in future. Three general approaches could lead to increased competitiveness in cultivated plants.

**i. Improved access to water, nutrients and light.** It is desirable that crop plants emerge quickly from the seedbed, and compete aggressively for water, nutrients and light. Central to this is the development of an aggressive, efficient root system and a large leaf area. In some situations, farmers may want their established crop plants to persist for more than one year, and crowd out weeds that way.

**ii. Crops which utilize allelopathy.** Allelopathy is the suppression of plant growth by neighbouring plants or their residues. Though little definitive proof of

the mechanisms involved is available, there is a growing body of evidence of real allelopathic effects which could be important for agriculture (Nelson 1996). Wu *et al.* (1999) identified several ways that allelopathy could be used in weed management: allelopathic cover or smother crops; allelopathic rotational or companion crops; toxic extracts from allelopathic plants; allelopathic mulch or incorporated crop residues; natural herbicides; and the breeding of allelopathic crop cultivars with weed-suppressive potential. For this latter approach to be useful, allelochemicals would need to be synthesized and effective early in crop development, adversely affecting surrounding weeds during crop establishment.

**iii. Exploitation of microorganisms.** Though practically challenging, it may be possible to better exploit microorganisms in agriculture. This could be achieved by enhancing the symbiotic interactions of crop plants and microorganisms, or by utilizing free-living microorganisms which specifically promote the growth of crop plants. Either way, the crop plants benefiting from the interaction would then be relatively more competitive.

Though practically challenging, it may be possible to improve crop competitiveness by enhancing the symbiotic interactions of crop plants and microorganisms, or by exploiting growth-promoting microorganisms.

#### *Improved biological control*

Biocontrol agents are regarded here as any organism which can colonize a target weed, killing it out-right or rendering it less fit. The agents required for better weed control could be bacteria, fungi, insects or other pathogens that specifically target weeds. Another contribution biological agents could make is to break down herbicide residues in the soil, in situations where this is a problem.

#### **Molecular biology hardware**

Molecular biology is the use of molecular approaches and techniques to characterize biological phenomena at the molecular level, leading to an increased understanding of how genes are expressed, give rise to a specific phenotype, and evolve. Recent technical advances provide new opportunities for study in three broad areas; namely, in all areas of genetics, in gene discovery, and in the manipulation of plant genomes through genetic transformation. In this section, an overview is provided of the molecular tools which allow study in these three areas and are relevant to weed management. A description of the recently proposed and much publicized 'Terminator' technology is also included, as an interesting example of molecular biology in action in agriculture.

#### *Developments in the detection of genetic variation*

Advances in molecular biology have seen the development of a variety of genetic markers based on polymorphisms detected at the DNA level; namely, Restriction Fragment Length Polymorphisms (RFLPs) (Botstein *et al.* 1980, Tanksley *et al.* 1989), Simple Sequence Repeat polymorphisms or microsatellites (SSRs) (Tautz 1989), Random Amplified Polymorphic DNAs (RAPDs) (Williams *et al.* 1990) and Amplified Fragment Length Polymorphisms (AFLPs) (Zabeau and Vos 1993, Vos *et al.* 1995).

These methods are routinely used for the analysis of plant genomes, in order to identify markers linked to genes of agronomic interest. Such markers may then be used in map-based cloning strategies (see below) or for marker-assisted plant breeding. In this latter approach, selection for desired genes in breeding programs is achieved by assaying for the genotype of linked marker(s), rather than for the trait of interest. This is especially useful when the trait of interest is recessive or difficult to assay.

An alternative application of DNA markers is in the detection of genetic variation within plant or animal populations. This can be useful for the identification of particular genotypes, in taxonomy, and in measuring levels of genetic diversity in plant or animal populations, as is relevant in the study of population dynamics.

#### *Approaches to gene discovery*

Four general approaches are used for gene discovery; namely, map-based (or positional) cloning, transposon/T-DNA tagging, protein-protein interaction cloning, and bioinformatics/genomics. The following summary is based on a review by Martin (1998).

**i. Map-based cloning.** A map-based approach to cloning is one in which molecular markers closely flanking a gene of interest are used to isolate a DNA fragment carrying both flanking markers and therefore the gene of interest. The development of genetic maps containing large numbers of such markers and the recognition that gene content and order is often conserved between related species greatly facilitates this process.

**ii. Transposons/T-DNAs.** A second approach to gene cloning is based on gene 'knockout' – the inactivation of a given gene by the insertion of a transposon or of *Agrobacterium* transfer DNA (T-DNA) within the gene sequence. Loss-of-function mutants so generated are then analysed to determine the exact location of the transposon or T-DNA insertion; the analysis of flanking DNA sequence then

enables researchers to link the interrupted gene sequence to a particular mutant phenotype.

