GM Science Update

A report to the
Council for Science and Technology

March, 2014

Professor Sir David Baulcombe, University of Cambridge
Professor Jim Dunwell, University of Reading
Professor Jonathan Jones, Sainsbury Laboratory
Professor John Pickett, Rothamsted Research
Professor Pere Puigdomenech, University of Cambridge/ Barcelona

GM Science Update

Executive summary

Background

Crop varieties have long been subject to steady improvement by selection of better-performing variants. The rate of improvement increased when Mendel's principles of genetics were applied to plant breeding early last century. These innovations underpinned increases in yields worldwide, and particularly contributed to the "green revolution" which prevented starvation in Asia and Central/South America, through the introduction of improved husbandry and high yielding varieties of wheat, rice and maize.

Continued innovation in crop breeding is required for food security in the face of a growing population, climate change and the need to minimise the environmental impact of agriculture. There are several innovative technologies to support crop breeding in meeting this challenge, but one of them, involving the use of GM crops, is controversial. Plant breeding depends on the capacity to select new useful variation. GM methods enable plant breeders to exploit additional variation that could not have been introduced by sexual hybridisation of two parental plants. GM crops were developed thirty years ago, and first grown commercially in the USA in 1994 (FlavrSavr tomato), and in Europe in 1998 (Bt Maize in Spain). They are now being grown on an increasing scale by farmers in both developed and developing countries. Nevertheless, there is still opposition to cultivation of GM crops in Europe and elsewhere.

At the request of the Council of Science and Technology, this paper considers the recent developments in the science of GM crops since the Royal Society published its report 'Reaping the Benefits – Sustainable Intensification of Global Agriculture' in 2009, which concluded GM crops (alongside other methods) have an important role to play in sustainable and productive agriculture globally. Since then, other more extensive studies have come to a similar conclusion (Foresight, 2010).

This paper comprises four sections. Section 1 summarises the findings of previous reviews that have assessed the impact, benefits, and trends of the cultivation of first generation GM crops worldwide. Section 2 reviews the potential applications of GM technologies in the research pipeline, and contributions that could be made by GM crops for UK, European and global agriculture if there were a more permissive regulatory and political process in Europe and elsewhere. Section 3 considers safety and risk assessment by reviewing the existing regulatory process in the EU and elsewhere, and exploring the consequences. Section 4 draws together the conclusions from Sections 1 – 3, with recommendations for potential actions that would allow a safe and sustainable agriculture to use GM crop varieties for the benefit of the farmer, the consumer and the environment.

Experience of GM crop cultivation

GM crops were first introduced in the USA in 1994, and are now grown in 28 countries worldwide. The acreage under GM cultivation is doubling every five years and now accounts for some 12% of global arable land. Most of the present GM crop acreage is maize, soybean, cotton and rapeseed (canola), with 81% of the global acreage of both soybean and cotton sown to GM varieties. The last crop to benefit from GM technology has been sugarbeet, with a herbicide resistant variety introduced in the USA in 2012, and now accounting for around 95% of the crop grown. The two principal traits introduced into GM crops are glyphosate herbicide resistance and Bt insect resistance. Other traits include drought tolerance in maize

(recently commercialised in the USA), virus resistance in papaya, flower colour in carnations and roses, and insect resistance in poplar trees.

The existing GM crops make it easier for farmers to control weeds or insects, by reducing not only the amount of chemical pesticides applied, but also the amount of diesel used for tractors to apply the pesticides. This can generate increased income for farmers, for example cumulatively since 1996, GM insect resistant varieties have added an estimated $25.8 billion to the income of global maize farmers, and 11.6% to the global value of the cotton crop. Herbicide tolerant crops allow seeds to be drilled into an unploughed field preserving soil structure and water retention, and preventing soil erosion. Once the seeds germinate, herbicides can be applied allowing the crop to grow without competition from weeds. The reduction in use of chemicals and soil tillage, also contribute to a lower carbon footprint and the reduction in greenhouse gas emissions.

The development of disease and insect resistant GM crops has been particularly successful. The GM virus resistant variety of papaya has allowed papaya to be cultivated again in Hawaii in regions where previously the crop could not be grown because of a virus disease. Protection of crops against the damage caused by viral, fungal and bacterial diseases, offers not only increased yields but also lower costs of crop protection, with environmental benefits through a lower carbon footprint from less chemical application. In addition, there will be less damage to non-target organisms compared with the use of chemicals, because the GM traits are normally specific for the pest or pathogen, whereas crop protection chemicals may affect beneficial organisms as well as the intended target.

It is likely that many more GM crops will be cultivated in the USA and other countries, with more permissive regulatory systems combined with a supportive political system. In the USA, between 500 – 1,000 field trial applications are approved per annum and 96 applications for commercialisation have been approved since 1990. Several North and South America countries have followed the USA. In contrast, in Europe there is only one GM crop approved for commercial cultivation, a Bt-insect-resistant maize. The total area of GM maize grown in the EU in 2012 was 129,000 hectares, of which more than 90% was grown in Spain. There are few commercial releases of GM crops in Africa (principally in South Africa) (IFPRI (International Food Policy Research Institute) 2013), while in Asia, there are several countries, including China, that have adopted GM crops with varying degrees of enthusiasm.

Although less than 0.1% of the global acreage of GM crops is cultivated in the EU, more than 70% of EU animal protein feed requirements are imported as GM crop products.

New scientific developments over the last five years

The potential for new GM crop varieties is likely to increase greatly, as combining genetics with high throughput genome sequencing reveals genes for important traits and mechanisms that could be moved from one plant species to another. The next generation of GM crops is expected to be improved not only by transfer of genetic elements between crop species but also from diverse organisms into crops.

The first generation of GM crops were developed with transgenes for the new trait integrated randomly into the plant genome, so that many breeding lines had to be tested to select those with stable expression of the new trait, from those expressing the trait at low levels or inconsistently. However in recent years, spectacular advances in basic science have led to new methods for transferring genes into defined locations in the recipient crop plant genome, allowing their expression to be more consistent. These methods also allow inactivation of

genes that are deleterious for crops and their products, and for the insertion of multiple genes into a single location.

In the short and medium term, there are many emerging GM traits with the potential for increasing photosynthetic efficiency, nitrogen use efficiency, aluminium tolerance, salinity tolerance and phosphate use efficiency in crop plants. These new GM traits will benefit major European crops including wheat, potato, rapeseed and tomato. They will also have benefits in crops for both farmers and consumers in developing countries. A striking example is Golden Rice, which has been developed over the last decade so that a modest portion of boiled rice contains sufficient β-carotene to provide a high proportion of an individual's daily vitamin A requirement. Introduction of Golden Rice could prevent blindness and health problems for many poor people who depend on rice-based diets.

In the longer term, transfer of genes for symbiotic nitrogen fixation from legumes to other crops, and for more efficient "C4" photosynthesis into "C3" plants such as rice, are likely. Once synthetic biology has been developed further, we expect more complex novel traits in GM crops including the production of novel compounds for biofuels and industrial use. Such GM crops could be key components of an expanding bio-economy.

Safety and risk assessment

Experimentation and commercial release of GM crops in the EU is subject to much more stringent regulation than conventionally bred plants, with a slow and inefficient approval process. As a consequence, multi-national companies (BASF and Monsanto) have withdrawn their research efforts to develop GM crops in Europe, and there has been a significant reduction in experimental field trials in the UK, with only one in 2012, compared with 37 in 1995. As in other countries, in the USA there is a similar stringent regulatory framework, but the approval process is more streamlined and effective. As a consequence of the regulatory process for commercial release, $7M-14M (2007 prices) is added to the cost of developing a new GM crop variety in the U.S.A. – an amount that is prohibitive for small and medium sized enterprises.

The European Academies Science Advisory Council (EASAC) and others have pointed out that there is no rational basis for the current stringent regulatory process. Stringent regulation of the technology would be justified if there were no benefits, if it was associated with inherent risks to the health of humans or animals or the environment, and if the technology was so poorly understood there was a high probability of unforeseen consequences. However, extensive studies over the nineteen years GM crops have been cultivated, have failed to reveal any of these risks from transgenes of any type. Notably, even in the highly litigious USA, there have been no successful lawsuits, no product recalls, no substantiated ill effects, and no other evidence of risk from a GM crop product intended for human consumption since the technology was first deployed commercially in 1994.

Conclusions and recommendations

Globally for crop varieties, resistance to pathogens and pests is of high priority (e.g. wheat take-all, foliar diseases, barley yellow dwarf virus, aphids, nematodes, slugs), along with improvements in genetic yield potential (total biomass production, nutrient use efficiency, and climate proofing traits – drought, heat tolerance) and crop quality (starch/oil/protein quality and functionality). GM has the potential to contribute substantially to advancing plant breeding to deliver these traits in crop varieties, more rapidly and in a more efficient manner; and to introduce many novel innovations that cannot be achieved using conventional breeding. This will not only help achieve sustainable and sufficient global food production in

the face of challenges from a growing population, climate change, and environmental degeneration; but also generate a successful bioeconomy (biofuels, fibre and other materials). Conventional plant breeding alone is not likely to meet this challenge because the cycle time for production of a new crop variety is long (usually a decade) and it is difficult to improve multiple crop varieties in the same way. In addition, conventional plant breeding is restricted to improvements that can be transferred between related species and it does not have the potential to take advantage of innovation in synthetic biology, with assembly of complex biosynthetic pathways, to enhance plant performance. To realize the full potential of GM crops we need to improve the European regulatory and approval process, and to strengthen the R & D pipeline.

Changes to the regulatory and approval process are essential, if GM crops are to be an integral component of the agri-tech approaches used by EU farmers to increase crop production, and contribute to the bio-economy. As there is no evidence for intrinsic environmental or toxicity risks associated with GM crops, it is not appropriate to have a regulatory framework that is based on the premise that GM crops are more hazardous than crop varieties produced by conventional plant breeding. We therefore endorse EASAC's proposal that a future regulatory framework should be product- rather than process- based. However, even if the safety assessment framework were revised, the approval process at the European level for cultivation of GM crops in Europe will remain an impediment. For that reason, to safeguard in part against the losses and damage to European agriculture that follow from the failure to adopt GM crops, we propose that approval for commercial cultivation of new GM crops is made at a national level, as happens at present with pharmaceuticals.

Plant breeding is a research-intensive and expensive. A competitive wheat breeding programme costs £1M to £1.5M p.a., but is only sufficient for a breeder to make incremental advances in crop improvement. Approximately, 30% of the limited breeders' royalty income (less than £20M p.a. for cereals, or £40M p.a. for all broad acre crops in the UK), derived from the sale of certified seed and from the use of farm saved seed is spent on R&D. Given the limited royalty stream, breeders must breed primarily for mainstream markets, and rely on good evidence that laboratory findings will translate into crops in the field. A well-functioning R&D pipeline is essential for translation of genomic research through the pre-breeding stage into the development of crop varieties for the marketplace. For these reasons we propose the establishment of an R & D programme (PubGM) that would allow evaluation of the practical application of academic research findings transferred to crops in the field. This would include capacity for field testing new GM crops either in partnership with companies or so that the public sector could validate traits before engaging in partnerships with the private sector. For crops where there is likely to be little or no market demand but where there are environmental or social benefits (for example, minor crops, energy crops, or break crops), the public sector is likely to need to undertake the fundamental research, and develop it further before it is ready for commercial evaluation.

Part 1: Experience of GM crop cultivation

Summary

- GM crops were first introduced in the USA in 1994 and **are now grown in 28 countries**. The acreage under GM cultivation is doubling every 5 years and now accounts for some 12% of global arable land.

  Most of the present global GM crop acreage is maize, soybean, cotton and rapeseed (canola), with the majority (81%) of both soybean and cotton acreage sown to GM varieties. However, there are many other commercialised products grown on smaller areas. In 2012, biotech crops represented 35% of the global commercial seed market.

- **Most of the commercially grown GM crops have one or both of two traits – glyphosate herbicide resistance and Bt insect resistance.** However, a drought tolerant maize was recently commercialised in the US and other traits include virus resistance in papaya, insect resistance in poplar trees, and flower colour in carnations and roses.

- **The existing GM crops are popular with farmers because they make it easier to control weeds or pests and lead to a reduction in inputs**. This can generate increased farm income. For example, cumulatively since 1996, GM insect resistant varieties have added $25.8 billion to the income of global maize farmers, and 11.6% to the global value of the cotton crop.

- **GM crops have also resulted in environmental benefits,** such as a lower carbon footprint, with reduced greenhouse gas emissions, through reduced use of chemicals (particularly insecticides) and reduced soil tillage. In addition, there will be less damage to non-target organisms compared with the use of chemicals, as GM traits are normally specific for the pest or pathogen, whereas crop protection chemicals may affect beneficial organisms as well as the intended target.

- **It is likely that many more GM crops will be cultivated in the US and other countries with permissive regulatory systems.** In the USA, between 500 and 1000 field trial applications are allowed per annum, and the 96 applications for commercialisation approved since 1990 are likely to proceed to full commercial development.

- **Only one GM crop is approved for commercial cultivation in the EU**: *Bacillus thuringiensis* (Bt)-insect-resistant maize. The total area of GM maize grown in the EU in 2012 was 129,000 hectares; with 90% grown in Spain.

- Whilst less than 0.1% of the global acreage of GM crops is cultivated in Europe, **more than 70% of EU animal protein feed requirements are imported as GM crop products.**

Introduction

In this section, we summarise recent reviews of the status of GM crops in different regions of the world. Our aim is to provide a context for the assessment of the future potential for GM crops as set out in Section 2 and to describe GM crops grown commercially or in trials and close to commercial cultivation.

A. Agriculture

GM crops were first introduced in the USA in 1994 (Flavr Savr tomato). In 2012, the 17th year of widespread commercialization, and the 15th year of consecutive increase in acreage of GM crops grown; a record 170 million hectares were planted in 28 countries. Compared with 1.7 million hectares sown in 1996, this is a 100-fold increase, making GM crops the fastest adopted crop technology in the history of modern agriculture (Figure 1). In 2012, seeds for GM crops represented just over a third of global commercial seed sales (James 2012).
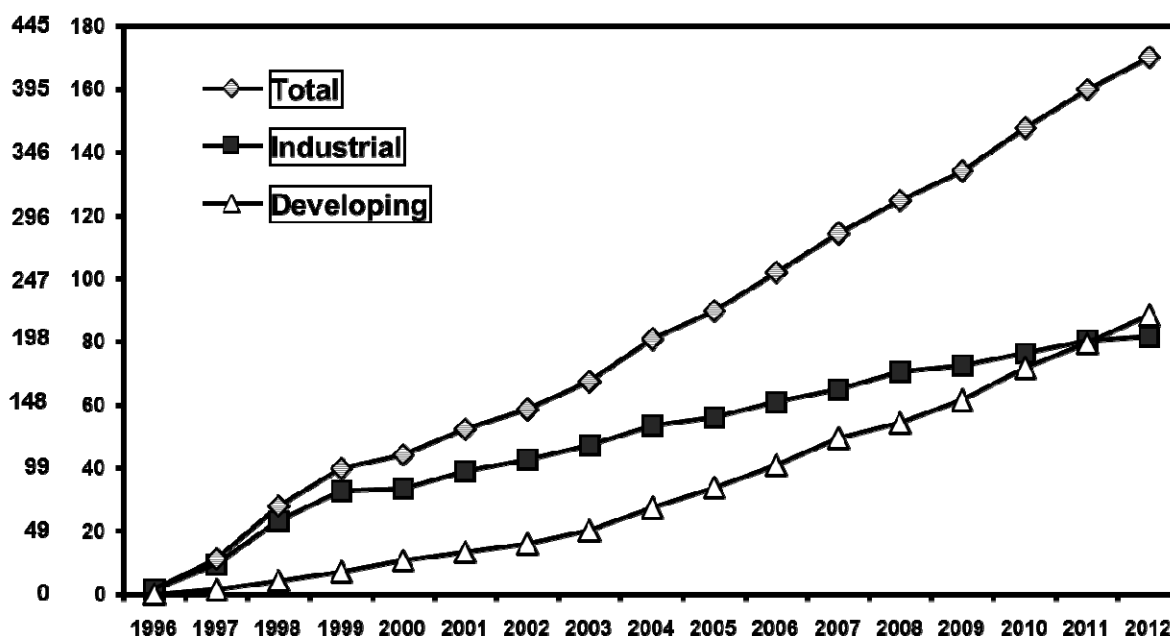
Figure 1.Global area of GM crops, 1996-2012: industrial and developing countries (M Has, M acres). Adapted from: (James 2012).

In 2012, GM crops were grown by a record 17.3 million farmers, of whom over 90%, or over 15 million, were resource-poor farmers in developing countries. In this year, the growth rate for GM crops was higher for developing countries, at 11% or 8.7 million hectares, versus 3% or 1.6 million hectares in industrial countries.

