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International Genetic Engineering Campaign

Background Information 06/02

GENETICALLY ENGINEERED WHEAT – CHANGING OUR DAILY BREAD

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1. Executive Summary

Wheat is grown on an enormous scale, with over 225 million hectares grown annually world wide between 1995-97. The 560 million tonnes of wheat produced each year makes up more than one quarter of the world cereal output. Wheat forms an important part of many people's diet in both the developed and developing world. It is not as high a value crop as soybean or maize and is technically difficult to genetically engineer, so has only more recently become a focus for the biotechnology industry and scientists in the public sector.

Monsanto hopes to commercialise Roundup Ready wheat in the USA and Canada in 2004. Other types of GE wheat being developed include wheat which is resistant to fungal and viral diseases, tolerant to drought, and which has better characteristics for baking and processing. GE wheat producing pharmaceuticals has also been proposed. However, all these applications have encountered difficulties associated with transgene silencing, instability and rearrangements as a result of the genetic engineering process. Therefore, with the possible exception of Roundup Ready wheat, there are no advanced GE wheats in development. Frequently, the consequences of gene silencing or instabilities do not become evident for several generations and although new techniques are being developed in an effort to overcome these problems, the future progress of GE wheat remains uncertain.

The growing of GE wheat could pose several threats to the environment most importantly that:

- Gene flow to related species could occur. The greatest risk would be in the Middle East where wheat evolved, related species are commonly found and the area is a centre of diversity. Related species are also found in areas outside the Middle East.
- Troublesome weeds may emerge rapidly and lead to more toxic herbicides being used to control them. Wheat can hybridise with jointed goatgrass, a weed of wheat in the USA which causes losses of $145 million annually.
- Volunteer wheat may be more difficult to control leading to the use of more toxic herbicides and increasing the potential for disease to spread between wheat crops

If herbicide tolerance genes were transferred from GE wheat into goatgrass which made it more difficult to manage, economic losses could be great. The transfer of disease or environmental stress resistance genes could lead to better survival of the plants acquiring the foreign genes, and disruption of ecosystems could result if they survived and displaced other species. The way in which disease controls natural populations of plants is very poorly understood so the effects are impossible to predict with any accuracy. With use of viral genes to produce virus resistant GE wheat, recombinations with infecting viruses and the evolution of new viruses is possible. Genes that alter the protein, carbohydrate or other biochemical pathways could alter the survival properties of wheat seed and the likelihood of germination. This could give either a positive or negative advantage to the plants acquiring the genes.

GE wheat could also have indirect negative impacts on biodiversity although, as with gene flow, there is little or no research taking place to address such concerns. For example, the use of Roundup wheat will increase the use of the broad spectrum herbicide, glyphosate. This could lead to even more efficient removal of weeds and loss of food supply for farmland insects and birds. Fungal resistant GE wheat could alter the normal plant/fungal interactions that take place. The introduction of genes coding for compounds toxic to insects may lead to adverse effects on non-target species. When used in potatoes, the snow drop lectin gene which has been introduced into wheat to give insect resistance against grain aphid, harmed beneficial insects (ladybirds) feeding on the pests (aphids) which had consumed the lectin.

Most wheat is used for human food consumption. Although the whole grain is rarely consumed, processing to produce flour or semolina involves milling and grinding rather than the use of high temperature or chemical treatments and this does not breakdown DNA. Whilst DNA may be degraded to some extent during cooking, intact DNA may be present in food and thus gene transfer to

microorganisms in the intestines is possible. In many GE wheat varieties being tested, genes giving resistance to the antibiotics neomycin and kanamycin are present and ampicillin resistance genes have been used in GE wheat in UK trials (although the resistance is not thought to be expressed). If these genes were transferred to disease causing organisms they may compromise antibiotic treatment.

The growing of GE wheat could also compromise the supply of non-GE or organic wheat. Wheat is self fertilising and produces less pollen than many other crops. There is little information about long distance movement of pollen but current separation distances of 3-10 metres may be insufficient to ensure seed purity. By comparison to barley, another self-fertilising species, a separation distance of 60 metres may be needed.

It is the vast scale upon which wheat is grown globally that means that the risks of GE wheat have to be taken seriously. Although gene flow is less likely than for more promiscuous species like oilseed rape, if GE wheat is grown commercially, it is inevitable at some stage. Currently there is no evidence that such are risks are being addressed in research programmes.