**iii. Protein-protein interaction cloning.** Having isolated one gene that plays a crucial role in some biological process, it is often of great interest to identify genes encoding proteins which interact with that key gene product. This can be achieved using the yeast two-hybrid system and protein-protein interaction cloning. In this process, the protein encoded by the gene of interest is introduced to yeast and expressed as 'bait'. Other sequences from a cDNA library are introduced to the same population of yeast cells, as 'prey'. Any physical interaction between 'bait' and 'prey' proteins activates the expression of a reporter gene and leads to the identification of clones encoding the 'prey'.

**iv. Genomics/bioinformatics.** The application of high volume information technology to biological databases has created a paradigm shift in gene discovery. Large numbers of cDNA sequences are publicly available for rice and *Arabidopsis*, and similar schemes are underway for a variety of other species. The ability to search for matches with cDNAs or genes in the database greatly simplifies the identification of transcribed regions within any candidate gene, and helps to identify or confirm putative functions.

There has also been exciting progress in the development of new techniques to analyse gene expression on a genome-wide basis. These new techniques are based on emerging DNA chip technology, which utilizes microscopic arrays of DNA molecules immobilized on solid surfaces for biochemical analysis (for reviews, see Lemieux *et al.* 1998, and Gerhold *et al.* 1999). Thousands of gene sequences, characterized or otherwise, are arranged on a microarray and hybridized to expressed sequences from biological samples of interest – for example, from roots synthesizing different levels of some allelochemical. The levels of expression of thousands of different genes can then be simultaneously compared, leading to the identification of genes which may play a role in the phenotypic difference – in this case, the synthesis of an allelochemical.

#### *Plant design through genetic transformation*

Practically all major crop plants can now be transformed, using either particle bombardment or *Agrobacterium*-mediated transformation. If the current trend towards transgenic crops continues, the majority of crops plants grown in future may well be transgenic. There are three different ways that genetic engineering can be used in crop improvement.

**i. The introduction of novel genes.** The first way that genetic engineering may be used in crop improvement is for the introduction of novel genes to crop plants. Initially, the genes introduced encoded simply inherited traits under single gene control – for example, for virus, herbicide or insect resistance. However, in recent years, complex traits under multi-gene control have been successfully introduced to plants, for resistance to high and low temperature, and to excess salinity (Grover *et al.* 1998). Provided ‘useful’ genes encoding other ‘useful’ multigenic traits can be identified, there is no reason why further multigenic traits should not be the subject of efforts in genetic engineering.

Importantly, tissue specific promoters of gene expression are now available in many crop plants (Gallie 1998), and libraries of plants expressing transactivators of transgene expression in a tissue-specific manner are available in *Arabidopsis* (<http://brindabella.mrc-lmb.cam.ac.uk/>) and may soon be available in other crop species. Thus, an era approaches where researchers will have access to the tools required for the spatial and temporal control of transgene expression in plants.

**ii. Gene knockout.** A second approach to plant design through genetic engineering is the gene knockout approach. Current strategies of effecting gene knockout are based on insertional mutagenesis using transposons or T-DNA vectors (see above).

A variation of gene knockout—or at least an alternative method of inducing some ‘loss-of-function’—is through gene silencing (Gallie 1998). In this approach, some fragment of the targeted gene is introduced, either in native (sense) or in reverse (anti-sense) orientation. Through some poorly understood mechanism, RNAs transcribed from these transgenic sequences suppress the expression of the target gene.

**iii. ‘Gain-of-function’ mutagenesis.** A final and more recent approach to plant design is that of ‘gain-of-function’ mutagenesis – an approach developed in *Drosophila* and now being tested in *Arabidopsis* (Paul Keese, personal communication). This approach seeks to effect genetic change not by introducing new genes to crop plants, or through loss-of-function mutagenesis, but by altering the levels of expression of genes already present in crop plants.

This is achieved by randomly inserting the genetic element recognized by a transcriptional transactivator into a crop plant of interest. When crossed with a plant expressing the transactivator in a tissue-specific manner, the expression of neighbouring sequences is up-regulated in a tissue-specific manner.

Advocates of this approach argue that the enormous species diversity evident in nature is based as much on variation in gene expression as on gene content. They argue that the genes already present in crop plant genomes offer enormous flexibility in plant design, and that the intelligent approach to plant design in future should be to alter the expression of these genes. An additional advantage of the approach is that it is more likely to be accepted publicly than other strategies in genetic engineering, since it can be argued that only gene ‘switches’, rather than genes themselves, are being manipulated (Paul Keese, personal communication).