The global area of the four major GM crops, soybean, maize (corn), cotton and canola (oilseed rape), and their relative adoption rates compared to conventional crop varieties, for the period 1996-2011, are shown in Figures 2 and 3 respectively. The adoption rate of GM maize is 90% in USA, 65% in Argentina, and 50% (65%) for summer (winter) maize in Brazil. GM soybean varieties are now planted for 90- 99% of the crop in the USA, Argentina and Brazil; and GM canola now accounts for 98% of the crop in Canada.
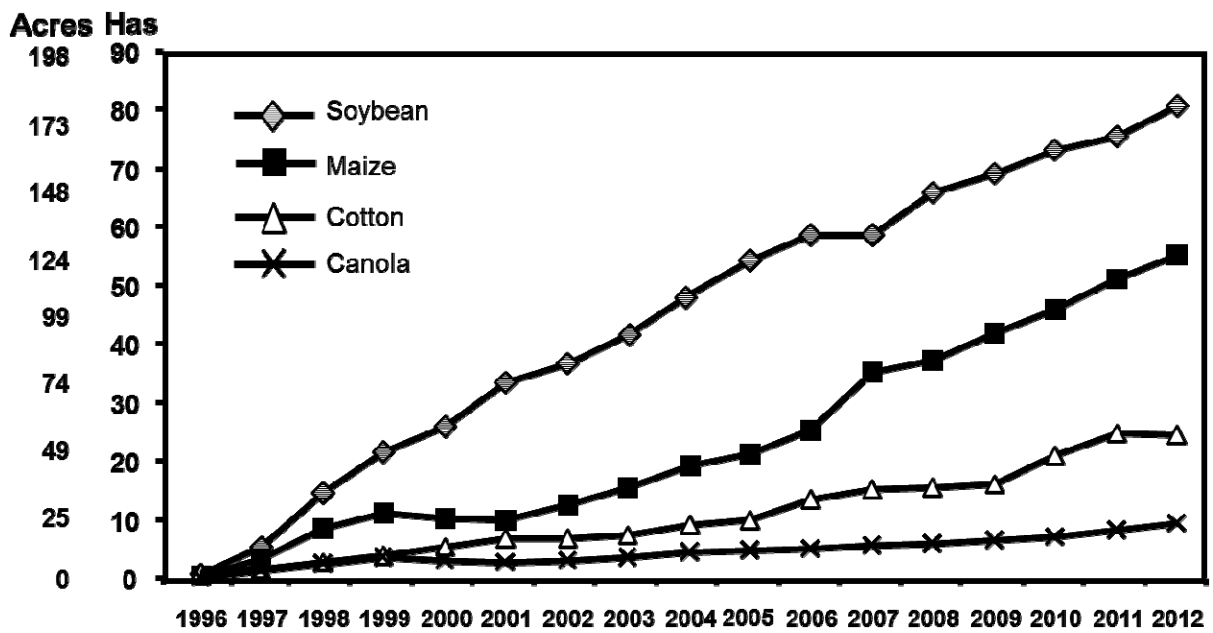
Figure 2. Global area of GM crops, 1996-2011: by crop (M Has, M acres). Adapted from: (James 2012).
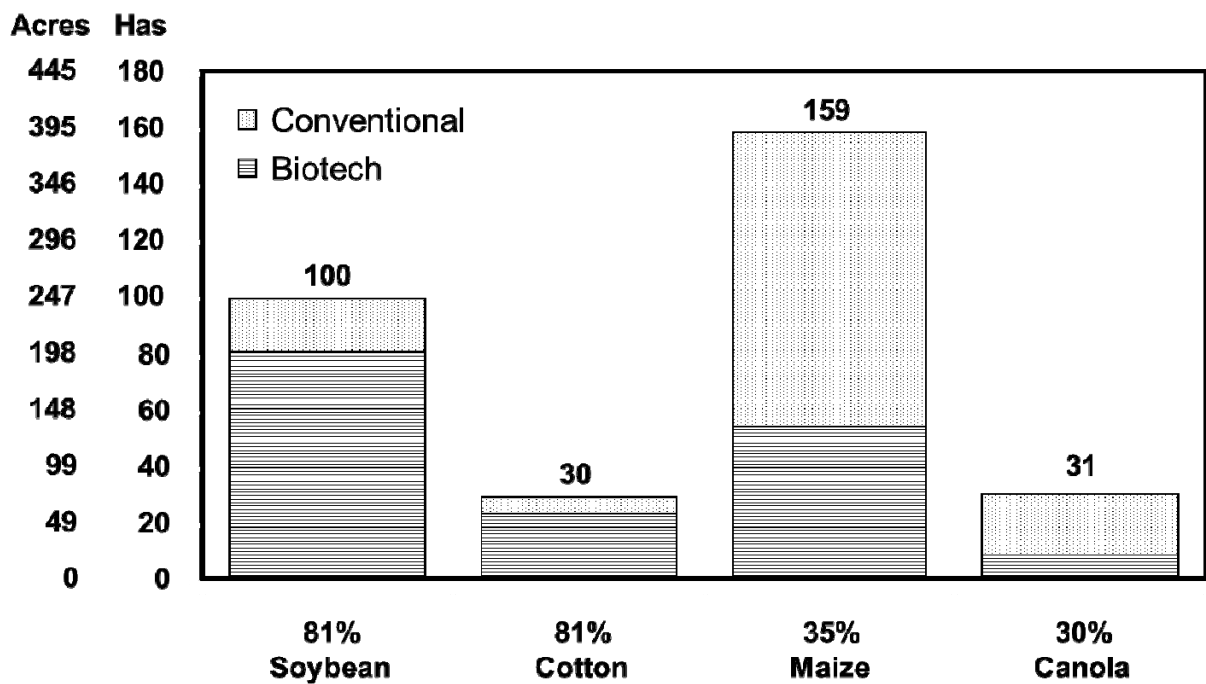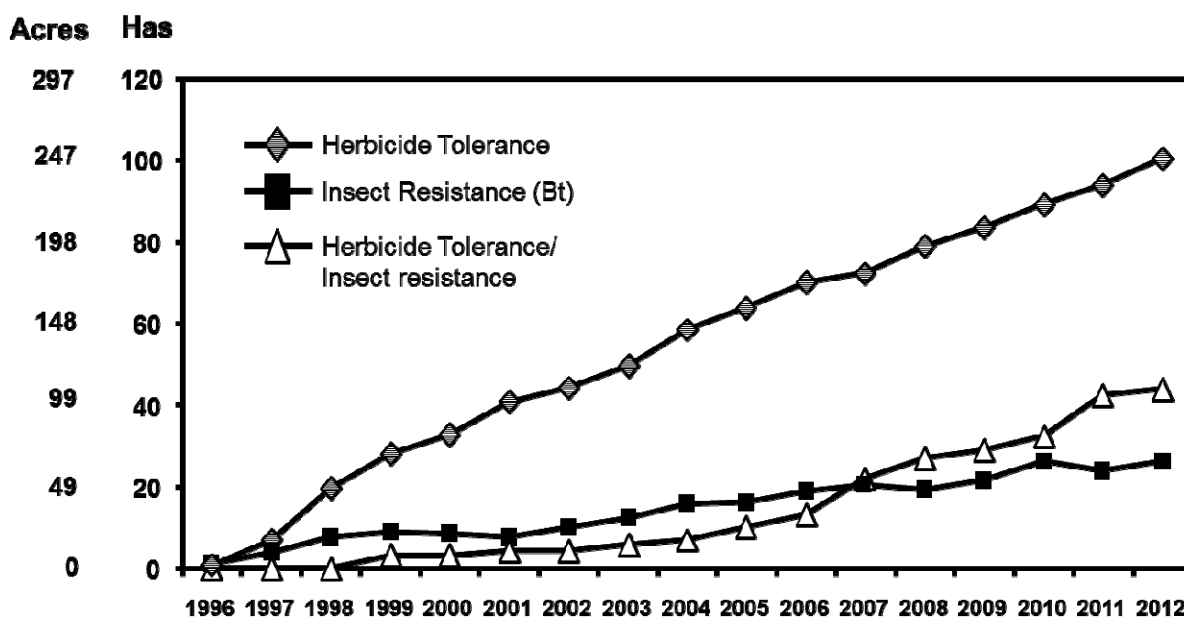

Figure 3. Global adoption rates (%) for principal GM crops (M Acres, M Has). Adapted from: (James 2012).

The two most important GM traits are herbicide tolerance and insect resistance, and these are now increasingly combined in the same product as shown in Figure 4.

Figure 4. Global area of GM crops, 1996-2012: by trait (M acres,M Has). Adapted from: (James 2012).

Of the 28 countries planting GM crops in 2012, 20 were developing and 8 were industrial countries (see Table 2 and Figure 1), with the top five countries (USA, Brazil, Argentina, Canada and India) each growing more than ten million hectares (James 2012).

A1. Socio-economic and environmental impacts

One advantage of the adoption of insect resistant GM crops, has been the decrease in environmental impact associated with insecticide use by 18.1% [as measured by the indicator the Environmental Impact Quotient (EI Q)]. It has also led to a significant reduction in the release of greenhouse gas emissions from the cropping area due to decreased fuel usage. The reduction in 2011 was equivalent to removing 10.22 million cars from the roads (Brookes & Barfoot 2013b).

A2. Farm income effects

During the period 1996-2011, the cumulative benefit of GM crops on farm incomes, derived from a combination of enhanced productivity and efficiency gains, has amounted to $98.2 billion, with US $49.6 in developing countries and US $48.6 billion in developed countries. Significant gains during this period, have been generated by GM insect resistant (GM IR) maize and Bt cotton which have generated an additional US $25.8 billion and US $32.5 billion for global maize and cotton farmers respectively.

In 2011, the benefit from GM crops of US $19.8 billion, is equivalent to adding 6.3% to the value of total global production of soybeans, maize, canola and cotton. The US $7.1 billion additional income generated by GM insect resistant (GM IR) maize in 2011 is the equivalent of an additional 3.3% to the US $214 billion value of the global maize crop.

Bt cotton has significantly increased the income of farmers, through a combination of higher yields and lower costs by halving the number of insecticide sprays. In 2011, gains in farm income from GM cotton were US $6.73 billion, equivalent to adding11.6% to the US $56

billion value of total global cotton production (Brookes & Barfoot 2013a). Data for Burkino Faso are given in Table 1.

Table1. Economic benefit from insect resistant Bt cotton in Burkino Faso. Compiled from (James 2012).

Year	Hectares of cotton planted (total)	Hectares of Bt cotton planted	% adoption of Bt cotton	National benefit from Bt cotton in million US$
2008	475000	8500	2%	no data available
2009	400000	123000	29%	35
2010	400000	260000	65%	80
2011	424810	247000	58%	70

Table 2. Global area of GM crops in 2012: by country (M Has). Source: (James 2012)**.**

Rank	Country	Area (M Has)	Crops
1	USA	69.5	Maize, soybean, cotton, canola, sugarbeet, alfalfa, papaya, squash
2	Brazil	36.6	Soybean, maize, cotton
3	Argentina	23.9	Soybean, maize, cotton
4	Canada	11.6	Canola, maize, soybean, sugarbeet
5	India	10.8	Cotton
6	China	4.0	Cotton, papaya, poplar, tomato, sweet pepper
7	Paraguay	3.4	Soybean. maize, cotton
8	South Africa	2.9	Maize, soybean, cotton
9	Pakistan	2.8	Cotton
10	Uruguay	1.4	Soybean, maize
11	Bolivia	1.0	Soybean
12	Philippines	0.8	Maize
13	Australia	0.7	Cotton, canola
15	Burkina Faso	0.3	Cotton
14	Myanmar	0.3	Cotton
16	Mexico	0.2	Cotton, soybean
17	Spain	0.1	Maize
19	Chile	<0.1	Maize, soybean, canola
18	Colombia	<0.1	Cotton
20	Honduras	<0.1	Maize
21	Sudan	<0.1	Cotton
22	Portugal	<0.1	Maize
23	Czech Rep.	<0.1	Maize
24	Cuba	<0.1	Maize
25	Egypt	<0.1	Maize
26	Costa Rica	<0.1	Cotton, soybean
27	Romania	<0.1	Maize
28	Slovakia	<0.1	Maize
Total		160.0	

A3. Commercial GM cultivation in the EU

In the EU, only two GM crops are approved for commercial cultivation: *Bacillus thuringiensis* (Bt)-insect-resistant maize and a potato variety with modified starch composition for industrial use (now withdrawn). In 2012, the total area of GM maize grown in the EU amounted to 129,000 hectares, with 90% of this total grown in Spain (EASAC 2013) (Figure 5). However, each year the EU imports more than 70% of its animal protein feed requirements for livestock as feed derived from GM crops (mostly soybean). This approximates to 20 million metric tonnes grown on 7 million hectares of agricultural area (EASAC 2013).
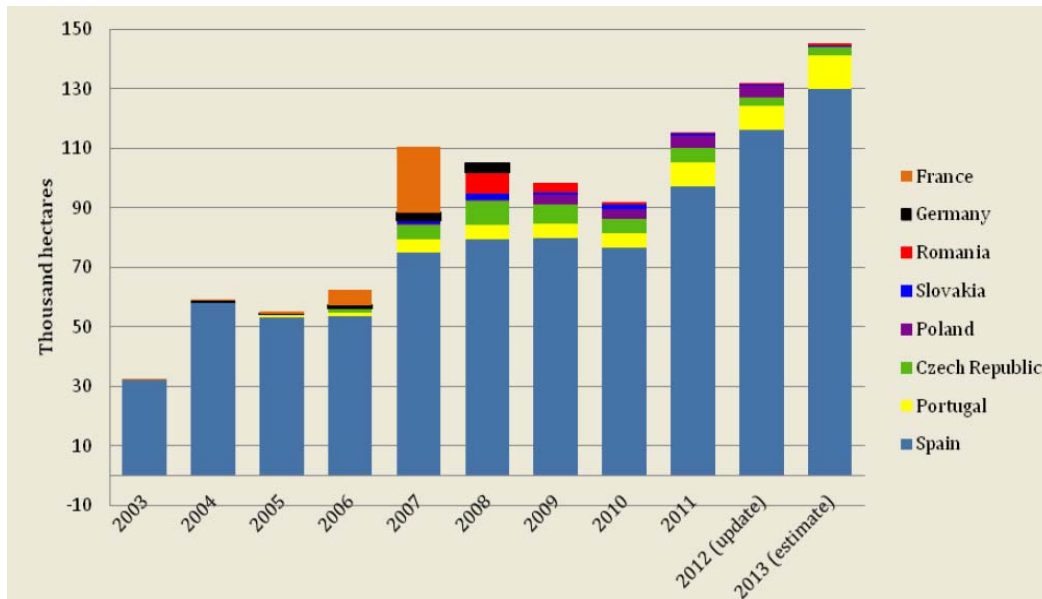

Figure 5. Area of GM maize grown in Europe 2003-2013. (USDA, 2013).

A4. GM crop regulation in the USA

The USA uniquely operates deregulation, allowing applications to request that a specific GM product is equivalent to the non-GM version and therefore should no longer be regulated and therefore not labelled. All GM crops on the USA market currently have achieved a deregulated status. There have been 96 approvals for deregulation since 1990; these comprise examples from alfalfa, canola, corn, cotton, flax, rose, papaya, plum, potato, rice, soybean, squash, sugar beet, tobacco, and tomato. Those not already on the market, are likely to proceed to full commercial development. There are also 13 applications pending decision which represent the next group of GM crop varieties available on the market; these include alfalfa (1), apple (1) (non-browning), corn (1), cotton (1), creeping bentgrass (1), eucalyptus (1) (cold tolerance), potato (1), and soybean (6) (Information Systems for Biotechnology 2013a). The most recently deregulated products are glyphosate tolerant canola (oil seed rape) (Pioneer/Monsanto), glyphosate tolerant corn and a novel F1 hybrid seed production system for corn (Monsanto) (USDA, 2014).

The numbers of US GM field trial applications are shown by year of application, crop and GM trait in Figures 6, 7 and 8 respectively. Each of these applications may cover a range of GM lines of a particular crop grown in one or more locations, and all commercial products will have been tested under this system over a period of several years and at several locations.

In 2013, there were 601 trials, with herbicide tolerant corn representing the largest category (Figures 7 and 8).
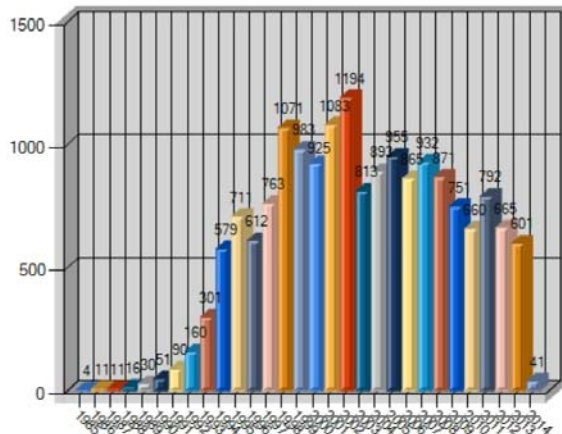
Figure 6. Number of US field trial applications by year 1985-2014. Data for Figures 6-8 from (Information Systems for Biotechnology 2013b)
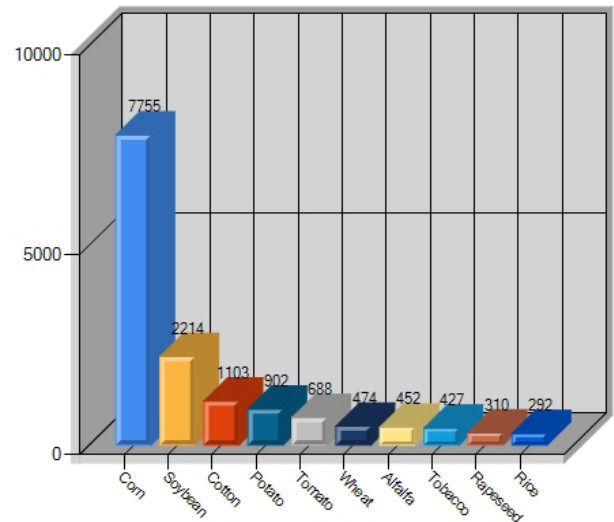
Figure 7. Numbers of field trial permits by crop.
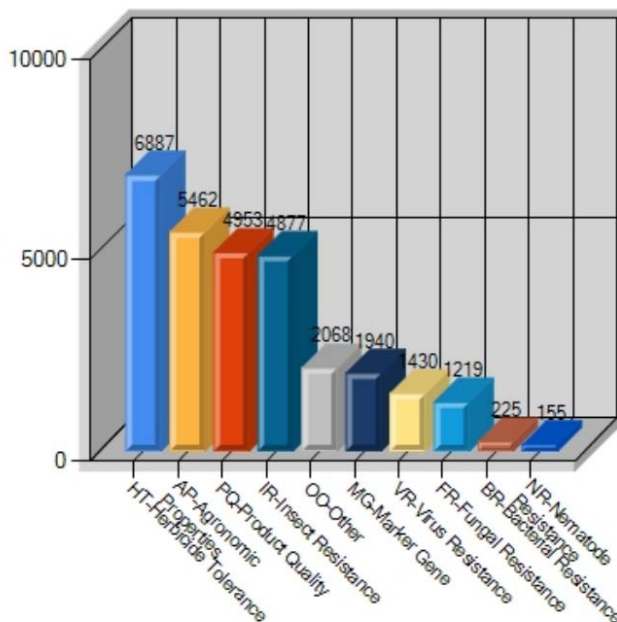
Figure 8. US field trial permits by trait.