2. Introduction

The news that Monsanto hopes to market wheat genetically engineered to tolerate the herbicide, glyphosate (known as Roundup Ready wheat) in 2004, has sparked considerable controversy. Wheat producers in both Canada and the US (where the GE wheat would be sold initially) expressed concern that its introduction could damage their export markets because of consumer resistance abroad. In response, the Canadian Wheat Board has called for changes to the variety registration process to include market impact as a criterion[1] which could block the approval of GE wheat.

However, there are also important environmental questions that need to be addressed. Could there be movement of the foreign genes into non-GM wheat or related wild species? How will agricultural practice be affected by an increased use of the broad spectrum herbicide, glyphosate? What other types of GE wheat might follow Roundup Ready wheat?

Wheat is grown on an enormous scale, with over 225 million hectares grown world wide annually between 1995-97 (see Table 1). The 560 million tonnes of wheat produced each year makes up more than one quarter of the world cereal output. Yields have risen dramatically from the 1950s onwards.

In the UK, the trebling of yields from 2.5 to 7.5 tonnes per hectare has been attributed to[2]:

- adoption of shorter (dwarf) varieties
- earlier sowing (winter sown wheat)
- high rates of fertiliser application
- herbicide and fungicide use.

In the future, the area of wheat grown is predicted to rise as a consequence of increasing incomes and westernisation of diets in developing countries, especially Asia.[3] GE of wheat is being proposed as one way of addressing increased demand. Further changes in practices in wheat farming could therefore have very profound impacts on the environment. In the UK, for example, the move from spring to winter sown wheat has been associated with a serious decline in farmland wildlife, particularly bird species which had fed over winter in the stubble of the previous crop.[4]

Table 1: Average annual growing area and yield of wheat 1995-97[3]

Country/Region	Average area grown 1995-97 (000 ha)	Yield (t/ha)
Eastern and Southern Africa	2,882	1.6
North Africa	6,037	2.0
West Asia	21,436	1.8
South Asia	35,240	2.4
East Asia	29,921	3.8
Mexico, Central America & Caribbean	850	4.2
Andean Region, South America	309	1.1
Southern Cone, South America	8,146	2.1
Eastern Europe and former Soviet Union	57,159	1.8
Europe	27,027	4.2
US	25,286	2.5
Canada	11,601	2.3
Australia	10,192	1.9
New Zealand	51	5.5
World	226,498	2.6

This report reviews the developments that are taking place in the genetic engineering of wheat, who is involved and what the consequences may be for the environment. Because there is no GE wheat on the market in any part of the world, there is an opportunity to prevent environmental harm developing.

3. Developments in GE wheat

Wheats (Triticum spp) are members of the cereal grass family (Gramineae). Two wheat species, common (Triticum aestivum L.) and durum wheat (T. turgidum L. var. durum), are the two major cultivated species. Common or bread wheat comes in two forms – soft and hard – according to its protein content.[5] Hard wheat has high protein content (10-17%) and soft wheat low-protein content (6-10%). Gluten, one of the major wheat proteins, has the high elasticity needed for bread making. Hard wheat gives flour with a high gluten content and is good for bread making and grows best in drier areas. Soft wheat is used in pastries, cakes and biscuits and grows in wetter regions. Durum wheat, although high in gluten, does not make good bread and is used in making pasta. About 80% of durum wheat is grown in the Middle East and North Africa region.

Wheat is widely grown in temperate climates with around 15-35 inches (36-84 cms) annual rainfall.[6] It does not tolerate warm, humid conditions because of its susceptibility to fungal disease. Around 75% of wheat is known as ‘winter wheat’ because it is sown in the autumn and the remainder is spring wheat sown in the spring.

Whilst there is no GE wheat being grown commercially in any part of the world, open field trials with GE wheat have taken place in 6 countries - the USA, Australia, Spain, Belgium, the UK and Canada (see Table 2). Laboratory and greenhouse based experiments with GE wheat are also taking place in many other countries, including China, Denmark, Egypt, Kenya, Germany and Switzerland.