#### *‘Terminator’ technology*

So called ‘Terminator’ technology is a recent invention of molecular biology of relevance to weed management, and is a specific example of a wider group of concepts called Genetic Use Restriction Technologies (GURTs). The technology is attracting a great deal of publicity at present and is worthy of consideration here.

In March 1998, a patent entitled ‘Control of Plant Gene Expression’ (US Patent Number 5, 723, 765; Control of Plant Gene Expression) was awarded to Delta and Pine Land Company (later to be purchased by Monsanto), covering a strategy whereby plants are engineered to kill their own seeds in the second generation. This technology has since been dubbed ‘Terminator’ technology by the Rural Advancement Foundation International. The following summary is based on a paper published on the internet by Associate Professor Martha Crouch (<http://www.bio.indiana.edu/people/terminator.html>). The approach is somewhat complex, and has three main steps<sup>A</sup>.

- i. A gene encoding a seed-killing toxin is introduced to the crop plant of interest, under the control of a seed-specific promoter which is only active late in seed development. To ensure that this transgenic plant can reproduce for enough generations to generate commercial quantities of seed, blocking DNA is introduced between the seed-specific promoter and the gene encoding the toxin. As long as the blocker DNA is present, the toxin is prevented from being expressed.
- ii. A gene encoding a ‘recombinase’ enzyme is introduced to the same plant. When expressed, the recombinase excises the blocker DNA, thereby enabling the expression of the toxin. To

exercise control over the expression of the recombinase, the promoter linked to this recombinase gene is repressible; i.e. able to be repressed by a ‘repressor’ protein.

- iii. A third gene, encoding just such a repressor protein, is introduced to the plant. It is expressed all of the time, but its repressive activity can be overridden by the addition of an ‘activator’ compound. In the example given, the activator compound is tetracycline, though other possibilities are available.

Thus, whenever tetracycline is absent, the repressor protein is expressed and represses the production of the recombinase enzyme. The blocker DNA remains in place and no seed-killing toxin is produced.

To activate the Terminator technology, seeds are treated with tetracycline. The repressor protein loses its repressive activity, and recombinase is expressed. This enzyme removes the blocker DNA, leaving the toxin gene functional and under the control of the seed-specific promoter.

One application seen for this technology is for the ‘biological’ protection of plant variety rights – any company utilizing the technology would be assured that farmers growing their crops could not save seed of their own to sow the following year, but would have to return to the crop’s developer to buy it from them. The developers of the technology also identify a second application, in preventing the escape of transgenic crops or their genes.

#### **Solving problems in weed management with molecular approaches**

In this section, areas where molecular approaches and technology might contribute to the general field of weed management are discussed. The areas identified to have potential fall into the three main categories, encompassing chemical, ecological and biological approaches. The potential of DNA chip technology to contribute to the discovery of genes relevant to weed management is also discussed, together with issues in intellectual property relevant to molecular biology.

#### *Herbicide efficiency*

The increased understanding of plant biology arising from advances in molecular biology should facilitate the development of new, safe, selective herbicides that do not persist in the environment for longer than is desired. This research area is assumed to be covered by public and private research programs the world over, and will not be explored in this review. Similarly, as more and more plant genes are isolated and characterized, new mechanisms of herbicide resistance are likely to be identified. Again, this area of research is

#### **Footnote**

<sup>A</sup> Subsequent to the preparation of this article, Monsanto made a public commitment not to commercialize sterile seed technologies such as Terminator (<http://www.monsanto.com/monsanto/gurt/default.htm>).

assumed to be well covered and will not be further discussed.

In the light of concerns about the release of herbicide-resistant crops, however, it may be worth discussing a related area of research – that of ensuring that herbicide resistance genes do not escape into the environment. Work is already underway to investigate the potential for herbicide resistant canola to outcross to wild radish (Rieger *et al.* 1999a,b). If this turns out to be a genuine possibility, or if other transgenic crops are found capable of outcrossing to weeds, new strategies for the prevention of herbicide resistance escape will be required.

One means of achieving this could be the inclusion of Terminator technology in herbicide resistant crops. Terminator seeds under cultivation will normally have been treated with tetracycline; the recombinase gene will have been expressed and the blocker DNA excised, so the toxin gene will be under the control of the seed-specific promoter. Seeds resulting from crosses between the herbicide resistant crop and some weedy relative would be heterozygous for an active toxin gene, which would express toxin late in development and render the seed non-viable.