A5. GM crop field trials in the EU and elsewhere

Applications for GM field trials in the EU during 2013 (European Comission Joint Research Centre 2013) included those from Spain, Poland, UK, Finland, Belgium, Sweden, Slovakia, Romania, France, and refer to trials of maize, wheat, poplar, sugar beet, cotton, and cucumber. Although commercial GM crops are grown in only four African countries - Burkina Faso, Sudan, South Africa and Egypt (Table 2) several more countries are now conducting field trials or are due to do so (FARA 2013) (Figures 9 and 10). For example, in 2013 Ghana granted permission for trials of GM rice, sweet potato, cotton and cowpea (Quandzie 2013) and Malawi established its first trial of GM cotton (NEPAD 2013).
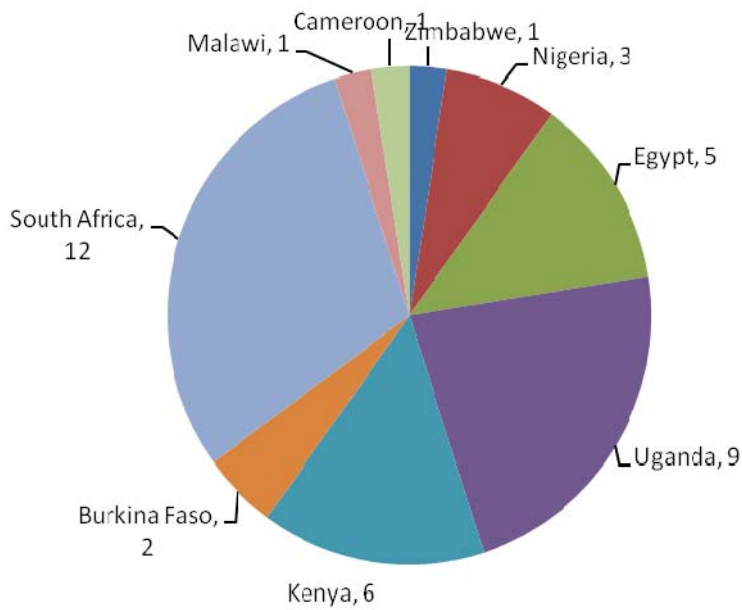
Figure 9. Summary of Confined Field Trials (CFT) of GM crops in Africa. (Data taken from (Savadogo 2010)).
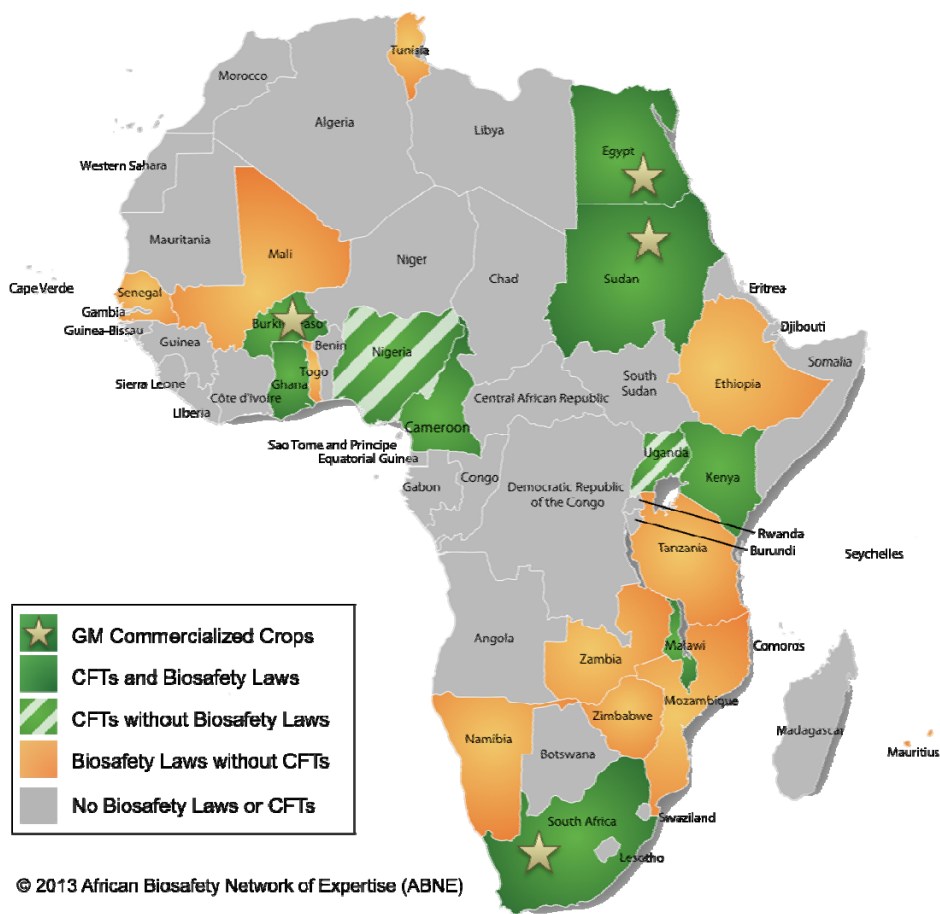
Figure 10. Status of GM Crops in Africa showing Confined Field Trials (CFT) and presence of biosafety laws (ABNE 2013).

B. Horticulture

B1. Papaya

GM papaya with resistance to papaya ringspot virus (PRSV) was not only the first GM tree and fruit crop, but also the first transgenic crop developed by a public institution to be commercialized (1998). It is also the first commercial GM product approved for direct consumption in Japan (Dec 2011) and China (Dangl et al. 2013).

B2. Other commercialized horticultural products

Figure 11. Colour modification in *Dianthus caryophyllus* (carnation). Flowers are shown from a control plant (right) and from a transgenic plant (left) expressing the flavonoid 3′5′-hydroxylase gene from *Viola tricolor* (pansy). (Chandler & Sanchez 2012).

Commercial GM flowers include one rose variety (*R. hybrida*) and eight varieties of carnations (*D. caryophyllus*) developed by Florigene Pty.Ltd. / Suntory Ltd (Florigene Flowers 2013) with modified flower colour generated by manipulation of the anthocyanin biosynthetic pathway. In nature, carnations and roses do not contain delphinidin derived anthocyanins, due to the absence of flavonoid 3'5'-hydroxylase. Introduction of this gene from *Petunia hybrida* (petunia) or *Viola tricolor* (pansy), in conjunction with other modifications to the endogenous anthocyanin biosynthesis pathway (to minimize substrate competition) results in an accumulation of delphinidin-related anthocyanins in flowers, conferring a unique colour (Figure 11). GM carnations were first marketed in Australia in 1997 and are now grown commercially in South America, Australia and Japan. Cut flowers are exported for sale primarily in North America, but also in Europe and Japan (Chandler & Sanchez 2012).

C. Forestry

The only known commercial-scale cultivation of a GM forest tree species is in China where two varieties of insect-resistant poplars have been planted since 2002 and by 2011 occupied a total of 490 ha, with one variety planted at eight sites in seven provinces (Häggman et al. 2013). One variety is *Populus nigra* transformed with the *cry1Ac* gene from *Bacillus thuringiensis* (Bt) and the second variety is a hybrid white poplar which is transformed with a fusion of *cry1Ac* and *API* (a gene coding for a proteinase inhibitor from *Sagittaria sagittifolia*). The transgenic *P. nigra* has also been hybridized with non-transgenic *Populus*

deltoides to generate insect-resistant germplasm for a breeding programme designed to generate new hybrid varieties, that could expand the planting area of Bt poplar (Häggman et al. 2013).

A summary of confined field trial data for GM forest tree species in various countries is given in Table 3. These include tests of a GM Eucalyptus variety with improved cold tolerance (Häggman et al. 2013) and designed for biofuel use (ArborGen 2013). Also of interest is the US trial of GM chestnut expressing an oxalate oxidase gene that provides resistance to chestnut blight (*Cryphonectria parasitica*) (B. Zhang et al. 2013), one of a number of fungal pathogens that represent an increasing threat to UK forestry.

Table 3. Summary of confined field trials approved for genetic engineering forest trees in different countries

Country or Region	Species and no. trials	Source
USA	*Populus* spp. (212), *Pinus* spp. (154) *Eucalyptus* spp. (77), *Liquidambar styraciflua* (37), *Castanea dentata* (15), *Ulmus americana* (5), *Picea glauca* (1)	http://www.aphis.usda.gov/brs/status /BRS_public_data_file.xlsx
China	*Populus* spp. (34), *Robinia pseudoacacia* (25), *Larix* spp. (16), *Paulownia* (3)	M.-Z. Lu, Chinese Academy of Forestry, (pers. comm..)
Brazil	*Eucalyptus* (65)	http://www.ctnbio.gov.br/index.php/ content/view/3509.html
Canada	*Populus* (28), *Picea mariana* (10), *P. glauca* (7)	http://www.inspection.gc.ca/english/ plaveg/bio/confine.shtml#sum
EU	*Populus* spp. (30), *Betula pendula* (6), *Eucalyptus* spp. (4), *Picea abies* (2), *Pinus sylvestris* (2)	http://gmoinfo.jrc.ec.europa.eu /gmp_browse.aspx, http://gmoinfo.jrc.ec.europa.eu/overview/
Japan	*Eucalyptus* (7), *Populus* (2)	http://www.bch.biodic.go.jp/english /e_index.html
New Zealand	*Pinus radiata* (5)	http://www.epa.govt.nz/new-organisms /popular-no-topics/Pages/GM-field-tests-in-NZ.aspx
Australia	N/A	http://www.ogtr.gov.au/internet /ogtr/publishing.nsf/Content/ir-1

Taken from: (Häggman et al. 2013).

Part 2: New scientific developments over the last 5 years

Summary

- **Recent laboratory science has identified genes with effects in experimental situations that could benefit crop plants** including: photosynthetic efficiency, nitrogen use efficiency, aluminium tolerance, salinity tolerance and other abiotic stresses, pest and disease resistance, and phosphate use efficiency. In the longer term, nitrogen fixation in GM crops is likely, and once synthetic biology has been developed, there is the potential for developing more complex novel traits in plants, including the production of novel compounds for biofuels or industrial biotechnology.

- **GM traits developed for specific use in major European crops including wheat, potato, rapeseed and tomato** to benefit crop production or product quality for food or biofuel use are available, if they could be introduced under a permissive regulatory and approval system.

- GM traits specifically for developing countries include nutritional enhancement (golden rice and others), as well as pest and disease resistance for tropical diseases.

- **The potential for more traits in GM crops is likely to increase greatly through the availability of high throughput genome sequencing with access to genes from plant and microbial sources.**

- **New technologies for targeted gene modification will also greatly enhance the potential of GM crops.**

Introduction

In this section we describe GM crops that could be commercialised in the short and medium term. The short-term examples involve either traits that have been validated by testing in model species or crops in the laboratory. The longer-term prospects will follow from current research that is identifying the genes that are responsible for important traits. The potential of GM crops is also influenced by technological advances. Progressive innovation in sequencing technology, for example, means that it is now much easier than ever before to identify genes and sets of genes affecting the traits of crop plants. Other innovations will allow genetic modification of crop plant genomes to be targeted to specific genomic locations. Current technologies, by contrast, are untargeted. The gene targeting or "genome-editing" technologies are important for two reasons. First, they will increase the range of genetic modifications that can be introduced in crop plants. Second, they blur the distinction between GM and non-GM: the end result of a gene targeted modification might be indistinguishable from a non-GM variety created by chemical or radiation mutagenesis.

Section A focuses on traits, whereas Section B focuses on the crops where these traits would be useful for Europe. Section C explores which traits would be useful in crops for developing countries; and Section D considers the recent developments in techniques and methods which will expand the list of available traits to those involving multiple genes.

A. GM traits "in the pipeline" to benefit many crops

Although there are relatively few types of first generation transgenes cultivated in the field (Section 1), there are many examples of traits "in the pipeline" that have been developed in the laboratory and that could be assessed in the field if the regulatory framework were not so restrictive. These second generation GM traits differ from the first generation in that they are more likely to involve genes from plants rather that microbes and they may involve multiple genes to confer a single trait. Scores, if not hundreds, of potentially useful genes have been added to plants using GM methods. For reasons of commercial secrecy, not all are in the public domain. Some are in advanced stages of trials in the private sector, primarily for the US market (CropLife International 2013). A selection of emergent and imminent second generation GM traits are described below.

A1. Enhanced photosynthesis

There is scope for enhancing the efficiency of photosynthesis, the remarkable process by which plants use light energy from the sun to convert carbon dioxide (CO_2) into sugars that can be used for plant growth. A process called photorespiration reduces this efficiency, and delivery of five bacterial genes greatly reduces losses to photorespiration and increases yield (Kebeish et al. 2007). Elevated levels of sedoheptulose bisphosphatase (one of the carbon fixation pathway enzymes), also increases photosynthetic efficiency and thus yields (Rosenthal et al. 2011).

Longer term innovations include exploitation of a carbon-concentrating mechanism that improves the photosynthetic efficiency of certain algae (Meyer et al. 2012). Finally, many laboratories are also seeking to bring the efficient C4 photosynthetic system from plants such as maize, to wheat, rice and other plants that carry a less efficient C3 photosynthetic system (Leegood 2013; Slewinski 2013). This is a more challenging, long-term, high-risk-high-reward goal, being addressed by an international consortium funded by the Gates Foundation (IRRI 2013).

A2. Stress tolerance

Plants are exposed to extreme variation in light intensity, temperature and water availability, and these stresses can impinge on the efficiency of photosynthesis and thus yields. Water stress can result in a state called "photo-oxidative stress", where the stomata close to avoid loss of water, and CO_2 levels in the leaf drop, and although the plants have plenty of energy for photosynthesis this cannot be used to fix CO_2, and causes damage to the plant. Several GM traits appear to increase yields by alleviating this damage. For example, an algal flavodoxin appears to rescue the damage caused to plant ferredoxin during this kind of stress (Zurbriggen et al. 2008).

Monsanto are most advanced with GM approaches to water stress tolerance, using a bacterial RNA chaperone protein (Castiglioni et al. 2008); and have recently commercialised a drought-resistant maize in the US. Drought tolerant transgenes are being made available by Monsanto through a public private partnership to improve maize varieties for African farmers (see Box 1). Several groups are engineering plant regulators called transcription factors to elevate plant stress tolerance, although it is not clear how close these are to commercialization (Nelson et al. 2007). Arcadia have also adopted a drought tolerance technology (pSARK:IPT) that also appears to show good promise (Peleg & Blumwald 2011).

A3. Aluminium tolerance

Acid soils comprise 30% of the Earth's ice-free land. At soil pH values above 5, aluminium exists in the soil in non-toxic forms. However, when soils are acidic, Al^{3+} ions are freed in the soil, and damage the root tips of susceptible plants, inhibiting root growth, and impairing the uptake of water and nutrients. A naturally occurring tolerance mechanism of several species to aluminium toxicity in soils, is the transport of organic anions, by proteins, from inside root cells to the external medium surrounding roots, where they chelate Al^{3+} into a non-toxic form, allowing the roots to grow unimpeded. The wheat TaALMT1 gene facilitates malate efflux from roots to elevate Al^{3+} tolerance, and can be used to genetically modify susceptible species for improved Al^{3+} tolerance. When expressed in barley, one of the most Al^{3+} sensitive cereal crops, TaALMT1 confers substantially improved grain yields in acid soil.

Engineering plants to overexpress citrate also elevates Al^{3+} tolerance(de la Fuente et al. 1997). In sorghum, barley, and maize, plant multidrug and toxic compound extrusion (MATE) transporters located at the root tip confer Al^{3+} activated citrate efflux and represent the primary Al^{3+} tolerance proteins. These proteins are also promising for GM approaches to elevating Al^{3+} tolerance (Schroeder et al. 2013).

A4. Salinity

Production in over 30% of irrigated crops and 7% of dryland agriculture worldwide is limited by salinity stress. Crop irrigation is increasing soil salinity, owing to trace amounts of salt in irrigation waters. Research in the reference plant Arabidopsis and rice has shown that the 'class 1' HKT plant plasma membrane transporters are sodium ($Na^{+)}$ selective and protect plant leaves from salinity stress by prohibiting toxic over-accumulation of Na^+ in the photosynthetic leaf tissue, through removal of excess Na^+ in the xylem vessels that carry nutrients and water to the leaves. Analogous mechanisms have been demonstrated in wheat for the HKT1;4 and HKT1;5 genes. The recent introgression of an ancestral form of the HKT1;5 gene from the more Na^+-tolerant wheat relative *Triticum monococcum* into a commercial durum wheat species which is susceptible to salinity, (*Triticum turgidum ssp durum*) has increased grain yields on saline soil by 25% (Schroeder et al. 2013).

Class 2 HKT transporters which mediate cation influx into the roots, together with transporters that sequester sodium and potassium in the vacuole (class 1 Na/H antiporters),

could improve the production of cereals such as barley which copes with high Na^+ loads in leaves by compartmentation in the vacuole. Combining HKT transporter traits with vacuolar Na^+ sequestration mechanisms provides a potentially powerful approach to improve the salinity tolerance of crops (Schroeder et al. 2013). In the short and medium term, as genes are identified that confer these traits, their introduction by GM methods, alone or in combination, should elevate salinity tolerance in other crop species.