The focus of wheat improvement using genetic engineering has been to reduce production costs, by making plants more resistant to stress such as disease and drought, and to increase the product value by making processing easier (e.g for bread making) or improving nutritional content. As with other crops, herbicide tolerance has been an early commercial focus of research. However, the technical difficulties of genetically engineering wheat (see Section 4) and the relatively low value of wheat compared to other crops, means that interest in genetic engineering of wheat has been limited and commercial involvement is lower. Monsanto and DuPont are the two companies with the most investment in GE wheat and much research is in the public sector. As with other GE crops, public-private partnerships are not uncommon and, for example, DuPont fund a wheat transformation laboratory at the Institute of Arable Crops Research in the UK. Wheat genes are also being patented by private companies. Table 3 lists the seven top companies and institutions who have applied for patents on wheat partial or complete gene sequences at the end of 2000.

However, the interest of the biotechnology industry in GE wheat may be declining. A recent report in the UK farming press states that Du Pont have pulled out of the development of wheat hybrids which they planned to use for delivery of GE traits because of concerns about GE food in Europe.[7]

The scientific literature and field trial data show wheat is most commonly being genetically engineered in the following ways:

- herbicide tolerance (the most advanced), where the wheat has been genetically modified to tolerate a chemical weedkiller, most commonly glyphosate (Roundup, Monsanto) or glufosinate (Liberty, Aventis).
- disease resistance – to fungal or viral diseases causing yield losses in wheat.
- quality improvements – changes to the starch or protein content to improve baking or other qualities

Past difficulties with hybrid production in wheat have also led to the development of GE systems for wheat which are at the laboratory stage and research is also taking place to improve resistance to environmental stress such as drought.

Each of these areas of research and their progress is reviewed below.

Table 2: Field trials with GE wheat

Country	Company/Institution	Trait
USA	AgrEvo	Herbicide tolerance – glufosinate
	Applied Phytologics	Altered composition – improved digestibility
	ARS	Fungal resistance (powdery mildew)
		Herbicide tolerance – glufosinate
		Altered composition – protein
	Cargill	Altered composition - protein
	Kansas State University	Fungal resistance (fusarium)
	Monsanto	Altered composition – starch/sugar
		Altered nitrogen metabolism
		Enhanced photosynthesis
		Increased yield
		Fungal resistance - fusarium
		Herbicide tolerance – glyphosate
		Virus resistance – BYDV and WSMV
	Montana State University	Drought tolerance
		Altered composition – starch/sugar
		Yield increase
		Virus resistance – WSMV
	Novartis Seeds (now part of Syngenta)	Fungal resistance – fusarium
		Fungal resistance – powdery mildew
		Fungal resistance – septoria
	Syngenta	Fungal resistance – fusarium
		Fungal resistance – powdery mildew
	University of Idaho	Virus resistance – BYDV
		Virus resistance - WSMV

Canada	BASF	Herbicide tolerance -
	Monsanto	Herbicide tolerance – glyphosate
	Syngenta	Fungal resistance -
	Novartis Seeds Seeds (now part of Syngenta)	Marker genes
		Fungal resistance
	Plant Biotechnology Institute (NRC)	Herbicide tolerance
		Altered composition
Australia	CSIRO Plant industry	Marker gene -GUS
		Altered composition – starch
		Altered composition – glutenin increased
		Herbicide tolerance - glufosinate
	University of Adelaide & Victoria Institute for Dryland Agriculture	Herbicide tolerance -
Spain	Compañía Navarra Productora de Semillas SA Senasa	Altered composition – starch + herbicide tolerance – glufosinate
		Altered composition - starch
	Consejo Superior de Investigaciones Científicas	Altered composition – glutenin increased
	Instituto de Agricoltura Sostenibile Consejo Superior de Investigaciones Cientificas	Altered composition – glutenin increased + herbicide tolerance – glufosinate
	Instituto Nacional de Investigaciones Agrarias y Alimentarias INIA (MAPA)	Dalapon tolerance
UK	Institute of Arable Crop Research (IACR)/John Innes Institute	Altered composition – improved baking quality + herbicide tolerance – glufosinate
		Altered composition – starch/sugar + herbicide tolerance – sulfonylurea
	Syngenta	Fungal resistance - fusarium

Key: BYDV – barley yellow dwarf virus; WSMV – wheat streak mosaic virus

Table 3. Top wheat gene patenters [8]

	COMPANY/INSTITUTE	SEQUENCES	% TOTAL (288)
1	DU PONT	117	40.6
2	MONSANTO	78	27.1
3	AVENTIS	14	4.9
4	NOVARTIS	12	4.2
5=	CSIRO	8	2.8
5=	GOODMAN FIELDER	8	2.8
5=	LIMAGRAIN	8	2.8
	TOTAL		**85.2**

