Next-Generation Crops Foresighting

Technology & Markets
September 2008

Life Sciences SCOTLAND

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Contents

Executive summary

- Despite the controversy surrounding the technology, genetically modified (GM) crops are planted on over 110 million hectares globally and generate around ~$7 billion in seed sales. Seed sales alone are predicted to grow to $12 billion by 2016, when over one-third of all seeds sold will be genetically modified.

- At present, almost all the value of GM is in just four commercial crops – maize (corn), canola, soybean and cotton. These incorporate simple traits, such as insect and herbicide resistance, which are of value only to the farmer.

- Future market growth will depend on the delivery of superior products in a broader range of food and feed crops. These will offer not only benefits for farmers (e.g. better yield) but also benefits to the environment (e.g. reduced dependence on nitrogen fertilisers), the ability to cope with impending climate change (e.g. drought resistance) and improved nutrition for humans and livestock (e.g. lipid profiles).

- The drivers are pressing: the world needs to feed more mouths with fewer resources, a consequence of global population growth, pressure on agricultural land and climate change. GM offers part of the solution. However, restoring confidence in the science and improving public perception of the value of the products will be necessary to realise this.

Executive summary (2)

- The market for GM seeds is dominated by a few major players, who maintain their position through control of the superior varieties that farmers prefer (so-called elite germplasm). Thus they prevent new entrants reaching the market.
- Although the leading companies invest heavily in novel trait identification and new crop development, much of the innovative input comes from collaborations with smaller players that offer novel tools, technologies or approaches – they provide the ‘R’ in the multinationals’ ‘R&D’.
- In this highly competitive industry, reducing the time and cost to bring new products to market is an imperative. Successful agbiotech startups have proprietary platforms or techniques that provide their partners with a competitive edge. In turn, these nascent businesses benefit from vital funding and a viable route to market, essential when venture capital funding is so restricted and direct access to market blocked.

Executive summary (3)

- Existing plant transformation techniques – primarily through *Agrobacterium* infection and biolistics – have changed little during the past decade. They work adequately for many crops but neither is well suited to the future needs of agricultural biotechnology.

- Our analysis suggests that there remains an opportunity to develop alternative strategies for the reliable and targeted insertion of multiple stacked genes across a broad range of cereals, fruits and vegetables. The key challenge here is in the delivery of larger DNA inserts across an intransigent plant cell wall and control of transgene expression.

- The mass screening of the phenotype of thousands of transformed plants (‘phenomics’) has become a bottleneck in the crop-development process. This is a time-consuming and costly process requiring assessment of many measures of phenotype (physical, chemical and biological) over time. Importantly, there is a need for automated, high-throughput, precision phenotyping techniques that can be applied in the environment where crops are grown – the field.

- Scotland is home to one of only three UK centres for applied crop research, many well-respected plant biologists, imaging experts and systems-modelling experts. Together they may answer some of the problems facing agriculture today and tomorrow.

Executive summary (4)

In conclusion

- ITI believes that this vital market is poorly served by innovation, but that this will be essential for its anticipated dramatic growth in the near future.
- ITI is concerned that public and political perceptions, which have served to constrain commercial opportunities in Europe to date, will need to be addressed and allayed, and that confidence in the technology needs to be rebuilt.
- ITI believes that Scotland is well placed to address the innovation needs – of both its local agriculture base and of the global market – by drawing on its wealth of basic and applied research.

Market analysis

Market analysis | Technology analysis | Scottish activity | Conclusion

- **Scope & Background**
- The Market
    - Size, products and dynamics
    - Drivers and restraints
- The Industry
    - Major players, performance and trends
    - The value of innovation in agbiotech
    - Case studies
- Conclusions

Scope

- This report looks specifically at the market for improved seeds for commodity crops, fruits and vegetables, the companies active in this area and the persisting technology needs.
- It does not examine trends in the development of agrochemicals for crop protection, although we recognise the importance of this market and the challenges that it faces.
- Research was focused on identifying the critical points in bringing new crops to market and to evaluate where existing techniques and strategies may not meet the future needs of agriculture.
- Although plant genomics has largely lagged that of human genetics, advances in gene sequencing are such that, at least on the basis of cost, this should not pose a bottleneck in new plant discovery. Instead, we have concentrated on two areas:
    - transformation techniques of plants;
    - precision phenotyping of the transformed plants.

Background

- Inadvertently, humans have been genetically modifying native species for centuries by selective breeding. This is a means of guiding the creation of new plant varieties by crossing, replanting and selecting only those plants with the desired trait or phenotype (e.g. height, yield, fruit colour).
- Even a rudimentary understanding of Mendelian genetics provides for greater direction. Modern genetics has enabled even more precise breeding programmes using genetic markers linked to desired traits, so-called **marker-assisted breeding**.
- However, it is only possible to introduce a trait into a plant variety if it already exists within the gene pool of that species. The process is cumbersome as crossing plants also introduces unwanted genetic material that take time and effort to eliminate through several back-crosses with parent plant.
- A faster and more precise method is to introduce only the necessary trait-related gene through genetic engineering.
- The first products of genetic modification include herbicide and pest-resistant crops, which have now been on the market for over a decade. GM crops are planted on over 100 million hectares globally and generate sales in excess of $6 billion.
- However, these were simple genetic transformations and existing technologies could not readily handle the complex genetic manipulation required for engineering of more complex traits such as yield and drought resistance.
- For further background please read **Plant Biotechnology, Environmental Scan**.

Abbreviations and terms used

Various terms and abbreviations are used throughout this document are explained here:

- **Phenotype**: The measurable characteristics of a plant, such as growth patterns, leaf shape, height, response to light. Some, but not all, are visually detectable characteristics. Phenotype is related to genotype but is also influenced by environmental and other factors.
- **Trait**: Refers to the distinct phenotype of a plant that might be inherited or determined by the environment. Agronomic traits of value might include, for example, insect or herbicide resistance, improved yield and delayed ripening. A trait is determined by a gene or several genes and can be patent protected and exploited commercially if this is known. Many companies cross-license traits for a royalty fee and/or might develop their own products containing the trait.
- **Conventional breeding** (also called selective breeding): This involves crossing an elite variety with another sexually compatible bearing a trait of commercial interest. Often, marker-assisted selection is used to improve the speed and accuracy of the process. The alternative is genetic modification.
- **Genetic modification (GM)**: The genetic complement of the plant is modified by the introduction of foreign genes that can come from a variety of sources.
- **Elite germplasm**: Plant material that has the preferred traits for growing commercially. It has often been developed over many years and is tightly controlled by suppliers as it is the product preferred by farmers.
- **Transformation**: In this report, 'transformation' refers to the process of introducing foreign material into plant genomes, resulting in a genetically modified plant.

Contents

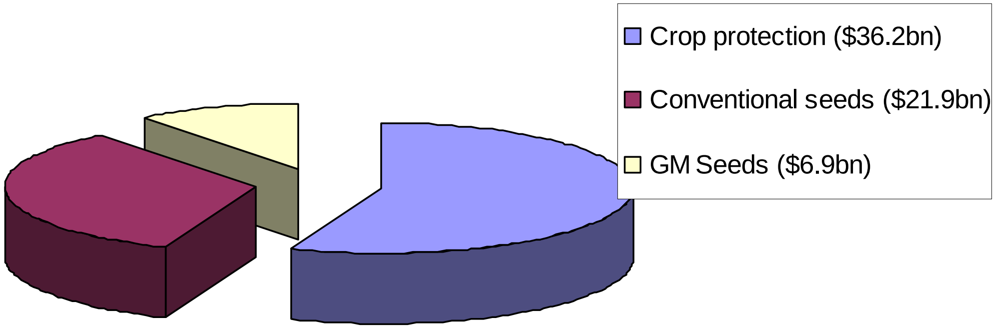
Market analysis | Technology analysis | Scottish activity | Conclusion

- Scope & Background
- **The Market**
    - **Size, products and dynamics**
    - Drivers and restraints
- The Industry
    - Major players, performance and trends
    - The value of innovation in agbiotech
    - Case studies
- Conclusions

Market size and growth

Agbiotech seeds and traits comprise 24% of branded seed sales.

Agrochemical/biotech sales (2007) $55 billion

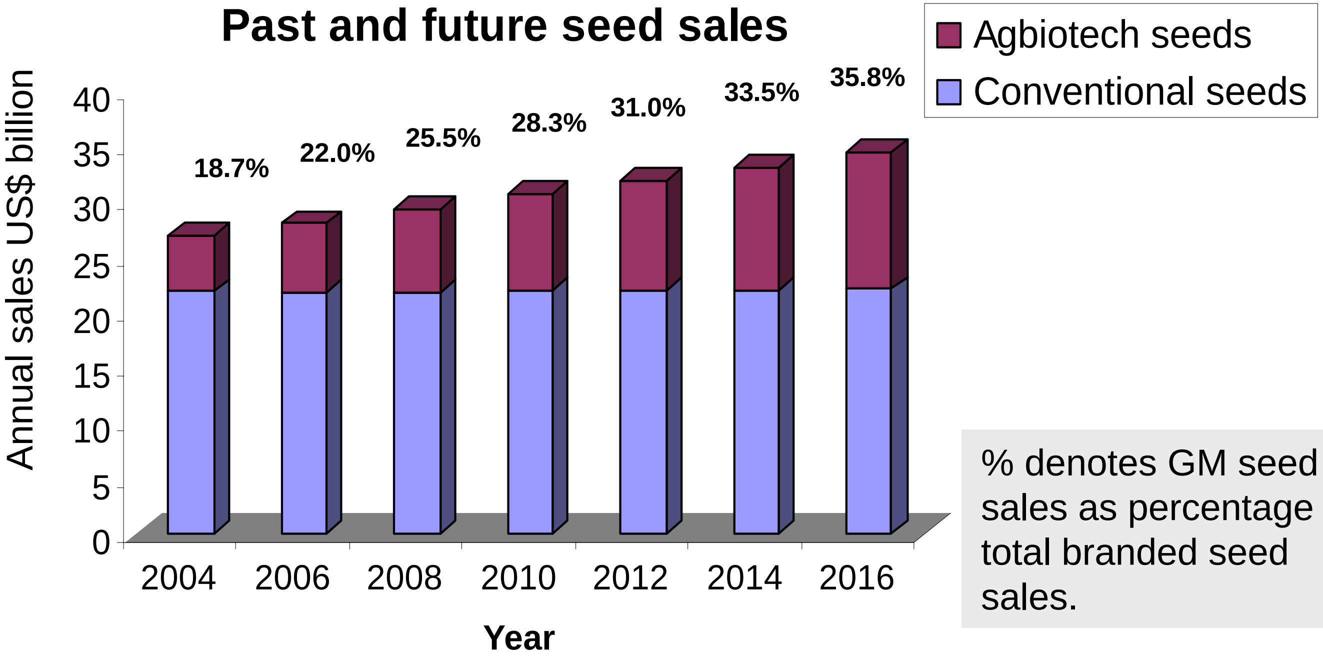


- The agrochemical/biotech market is valued at around $55 billion, posting annual growth rates of around 12% (2007 figures). (This excludes the $153 million sales of fertilisers.)
- The quoted market includes crop protection products, branded seeds for crops, fruit and vegetables, and a range of speciality products (e.g. for gardens and nurseries) to protect and optimise plant growth and yield.
- At present, the bulk of sales (66%) is for crop-protection products. Increasing pressure on agrochemical sales following tighter government controls (e.g. the recent EU Common Agricultural Policy directives) and impending patent expiry of many of the leading active ingredients will contribute to slowing of sales over the next 5 years to around 5% CAGR.

Source: Cropnosis

Market size and growth (2)

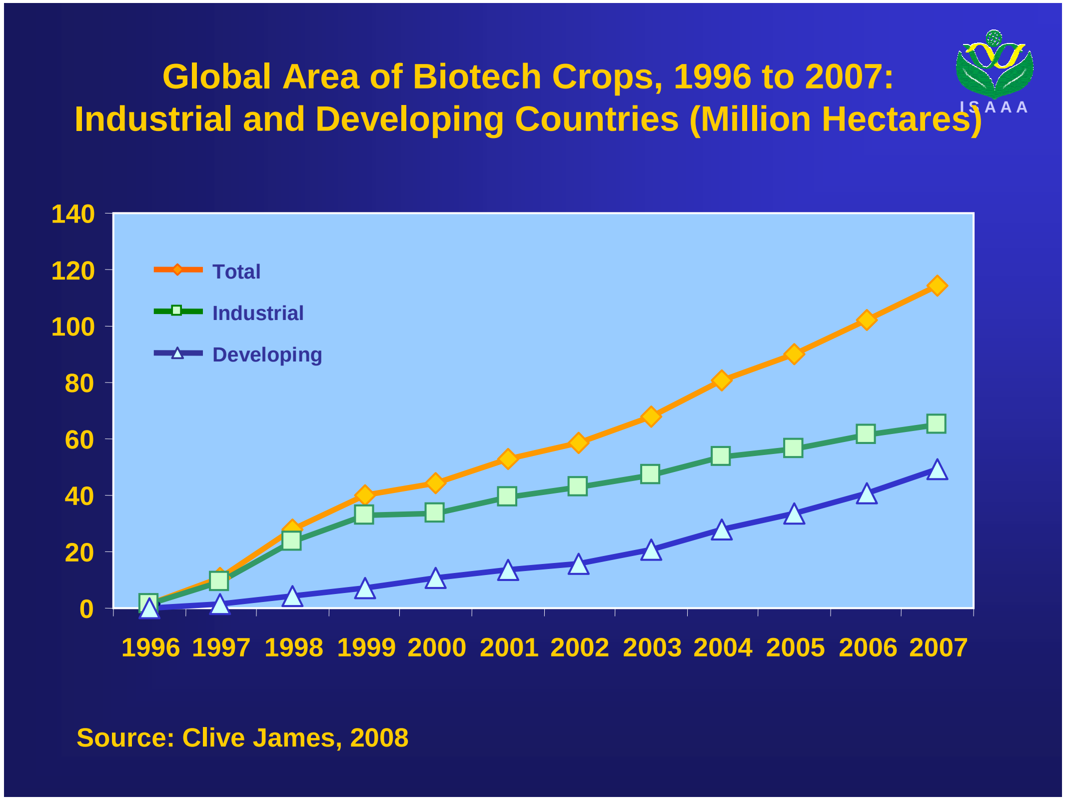
- Today, around 24% of all seeds sales (this includes seeds sold via state-owned companies, cooperatives and small family businesses) incorporate agbiotech traits, generating a turnover of around $6.9 billion (2007). The predicted CAGR over the next 5 years is around 7.5%.
- By 2016, agbiotech seeds and traits is predicted to have a market potential of around $12 billion, comprising around 36% of the total. This growth will be driven by the entry of indigenous traits from India and China and the increasing uptake of combined-trait products.



% denotes GM seed sales as percentage of total branded seed sales.

Source: Cropnosis

Market size and growth (3)



Global Area of Biotech Crops, 1996 to 2007:
Industrial and Developing Countries (Million Hectares)

Source: Clive James, 2008

Reproduced by courtesy of International Service for Acquisition of Agro-Biotech Applications (ISAAA)

- Despite European anxieties about GM crops, the first-generation products have been highly successful globally.
- In total, 114 million hectares of GM crops were planted across 23 countries during 2007, an increase of 12% from 2006.
- The global market value of GM crops was $6.9 billion in 2007 and is projected to be around $7.5 billion during 2008. (Source: Cropnosis)

The fastest rate of uptake has been in Brazil and India, where the area planted has more than doubled over the past 2 years.

Source: ISAAA, 2008

First-generation products

- The first genetically modified (GM) crops that came to market benefited the farmer by improving a crop's resistance to common pests and their tolerance to herbicides, so-called **input traits**. The goal here was to reduce loss of yield and the need for costly spraying of herbicides and pesticides. The products incorporated single-gene (or single-event) traits. The best-known examples include:
  - Bt maize and cotton (YieldGard^{TM}, Bollgard^{TM}): These crops possessed a gene for a fungal endotoxin – the Cry protein – derived from *Bacillus thuringiensis.* The plant synthesises toxins that are effective against larvae and beetles but harmless to native wildlife.
  - Herbicide-resistant corn, soybeans, cotton and canola (e.g. RoundUp Ready^{TM}). Glyphosphate is a broad-range herbicide that targets enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which plants require to synthesise key metabolites. Plants were engineered to carry a gene encoding an EPSP resistant to glyphosphate derived from *Agrobacterium tumefaciens*. Resistance to two other broad-range herbicides is conferred by the introduction of pesticide detoxifying enzymes, again both derived from bacterial sources.
  - Apart from a resistance to ringspot virus, there are no other virus- and fungal-resistant crops.
- GM crops offer advantages to the farmer:
  - Reduced numbers of pesticide sprays.
  - Reduced tilling of fields (i.e. the land remains untilled during the winter reducing erosion and leach of pesticides into the soil).
  - Improved yields of crops through reduced losses.

Agrisure®
CORN TRAITS

Roundup Ready
CORN 2

HX

First-generation products – region

Global area of biotech crops in 2007 by country (ISAAA, Clive James, 2008)

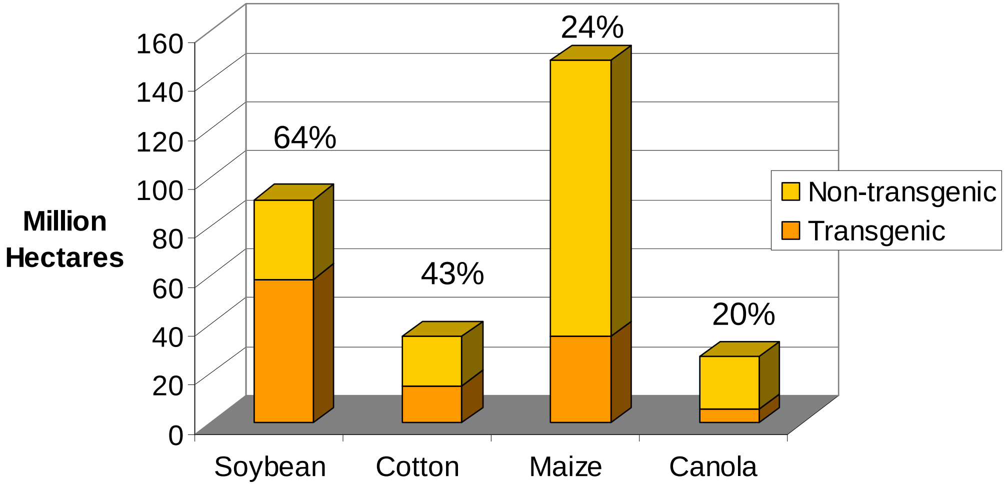
Country	Area (million hectares)	Biotech crops planted
US	57.7	Soybean, maize, cotton, canola, squash, papaya, alfalfa
Argentina	19.1	Soybean, maize, cotton
Brazil	15.0	Soybean, cotton
Canada	7.0	Canola, maize, soybean
India	6.2	Cotton
China	3.8	Cotton, tomato, poplar, petunia, papaya, sweet pepper
Paraguay	2.6	Soybean
South Africa	1.8	Maize, soybean, cotton
Uruguay	0.5	Soybean, maize
Philippines	0.3	Maize

The majority of GM planting are of commodity crops such as soybean, canola, cotton and maize. Indeed, around 98% of the value of GM crops resides in these four crops. A small range of GM fruits and vegetables is grown in the US and China, but there is substantial scope for growth in this sector.

First-generation products – traits

- All the GM products on the market bear simple herbicide- and/or insect-resistant traits. In some regions, the GM variety is the preferred choice for farmers: In the US, almost all soybean planted is now genetically modified.
- By global planted area, the preferred traits are as follows: herbicide resistance (72%); insect resistance (18%) and two or more traits in the same plants (i.e. stacked traits, 19%). A smaller area of virus-resistant crops (squash, papaya) has been planted (<1%). (Source: ISAAA data)
- Crops bearing Monsanto's traits now account for 91% of global GM crop acreage, followed by Syngenta (3.3%), Bayer CropScience (2.6%) and DuPont/Dow AgroSciences (2%). Many of the leading brands are now multimillion dollar products.

Global adoption rates for principal GM Crops



Source: ISAAA , 2008

First-generation products – brands

- The sales of leading traits in commercial crops (2007 figures). It is not possible to provide data for each seed brand individually due to the complex nature of the market for traits and seeds. Several traits are licensed to other seed suppliers and therefore provide income in the form of royalties from the sale of other brands.

Brand	**Global sales (2007)**
RoundUp Ready Soybean	$2,796 million
RoundUp and YieldGard Maize	$673 million
Bt Maize	$634 million
Bt Cotton	$499 million
Bollgard and RoundUp Ready Cotton	$410 million

Source: Cropnosis 2008

Next-generation products

- There is a growing market for products possessing stacked traits – three or more transgenes – that offer the **convenience of resistance to multiple pests and herbicides in a single variety**. Several large cross-company licensing deals and collaborations have been struck to facilitate the creation of multiple stacked-gene crops (see later).

- Second-generation crops that might benefit the consumer and the environment – so-called **output traits** – are also in development. New products under development will:
  - **limit the potential impact of climate change** (e.g. crops with improved drought resistance and tolerance to higher temperatures);
  - **provide additional nutritional benefit** for animal feedstock and for human consumption;
  - **better protect the environment** (e.g. improved use of nitrogen fertilisers);
  - **combat existing and emerging pathogens and pests (both new pests and resistant pests)**.

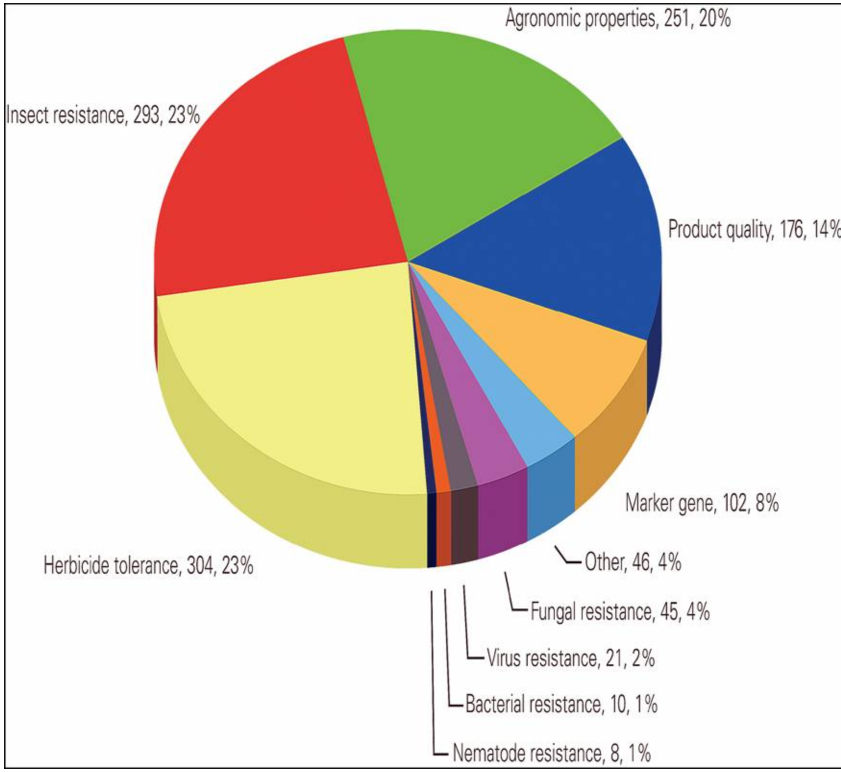
- Many agrochemical companies are also investing in developing crops suitable for the biofuel industry and for industrial chemicals thereby further diversifying their portfolios.

- **However, many of these second-generation products are complex traits that most likely will not be solved by the simple addition of a single gene. This is the challenge for new crop development in the 21st century.**

Next-generation products (2)

- A snapshot of current products in the pipeline, the US Animal and Plant Health Inspection Service approved 671 field trials during 2007.
- Around half of traits pertain to crop protection through biotic stress resistance or tolerance.
- The ‘agronomic properties’ phenotype includes traits such as resistance and tolerance to abiotic stress, altered amino acid composition, fertility, lignin content, oil profile and germination.
- ‘Product quality’ includes altered secondary metabolites including caffeine, production of industrial enzymes, among others.
- ‘Other’ includes pharmaceutical protein production.
- The broad spectrum of phenotypes highlights the diversification ongoing in the industry.