A5. Pest and disease resistance

Infectious disease is one of the biggest threats to crop production in all environments (Oerke, 2005). Of the available control methods, genetically resistant crop varieties are the most preferable, as they do not damage non-target organisms, reduce exposure of farm workers to chemicals, and do not incur CO_2 emissions from applications of chemicals by tractors.

The available strategies for GM disease resistance can be considered as either artificial or based on natural mechanisms.

Artificial approaches include:

1) Bt traits (referred to in Section 1) in which a bacterial gene conferring resistance to some insect pests is transferred into the crop variety.

2) Interfering RNA (RNAi) technology, which exploits an RNA silencing mechanism with small RNA (sRNA) molecules blocking the activity of RNA molecules that are complementary to the sRNA. The first examples of RNA silencing mediated resistance have been the design of transgenes to target a viral gene that is essential for the viral life cycle, enabling plants to be highly resistant to the virus disease. A recent example of such a trait provides resistance in Pinto beans to bean golden mosaic virus (Bonfim et al. 2007) with the potential to increase production by 10-20%, and was approved in 2011 by the Brazilian National Technical Commission on Biosafety (CTNBio) (Tollefson, J., 2011). The silencing RNAs are incredibly mobile, moving not only between cells of the plant but also from a plant to an invertebrate pest or into a fungal pathogen. Transgenic plants producing sRNA targeted to an essential gene of a pest or pathogen can, therefore, be resistant against this pest (e.g. cotton bollworm) (Mao et al. 2007). As a safeguard the transgene is designed so that the sRNA would have no effect on mammals. RNAi against a corn rootworm transporter gene Snf7 is currently in the Monsanto pipeline with commercial production expected by the end of this decade.

3) Engineering a metabolic pathway as in wheat to produce β-farnesene, an insect alarm pheromone, making wheat plants resistant to colonization by aphids has been carried out by Rothamsted Research in the UK (Beale et al. 2006).

Natural mechanisms involve taking genes involved in natural disease resistance and transferring them as transgenes into disease-susceptible varieties (Dangl et al. 2013).

A6. Nitrogen use efficiency

Technologies developed by Arcadia (Arcadia Biosciencs 2013) for nitrogen use efficiency based on alanine aminotransferase, enhance absorption of nitrogen by crops so that lower levels of N fertilizer can be applied without compromising yield (McAllister et al. 2012).

A7. Phosphate use efficiency

Phosphorus (P) is a macro-element that is essential for plant growth and crop yield. The availability of inorganic P, or orthophosphate (the only form of P directly accessible to plants), is influenced by soil chemistry and limits crop production on most soils. Consequently, crop production depends on orthophosphate fertilizers, produced from rock phosphate, a finite, non- renewable mineral resource. Only 20–30% of the P fertilizer applied is absorbed by cultivated plants and at the current rate of use. It is estimated that rock phosphate reserves will be consumed within the next 70–200 years, so sustainable use of orthophosphate is important. Improving orthophosphate acquisition and use-efficiency in plants is a complex problem and recent solutions have included modifications to root growth and architecture, and novel engineering strategies to use alternative sources of P. Plants possess several families of orthophosphate transporter proteins, with both high- and low-affinity phosphate transporters, important for orthophosphate uptake into roots, and also critical for orthophosphate distribution throughout the plant, and for remobilization between source and sink tissues. The rice *Pstol1* gene confers tolerance of phosphorus deficiency, and would be expected to confer this trait if moved to other species by GM methods (Gamuyao et al. 2012).

Plants cannot utilize phosphite as a P source, but GM plants carrying a bacterial phosphite-utilization gene *PtxD* enables reduction in the level of P application required for full growth, and with phosphite as sole source of P, enables effective selection against weeds, and offers a potential control method for blackgrass (see next section) (López-Arredondo & Herrera-Estrella 2012).

Seeds store phosphate in the form of inositol 6 phosphate (phytic acid). When eaten by domestic animals such as pigs and chickens, phytic acid passes through the gut unprocessed and the resulting PO4-rich effluent promotes water eutrophication. China has approved deployment of a maize variety that expresses the enzyme phytase in maize seed, breaking down phytic acid by hydrolysis during digestion, and making phosphorus available to the animal, which on excretion does not pollute watercourses (Bohn et al. 2008).

A8. Nitrogen fixation

Nitrogen fertilizer is produced via the energy-intensive Haber-Bosch process that combines hydrogen and nitrogen to make ammonia. For farmers in Africa, synthetic fertilizer is much more expensive than for farmers in the US, largely because of transportation costs and economies of scale. Some plants recruit nitrogen-fixing bacteria into specialized structures-"nodules"- within which, in return for plant-derived sugars, bacteria supply the plant with biologically fixed nitrogen-containing compounds. If crops like wheat, maize and sorghum could be modified to accommodate these nitrogen-fixing bacteria, yields of these staple grains in Africa could be significantly elevated. However, the process of nodule development in response to these bacteria is complex and involves many plant genes. The target of nitrogen fixing cereals is another long-term and high-reward programme funded by the Gates Foundation (ENSA 2013).

B. GM traits for European crops

This section outlines which European crops could benefit from GM technology and which traits would be most useful. Table 4 gives an overview.

B1. Wheat

In the UK, 20,000 farms in England are sprayed against black-grass (on at least one field) and the total area sprayed with grass-weed herbicides is about 1 million ha. A herbicide-resistant wheat, would be very useful. Alternatively, the phosphite utilization trait conferred by *PtxD* (see above) could favour the crop against weeds. Both these traits could be developed in the short/medium term.

A major disease of wheat is "take-all". Oats are resistant to wheat strains of the disease, because unlike wheat, they make avenacin, an antibiotic. The oat genes for avenacin biosynthesis are close to being fully characterized (Qi et al. 2004; Geisler et al. 2013); and work is well advanced by the John Innes Centre in the UK to move these genes into wheat varieties for take-all control in the short/medium term.

There is scope for nutritional enhancement of wheat. Coeliac disease in humans results from an allergic response to certain wheat proteins. Lines of wheat have been generated using RNAi, to eliminate these proteins without reducing bread-making quality or animal feed utility of the grain(Gil-Humanes et al. 2010; van den Broeck et al. 2009). Compared with wheat, oats have a lower glycaemic index (GI) for the same amount of carbohydrate, due to elevated levels of a *CSLH* gene promoting (1,3;1,4)-β-D-glucan accumulation (Burton et al. 2006; Burton et al. 2011). Low GI foods release sugar more slowly, whereas a diet rich in high GI foods is associated with larger pulses of sugar that may promote type II diabetes. Compared to starch, (1,3;1,4)-β-D-glucan is digested more slowly, with the help of specific human gut microflora (Brennan & Cleary 2005). If one were to express this class of *CSLH* gene in wheat, potatoes and tomatoes, the resulting product would be expected to promote healthier diets and lower GI food.

Worldwide losses to wheat rusts are substantial. Programmes are underway in the UK and elsewhere to clone multiple genes for wheat rust resistance from wild or cultivated relatives of wheat, and to "stack" multiple different rust resistance genes on the same transferred DNA (T-DNA) to confer disease resistance (Periyannan et al. 2013; Saintenac & Zhang 2013; Two Blades Foundation 2013).

B2. Potato (and apple)

Potato late blight was the biotic cause of the Irish potato famine and is still the major worldwide disease of potato. The disease organism (*Phytophthora infestans*) evolves rapidly, but several research groups, including the Sainsbury Lab Norwich, are isolating multiple new resistance genes against this disease. Multiple resistance genes can now be delivered on the same T-DNA ("stacking"), which will make it more difficult for the pathogen to overcome such genetic resistance. An early example of a potato variety produced using this approach is Fortuna, which carries two resistance genes (*Rpi-blb1* and *Rpi-blb2*) but was withdrawn by BASF as a result of the hostile EU market conditions. In 2010, Amflora was authorised for cultivation and industrial processing in the EU however, for strategic/marketing reasons BASF withdrew it from commercial sale and it has no longer been cultivated in the EU since 2011. In 2013, a case was taken to the EU court claiming that the EC had not followed its

own guidelines, and won. The EC annulled the authorisation in Dec 2013 (General Court of the European Union, 2013). This provides one of the most conspicuous examples of the impact the European regulatory regime has in deterring deployment of improved GM crops in Europe (BASF 2012; Turley 2013).

When potatoes are processed into French fries or potato crisps, chemical reactions occur during cooking between the natural amino acid, asparagine and naturally present or added reducing sugars, resulting in the formation of acrylamide which is considered to be a genotoxic carcinogen (Joint FAO/WHO Expert Committee on Food Additives 2005). Absent in raw food, or the raw materials used to make food, acrylamide is a chemical substance produced naturally when processed foods are subjected to temperatures greater than 120ºC during manufacture. An American company, Simplot, has initiated regulatory approval for potato varieties in which GM has been used to reduce levels of genes that result in high arginine and reducing sugar levels, and also reduced the tendency for bruising via silencing of polyphenol oxidase (PPO). This trait would also be likely to benefit European consumers.

Potato is also susceptible in warmer, wetter conditions to bacterial wilt caused by *Ralstonia* sp. A bacterial resistance gene, EFR, from the plant Arabidopsis, introduced into tomato by GM, confers enhanced resistance to this disease and is expected to work the same way in potato(Lacombe et al. 2010). There are also credible GM solutions to potato cyst nematode (eelworm), an extremely important constraint on yield, and to several potato viruses.

Bruising in apples, as in potatoes, results in browning, and requires the activity of polyphenol oxidases (PPOs). A Canadian company, Okanagan Speciality Fruits, is seeking to commercialize an engineered apple in which RNAi suppresses the PPO genes, resulting in reduced browning once it has been cut/sliced, thereby prolonging shelf life and reducing waste (Arctic Apples 2013).

B3. Rapeseed (Brassica napus) and other oilseeds

Plant oils lack the health-beneficial omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) which are found in fish oils and those from other marine organisms such as algae and krill. There has been considerable interest and effort in developing oilseed crops in which the omega-3 LC-PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are produced. Such fatty acids can reduce the risk of cardiovascular disease (*Flock et al. 2013*) but natural stocks from the oceans are a diminishing resource. GM oilseed crops such as *Brassica napus* and *Camelina sativa* have been developed to accumulate EPA and DHA in their seed oils, representing a potential sustainable, terrestrial source of fish oils.

B4. Tomato

Tomato is subject to many diseases and with the limited diversity in germplasm available for breeding, resistance for some of these, is best introduced by GM methods. The Arabidopsis EFR gene confers *Ralstonia* resistance to tomato as well as potato (Lacombe et al. 2010).

GM tomatoes developed at the John Innes Centre in the UK, have elevated levels of health-promoting flavonols and flavonoids in the fruit, and the juice has been shown to extend the life of cancer-prone mice by 30% (Butelli et al. 2008). These lines also show an extended shelf life, which could reduce post-harvest losses, and allow harvesting after more flavour has developed (Y. Zhang et al. 2013). This potentially valuable technology is being commercialized in North America, long before it is commercialized in Europe.

Table 4. GM traits for European crops

Species	Problem	Potential solution / desirable traits	Progress	Publications
Wheat (*Triticum aestivum*)	Take-all disease which is a fungal rot	Oat genes for avenacin biosynthesis which make oat resistant to wheat strains of take-all.	Oat genes for avenacin biosynthesis are nearly fully characterised at JIC.	(Papadopoulou et al. 1999; Mylona et al. 2008)
	Wheat rust	Clone and transfer multiple wheat rust resistance genes from wild and cultivated wheat relatives		
	Blackgrass infestation of wheat and barley fields can significantly reduce the yield	Glufosinate resistant wheat as farmers use glyphosate to get rid of weed wheat.	Implementable immediately.	
		Hybrid seed production: Transgenic methods for hybrid wheat seed production could lead to yield increases of up to 10%		
	Cereal aphids causing direct feeding damage and transmission of barley yellow dwarf virus	Engineer wheat to produce the aphid alarm pheromone to repel aphids and attract beneficial organisms such as aphid parasitoids.	The elite wheat variety Cadenza has been engineered accordingly and works well against aphids in the laboratory and increases parasitoid foraging. Confined field trials are in progress at Rothamsted Research.	(Beale et al. 2006) in model crop Arabidopsis thaliana. No publications on confined field wheat trials until complete
Potato (*Solanum tuberosum*)	Potato late blight (caused by *Phytophtora infestans* the causal agent of the Irish potato famine)	Multiple new resistance genes from wild potato species are being isolated and by introducing multiple genes, in a single event, into the potato it will be harder for the pathogen to overcome this resistance.	Several labs in the UK and elsewhere are isolating new resistance genes Field trials in Norwich.	(Tan et al. 2010; Foster et al. 2009)
	Acrylamide formation during frying of potatoes	GM potato varieties with reduced levels of arginine and reducing sugar which are the cause of acrylamide formation.	Regulatory approval initiated by American company Simplot.	http://www.simplotplantsciences.com/
	Bruising of potatoes and apples result in browning and a loss of commercial value	Silencing of polyphenol oxidase (PPO).	Regulatory approval initiated by American company Simplot. A Canadian company is trying to commercialise an apple with suppressed PPO.	http://www.simplotplantsciences.com/ http://www.arcticapples.com/

	Bacterial wilt caused by Ralstonia bacteria	A bacterial resistance gene, EFR, from Arabidopsis has been introduced into tomato and confers enhanced resistance. Expected to work the same way in potato and bananas.	Analysed under controlled laboratory conditions.	(Lacombe et al. 2010)
	Potato Cyst nematode (eelworm) feed on and infest potato roots and can lead to significant yield losses.	Potato cyst nematodes are difficult to fight naturally although some cultivars show some resistance. If this resistance is combined with an expression of cystatin in the roots, good resistance can be achieved. Cystatin is a plant protein found for example in rice that interferes with feeding behaviour in nematodes. Further studies have investigated a peptide that interferes with the chemoreception of the nematodes.	Field trials for both the cystatin and the peptide have been conducted and shown that they lead to good resistance without impacting on soil quality.	(Green et al. 2012; Urwin et al. 2003; Lilley et al. 2004)
Tomato (*Solanum lycopersicum*)	Bacterial wilt caused by Ralstonia bacteria	A bacterial resistance gene, EFR, from Arabidopsis has been introduced into tomato and confers enhanced resistance.	Analysed under controlled laboratory conditions.	(Lacombe et al. 2010)
		Concentrations of health promoting flavonols and flavonoids have been increased in tomatoes by a group at JIC and it has been shown that juice from these tomatoes extends the life span of cancer-prone mice by 30%. An increase in anthocyanin production also increases the shelf-life of the fruits.	Grown in the greenhouse. Commercial field trials in the USA.	(Butelli et al. 2008; Y. Zhang et al. 2013)
Oilseed rape (*Brassica napus*) And Camelina (*Camelina sativa*)		Oilseed crops are being developed to accumulate omega-3-LC-PUFAs such as EPA and DHA, representing a potential sustainable, terrestrial source of fish oils. Such fatty acids can reduce the risk of cardiovascular disease.	Could be available within a decade.	(Flock et al. 2013)

B5. Bio-fuels and industrial biotechnology

Plants can provide a renewable alternative to fossil fuels, as feedstocks for biofuels e.g. sugar (sugar beet, sugar cane), starch (corn, wheat), oil (rape seed) and woody ligno-cellulose (willow).

In Brazil, sugarcane is an important source of bioethanol production. Sugarcane productivity can be limited by availability of "sinks" to receive sugars resulting from photosynthesis. GM sugarcane that expresses a vacuole sucrose isomerase, can increase photosynthetic rates by diverting sugars into the high value sugar isomaltulose and preventing photosynthesis "backing up" (Wu & Birch 2007). Sugarcane production is constrained by water and mineral availability, and by pests and diseases, so in principle many of the technologies explored in this section could be applied to sugarcane, although currently such deployment is not imminent.

To date, the only commercialised GM cereal with a biofuel-related trait is Enogen™, a maize hybrid developed by Syngenta (Syngenta 2012), expressing a thermostable alpha amylase for efficient starch hydrolysis and higher bioethanol yields. Ethanol throughput during fermentation is increased by 5.2% and the financial benefit is between 8-15 US cents per gallon.

Various approaches are being taken to improve the efficiency of biofuel production (Zhang 2013). These include the production of bacterial amylopullulanase in maize grain (Nahampun et al. 2013), thermostable xylanases in maize stover (Shen et al. 2012); glycoside hydrolases (Brunecky et al. 2012) and an *Acidothermus cellulolyticus* endoglucanase in transgenic rice seeds (Q. Zhang et al. 2012). Additionally, decreasing expression of the regulation of the enzyme cinnamyl alcohol dehydrogenase in maize has been shown to reduce lignin levels and thus produce a higher amount of biomass and a higher level of cellulosic ethanol in assays (Fornalé et al. 2012).

Future sustainable biofuel systems are more likely to rely on high yielding ligno-cellulosic feedstocks, rather than the current oily, starchy or sugary feedstocks. This should ease the tension created by direct use of foodstuffs, improve land use efficiency (particularly as these can be supported on lower grade land) and add flexibility of end-use options(Thornley 2012). The most likely European woody biomass crops are willow and Miscanthus; but GM technology to improve these is not expected to be commercialized in the next decade. Judgements will be needed on the best use of the biomass given its suitability for conversion to heat, electricity, transport fuel or renewable chemicals.

C. GM traits for developing countries

Many of the traits described above (improved photosynthesis, and tolerance to acid/saline soils and drought) could be useful worldwide. In particular, pest and disease control is a problem everywhere, and indeed, is more problematic for poor farmers who cannot afford pesticides or fungicides, or the equipment to safely deploy them. For example, a public-private partnership between Syngenta and the International Maize and Wheat Improvement Center (CIMMYT) in Mexico will focus on the development and advancement of technology in wheat through joint research and development in the areas of native and GM traits, hybrid wheat and the combination of seeds and crop protection to accelerate plant yield performance. The partnership will leverage Syngenta's genetic marker technology, and advanced traits

platform, along with CIMMYT's access to wheat genetic diversity, global partnership network, and wheat breeding programme targeted at low-income countries.