Another possibility could be the engineering of herbicide resistant plants with *increased* susceptibility to a second herbicide or control agent. In this scenario, any instances of outcrossing to weedy species would 'pollute' the weed population with some susceptibility factor. Volunteer transgenics and/or their hybrid progeny could then be selectively removed by the second control agent.

Further options available for the prevention of gene transfer to weed species are presented in a review by Gressel (1999), published after the preparation of this report.

One final subject to raise in this section must be the possibility that chemical control of the future could target plant reproductive as well as vegetative processes. In combination, these two strategies might enable managers to effectively deplete the weed seed bank, and so achieve the ultimate goal of population management. The approach of targeting reproductive processes is already under investigation for the control of wild oats and selected broad-leaf weeds, with an inhibitor of meristematic growth being used to inhibit floral development (Dick Medd, personal communication). Researchers at CSIRO Plant Industry are also gaining new insights into the processes involved in floral initiation and development (Richard Richards, personal communication), thereby raising the question of whether this knowledge could be exploited to develop broad spectrum or specific inhibitors of flowering. Conceivably, crop

plants could be engineered to be resistant to this inhibitor if necessary.

#### *Increased competitiveness*

It may be possible to improve weed management by enhancing the ability of crop plants to compete with weed species. The three main approaches identified earlier which could achieve this were improving the plant's access to water, nutrients and light (either through increased plant vigour or changed morphology), the utilization of allelopathy, and the exploitation of microorganisms. An additional approach, that of reducing levels of 'fitness' in weed populations, is also discussed.

**i. Improving access to water, nutrients and light.** Improving plant access to light, water and nutrients is an obvious way to increase the ability of crop plants to compete with weeds, but has never been applied to any great extent in breeding programs. In fact, selection for some of the traits these programs have sought to develop – for example, for dwarf habit – has actually served to reduce plant vigour and competitiveness. The study of factors affecting competitiveness is therefore more important than ever. Wheat is the only crop species the author is aware of in which attributes contributing to plant vigour have been studied in detail (for examples, see Richards 1991, Lemerle *et al.* 1996, and Rebetzke and Richards 1999); however, the principles developed should apply to at least some other crop species.

Considerable variation in embryo size and leaf thickness has been identified in the wheat germplasm (Richard Richards, personal communication). Research has shown that genotypes with large embryos and relatively thin leaves give rise to more vigorous seedlings with larger first leaves and roots (Lopez Castanedo *et al.* 1996). These seedlings are therefore well placed to exploit moisture and nutrients early in development and are likely to be more competitive with weeds. No genes controlling these traits have yet been mapped. However, a population segregating for both embryo size and leaf thickness is being developed for mapping purposes. Once identified, linked markers could then be used to assist in the selection of these traits.

Coleoptile length is another trait relevant to early vigour and competitiveness (Liang and Richards 1994). Dwarf habits have been bred into modern cultivated wheats, improving markedly the efficiency with which wheat plants yield grain. However, the genes traditionally introduced to reduce plant height also reduce coleoptile length and, as a consequence, dwarf wheats often suffer poor emergence and are compromised in their competitive ability. Alternative dwarfing genes have been identified which offer the

potential for the development of dwarf wheats that still produce a long coleoptile (Rebetzke *et al.* 1998). Such plants are expected to emerge vigorously from the soil and compete better with weeds than current wheat cultivars. A DNA marker for coleoptile length has already been identified, again offering the potential for marker-assisted selection. While selection for the trait may be made relatively easily using a visual score, it is envisaged that the linked marker would still be very useful for the pyramiding of different genes encoding high seedling vigour – for example, for the development of genotypes with both large embryos and long coleoptiles.

Plant root morphology must be just as important to overall plant vigour as the above-ground morphology of crop plants, and represents another aspect of plant phenotype which could be manipulated using methods in molecular plant breeding. However, it is not clear whether there exists significant variation in root morphology within the different crop species. Resolving this question will be an important first step towards determining the potential of improving overall plant competitiveness through root morphology. A comprehensive review of plant competition underground has been published recently (Casper and Jackson 1997), and serves to highlight the complexity of the field. The authors conclude by saying that, while much is known about the ways roots respond to their environment, we are far from linking these responses to the ways plants affect each other.

A related area of research is that of nutrient-use efficiency. In general, 'weedy' species take up and use soil nutrients more efficiently than most crop plants (Robin Graham, personal communication), contributing to their relative competitive ability. Genetic variation in the ability of crop plants to tolerate and utilize low levels of particular nutrients does exist in a variety of crop plants (for example, in cereals; Graham 1984). Therefore, there would appear to be considerable scope for improving crop competitiveness *via* improved nutrient acquisition and use efficiency. However, the area is already attracting considerable interest in the general context of production agriculture, and is not a focus of this review. Interested readers are referred to a recent review by Hirsch and Sussman (1999).