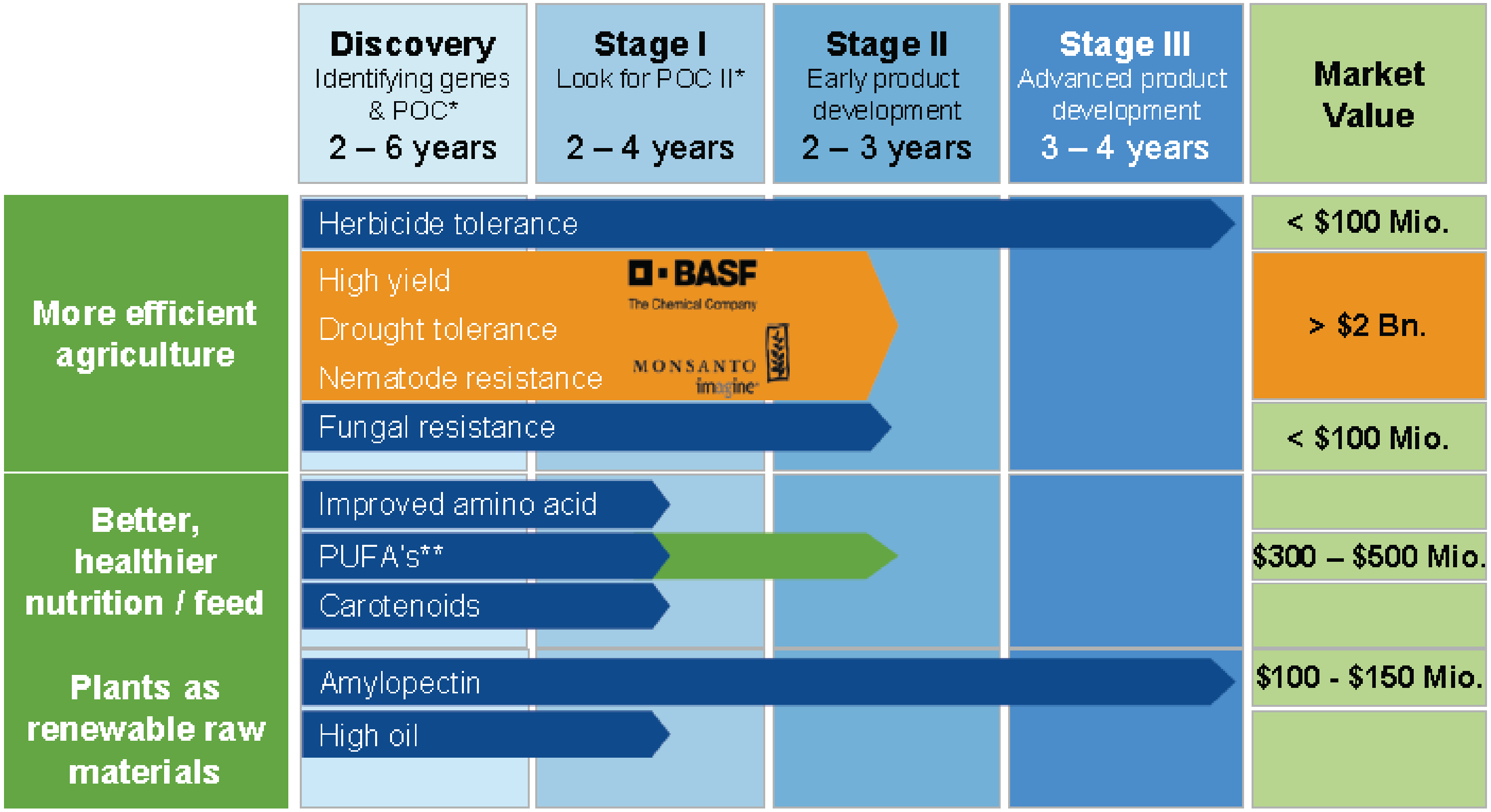
Phenotype, number of permits issued, percentage of total traits tested during 2007



Data from www.isb.vt.edu/cfdocs

Next-generation products (3)

The variety of traits being pursued is also revealed in the pipelines of the multinationals. For example, BASF's pipeline shows the shift from input to output traits in the form of traits addressing abiotic stress, improved nutrition and non-food applications, which will drive market growth during the next decade.



Discovery
Identifying genes & POC*
2 – 6 years

Stage I
Look for POC II*
2 – 4 years

Stage II
Early product development
2 – 3 years

Stage III
Advanced product development
3 – 4 years

Market Value

More efficient agriculture
- Herbicide tolerance – < $100 Mio.
- High yield, Drought tolerance, Nematode resistance – > $2 Bn.
- Fungal resistance – < $100 Mio.

Better, healthier nutrition / feed
- Improved amino acid
- PUFA's**
- Carotenoids
- $300 – $500 Mio.

Plants as renewable raw materials
- Amylopectin – $100 - $150 Mio.
- High oil

* POC = 'Proof of Concept' in model crop / POC II: 'Proof of Concept in Target Crop' // ** PUFA = PolyUnsaturated Fatty Acids

The market - summary

- Despite persisting anxieties about the risks of GM crops in many countries, they are a $billion business and growing. GM seeds will generate sales in excess of $12 billion within the next 8 years and even then have further future growth potential.

- Many agrochemical/biotech companies will see most growth in sales of agbiotech seeds and traits in the coming decade with the trend for a shift from simple single-gene events to more complex multi-gene stacking, which offers multiple benefits to the farmer, the consumer and the environment.

- However, the 21st-century problems of impending climate change, new and resistant pests and pathogens, and the need to improve agricultural productivity must be addressed. This poses a much greater challenge for the industry, as such products are not readily generated from simple single-gene insertions.

Contents

Market analysis | Technology analysis | Scottish activity | Conclusion

- Scope & Background
- The Market
    - Size, products and dynamics
    - **Drivers and restraints**
- The Industry
    - Major players, performance and trends
    - The value of innovation in agbiotech
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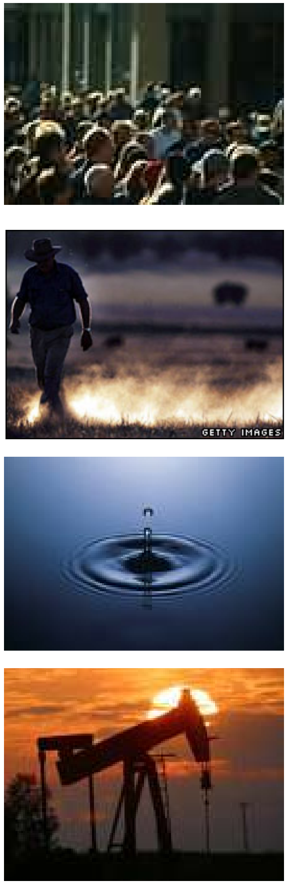
Drivers – the global imperative

The recent hike in food prices has served to highlight the pressures on today's agriculture and therefore the drivers for the market for seeds and traits.



Increasing global population:

- The global population is predicted to increase from 6.7 billion to 9.2 billion by 2050. (Source: United Nations)
- Increase in meat consumption in developing countries with growing wealth drives feedstock needs.

Climate change:

- As 70% of water usage is for irrigation, droughts hit agriculture hardest.
- Changes in climate adversely affect growing seasons and crop yields.
- Climate change might increase the virulence of pests, as well as their spread.

Renewable energy sources:

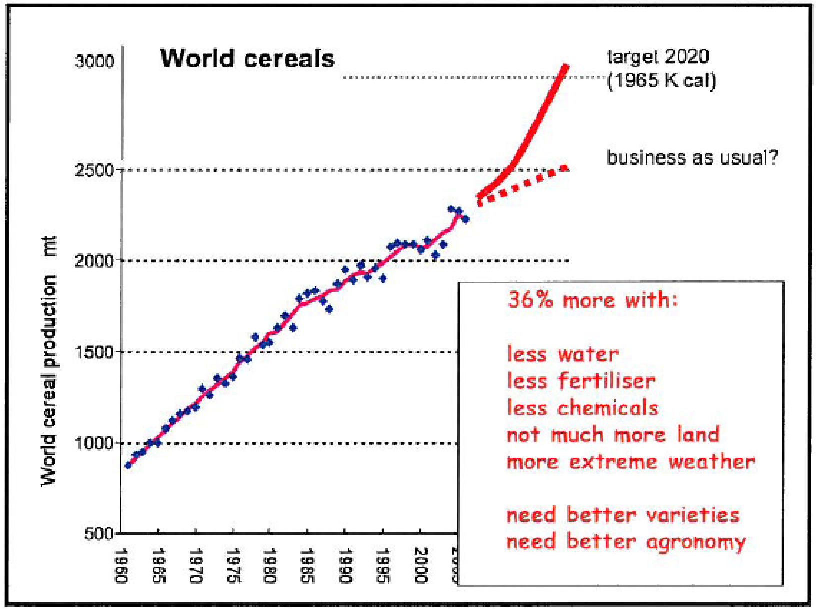
- Policy-driven demand for greener, renewable sources of biofuels from crops will impact on crops for food and feed.

Drivers – demand and supply

- Population growth – and the resulting increased demand for food and animal feed – poses a serious challenge to agriculture.

- Although agricultural productivity has been increasing, when computed as kg grain per capita in global terms there has been a decline over the past 20 years (CGIAR data). After deducting the grain fed to animals, the actual available crop remaining is currently below the 1965 kcal designated as necessary for healthy life.

- As the population grows, the demands on agriculture to produce 36% more, by 2020, with ever fewer resources suggest that this ‘yield gap’ will grow even wider in future years.



Courtesy: Mike Gale, John Innes, and Consultative Group on International Agricultural Research (CGIAR)

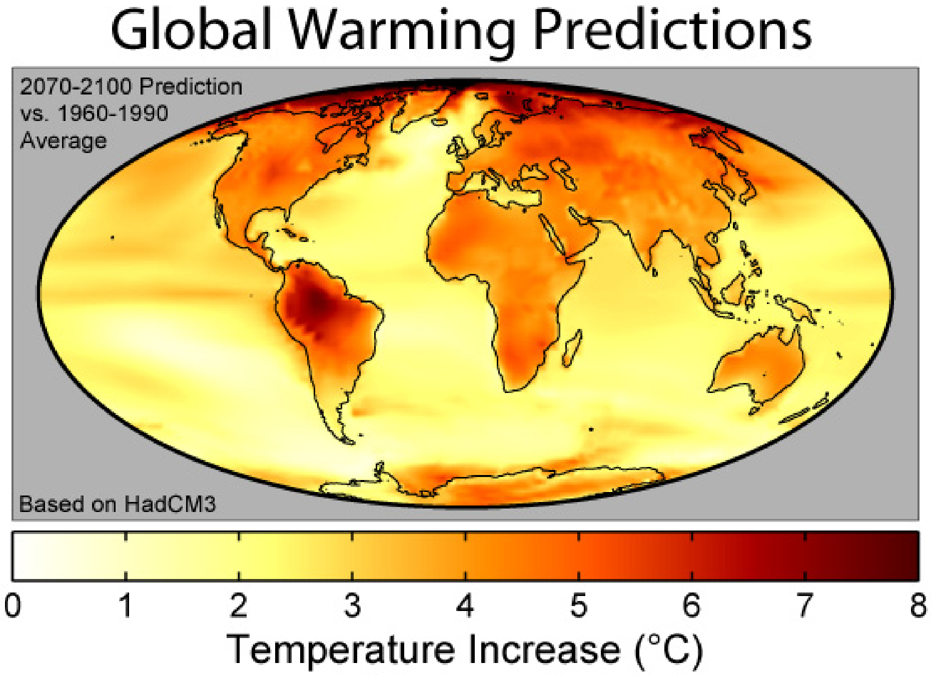
There is a pressing need to enhance harvest yield using the same or fewer resources. This is the grand challenge for plant breeders and biotechnologists.

Drivers – climate change

- Although predictions as to the forthcoming increase in global temperature vary, any prolonged increase in temperature (with associated changes in precipitation) will inevitably have some impact on plant growth and therefore on agricultural productivity.

- Plants not adapted to intense light might also suffer in the longer term.

- Different pests and pathogens might also thrive (with predictions that global warming could make them grow more aggressively), whereas more unpredictable and severe weather events pose challenges for farming. Indeed, crop destruction via flooding or drought has been a key contributor to the recent hike in commodity crop prices (see next slide).



Credit: www.globalwarmingimages.net

Drivers – climate change (2)

- Australia is currently experiencing a taste of the potentially devastating impact of climate change on agriculture. Below-average rainfall for the past 6 years has left the water table at its lowest for many decades.
- Many agricultural towns are threatened and Aus$20 million has been wiped off the economy since the dry spell started in 2002. Domestic water supplies are also at risk. The Murray-Darling river basin provides around 75% of Australia's water supply and further water shortages seem inevitable.
- The future looks bleak. A joint assessment by the Bureau of Meterology and the CSIRO warned the Australian government that the frequency of such droughts would increase from once every 20–25 years to once every 2–3 years.
- The impact is global not just local: Australia is one of the world's bread baskets and a shortfall in exports from this region has contributed directly to the reduction in global stocks and elevation of wheat prices. Australia is currently producing less that half its normal tonnage.

While many debate on the exact nature and degree of future climate change, the potential impact is evident *today*. Modifying plants to cope with changes in their local environment is a significant challenge that needs to be addressed swiftly.

Drought in Australia's agricultural area.

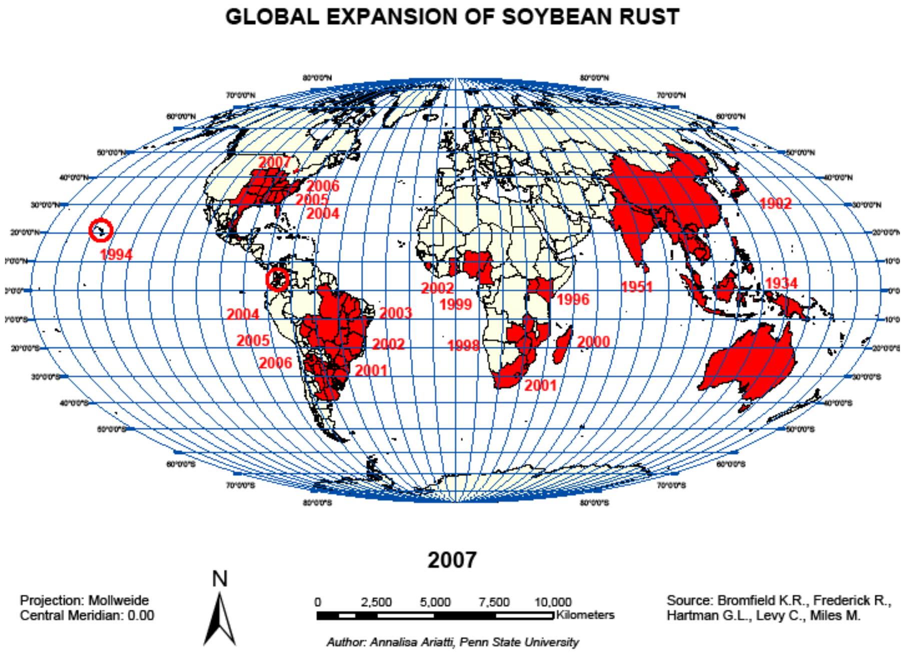
Drivers – pathogens and resistance

- One of the challenges for agriculture is the spread of pests between continents, which poses a threat both to crops and to the environment (e.g. recent outbreaks of Sudden Oak Death – originally from California – in rhododendrons and native oak trees in the UK).

- Various factors contribute to the global spread: greater international movement of people and goods, extreme weather events.

- Pathogens are also becoming resistant to existing crop protectants, which confounds the problem.

- Plant pathogens can have a devastating affect or agricultural productivity. For example, potato blight can completely destroy a crop within 2 weeks without prompt action. In the UK, the wet summer in 2007 resulted in blight that hiked potato prices significantly.



Projection: Mollweide
Central Meridian: 0.00

Source: Bromfield K.R., Frederick R., Hartman G.L., Levy C., Miles M.

Author: Annalisa Ariatti, Penn State University

- The spread of soybean rust highlights the mobility of pests and pathogens.

The battle to protect crops against existing pathogens and pests is not over and new solutions are still needed.

Restraints

- Whereas the drivers for innovation within agriculture seem acute and topical, the restraints have become much more chronic and entrenched. These stem from almost a decade of negative public sentiment around genetic modification and its products, which has influenced policy and driven both public and private funding out of this sector.

- Restraints come from several quarters:

  - the regulators;
  - the public;
  - the intellectual property landscape.

Restraints – regulatory barriers

- The main barrier for GM plants for food and feed are the regulatory barriers, which were erected in part in response to the vehement public reaction to the launch of GM crops in the late 1990s.

- In Europe, **no *new* GM crops or plants have been approved for commercial cultivation in the EU for almost a decade.** In part, this stems from several EU member states having zero tolerance to GM: a qualified majority is needed for approval.

- In 2003, the US, Canada and Argentina went to the World Trade Organization (WTO) to argue that the EU was in breach of fair trade rules in preventing imports of GM products. In November 2006, the WTO concluded that during 1998–2004 the European Union had breached fair trade rulings.

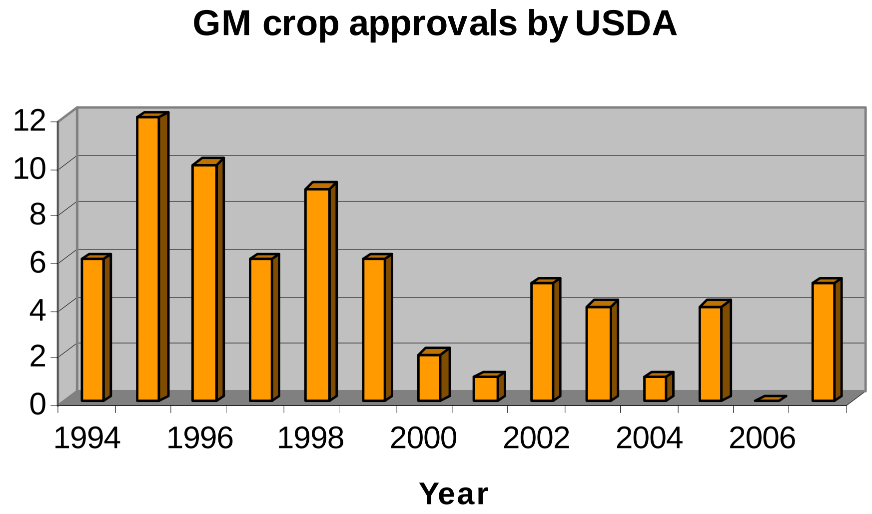
- In reaction to a complaint by the US, Canada and Argentina to the WTO, Europe began to allow importation of limited GM crops but implemented new regulations that required the labelling of all products containing more than 0.9% approved GM crops and also if the genetic modification has been used in the *production* of the food (e.g. if an oil was derived from a GM crop). The US argues that the new regulations have provided even higher hurdles for GM crop producers.

- The situation looks set to shift. Austria, one of the EU member states most resistant to GM, lifted a ban on importing and processing GM corn on 27 May 2008. Several prominent EU leaders have publicly been in support of reconsidering GM crops in the light of recent food price increases and growing global hunger.

Restraints – regulatory barriers (2)

- The US continues to be supportive of GM technology. A GM crop destined for food and feed must be approved by both the US Department of Agriculture (USDA) and the Food and Drug Administration (FDA).
- There is no mandatory labelling of GM foods required by the US FDA. The FDA regards GM products (on approval) to be **not substantially different** to the non-GM equivalent.
- However, concerns have been raised about the number of GM approvals by the FDA and the time taken for the consultation process. GM crop approvals continue to fall and the time for consultation during the 2005–2007 period was around 14 months (range 9–24 months). Approval rates are low. (Source: www.cspinet.org and ITI LifeSciences)

The barriers posed by regulators internationally has meant that GM products are approved at best slowly and at worst not at all. With lack of confidence in the market for GM products, innovation has slowed and investment restricted.



Restraints – freedom to operate

- Consolidation within the industry created a **dominant position over key intellectual assets,** including transfection strategies, expression vectors, selectable markers and genes linked to useful traits, etc.
- The cost of licensing can have a constraining effect on newcomers to the sector, including those within the public sector.
- However, some of the core patents are close to expiry and this could create an environment conducive for creation, protection and adoption of new technologies, in particular by the smaller players.
- New varieties of plants are also protected for 20–25 years under **Plant Breeders Rights** (or Plant Variety Rights). The new plant must be tested at a specialist centre and shown to be distinct from other varieties, uniform and stable.
- The breeder of the plant can register the plant as its own property and can sell it exclusively or offer licenses to other users.
- There are various exemptions under Plant Breeders Rights that, at times, can clash with rights under patent law. For example, a farmer can save the seeds from harvested plants and replant them the following year; a grower can also use a protected plant variety as a source for further plant breeding. Both would contravene patent rights.

The key obstacle to any smaller player taking a new trait to market is access to **elite germplasm**. These varieties, tried and tested over many growing seasons, are preferred by farmers and are owned by the larger seed companies. Without access to this superior genetic starting material it is not possible to take new products to market.

Restraints – cost and investment

- Although generating a new plant variety is not as costly as developing a new drug, it is still not without substantial financial risk. It can cost upwards of $100 million to bring a new GM variety to market and around 10% of these costs are associated with compiling the appropriate regulatory dossiers (*Nature Biotech*. 25, 2007 p. 509).

	Proof of concept (Phase 1)	**Early product development (Phase 2)**	**Advanced development (Phase 3)**	**Pre-launch (Phase 4)**
Key activities	• Gene optimisation • Crop transformation • Field testing	• Large-scale transformation • Trait development • Pre-regulatory data • Field testing	• Trait integration • Expanded field testing • Regulator data generation	• Regulatory submission • Seed bulk-up • Pre-marketing
Average duration	1 to 2 years	1 to 2 years	1 to 2 years	1 to 3 years
Average probability of candidate getting to market	25%	50%	75%	90%

Restraints – public acceptance

- One of the greatest risks for the production of any GM is the public perception and acceptance of such products. To date, in Europe, the vehement reaction against GM products has been championed by reputable and well-organised non-government organisations such as Greenpeace and Friends of the Earth.

- There has been less anxiety around GM products in the US, although studies suggest that education on the topic has heightened rather than alleviated anxieties.

- Almost every American will have consumed at least one GM-derived product. However, notably, few GM fruits and vegetables have reached the market and companies have avoided modifying some major crops (e.g. wheat) largely because of their fundamental role in key staples (i.e. bread and pasta). So have public perceptions of GM changed over the past decade and why?

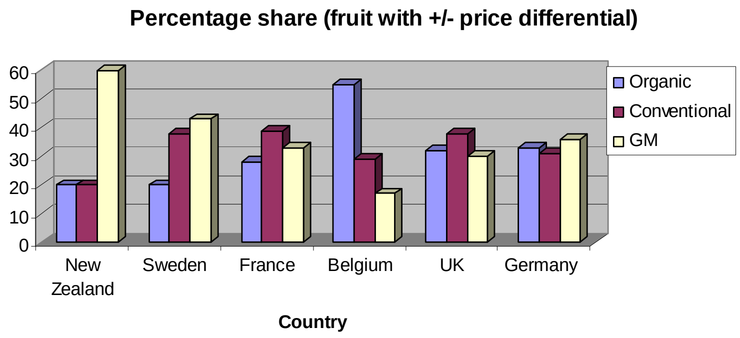
- In the last Eurobarometer Survey of Biotechnology (2006), only 27% of the 25,000 participants thought GM applied to food should be encouraged, seeing little benefit for consumers and high risks for health and the environment. When asked what might influence a choice to buy GM food, health benefits and reducing pesticide use were identified. However, consumers would not choose GM products to save money.

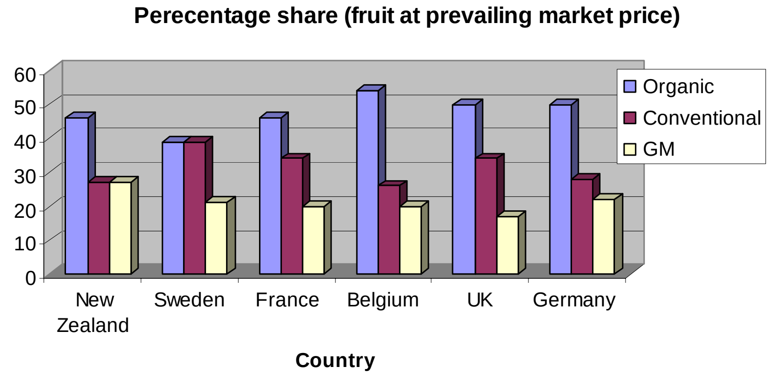
Anti-GM protest outside the Berlin parliament

Restraints – public acceptance

- Interestingly, other – less theoretical – approaches have contradicted this finding. A New Zealand team carried out an interesting experiment on consumers in six EU countries. On each stall were organic fruit (treated with organic pesticides, e.g. a bacillus), conventionally grown fruit (with conventional spraying) and GM fruit (pesticide free). These were sold either at the same price, or later with a price differential with organic more costly (+15%) than the conventional and GM products at a lower cost (–15% of conventional).
- When costs were equal, only 20% of people bought GM products. However, when organic prices were raised, more people chose GM. This would suggest that GM products would be viable if cheaper and if health benefits were clearly identified.
- With current rises in food prices and a global credit crunch, along with better communication of health and environmental advantages, it seems that a door may open for GM products.

If GM foods were cheaper than conventional products, and their benefits better highlighted, consumers might soften their anti-GM stance.