Some specific problems of the developing world that could be alleviated with GM traits are described below.

C1. Nutritional enhancement (biofortification) – vitamin A, iron and zinc

Micronutrient malnutrition, or hidden hunger, is caused by a lack of micronutrients in the diet and can result in blindness, stunting, disease and even death. Fruits, vegetables, and animal products are rich in micronutrients, but these foods are often not available to the poor who rely on a daily diet of inexpensive staple foods, such as rice or cassava, with few micronutrients. Enhanced nutrient content is a crucial goal in the light of the world's growing population and the central roles of staple crops in human diets. Developing crop varieties with increased micronutrient concentrations is an approach known as biofortification, and much of this work has been carried out in rice. Similar studies and field trials are underway in banana and cassava (Welsch et al. 2010).

Vitamin A is made in the human body from β-carotene, and vitamin A deficiency is the leading cause of irreversible blindness in children. The World Health Organisation estimates that this results in up to 500,000 children going blind per year, 250,000 of whom will lose their lives within a year. The problem is particularly severe in South East Asia. Rice is a staple food consumed by half the world population every day, but none of the existing cultivated varieties contain β-carotene in the grain. The International Rice Research Institute working with Asian farmers has developed locally adapted Golden Rice varieties, which produce β-carotene in their grain (giving the golden colour). Golden Rice was only possible as a result of genetic engineering developed in the late 1990s at ETH in Zurich by German Professors Potrykus and Beyer and a not-for-profit independent research institute (Golden Rice Humanitarian Board 2013). Fifteen years later Golden Rice is still not authorized for cultivation, to the dismay of scientists (Potrykus 2010). The β-carotene produced is of equivalent nutritional utility to other sources (Tang et al. 2012). This technology is also being applied to banana, and "Golden bananas" are undergoing field trials in Uganda.

Over two billion people suffer from iron and zinc deficiencies. Biofortification of crop varieties with these micronutrients is challenging because metal ion concentrations in various tissues and compartments are maintained within narrow physiological limits by coordinated uptake, translocation and storage. Furthermore, for crops like rice, removal of the outer layers of the grain during polishing, removes many micronutrients, leaving only the starchy endosperm.

Scientists are expressing key genes involved in the mobilization of micronutrients from the soil to the seed in rice through three different approaches: 1) enhancing iron translocation through overproduction of the metal chelator nicotianamine and phytosiderophores; 2) enhancing iron influx into the endosperm by means of the iron-nicotianamine transporter *Oryza sativa* yellow-stripe-like-2 (OsYSL2); and 3) enhancing expression of the iron storage protein ferritin. Combining 1) and 2) has resulted in greenhouse-grown rice with three- to fourfold higher levels of iron (Fe) in polished grain (Schroeder et al. 2013). Combining 1) and 3) has increased the iron content more than sixfold; and combining all three approaches has resulted in paddy-field- grown polished rice with Fe concentrations 4.4-fold higher than those found in non-transgenic seeds, with no yield penalty. Although these results bring iron levels close to those recommended by nutritionists, only a handful of studies have tested whether these enhanced levels of nutrients are available on consumption. Enhancing the nicotianamine concentration does increase the levels of bioavailable iron and zinc in polished rice.

Vacuolar sequestration also enhances the concentrations of iron and zinc (Zn) in seeds. Metals are transported between the cytoplasm and the vacuole by transporters, including the Arabidopsis vacuolar iron transporter VIT1 protein, which is highly expressed in developing seeds and transports iron and manganese. Disruption of the rice VIT orthologues (OsVIT1 and OsVIT2) increases Fe/Zn accumulation in rice seeds and decreases Fe/Zn in the source organ flag leaves. Metal tolerance protein 1 (MTP1) also transports divalent cations into the vacuole. Thus several strategies are being used to enhance iron and zinc micronutrients in edible plant tissues, but more improvements are needed using our increasing knowledge of the transporters that take up micronutrients from the soil, such as iron-regulated transporter 1 (IRT1), the major entry point for Fe in many plant species.

C2. Banana diseases

There are four major pests and diseases of banana:
  o Black Sigatoka, caused by *Mycosphaerella fijiensis* is controlled by enormous fungicide application rates- usually from aeroplanes over plantations, with limited efficacy. Credible GM strategies for resistance are underway but of unproven effectiveness.
  o *Xanthomonas* bacterial disease causes considerable losses in bananas in Central Africa. Credible GM solutions exist from expression of a sweet pepper-derived gene (Tripathi et al. 2010) but still need to be tested further.
  o Nematode infection of roots also suppresses yield. A collaboration between University of Leeds with Uganda has led to nematode-resistant bananas (Roderick et al. 2012), which are being grown in field trials (Figure 12).
  o A new strain (tropical race 4, TR4) of *Fusarium oxysporum* f. sp. *cubense* has emerged that causes Panama disease on the widely-planted Cavendish variety, which was previously resistant. Solutions, GM or otherwise, are awaited (Ploetz 2005).

Figure 12. Field trial of GM banana at the National Agricultural Research Organisation in Uganda.

C3. Bt brinjal /eggplant /aubergine

In India and the Philippines, *Solanum melongena* (aubergines, eggplant or brinjal) is an important crop, but very susceptible to losses from lepidopteran insects such as the brinjal Fruit and Shoot Borer (FSB), *Leucinodes orbonalis*, and the Fruit Borer *Helicoverpa armigera*. Control of these pests is currently only achieved by extensive use of insecticides. GM Bt brinjal has been developed by Mahyco Seed Company (affiliated to Monsanto) by the introduction of a Bt gene (Cry1Ac) and is fully resistant to FSB. Permission is being sought for deployment, but has not yet been granted. Recently, Bt brinjal has been approved for cultivation in Bangladesh and is expected to be grown commercially next year.

C4. Bt cowpea

Cowpea (black-eyed peas) is the most important indigenous African legume (especially for small-scale, low-income farmers) due to its ability to grow in drought-prone areas and improve soil fertility. However, losses to pod-boring insects can be severe, with the cowpea pod borer (*Maruca vitrata*) causing yield losses as high as 70-80%. Insecticides against the Maruca pod borer exist but have not been widely adopted by farmers due to prohibitive costs and significant health hazards. GM BT-resistant cowpea has been developed by an international agbiotech public-private partnership (PPP) coordinated by the African Agricultural Technology Foundation (AATF), a not-for-profit organization that facilitates and promotes PPPs for the access and delivery of appropriate agricultural technology for sustainable use by smallholder farmers in sub-Saharan Africa. Monsanto donated the Bt gene to AATF on a humanitarian basis under a royalty-free license. The Institute for Agricultural Research (IAR) in Zaria, Nigeria is responsible for the Bt cry1Ab gene introgression into local cowpea varieties. Field-testing was then carried out in specific locations in Nigeria (Ezezika & Daar 2012). Deployment is expected by 2017.

C5. Cassava diseases

A major challenge for cassava farmers are cassava mosaic virus and cassava brown streak virus. Research funded by the Gates Foundation is exploring the use of RNAi against these viruses to reduce susceptibility (Taylor et al. 2012) (Figure 13). In addition, Xanthomonas bacterial disease of cassava can also cause extensive losses (Bart et al. 2012), and GM disease resistance traits being developed are likely to be available in the short/medium term.

Figure 13. Sections of cassava roots from conventional variety (left) and GM brown streak virus resistant material (right) (Photos from NEPAD).

D. New enabling techniques and methods

D1. Synthetic biology

Many of the opportunities highlighted above (C4 rice, nitrogen-fixing wheat, avenacin-producing wheat) are now technically feasible due to the availability of new methods in synthetic biology, gene synthesis and multigene vector cloning.

New methods greatly facilitate assembly of T-DNAs that carry many genes (Weber et al. 2011), enabling assembly of complex biosynthetic pathways to enhance plant performance. This type of "Synthetic biology" offers a major new opportunity and extraordinary potential for breeding GM crops. If multiple genes can be delivered into a plant at one locus and confer multiple traits (herbicide, insect, disease, stress resistance, and nutritional enhancement), plant breeding would be greatly simplified compared to current breeding methods where each trait is delivered in distinct GM events with inserted DNA at different chromosomal locations, which then segregate independently during breeding.

Many pests are specialized to cope with the chemical defences of their plant hosts, and plants engineered to produce the chemical defences from a different plant may have increased pest resistance. An early example of the opportunities in this kind of engineering was elevation of insect resistance in Arabidopsis by delivering three enzymes for the biosynthesis of the sorghum-derived cyanogenic glycoside dhurrin (Tattersall et al. 2001).

Adding multiple genes to a crop in one GM event provides a challenge for the current EU regulatory framework, and potentially means GM crops developed by these new methods could not be deployed. For example, individual toxicity tests need to be carried out on each added protein, even if it has been part of the diet in a non-transgenic plant.

D2. High throughput sequencing technologies

One of the most important bio-technological developments in the last ten years, together with advances in bioinformatics, is the availability of high throughput DNA sequencing methods at very affordable prices. These techniques allow the production of Gigabases of DNA sequences for around US$1000, and as a consequence, the amount of information present in DNA databases has doubled every 18 months (Figure 14).

As a result of these high throughput methods, the genome sequences of the main plant species are now known (Figure 15). The resulting datasets include the genomes of model species, such as *Arabidopsis thaliana*, those of the main crops - rice, maize, and soybean, and other important species such as poplar, cotton, grapevine, apple, cassava and sorghum.

At the same time, the genomic variation within a species is becoming accessible due to resequencing of different breeding lines (cultivars). In the case of Arabidopsis but also rice, more than 1000 sequences from different cultivars have been obtained and published (1001 Genomes Project n.d.; Huang et al. 2012). This genome sequence data is helping to identify the genetic basis of domestication of the main crop species, and the many major genes affecting the performance of crops, including yield and disease resistance. Progress is also being made towards the identification of minor genes affecting quantitative traits that would have been more difficult to identify using classical molecular biology and genetics.

The same technologies applied to collections of microorganisms allow metagenomic analysis of the species populating oceans (Venter et al. 2004), soils, human or ruminant intestine and

other environments. These data are generating information about large numbers of microbial genes.

The current and imminent innovations in GM crops described above in Sections 1 and 2A-C were developed in an era when DNA sequencing was slower and less efficient than at present, and it was relatively difficult to identify genes that control a particular trait. The availability of these large amounts of sequence data linked to innovations in bioinformatics has increased the potential list of traits for GM. It will extend beyond the list of traits dependent on single genes to those involving multiple genes.
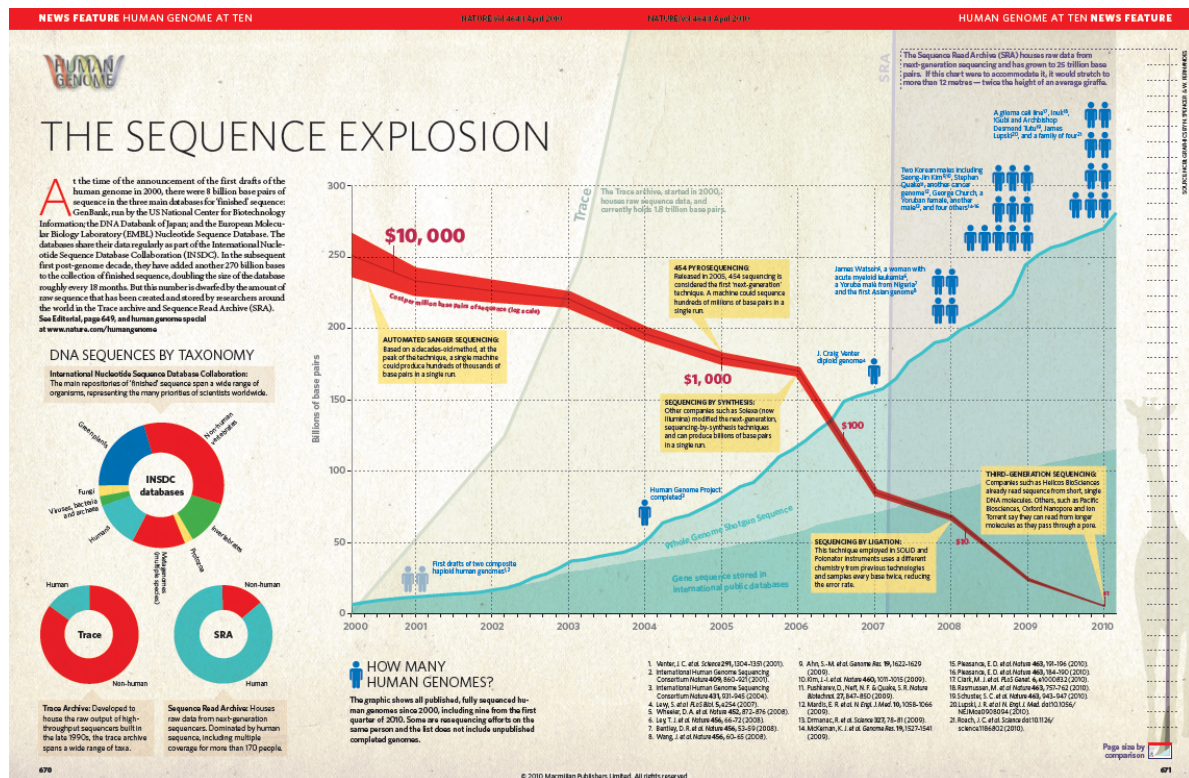
Figure 14. Comparison of the information present in databases and the price for sequencing in relation to the human genome (Lupski et al. 2010).

D3. Directed methods for producing mutations and for plant transformation

Genetic variability relies on the continuous production of gene variants within a population of a given species, through different mutagenic processes. In order to accelerate breeding, mutagenesis has been applied to crops using similar methods, based on the action of radiation, chemicals or the insertion of transposons and T-DNA. These methods essentially act at random and a subsequent search is needed to identify the desired mutants within a population of plants. The approaches of reverse genetics consist in looking for mutants in a gene of interest, with methods such as tilling(McCallum et al. 2000) developed to detect mutations in any gene of a plant. Genetically modified plants have been developed as an alternative to mutagenesis that enables completely new traits to be established in a plant.

New methods to produce genetically-modified plants have increasingly been developed during the last five years that enable mutations at very specific locations or to target gene sequences at specific sites. Classical methods of GM cannot predict the location of the new gene in the plant genome, and the insertion may cause the new gene to have low levels of

expression or to insert into another useful gene. The targeted GM methods in contrast insert the new gene in a specific location to avoid unforeseen effects.
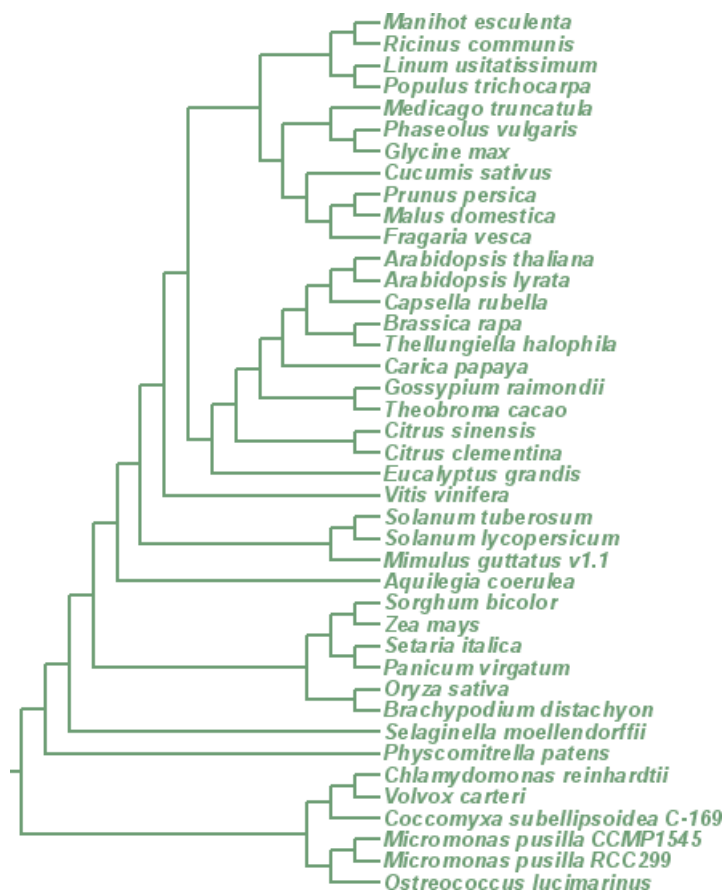
Figure 15. Sequences of plant genomes available in 2013 in Phytozome, a database for public plant genomes (Goodstein et al. 2012)**.**

These new methods are based on the use of site-directed nucleases (SDN) (Podevin et al. 2013) (Figure 16) to prepare the target site DNA for modification or insertion of a new sequence. Two examples of SDNs are Zinc-finger nucleases and TAL-nucleases in which a hybrid protein comprises a nuclease domain from a bacterium to cleave DNA and a sequence-specific DNA binding domain with a motif from a plant pathogen *Xanthomonas*. The SDN is expressed in cells of the crop to be engineered by a break at a defined location in the genome, where a mutation or foreign DNA can be introduced.

Other SDN systems include:

- The Cre-lox recombination system - a bacterial system directing the insertion of genes to a sequence that has been previously introduced in the genome (Gilbertson 2003).

- CrispR-Cas9 gene disruption system - using RNA sequences to direct the Cas9 nuclease to a specific DNA sequence has recently been shown to efficiently produce targeted mutations in plants (Nekrasov et al. 2013) and even in primates (Niu et al. 2014).

