Plant Biotechnology: Global Perspectives and Irish Priorities

E. Guiney, D. Milbourne, E. Mullins, S. Barth, and J. I. Burke.
Teagasc, Crops Research Centre, Oak Park, Carlow.

Introduction

The agri-food industry in the next decade will operate in a rapidly changing world environment due to increased competitiveness, globalisation of prices, and consumer demands for food quality, safety, health enhancement and convenience. It is therefore imperative to adopt new and innovative techniques to improve the competitiveness and efficiency of the crop sector. Innovation is essential for sustaining and enhancing crop productivity, and has always involved new, science-based products and processes which have in the past contributed reliable methods for increasing productivity and environmental sustainability. Irelands' capacity to compete will be dependent on the quality of our technology and the capacity of producers and processors to apply that technology. The set of techniques commonly referred to as biotechnology has introduced a new dimension to such technology.

From Genomics to Systems Biology

Genetic research over the last decade has experienced a paradigm shift, driven largely by the global initiative to characterise the DNA sequence of the human genome. The volume of work required for this task has stimulated the development of an array of technologies and experimental methodologies that form the basis of what we now refer to as genomics. Genomics could be viewed as having two distinct (but potentially overlapping) phases. The first phase comprises high throughput gene discovery and genome sequencing. The second phase involves exploitation of data from the first phase to examine the expression and effect of many genes simultaneously. This prospect is enormously attractive to biologists given the complexity of most biological processes. Plants contain an estimated 25,000 to 60,000 genes (Bennetzen, 2000), and the products of hundreds of these genes may contribute to the expression of a single character or trait. Up until recently, a limiting factor in understanding such complex processes has been the necessity to identify the underlying genes and characterise their expression patterns and interactions on an individual basis.

The primary method of high throughput gene discovery that has been adopted for both plant and animal systems is EST (expressed sequence tag) sequencing (WWW Ref1). Active genes make up as little as one percent of the total complement of DNA in a chromosome, and thus, sequencing the entire chromosome to identify the sequence and location of the genes on that chromosome could be viewed as a radically inefficient process. However, during their expression, genes are 'copied' from the chromosome by the cellular machinery, and at any one point in time, the majority of the genes also exist in a 'free form', called mRNA, in the cell. These mRNA molecules can be converted back into individual DNA molecules representing the genes, and the partial DNA sequence of large numbers of the genes can be determined very rapidly using modern DNA sequencing technology (Fig.1). The resulting sequences are archived in databases that are publicly accessible via the internet. Hundreds of thousands of EST sequences from the world's most significant agricultural crop species are now available in these databases.

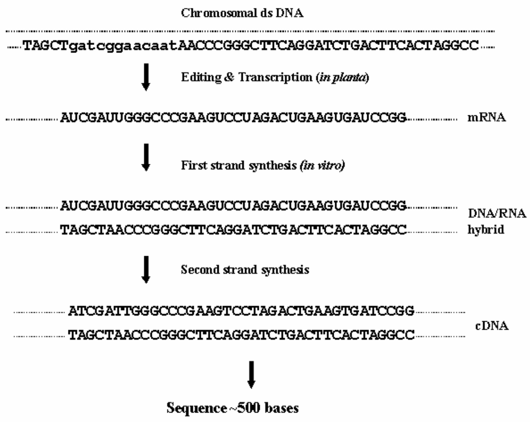

Fig.1: During gene expression, one strand of the double stranded chromosome is edited and transcribed into mRNA in the cell. RNA is distinguishable from DNA because it has the base uracil (U) in place of thymine (T). The total mRNA from a tissue or organ is extracted, and two in vitro steps produce a mixture of complementary DNA (cDNA) molecules representing every gene that was being expressed in the tissue. These cDNA molecules are cloned and partially sequenced to produce ESTS.