Drivers and restraints - summary

- There are clear challenges to achieving sustainable food production for the growing global population in an environmentally friendly fashion.
- The drivers to improve agricultural productivity include the need to address climate change, the advent of new and resistant pests on less land and with a lower burden of fertiliser and pesticide use.
- The application of agbiotech to address these issues would seem a viable solution but there are many restraining factors. The time and cost to get new products to market will continue to deter smaller players, as will the question over the public acceptance of, and therefore the market for, the resulting products. The position of regulators needs also to be more permissive to encourage growth in the sector.
- Whether the recent rise in food commodity prices will spur on regulators, policy makers or consumers to take another look at GM remains to be seen, but certainly the GM debate has been reignited and the benefits of GM explored afresh.

Contents

Market analysis | Technology analysis | Scottish activity | Conclusion

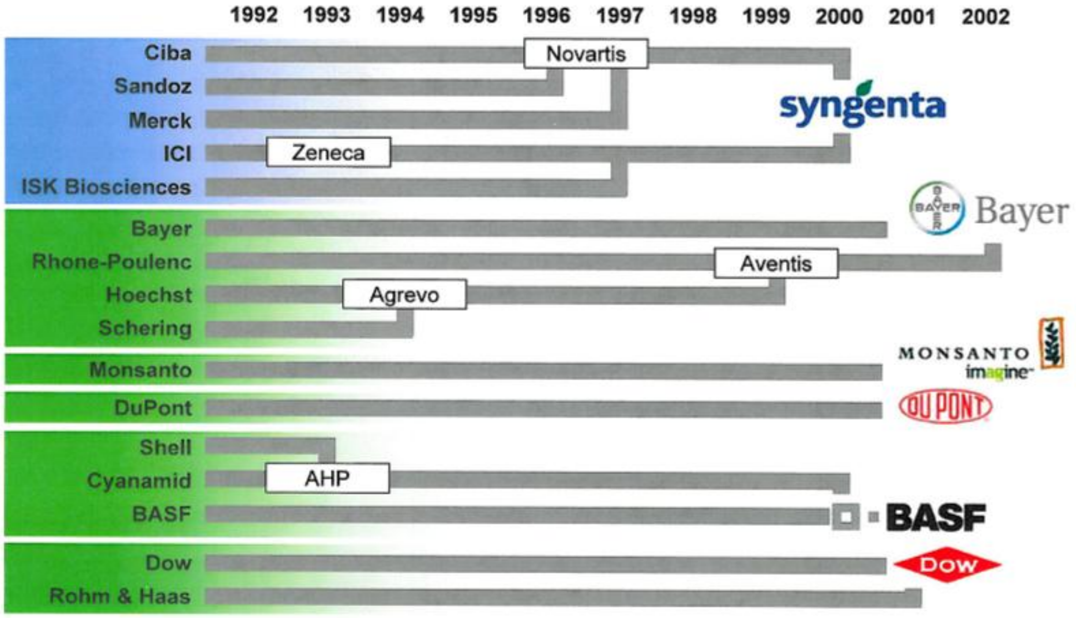
- Scope & Background
- The Market
    - Size, products and dynamics
    - Drivers and restraints
- **The Industry**
    - **Major players, performance and trends**
    - The value of innovation in agbiotech
    - Case studies
- Conclusions

State of the industry

- The current agrochemical industry emerged from several waves of consolidation within the chemical/pharmaceutical industry. A key feature of these was the aggressive consolidation between agrochemical ‘spin offs’ during the 1990s, which generated the dominant larger multinationals we see today.

- Today, around 95% of the global agrochemical market is owned by 20 companies, and the top six players hold over 80%. This agrochemical industry is not examined further in detail in this report but, as many companies serve both markets, its contribution can’t be excluded.

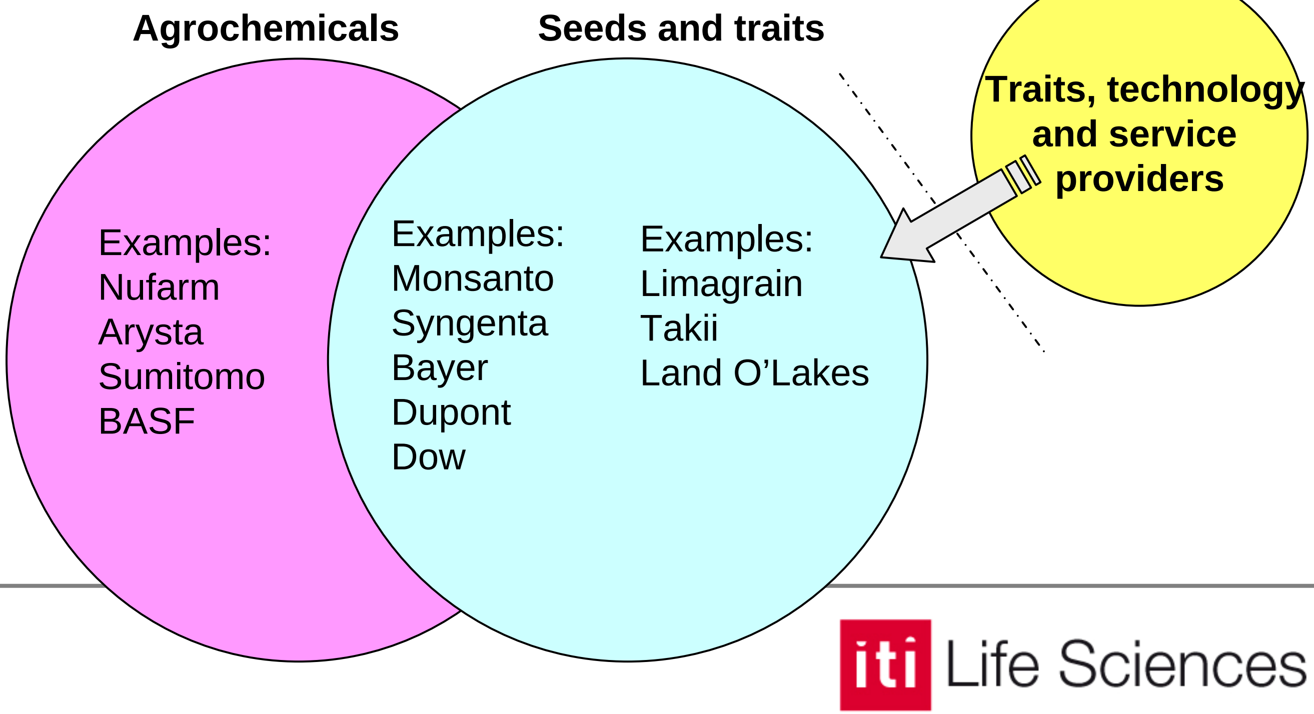
- The seeds and traits business is, by comparison, more fragmented, although the top six companies hold around 40% of the total (branded and non-branded) seeds market. This is the most direct market for tools and technologies for crop modification.



State of the industry (2)

- The chemicals and seeds/traits businesses are quite distinct (with different investment, time and regulatory barriers for each product type), although they can both go hand in hand in agriculture (e.g. RoundUp Ready seeds and herbicides). Some larger multinationals continue to be successful in both, but there has been room for mid-tier companies to specialise and gain a foothold in either market.

- A growing group of service providers offers seed developers access to enabling technologies. Note, however, that a barrier exists, preventing them from accessing the seeds and traits marketplace directly – access to elite germplasm.



Agrochemicals

Seeds and traits

Traits, technology and service providers

Examples:
Nufarm
Arysta
Sumitomo
BASF

Examples:
Monsanto
Syngenta
Bayer
Dupont
Dow

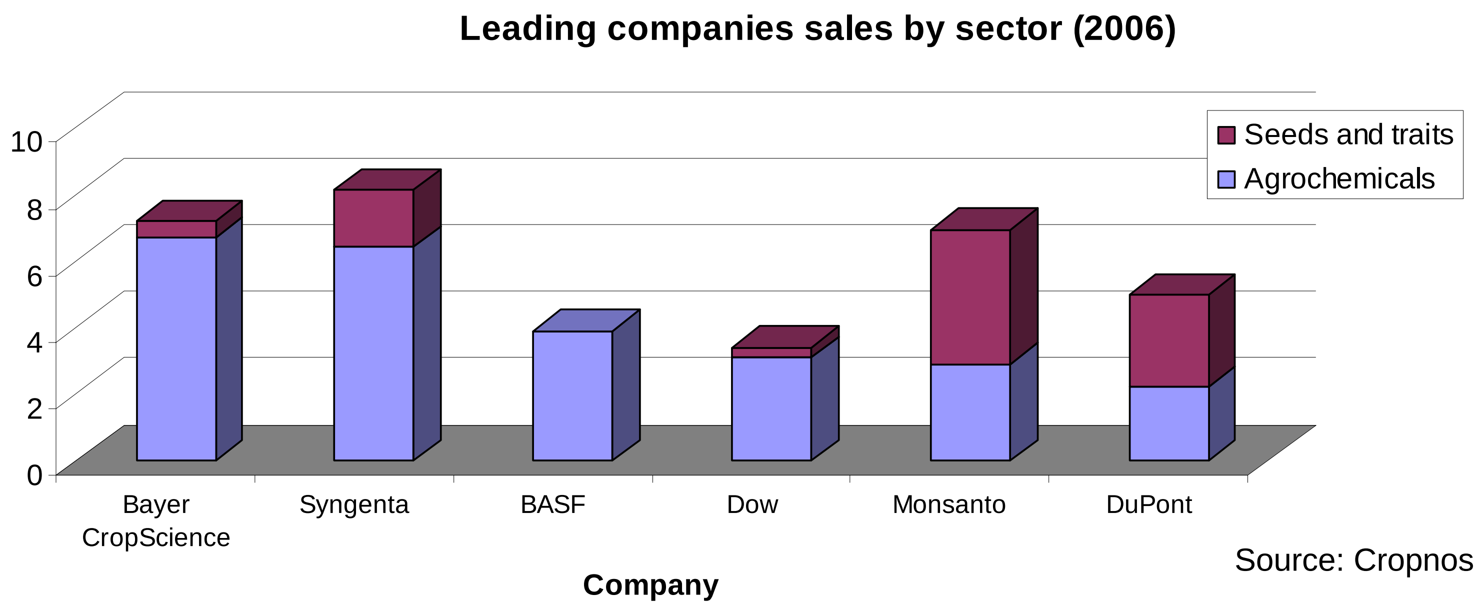
Examples:
Limagrain
Takii
Land O'Lakes

Seeds and traits – fragmented market

The market for seeds has been highly fragmented, with many small suppliers. However, strategic acquisition is now enabling a few multinationals to dominate: Monsanto, DuPont and Syngenta now capture more than 50% of the global branded seed and trait market (equating to 20% of total seed sales).

	Branded seed and traits (2006) US $ million	**Seeds and traits (2007 estimate) US $ million**	**% Market share**
Monsanto	4,028	4,971	25
DuPont/Pioneer	2,781	3,285	16
Syngenta	1,743	1,936	10
Limagrain	1,475	1,363	7
Land O'Lakes	550	769	4
KWS SAAT AG	621	686	3
Bayer CropScience	465	510	3
Sakata	410	448	2
Takii	342	371	2
DLF Trifolium	365	377	2
DeltaPine&Land	417	Now part of Monsanto	
Dow/Mycogen	302	389	2
Others	4,571	4,882	24
Total	**18,070**	**19,987**	**100**

Seeds – the traits have it!



Source: Cropnosis

- Over the past decade, many of the major agrochemical/biotech players have begun to concentrate efforts on the application of biotechnology to the creation of novel agbiotech products.

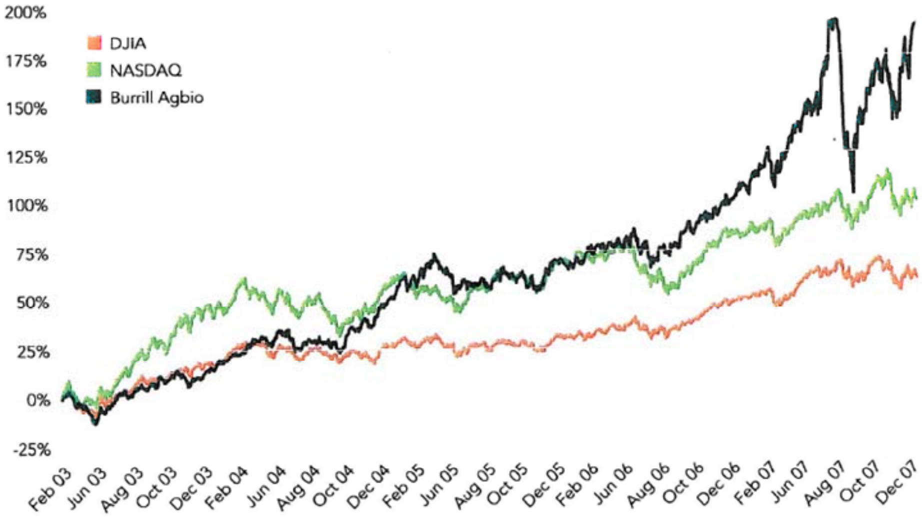
- The clear leaders in agbiotech are Monsanto and Dupont, deriving more than half of annual sales from seed and traits rather than crop-protection products (see next slide).

- Although Dow and BASF remain predominantly agrochemical providers they are both investing heavily in agbiotech and will begin start to make their mark in this market sector in the near future (see later).

Agbiotech – posting growth

- The sector's performance is strong. Agbiotech companies have posted good growth over the past few years. During 2007, the Burrill AgBiotech Index rose 40% compared with the FTSE Global Pharmaceutical Index, which rose just 1.3% (Burrill & Co, and see graph).

- The large multinationals are reaping the rewards of the demand for crops for food and fuel. For example:
    - Monsanto's share price rose 113% during 2007, generating a net profit of almost $1 billion on sales of $8.5 billion.
    - Syngenta's share price rose 70% on a turnover of $9.2 billion and a profit of $1.1 billion.

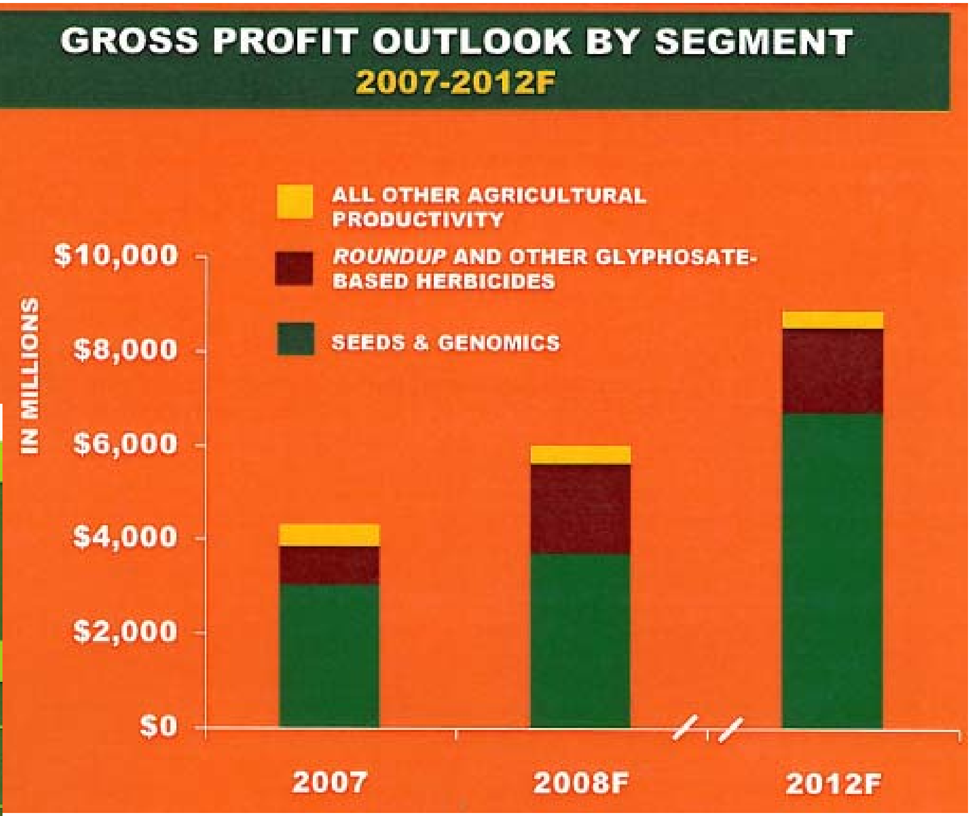


Source: Burrill & Company

Burrill & Company. AgBiotech Index performance 2003–2008

Seeds and traits – bullish forecast

Companies expect double-digit growth in revenue over the next few years, especially in seeds and traits (see right). Monsanto recently forecast that sales of seeds and traits would generate $7 billion in gross profit for the company by 2012.



Monsanto presentation for 2008 Q3 financial results

Gross Profit

2008 STATUS▼

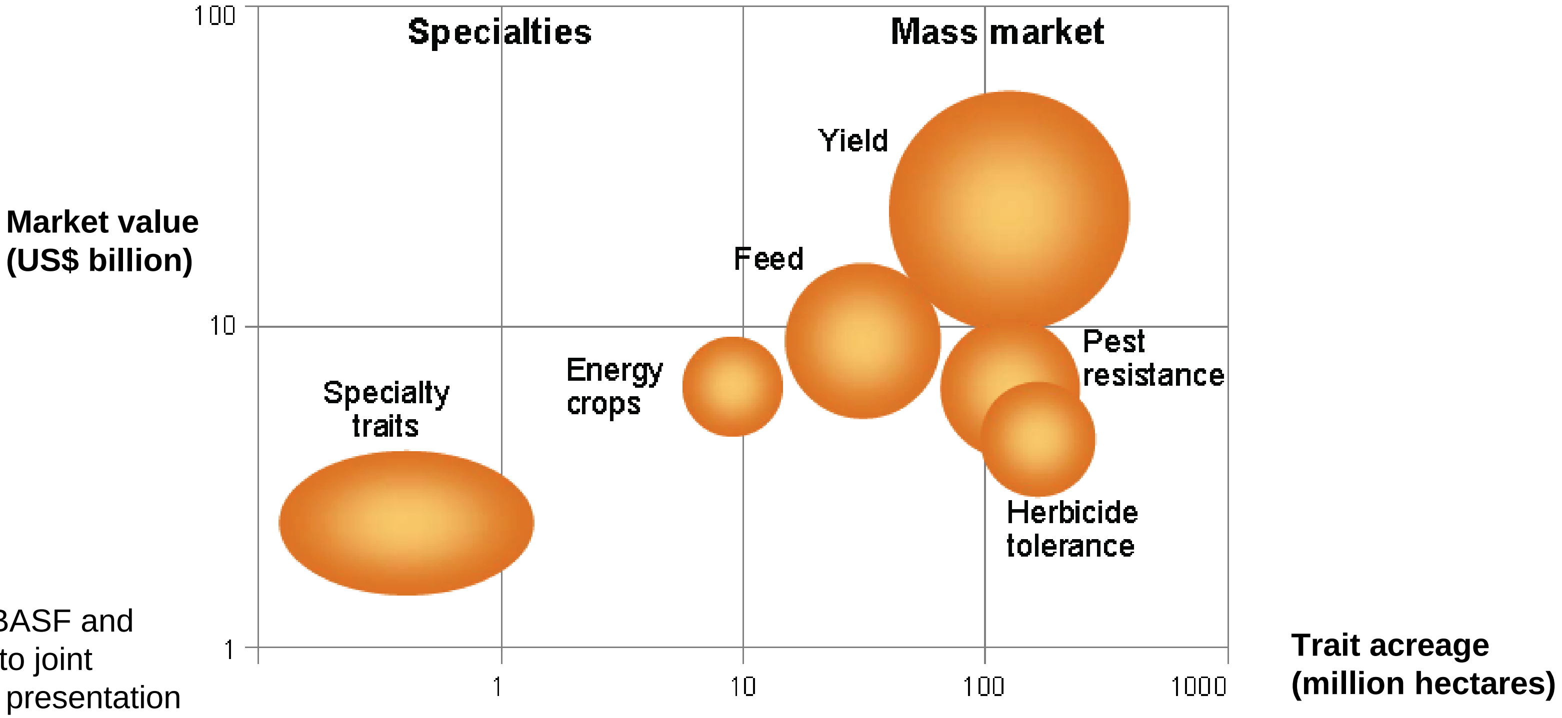
- New FY2008 targets of $3.7B gross profit for Seeds and Genomics; $1.9B for *Roundup* and other glyphosate-based herbicides

2012 OUTLOOK▼

	2012F
SEEDS & GENOMICS	$6.5-7.0B
ROUNDUP AND OTHER GLYPHOSATE-BASED HERBICIDES	$1.8B
ALL OTHER AG PRODUCTIVITY	$350M

Seeds and traits – 2025 vision

- Recent estimates predict an estimated global value of seeds and traits of $50 billion by 2025.
- The market will be dominated by agronomic traits and commodities with improved yield the single largest market.



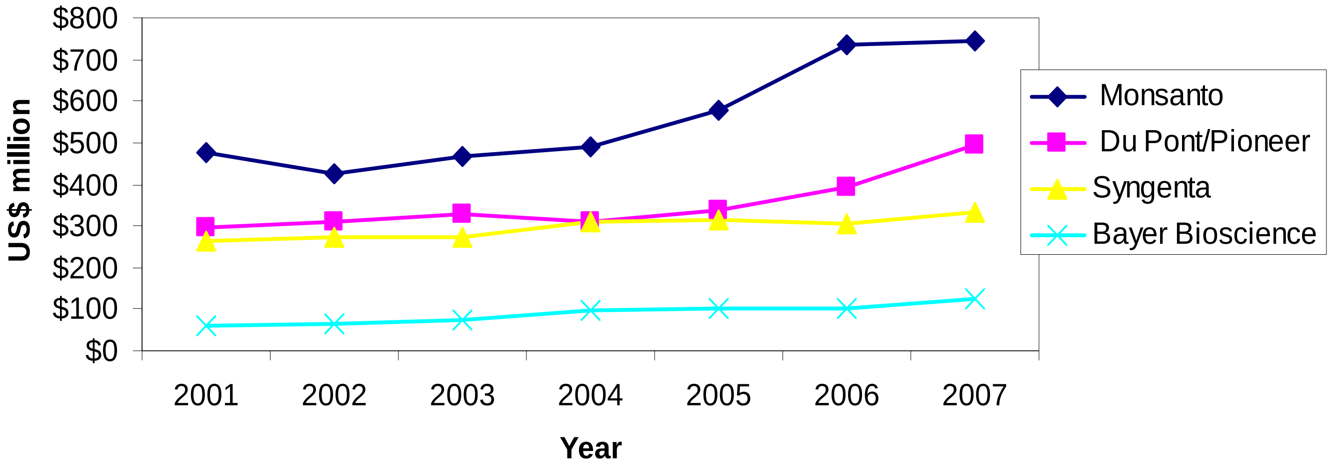
Credit: BASF and Monsanto joint investor presentation (September 16, 2008)

Industry trends – R&D investment

The agbiotech industry invests heavily in R&D

- Companies have been investing heavily in seed and trait development, ploughing up to 24% of sales back into R&D (see graph). On average, during 2007, agbiotech companies spent 13% of sales on R&D.
- A total of ~ $2.9 billion is estimated to have been invested by seeds and traits R&D during 2007 (Source: Cropnosis). For example, Monsanto's budget was around US$745 million, whereas DuPont spent US$495 million. This is good news for potential collaborators.

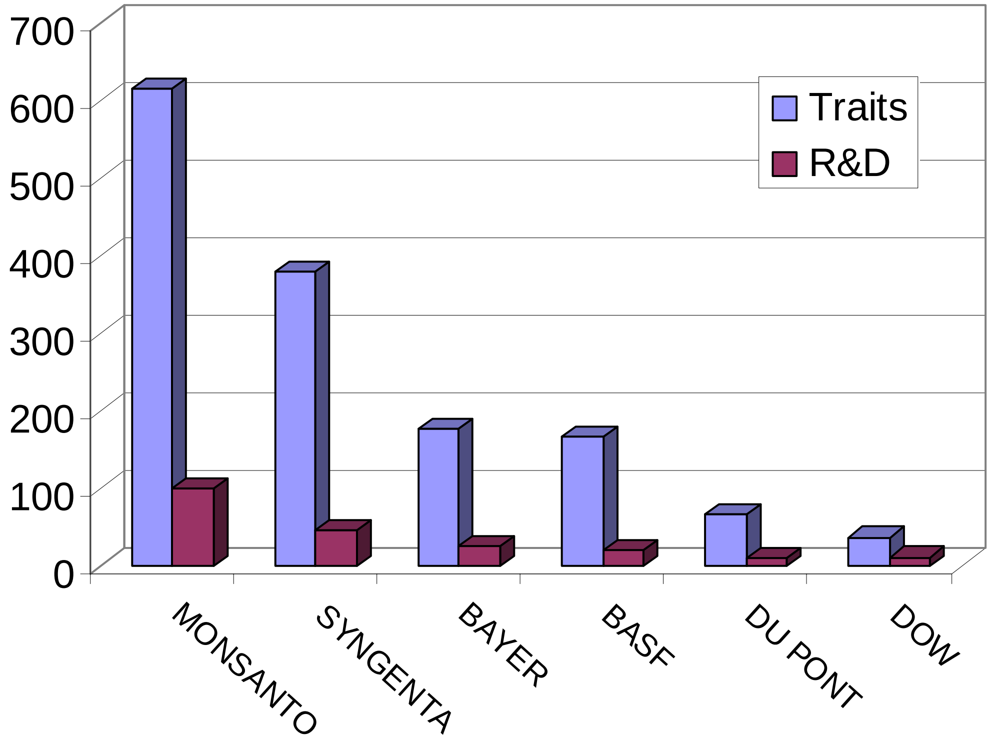
Investment in seed and trait R&D (2001- 2007)



Industry trends – patent filings

- Analysis of patent filings confirms that agbiotech companies concentrate more efforts on development (seeds and traits) than on new methods.