3.1 Herbicide tolerance

The involvement of the agrochemical industry in crop genetic engineering has led to the development of herbicide tolerant wheat being most advanced. In numerical terms, field trials with Roundup Ready wheat dominate in North America. Monsanto has used the same genetic systems it uses in many of its other GE Roundup Ready crops to develop Roundup Ready wheat. Glyphosate acts by inhibiting a key enzyme in plant metabolism, 3-enolpyruvylshikimate-5-phosphate synthase (EPSPS). To make glyphosate resistant wheat, Monsanto have used both a glyphosate-tolerant EPSPS gene (CP4) from an *Agrobacterium* and another bacterial gene which codes for glyphosate reductase (GOX), which breaks down glyphosate.[9]

Other forms of herbicide tolerance, mainly to glufosinate, have been widely used in GE wheat by the private and public sector researchers, but often this is as a marker gene to identify when the genetic engineering process has been successful. Only Monsanto has a clear strategy to bring herbicide tolerant wheat to the market as soon as possible.

3.2 Disease and insect resistance

Fungal and viral diseases are among the most important causes of yield loss in wheat and the susceptibility of wheat to certain diseases restricts the areas in which it can be grown. Resistance to fungal and viral diseases has, therefore, been an active area of research and there have been some attempts to introduce insect resistance.

3.2.1 Fungal resistance

The most common approach to engineering fungal resistance is to transfer genes from other plants coding for pathogenesis-related proteins (P-R proteins).[10] P-R proteins are produced in response to disease or insect attack and they are thought to be associated with increased resistance. Engineering resistance to fungal diseases such as Scab (*Fusarium* head blight, FHB), a very damaging disease which can cause losses of up to 40%, powdery mildew (*Erysiphe graminis*), leaf rust (*Puccinia recondita)* and stinking smut (*Tilletia tritici*) have all been attempted. Approaches have included the use of a rice chitinase gene,[11] both rice chitinase and thaumatin genes,[12] an anti-fungal protein, Ag-AFP, from *Aspergillus giganteus*, barley chitinase and ribosome inactivating proteins (RIPs)[13,14] and a viral (*Ustilago maydis* – infecting virus) antifungal protein, KP4.[15] It is hoped that using by using multiple P-R proteins with different mechanisms of action, resistance should be increased. However, despite *in vitro* activity against the fungi, protection of the wheat plant tends to be limited. Protection may involve delaying the onset of symptoms[12] or decreased susceptibility[13,14] but is not absolute.

When the cauliflower mosaic virus (CaMV) promoter is used to activate the resistance gene, transgene silencing is common[11,12] and this has led to the maize ubiquitinin maize promoter being used instead. This promoter can be induced by wounds, infections or environmental stress.

3.2.2 Viral resistance

To introduce resistance to the viral diseases, wheat streak mosaic virus and barley yellow mosaic virus, viral replicase or coat protein genes have been used.[16,17,18,19] Only a small proportion of transformed plants show resistance and resistance tends not to be absolute. GE wheat with the WSMV replicase gene were resistant to one strain of the virus but not another[18]. One line of GE wheat with the WSMV coat protein gene showed reduced symptoms in laboratory studies.[19] However, in other trials, GE wheat was resistant to WSMV in the laboratory but not under field conditions and yields were lower than wheat with traditional sources of resistance.[17]

Another approach to resistance against another RNA virus, barley stripe mosaic virus (BSMV), has been to introduce a bacterial ribonuclease gene (from *Escherichia coli*) into wheat. The GE wheat plants became infected but did not show symptoms of disease.[20]

3.2.3 Insect resistance

Insect resistance genes have been introduced into wheat in an effort to reduce losses caused by insects feeding on stored grain. Approaches have included the use of the lectin gene from the snowdrop[21] and the barley trypsin inhibitor[22] but protection was not complete. Snowdrop lectin reduced the reproduction of the grain aphid, *Sitobion avanae*, but not its survival. The barley trypsin inhibitor inhibited early larval stages but not adults of the Angoumois grain moth (*Sitotroga cerealella*).

3.3 Improved quality

The main ways in which the quality of wheat is being genetically altered is to modify protein content to improve bread making qualities and carbohydrate composition to meet processing needs. Some work has also been undertaken to improve nutritional quality for humans and animals.