EST sequencing as a gene discovery method has several drawbacks. Foremost amongst these is the fact that it is impossible to sequence all of the genes in an organism using this method. Many genes are expressed at a very low level or only in a specific tissue at a specific time in complex organisms. Even using different tissues and developmental stages as a source of ESTs, the practical upper limit for the proportion of genes that can be identified in an organism by this method is in the region of 70%. Thus, while sequencing the entire genome (i.e. the DNA in all of the chromosomes) of an organism is not an efficient method of gene discovery, it is probably the most effective. Further advantages of whole genome sequencing include the ability to identify the chromosomal positions and associated regulatory regions of genes. Sequencing entire plant genomes is not a trivial task. The publicly funded initiative to sequence the human genome involved 16 major research centres, and a working draft of the sequence consisting of 90% of the genome took a total of ten years to complete at a cost of $300 million (WWW Ref2). While advances in technology have reduced the time and cost inputs, sequencing significant portions of plant genomes is still an extremely expensive process. This is further exacerbated by the fact that many plant genomes are similar in size or even larger than the human genome. Of the three most agronomically significant crop plant species worldwide, only rice has a genome significantly smaller (approximately one sixth) than the human genome, while maize has a genome of almost the same size, and wheat a genome that is five to six times the size of the human genome. Given the enormity of the task, the plant genetics community initially focused on the concept of sequencing a very small plant genome in order to ascertain the basic set of genes for a plant species. The species chosen for this task was not a crop plant, but *Arabidopsis thaliana*, a small member of the mustard family with a genome approximately 25 times smaller than the human genome. The basic assumption was that, given the similarities of all plants, many of the genes found in *Arabidopsis* will also be present in crop plants. An obvious limitation of the *Arabidopsis* sequence is that while *Arabidopsis* is a dicot (broad-leafed) species, many of the most important crop species in the world are monocotyledonous grass species with significant differences in morphology and physiology to dicots. Thus, sequencing of the rice genome (one sixth the size of the human genome) was initiated soon after the *Arabidopsis* genome to provide a model genome sequence for grass (and cereal) species.

The publicly funded *Arabidopsis* and rice sequencing projects were completed in 2000 and 2002 (The Arabidopsis Genome Initiative, 2000, Bennetzen et al. 2002) respectively and the availability of the complete sequences of these species has given real impetus to one aspect of the aforementioned second phase of genomics sometimes referred to as comparative structural genomics. Species that have evolved from a common ancestor relatively recently in evolutionary time tend to share the same basic set of genes, organised in the same order along their chromosomes. Thus the position and sequence of genes in the rice genome can be used to predict the position and sequence of genes in related species such as wheat, barley, maize and other grasses (Gale and Devos, 1998). This relationship breaks down as the relatedness of the species decreases, and the predictions must be based on comparative maps of the chromosomes of the species in question. These maps indicate which chromosome regions in the crop species are equivalent to which chromosome regions in the model species. While rice is an excellent predictive model for cereals, *Arabidopsis* has a similar level of utility for crops of the genus *Brassica* (eg. oilseed rape, cabbage) (Lagercrantz, 1998), but its utility is limited in other dicotyledonous crop species. This has prompted the initiation of plans to sequence significant portions of the genomes of other crop plants that can act as models for closely related species, eg tomato as a model for other solanaceous crops such as potato, pepper, and aubergine.

Another aspect of the second phase of genomics, referred to as functional genomics, deals largely with elucidating the roles of genes identified during the types of gene discovery programmes described above. Approximately 50% of the genes discovered in all plant species to date currently have no known function. Gene discovery programmes have allowed the development of powerful tools for what is referred to as massively parallel gene expression analysis, where the level and timing of the expression of thousands of genes can be examined simultaneously for a given set of environmental conditions. For over two decades, molecular biologists have taken advantage of the fact that, under certain conditions, DNA molecules with the same sequence will hybridise (or stick to each other), to discover whether the DNA extracted from an organism of interest has the gene they are interested in or not. One of the most recent developments of this DNA hybridisation technology are microarrays, or DNA chips (WWW Ref3), where thousands of individual genes identified during gene discovery programmes are deposited in arrays of highly concentrated spots on to the surface of a glass microscope slide. mRNA (or its DNA dervative), isolated from an organism that has been exposed to a certain set of environmental conditions (eg a plant that is being infected by a pathogenic fungus) can be hybridised to the DNA on the chip. A detection system involving fluorescent molecules that have been attached to the DNA makes it possible to identify which genes on the chip are present in the DNA from the organism, and how active the genes are for that particular environmental condition (Fig.2). The process is repeated for various environmental conditions, and for different tissues and organs in the organism, yielding a powerful insight into which genes are active during certain processes.