Analysis of patent filings of the major players over past 3 years



Key: Traits include novel varieties while R&D includes new methods and improvements.

Industry trends – collaboration

The industry is currently undergoing unprecedented levels of collaboration

- The major players have been joining ranks to share expertise and traits to develop next-generation products containing combinations of multiple traits that would not be possible for either company to do alone.
  - Monsanto has a collaboration in place with Dow AgroSciences for SmartStax, a new corn variety that will bear eight genes (four provided by each partner). It secured a similar agreement with BASF during 2006 to develop crops with enhanced stress resistance and yield, a project that will receive around $1.5 billion in investment.
  - Syngenta too has entered into industry collaborations with DuPont. In April 2006, the two companies set up a joint venture – GreenLeaf Genetics – and a licensing agreement to offer a broad access to proprietary corn and soybean genetics and biotech traits. In 2008 they further strengthened their business collaboration through exchange of crop-protection technology.
- Cross-industry collaborations will be a continued feature of future deal making, although the resulting ‘monopolisation’ of the commodity seeds and traits market could make penetration of the market by new players increasingly difficult.

As explored later in this foresight report, whereas these collaborations compound the dominance of a few major players in existing traits, the larger companies are looking to the more innovative smaller agbiotech players for traits for the future.

Industry trends – M&A activity

The industry remains acquisitive

- The bigger players continue to make strategic acquisitions to strengthen their market position, in particular in high-growth regions and to strengthen dominance in seeds and traits markets, as exemplified by Monsanto’s merger and acquisition (M&A) history (see table).
- As yet, there is little acquisition of technology platforms, exposure to which is mostly achieved via R&D collaboration. However, BASF’s 2006 acquisition of Crop Design could suggest a shift in strategy.

Monsanto’s recent acquisitions

Acquisition (date)	Deal value	Comment
Emergent Genetics (2005)	$300m	Third largest cotton company in US
Seminis (2005)	$1.4bn	Seminis controlled 40% of the US vegetable seed market
Delta Pine and Land (2006)	$1.5bn	Cotton and soybean genetics and large breeding programme
De Ruiter Seeds Group (2008)	€546m	Global vegetable seeds company
Marmot SA (2008)	ND	Marmot controls Latin American seed company Semillas Cristiani Burkard (corn, sorghum, soybean)
Various	$348 (total invested to date)	Monsanto also indirectly owns 15 small seed companies through its wholly-owned holding company American Seeds, which was incorporated in 2004. Most of the acquisitions are of smaller local, family business in corn and soybean seed provision

Industry news – regional focus

A growing focus on Brazil and other Latin American countries

These countries have fast-growing agricultural sectors and are increasingly attractive targets for agrochemical and seed sales, a trend not gone unnoticed by the bigger players.

For example, during 2007, Syngenta reported a 37% increase in sales in Latin American compared with just 7% in NAFTA regions. Equally, there were high increases in GM crop plantings during 2005–2006 in Brazil, Uruguay and Paraguay, which helped sales in the Latin American region.

Maize crop growing in Brazil

Industry news – regional focus

Companies are going East for R&D collaborations and commercial partners

India and China are investing heavily in agbiotech, which they see as a means of securing their national food security. Regulations around GM are being relaxed and it is predicted that these regions will help drive growth in agbiotech over the next 10 years. Western players have been quick to spot this trend:

- In April 2008, Syngenta announced it would invest $65 million in creating a new research centre in Beijing to evaluate GM and native traits for yield improvement, abiotic stress resistance and biofuels. In 2007, it acquired a 49% equity stake in a Chinese corn seed business, Sanbei Seed Co Ltd; it also has a 5-year research collaboration with the Institute of Genetics and Developmental Biology in Beijing.
- DuPont created its first biotech research centre outside the US in Hyderabad, India, and recently secured a joint venture with a Beijing company, Weiming Kaituo Agriculture Biotechnology (associated with Peking University) to study novel traits combinations to combat abiotic stressors. DuPont has had agribusiness interests in China since 2002.
- German company BASF joined the trend more recently by establishing a research collaboration in rice with Academia Sinica in Taipei, Taiwan (May 2008), the National Institute of Biological Sciences, Beijing, China (January 2008) and the South Korean Crop Functional Genomics Centre (October 2007).

‘Asia is emerging as a key player in plant biotechnology both in research and cultivation and we are striving to intensify partnerships in this dynamic region. Europe, on the contrary, is losing its competitiveness due to slow and contradictory political decisions.’

(Dr Hans Kast, President and CEO of BASF Plant Sciences)

The industry - summary

- Increasingly, agbiotech players are shifting from their agrochemical industry roots into seeds and traits, and are investing large portions of turnover into R&D in this area. This strategy is increasingly being rewarded with strong growth in profits and share prices.
- The healthy R&D budgets provide an opportunity for innovators to supply novel solutions for the larger players.
- An increasingly dominant group of a few multinationals has – through acquisition and major collaborations – an increasing degree of control over the existing seed and trait market.
- Europe is increasingly missing out on the agbiotech play as the larger multinationals seek both commercial and research collaborators in the East – India and China – in the face of restrictive policy in the EU.

Contents

Market analysis | Technology analysis | Scottish activity | Conclusion

- Scope & Background
- The Market
  - Size, products and dynamics
  - Drivers and restraints
- The Industry
  - Major players, performance and trends
  - **The value of innovation in agbiotech**
  - Case studies
- Conclusions

Putting a value on agbiotech

- As we have seen, the agricultural marketplace is quite different in size and dynamics to that, for example, of the biopharmaceutical arena. So as there are clear social and economic drivers for the future of agriculture, and genetic engineering might well address these, what return might one get on an investment in agbiotech?

- The value for tools and technologies in agriculture are difficult to estimate because deals are few in number and the terms rarely disclosed.

- Instead, it is worth looking at three indirect measures of the perceived value of agriculture biotechnology and crop science:
  - the value and impact of publicly funded research in this area;
  - venture capital interest and investment in agbiotech;
  - the nature of the deals or collaborations between players within the sector.

The impact of research in agriculture

- Anecdotally, the transfer of agricultural technology from public to private sector has been difficult because much of plant biology is carried out on model plants (e.g. *Arabidopsis*) and so the knowledge gained is not readily transferred to the commercial crops of interest to industry.

- More fundamentally, the true value of improvements in crop technology can be hard to evaluate as the real benefits are often indirect. For example, the development of a pest-resistant wheat cultivar might generate *de novo* seed sales of $10 million but might indirectly reduce predicted annual losses of $30 million in wheat sales.

- Several organisations have attempted to place a value on agbiotech and found that both direct and indirect returns are substantial.

Return on public investment

Two publicly-funded UK organisations that lead in crop research have attempted to make a quantitative analysis of the outputs (direct and/or indirect) from public investment in applied crop sciences:

- Independent consultants estimated that research outputs from the technologies generated by the **John Innes Centre** (Norwich), which receives around £27 million in funding annually, has made a >£8 billion annual impact (both direct and indirect) on world wheat and cereal markets.

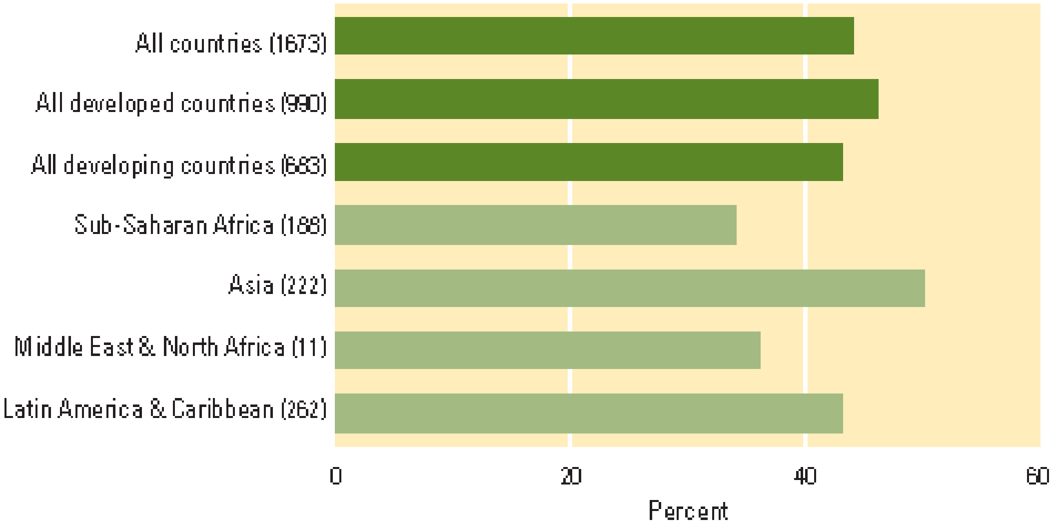
- The direct impact of the **Scottish Crop Research Institute** (Invergowrie) was evaluated at around £160 million per annum – a 14-fold multiplier of the annual research funding. By any estimate, this is an excellent return on investment.

The impact of research in agriculture (2)

The World Bank would agree in the value of investing in agriculture as examined in the 2008 World Development Report, the first update on the status of global agriculture in 25 years…

> ‘Agricultural productivity improvement have been closely linked to investment in agricultural R&D … there is little doubt that investing in R&D can be a resounding success.’

Figure 7.2 Estimated returns to investment in agricultural R&D are high in all regions[a] —averaging 43 percent



Credit: World Development Report 2008

Where angels fear to tread

Is the potential value of agbiotech being realised within the venture capital (VC) sector?

- It has been stated that **just US$5 billion has been invested over the past 10–15 years in agbiotech, compared with US$316 billion for lifescience companies** (Ganesh Kishore, Burrill & Co, ABIC meeting, Cork 2008).
- Indeed, fewer than 1 in 20 companies in most VC portfolios could be classified as agriculture/ agbiotech, and often these the more currently fashionable nutraceutical or functional food operations (ITI Life Sciences research).
- The situation is particularly acute in the UK, where very few agbiotech startups have been launched in the past few years. US startups have fared better but, anecdotally, have found securing private investment very difficult.
- A few VC companies have bucked that trend and taken an educated bet on agbiotech including:
  - **Burrill & Company**: manages an AgBio I and II fund that have a current capital value of ~ US$100 million. Fund II is still active. Portfolio companies include Chromatin, Crop Design and CreAgri.
  - **Life Science Partners**: manages the US$100 million LSP Bioventures launched by Syngenta during 2006 to invest in agbiotech, crop protection, biomaterials and biofuels, and health and wellness. Syngenta adopted this approach to get not only a return on the investment but also 'early cooperation with emerging innovators ...'
  - Smaller players, including **AquoAgro** (Israel), **Ceres Agri** (Toronto, Canada) and **Foragen** (Saskatoon, Canada), have ag-targeted funds.

So why not agbio?

Why have private investors shirked from making agbiotech investments? There are many confounding factors:

- **Limited agbiotech experience** among VC personnel means there is less confidence in making investments outside the individual's 'comfort zone'.
- **Difficult business valuations**: process of a new plant discovery and regulatory approval is very different to new drug development, where the likely attrition rates, costs of development and value inflexion points are well understood by VCs and used when making valuations.
- **Unclear markets and ROI**: the overall market for agrochemical products (currently around US$55 billion) is a small fraction of that of medicines (US$720 billion; source IMS Health Data), providing smaller potential returns. In the past decade, anti-GM lobbying will certainly have made investors question the viability of proposed end markets for GM products and technologies.
- **Limited exits**: unlike the many thousands of biopharmaceutical players around, from large-cap potential acquirers to smaller 'merger' possibilities, the landscape of possibilities for agbiotech companies are more limited. There are only six major players and a smaller group of mid-tier companies that offer acquisition potential or licensing opportunities.

It has yet to be established whether the renewed interest in agricultural biotech and the emerging biofuels business will spur private investors to take a serious look at agbiotech propositions in the future.

Licensing deal value

There are few publicised data on the deal sizes in agbiotech but, from the limited deals values available, it is clear that upfront and milestones are low compared to, for example, lifesciences deals.

Licensor	Licensee	Date	Technology	Exclusive	Upfront	Royalty
Cornell Research Foundation	Eden Bioscience	1995	Genes linked to plant hypersensitivity	Yes	No	2%
Monsanto	Calgene	1996	CMV application for fruit and vegetable development	No	ND	6%
Mycogen	Dowelanco Canada	1997	Improved crops incorporating Bt	Yes	No	5%
Rutgers Uni	Undisclosed	1999	Production novel grass cultivars	ND	No	5%
AERC (Canada)	Summit Seeds	2003	Feasibility study for new hybrid seeds	Yes	No	10%
Agriculture Canada	Monsanto	2003	GM wheat	No	ND	5%
Korean Research Institute	Penn Biotech	2004	GM seed potatoes	ND	~$30,000	1%
National Botanic Research Institute	JK Agri Genetics	2006	New Bt cotton technology to provide broader disease resistance	?	Rs 20 lakh upfront Rs 15 lakh on launch	3%

Data from KnowledgeExpress

R&D deals have higher value

Although deal values are hard to identify, the value that larger players put on innovative technologies is both more obvious and more significant. Many smaller agbiotech tools and technology developers have secured substantial deals with bigger players, providing all important cash-flow for emerging biotech startups.

Date	Deal	Source	Partner	Deal type(s)	Details
2008	$35m	Monsanto	Evogene	R&D and commercialisation	A 5-year research and development collaboration to identify yield, stress and fertiliser utilisation genes
2006	$20m	Landec	Monsanto	License, Marketing, Option, Sales	A 5-year global technology license agreement for Landec's Intellicoat polymer seed-coating technology
2006	$10m	Monsanto	Nufarm	R&D and Commercialisation	Nufarm acquires an Australian License for RoundUp Ready canola
2005	$10m	National Research Council of Canada	Dow Chemical	Development, Research	A 5-year strategic alliance to produce new oil profiles in canola for industry and health, and for animal feed. The agreement also includes research into the development of plant lines that produce valuable proteins, such as vaccines
2002	$137m	Monsanto	Ceres	Development, Research	Applying genomics technologies to provide improvements in certain agricultural crops
2001	$20m	Monsanto	Mendel Biotechnology	Commercialisation, Research	High-value plant traits extending 1997 deal (the value of which was not disclosed)

The value of innovation - summary

- Agbiotech businesses are few in number relative to their medical biotech peers. In part, the cause of this is the small amount of VC funding that they have attracted.

- There are many valid reasons why only niche VCs choose to invest, and possibly the most challenging has been the tenuous nature of the markets for the resulting products.

- However, there is good evidence from the public sector that significant value can be created from novel crop research technologies and end products.

- Although deal values are hard to come by in this sector, the attractiveness of novel platforms – as evidenced by the substantial R&D collaborations that many of the larger multinationals companies have engineered with innovative startups – is clear.

Contents

Market analysis | Technology analysis | Scottish activity | Conclusion

- Scope & Background
- The Market
    - Size, products and dynamics
    - Drivers and restraints
- The Industry
    - Major players, performance and trends
    - The value of innovation in agbiotech
    - **Case studies**
- Conclusions

Business models that work

- Clearly, there are several challenges for any company that intends to generate a viable business in agricultural biotechnology. The limited access to private investment, along with the increasing dominance of a few major players, makes achieving a foothold a challenge.

- However, many small companies have managed to gain traction, and it is interesting to assess how companies have made a mark on the sector.

- The following slides provide an overview of what might be considered a success in agbiotech, and provide some comfort that there is both need and room for innovation within the sector.

- The companies examined here are:
    - Mendel Biotechnology
    - Crop Design
    - Evogene
    - Performance Plants

Case study: Mendel Biotechnology

- A leading agbiotech company is Californian company Mendel Biotechnology, founded in 1997.
- Mendel scientists have systematically studied all 2000 transcription factors in the model plant *Arabidopsis* to identify those that control complex traits, such as freezing and drought tolerance, growth rate, disease resistance and nitrogen use efficiency.
- The company has a strong position in the area of transcription factors, with a broad portfolio of patents that it has used in a variety of markets to improve yield and yield stability in commercial crops, such as corn and soybean, with its most significant commercial partner Monsanto.
- In 1997, Monsanto and Empressa La Moderna (a supplier of premium fruit and vegetable seeds) both paid US$15 million for a 5-year research contract with Mendel and a 20% equity stake. They also secured rights to license outputs and a future 10% equity stake. **By any measure this is an impressive collaboration, providing Mendel visibility and credibility from its peers.**
- Mendel also has a collaboration with Bayer CropScience to identify chemicals that can induce stress tolerance via transcription factors. Mendel has collaborations with a wide range of other players (see next slide), which provide breadth of application for its core transcription technology.
- Another interesting application that Mendel is developing alone is the transcriptional regulation of the production of various natural plant metabolites, such as Taxol (from Yew) and rubber (from Guayule plant), as well as enhanced disease resistance.

Mendel Biotechnology

Case study: Mendel's R&D portfolio

Partner	Announced	Topic	Nature of deal
MMR Genetics & Richardson Seeds	June 2008	Sorghum biofuels	R&D
Monsanto	April 2008	Cellulosic biofuels	R&D
Bayer CropScience	Feb 2008	Chemicals that can modify stress response in crops	R&D
BP	June 2007	Biofuels	R&D, BP also took equity stake
Tinplant	March 2007	Miscanthus	Mendal acquired Miscanthus breeding program
Selecta Klemm	Sept 2006	Transgenic ornamental plants	JV established, Ornamental Bioscience
Monsanto	July 2006	Yield controlling transcription factors	Extension of 1997 collaboration
SweTree Technologies	Oct 2004	Forestry genes	Collaboration
Monsanto	Nov 2001	Crop improvements	Extension of collaboration
Seminis	April 2001	New tool development	$4.2 million NIST funding
Monsanto, Empressa La Moderna	Nov 1997	Crop improvements	$30 million funding in exchange for 20% stake each and rights to commercialize outputs (+10% option each)

Mendel Biotechnology

Case study: Mendel Biotechnology

- In 2005, Mendel took a strategic decision to branch out into biofuels and secured a 5-year collaboration with British Petroleum (BP) to develop proprietary varieties of cellulosic biofuels. BP also led an investment round and took an undisclosed equity stake in Mendel.

- Mendel's BioEnergy Seeds and Feedstocks business was further strengthened by the acquisition of a Miscanthus breeding programme from TINPLANT Biotechnik und Pflanzenvermehrung Gmbh (Berlin, Germany) and a further deal in June 2008 with MMR Genetics to develop sorghum biofuels.

- Mendel also has a collaboration with two forest genetics companies, Swedish SweTree Technologies and Arborgen (based in South Carolina).

- Today, Mendel is focused on applying a systems biology approach to map genetic and regulatory circuits, which will better enable the company to understand and modify the complex pathways involved in controlling complex traits. Mendel also has a close relationship with Edinburgh's Centre for Systems Biology (with Andrew Millar). Better understanding of the mode of action of transcription factors (their interaction with other cell proteins and DNA sequences) will also provide new intellectual property and targets for intervention.

Mendel's success proves that a novel and versatile platform technology can attract partnerships from many sectors providing cash flow to build a young business.

Mendel Biotechnology

Case study: Crop Design

- Ghent-based company Crop Design (Belgium) emerged as a success story for European agbiotech. Crop Design was founded in 1998 as a spin out from VIB, the Flanders Institute for Biotechnology. It raised €4.5 million in first round of funding from GIMV (then a publicly funded investment group), Atlas Venture and Sofinnova, and a further €41.7 million in later rounds.

- Crop Design's core technology was on key genes in cell-cycle regulation, which are fundamental to plant growth and development, and how these are influenced by the environment. It developed a high-throughout functional genomics platform that enabled automated analysis of gene function in *Arabidopsis* and rice – the so-called TraitMill.

- Wim Van Camp, Crop Design's Director of Technology Management and Business Development, says that the company's success is its focus on enhancing yield in cereal crops, which was identified early as a longer-term agricultural need well in advance of the competition.

- Crop Design secured several R&D collaborations over its first 7 years with companies such as Plantech, Henkel and Korean company CFGC, but made a breakthrough during December 2005 with a collaboration with BASF Plant Sciences. **Impressed with Crop Design's capabilities, BASF acquired the company less than 6 months later for an undisclosed sum** (industry insiders claim this was around $100 million).

- Arguably, Monsanto and BASF Plant Sciences $1.5 billion collaboration to develop yield and stress tolerance traits in major crops was largely to access Crop Design's capabilities.

High-throughput phenotyping made CropDesign an attractive acquisition target.

Case study: Arcadia Biosciences

- Arcadia Biosciences (Davis, CA, US) uses high-throughput screening, advanced plant breeding and genetic engineering encompassing **TILLING®** - a type of plant ‘knockdown’ or ‘knockout’ technology that enables it to identify genes related to specific traits. The mutant plants can be developed or a GM variant of the native species generated.
- Arcadia accessed the proprietary technology through acquisition of Anawah in 2005.
- The company’s main focus is to provide a new generation of crops that benefit not only growers but also the environment and human health.
- Arcadia has carried out field trials of a canola that uses just a third of the normal amount of nitrogen fertiliser used by conventional varieties to achieve similar yields (so-called Nitrogen Use Efficiency, NUE). It is also investigating salt-tolerant varieties of canola, rice, cotton and tomatoes.
- The company is also identifying tomato varieties with enhanced lycopene and natural antioxidants levels.
- The US Dept of Defense funded a $2.9 million project with Arcadia in 2005 to look at improving shelf life of tomatoes and lettuce
- DuPont, Monsanto, SES VanderHave Seeds and Cal/West Seeds have taken commercial licenses to NUE during 2005.

Arcadia
BIOSCIENCES

A novel approach to high-throughout trait identification put Arcadia on the agbiotech map.

Case study: Evogene

- Evogene (Rehovot, Israel) was founded in 2002 as a ‘spin off’ of the agricultural biotechnology division of the computational biology company Compugen.
- Evogene’s core competence is derived from its so-called ATHLETE gene-discovery platform, a computational biology (‘*in silico*’) approach to novel gene discovery. The platform is a tool kit that enables the compiling, comparing, analysis and prioritising of large (public) genomic datasets to sift through tens of thousands of genes to identify a core target set of just tens that could be related to a specific trait. These genes can then be screened through various biological assays and then tested in various model plants before being applied to crops. Evogene could test up to 200 genes each year using this system.
- The strategy adopted by Evogene provides it with a broad scope that avoids it being limited to a specific subset of genes or gene families – a limitation suffered by many other agbiotech companies.
- Evogene has programmes to study a range of output traits, including drought, salinity, NUE and improving yield and is also improving non-edible feedstock sources for biodiesel.
- Ofer Haviv, CEO of Evogene, says that the company operates ‘like a factory’ to validate up to 150 prioritised genes a year in both *in-vitro* assays and in two model crops. Today, confidence in the company’s technology platform allows it to negotiate deals earlier (at model crop stage) and for higher value than at earlier stages of development.
- Since 2002, the company has raised $11.5 million from private investors and in July 2007 another $8 million from an IPO on the Israeli stock exchange. It has partnerships with almost all the big agbio players and many specialist outfits, such as Biogemma (corn), Rahan Meristem (bananas), Cirad (cotton).

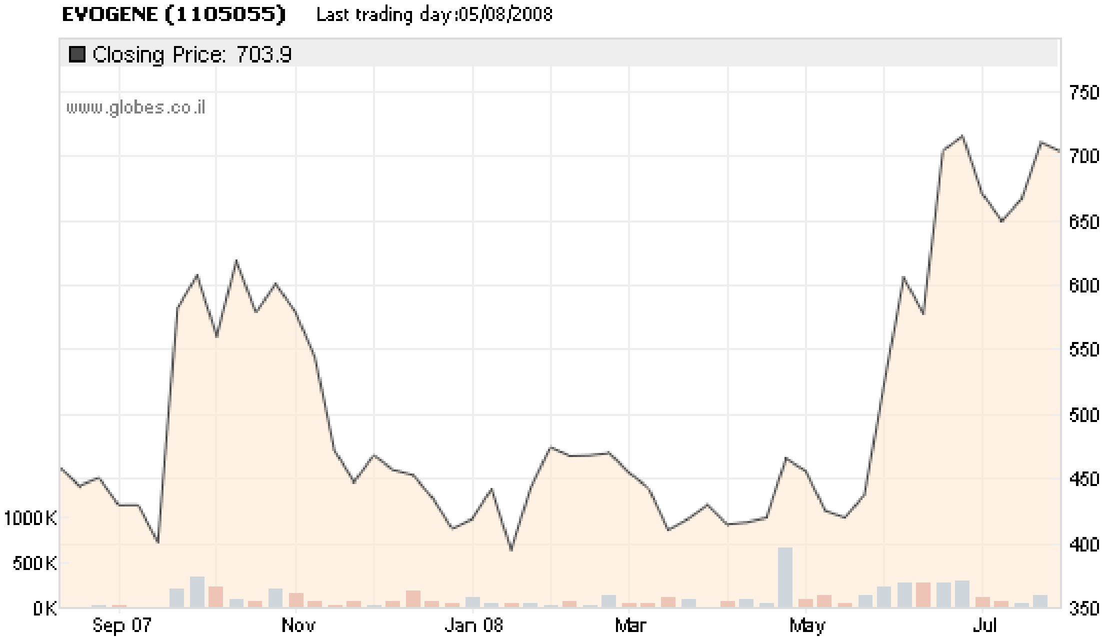
evogene

Case study: Evogene

- As one of the few public agbiotech companies, Evogene's performance on the Israeli Stock Market is of interest. Evogene's share price has increased significantly over the past year and the company has a market cap of NIS 142 million (approx £2 million).