3.3.1 Baking quality

The proteins stored in the wheat grain interact with water to form gluten, a highly elastic compound which gives wheat dough its unique properties. The majority of proteins in wheat participate in this reaction but the high molecular weight glutenin subunit (HMW-glutenin) plays a major role in determining bread making quality.[32] Strong doughs, which make the best bread, have higher levels of HMW-glutenin which gives the consistency that traps the bubbles of carbon dioxide produced by yeast fermentation. Efforts have focussed on increasing the expression of the HMW 1-Ax 1 gene by introducing extra copies[33] of the gene. These have succeeded in increasing levels of HMW-glutenin in some cases, but bread quality remains to be tested.

3.3.2 Carbohydrate composition

Starch forms the majority of the dry weight of the wheat grain. Altering the composition of starch in the grain is of interest to the bakery and grain processing industries and the ratio of amylose to amlypoectin determines the properties and end-uses of wheat starch. For example, wheat flour low in amylose is good for noodle making and increasing the level of amylopectin should reduce levels of amylose. The starch branching enzymes play an important role in determining the final amylose content and have become a target of genetic engineering efforts.[32] Efforts to genetically engineer the starch composition of wheat in this way are at an early stage and have included altering the activity of enzymes in the starch synthesis pathway – starch branching enzyme, soluble starch synthase and granule bound starch synthase.[33]

3.3.3 Nutritional quality

Attempts have been made to GE wheat so that is has improved nutrition for both people and animals. To address problems of iron deficiency in diets which are low in this important micro-nutrient, scientists at the John Innes Centre in the UK, have introduced a soybean ferritin gene under the control of the maize ubiquitin promoter into both rice and wheat.[23] In both cases, levels of iron were increased in the leaves but not in the grain and, therefore, could not address nutrient deficiencies.

Scientists in Denmark have genetically engineered wheat with a phytase gene (*phaA*) from *Aspergillus niger*[24] so they produce the enzyme, phytase. The intention is to make wheat a more suitable feed for some animals. Pigs and other monogastric species cannot digest phosphorous as phytate, the form it takes in plants, so the plant phosphorous in their diets is excreted. In order to maximise growth, pigs have increasingly been fed mineral phosphate supplements. Nutrient enrichment of waterways with phosphate and nitrate, or eutrophication, is recognised as a national and international environmental problem.[25] If GE wheat contains phytase, phytate will be broken down, the phosphorous will be available to the animals eating it and phosphorous supplementation would not be necessary.

In another GE approach to the problem, a group at the University of Guelph in Ontario has introduced a gene from the bacteria *Escherichia coli* coding for the phytase enzyme into the salivary glands of pigs so that they can digest plant phytate – the so-called *Enviro-pig*. A reduction of ~ 65% in phosphate content of pig manure was reported in transgenic pigs expressing phytase, and virtual removal of the need for phosphate supplements.[26]

3.4 Others

3.4.1 Hybrid production

Developing reliable wheat hybrid systems has proved very difficult. Most approaches are directed towards establishing male sterility to ensure one plant is pollinated by another (rather than self-pollination which is the norm), but conventional systems tend to leave some male fertility so hybrid seed is not pure. The GE approach has been to utilise the GE oilseed rape system involving the bacterial *barnase* gene under the control of a promoter which targets its expression to the tapetal cell layer of the anther responsible for pollen production.[27] The *barnase* gene codes for the enzyme, barnase, which damages the tapetal layer and thus prevents pollen production. The system, developed by Plant Genetic Systems, now part of by Aventis following the merger of Rhône Poulenc and Hoechst which owned AgrEvo.

3.4.2 Environmental stress

Scientists in the UK, Egypt and the USA have been investigating methods to develop salt and water deficit tolerance in wheat. The UK work has used sense and antisense methods to interfere with the enzymes, argenine decarbolxylase and S-adensolymethionine decarboxylase, which are important in polyamine metabolism and involved in salt tolerance.[28] Researchers in the US and Egypt, have genetically modified spring wheat with a barley gene, HVA1, coding for a protein which protects against water deficit.[29] Compared to non-modified wheat, water use efficiency was increased in laboratory studies.

3.4.3 Pharmaceutical production

Using GE wheat as a production and storage system for pharmaceuticals has been investigated by scientists in the UK and Germany.[30] A gene coding for a mouse antibody fraction was transferred into the wheat and the protein produced in the GE plants but at lower levels that in rice with the same genetic transformation. The experiments were used as ‘proof of principle’, that high-value pharmaceuticals could be produced in wheat.