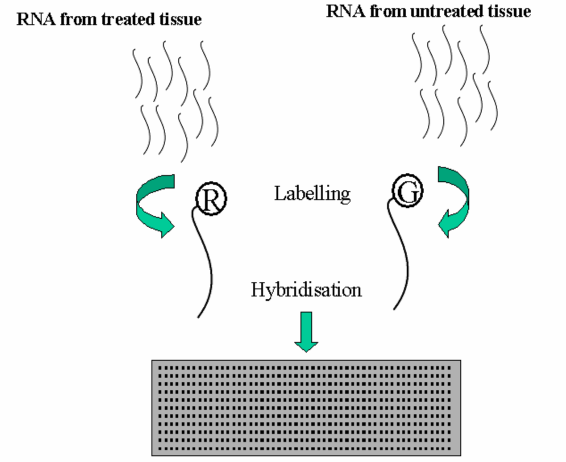

Fig. 2: Depending on the process used, tens or hundreds of thousands of genes can be 'spotted' on to a microarray the size of a microscope slide. mRNA is then extracted both from tissues or organs subjected to a certain treatment (eg disease), and untreated tissues. The RNA molecules are labelled with dyes that fluoresce red (treated) or green (untreated) when scanned with light of a certain wavelength. Both sets of labelled RNA solutions are incubated with the genes on the slide. When scanned, the spots will appear: red, indicating a gene that is only active in the treated sample, or; green, indicating a gene active only in the untreated sample, or; yellow (red + green) indicating a gene active in both samples. No fluorescence of a spot indicates the gene is not active in either sample.

Several commercial companies produce microarrays comprising standard sets of genes, or custom microarrays containing genes of interest to individual research groups. However, the high cost of these services has prompted the same consortia involved in high throughput gene discovery programmes in plant species to produce and make microarrays available to the plant genetics community on a cost recovery basis, at a significant reduction in price compared to commercially available arrays. Microarrays comprising tens of thousands of genes are now either available from, or being developed by such consortia for plant species for which there are significant numbers of sequenced genes. In addition to microarrays, several other methods of massively parallel gene expression analysis have been developed and are being used in plant species.

Another approach traditionally used by plant geneticists to identify gene function is the use of mutants, or gene knockouts, in which individual genes are rendered non-functional, and the effect on the plant is observed. Mutations can arise naturally or be induced by deleting or damaging a gene artificially via chemical or biological mutagens, or ionising radiation. Large scale gene discovery programs have galvanised the initiation of several programmes aimed at producing large collections of mutant or knockout plants with the eventual goal of producing one or more gene mutants for every plant gene. The availability of mutants for almost every gene identified in a gene discovery programme for any crop species would constitute a powerful resource for defining the function of these genes in that species. The closest to this ideal scenario currently exists in *Arabidopsis* where mutant have recently been generated for over 75% of genes (Alonso et al. 2000)

Over the last several years, the genomics paradigm shift has also been applied to areas such as protein and metabolic biochemistry, spawning disciplines such as proteomics and metabolomics. High throughput and massively parallel identification in the analysis of proteins and complex biochemical molecules are yielding insights into biological systems at a point further downstream to gene expression. Genomics, proteomics, and metabolomics, along with allied disciplines such as bioinformatics (which deals with the databasing and computational analysis of the vast amounts of data produced by these approaches), have come to be collectively referred to as "systems biology". Over the next decade, systems biology approaches will be of primary importance in providing the basic knowledge that will allow advances to be made in the applied areas of plant biotechnology discussed in the remainder of this article. Given this fact, it is essential for the Irish plant biology community to establish and maintain a competence in this area, with a focus on nationally significant crop species such as perennial rye-grass, clover, potato and cereals. With reference to the first 'phase' of genomics discussed above, Ireland has made little contribution to high throughput gene discovery in plant species. The relatively high cost of participating in gene discovery consortia, coupled with the lack of an appropriate funding mechanism, as well as the limited size of the country as a whole and the plant molecular biology community in particular have all contributed to this. Nevertheless, participation in such international efforts may be central to ensuring that Irish scientists have unfettered and timely access to the resources developed in these programmes. The second phase of genomics is based largely on exploiting those resources that are made freely available as a result of the first phase. This area is currently more suitable for exploitation in the Irish context, and both universities and state-sponsored research bodies such as Teagasc are beginning to apply the techniques described above to nationally important crop plant species.