- In what will be a transforming event for the company, at the end of August 2008 it signed a $35 million R&D deal with Monsanto to look at yield, fertiliser use and other environmental stresses. Monsanto has purchased an additional $18 million in equity (with an option for a further $12 million).

Evogene's reliable bioinformatics and gene-prediction software set it apart from its competitors.



Case study: Performance Plants Inc.

- Canadian company Performance Plants, based in Kingston, Ontario, is of note among Canadian agbiotech players having developed several ‘weatherproofed’ crops and securing VC investment.

- The company was founded in 1995 by David Dennis and other members at the Biology Department at Queen’s University, Kingston. It was originally a technology-transfer vehicle with the goal of exploiting university-discovered traits but soon built in-house capabilities to generate its own leads.

- Its most advanced work on drought-resistant crops arose from a serendipitous discovery of an *Arabidopsis* mutant in the lab. of Peter McCourt, University of Toronto. A student forgot to water the lab. specimens before the weekend and the single surviving plant was found to have a mutation in the *ERA1* gene that rendered it more sensitive to the hormone abscisic acid (ABA). ABA shuts plant stomata, reducing water loss and improving a plant’s drought-resistance.

- Performance Plants developed the concept by engineering into plants a promoter activated under drought conditions that reduced *ERA1* production, thereby making stomata close earlier and tighter. The advantage of the system is that the protective mechanism is activated only during drought stress, otherwise the plant grows normally.

- In the field, this so-called Yield Protection Technology (YPT), when applied to various crops, shows that the yield during drought can be as high as under control conditions, while non-modified crop yield declines by up to 15%. This is significant, as stress-resistant genetic mutations frequently impact negatively on yield (so-called ‘yield drag’).

performance
PLANTS

Case study: Performance Plants Inc.

- Kevin Gellatly, VP of Alliances, says that much of the company's success has been its focus on plant physiology and 'old-fashioned' mutation analysis. The company employs a large number of experienced plant physiologists. Gellatly argues that the move to large-scale genomic analysis just increases a company's burn rate without necessarily delivering validated traits. A fundamental understanding of plant physiology is key for targeted intervention.

- Performance Plants is evaluating YPT and related abiotic stress systems in various food crops and has deals with DuPont, Syngenta, Stine Seed, RiceTec Inc and The Scotts Miracle-Gro Company.

- Like many other agbiotech companies, it has branched out into biofuels, buying a biotech research facility in Waterloo, New York, and an agreement with Lafarge Canada to develop and grow clean energy biomass in a locally sustainable manner.

- Performance Plants has raised a total of C$55 million in a series of fund raisings, the largest being C$15 million in July 2008.

Insight and expertise in plant physiology and breeding of complex traits has provided Performance Plants with a competitive edge.

Case studies - summary

Several criteria are key to success in an emerging agbiotech company

- A versatile platform technology that can be applied broadly to generate a range of value-added products (food, feed, biofuels, etc.).
- A platform that offer efficiencies in cost and time to identify novel genes/traits.
- The ability to rapidly secure partnerships and collaborations with the leading players to access elite germplasm, thus gaining access to the end market.
- A technology that is versatile and can apply to multiple markets (food, feed, biofuels, chemical feedstocks).
- Some means of differentiation from the competition.

The challenges for agbiotech survival are primarily

- The lack of access to venture capital.
- Inability to take products to market direct because of lack of access to elite germplasm.
- The relatively small pool of potential licensees, although arguably ones with deep purses.

The most viable option for creating value from investment from agbiotech innovation is in the form of a technology platform that can generate cash flow from fee-for-service provision or R&D collaboration for both the large multinationals and businesses in emerging markets. IP alone is not sufficient to generate a successful business.

Contents

Market analysis

Technology analysis

Scottish activity

Conclusion

- Scope & Background
- The Market
    - Size, products and dynamics
    - Drivers and restraints
- The Industry
    - Major players, performance and trends
    - The value of innovation in agbiotech
    - Case studies
- **Conclusions**

Conclusions

- Agricultural biotechnology as applied to seeds and traits is a growing billion-dollar business that is increasingly required to address some of the issues posed by the global challenges of population growth, climate change and renewable energy demands.

- At present, most of the value of genetic modification lies in just four crops bearing simple improvements such as herbicide and pest resistance. However, if successful, even combinations of these traits will not be adequate to address the impending yield gap, nor will they help reduce the impact of agriculture on the environment.

- Much of the value of the seeds and traits industry lies in the hands of a few companies, which dominate the seed landscape and invest heavily in protecting this position through R&D. But they are not sufficiently innovative, as evidenced by their IP portfolios, and depend on external collaborations with smaller tools and technologies players.

- Largely as a result of public distrust of the technology, and the resulting policy changes, the market for genetically modified products has been curtailed. Strict regulations, lack of private and public investment (a small fraction of that invested in medical innovation) and the resulting loss of expertise from the sector have contributed to low activity in this sector.

- Yet it is proven that investment in agricultural R&D reaps a good harvest and there is an increasing awareness that funding is needed to bridge the gap between lab. and field.

Contents

Market analysis
- Scope
- The Market
- The Industry
- Conclusions

Technology analysis
- **Transformation**
- Phenotyping

Scottish activity
- Agriculture
- Research
- Crop science

Conclusion
- Conclusion
- ITI Opportunity

Technology analysis

Transformation

Phenotyping

- **Background and scope**
- Existing technologies – benefits and limitations
- Outstanding needs and solutions
- IP landscape
- Unmet need

Background and scope

- The unprecedented increase in agricultural productivity during the last century (the so-called ‘green revolution’) arose from the selection of crops – through selective breeding – that offered better yield, disease and stress resistance, and adaptability to different environments.

- However, whereas selective breeding can introduce a new desired trait, it can also ‘drag along’ many undesirable traits that must then be eliminated by back-crossing with the elite germplasm. As a result, such conventional breeding methods can be slow and unpredictable. Also, the traits introduced are limited to those available within the native gene pool.

- By contrast, genetic engineering offers a faster and more accurate means of altering the plant’s genetic makeup. Furthermore, traits can come from any source providing greater diversity. Despite early resistance to this technology, GM crops are a multi-US$ billion business.

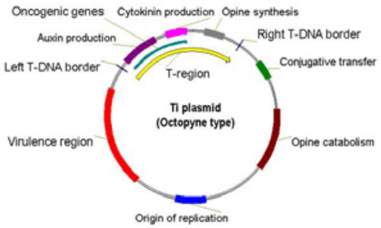
- The development of new plant varieties in an industrial setting, using medium- and high-throughput discovery platforms, is not so dissimilar to that of drug discovery – traits must be identified, validated and new products generated. Any means to improve efficiency and accuracy, while reducing costs, become differentiators within a highly competitive marketplace.

- This section examines plant transformation – the means by which novel genetic information (traits) are introduced into plants. The benefits and disadvantages of each technique are assessed along with emerging solutions.

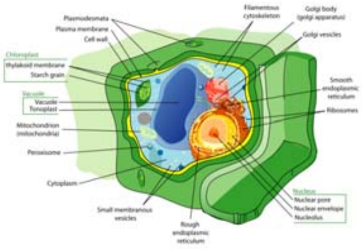
- Note that this section does not attempt to address all the potential applications of transformation, which are outside the scope of this document.

New plant discovery

This diagram summarises the basic process of creating a transgenic plant.



1. The transgene to be inserted is linked to a promoter and a marker to regulate its expression and selection, respectively.



2. The vector is inserted into the infective plasmid (Ti) of plant pathogen *Agrobacterium tumefaciens* and plant infected OR the vector may be delivered using bolistics.

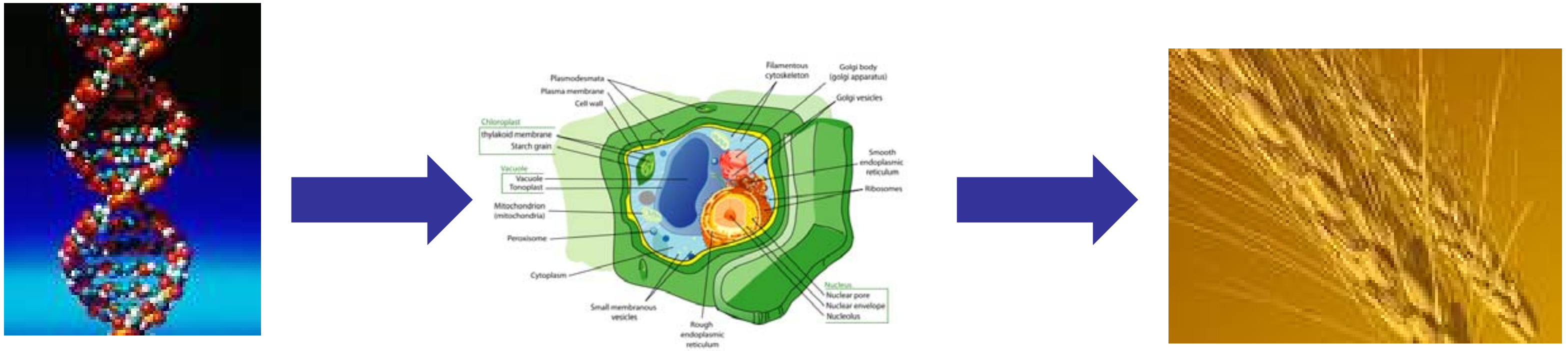
3. Cells are grown in tissue culture and successfully transformed cells selected using the marker (often using antibiotic incubation). Plants are regenerated from the transformed cells.

4. Transgenic plants are grown in greenhouse and their phenotype studied. Transgenics are then crossed to establish a stable line.

5. Extensive field trails and safety studies are needed to establish the stability of line and provide data for regulatory dossiers.

Transforming plants

In principle, there are four key hurdles to clear to achieve efficient and stable plant transformation.



The first challenge is to get the transgenic material across the plant cell wall, which offers a formidable barrier.

Second, the transgene must be integrated into the nucleus, preferably at a desired location and as a single copy.

Third, transgenic plants must be regenerated from transformed cells (using selection markers). The transgene must be stably expressed and inherited through future generations.

1. Delivery

2. Integration

3. Stable and inherited expression

4. Tissue culture

Underpinning the entire process is the success of the tissue culture – to enable gene delivery, maintain cell or explant health and to optimise regeneration

A transformation wish list

It is possible to create a 'wish list' for the ideal transformation technology(ies). In this report, we examine whether existing and emerging technologies meet these stringent criteria.

The ideal transformation system might:

- ✓ Breach the barrier of the plant cell wall.
- ✓ Be effective on a wide range of species and different genotypes of that species.
- ✓ Provide a high efficiency of transgene integration into the genome.
- ✓ Ensure targeted insertion of single copy with limited risk of gene silencing.
- ✓ Permit the transfer of large lengths of DNA for stacking complex traits.

Technology analysis

Transformation

Phenotyping

- Background and scope
- **Existing technologies – benefits and limitations**
- Outstanding needs and solutions
- IP landscape
- Unmet need

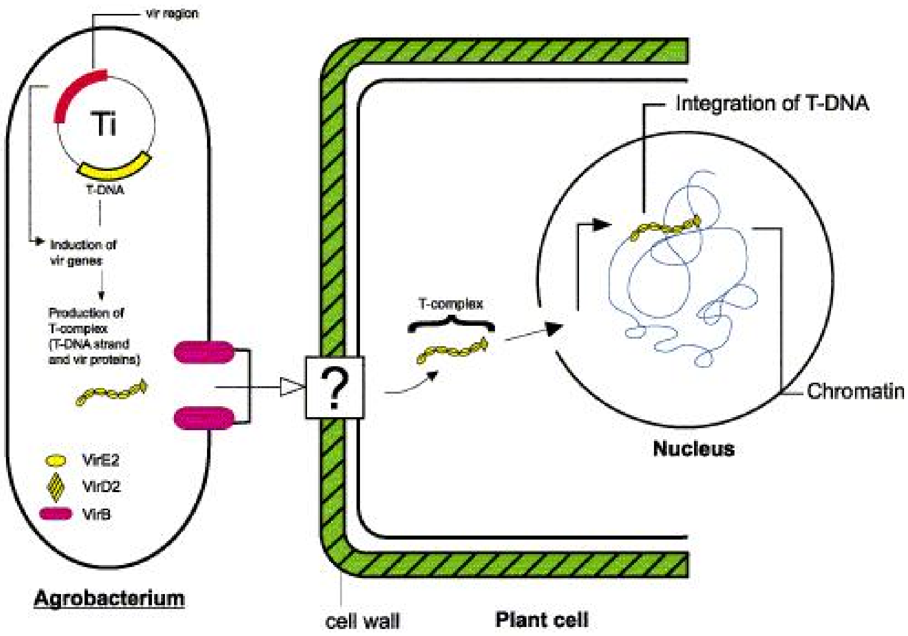
Transformation today

The gold standard techniques used for plant transformation today include:

- **Indirect methods**: by harnessing the natural infective mechanisms of plant pathogens to inject DNA into plant cells. The most well-established method is with *Agrobacterium* spp.
- **Direct methods**: by introducing naked DNA into plant cells directly using physical or chemical assistance via:
  - microinjection into protoplasts;
  - electroporation of protoplasts;
  - polyethylene glycol (PEG) method;
  - use of silicon carbide fibres (to temporarily disrupt the plant cell wall);
  - calcium alginate microbeads.
- The most common application is through high-pressure bombardment with gold particles (i.e. **biolistics**) bearing the DNA.

Agrobacterium transformation

- *Agrobacterium tumefaciens* has been used for over three decades for plant transformation – it is nature’s genetic engineer!
- The bacterium naturally infects dicotyledons (dicots) to produce tumours, so-called ‘crown galls’, by injecting the **T-DNA** portion of its **T**umour-**i**nducing (Ti) plasmid into the plant cell.
- The T-DNA would normally bear the oncogenes and genes that hijack the plant’s metabolic machinery. However, these genes can be removed, disarming the bacterium, and replaced with transgenes of interest.
- Several virulence (*vir*) genes are retained to ensure the T-DNA can be transferred.
- A promoter and selection marker (normally antibiotic resistance) is introduced along with the transgene to ensure its expression and the ability to select for transformed cells, respectively.



T-DNA transfer into the Plant's Genome

Adapted from Zupan et al 2000

Agrobacterium transformation

In summary the advantages and disadvantages of using *Agrobacterium* are as follows:

The advantages

- *Agrobacterium* transformation techniques are well established, cheap and easy to apply. This is the transformation method of choice.
- The technique has been used to transform a range of dicots, including tomato and potato and, with refinement, has been used successfully to transform several commercially valuable monocotyledons (monocots), such as maize and rice.
- Many GM cereals have been developed using this method.

The limitations

- *Agrobacterium* is not efficient on all commercially valuable crops (e.g. soybean, tea crops) and, more importantly, not all genotypes of even transformable species (e.g. only certain wheat and barley cultivars can be transformed readily).
- Only a limited amount of transgenic material (<120 kb) can be stably carried on the Ti plasmid, which restricts its application for gene stacking.
- On occasions, unexpected rearrangements of the T-DNA sites can occur resulting in the inadvertent introduction of some of the Ti–plasmid backbone DNA. Indeed, some 1 in 250 transformed plants may carry some bacterial chromosomal DNA integrated along with the T-DNA during transformation (*Nature Biotech*, September 2008). This poses concerns for food safety and horizontal gene transfer.
- Finally, the need to introduce a selectable marker (often antibiotic resistance) is a disadvantage of *Agrobacterium* transformation largely because of its low efficiency. However, as explained later, this can be readily resolved.

Circumventing IP Agro

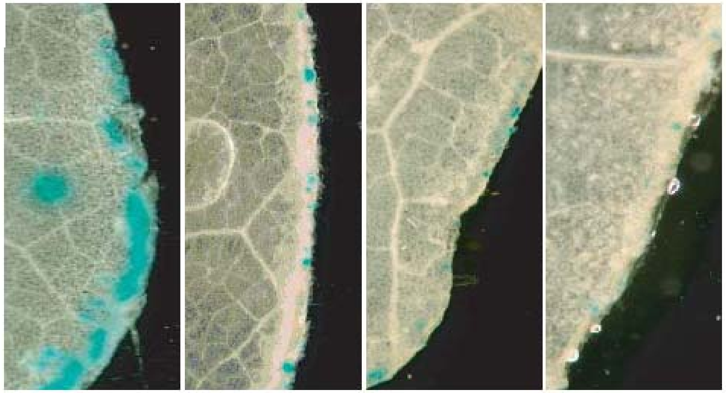
- The intellectual property around *Agrobacterium* transformation is tightly controlled by commercial entities, which has spurred academic researchers to find alternatives. One solution was to use other plant pathogens, with differing host specificity, as genetic engineers.

- CAMBIA researchers discovered that if they engineered the Ti plasmid, and other essential vectors, into *Rhizobium* spp., *Mesorhizobium loti* and *Sinorhizobium meliloti*, these plant pathogens could be used as vehicles for gene transfer (*Nature* (2005) 433: 629–632).

- The pathogens could infect monocots and dicots and a range of tissues with good efficiency (see figure, blue stain).

- Many of these bacteria are available from CAMBIA via an open-source model akin to that adopted in software licensing, which is to encourage their uptake for both research and commercial purposes.

- Further screening could identify other plant pathogens suited for plant transformation application.

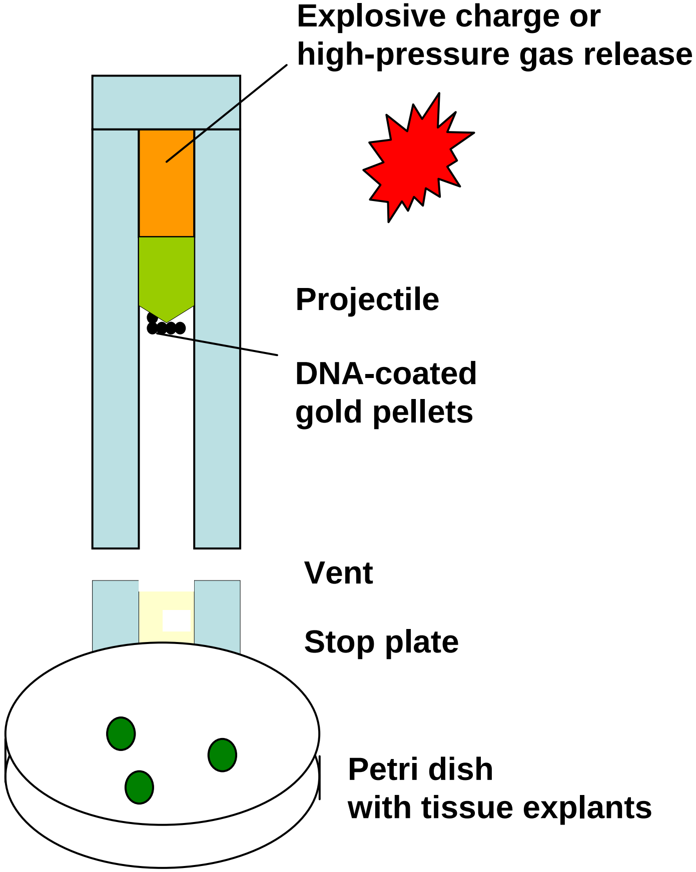


CAMBIA

Biolistics

Biolistics

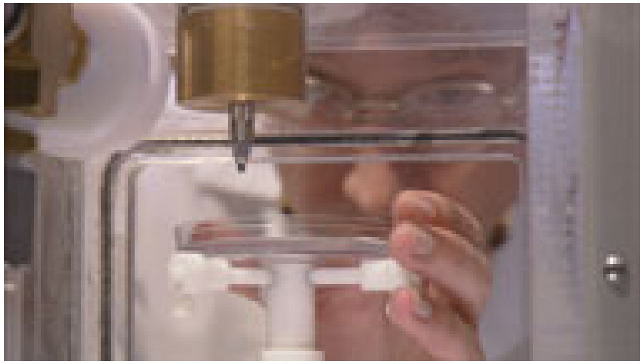
- The alternative to bacterial transformation is biolistics – shooting plant cells with DNA!
- A DNA construct, containing a transgene flanked by expression regulators and a selectable marker coated on gold particles, is forced down the barrel of a ‘gene gun’ (see right) by either an explosive charge or a release of high pressure.
- The particles are forced through the plant cell wall where the DNA is released and some will integrate into the nucleus.
- The plant tissue is grown in tissue culture, the transformed cells are selected and the transgenic plant is regenerated.



Biolistics

- The helium-driven gene gun was developed and patented by DuPont, and subsequently marketed by BioRad in various formats (see below).
- Several variations on the biolistic theme have been employed, in part to circumvent the intellectual property of the gene gun concept, including:
    - The Particle Inflow Gun (PIG) was based on the acceleration of DNA-coated tungsten particles in a helium stream.
    - ACCELL^{TM} system: electrical discharge technology was used to accelerate the DNA–gold particles.
    - Stine Seeds (Ames, Iowa) has a patented Aerosol Beam Injector system that uses an aerosol solution of the DNA without a carrier. The aerosol is forced through a fine nozzle into a vacuum, where it is accelerated to supersonic speeds through the plant cells. No carrier is used so the particles are smaller and are claimed to cause less damage and to improve transformation rates.

BioRad's PDS 1000He mostly commonly used in transformation labs



The Aerosol Beam Injector in action

Biolistics

In summary the advantages and disadvantages of biolistics are as follows:

The advantages

- Biolistics are well established and work on a broad range of plants, both monocots and dicots, many of which are recalcitrant to *Agrobacterium* transformation.

The limitations

- Transformation efficiency is low, often lower than with *Agrobacterium*.
- The main limitation of biolistics is the increased risk of the introduction of multiple copies of the transgene, which increases the risk of gene silencing.
- Biolistics can delivery large pieces of DNA but transgene instability arises at longer lengths.
- To a large extent the limitations of biolistics are not dissimilar to those of *Agrobacterium*.

Technology analysis

Transformation

Phenotyping

- Background and scope
- Existing technologies – benefits and limitations
- **Outstanding needs and solutions**
- IP landscape
- Unmet need

Outstanding needs and solutions

Improving on gold standards

- Standard *Agrobacterium* and biolistics methods are widely and successfully used but there is still room for improvement. Four key challenges remain:
    - **Improving transformation efficiency**: the recalcitrance of many species and genotypes, the temperamental tissue culture techniques, and innate plant responses to foreign gene intrusion all reduce efficiency, increasing time and cost!
    - **Achieving true gene targeting**: it is still not possible to precisely control either the number of copies or position of the transgene inserted within the plant genome.
    - **Gene stacking**: complex traits may require the introduction of several traits under coordinated control.
    - **Safety**: the elimination of selection markers and non-plant transgenic material would address concern of regulators and the public.
- This section examines various strategies and improvements currently used to mitigate each of these limitations in turn.

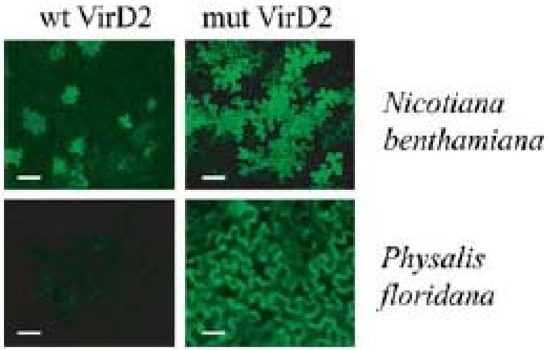
Transformation efficiency – tissue culture

Improving transformation efficiency

- Tissue culture is a time-consuming, laborious and costly process but it is crucial for efficient plant transformation. Explanted plant tissue or cells must be kept in a condition that permits selection of transformed cells and their regenerate into transgenic plants.

- Patent landscaping reveals that tissue culture improvements are actively sought: some examples include cold storage of the *Agrobacterium* in advance of infection and the treatment of explants with low oxygen, nitric oxide modulators or copper during transformation. The use of more virulent forms of *Agrobacterium* has also been explored and various modifications to the biolistics technique.