One possible ecological approach which has already attracted some attention, again in wheat, is the possibility that annual crops could be grown as biennial or perennial plants. The major advantage of this extended life-span would simply be that crops would not need to be established every year – something that might give them a relative advantage over weeds.

In wheat, related species with perennial growth habit do exist, and programs to cross these species to cultivated wheats are already underway at Washington State University (McGill 1998). The next step must be to determine the genetic basis of the perenniality; however, once determined, methods in molecular plant breeding could be used to develop perennial wheat or other cereals of commercial quality.

Other more drastic changes to patterns of crop plant growth may be considered in future, as functions are determined for more and more plant genes. One obvious example would be to engineer plants to grow in a prostrate pattern at first, to gain maximum ground cover for weed control and water use efficiency, before bolting to producing seed heads which could be readily harvested – in the manner of some Brassicaceous plants, for example. Many genes are known that control plant height (Gressel 1999, and references therein), and it is possible that these could be further exploited in future.

If currently C3 plants are found to possess much of the genetic hardware for C4 photosynthesis, then it may be possible to alter the photosynthetic pathways of C3 plants too, through gain-of-function mutagenesis or genetic transformation. C4 plants are especially well adapted to hot, dry environments with a high light intensity (Bjorkman and Berry 1973). In these conditions, the C4 pathway could increase the relative competitiveness of crop plants currently utilizing C3 photosynthesis.

**ii. Allelopathy.** The utilization of allelopathy in crops and pastures provides another opportunity for ecological solutions to be developed in weed management, and represents an area where molecular biology could make an important contribution. Significant variation exists in the allelopathic potential of a variety of crop and pasture species, including cucumber, rice, wheat, barley, *Brassica* and *Phalaris*. In most cases, the genetic basis of these effects has not been determined, but is likely to be multigenic.

There are a number of questions which need to be addressed when considering the relevance of molecular biology to the development of allelopathic crops (John Kirkegaard, personal communication). Firstly, can the allelopathic potential of existing crops be better exploited through changes in farm management strategies? Allelopathy in crops is by no means completely understood, and may be better utilized simply by changing the way existing crops are managed (i.e. through changed rotations, stubble retention etc.). Such strategies may provide results ahead of more expensive genetic solutions.

Secondly, can crops with greater allelopathic potential be developed using

a conventional breeding approach? The transfer of genes encoding allelochemicals from crop progenitors to current crop cultivars has already been demonstrated (Wu *et al.* 1999). In some cases, crops with greater allelopathic potential could be bred using existing genetic material and a conventional approach to breeding. Given the likelihood that allelopathic potential is inherited as complex trait, marker-assisted selection could hasten the process considerably.

If existing genetic material is deemed insufficient for effective allelopathy, researchers may consider genetic engineering. Approaches taken could be to manipulate one step in a complex pathway, resulting in increased levels of a desired compound, or to introduce whole new pathways into crop plants. Still more questions would need to be addressed: What is the allelochemical? How is its synthesis controlled? How many genes are required for its synthesis? Can these be cloned? Can the crop plant be transformed in such a way as to be acceptable agronomically, with the allelochemical expressed in the right tissue at the right time? Will the allelochemical be toxic to the crop plant itself, or even to consumers (human or other)? Will the continuous production of the allelochemical prove expensive physiologically, and so reduce yield?

The only example the author is aware of where answers are possible for several of these questions is the glucosinolate biosynthesis pathway in Brassicaceous plants. Glucosinolates are known allelochemicals (Brown and Morra 1997) with recognized potential for use as bio-herbicides. The pathway leading to their synthesis is well characterized and the cloning of the genes responsible in *Arabidopsis* is imminent. These genes could, therefore, be a target for manipulation in future (Halkier and Du 1997).