Genetic Transformation

Undoubtedly the best known application in plant biotechnology, genetic transformation (genetic modification, GM) has become the system of choice for the introduction of traits and characteristics with the potential to significantly increase productivity of agricultural crop production. Transformation offers vast improvements over traditional plant breeding methods in terms of both the speed with which new varieties can be brought to the market, and the overall level of improvement that can be achieved for specific plant characteristics.

Genetic transformation of plants is essentially a three stage process. The first stage involves developing an appropriate 'expression cassette' by fusing the gene of interest (GOI) with other DNA sequences essential to the transformation process. Regulatory sequences, in front and behind the GOI, determine when and where the resulting protein is produced in the plant. A selectable marker gene may be included in the expression cassette, allowing cells that have successfully been transformed to be identified later in the process. The second stage of the transformation process involves delivery of the expression cassette into plant cells via one of two very different systems (Fig.3).

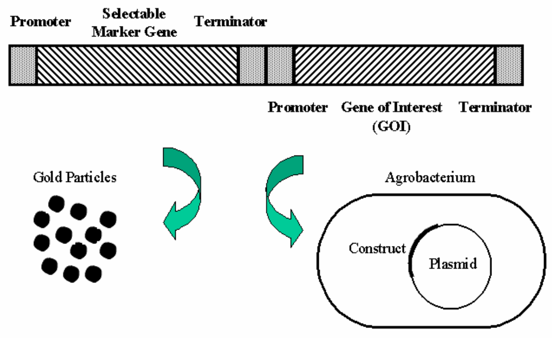

Fig. 3: The transgene construct consists of the gene of interest, fused to a selectable marker gene, both of which may be flanked by promoter and terminator sequences that control the expression of the genes in the plant. The construct is then coated onto gold particles, which are fired into plant cells using a high pressure delivery device called a biolistics-gun. Alternatively, the construct may inserted into be a closed circular DNA molecule (called a plasmid) carried by the bacterium *Agrobacterium tumefacians*. In nature, when *Agrobacterium* attacks plant cells, it inserts some of its plasmid genes into the plant chromosomes. In this transformation technique, these plasmid genes have been replaced by the construct, which is then inserted into the plant chromosomes instead.

The most commonly used system takes advantage of a bacterium (*Agrobacterium tumefaciens*) that naturally infects plants by inserting its own DNA into them, effectively causing the plant to manufacture food for the bacteria. This microbe is re-engineered by removal of genes normally transferred to the plant in nature, replacing them with the expression cassette containing the GOI. A brief co-cultivation step between the altered bacteria and some cultured plant cells allows the bacteria to deliver the expression cassette into a fraction of the cells in the mixture. The alternative delivery system involves the introduction of the expression cassette into a plant tissue under high pressure via a biolistics gun, which is predominantly associated with the transformation of monocotyledonous plants (e.g. maize, grass etc). The third stage involves selection of the cells that have successfully taken up the gene, and regeneration of whole plants from these cells. This is achieved by placing the plant cells on a culture medium containing a mixture of biochemical compounds and nutrients. One of the components of the culture medium may be a compound such as an antibiotic or herbicide, and selection of the successfully transformed cells is facilitated by the selectable marker gene in the expression cassette, which renders cells containing the cassette immune to this compound, while untransformed cells die. Plant growth inhibiting sugar sources can also be used to select for transformed cells if the expression cassette contains a gene to metabolise this sugar (Joersbo et al. 1998). Individual plant cells have the capacity to develop into entire plants in the presence certain biochemical compounds, and these compounds are also included in the medium, allowing each transformed cell to develop into a plant in which the GOI will be expressed.

The first generation of GM crop plants released have almost exclusively contained genes (frequently originating from bacteria) that confer resistance on the plant to proprietary herbicides or insect pests. Effectively any gene can be targeted for introduction in to crop species. The following are some candidate genes and transformation targets that have been proposed to be of significance in an Irish context:

- Genes for tannin expression in white clover to prevent bloat.
- Genes conferring male sterility to facilitate hybrid seed production (target species, white clover, ryegrass)
- Biotic stress resistance (e.g. the enzyme oxalate oxidase to confer resistance to S*clerotinia* in white clover, genes conferring resistance to *Globodera pallida* and tuber moth in potatoes)
- Abiotic stress resistance (e.g. genes increasing the frost, drought and UV tolerance of plants)
- Yield increase by manipulating genes involved in senescence and photo-assimilate production and utilisation
- Genes encoding novel products not normally produced by plants (e.g. therapeutics/vaccines, nutraceuticals, novel oils, biopolymers)