These directed methods for GM are very new and have not yet had a big impact on practical application. However, in one example a TAL –nuclease was used to introduce a mutation in a plant gene that is normally harnessed by pathogenic bacteria when they infect a plant and cause disease. TAL nucleases are based on bacterial transcription Factors (called TAL effectors) which when transferred into the plant activates plant genes. The TAL nucleases

targeted to one of these genes was used to introduce mutations in "susceptibility genes" resulting in resistance to *Xanthomonas* bacterial blight (Li et al. 2012).
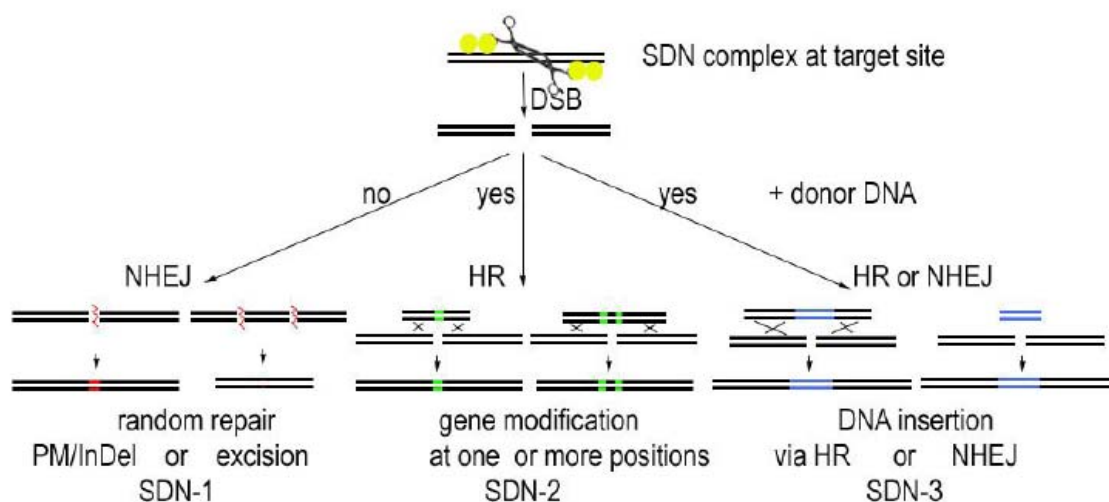
Figure 16. Mode of action of Site-Directed Nuclease (EFSA opinion) (EFSA GMO Panel, 2012)**.**

Methods using SDNs are likely to become the normal method of GM. This change from current practice will have practical and regulatory consequences. At the practical level these methods will allow:

- GM at defined sites in the genome that favour transgene expression, reducing the need to screen multiple GM lines for those in which the transgene is expressed.

- targeted mutation of a specific gene; this is useful if the target gene encodes a gene that adversely affects the yield or quality of a crop.

- replacement of an endogenous gene with a similar but improved version from a related species or one that has been modified in vitro using knowledge of the gene's function.

- insertion of a completely novel gene into a site in the genome that is known to favour foreign gene expression and where there is no effect on expression of other genes.

- insertion of "cassettes" that contain multiple genes allowing the introduction or modification of complex traits such as metabolic pathways in a single step, and placement of the whole set of genes in the same locus in the plant avoiding any effect from multiple insertions of new genes in different places in the genome. Multi-gene cassettes of up to 24 genes can be assembled using GoldenGate or Goldenbraid systems (Weber et al. 2011; Sarrion-Perdigones et al. 2011).

These new methods will also have regulatory consequences for GM crops. They will, for example, facilitate characterisation of the transgene insert in any one line because the site of insertion will be known, and this will negate the criticism of GM crops that insertion of DNA is currently random with "potentially unforeseeable consequences". In addition, these methods will eliminate the need to introduce a selectable marker gene together with the useful insert of a GM crop. In extreme instances the difference between a targeted GM and an equivalent non-GM line will be as little as a single nucleotide, and the practical distinction between GM and non-GM lines will be blurred.

Part 3: Safety and risk assessment

Summary

- **Experimentation and commercial release of GM crops is subject to considerably more stringent regulation than are conventionally bred plants. There are similar stringent frameworks in other countries but the approval process is more streamlined and effective in the Americas and Australia.**

- **The consequence of the regulatory process for commercial release adds between €10 and 20 millions to the cost of developing a new GM variety** – an amount that is prohibitive for small and medium sized enterprises and the public sector. Large multinational companies have withdrawn their research effort to develop GM crops in Europe because the approval process operates slowly and inefficiently. Also, there is a consequence for research, in the significant reduction in number of experimental field trials in the UK with only one field trial in 2012, compared with 37 in 1995.

- **EASAC and others have pointed out that there is no rational basis for the current stringent EU regulatory process.** There are no reliable data indicating inherent risk for human or animal health, for the environment or from unforeseen effects.

Introduction

Section A provides details on the different regulatory frameworks for GM crops in the EU and other countries; while Section B explores the consequences of the EU regulatory framework on the development of GM crops for Europe; and Section C reviews the scientific evidence available to assess the risks of GM crops to human or animal health, and the environment.

A. Regulation of GM crops in the EU and elsewhere

Growing GM crops in the EU is controlled by two pieces of EU legislation:

- Part B of Directive 2001/18/EC (the 'deliberate release' directive) is used to regulate field trials on a national basis; and

- Regulation 1829/2003/EC (the 'GM food and feed regulation') is used to control the commercial cultivation of GM crop plants and operates at the EU-level.

Both sets of legislation adopt the same basic principles i.e. that each GM crop should be assessed on a case by case basis and decisions on whether to authorise their use should be founded on an assessment of the risks to human health and the environment. Benefits are not taken into consideration in the assessment. The legislation requires indirect effects (e.g. the consequences of gene flow to sexually compatible wild species or to bacteria in animal guts) as well as direct effects (impacts on organisms that come into contact with the GM crop) to be taken into account. It also requires delayed (e.g. the evolution of insect resistance) as well as immediate effects to be assessed. Post-market environmental monitoring (PMEM) of every GM crop authorised for commercial cultivation must be carried out to look for unforeseen impacts. Table 5 summarises the safety assessment of GM crops in the EU.

The EU's approach to assessing the safety of GM crops is widely viewed as the most stringent system in the world. Applicants are required to submit risk assessments, risk management options and plans for PMEM. This information is scrutinised by experts in the 28 EU member states and by the Commission's expert advisory committee on GMOs (EFSA GMO Panel, 2013). The assessment process also includes public consultations.

Table 5. Overview of studies required for the safety assessment of GM crops in the EU. (Raybould & Poppy 2012).

Safety Area	Safety Study Performed	Safety Parameter Measured
Molecular characterization	Southern and sequence analysis to assess where the gene has been inserted into the plants DNA. Southern analysis to assess the stability of the insertion over multiple generations. Sequencing of the inserted gene. Sequencing of the insertion site. Bioinformatic searches.	To check what DNA has been inserted into the plant and to ensure this insertion is stable over multiple generations. To check no essential gene has been disrupted and that no unintended genes have been created.
Protein expression	Protein expression of the introduced protein over multiple generations. Protein expression of the introduced protein during development of the plant. Protein expression in plants grown at different locations.	To show that the protein is stably expressed over multiple generations. To assess levels of exposure to humans and the environment from field trials performed at different locations.
Protein toxicity and allergenicity studies.	Stability studies. Acute toxicity in mouse (high dose). Repeat dose toxicity study. Homology searches to known toxin/allergens. Allergencity studies (sera studies performed on a case by case basis). Ecotoxicology studies on a wide range of non target species (for cultivation, mainly for insect tolerant proteins).	To assess stability to gastric fluid. To assess stability to processing. To check the protein is not toxic. To ensure there are no homologies to known toxins or allergens. To assess the toxicity to non-target organisms (cultivation only).
Compositional and phenotypic analysis	Compositional analysis. Phenotypic (agronomic) analysis.	To compare the GM to the non GM plant to assess if there are difference and if the differences are likely to be of biological relevance (compared to ranges of commercially grown plants).
Whole plant studies	Comparative analysis (as described above). Protein expression (as described above). 90 day rat study (case by case basis). Processing study. Ecotoxicology studies on plant material (for cultivation, for all traits). Processing study. Ecotoxicology studies on plant material (for cultivation, for all traits). Non-target organism field studies.	To assess the safety of the whole plant and the likelihood of adverse effects.
Supporting information	Event specific detection method. Certified reference material. Unique identifier. Post market monitoring plan.	Event specific detection method to distinguish the event from other events. Reference material is required as part of the detection procedure. Unique identifier is the unique code for the event. A post market monitoring plan is required in the EU to monitor for unanticipated adverse effects or to confirm the risk assessment.

Whereas the regulatory framework is broadly similar to other countries e.g. Australia, Brazil and Argentina, it has been less efficient in processing applications to commercially cultivate GM crops. Since the system was adopted in 1990, only three decisions[1] have been taken in the EU; and only one since 1998. The latter was for a GM potato with altered starch content (EH92-527-1) authorised in 2010, but only grown on a very limited scale before commercial cultivation was suspended due to difficulties for the Company (BASF) associated with operating in the EU. Approximately 20 applications for commercial cultivation have been submitted to EU regulators over this period, with most of these held in the regulatory pipeline for at least five years. Applications to commercially cultivate two types of GM insect resistant maize (Bt11 and 1507), have been in the system the longest, with submissions in 1996 and 2001 respectively. This situation compares with Australia, which has authorised the commercial cultivation of 12 GM crops since 2002, five of which have since been surrendered (AU Department of Health 2013). The USA with a different system has approved the commercialisation of 96 GM crops since 1990 (See section A4). In a recent decision (November 2013) the General Court of the European Union ruled against the European Commission for not presenting to the Regulatory Committee a proposal to allow a GM maize from the seed company Pioneer (maize 1507) that was submitted in 2001 and approved by EFSA in 2005. As a consequence the European Commission has remarked on the inability of member States to agree to a process that would allow each one of them to take their own specific decision.

B. The consequence of a stringent EU regulatory framework

Compliance with the GM regulatory system, based on the costs for a GM insect resistant maize in the USA, is estimated to add €5.5-11.5M to the cost of developing a new GM crop relative to a non GM variety. Costs in the EU may be higher[2], particularly since GM crop approvals in the EU must be renewed at least every 10 years (See Tables 5 and 6).

This regulatory burden is prohibitive for all but large companies (EASAC 2013) and only certain crop/trait combinations are economically viable if they rely on seed sales to capture profit. Otherwise, regulatory costs need to be vertically integrated into the production of a high value product (e.g. Del Monte's pink-fleshed GM pineapple grown in Costa Rica, which will be imported into the USA once USDA approval is received). In general, these two routes for capturing profit to offset regulatory and development costs do not exist in developing countries.

Even large multinational corporations experience difficulties with the EU regulatory process. BASF and Monsanto withdrew applications to commercially cultivate GM crops and have closed down their GM crop programmes in the EU this year. The result is that only one GM crop (another insect resistant maize) has been cultivated on any significant commercial scale in the EU (see section A3).

The overly burdensome and slow regulatory framework means that Europe is losing out to international competition in the development and use of GM crops, most notably from the Americas and China. It is also moving away from a knowledge based bio-economy and losing international competitive advantage (EASAC 2013).

---

[1] T25 maize (tolerant to glufosinate ammonium herbicides), BASF's Amflora potato and MON810 maize.
[2] There have been no authorisations of equivalent GM plants since 1998.

The number of GM crop field trials in the UK has decreased dramatically since the 1990s, with typically only one or two applications in recent years. In 1995, there were 37 applications to trial GM plants in the UK (compared with 684 in the USA and 213 in the EU). In 2012, one field trial application was received in the UK (compared with 664 in the USA and 45 in the EU).

Table 6. Compliance costs for insect-resistant maize (Kalaitzandonakes et al. 2007).

Cost categories	Range of costs incurred ($)
Preparation for hand-off of events into regulatory	20,000–50,000
Molecular characterization	300,000–1,200,000
Compositional assessment	750,000–1,500,000
Animal performance and safety studies	300,000–845,000
Protein production and characterization	162,000–1,725,000
Protein safety assessment	195,000–853,000
Nontarget organism studies	100,000–600,000
Agronomic and phenotypic assessments	130,000–460,000
Production of tissues	680,000–2,200,000
ELISA development, validation and expression analysis	415,000–610,000
EPA expenses for PIPs (e.g., EUPs, tolerances)	150,000–715,000
Environmental fate studies	32,000–800,000
EU import (detection methods, fees)	230,000–405,000
Canada costs	40,000–195,000
Stewardship	250,000–1,000,000
Toxicology (90-day rat)—when done	250,000–300,000
Facility & management overhead costs	600,000–4,500,000
Total	*7,060,000–15,440,000*
ELISA, enzyme-linked immunosorbent assay; EPA, US Environmental Protection Agency (Washington, DC); EUP, experimental use permit; PIP, plant-incorporated protectant.	

Extraordinarily, in Romania before they joined the EU, GM soybeans were extensively grown and exported to Europe. Since they joined the EU, Romania is now forbidden to grow GM soy as it is not authorized for cultivation in Europe. Instead, the EU pays farmers in Brazil, Argentina and US to grow GM soy, and provides subsidies to Romania from regional funds.

The costs of regulation and the delays associated with having to gain all necessary approvals for GM crops, also have impacts outside the EU. In particular, they deter innovation and technology transfer in the public sector and in developing countries. An economic study of GM eggplant, tomato, rice and papaya showed that a three year delay in reaching the market reduced the Net Present Value of these products by between 34% and 93% (Falck-zepeda et al. 2012).

C. Is GM regulation necessary?

A special GM crop regulatory process would be justified if GM technology is especially dangerous. If, for example, it introduced inherent risks to human or animal health, the environment or if the technology is so poorly understood that there are extraordinary uncertainties then special measures would be required that do not apply to crops improved by

conventional breeding. In this section the scientific evidence is reviewed to consider these possibilities.

C1. Human and animal health

There are a few high profile and controversial studies that claim to refute positive assessments of GM crops. For example, a study by Séralini et al concluded that a herbicide-tolerant maize caused tumours and early death in rats (Séralini et al. 2012), but this was widely dismissed by scientists not involved with the study, including EFSA (European Food Safety Authority 2012). In November 2013, this article was retracted by the journal concerned (Elsevier, 2013). Séralini had published other papers linking health risks to GM food/feed previously (Séralini et al. 2007). In each case, the scientific community and food safety authorities found that that the evidence did not support the conclusions. There are other examples of this type where claims have been made based on poor science (e.g. (Ewen & Pusztai 1999); (Carman et al. 2013)). In other cases, claims are based on hypotheses that harm could occur under certain scenarios. For example, Zhang et al., 2012 suggested that plant-derived RNA might transfer from plant-based feed and have regulatory effects on genes in animal organs (Y. Zhang et al. 2012; L. Zhang et al. 2012). Despite the fact this phenomenon has been described for non-GM plants, it has formed the basis for claims (Heinemann et al. 2013) that transgene RNAs (dsRNAs) create biosafety risks which are not being adequately assessed by regulators.

Snell et al. reviewed 24 studies of the health impacts of GM and found that there were no statistically significant differences between GM and non-GM crops within the parameters observed (Snell et al. 2012). Other studies have been inconclusive about risks associated with health (Domingo & Giné Bordonaba 2011). Notably, even in the highly litigious USA, there have been no successful lawsuits, no product recalls, no substantiated ill effects, and no other evidence of risk from a GM crop product intended for human consumption since the technology was first deployed commercially in 1994 (Masip et al. 2013).

C2. Environmental damage

One of the prominent concerns about GM crops is that their release into the environment "releases the genie out of the bottle". Like alien species, it is feared, the GM crops or their genes will invade and damage existing ecosystems and once released, it will be difficult to eliminate them. However, as described below, there is no evidence or justification for this fear, as any observed environmental effects of GM crops are no different to those experienced with equivalent conventionally bred crops.

There is no question, for example, that widespread cultivation of GM herbicide (glyphosate) tolerant crops in the US has selected for herbicide tolerant weeds. However, this is not due to the GM crop *per se*, but the crop protection regimes relying on glyphosate used by farmers, and the result would be the same for conventionally bred plants had they been cultivated on the same scale with limited herbicide rotation. Once the herbicide-tolerant weeds are present in both conventional and GM crops, and the usefulness of glyphosate is compromised, farmers need to apply higher levels of herbicide or rotate other products or use new and different integrated weed protection strategies (Heap 2013).

It is likely that cultivation of herbicide tolerant GM crops does have an effect on the biodiversity of agricultural ecosystems, as their use allows for highly effective weed control so the cultivated environment contains fewer plant species with inevitable consequences on other layers of the ecosystem in that environment. There would be the same effect with

herbicide tolerant crops produced by conventional breeding, and moreover, there is evidence to demonstrate how this effect can be mitigated (Pidgeon et al. 2007).

Conversely, there is evidence that demonstrates that the use of herbicide tolerant GM crops has resulted in a number of environmental benefits, such as reduced greenhouse gas emissions, reduced soil tillage and associated nutrient loss, and the use of more benign herbicides which degrade quicker in the environment (Brookes & Barfoot 2013b).

An early scare with GM maize producing insecticidal (Bt) proteins was of claimed damage to non-target insects including Monarch butterflies (Losey et al. 1999). Whilst ongoing research is still trying to pinpoint the precise cause of the insect decline, a large body of peer-reviewed work on Monarch butterflies and Bt corn was published in the Proceedings of the National Academy of Sciences (Sears et al. 2001). These studies concluded that populations of monarch butterflies would not be significantly affected by the cultivation of this GM maize. A separate Chinese study reported that the use Bt transgenic crops including rice, with reduced applications of systemic insecticides led to increased levels of non-target insects and protection for farmers applying less of the toxic chemicals (Huang et al. 2005; Chen et al. 2011). However, these effects were not generic effects of GM but consequences of the trait and would have arisen both with GM and non GM traits.