**iii. Interactions with microorganisms.** Except for a few well studied examples (*Rhizobium*, mycorrhizal fungi and *Frankia*), the symbiotic relationships between plants and microorganisms in the soil are poorly understood. They can involve free-living, root-associated or endophytic fungi or bacteria, and can result in any of the following (Maarten Ryder, personal communication):

- i. the antagonism of pathogens;
  - ii. the induction of 'systemic acquired resistance' (SAR);
  - iii. increased production of growth-promoting hormones;
  - iv. nitrogen fixation;
  - v. increased access to nutrients; and
  - vi. growth promotion through some unknown mechanism (Ogoshi *et al.* 1997).
- There is no doubt that molecular biology could one day enable the manipulation of

some of the microorganisms involved to specifically promote the growth of crop plants, but not weeds (for example, Phillips and Streit 1998). Further, molecular biology could enable the design of new symbiotic relationships, or even systems whereby microorganisms are employed to deliver advantageous genes (for example, herbicide resistance) to target plants. However, the release of genetically-modified microorganisms into the environment would no doubt be controversial, and it would be difficult to engineer and control the spread of the microorganisms in the complex ecology of the soil. A more feasible approach might be to identify microorganisms which already have a beneficial effect, and then manipulate their interaction with the plant via the plant; i.e. improve the plant's ability to attract and sustain an association with beneficial microorganisms.

**iv. Reducing 'fitness' in weed populations.** The idea of 'polluting' weed germplasm with some gene which could make weeds more susceptible to herbicides was mentioned briefly in the section on chemical management. A logical extension of this approach could be to 'pollute' weed populations with some other 'unfitness factor'. This could be spread by the pollen of unfit weeds planted in the population for the purpose. A logical conclusion of the idea could be to use the Terminator technology discussed previously as the lack-of-fitness factor. Pollen carrying the Terminator gene could be spread throughout the weed population, and any seeds arising from fertilization by such pollen would be killed.

A slight modification of the strategy could result in the triggering of 'termination' some time after the initial release of pollen carrying the Terminator gene. Under the current system, termination is triggered by the application of an 'activator' compound (tetracycline) to seeds. In weed populations, why not apply the activator some years down the track? (The activator would need to be something besides tetracycline, since the broad-scale application of the antibiotic could be unrealistic and unwise). This would give the Terminator gene a number of years to spread through the population. And finally, why not add a 'fitness' factor to the cassette carrying the Terminator gene? This could help the Terminator to spread before its expression was triggered.

Another approach, similar in principle, has been proposed for reducing fitness in insect populations (Pfeifer and Grigliatti 1996) and could conceivably be applied in weed management. In this approach, a lack of fitness gene, under the control of an inducible promoter (again, the use of the tetracycline-based system would seem unwise), would be linked to a transposon.

This construct would be introduced to the targeted species, for planting amongst populations in the field. Over several generations, the construct would be transmitted to the majority of their offspring due to the nature of transposon spread. Expression of the lack of fitness factor could finally be induced by the application of the activator compound.

The serious inadequacy of the concept of spreading 'unfitness' in a weed population is that it is highly unlikely that the unfitness factor could be introduced to every plant in the population. Since affected plants would ultimately be less fit than their wild-type relatives, they would be out-competed and lost from the population. The factor would therefore need to be repeatedly introduced to the population, and it is hard to envisage a scenario where this would be practicable on a commercial scale.

### Biological control

A most important role for molecular biology is in the taxonomy of weeds and their biocontrol agents, as this information helps match control agents to different weed biotypes in genetically diverse populations. Information on the genetic diversity present in weed populations is also crucial to predicting population responses to biocontrol strategies. These roles for molecular biology in weed management are already recognized, for example in skeleton weed (Chaboudez 1994), and will not be discussed further.

The only other aspect of molecular taxonomy which might be worthy of consideration could be commercial-scale diagnosis of weed genotypes. This could be conducted on weed plants collected from the paddock, or on weed seeds collected from grain samples or the soil. If ever there was a commercial need to distinguish morphologically similar samples, or to detect undesirable genetic material – such as some herbicide resistance gene – a simple molecular assay would be ideal.

A controversial application for molecular biology in weed management could be in the genetic engineering of more effective biocontrol agents. Plant-pathogen interactions have already been extensively studied and characterized at the molecular level, mostly in response to demand for disease resistant crops. However, this understanding of the determinants of pathogen virulence could also be used to develop more virulent pathogens, and the author has no doubt that this would be possible. Indeed, a number of interesting strategies for the development of more effective and safe biocontrol agents have been proposed (Sands and Miller 1993). A major concern would be that the super-virulent pathogen be specific for the target weed yet never infect it in its native environment. The agent should also be

incapable of infecting non-target plants, and of crossing to other microorganisms to create new transgenic organisms. These assurances would be difficult to give and, given the level of community concern about the release of transgenic crops, it seems unlikely that transgenic pathogens will be released in the foreseeable future.

In any case, fungal and bacterial bioherbicides already exist for many weed species, but cannot be exploited because of limitations in formulation technology. This is already recognized and work is already underway to find ways of applying bioherbicides so that they can actually infect the target plant (Dick Medd, personal communication).