It is anticipated that a second and third generation of highly useful and high value GM crops will emerge from the last category. Already, several crops (e.g. potato, tomato) have been modified to produce human antibodies/vaccines (Richter et al. 2000) and biodegradable plastics (Snell and Peters 2002). This "Cell Factory" application of GM technology is significantly more challenging than the relatively 'simple' incorporation of a herbicide/insect resistance trait into a crop variety. These herbicide and insect tolerance genes are inserted into the chromosomes in the nucleus of the plant, however, because there is only one nucleus per cell, the overall quantity of protein that an introduced gene can produce is limited. Chloroplasts, the chlorophyll containing bodies in plant cells, contain an independent chromosome. In second and third generation GM plants, it will be desirable to insert the GOIs into these chloroplast chromosomes, primarily because of the spectacular amounts of recombinant protein they can produce but also because each plant cell may have several hundred chloroplasts, increasing the rate of production protein several fold. Limitations do exist though with the principal one being the effect that this large-scale production of a recombinant protein can exert on the host plant/cell. In extreme cases, the stockpiles of protein can reach toxic levels, which can result in poor growth and ultimately, plant death. As an alternative, the adoption of inducible promoters could provide a means to exert greater control on the rate of protein production. In the majority of GM plants released to date, constitutive promoters have been used, and these tend to permanently express the GOI, resulting in the aforementioned toxic effect. An inducible promoter has the capacity to switch on/off the GOI following exposure to a specific chemical or environmental stimulus, thereby limiting the expression levels to manageable amounts.

Several laboratories in Ireland already have extensive experience in the production and characterisation of genetically engineered plants (including via chloroplast transformation). At Teagasc's Oak Park Research Centre, for instance, programmes are under way to modify crops including clover and potato to produce GM plants that will be utilised to achieve the objectives of a functional genomics programme. These plants will also be used in experiments aimed at designing a robust risk assessment strategy for the potential use of each particular GM crop.

Environmental safety of GM crops

The worldwide acreage of GM plants has increased steadily since the first commercial plantings in 1995-1996. Equating to a 35-fold increase, no other crop technology has achieved such a rapid rate of adoption. Cultivated primarily in the USA (39 million ha, 66% of world total), Argentina (13.5 million ha, 23%), Canada (3.5 million ha, 6%) and China (2.1 million ha, 4%), the principal crops include GM soybean, maize, cotton and oilseed rape with the corresponding dominant traits being herbicide tolerance and insect resistance (James, 2001).

Among the ecological issues associated with GM crops is the possibility that some newly introduced traits, such as pest or pathogen resistance, could 'flow' into an adjacent conventional crop (a concept termed 'gene flow'), thereby conferring upon it an unanticipated fitness. As a result, the crop may gain characteristics that significantly enhance its ability to survive and spread outside of the parameters of its cultivation. A second issue arises if a GM crop is grown in the vicinity of a compatible wild or weedy related species; transfer of the trait by natural hybridization may produce hybrid progeny that are more aggressive or more difficult to control. In Ireland, farmers cultivate a variety of indigenous and non-indigenous crops, which may or may not have an interfertile wild relative growing on the island. So, while wheat, potatoes, peas, runner beans and maize are all alien species without interfertile wild relatives, ryegrass, clover, sugar beet, oats, carrots, oilseed rape and apples are all either native or interfertile with other wild natives.

Clearly, this raises the possibility that commercial GM crops will interbreed with other varieties already growing in Ireland. The adoption of large-scale risk assessment strategies is therefore essential so that when introgression of the transgene into a wild, related species does occur, its impact is no more significant than if occurred between a non-GM version of that crop.

However, assessing the potential for transgenic pest resistant crops to become problem weeds, or to enhance the weediness of nearby sexually compatible relatives, is a complex task. From the onset, a multi-disciplinary approach must be adopted. Information is required from many disciplines e.g. weed science, agronomy, population biology and genetics, entomology, plant breeding, ecology, plant pathology, molecular biology, and more. Once achieved, the information must then be collated and disseminated into a format that educates the public on the issue at hand.