- Further improvements in transformation efficiency can be achieved by modifying **hypersensitivity reactions** of a plant triggered by *Agrobacterium* infection. Hypersensitivity can lead to apoptosis, reducing the probability of both transformation and regeneration. Plant proteases are implicated and by mutating a bacterial target (VirD2) of these enzymes transfection efficiency has been improved (Reavy et al. (2007) *Plant Cell Reports* 26: 1215 and see below). This approach warrants further investigation.



Efficiency of GFP expression (green) in transformed plants with wild-type bacterium (left) and one carrying a mutant VirD2 protein (right), the target of plant enzyme action.

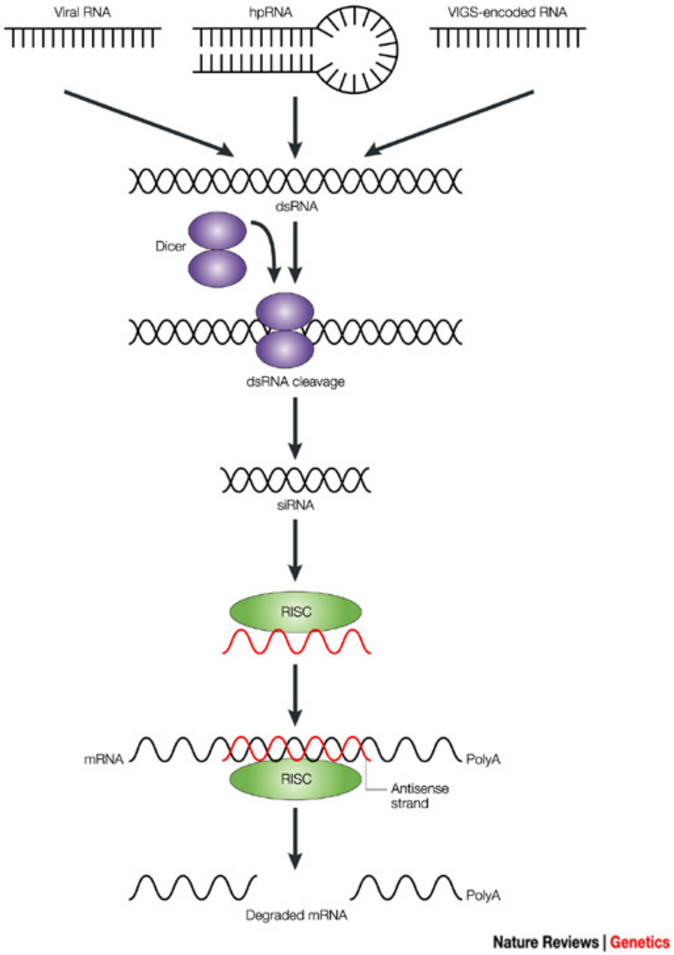
Transformation efficiency – gene silencing

Gene silencing

- The integration of multiple transgene copies can lead to silencing, a phenomenon in which the expression of the transgene (or an endogenous gene with sequence homology) is partially or completely reduced. The degree of silencing is related to the dosage of mRNA transcripts and so is more problematic with biolistics (and with transient viral transformation) where multiple transgene insertions can take place.

- Gene silencing is not an issue for many crops but may contribute to the recalcitrance of many other species.

There is, and will continue to be, much improvement in efficiencies in existing plant transformation methods. Much remains to be learnt about the physiological basis for recalcitrance of many genotypes of commercial crops along with effective means to overcome these barriers.



Gene targeting

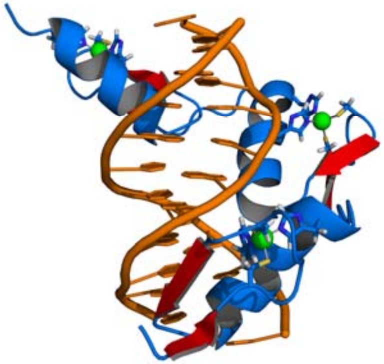
Location, location, location

- With current transformation technologies, the transgenes are inserted randomly into the plant genome. They can be influenced by the nature of this location. For example, the transgene may:
  - insert and disrupt an endogenous gene potentially with a critical function;
  - be influenced by regulatory elements up or downstream the insertion site (the so-called position effect).
- The problem is the high frequency with which transgenic DNA integrates at non-homologous sites by illegitimate recombination (typically 10^5 to 10^7 illegitimate recombination events for every homologous recombination).
- To further complicate matters, around 30–80% of plants have more than two sets of chromosomes (**polyploidy**). For example, maize and potato have four sets and wheat and oats have six. As a transgene can insert at *any* position on *any* chromosome, coordinated expression of several transgenes (e.g. for gene stacking) is problematic.
- In an attempt to mitigate the position effect, attempts have been made to shield transgenes by so-called **matrix attachment regions (MARs)**. MARs elements constitute anchor points on the DNA for the chromatin scaffold and play an important role in the regulation of gene expression. Although promising, results are highly variable and depend on the specific transgene construct and the MARs element used.

Gene targeting

The numbers game

- The other problem is lack of control over the number of transgenes inserted: the transgene often integrates as multiple copies, especially when delivered through biolistics (sometimes in up to 90% of transformants).
- Multicopy insertions are often associated with reduced or unstable transgene expression, or might trigger transgene silencing.
- The current solution is to use quantitative real-time PCR to screen stably transformed plants to check for a few with simple integration structures that express the transgene at the desired level. This is time consuming.
- True gene targeting, whereby a gene can be inserted at a designated site on a designated chromosome, is currently not achievable although several tools show promise. These include:
  - site-specific homologous recombination;
  - zinc fingers proteins;
  - oligonucleotide transformation.



A zinc finger protein (blue) sitting within the major cleft of the DNA helix

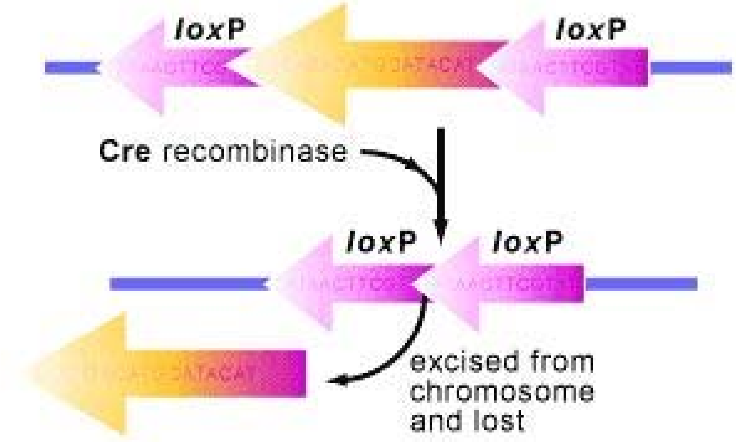
Gene targeting – site-specific recombination

Site-specific recombination

This is a means of manipulating transgene at a pre-defined site determined by the site and orientation of the components of the system. Note that it is not a true gene-targeting system.

Three different systems have been tested so far:

- Cre-*lox* from bacteriophage P1;
- FLP/*FRT* from yeast;
- INT from phiC31 *Streptomyces* phage.



- The Cre-*lox* system is the best understood. The key components are a Cre protein, a site-specific DNA recombinase, which catalyses the recombination of DNA between specific sites known as *loxP* sequences.
- When all components are present, the *loxP* sites recombine and the Cre enzymes cut the double-stranded DNA and ligate the ends. Anything lying between the *loxP* sites is eliminated.
- For the system to work in plants two stable lines must be generated: one carrying the transgene flanked by the *loxP* sites and the other carrying the *cre* gene. These can then be crossed to eliminate the transgene.

Gene targeting – site-specific recombination (2)

New developments

- Site-specific recombination was first used to remove marker genes (see Box) but the technique is more versatile.
- It is now possible to conditionally express recombinase genes that can induce deletion not only of the selection marker but the recombinase gene itself. This could enhance public and regulator confidence in GM crops for the future.
- Flanking a transgene with inversely oriented *loxP* sites can be used to cut down multiple transgenes into single-copy versions – proven in wheat, rice and maize.
- Once a suitable target site is found, the plant line can be used for predictable insertion and expression of a variety of genes and the technique can be adapted for sequential gene addition.

Limitations

- Recombinase-based systems require the creation of well-characterised lines carrying target sites that will permit stable and predictable transgene expression. This has to be done using standard methods and so suffers from the same inefficiencies.

Site-specific recombination gets to market

During 2006, Renessen, a joint venture between Cargill and Monsanto, received US regulatory approval for LY038 (Mavera^{TM}), a variety of maize with high lysine levels designed for the poultry and pig feed industry. Here the Cre-*lox* system was used to excise the marker selection gene (here antibiotic resistance) leaving the transgene of commercial value.

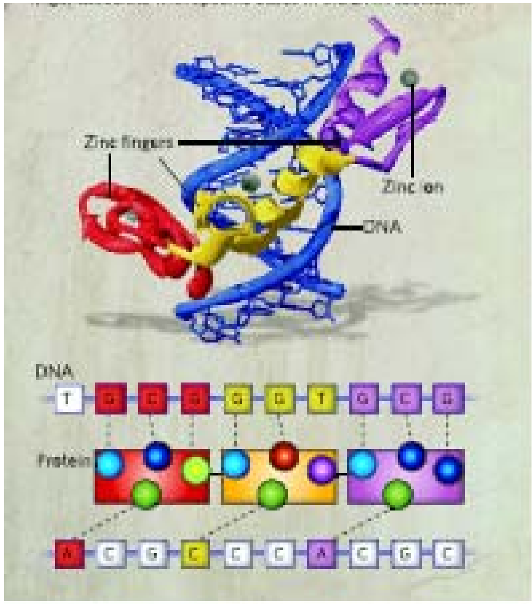
Gene targeting – zinc fingers

Zinc finger proteins

- Zinc finger (ZF) proteins are a class of naturally occurring transcription factors that bind to DNA to alter gene expression. Over 700 ZF proteins have been identified in humans and over 170 have in *Arabidopsis*.

- ZF proteins have highly specific DNA-binding motifs created from their ‘fingers’ – a stretch of ~30 amino acids stabilised by a zinc ion. Three fingers (a triplet) lie adjacent to one another along the major groove of the DNA helix. Each finger is associated with three DNA bases, giving each ZF protein its high degree (nine bases) of specificity. (see diagram).

- It was quickly spotted that ZF proteins would make excellent scaffolds for designer enzymes!

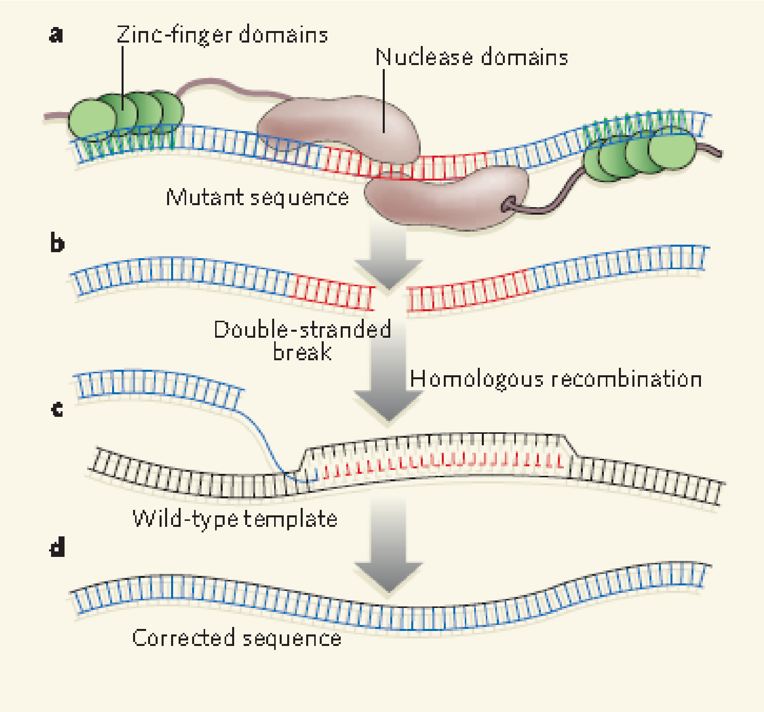


X-ray structures showed that three zinc fingers sit within the major groove of the DNA helix. Each is associated with a distinct triplet of base pairs.
Credit: *Nature* (2008) 455: 162

Gene targeting – zinc fingers (2)

Zinc finger nucleases

- The application of ZF nucleases (ZFN) in plant genetic engineering has been successfully demonstrated in *Arabidopsis,* tobacco and maize.
- Engineered ZFNs have a high degree of specificity for the desired gene target but a non-specific nuclease domain, such as the one from endonuclease Fok I. The nuclease cuts the double-stranded DNA, which induces homologous recombination (a genome repair mechanism).
- Unlike other forms of genome repair, homologous recombination is accurate. It normally uses a homologous sequence from an undamaged sister chromatid but can also apply a transgene of interest for repairing the break.
- Other designer ZFs have been developed to modify, delete or add genes in a site-specific fashion.



Basic mechanism of action of zinc finger nucleases.
Credit: *Nature* (2005) 435: 579

Gene targeting – zinc fingers (3)

- Much of intellectual property around the design and application of ZFs resides with Californian company Sangamo Biosciences. In 2005, Dow and Sangamo signed an exclusive R&D deal and license option around the application of ZF transcription factors and nucleases in plants and industrial products.

- The collaboration has been highly successful and in June 2008 Dow exercised its option to the technology.

- ZF technology has been used to produce a maize variety with reduced phytase metabolism with unprecedented accuracy and speed (ABIC meeting 2008, Cork).

- To fully exploit the value of ZFs for agriculture, Dow intends to sublicense the technology to third parties through a range of products trademarked as **EXZACT™ Precision Traits**.

- Sangamo's main business focus is on application of ZFs for therapeutic gene regulation. The company currently has a Phase 2 trial of a ZF able to upregulate endogenous VEGF-A in diabetic neuropathy and Phase 1 trial in peripheral arterial disease.

Sangamo BioSciences, Inc.

Gene targeting – zinc fingers (4)

Limitations

- Probably the greatest limitation of the technology is the time and effort required to achieve the necessary specificity. This is done through randomly generating coding sequences and testing these using phage display – a lengthy and expensive process. In practice, it might not be possible to design a ZF to target every gene of interest.
- Another limitation is off-target cleavage of chromosomal DNA by nucleases, which can lead to mutations and cellular toxicity. Several groups are working to address this issue.
- As with other site-directed recombination techniques, the introduction of ZF proteins requires *Agrobacterium* and biolistic transformation methods for delivery.
- Plants possess zinc fingers but it is not known if they contain ZFNs. However, if present, these might be interesting sources of intellectual property.

Site-specific gene targeting methods are versatile with a range of applications but still fall short of the ideal for true gene targeting. However, they are already providing leverage for some of the bigger players to develop new products with greater precision.

Gene targeting – *in situ* modification

In situ modification

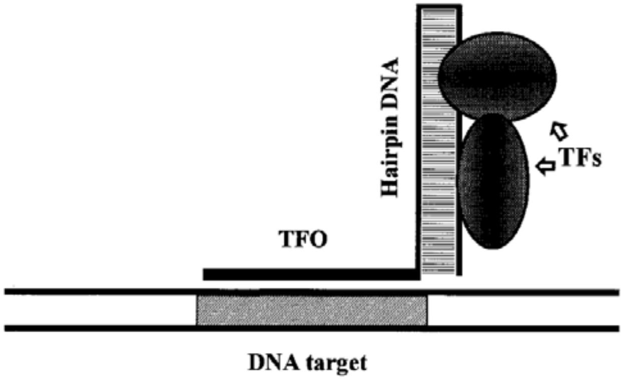
- The idea behind *in situ* modification is that genes are modified where they occur naturally – in the genome of a plant.
- No new transgenes or marker genes are required but modifications are limited to the gene pool of the plant and only a single gene can be targeted at a time. Methods employed include:

Triple-helix-forming oligonucleotides

The use of triple-helix-forming oligonucleotides (TFOs) was developed first in mammalian cells to switch off genes. Chemicals linked to the TFO bring about the exchange or loss of individual genes.

Bi-functional oligonucleotides

These oligonucleotides contain both a triple-helix-forming section and a sequence with the desired modification (see diagram). The triple-helix-forming section 'anchors' the oligo- to the double helix and when the DNA double helix is opened, the modified section can bind to one of the two strands, thereby triggering the desired modification, e.g. during repair processes.



Gene targeting – addressing safety

Much of the public distaste and regulatory distrust surrounding the first-generation GM products was centred on the bacterial origins of both transgene and selection marker. There are several viable solutions.

Eliminate the of selection markers

- As described earlier, site-direct recombination systems can be used to excise the selection marker from a transgenic plant.
- Another technique is co-transformation. Here the transgene and selection marker are delivered on separate T-DNAs either on the same or different plasmids in *Agrobacterium*. As the two transgenes are not physically linked they can be segregated later during breeding.
- Multi-auto-transformation (MAT) vectors can remove the marker genes and increase the regeneration frequency of transgenic crops without antibiotic selection. The system uses agrobacterial oncogenes (in conjunction with site-specific recombination system) to control the endogenous levels of plant hormones and plant growth regulators, to differentiate transgenic cells, and to select marker-free transgenic plants.

Gene targeting – addressing safety (2)

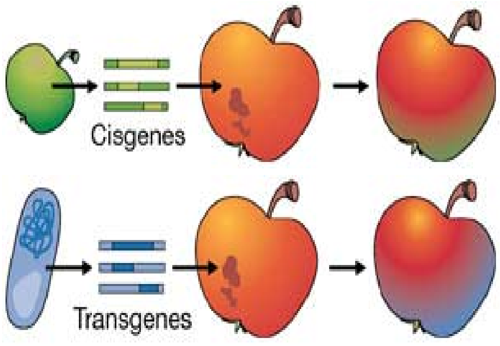
Eliminating transgenes

- A promising strategy for eliminating bacterial transgenes is to use plant-derived genes from sexually compatible species instead. These are often called **cisgenes**.

- Cisgenics offers a means of using the natural genetic variation core to classical plant breeding but with the speed and accuracy of genetic engineering. Novel traits can be introduced without the so-called linkage drag, whereby non-desirable traits were transferred along with the desired trait during traditional cross-breeding.

- Researchers at Simplot (Idaho, US) have developed methods of transformation that use plant-derived vectors and genes to replace the agrobacterial elements essential for gene transfer into plants.

- Whether the ‘all-plant transgenic’ will pacify regulators remains untested. It is not clear that they would view a cisgenic GM plant more leniently than a transgenic.



> The production of a marker-free transgenic plants is a reality today and will possibly become standard practice in the future. Whether cisgenics will supersede transgenics remains to be seen.

Gene stacking

There are various strategies under review for stacking multiple genes in plants. Some involve novel constructs to enable large gene inserts whereas others are novel approaches to overcoming several of the limitations of existing techniques. These include:

1. Gene-stacking systems.
2. Mini-chromosomes – designer chromosomes.
3. Nanoparticles – useful for delivery of both transgene and other bioactives.
4. Plastid transformation – for gene stacking and transgene containment.

Gene stacking – systems

Approaches to gene-stacking using existing transformation methods

Commercially, gene stacking is achieved by crossing a transgenic plant with one trait with a transgenic plant bearing a second trait and selecting plants carrying both. This is then repeated to add further genes. However, as the transgenes are not co-located in the genome there is a high risk of gene segregation, making the process very time consuming. Various other strategies can be used to improve the efficiency of gene stacking including, for example:

- **Co-transformation**: when transgenes are delivered on separate plasmids they integrate at the same chromosomal position in a large percentage of cases, circumventing segregation in later generations.
- **Linked-gene cassettes**: two or more genes, each with its own promoter and terminator, are positioned contiguously (within the same T-DNA of construct if biolistics used) and transfer as a single entity.
- Other systems that enable **co-ordinated expression** of introduced genes include:
  - Chimeric polycistronic constructs that incorporate internal ribosome entry sites (IRESs).
  - Polyproteins with different protein-coding sequences connected in a single open reading frame via short linker sequences that are substrates for a host cell or introduced proteinase.
  - Ubiquitin-based vectors in which genes are linked by ubiquitin moieties. The chimeric protein expressed is then cleaved by endogenous deubiquitinating enzymes.
  - Single transgenes containing fused partial sequences can be used to simultaneously suppress multiple endogenous genes.
  - Cloning systems such as Invitrogen’s GATEWAY technology, which allows for ease of gene cloning for gene stacking using recombination systems.

Gene stacking – systems (2)

Controlling expression

- One of the other challenges in plant genetic engineering is to design a construct that enables the precise control of transgene expression.

- On key determinant is the choice of **promoter**. Most promoters used commercially have been constitutive (e.g. cauliflower mosaic virus [CaMV] 35S promoter) but high levels of transgene expression can compromise plant growth and development. Indeed, in many instances expression is needed only at certain times (e.g. during infection) and certain tissues. Temporal and tissue-specific control is therefore highly desirable in particular inducible expression (e.g. in response to pathogen attack).

- Advances in promoter technology have lagged behind those of gene discovery and there is a dearth of suitable available promoter candidates. Systems biology and bioinformatics could help in the identification and/or design of synthetic promoters capable of providing such versatility.

- Other means of optimising transgene expression lie in the transgene construct itself. For example, it was found that the inclusion of introns within the transgene can greatly enhance expression – so-called **intron-mediated enhancement of gene expression (IME)**. Various other regulatory sequences can be applied to manipulate expression of the transgene.

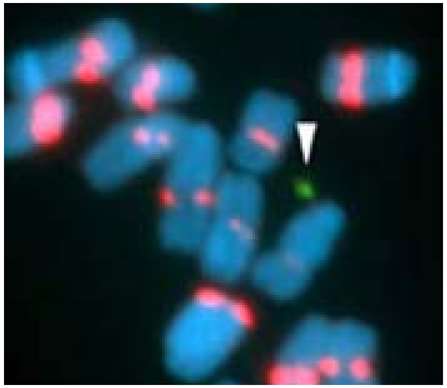
Gene stacking – mini-chromosomes

Plant artificial chromosomes

- In theory, a mini-chromosome could be engineered to carry several genes suited not just for crop improvement but also metabolic engineering and molecular pharming.

- Mini-chromosomes could overcome the transgene position being physically independent of the native genome. They could also address the existing size restriction on transgenes transferred by other means. Much of the work to date has been in mammalian systems (e.g. Yeast Artificial Chromosomes) but some advances have been made recently in plants.

- Strategies employed to construct mini-chromosomes in plants include:
  - *In situ*, by paring down an existing chromosome using a telomere-chromosomal truncation system, that effectively chews down the chromosome arms leaving the centromere region. This has been reasonably successful to date.
  - *De novo* construction of artificial mini-chromosomes, using known chromosomal elements. This has had some success but is still in its infancy. An *in vitro* assembled autonomous mini-chromosome vector has been demonstrated in maize (*PLoS Genetics* (2007) 3: e179).



Mini-chromosome highlighted by arrow. Credit: Chlorogen Inc.

Mini-chromosomes go commercial

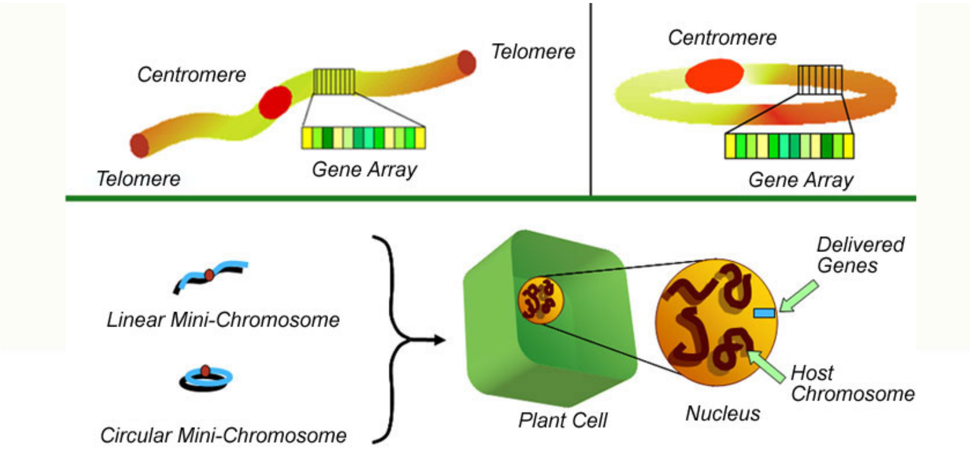
- A pioneer of mini-chromosome technology is Dr Daphne Preuss at the University of Chicago, who founded Chromatin, Inc. (Chicago, US) in 2000.

- Chromatin's proprietary technology includes an understanding of the centromeres that provide stability for the chromosomes and ensure that the genes are inherited in subsequent generations.

- Chromatin claims that its technology could accelerate the speed-to-market for single gene modifications by 2–3 years and offer a greater time saving for multiple gene transformations.