### DNA chip technology

A striking contribution that the field of molecular biology could make to weed management would be the identification and cloning of genes important in weed management using the emerging DNA chip technology. The genes which stand out in this review as amenable to the approach are those encoding aspects of plant vigour, perennial habit, and the ability to synthesize allelochemicals.

The tools required for the work would be libraries of DNA representing the relevant crop plants, arranged in a microarray. These would then be screened with expressed sequences from plants or tissues expressing the phenotype of interest, so that hybridization patterns could be compared with those obtained using plants or tissues lacking the desired phenotype. How soon such screens could be conducted will depend on the commercial availability of chips, detection systems, software and reagents. Though prohibitively expensive at the moment, enthusiasm in the private sector suggests affordable systems may be available soon (Lemieux *et al.* 1998).

### Intellectual property issues

Many of the techniques developed in molecular biology are patented inventions. This is something often ignored by researchers in the agricultural sector and, in the many cases where research findings have no great commercial implications, this is of little practical importance.

Occasionally, however, a research outcome is identified with far-reaching commercial implications. This is when serious problems can arise, with the owners of a patented technology demanding fees for its use, a share of any profits, or prohibiting the commercialization of the invention altogether. In these situations, researchers regret not having investigated intellectual property issues more thoroughly, or regret using a patented technique when a non-patented alternative was available. For this reason, researchers are urged to

consider issues in intellectual property whenever they consider using tools in molecular biology or, indeed, any patented technology.

### Conclusions

Molecular biology is clearly important to the development of new herbicides and herbicide resistant crops, and in measuring genetic diversity in populations of weeds and their various biocontrol agents. These roles are already recognized in the scientific community and will continue to be important in future. Important roles also exist in the development of safe strategies for herbicide resistant cropping, and in the breeding of factors for increased crop competitiveness. While these areas are under current investigation, there is considerable scope for the effort to be expanded. There seems little doubt that the marker-assisted selection of traits for increased crop plant vigour and/or allelopathic potential will become a reality.

The imminent revolution in gene discovery is one major factor identified in this review which could have far-reaching implications for weed management. The isolation of various genes which affect crop competitiveness, together with refined techniques for plant transformation, probably will enable the development of crops better able to compete with weeds. Provided they can be grown commercially and are accepted by the community, these crops will have a major impact on weed control in future.

### Acknowledgments

The author gratefully acknowledges the input of the following people during the preparation of this review: Rick Roush, Steve Powles, Peter Langridge, Jill Karotam, Mary Rieger, Kathy Evans, Richard Richards, Deirdre Lemerle, Max Tate, David Coventry, David Briese, Maarten Ryder, Mat McCallum, Greg Rebetzke, John Passioura, Richard Groves, John Curran, Jeremy Burdon, John Kirkegaard, Paul Keese, Richard Jefferson, Tony Pryor, Jeff Ellis, Bob Symons, Uli Theopold, Glen McDonald, Dick Medd, Bryan Whan, Gurjeet Gill and Robin Graham. Special thanks go to Rick Roush and Glen McDonald for their critical reviews of the manuscript. The author also thanks two anonymous reviewers for their suggestions.

### References

- Bjorkman, O. and Berry, J. (1973). High-efficiency photosynthesis. *Scientific American* 229 (4), 80-93.
- Botstein, D., White, R.L., Skolnick, M.H. and Davis, R. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32, 314-31.