Like many states in the European Union, Ireland has yet to fully commit itself to the adoption of GM crop technology, with the general position stated as 'positive but precautionary'. However, with the European wide moratorium on commercial production of GM crops close to ending, many strategically important decisions regarding the commercial deployment and co-existence with conventional/organic crops need to be considered. To date little research on the environmental impact of GM crops has been carried out in Ireland, and provision of relevant local information lags far behind other countries in the EU.

As such, background research and development is imperative if we are to put in place an effective and acceptable framework to test and monitor the relative impact of the products of plant biotechnology in non-target areas. Examples include:

- Compiling inventories of pest infestations in related weed species
- Determining the presence of pest resistance traits in weed populations
- Quantifying the impact of pests on weed populations dynamics in the absence of resistance
- Creating databases of sexually compatible species and varieties
- Designing modelling systems with which to synthesise available knowledge and direct future research.

Combined, these would complement any possible outcome from a risk assessment project, thereby providing an insight into what society could expect if GM crops were to be incorporated into Irish cropping systems

Marker Assisted Selection (MAS)

Less well known to the public than transformation, another DNA-based technique called marker assisted selection (MAS) has the potential to increase the speed and efficiency with which new plant varieties are bred, a process that can currently take up to 15 years per variety, depending on the crop species in question. In plant breeding the major objectives are to breed improved varieties with superior response to abiotic and biotic stresses and improved quality. The introduction of these traits is achieved by crossing an elite line or commercial variety (the recipient) with a donor plant that possesses the desirable trait. Essentially, the goal is move (alleles of) the gene or genes conferring that desirable trait from the donor to the recipient, producing an improved variety. Sources of valuable genes (i.e. donors) can include other elite lines, land races, strains, ecotypes and wild relatives from other species. However, sexually crossing two plants results in progeny that derive half of their genes from each parent, and this may result in transfer of undesirable genes (and traits) from the donor, as well as the target genes. A process called backcrossing, where progeny individuals with the target gene are crossed with the original recipient parent, is used to reduce the amount of the donor genome and increase the proportion of the original elite line (recipient) genome present. The process is repeated with progeny plants from each successive generation, gradually eliminating unfavourable donor genomic regions. During the backcross process, selection for the target gene/genes is combined with selection against the undesirable traits of the donor. This process can take up to ten generations and is highly time consuming and demanding in resources, and in addition, the selection process is generally based on a visual assessment of the plants characteristics rather that the desirable and undesirable genes themselves. The efficiency of this process can be low because (1) the target trait has a low heritability (i.e. its expression is unpredictable) because it is influenced by environmental conditions, (2) the trait is controlled by multiple genes, (3) the screening is expensive and inconvenient for large scale approaches.

In many cases, it would be useful to select directly for and against the genes underlying the traits of interest in a breeding programme, rather than having to visually assess these traits in the plants. These genes would constitute molecular markers, by which the presence or absence of the trait encoded by that gene could be tracked and selected for in the breeding programme. This principle is called marker assisted selection (MAS). While the ultimate molecular markers for a trait would be the sequence of the genes underlying that trait, this could only be a broadly applicable strategy in plants with completely sequenced genomes. As previously mentioned, this situation is currently rare for crop plant species. It is, however, possible to track a gene in relation to almost any piece of DNA that is near the gene on that chromosome – a concept known as genetic linkage. Genetic fingerprinting technology similar to that used in forensic analysis can detect certain DNA motifs spread about the genome, and these can be used as markers for genes to which they are linked. There are several strategies to determine whether a marker is linked to a target gene of interest in a breeding program, but all operate on basically the same principle of testing how frequently the marker occurs with the trait in populations of individuals where the trait is segregating (i.e. present in some individuals, not in others). When a marker occurs in the same individuals that the trait occurs in at a high frequency, this indicates that the marker is near the gene in the chromosome, and can be used to track that gene in a breeding program. Thereafter, in other words, one does not have to look for the trait, but only for the molecular marker. This is particularly useful in those aforementioned situations where screening for the trait is difficult or expensive, or where the trait is controlled by several partially effective genes instead of one single gene. A trait controlled by several genes is referred to as a quantitative trait (Liu, 1998), and the genes contributing to that trait as quantitative trait loci (QTL). This is significant, as many of the most important traits in breeding programmes are controlled by QTL, and developing markers to detect them is central to the success of MAS in plant breeding.