At the University of North Carolina, scientists are trying to introduce a herpes virus gene into wheat in an effort to see if it will make the protein and then be used as a vaccine.[31] The aspiration is to produce a vaccine which can be given my mouth and which would be cheap and easy to produce.

4. Technical problems with genetic engineering of wheat

All applications of GE to wheat have encountered problems of success and reliability because there appear to be particular technical difficulties in the genetic transformation of wheat. Two main techniques have been used. The most common approach is via the use of 'biolistics', where genes are coated onto microscopic gold particles and fired into wheat cells. In a small proportion of cells, the genes become incorporated into the DNA of the cell and these are then grown into plants and multiplied by conventional means. The other, more recently developed method, uses the *Agrobacterium tumifaciens* organism to 'smuggle' in the DNA. *Agrobacterium* causes a type of tumour (crown gall) plants and can infect cells and transfer DNA into the cells' genes.[32] This technique was only developed more recently because initially it was thought that *Agrobacterium* could not infect the moncot grass species such as wheat, rice and maize.

The genome of wheat is some 10-20 times larger than that of cotton or rice making it much more difficult to reliably genetically modify[32] and transgene silencing, instability and rearrangements are common problems with GE wheat.[32,33,34] The insertion of multiple copies of the transgene appears to underly many of the problems which are experienced. These are most evident with the biolistic method where it is less easy to control the extent of DNA integration compared with use of the *Agrobacterium* method. Although it was thought that the *Agrobacterium* method may allow delivery of a more well defined piece of DNA, both methods have been shown to result in transgene rearrangements[36] and which is the method of choice remains to be established.

Transgene silencing, where the activity of the gene is reduced or abolished, is a particular problem with multiple copies of genes. Silencing in wheat may be progressive over several generations and arises both from methylation of the genes so they are not transcribed and at the post-transcriptional stage. Not all transgenes transferred in one event are affected in the same way (one may be silenced while another may be expressed) and environmental changes may trigger silencing. Even supposedly stable GE lines of wheat have shown such effects over several generations.[35] As a result of such problems, new systems of genetic modification which allow only one copy of a gene to be transferred are being developed.[34] The type of promoter used also influences transgene silencing with the CaMV promoter being particularly vulnerable to transgene silencing effects in wheat.[11,12]

Transgene rearrangements, where the order of introduced genes alters during integration so genes may be reversed for example, appears to be similar in both methods according to studies in rice.[36]

These technical problems raise practical questions both for the success of GE wheat in the short term, but also about whether gene stability can be guaranteed under the varying environmental conditions which will be experienced. It will not be possible to mimic all possible situations in field trials and therefore elements of doubt about performance will remain.

5. Environmental impacts

There are several areas where the use of GE wheat could bring detrimental ecological impacts. These include gene flow to wild related species (altering the gene pool), non-GE wheat or unrelated organisms; secondary impacts on biodiversity as a result of altered management practices; and impacts on non-target organisms. Concerns about the human safety aspects of using GE wheat and associated herbicides have also been raised and these are also considered in this section.

5.1 Gene flow

The potential for gene flow is important for two reasons:

- wild related plants may acquire foreign genes leading to the evolution of more problematic weed species or damage to ecosystems;
- neighbouring organic or conventional non-GE farmers may be unable to sell their crop if it becomes contaminated.

Movement of the introduced, foreign gene(s) from GE wheat could take place through crossing with related wild species, with other non-GE wheat plants in the vicinity or via non-sexual transfer to unrelated species such as soil microorganisms. If GE wheat persisted as a volunteer weed in subsequent crops, this would also add to the potential for gene flow. The factors affecting the likelihood and impact of such gene flow in each situation are considered below.

5.1.1 Related species

For gene flow to occur there has to be cross pollination of the related plant with the production of viable hybrids. The likelihood of hybridisation occurring depends on the compatibility of the two species involved and therefore has to be assessed at a local level. Whether the foreign gene persists and becomes established within a gene pool (known as introgression) depends on the performance of the hybrid and whether it is fertile in following generations. There is a paucity of research data on the potential for gene flow from GE wheat to related species. As will be seen below, it is only in the case of jointed goatgrass, an economically important weed in the USA, that any systematic investigation is being undertaken. In all other instances, information has to be gained from extrapolation, assumption and educated guess work.