- Brown, P.D. and Morra, M.J. (1997). Control of soil-born pests using Glucosinolate-containing plants. *Advances in Agronomy* 61, 167-231.
- Casper, B.B. and Jackson, R.B. (1997). Plant competition underground. *Annual Review of Ecology and Systematics* 28, 545-70.
- Chaboudez, P. (1994). Patterns of clonal variation in skeleton weed (*Chondrilla juncea*), an apomictic species. *Australian Journal of Botany* 42, 283-95.
- Gallie, D.R. (1998). Controlling gene expression in transgenics. *Current Opinion in Plant Biology* 1, 166-72.
- Gerhold, D., Rushmore, T. and Caskey, C.T. (1999). DNA chips: promising toys have become powerful tools. *Trends in Biochemical Sciences* 24, 168-73.
- Graham, R.D. (1984). Breeding for nutritional characteristics in cereals. *Advances in Plant Nutrition* 1, 57-102.
- Gressel, J. (1999). Tandem constructs: preventing the rise of superweeds. *Trends in Biotechnology* 17, 361-366.
- Grover, A., Pareek, A., Singla, S.L., Minhas, D., Katiyar, S., Ghawana, S., Dubey, H., Agarwal, M., Rao, G.U., Rathee, J. and Grover, A. (1998). Engineering crops for tolerance against abiotic stresses through gene manipulation. *Current Science* 75, 689-96.
- Halkier, B.A. and Du, L. (1997). The biosynthesis of glucosinolates. *Trends in Plant Science* 2, 425-31.
- Hirsch, R.E. and Sussman, M.R. (1999). Improving nutrient capture from soil by the genetic manipulation of crop plants. *Trends in Biotechnology* 17, 356-61.
- Lemerle, D., Verbeek, B., Cousens, R.D. and Coombes, N.E. (1996). The potential of selecting wheat varieties strongly competitive against weeds. *Weed Research* 36, 505-13.
- Lemieux, B., Aharoni, A. and Schena, M. (1998). Overview of DNA chip technology. *Molecular Breeding* 4, 277-89.
- Liang, Y.L. and Richards, R.A. (1994). Coleoptile tiller development is associated with fast early vigour in wheat. *Euphytica* 80, 119-24.
- Lopez-Castaneda, C., Richards, R.A., Farquhar, G.D. and Williamson, R.E. (1996). Seed and seedling characteristics contributing to variation in early vigor among temperate cereals. *Crop Science* 36, 1257-66.
- Lovett, J.V. and Knights, S.E. (1996). Where in the world is weed science going? Proceedings of the Eleventh Australian Weeds Conference, Melbourne, pp. 3-13.
- McGill, S. (1998). Perennial wheat: getting closer. *The Furrow*, Issue 2, pp. 8-9.
- Martin, G.B. (1998). Gene discovery for crop improvement. *Current Opinion in Plant Biotechnology* 9, 220-26.
- Nelson, C.J. (1996). Allelopathy in cropping systems - Forward. *Agronomy Journal* 88, 853.
- Ogoshi, A., Kobayashi, K., Homma, Y., Kodama, F., Kondo, N. and Akino, S. (Eds) (1997). Plant growth-promoting rhizobacteria: present status and future prospects. Hokkaido University, Sapporo, Japan.
- Pfeifer, T.A. and Grigliatti, T.A. (1996). Minireview: Future perspectives on insect management: engineering the pest. *Journal of Invertebrate Pathology* 67, 109-119.
- Phillips, D.A. and Streit, W.R. (1998). Modifying rhizosphere microbial communities to enhance nutrient availability in cropping systems. *Field Crops Research* 56, 217-21.
- Rebetzke, G.J. and Richards, R.A. (1999). Genetic improvement of early vigour in wheat. *Australian Journal of Agricultural Research* 50, 291-301.
- Rebetzke, G.J., Richards, R.A., Fischer, V.M. and Mickelson, B.J. (1998). Breeding long coleoptile, reduced height wheats. *Euphytica* 106, 159-68.
- Rieger, M.A., Preston, C., Potter, T. and Powles, S.B. (1999b). Gene flow from transgenic canola to wild radish - a model system to determine the risks. Gene Flow and Agriculture: Relevance for Transgenic Crops. British Crop Protection Council Symposium Proceedings No. 72, 131-36.
- Rieger, M.A., Preston, C. and Powles, S.B. (1999a). Risks of gene flow from transgenic herbicide-resistant canola (*Brassica napus*) to weedy relatives in southern Australian cropping systems. *Australian Journal of Agricultural Research* 50, 115-28.
- Richards, R.A. (1991). Crop improvement for temperate Australia: future opportunities. *Field Crops Research* 26, 141-69.
- Sands, D.C. and Miller, R.V. (1993). Evolving strategies for biological control of weeds with plant pathogens. *Pesticide Science* 37, 399-403.
- Tanksley, S.D., Young, N.D., Paterson, A.H. and Bonierbale, M.W. (1989). RFLP mapping in plant breeding: new tools for an old science. *Bio/Technology* 7, 257-64.
- Tautz, D. (1989). Hypervariability of simple sequences as a general source of polymorphic DNA markers. *Nucleic Acids Research* 17, 6463-71.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23, 4407-14.
- Williams, J.D.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18, 6531-35.
- Wu, H., Pratley, J., Lemerle, D. and Haig, T. (1999). Crop cultivars with allelopathic capability. *Weed Research* 39, 171-80.
- Zabeau, M. and Vos, P. (1993). Selective restriction fragment amplification: a general method for DNA fingerprinting. *European Patent Application* 92402629.7 (Publication No. 0 534 858 A1).