- The company has raised more than $12 million in a series A and B round and $3 million in grants. In 2007, Chromatin entered into a research and commercial license agreements with both Syngenta and Monsanto.

Chromatin, Inc.



Gene stacking – mini-chromosomes (2)

Expectations are high for this new technique but there is still much work to be done and limitations addressed:

- The introduction of artificial mini-chromosomes still requires *Agrobacterium* or biolistic transformation. Mini-chromosomes can be very large (>1 megabase pairs) – a challenge for *Agrobacterium* delivery and with a high risk of transgene instability.
- Removing large sections of genetic material from native chromosomes could lead to instability or the loss of essential genes key in plant growth and development. Equally, the addition of large sections of new genetic material could interfere with native gene expression.

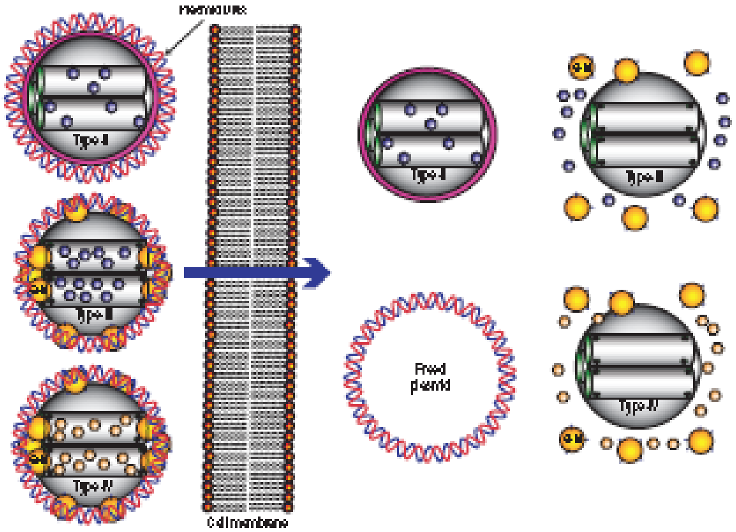
Mini-chromosomes are an engaging concept and could solve the gene-stacking challenge, but the jury is still out on their viability.

Gene stacking – nanoparticles

- Nanoparticles have been used to deliver DNA and drugs into animal cells. Recently, mesoporous silica nanoparticles (MSNs) have been effective in plant protoplasts.
- The honeycomb-like MSNs can encapsulate chemicals in their pores, and their surface can be coated with DNA molecules (see diagram). The most distinct advantage of this nanoparticle system over the current plant transformation methods is its ability to deliver more than one biological species to cells.
- The chemicals incorporated are encapsulated with covalently bound caps and so can be released, on demand, using specific triggers such as chemicals or environmental changes.

Limitations

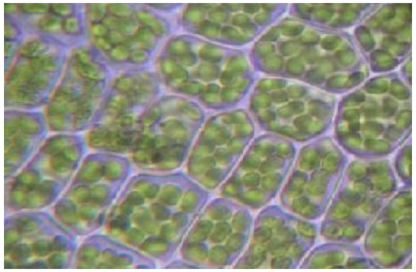
In principle, nanoparticles could address the problem for stacked genes, site-specific recombination and more. However, delivery is again the key obstacle, as particles cannot cross the plant cell wall. New techniques/systems will need to be developed.



(Torney et al., *Nature* 2007)

Gene stacking – plastid transformation

- All approved commercial transgenic crops are nuclear transformants but there is increasing interest in targeting genes to plastids, most commonly chloroplasts. Plastids have several characteristics that make them highly attractive targets for transformation:
  - Plastid genomes are of prokaryotic (e.g. bacterial) origin and so can express several genes from a single operon (i.e. from a single unit), which is desirable in gene stacking where coordinated expression is needed.
  - Epigenetic interference (due to lack of promoter silencing) is reduced in plastids leading to reproducible and stable transgene expression.
  - A single cell in higher plants can contain thousands of copies of a single plastid gene, which means that transgenesis can produce high protein expression – up to 45% of total soluble protein – which is of value for many applications (e.g. protein production, metabolite concentration, etc.).
  - Plastids are inherited maternally and thus transgenes are less likely to be disseminated through cross-pollination. Containment is particularly important if transplastomics are to be used for non-food bioactive production (e.g. pharmaceuticals or industrial enzymes).



Chloroplasts in plant cells

Chlorogen's business in plastids

- Chlorogen (St Louis, MO, US) specialises in the production of pharmaceutical proteins within the chloroplasts of tobacco plants.
- Chloroplasts are the organelles that carry out photosynthesis. Each plant cell may carry around 100 chloroplasts and each of these can hyperexpress genes (up to 100 copies) during plant leaf development.
- Chlorogen's intellectual property resides in a genetic regulatory signal that controls chloroplast gene expression in seeds only.
- Chlorogen's technology is less well developed than many of its competitors but it captured the interest of Dow Agrosciences for application in plant cell culture and animal healthcare. It has raised ~$12 million to date (Burrill & Co is an investor).
- First product is a member of the TGF-beta superfamily for application in gynaecological cancers with possible entry to the clinic during 2009–2010. Chlorogen is also looking at cholera vaccines and insulin-like growth factors for diabetes.
- Processing and extraction remain obstacles and Chlorogen has established an alliance with a local bioprocessing facility.

Chlorogen *The future of protein production*

Gene stacking – plastid transformation (2)

Limitations of plastid transformation

- Plastid transformation requires delivery by biolistics as *Agrobacterium* cannot access the plastid genome.
- The difficulty of engineering the plastid genome lies in the large number of copies of the plastid genome per cell. There can be 10–100 plastids per cell, each plastid containing multiple copies of its genome. The transgene may be incorporated into just a few copies of the plastid genome and it may require many rounds of selection to ‘dilute’ wild-type plastid genome copies.
- Proteins expressed in the plastids cannot always be modified appropriately (e.g. glycosylation) as they lack access to the necessary enzymes and they are not secreted.
- There is a need to find good promoters for use in plasmids to optimise expression.

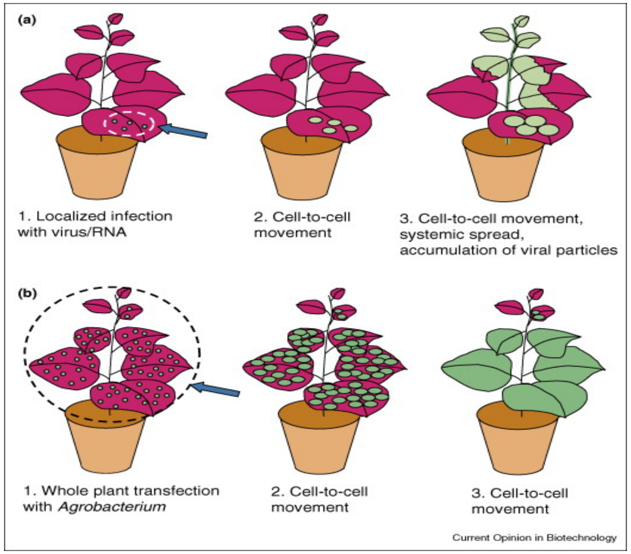
Plastid transformation appears a viable means of stacking multiple genes but the low transformation efficiency could be a barrier for commercial application.

Viral transformation

Viral transformation?

- As infective agents, plant viruses might be thought to be of some use as natural genetic engineers. However, many plant virus genomes are transmitted through RNA not DNA and this is not inserted into the plant's genome – so is of no use for creating nuclear transformants.

- In principle, a virus capable of integrating genetic material into a plant's genome would be a highly attractive transformation tool but there is no means of doing this. Undoubtedly, host specificity, as with *Agrobacterium*, would still be an issue. For now, viral vectors are used for molecular pharming, to trigger rapid and high-level expression of, for example, viral-like particles and antibodies.



Schematic description of infection and spread of replicons based on (a) first-generation and (b) second-generation viral vectors.
(Gleba et al. (2007) *Current Opinion in Biotechnology*)

Technology analysis

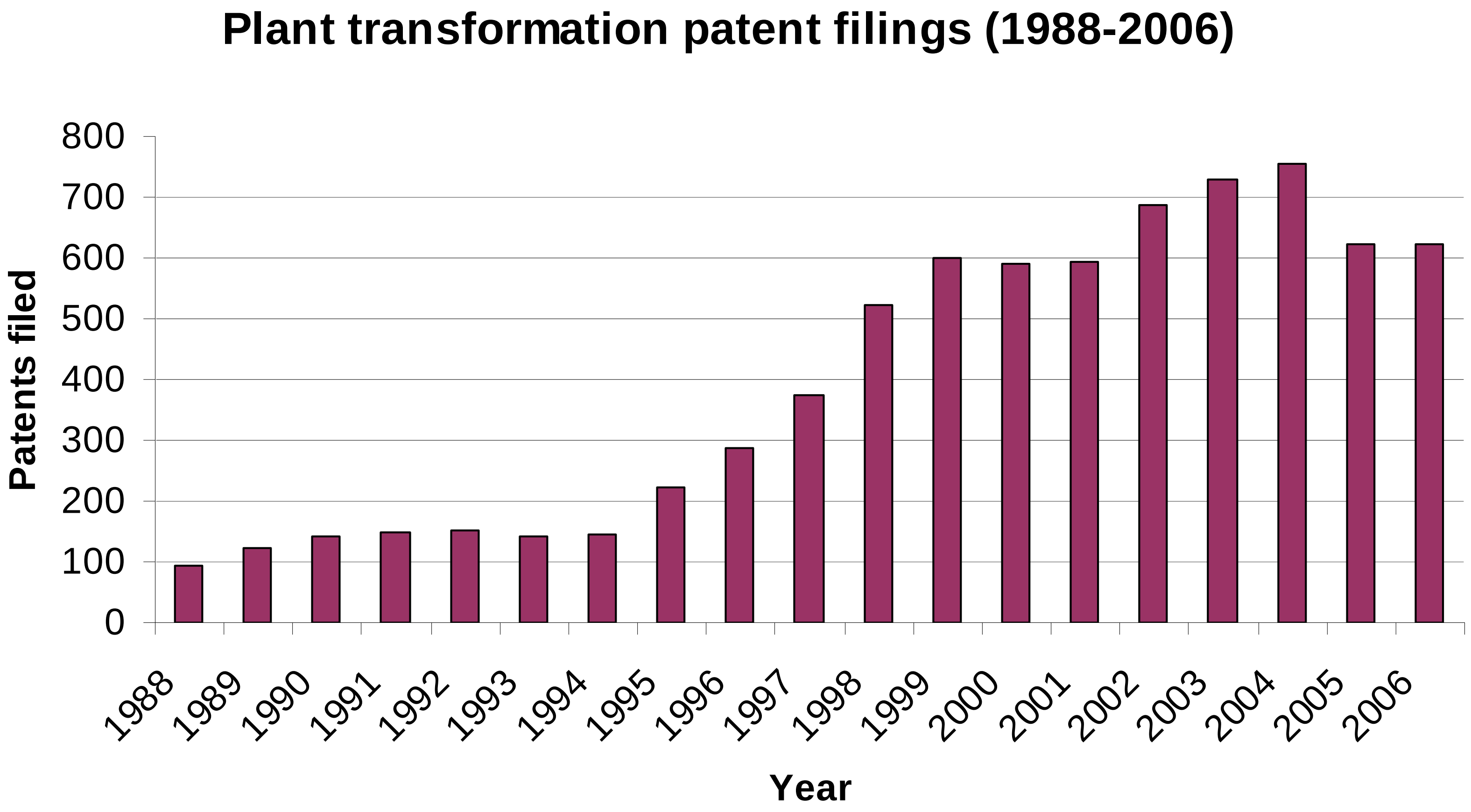
Transformation

Phenotyping

- Background and scope
- Existing technologies – benefits and limitations
- Outstanding needs and solutions
- **IP landscape**
- Unmet need

IP landscape

There has been a significant increase in patent filing around plant transformation over the past 10 years with a peak around 2005 (probably correlating with the rise in interest around the application of siRNA and other gene-silencing approaches).



IP landscape

Patent map of transformation technologies filed from 2003 to 2008. It highlights the advent of zinc finger, recombination techniques and mini-chromosomes.

Keywords within an identified set of patents and patent applications in the field of plant transformation are grouped into topics to produce a ‘map’. Collections of documents that share common elements are geographically close together whereas collections with less similarity are further away. The patent landscape is therefore displayed as a series of technology 'mountain tops' and ‘valleys’, with the higher ‘mountains’ representing the larger patent collections.

Technology analysis

Transformation

Phenotyping

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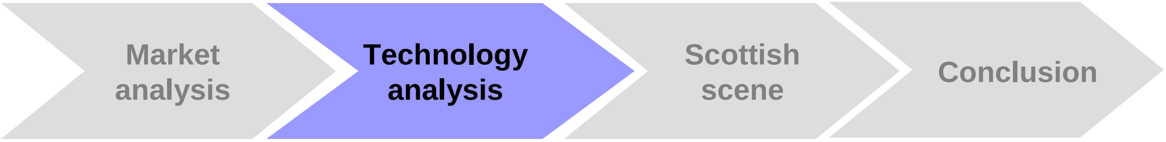
- **Conclusion**

Conclusion

- The transformation of plants requires that the novel genetic material (transgenes or cisgenes) cross the plant cell wall, are integrated into the plant genome and are expressed in a predictable, controllable and inheritable manner.
- Existing transformation methods – using *Agrobacterium* and particle bombardment – work well on many commercial crops. However, despite their popularity, they are unlikely to meet the needs of plant transformation in the 21st century.
- More complex genetic manipulations will need more sophisticated transformation systems to enable:
  - precision gene targeting;
  - controlled expression;
  - gene stacking;
  - marker removal;
  - and – above all – improvements in tissue culture to optimise success rates.
- Techniques such as zinc finger proteins and site-specific recombination go some way towards achieving site specificity but, as yet, true gene targeting is not possible.
- The introduction of multiple genes (gene stacking) could be achieved through novel constructs or through the newly emerging field of mini-chromosomes. Targeting genes to plastids might enhance the expression of stacked transgenes and ensure their containment.
- Improvements in delivery methods would appear fundamental to getting larger DNA constructs across the tough plant cell wall. Nanotechnology might provide solutions.
- Finally, a clearer understanding of the physiological basis of the recalcitrance of many commercial crop genotypes to transformation might suggest ways to overcome this problem.

Contents



Market analysis

Technology analysis

Scottish scene

Conclusion

- Scope
- The Market
- The Industry
- Conclusions

- Transformation
- **Phenotyping**

- Agriculture
- Research
- Crop science

- Conclusion
- ITI Opportunity

Technology analysis

Transformation

Phenotyping

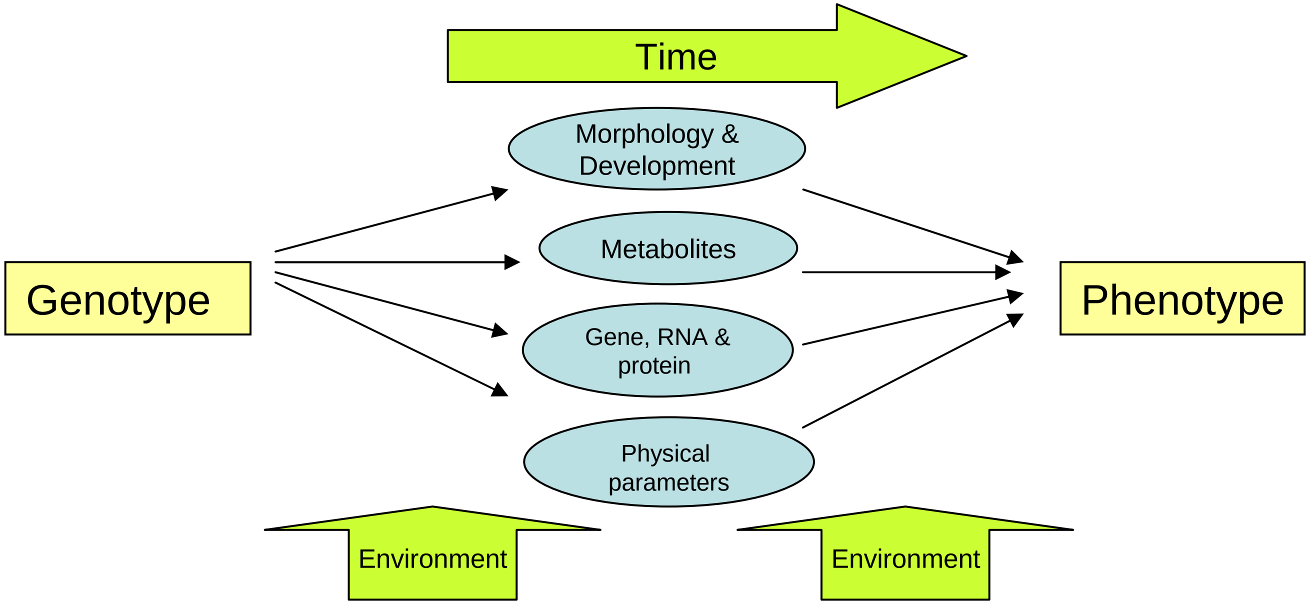
- **Background**
- Current approaches
- Emerging techniques
- Conclusion

From genotype to phenotype

- Making the link between genotype and phenotype (so-called phenomics) has become the bottleneck in the process of identifying commercially useful traits and new plant varieties, regardless of whether these are created though traditional breeding or genetic modification.

- Measuring the impact of changes in genetic makeup requires the measurement of a complex array of parameters – anatomical, physiological, biochemical – over time and in response to biotic and abiotic stresses.

- The **grand challenge** in new trait and plant discovery **is to generate a precise picture of a plant's phenotype:**
  - under controlled and field conditions;
  - throughout the plant's lifecycle;
  - in a high-throughput manner.



Precision phenotyping needs

- Various key parameters need to be measured to provide a comprehensive assessment of the phenotype of a plant. These include:
    - Visual measures (leaf and root size and shape, plant height, development of seeds, stems and tubers, etc.).
    - Physical measures (hardness, etc.).
    - Metabolite complement (metabolomics) and also mineral analysis (ionomics).
    - Photosynthetic and respiratory activity.
    - Response to disease and infection.
- **The ideal precision phenotyping system/s would therefore be able to:**
    - ✓ **Non-invasively image the intact plant *in situ*, both in a greenhouse and in the field.**
    - ✓ **Measure a variety of parameters in parallel and reproducible manner.**
    - ✓ **Process large numbers of plants quickly.**
    - ✓ **Be cheap, fast and – ideally – portable.**
- Most crucial will be the need to collect, store and analyse these large and complex datasets efficiently, and to make a meaningful interpretation of the results using bioinformatics, systems biology and modelling strategies.

Industrial solutions

- The seed and trait industry is highly competitive and therefore the speed of delivery of new varieties becomes a critical factor in business success. Gene sequencing and identification is no longer a technical or financial obstacle and so companies are looking for improvements in phenotyping as a differentiator.

- Precision phenotyping is increasingly important to industry:
    - In March 2008, DuPont opened a new facility embedding its FAST (Functional Analysis Systems for Traits) for corn, which enables it to speed selection and growing of new maize varieties. The system relies on an automated digital analysis system that continuously monitors plant growth to better spot desirable plants.
    - BASF’s acquisition of Crop Design, and – arguably – Monsanto’s subsequent partnership with BASF, is testament to the perceived value of the TraitMill platform.
    - All the larger agbiotech companies now employ some form of automated plant screening process and many are becoming interested in a systems biology approach (e.g. Monsanto, Syngenta).

The automated FAST system in action. Credit: DuPont

Crop Design’s high-throughput phenotyping platform TraitMill. Credit: Crop Design

Relevant initiatives – Europe

- In June 2007, the European Commission published its Strategic Research Agenda for Plant Technology (2007–2012). This recognised the need for more comprehensive genome sequences for European crops, along with precision phenotyping platforms. The authors specified that such a platform would have to make use of non-destructive advanced imaging and analysis systems, e.g. fluorescence measurements of photosynthesis, hyperspectral imaging, nuclear magnetic resonance (NMR) or X-ray imaging of root architecture, or functional tomography like positron emission tomography (PET). They emphasised that this will require **major investments and expertise support.**

- The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben, Germany) has recently invested in a phenotyping system for barley and maize.

European Technology
Platform

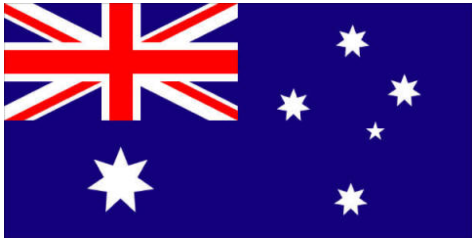
Plants for
the Future

Strategic Research
Agenda 2025
Summary

Some of this work will be funded through EC FP7 programmes under the Food, Agriculture and Fisheries and Biotechnology theme, which has an overall budget of €1.9 billion.

Relevant initiatives – Australia

- Australia is already feeling the potential damage of climate change on its Aus $27 billion agricultural export sector. In June 2008, work started on the Aus$40 million Australian Plant Phenomics Facility, a national resource to study how the genetic makeup of a plant affects its appearance, function and performance.
- The facility comprises the ‘Plant Accelerator’, based at the University of Adelaide, which will use robotic techniques to make 3-D images of up to 160,000 plants every year. The High-Resolution Plant Phenomics Centre in Canberra will further adapt and apply next-generation research tools to probe plant function.
- The technologies used for screening will include various imaging approaches, including morphological growth and colour analysis, leaf gas-exchange studies, chlorophyll fluorescence and hyperspectral reflectance.



Technology analysis

Transformation

Phenotyping

- Background
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- Conclusion

Measuring multiple parameters

- A wide variety of quite different parameters needs to be measured in parallel and over time for effective precision phenotyping.
- The challenges vary with the type of plant of interest. Many existing systems were developed with *Arabidopsis* in mind, a plant with a relatively simple architecture, and are not readily transferred to commercial crops such as wheat and barley. Potatoes pose an even greater challenge, as they are a bushy, spreading crop, the commercially interesting region of which grows below ground.
- No single system is able to measure all parameters but several could be used to measuring several useful indicators in parallel. Some of these are explored in the following slides.

Techniques employed to measure various phenotyping parameters

Morphology	Composition of matter	Photosynthetic and respiratory activity	RNA profiling, gene expression, proteomics
Digital imaging (*in situ*) Optical projection tomography (*ex vivo*) X-ray and other imaging techniques	High performance liquid chromatography (HPLC) and other standard techniques (in the lab.) Near-infrared spectroscopy (in the field)	Thermal and chlorophyll fluorescence	Standard techniques Optical projection tomography (*ex vivo*) – 3-D gene expression

Visual parameters – digital imaging

Digital imaging

- Digital imaging of plant architecture during growth and development is the most commonly used measure for a wide variety of morphological parameters, including root and leaf morphology, leaf colour and root architecture; it can also be used to look for disease.

- The advantage of digital imaging is that it can be automated and can look for changes over the entire lifecycle of a plant. However, the images are captured at a single time point only, which does not reflect the dynamic changes of the expression of the trait in question.

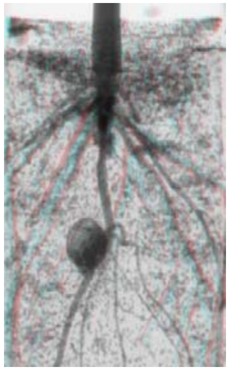
- Examples of automated platforms include Lemnatec's Scanalyzer (right). Crop Design has been the most successful high-throughput phenotyping company to date and its proprietary platform TraitMill is based on automated, high-throughout digital imaging. Digital imaging as the basis of most commercial phenotyping platforms available.

Lemnatec's automated image analysis system

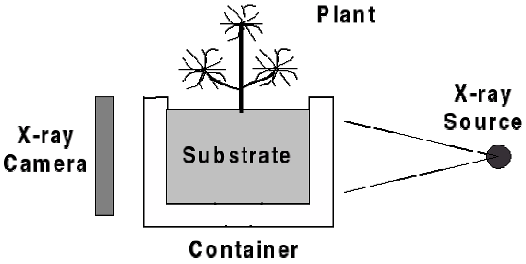
Imaging root systems

Imaging the root

- Plant root systems are complex 3-D structures and are not readily visualised using conventional systems such as digital imaging. Yet the health and growth of the root system is vital for key agronomic properties such as water uptake, nitrogen use efficiency and the prevention of soil erosion.
- Existing techniques tend to provide 2-D images of the root architecture of plants grown hydroponically between glass plates – not properly replicating the structural support provided by soil or, indeed, the important microbial ecology of the root system.
- Several imaging modalities have been tested, including X-ray computed tomography, NMR and MRI. However, these are very costly techniques and not readily applied in the field.
- For example, the Phenotype Screening Corporation (Seymour, Tennessee) uses X-rays to monitor roots growing in solid substrate but the resulting images are in 2-D.
- A remaining challenge here is the software analysis of the highly complex image generated.



Phenotype Screening
CORPORATION
enabling discovery



Both Container and Substrate Are Virtually Transparent To X-rays.