T. aestivium is a hexaploid with a total of 42 chromosomes (AABBDD). This means it has six sets of seven chromosomes. Durum wheat is tetraploid (AABB), having four sets of seven chromosomes. Wheat originated in the middle east in the area of Iran, Iraq, Syria and Turkey, with modern forms being derived through crosses between the original wild einkorn wheat, *T. monococcum,* and emmer wheat, *T. turgidum*. Eikorn wheat is still found in southeastern Turkey as is wild emmer wheat which also extends to Mediterranean areas of the middle east.[37] Many spontaneous hybrids and backcrossed progeny are reported to have been found with both bread and durum wheat in Greece, Turkey and Israel.[38]

There are only a few reports of natural hybridisations with others in the *Triciticum* genus. However, in breeding programmes, *T. aestivium* can be crossed with other members of the *Triticum* species sharing the same number of chromosomes (i.e. that are hexaploid) – *T.aestivium* ssp. *vulgare*, *T. compactum, T. spharaerococcum*, *T. vavilovii, T.macha* and *T. spelta*.

Wheat can also cross with some other closely related species including jointed goatgrass, *Aegilops cylidrica*. Jointed goat grass is common in the middle east and the Mediterranean region and is occasionally found in more northern European countries such as Switzerland as a result of seed spread by trucks, trains or birds. In the USA, jointed goat grass is an introduced species which has become a major weed of winter wheat infesting 5 million acres of winter wheat and 2.5 million acres of fallow land and is spreading at the rate of 50,000 acres per year. Yield losses can be up to 50% and total costs of goatgrass in the USA are estimated to be $145 million a year.[39,40]

Studies in the USA and Switzerland have shown that natural hybridisations can take place in the field between wheat and jointed goatgrass.[41, 42,43,44] The likelihood of hybridisation between wheat and goatgrass in the field is underestimated in greenhouse conditions where the levels of pollen are much lower than those found in a field of wheat[41,44] and hybrids could arise very rapidly. In one of the US studies,[43] herbicide tolerant wheat/goatgrass hybrids were found the year following a trial with non-GE imazamox tolerant wheat. A survey of Oregon wheat fields in 1998, 1999 and 200, identified wheat/goatgrass hybrids in over 50% of the cultivated fields examined and 20% of non-field sites[40] and these are thought to be becoming more common in the Northwestern USA.[45] Although the fertility of hybrids produced is low, in the order of 2-7% viable seed produced, self-fertility increases in subsequent generations.[44] Because the area over which wheat is grown and where jointed goatgrass also occurs is vast, low frequency events will inevitably arise. Swiss researchers concluded in relation to GE wheat that '*the possibility of gene transfer from wheat to jointed goatgrass under natural conditions (e.g. agroecosystems) is likely*' and that '.. *even a small number of plants receiving an herbicide resistance gene could have important consequences on wheat cultivation in regions where both species grow intermixed*'[41].

Gene flow may not be restricted to species that are already considered weeds. In northern Europe, there are two wild relatives that wheat have been crossed with in controlled breeding studies, sea barley (*Hordeum marinum*. Huds.) and bearded wheat grass (*Elymus caninus* L.). In a study of English and Austrian populations of these plants, one bearded wheat grass plant found in Sounthy Wood near Peterborough in England, showed evidence of wheat specific genes, demonstrating that natural hybridsation and introgression of wheat genes into wheatgrass is possible.[46]

It is unclear what the impact of the transfer of genes from GE wheat would be but if there was some kind of competitive advantage as a result, the plants could disturb natural ecosystems or become a problem weed. It is quite easy to see how the acquisition of a herbicide tolerant gene could lead to the evolution of weeds which are more difficult to manage. If herbicide tolerance genes were transferred from GE wheat into goatgrass which made it more difficult to manage, economic losses could be great. There are potentially harmful impacts of the transfer of other genes which are being used in GE wheat. The transfer of disease or environmental stress resistance genes could lead to better survival of the plants acquiring the foreign genes, and disruption of ecosystems could result if they survived and displaced other species. The way in which disease controls natural populations of plants is very poorly understood so the effects are impossible to predict with any accuracy.[47] With use of viral genes, recombinations with infecting viruses and the evolution of new viruses is possible. The use of viral genes as sources of resistance has also been noted to '*introduce a substantially new dimension into the dynamics of plant/virus coevolution...'.* [47]