Pollen-mediated gene transfer from GM crops to sexually compatible weeds and wild relatives is frequently conjectured as a hazard of GM crops, as it is considered likely that the transgenes with major effects in the GM crop will have similar effects in weeds (Warwick et al. 2009). However, gene flow between plants is a natural phenomenon with both GM and non GM crops especially with wild or weedy relatives. A few research studies have been cited as evidence that gene flow from GM crops results in so-called 'super weeds'. In a three year study in the UK, researchers claimed to have found two hybrid plants resulting from the cross-pollination of charlock with GM herbicide tolerant oilseed rape (Hopkin 2005); one was sterile and the other did not survive so could not be analysed fully to determine the true hybrid status. Snow et al. crossed a weedy relative with GM sunflowers that expressed an insecticidal protein, and the resulting GM plants produced more seed than their non-GM counterparts, but it was unclear whether this would increase fitness under field conditions (Snow et al. 2003). The only report of the occurrence of 'super weeds' resulting from the commercial cultivation of GM plants was in Canada, where cross-pollination between GM oilseed rape plants containing different herbicide tolerance genes resulted in plants with tolerance to multiple herbicides. This effect has also been observed with conventionally bred herbicide tolerant plants, and since 2004, stewardship plans have included strategies to reduce multiple herbicide-tolerant volunteer rape. These include measures such as post-harvest tillage, appropriate crop rotation, use of certified seed and wherever possible avoiding, successive cultivations of varieties with different herbicides tolerances (Beckie et al. 2006).

Agriculture is an environmentally invasive activity with ecosystem effects that may be as profound as with any other industry. These various examples with GM crops illustrate the need to manage innovation in agriculture, but that this management should be no greater with GM than with any other type of crop. The use of herbicide tolerant crops of both GM and non GM types, for example, should be managed to mitigate selection of herbicide tolerant weeds. Similarly as disease-resistant GM crops are developed, as with non GM types, their use needs to be managed to prevent selection of pests and pathogens able to overcome the resistance.

A good example of such a management strategy is with Bt insect resistant GM crops that have been grown alongside reservoirs of non GM crops providing refuges where the insect pests of the crop can persist in the agricultural environment so that selection pressure for Bt resistance is minimised (Gould 1998). This strategy has mostly been successful until now and similar

strategies could be developed for other types of disease resistance. Indeed, the application of a refuge strategy could have increased the durability of non GM forms of disease resistance. The history of plant breeding includes many examples of good disease resistance genes that have been rapidly overcome by the pathogen once they were grown widely without attempts to mitigate selection of resistance-breaking forms. A refugia policy is only successful with farmer compliance (Tabashnik et al. 2013; Kruger et al. 2009; Kruger et al. 2012; Dangl et al. 2013).

C3. Unknown unknowns

GMO-sceptics continue to claim that GM crops are harmful because they are likely to contain more unintended changes to their DNA compared with plants produced by traditional breeding techniques (van den Belt 2003). Extensive use and study of GM plants since the EU's GMO legislation was put in place, demonstrates that this is not the case. The objective of plant breeding in general is to generate genetic variation and to select variants with desirable characteristics. Consequently, plant breeding produces a vast number of off-types that have to be discarded during the process of producing a new variety.

New technologies are being developed that aid precision and thereby efficacy, both in GM and non-GM plant breeding e.g. site-specific insertion of DNA and site-directed mutagenesis. This goal has been facilitated by dramatic advances in genomics (Section 2D). Genome sequencing projects have demonstrated the extreme plasticity of plant genomes (Bevan 2011; Weber et al. 2012). Given the high degree of natural plasticity and variability between genomes and epigenomes of individuals of the same species as well as within any single individual, focussing on differences between genomes in regulatory assessments is not helpful, unless the significance of difference is understood and is meaningful in terms of the observable physical or biochemical characteristics of the plant (as determined by both the genetic make-up and environmental influences).

Part 4: Conclusions and recommendations

Summary

- **GM crops have the potential to contribute substantially to advances in agriculture** that are necessary to achieve sustainable and sufficient global food production in the face of challenges from population, climate change, and environmental degeneration. Conventional plant breeding is not likely to meet this challenge and cannot take advantage of innovation in synthetic biology.

- **To realize the potential of GM crops, the R & D pipeline needs to be strengthened and the European regulatory process improved.**

- The R & D pipeline would benefit from **a programme that promotes preliminary evaluation in the field of the practical potential of genes defined in academic laboratories that could be useful in crops.** This programme referred to as PubGM would enable and facilitate **field testing of new GM crops** either in partnership with companies or so that the public sector could validate traits before commencing partnerships with companies.

- Many new plant breeding techniques developed since the EU GMO definitions were adopted in 1990, were not foreseen, and some plants with a particular novel trait will be captured by the legislation, whilst others will not. Given that there is no evidence for intrinsic risks associated with GM, it is not useful to have a regulatory framework that is based on the premise that GM crops are more hazardous than those produced by conventionally bred plants. As proposed by EASAC, **a future regulatory framework should be product rather than process based so that it is consistent and applies to the novelty of the characteristics of new plant varieties.**

- **Approval for commercial cultivation should be made on a national level as happens at present with pharmaceuticals.** This would safeguard against potential losses and damage to European agriculture that follow from the failure to adopt GM crops, and enable appropriate regulation of new technologies such as genome editing and synthetic biology for crops,

Introduction

The first two sections of this report illustrate how the advances in GM science for crop improvement promise effective ways to develop GM crops that improve the efficiency of agricultural production by protecting or increasing crop yields, combating the damaging effects of unpredictable weather and disease on crops, reducing fertiliser and chemical use, and reducing losses to pests and disease, both before and after harvest. Equally important is the benefit from using cultivated land more efficiently, to provide more space for biodiversity, nature and wilderness. Research at Rockefeller University suggests that over next 50 years new technology, combined with improved agricultural practices across the world, could release an area 2.5 times the size of France from agricultural cultivation (Asubel et al. 2013).

Increasing crop productivity solely from conventional plant breeding is becoming more difficult. Meeting the global challenges of food security in the context of increasing population, economic growth, predictions of faster climate warming, biodiversity action plans

and farming and environmental regulations will be tough. We need every tool in the toolbox (The Royal Society 2009).

To realise the potential of GM crops we propose strengthening the crop science research and development pipeline in the UK and modifying the regulatory process for GM crops at the EU-level.

A. Research and Development

The UK has world leading basic research in plant genomics (both GM and non-GM), but this is not currently being exploited to its best advantage to enable the commercial crop breeding sector to respond to these challenges. A well-functioning R&D pipeline is essential for the translation of genomic research through the pre-breeding stage into the development of crop varieties for the marketplace. However, the current pipeline in UK crop improvement fails to provide effective links between much needed innovation in crop breeding, investment in new technologies at the translational R&D stage, and public sector support in R&D. This failure is because the potential for profit in agriculture is more limited than in the parallel discipline of biomedicine, and commercial sponsors or investors are less likely to adopt crop improvement programmes, unless there is sufficient evidence that laboratory findings will translate into commercial crops in the field.

There are two interlinked R&D pillars needed to strengthen the pipeline in UK crop improvement using GM.

A1. Public Enterprise GM (PubGM)

Researchers in Universities and Institutes are continually discovering genes for promising crop traits, often using experimental species (see Section 2). Assessing the commercial potential of these discoveries is beyond the reach of standard academic laboratories. The costs are high both for field trial regulation and to support the field scale infrastructure. The likelihood of commercial reward is too low at early stages to gain commercial backing. Therefore new approaches are needed to realize the value of the backlog of innovations from public and private sector scientists, and assess these available traits in UK varieties and environments within a standardized framework on GM trial plots for possible commercialization. In addition, PubGM could engage with the private sector and test "company traits" via contract research, and could make DNA constructs and multiple transgenic events that are screened, tested, selected for deregulation, assessed by DEFRA and FSA, and then returned to the private sector in return for a suitable license fee. It could also develop, test and deploy state-of-the-art genome editing and multigene T-DNA methods. Particularly useful events might be auctioned, resulting in revenues that could defray costs of the programme. This approach also has the benefit of generating data to assess the utility of GM approaches, enabling both consumers and regulatory authorities to make decisions about usefulness of particular GM traits in the UK, based on evidence.

A2. Next-generation farm scale crop evaluation platforms

Multiple factors, including pests and diseases, can strongly and adversely influence crop productivity. New methods are needed to understand these factors and their influence on diverse crop genotypes to guide science and inform breeding decisions. This knowledge directly underpins sustainable production and links breeders, growers and farmers with R&D in new productive ways. A small network of high precision, high throughput farm-scale crop assessment centres in the UK would develop a key new interface between academia, plant

breeders, producers and farmers to provide a platform for integrating multiple R&D activities. These include high precision remote sensing of the growing environment and crop growth, high-throughput and high precision assessment of new crop varieties produced by advanced breeding and GM methods, disease and epidemiology, soil science, different agronomy practices (e.g. organic/low input approaches), environment and biodiversity monitoring. Crops for food, forage, bioenergy, and novel biomaterials could all be assessed according to their different output traits.

A fully optimised research pipeline for crop improvement would be reinforced by enhanced UK capacity in crop plant genomics that could be applied to both GM and non GM approaches. For crops where there is currently little or no market demand but where there are environmental or social benefits (for example new environmentally friendly varieties, or energy crops, break crops), the public sector will need to undertake the fundamental research, but may also need to develop it further to a more commercial stage.

The Government's recently published 'Agri-Tech' Strategy looks at how the UK can capitalise on its world-class science base by turning new ideas into practical applications. Crop improvement (and the role of GM) should be an integral component of the new Agricultural Innovation Centres proposed.

B. EU regulation

A number of recent reports have considered the suitability of the EU regulatory process (EASAC 2013; European Policy Evaluation Consortium (EPEC) 2011; ACRE 2013a). A key question is whether the EU should continue to adopt a regulatory framework based on how new plant varieties are generated (i.e. a 'process-based' approach) rather than on the novelty of their characteristics ('product-based' approach)[3]. This issue has become more prominent in recent years, as many new plant breeding techniques that have been developed since the GMO legislation was adopted in 1990 were not foreseen, and it is not clear whether the definition of a GMO applies to the plants produced by them. This uncertainty will increasingly inhibit innovation in biotechnology. EASAC and ACRE (UK Advisory Committee on Releases to the Environment) have questioned the scientific validity of a process-based approach to regulating novel plants. Inevitably it results in inconsistency; some plants with a particular novel trait will be captured by the legislation whilst others will not. In some of these cases, the plants produced by GM or conventional breeding may contain the same genetic alteration and have the same properties but only the GM version will be subject to regulation.

Even if the EU were to adopt a 'product-based' approach to regulating crops with novel traits in the medium to longer-term, an efficient approach to risk assessment will be crucial. In discussing its experiences of working within the current GMO regulatory system, ACRE has highlighted concerns about how the system is implemented (ACRE 2013b; ACRE 2013c; ACRE 2013d). There is no consensus between EU regulators on what constitutes environmental harm and where there is no clearly defined risk hypothesis linking a characteristic of the GM crop to harm, information requirements are potentially open-ended and do not inform effective decision-making. This also results in assessments that lack perspective and confuse change with harm. The last three-yearly evaluation of the EU's regulatory framework for GM crop cultivation (European Policy Evaluation Consortium (EPEC) 2011) made a number of recommendations for improving implementation of the

---

[3]The Canadian Plant Biosafety Office (PBO) controls plants with novel traits (PNTs) regardless of the techniques used to produce them. This is the only example of a product-based approach to regulating novel crops. However, the assessment process is very similar to that of the EU's i.e. it does not take benefits into account.

legislation, including restricting the number of requests for further information from applicants. There is also concern that the scientific risk assessment is being influenced by political considerations that do not have a scientific basis. For example, the EU has made animal feeding studies a requirement in GM crop risk assessments against the advice of its expert scientific advisory committee (Kuiper et al. 2013).

It is unlikely that more than a handful of GM crops will be approved in the EU in the short term due to the dysfunctional approval process. One option to remedy this situation would be to continue with EFSA having an EU wide advisory role on risk and safety, but for approval of commercial cultivation to be made on a national basis. This would be similar to the current situation in pharmaceuticals, where the European Medicines Agency assesses new pharmaceuticals for humans and animals and, if they recommend use, a national decision is made by the National Institute for Clinical Excellence, the UK national competent authority. In the UK, decisions at a national-level to approve field trials of GM crops typically occur within statutory timeframes (3 months) and a related process could be used for commercially grown crops for which EFSA had made a positive recommendation.

References

1001 Genomes Project, 1001 Genomes Project. Available at:
http://www.1001genomes.org/about.html.

ABNE, 2013. Status of Crop Biotechnology in Africa. Available at:
http://www.nepadbiosafety.net/subjects/biotechnology/status-of-crop-biotechnology-in-africa.

ACRE, 2013a. Advisory Committee on Releases to the Environment. Available at:
http://www.defra.gov.uk/acre/.

ACRE, 2013b. Report 1: Towards an evidence-based regulatory system for GMOs . , pp.1–13.
Available at: http://www.defra.gov.uk/acre/files/Report-1.pdf.

ACRE, 2013c. Report 2: Why a modern understanding of genomes demonstrates the need for a new
regulatory system for GMOs . , pp.1–14. Available at: http://www.defra.gov.uk/acre/files/Report-
2.pdf.

ACRE, 2013d. Towards a more effective approach to environmental risk assessment of GM crops
under current EU legislation. , pp.1–23. Available at: http://www.defra.gov.uk/acre/files/Report-3.pdf.

ArborGen, 2013. Superior Hardwood Seedlings for Multiple Uses. Available at:
http://www.arborgen.us/index.php/products/hardwoods.

Arcadia Biosciencs, 2013. Agricultural products that benefit the environment and human health.
Available at: http://www.arcadiabio.com.

Arctic Apples, 2013. Nonbrowning Apples. Available at: http://www.arcticapples.com.

Asubel, J.H., Wernick, I.K. & Waggoner, P.E., 2013. Peak farmland and the prospect for land sparing.
Population and Development Review, 38, pp.221–242.

AU Department of Health, 2013. Current licences for commercial releases of GM crops (subset of list
of licences involving Intentional Release).

Bart, R. et al., 2012. High-throughput genomic sequencing of cassava bacterial blight strains identifies
conserved effectors to target for durable resistance. *Proceedings of the National Academy of Sciences*,
109(32), pp.1972–1979.

BASF, 2012. Fortuna by BASF Plant Science. Available at:
http://basf.com/group/corporate/en/function/conversions:/publish/content/products-and-
industries/biotechnology/images/Fortuna_VC.pdf.

Beale, M.H. et al., 2006. Aphid alarm pheromone produced by transgenic plants affects aphid and
parasitoid behavior. *Proceedings of the National Academy of Sciences of the United States of America*,
103(27), pp.10509–10513.

Beckie, H.J. et al., 2006. A decade of herbicide-resistant crops in Canada. *Canadian Journal of Plant
Science*, 86(4), pp.1243–1264.

Bevan, M., 2011. Endless variation most beautiful. *Nature*, 477, pp.415–416.

Bohn, L., Meyer, A.S. & Rasmussen, S.K., 2008. Phytate: impact on environment and human
nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science B*, 9(3), pp.165–
191.

Bonfim, K. et al. 2007. RNAi-mediated resistance to bean golden mosaic virus in genetically
engineered common nean (*Phaseolus vulgaris*). *Molecular Plant-Microbe Interactions*, 20,
pp.717–726. Available at: http://apsjournals.apsnet.org/doi/abs/10.1094/MPMI-20-6-0717.

Brennan, C.S. & Cleary, L.J., 2005. The potential use of cereal (1→3,1→4)-β-d-glucans as functional
food ingredients. *Journal of Cereal Science*, 42(1), pp.1–13.

Brookes, G. & Barfoot, P., 2013a. GM crops: global socio-economic and environmental impacts 1996-2011. *PG Economics Ltd, UK*, (April). Available at: www.pgeconomics.co.uk/pdf/2013globalimpactstudyfinalreport.pdf.

Brookes, G. & Barfoot, P., 2013b. Key environmental impacts of global genetically modified (GM) crop use 1996-2011. *GM Crops and Food: Biotechnology in Agriculture and the Food Chain*, 4(2), pp.109–119.

Brunecky, R. et al., 2012. Chapter 18: Analysis of Transgenic Glycoside Hydrolases Expressed in Plants: *T. reesei* CBH I and *A. cellulolyticus* EI. In M. E. Himmel, ed. *Biomass Conversion: Methods and Protocols, Methods in Molecular Biology*. Totowa, NJ: Humana Press, pp. 197–211.

Burton, R. a et al., 2011. Over-expression of specific *HvCslF* cellulose synthase-like genes in transgenic barley increases the levels of cell wall (1,3;1,4)-β-d-glucans and alters their fine structure. *Plant Biotechnology Journal*, 9(2), pp.117–135.

Burton, R.A. et al., 2006. Cellulose synthase – like *CslF* genes mediate the synthesis of cell wall (1,3;1,4)-ß-D-glucans. *Science*, 311(March), pp.1940–1942.

Butelli, E. et al., 2008. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nature Biotechnology*, 26(11), pp.1301–1308.

Carman, J.A. et al., 2013. A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet'. *Journal of Organic Systems*, 8(1), pp.38–54.

Castiglioni, P. et al., 2008. Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. *Plant Physiology*, 147(2), pp.446–455.

Chandler, S.F. & Sanchez, C., 2012. Genetic modification; the development of transgenic ornamental plant varieties. *Plant Biotechnology Journal*, 10(8), pp.891–903.

Chen, M., Shelton, A. & Ye, G., 2011. Insect-resistant genetically modified rice in China: from research to commercialization. *Annual Review of Entomology*, 56, pp.81–101.

CropLife International, 2013. CropLife Ressources. Available at: http://www.croplife.org/Resources.

Dangl, J.L., Horvath, D.M. & Staskawicz, B.J., 2013. Pivoting the plant immune system from dissection to deployment. *Science*, 341(6147), pp.746–751.

Domingo, J.L. & Giné Bordonaba, J., 2011. A literature review on the safety assessment of genetically modified plants. *Environment International*, 37(4), pp.734–742.

EASAC, 2013. *Planting the future: opportunities and challenges for using crop genetic improvement technologies for sustainable agriculture*,

EFSA GMO Panel, 2012. Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. *EFSA Journal*, 10(10), p.2943.