Phenotype Screening Corp. has an X-ray system for measuring root growth

Visualising in 3-D

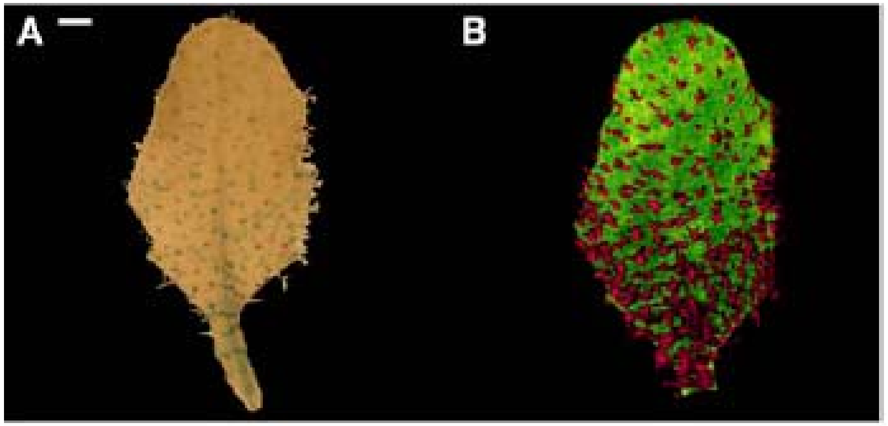
Optical projection tomography

- Optical projection tomography (OPT) is a method for visualising plant morphology and gene expression in 3-D by projecting light rays through a specimen and analysing the rays transmitted.

- The advantage of OPT is that it is cheaper than comparable alternatives (e.g. the scanner is around a quarter of the cost of a confocal microscope) and can work with established stains and dyes using appropriate fluorescence light sources.

- OPT provides a means of looking at morphology in large tissue masses (seeds, leaves, meristems), although not at a subcellular level. It does not work on living tissue, although there has been some success applying it within the living roots of *Arabidopsis*.

- OPT technology was developed in Edinburgh by the MRC Human Genetics Unit, which has patents filed on the device. There are no commercial devices as yet.



Visualising gene expression in 3-D using OPT. The red areas show leaf hairs expressing a label indicating a specific promoter activity.

Source: *The Plant Cell* (2006) 18: 2145

Real-time phenotyping

Controlled environment systems

- To evaluate the impact of environmental factors on phenotype, it is necessary to be able to keep such parameters relatively stable during sequential measurements, using a climate-controlled environment.
- One such platform is the Phenopsis automaton – a digital imaging Y prototype robot built by Optimalog (France). It can weigh and irrigate ~ 500 *Arabidopsis* plants in a computer-controlled environment.
- The Phenopsis platform was used in the GABI-GENOPLANTE (EU Framework 7) project to analyse leaf growth, transpiration and water-use efficiency in a collection of 24 accessions subjected to various water-deficit treatments.
- The platform enabled the identification of an accession with drought resistance and revealed compensation mechanisms in leaf growth.
- Various other related platforms (e.g. Phenodyn) look at slightly different variables but work largely on the same principles. There are few commercial versions of these platforms suitable for crop research.

Granier C et al. *New Phytologist* (2006) 169 (3): 623–635

http://www.mri.cns.fr

Imaging in the field

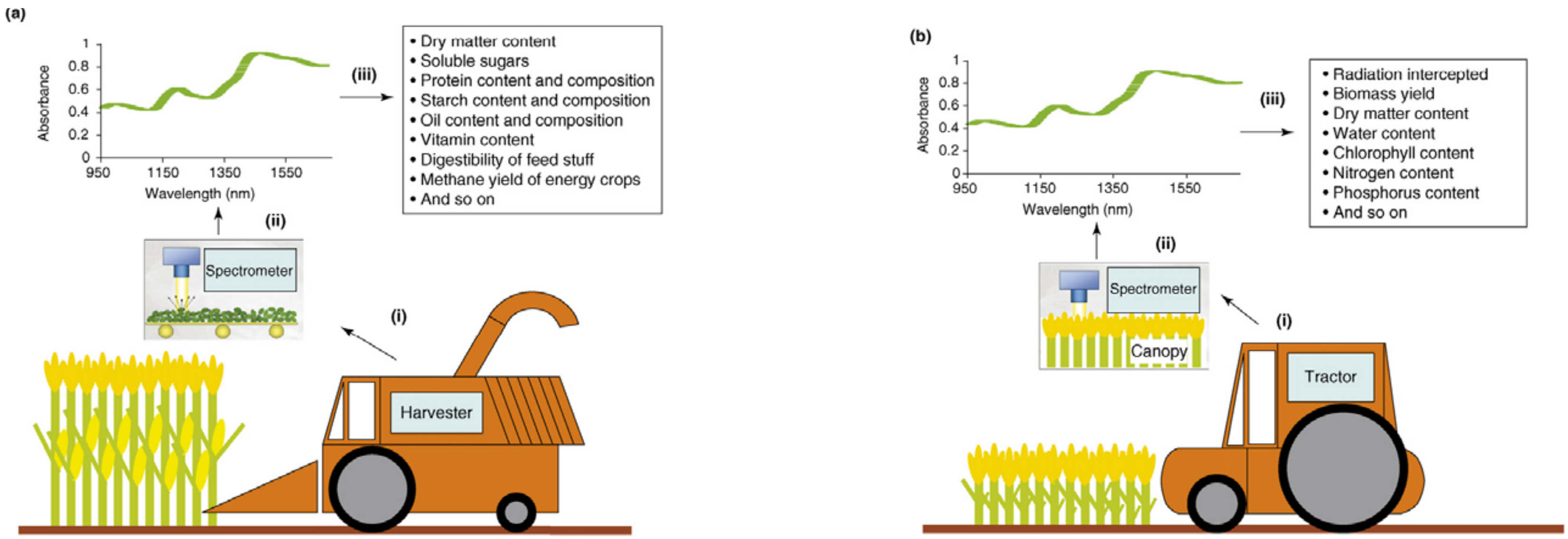
Composition analysis

- The near-infrared (NIR) spectrum sits at the top end of the light spectrum (780–2526 nm) and the absorbance at each wavelength (by various functional groups such as –CH, – NH, – OH) can provide a measure the biochemical status of compounds such as water, carbohydrates and proteins.
- **Near-infrared spectroscopy**, using a mounted spectrometer on farming equipment, can be used to measure a variety of parameters, over large areas, that would otherwise be labour-intensive using conventional sampling (see next slide). The crop can be analysed *in situ* or during harvesting.
- The alternative is to measure **near-infrared spectral reflectance** of ambient light from the plant canopy. Sensors mounted on tractors can pick up the reflected light and provide a measure of physical and chemical characteristics such as plant architecture, water status and nitrogen concentration.
- Bench-top NIR analysers are available, e.g. Perten's DA 7200 NIR analyser (below), used for very fast (< 10 s) automated analysis of grain composition.

Limitations

NIR analysers take a static measure at a specific point in time. Reflectance methods are superior on broad- rather than narrow-leafed plants. Moreover, they are highly dependent on correct calibration of the systems; improved models are needed.

Imaging in the field (2)



- Dry matter content
- Soluble sugars
- Protein content and composition
- Starch content and composition
- Oil content and composition
- Vitamin content
- Digestibility of feed stuff
- Methane yield of energy crops
- And so on

- Radiation intercepted
- Biomass yield
- Dry matter content
- Water content
- Chlorophyll content
- Nitrogen content
- Phosphorus content
- And so on

Near-infrared spectroscopy can be used in two formats. If mounted within agricultural harvesters it can be used to irradiate and analyse harvested plant material (left). Phenotypic values are then predicted by using calibration models that relate spectral and phenotypic information.

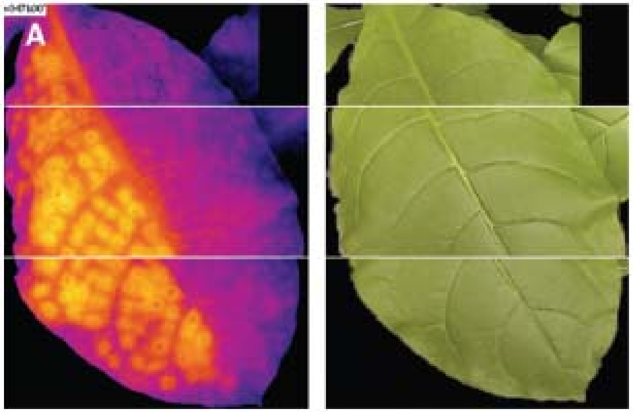
The alternative is to measure the spectral reflectance of ambient light from the intact plant canopy using a tractor equipped with sensors (right).

Source: *Trends in Plant Science* (2007) 12 (10)

Natural outputs

Thermal imaging and chlorophyll fluorescence

- Thermal and chlorophyll fluorescence imaging are useful tools in the study of leaf transpiration and photosynthetic performance, respectively.
- Thermal imaging, or thermography, measures leaf surface temperature, which is a proxy for transpiration and stomatal conductance. No illumination or radiation is necessary.
- Chlorophyll is a natural, light-absorbing dye and its fluorescence (at 690 and 740 nm) provides an indicator of photosynthetic efficiency. It has been used with success to predict yield before crop maturation. The value of this technique is that it can be done continuously and needs only illumination.
- When combined, thermal and chlorophyll fluorescence imaging can produce a ‘signature’ indicative of various abiotic and biotic stressors. This can be useful, e.g. for screening for drought-tolerant varieties.
- Other imaging techniques include UV-induced blue–green fluorescence of innately fluorescing compounds that accumulate under stress.



Thermal imaging shows hot spots correlating with leaf infection. *J Exp Botany* (2007) 58: 773

Data handling and processing

- One general problem in dealing with volumetric data obtained from phenotyping experiments is the need to analyse large and complex datasets that might have been derived using different techniques. Software tools need to be user friendly, but this can make them less flexible as a result.
- Another problem is the lack of standardisation in the parameters to be measured, which makes cross-comparisons with different studies difficult. However, various initiatives to derive some common ontology for plant phenotyping are underway (see below).
- More sophisticated measurement tools and analysis programmes need to be developed so that a systems approach to crop phenomics can be fully realised.

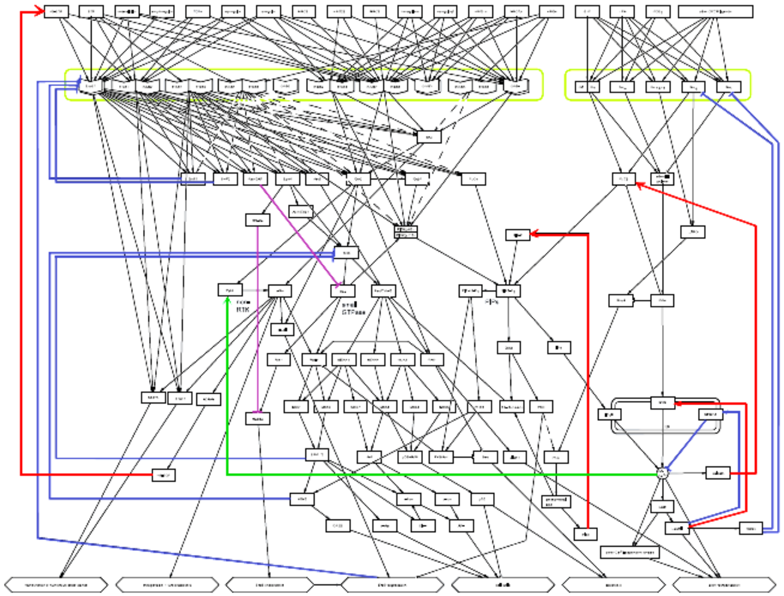
Data curation

The North Carolina Biosciences Organization (NCBiO) has designed a curation tool to annotate phenotypes using an EQ model (entities represented by gene ontology or plant ontology terms combined with qualities such as absent, abnormal, decreased length, etc. represented by the PATO ontology).

The role of systems biology

- Precision phenotyping is important for speeding new crop development but the data gained through experimentation can also be used, via **systems biology** approaches, to generate a predictive model of the complex and dynamic interactions between genes, proteins, metabolites and regulatory elements, and other biological components. This requires a multidisciplinary approach involving plant biologists, bioinformaticians, mathematicians and engineers, and is the remit of the emerging discipline of systems biology.

- Ultimately, a systems approach would allow the generation of a computer model – or at its most sophisticated an ‘*in silico* plant’ – which could be used to predict the genetic changes needed to generate new plant phenotypes. Such as model would have a dramatic effect by eliminating the need to screen thousands of plants to identify the desired phenotype.



Complex interrelated networks can be used to model the living plant.

The role of systems biology (2)

- The Centre for Systems Biology in Edinburgh (CSBE) has several programmes already focused on plant biology, including one investigating the networks involved in circadian rhythms and another examining the effect of temperature on biological signalling (ROBusT, see below).
- However, the challenge will be in translating what is known in model species into the field environment, and this is where interlinking model plant research and applied crop science will prove powerful.

ROBusT systems approach to climate change

Dr Karen Halliday of Edinburgh University's Institute of Molecular Plant Sciences is leading a £5 million BBSRC-funded project to develop a model of how temperature change can influence plant growth and development using systems biology. The project is in collaboration with researchers from the universities of Liverpool, Warwick and York; the Scientific Advisory Board includes some leading agbiotech players.

Plants become intimately adapted to their local environment and so plant growth, and therefore harvest yields, are highly sensitive to ambient temperature during the growing season. A better understanding of the factors that moderate this interaction could be used by breeders to develop crop varieties better suited to withstand future climate changes.

csbe
centre for systems biology at edinburgh

Technology analysis

Transformation

Phenotyping

- Background
- Current approaches
- Emerging techniques
- **Conclusion**

Conclusion

- Current developments in precision phenotyping rely on the automation of existing imaging and analysis techniques.

- However, almost all platforms fail to address the need for monitoring more dynamic changes in a plant’s phenotype in response to environmental influences. Many are also poorly suited to studies of commercial.

- There remains a critical need for capacity building for high-throughout precision phenotyping in the UK – we are now lagging behind our competitors.

- Facilities to provide high-throughput and controlled-environment studies are needed but equally important is the ability to measure phenotype ‘in the field’, for which there are few commercial solutions. A viable next step would be to create automated hybrid devices able to measure several parameters over time.

- Another bottleneck is the need for systems to store and interpret data from a wide variety of platforms.

- Systems biology may offer another approach to addressing the precision phenotyping challenge by making better predictions of the impact of genes on phenotype.

Contents

Market analysis

- Scope
- The Market
- The Industry
- Conclusions

Technology analysis

- Transformation
- Phenotyping

Scottish scene

- **Agriculture**
- **Research**
- **Crop science**

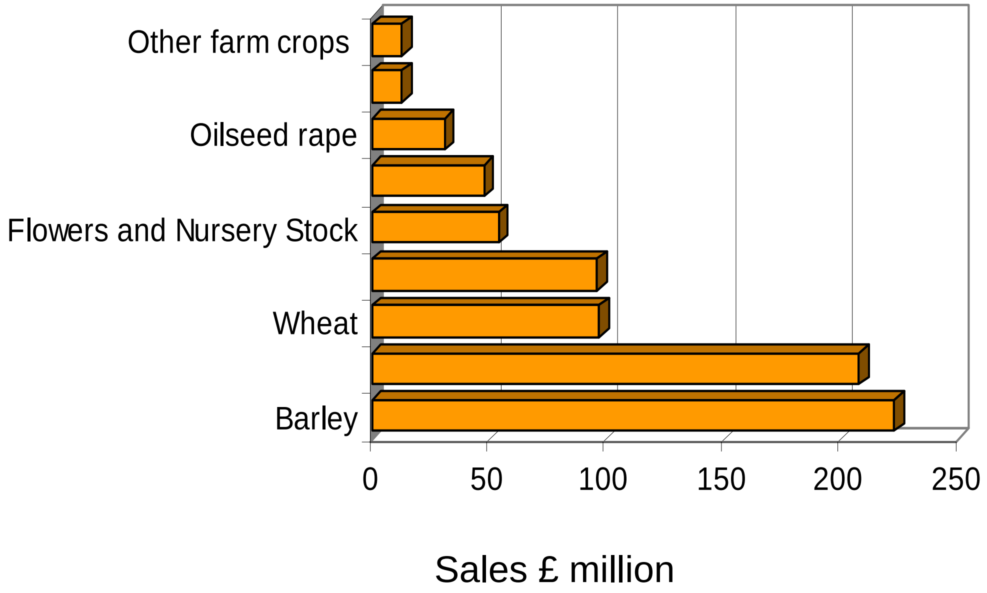
Conclusion

- Conclusion
- ITI Opportunity

Scottish agriculture

- According to the Scottish government, agriculture accounts for 5 per cent of the rural workforce and contributes 1.3 per cent Gross Value Added to the Scottish economy.
- During 2007, Scotland's agricultural output was £2.1 billion, around 13% of the total UK output. However, this is a broad definition and includes livestock and dairy products.
- The main crops grown in Scotland are wheat, barley, potatoes and oilseed rape; soft fruits and vegetables make an important contribution. Their total market value in 2007 was around £780 million.

Market value of crops (2007)



Source: Scottish Agriculture

Scottish agriculture - berries

- Soft fruit production in Scotland is a niche but high-value market.

- The market for Scottish raspberries is worth around £52 million annually, for fresh blackcurrants around £8 million and up to £200 million for blackcurrant processing crop. UK sales of vitamin-rich ‘superfoods’, like strawberries, raspberries, blueberries and blackcurrants, totalled £204 million during 2006, up more than 25% over previous years.

- Scottish-bred varieties have high global impact:
  - Scottish blackcurrants account for more than 50% of the total global crop.
  - The Glen Lyon raspberry, developed at the SCRI, also makes up to half the raspberry crops grown in some southern European countries (e.g. Spain) where the climate is well suited for this variety.

Blackcurrants (left) and the Glen Lyon raspberry (right).

Scottish agriculture – barley

- Barley is another major Scottish crop and is used for cattle feed and for the production of whisky (and other fermented alcohols).

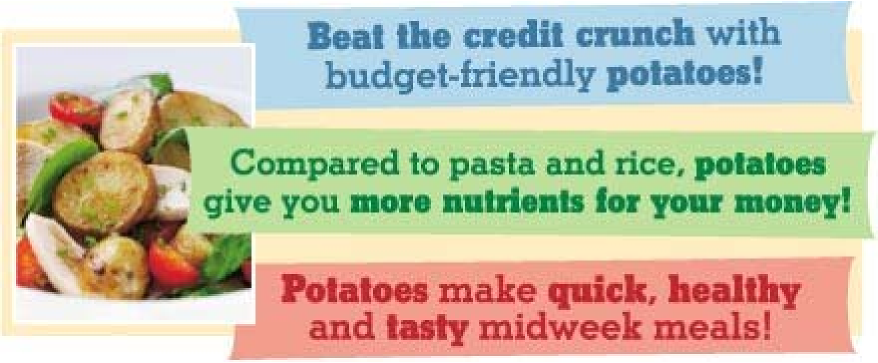
- Almost 90% of Scotch whisky is now exported, with a market value of over $5 billion. The home market for whisky has largely stagnated.

- Although Scottish barely is not exclusively used in Scotch production (distillers buy malted barley in from suppliers who source the barley independently) much is local and this provides a large market opportunity. For example, a report by DTZ Pieda Consulting for the Scotch Whisky Association estimated that whisky production generated around £90 million in purchases from cereal suppliers in 2000.

- Scotland’s barley is worth around £223 million. (Source: Scottish Agriculture)

Scottish agriculture - potato

- The Food Agency Organisation nominated 2008 the ‘Year of the Potato’ to highlight the often unrecognised value of this humble crop.
- World potato production has changed radically during the past decade. Until the early 1990s, Europe and North America and Russia were among the largest potato producers (and consumers) but now China and India are taking the lead. Developing countries now grow more than half of the world’s potato crop, although yields per hectare are still below Western yields.
- Potatoes are around the fourth largest crop globally, with production of around 320 million tonnes and worth around £1.5 billion.
- Scotland’s contribution to the global potato crop is worth around £200 million. More importantly, Scotland has built a reputation for high-quality seed potatoes, largely because of its tight phytosanitation regulations, providing more than 90% of the seed exports outside of the EU and around seeds for around 75% of the UK’s potato production.



Beat the credit crunch with budget-friendly **potatoes!**

Compared to pasta and rice, **potatoes** give you **more nutrients for your money!**

Potatoes make **quick, healthy** and **tasty** midweek meals!

The UK’s Potato Council promoting potato solutions to the credit crunch

Scottish research

- Plant sciences and biotechnology is a **particular area of strength in Scotland**, with research teams at universities in Glasgow, Edinburgh, Dundee and Abertay, Strathclyde and Aberdeen. Areas of expertise exist in:
  - basic plant biology – control of development, metabolism, photosynthesis, lipid biology, etc.
  - interaction of plants with pathogens
  - plant ecology
  - microbiology
  - applied crop technology
  - forestry and phytoremediation
- The Scottish plant science community has in recent years worked hard to provide a strong cohesive and supportive network. Funding was recently sought to create a ‘Plant Pool’ for Scotland, for which a communal plant transformation resource would be created at SCRI.
- Scottish plant scientists are well networked and profitably use their contacts oversees to help carry out field work not possible in the UK.
- Although there is not a critical mass of agbiotech companies in Scotland (indeed, this is a UK-wide issue), there is some expertise within the various markets and this could provide a foundation for growth in the future (see next slide).

PSS Plant Science Scotland

Scottish applied research

- The Scottish Crop Research Institute (SCRI) is one of the three remaining applied crop science centres in the UK (alongside the John Innes Centre and Rothampstead). It undertakes research, largely for the Scottish government, with a focus on the nationally relevant crops: barley, potato, blackcurrant and raspberry. The research spans processes that regulate the growth of plants, their interaction with pathogens and what influences food quality and nutritional value within four key areas:
  - efficient use of resources for sustainable agriculture;
  - new crop breeding, using state of the art breeding and genetics;
  - plant pathogen interactions to provide durable and sustainable host resistance;
  - plant products looking at quality and bioactivity of plant-derived foods and products.
- SCRI has over 170 hectares of land for conducting filed trials, 11,000 m^2 of greenhouses and laboratory space. It receives around £11 million in funding annually and has been highly effective at developing commercially valuable crops such as blackcurrant and raspberry.

Contents

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Technology analysis

- Transformation
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Scottish scene

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Conclusion

- **Conclusion**
- **ITI Opportunity**

Conclusion

- GM seeds are a multi-billion dollar business and the market is projected to grow steadily over the next decade. The range of GM crops will broaden and the complexity of the new traits incorporated increase. In parallel, improvements in efficiency and versatility of the underpinning development platforms must be engineered to ensure such products can come to market in a timely fashion.

- The GM crop seed markets are dominated by a few multinational players, but these companies are open to any innovation that might offer them a commercial advantage is this highly competitive environment.

- Plant transformation for the 21st Century will require developing methods to overcome the recalcitrance of many commercial food crops to genetic modification and to introduce multiple genes in a predictable and controllable fashion.

- Following on from transformation is the need for high-throughout precision phenotyping in both controlled and “real world” scenarios. Whether derived through conventional breeding or genetic modification, thousands of transformed plants must be screen - a costly and time-consuming process. There are few commercial systems that are capable of carrying this out in a high-throughout manner in the field.

- Areas identified within transformation and precision phenotyping that are open to further innovation are highlighted on the following slides.

ITI opportunity – transformation

Existing transformation methods are widely used and largely effective but remain time consuming and costly. Moreover, they do not work robustly on all genotypes of all species, many of which are of commercial interest. As GM applications spread beyond the four main commodity crops there is scope to develop new methods that can work on a more diverse range of food and feed crops. Areas that would welcome further innovation include:

- Further refinements in tissue culture protocol to increase transformation efficiency especially in recalcitrant genotypes. This may be guided by new insights into the plant processes that impede transformation.
- True gene targeting is not yet possible although several methods are moving towards better control over the site of insertion. Further advances are needed.
- Suites of promoters suited for regulating novel genes of interest are still needed along with more reliable means of designing these.
- Whatever form of gene stacking methodology proves most successful, the delivery of large DNA fragments remains a key obstacle. Methods to breach the tough barrier of the cell wall would a valuable aid in transformation. Nanotechnology may provide solutions.

ITI opportunity - phenotyping

- Facilities to provide high-throughput and controlled-environment studies are needed but equally important is the ability to measure phenotype ‘in the field’, for which there are few commercial solutions. Despite the challenges, several areas are open to innovation including:
    - The development of automated hybrid devices able to measure several parameters of phenotype over time.
    - Non-invasive imaging of root systems *in situ*, along with applicable software analysis tools.
    - Advances in data handling, archiving, storage and analysis, with improved interfaces between what can be quite different types of datasets. Existing systems do not handle this adequately.
    - Dynamic models of plant phenotypes using a systems approach that might be used to more efficiently predict the genetic basis of commercially important traits. This may be addressed in the future using systems biology to create the *in silico* or virtual plant.

Please talk to us!

We would welcome further dialogue with our Members on any issues or ideas stimulated by this Foresighting. If you would like to discuss the report findings and associated opportunities with us further, please contact ITI Life Sciences at:

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