Fig. 4: A molecular marker linked to a gene conferring resistance to potato cyst nematode (PCN) in potato. DNA was isolated from the donor wild species that carries the resistance gene (P), four resistant breeding lines developed from this wild species (R), and ten other non-resistant potato lines (S). The DNA was subjected to the polymerase chain reaction (PCR) to verify if the marker was present or not. The marker is visualised as a band on an agarose gel after electrophoresis. It can be seen that the marker is only present in the donor parent and the resistant lines developed from this parent, and not in any of the other lines. Thus, this marker may be useful in selecting for this form of resistance in a potato breeding programme.

A major attraction of MAS is that it does not involve genetic modification, and thus it is not subject to the concerns associated with genetically modified organisms expressed in the previous section. Priority areas for the application of MAS in breeding programmes aimed at providing crops tailored to Irish conditions are listed below:

Forage. Plant breeding programmes carried out by Teagasc at Oak Park are focused on perennial ryegrass, white clover and potato. In these crops research on linkage mapping is for a variety of traits is currently being carried out to underpin the existing breeding programmes and to help the breeding process for new and improved varieties suited to Ireland.
Cereals. Cereals, mainly wheat and barley, constitute by far the largest section of arable crops in Ireland. Development of varieties with durable resistance to *Septoria* which is the major disease of wheat, the novel disease *Ramularia* in barley and barley-yellow-dwarf virus in both crops would reduce chemical usage considerably resulting in lower costs of production and much less risk of chemical residues in grain. This objective could be greatly enhanced through marker assisted selection in breeding.
Forestry. In forestry species quantitative traits, such as wood yield or wood quality can also be improved using molecular markers closely linked to, or located within, one or more QTL. The potential benefits of MAS are greatest for traits that are difficult, time-consuming or expensive to measure (for example, stem length/ girth and wood quality). MAS may be justified for high-value hardwoods and have most potential when integrated with the development of selected varieties in an improvement programme, where additional genetic gains can be rapidly multiplied.
Nursery Stock. Nursery stock and ornamental plants are a growing sector of horticulture in Ireland. There is need to reduce imports and compete in the export market. Hence, the primary focus of biotechnology research in the horticulture sector will be generation and identification and development of new genotypes of ornamental plants and shrubs by using molecular markers in conjunction with other biotechnology-based approaches such as induced mutations and tissue culture.

Disease Diagnostics

Damage by plant pathogenic organisms remains one of the major sources of loss of potential yield in modern crop production systems. Monoculture and high chemical usage are placing enormous selection pressures on pathogen populations, resulting in the continuing development of more virulent strains capable of overcoming natural and chemical barriers to infection. Because of this, the ability not only detect various pathogens, but to identify the different strains of any one pathogen present is gaining increasing importance in knowledge-based control strategies.

Molecular biology-based techniques such as the polymerase chain reaction (PCR) can be used to detect and distinguish between different strains of the major crop pathogens such as fungi, bacteria, viruses and viroids. For example, this technology has recently been applied in the UK (Fraaije et al. 2002) and Ireland (at Teagasc, Oak Park), to detect a novel strains of the cereal pathogens, *Erysiphe graminis* and *Septoria tritici,* which have developed strong resistance to the primary fungicides typically used to control them. Using the PCR-based approach, a comprehensive survey of the Irish wheat crop was completed in 2003 (O' Sullivan, 2004). It was established that this new strain of *Septoria* has taken hold in the cereal growing regions, thereby rendering this class of fungicides completely ineffective in controlling this disease. In the future it is envisaged that this technology will also be used to study the epidemiology of new or emerging plant pathogens such as *Ramularia* and to quantify epidemiological or genetic changes in existing pathogens (e.g. *Phytophthora infestans*, the causal agent of late blight). Accurate identification of the presence of latent infection is critical for some disease control studies and can be an integral part of decision support systems.

Conclusions

All of the aspects of plant biotechnology examined here will play an increasingly important role in many arenas of crop production in the coming decades. Thus it is important for Ireland as a nation to acquire and maintain the core skills and competences in this area to ensure the continued competitiveness and existence of Irish tillage-based agriculture in the 21st century. The benefits of biotechnology for the crops sector and consumers will be far-reaching. Biotechnology will bring cost savings to farmers by lowering their input costs to control insects and plant diseases. It will also help them obtain premiums for crops with improved traits. It offers the opportunity to minimise environmental risks by lowering the need for agricultural chemical applications and will be of benefit to the consumers by making future food products safer, more nutritious, longer lasting and less costly.

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