EFSA GMO Panel, 2013. GMO. Available at: http://www.efsa.europa.eu/en/panels/gmo.htm.

Elsevier, 2013. Elsevier announces article retraction from *Journal Food and Chemical Toxicology* November 28, 2013. Available at: http://www.elsevier.com/about/press-releases/research-and-journals/elsevier-announces-article-retraction-from-journal-food-and-chemical-toxicology.

ENSA, 2013. Engineering Nitrogen Biosynthesis for Africa. Available at: www.ensa.ac.uk.

European Comission Joint Research Centre, 2013. Deliberate Release and Placing on the EU Market of GMOs. *GMO Register*. Available at: http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx.

European Food Safety Authority, 2012. Final review of the Séralini et al . (2012a) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology 1. *EFSA Journal*, 10(11), pp.1–10.

European Policy Evaluation Consortium (EPEC), 2011. Evaluation of the EU legislative Framework in the field of cultivation of GMOs.

Ewen, S.W.B. & Pusztai, A., 1999. Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine Differential binding of the insecticidal lectin GNA to human blood cells. *The Lancet*, 354, pp.1353–1354.

Ezezika, O.C. & Daar, A.S., 2012. Overcoming barriers to trust in agricultural biotechnology projects: a case study of Bt cowpea in Nigeria. *Agriculture & Food Security*, 1(Suppl 1), p.S5.

Falck-zepeda, J. et al., 2012. Estimates and implications of the costs of compliance with biosafety regulations in developing countries. *GM Crops and Food: Biotechnology in Agriculture and the Food Chain*, 3(1), pp.52–59.

FARA, 2013. Forum for Agricultural Research in Africa - SABIMA Publications. Available at: http://www.fara-africa.org/our-projects/sabima/publications/.

Financial Times, 2014. Purple tomato juice from Canadian GM crop heads for trial in UK. http://www.ft.com/cms/s/0/5d12b152-8504-11e3-a793-00144feab7de.html#axzz2v6lLf2rd - 24 January 2014.

Flock, M.R., Harris, W.S., & Kris-Etherton, P.M. 2013. Long-chain omega-3 fatty acids: time to establish a dietary reference intake. *Nutrition Reviews* Vol.71 (10): 692-707.

Florigene Flowers, 2013. Florigene Flowers. *Suntory Holdings Limited.* Available at: http://www.florigene.com/.

Fornalé, S. et al., 2012. Altered lignin biosynthesis improves cellulosic bioethanol production in transgenic maize plants down-regulated for cinnamyl alcohol dehydrogenase. *Molecular Plant*, 5(4), pp.817–830.

Foster, S.J. et al., 2009. *Rpi-vnt1.1*, a *Tm-22* homolog from *Solanum venturii*, confers resistance to potato late blight. *Molecular Plant-Microbe Interactions*, 22(5), pp.589–600.

Gamuyao, R. et al., 2012. The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature*, 488(7412), pp.535–539.

General Court of the European Union, 2013). Annulment the Commission's decisions concerning authorisation to place on the market the genetically modified potato Amflora. Available at: http://europa.eu/rapid/press-release_CJE-13-160_en.htm.

Geisler, K. et al., 2013. Biochemical analysis of a multifunctional cytochrome P450 (CYP51) enzyme required for synthesis of antimicrobial triterpenes in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 110(35), pp.3360–3367.

Gilbertson, L., 2003. Cre-lox recombination: Cre-ative tools for plant biotechnology. *Trends in Biotechnology*, 21(12), pp.550–555.

Gil-Humanes, J. et al., 2010. Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. *Proceedings of the National Academy of Sciences of the United States of America*, 107(39), pp.17023–17028.

Golden Rice Humanitarian Board, 2013. Golden Rice Project. Available at: http://www.goldenrice.org/.

Goodstein, D.M. et al., 2012. Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research*, 40(Database issue), pp.1178–1186.

Gould, F., 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology*, 43, pp.701–726.

Green, J. et al., 2012. Transgenic potatoes for potato cyst nematode control can replace pesticide use without impact on soil quality. *PloS one*, 7(2), p.e30973.

Häggman, H. et al., 2013. Genetically engineered trees for plantation forests: key considerations for environmental risk assessment. *Plant Biotechnology Journal*, pp.785–798.

Haslam, R.P. et al., 2013. The modification of plant oil composition via metabolic engineering-better nutrition by design. *Plant Biotechnology Journal*, 11(2), pp.157–168.

Heap, I., 2013. The International Survey of Herbicide Resistant Weeds. Available at: http://www.weedscience.org/summary/home.aspx.

Heinemann, J. a, Agapito-Tenfen, S.Z. & Carman, J. a, 2013. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. *Environment International*, 55, pp.43–55.

Hopkin, M., 2005. Transgenic crop may have bred with wild weed. *Nature*. Available at: http://www.nature.com/news/2005/050725/full/news050725-2.html.

Huang, J. et al., 2005. Insect-resistant GM rice in farmers' fields: assessing productivity and health effects in China. *Science*, 308(5722), pp.688–690.

Huang, X. et al., 2012. A map of rice genome variation reveals the origin of cultivated rice. *Nature*, 490(7421), pp.497–501.

IFPRI (International Food Policy Research Institute), 2013. *Genetically Modified Crops in Africa. Economic and Policy Lessons from Countries South of the Sahara.*,

Information Systems for Biotechnology, 2013a. Crops No Longer Regulated By USDA (Petitions for De-Regulation). Available at: http://www.isb.vt.edu/search-petition-data.aspx.

Information Systems for Biotechnology, 2013b. Release Summary Data and Charts(1987-Present). Available at: http://www.isb.vt.edu/release-summary-data.aspx. Accessed 30 January 2014.

IRRI, 2013. C4 Rice. Available at: http://c4rice.irri.org/.

James, C., 2012. Global Status of Commercialized Biotech/GM Crops: 2012. *ISAAA Brief No. 44. ISAAA: Ithaca, NY.*

Joint FAO/WHO Expert Committee on Food Additives, 2005. List of Substances Scheduled for Evaluation and Request For Data. *JECFA/64/CD*.

Kalaitzandonakes, N., Alston, J.M. & Bradford, K.J., 2007. Compliance costs for regulatory approval of new biotech crops. *Nature Biotechnology*, 25(5), pp.509–511.

Kebeish, R. et al., 2007. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana. Nature Biotechnology*, 25(5), pp.593–599.

Kruger, M., Van Rensburg, J.B.J. & Van den Berg, J., 2009. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. *Crop Protection*, 28(8), pp.684–689.

Kruger, M., Van Rensburg, J.B.J. & Van den Berg, J., 2012. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. *Journal of Applied Entomology*, 136(1-2), pp.38–50.

Kuiper, H. a, Kok, E.J. & Davies, H. V, 2013. New EU legislation for risk assessment of GM food: no scientific justification for mandatory animal feeding trials. *Plant Biotechnology Journal*, pp.781–784.

De la Fuente, J.M. et al., 1997. Aluminum tolerance in transgenic plants by alteration of citrate synthesis. *Science*, 276(5318), pp.1566–1568.

Lacombe, S. et al., 2010. Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nature Biotechnology*, 28(4), pp.365–369.

Leegood, R.C., 2013. Strategies for engineering C4 photosynthesis. *Journal of Plant Physiology*, 170(4), pp.378–388.

Li, T. et al., 2012. High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nature Biotechnology*, 30(5), pp.390–392.

Lilley, C.J. et al., 2004. Preferential expression of a plant cystatin at nematode feeding sites confers resistance to *Meloidogyne incognita* and *Globodera pallida*. *Plant Biotechnology Journal*, 2(1), pp.3–12.

López-Arredondo, D.L. & Herrera-Estrella, L., 2012. Engineering phosphorus metabolism in plants to produce a dual fertilization and weed control system. *Nature Biotechnology*, 30(9), pp.889–893.

Losey, J.E., Rayor, L.S. & Carter, M.E., 1999. Transgenic pollen harms monarch larvae. *Nature*, 399, pp.6–7.

Lupski, J.R. et al., 2010. Human genome at ten: The sequence explosion. *Nature*, 464, pp.670–671.

Mao, Y. et al., 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature Biotechnology*, 25(11), pp.1307–1313.

Masip, G. et al., 2013. Paradoxical EU agricultural policies on genetically engineered crops. *Trends in Plant Science*, 18(6), pp.312–324.

McAllister, C.H., Beatty, P.H. & Good, A.G., 2012. Engineering nitrogen use efficient crop plants: the current status. *Plant Biotechnology Journal*, 10(9), pp.1011–1025.

McCallum, C.M. et al., 2000. Targeting Induced Local Lesions In Genomes (TILLING) for plant functional genomics. *Plant Physiology*, 123(2), pp.439–442.

Meyer, M.T. et al., 2012. Rubisco small-subunit α-helices control pyrenoid formation in *Chlamydomonas*. *Proceedings of the National Academy of Sciences of the United States of America*, *PNAS*, 109(47), pp.19474–19479.

Mylona, P. et al., 2008. *Sad3* and *sad4* are required for saponin biosynthesis and root development in oat. *The Plant Cell*, 20(1), pp.201–212.

Nahampun, H.N. et al., 2013. Ectopic expression of bacterial amylopullulanase enhances bioethanol production from maize grain. *Plant Cell Reports*, 32(9), pp.1393–1405.

Nekrasov, V. et al., 2013. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nature Biotechnology*, 31(8), pp.691–693.

Nelson, D.E. et al., 2007. Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proceedings of the National Academy of Sciences of the United States of America*, 104(42), pp.16450–16455.

NEPAD, 2013. The New Partnership For Africa's Development - Biosafety. Available at: http://www.nepadbiosafety.net/about.

Niu et al. 2014. Generation of Gene-Modified Cynomogus Monkeys via Cas9/RNA-mediated Gene Targeting in One-Cell Embryos. *Cell*,156, pp.1-8.

Papadopoulou, K. et al., 1999. Compromised disease resistance in saponin-deficient plants. *Proceedings of the National Academy of Sciences of the United States of America*, 96(22), pp.12923–12928.

Peleg, Z. & Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology*, 14(3), pp.290–295.

Periyannan, S. et al., 2013. The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. *Science*, 341(6147), pp.786–788.

Pidgeon, J.D. et al., 2007. Mitigation of indirect environmental effects of GM crops. *Proceedings. Biological sciences / The Royal Society*, 274(1617), pp.1475–1479.

Ploetz, R.C. 2005. Panama Disease: An Old Nemesis Rears its Ugly Head Part 2: The Cavendish Era and Beyond. *Online. APSnet Features. doi: 10.1094/APSnetFeature-2005-1005.* See: http://www.apsnet.org/publications/apsnetfeatures/Pages/PanamaDiseasePart2.aspx

Podevin, N. et al., 2013. Site-directed nucleases: a paradigm shift in predictable, knowledge-based plant breeding. *Trends in Biotechnology*, 31(6), pp.375–383.

Potrykus, I., 2010. Regulation must be revolutionized. *Nature*, 466, p.561.

Qi, X. et al., 2004. A gene cluster for secondary metabolism in oat: implications for the evolution of metabolic diversity in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 101(21), pp.8233–8238.

Quandzie, E., 2013. Ghana approves first crops to undergo GMO confined field trials. *Ghana Business News*. Available at: http://www.ghanabusinessnews.com/2013/07/17/ghana-approves-first-crops-to-undergo-gmo-confined-field-trials/.

Raybould, A. & Poppy, G.M., 2012. Commercializing genetically modified crops under EU regulations: objectives and barriers. *GM Crops and Food: Biotechnology in Agriculture and the Food Chain*, 3(1), pp.9–20.

Roderick, H. et al., 2012. Generation of transgenic plantain (*Musa* spp.) with resistance to plant pathogenic nematodes. *Molecular Plant Pathology*, 13(8), pp.842–851.

Rosenthal, D.M. et al., 2011. Over-expressing the C3 photosynthesis cycle enzyme Sedoheptulose-1-7 Bisphosphatase improves photosynthetic carbon gain and yield under fully open air CO_2 fumigation (FACE). *BMC Plant Biology*, 11(1), p.123.

Saintenac, C. & Zhang, W., 2013. Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. *Science*, 341, pp.783–786.

Sarrion-Perdigones, A. et al., 2011. GoldenBraid: an iterative cloning system for standardized assembly of reusable genetic modules. *PloS one*, 6(7), p.e21622.

Savadogo, M., 2010. Confined field trials in Africa: a key step to safely perform experiments with genetically modified crops. *NEPAD*. Available at: http://www.nepadbiosafety.net/abne/wp-content/uploads/2010/10/Env.-Biosafety-Policy-Brief-N0-2.pdf.

Schroeder, J.I. et al., 2013. Using membrane transporters to improve crops for sustainable food production. *Nature*, 497(7447), pp.60–66.

Sears, M.K. et al., 2001. Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proceedings of the National Academy of Sciences of the United States of America*, 98(21), pp.11937–11942.

Séralini, G.-E. et al., 2012. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food and Chemical Toxicology*, 50(11), pp.4221–4231.

Séralini, G.-E., Cellier, D. & de Vendomois, J.S., 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Archives of Environmental Contamination and Toxicology*, 52(4), pp.596–602.

Shen, B. et al., 2012. Engineering a thermoregulated intein-modified xylanase into maize for consolidated lignocellulosic biomass processing. *Nature Biotechnology*, 30(11), pp.1131–1136.

Slewinski, T.L., 2013. Using evolution as a guide to engineer Kranz-type C_4 photosynthesis. *Frontiers in Plant Science*, 4(July), p.212.

Snell, C. et al., 2012. Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: a literature review. *Food and Chemical Toxicology*, 50(3-4), pp.1134–1148.

Snow, A.A.A. et al., 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecological Applications*, 13(2), pp.279–286.

Syngenta, 2012. Enogen. Available at: http://www.syngenta.com/country/us/en/enogen/Pages/Home.aspx.

Tabashnik, B.E., Brévault, T. & Carrière, Y., 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nature Biotechnology*, 31(6), pp.510–521.

Tan, M.Y.A. et al., 2010. The effect of pyramiding Phytophthora infestans resistance genes RPi-mcd1 and RPi-ber in potato. *TAG. Theoretical and Applied Genetics*, 121(1), pp.117–125.

Tang, G. et al., 2012. ß-Carotene in Golden Rice is as good as ß-carotene in oil at providing vitamin A to children. *American Journal of Clinical Nutrition*, 96, pp.658–664.

Tattersall, D.B. et al., 2001. Resistance to an herbivore through engineered cyanogenic glucoside synthesis. *Science*, 293, pp.1826–1828.

Taylor, N.J. et al., 2012. The VIRCA Project Virus resistant cassava for Africa. *GM Crops and Food: Biotechnology in Agriculture and the Food Chain*, 3(2), pp.93–103.

The Royal Society, 2009. *Reaping the benefits: science and the sustainable intensification of global agriculture.*

Thornley, P., 2012. Biofuels Review Report for Government Office for Science. *Tyndall Centre for Climate Change Research and Sustainable Consumption Institute, University of Manchester.*, pp.1–39.

Tollefson, J., 2011. Brazil cooks up transgenic bean - Approval draws criticism over transparency and safety tests. *Nature,* 478, pp.168. (Available at: http://www.nature.com/news/2011/111012/full/478168a.html)

Turley, A., 2013. BASF drops GM potato projects. *Chemistry World*. Available at: rsc.org/chemistryworld/2013/02/basf-gm-potato-amflora.

Two Blades Foundation, 2013. Wheat Stem Rust Resistance. Available at: http://2blades.org/wheat-stem-rust.php.

Urwin, P.E., Green, J. & Atkinson, H.J., 2003. Expression of a plant cystatin confers partial resistance to *Globodera*, full resistance is achieved by pyramiding a cystatin with natural resistance. *Molecular Breeding*, 12, pp.263–269.

USDA, 2014. Animal and Plant Health Inspection Service (APHIS). Available at: http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml. - Accessed 30 January 2014)

van den Belt, H., 2003. Debating the precautionary principle: "Guilty until proven innocent" or "Innocent until proven guilty"? *Plant Physiology*, 132(3), pp.1122–1126.

van den Broeck, H.C. et al., 2009. Removing celiac disease-related gluten proteins from bread wheat while retaining technological properties: a study with Chinese Spring deletion lines. *BMC Plant Biology*, 9, p.41.

Venter, J.C. et al., 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, 304, pp.66–74.

Weber, E. et al., 2011. A modular cloning system for standardized assembly of multigene constructs. *PloS one*, 6(2), p.e16765.

Weber, N. et al., 2012. Crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. *Plant Physiology*, 160(4), pp.1842–1853.

Welsch, R. et al., 2010. Provitamin A accumulation in cassava (*Manihot esculenta*) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. *The Plant Cell*, 22(10), pp.3348–3356.

Wu, L. & Birch, R.G., 2007. Doubled sugar content in sugarcane plants modified to produce a sucrose isomer. *Plant Biotechnology Journal*, 5(1), pp.109–117.

Zhang, B. et al., 2013. A threshold level of oxalate oxidase transgene expression reduces *Cryphonectria parasitica*-induced necrosis in a transgenic American chestnut (*Castanea dentata*) leaf bioassay. *Transgenic Research*, 22(5), pp.973–982.

Zhang, L. et al., 2012. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Research*, 22(1), pp.107–126.

Zhang, Q. et al., 2012. Expression of an *Acidothermus cellulolyticus* endoglucanase in transgenic rice seeds. *Protein Expression and Purification*, 82(2), pp.279–283.

Zhang, Y. et al., 2012. Analysis of plant-derived miRNAs in animal small RNA datasets. *BMC Genomics*, 13(1), p.381.

Zhang, Y. et al., 2013. Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. *Current Biology*, 23(12), pp.1094–1100.

Zurbriggen, M.D. et al., 2008. Combating stress with flavodoxin: a promising route for crop improvement. *Trends in Biotechnology*, 26(10), pp.531–537.