Genes that alter the protein, carbohydrate or other biochemical pathways could alter the survival properties of seed and the likelihood of germination. This could give either a positive or negative advantage to the plants acquiring the genes. If the transferred gene gave a disadvantage, there could be local populations extinctions as have been recorded following transfer of genes from cultivated rice to wild species.[48]

5.1.2 Non-GE wheat

There are no compatibility barriers between GE and non-GE wheat so gene transfer is not constrained by biological compatibility - separation distance will be the major factor influencing whether cross-pollination takes place. Wheat is mainly self-pollinating (with outcrossing rates thought to be less that 1%) and produces a relatively small amount of pollen compared to other species - 2.5% of that produced by maize and 10% of that by rye – which has a short viability period. Therefore, small separation distances of 1.5 – 3.0 metres are used for the production of certified seed and 3 – 10 metres for pedigreed seed. However, studies of outcrossing rates of 10 Canadian spring wheat cultivars showed that outcrossing can be over 6% and the author suggested that separation distances may need to be greater to minimise contamination through outcrossing.[49] There seems to be no specific data on the potential for long-distance wheat pollen transfer but it has been suggested that, by comparison to another predominately self-fertilising species, barley, that significant levels of outcrossing could occur up to 60 metres.[50]

The consequences of gene transfer to non-GE wheat are largely associated with the ability to supply a non-GM market. However, if pharmaceutical proteins were produced in wheat or other potentially harmful proteins were found in GE wheat, these could pose health threats.

5.1.3 Horizontal gene flow

Movement of foreign genes from the GE wheat to unrelated species such as bacteria may occur either in the soil or following ingestion although this is much less likely than gene flow via pollination. In the soil, as the plant remains degrade at the end of the growing season, bacteria could take up and incorporate the foreign genes into their own DNA. Laboratory studies have shown that that microorganisms can take up plant DANN.[51] However, despite persistence of transgenic DNA following field releases of GE sugar beet, no horizontal gene transfer to bacteria was detected.[52] There are no specific data available about GE wheat.

What the impact would be if horizontal transfer from GE wheat to soil microorganisms did occur is difficult to predict but would depend to some extent on the gene involved. Microorganisms with herbicide tolerance genes may be present in soil organisms already and therefore have little net effect. However, with antibiotic resistance genes the potential for harm may be greater as the genes may be passed to disease causing organisms and compromise the effectiveness of treatment.

Antibiotic resistance genes are used as ‘marker genes’ in GE crops. The process of genetic modification is inefficient and only a small number of cells incorporate the foreign genes (to make them insect resistant, for example). Therefore, an antibiotic resistance gene is linked to the gene of interest and, if the genetic modification is successful, the plant cell will grow in the presence of antibiotic: if not, it will die. In many GE wheat varieties being tested, genes giving resistance to the antibiotics neomycin and kanamycin are present and ampicillin resistance genes have been used in GE wheat in UK trials (although the resistance is not thought to be expressed). If these genes were transferred to disease causing organisms they may compromise antibiotic treatment. This could happen if the genes were taken up by microorganisms in soil or in the intestines of a person or animal eating the wheat and were then passed to harmful bacteria. Transfer of genes from plant material to bacteria is rare, but gene exchange between microorganisms is common.

Most wheat is used for human food consumption. Although the whole grain is rarely consumed, processing to produce flour or semolina involves milling and grinding rather than the use of high temperature or chemical treatments. It has been shown that milling and grinding of wheat does not breakdown DNA and that temperatures of at least 95^{o}C is required to degrade DNA in air-dried wheat grain.[53] Whilst DNA may be broken down to some extent during cooking, intact DNA will therefore be present in food and thus gene transfer to microorganisms in the intestines possible following ingestion. The antibiotics neomycin and kanamycin are not widely used[*] ampicillin is commonly used. The antibiotic marker genes have no function in the plant and could have been removed during a later stage of the genetic modification procedure but this would have delayed their commercialisation. Some medical organisations, such as the British Medical Association, have called for a ban on the use of antibiotic resistance marker genes.[54] However, the risk posed by antibiotic marker genes in GE crops is much smaller that posed by the abuse of antibiotics in human and veterinary medicine.

5.2 Volunteer weeds

Grain shed from wheat before or during harvesting many remain in the soil, germinate and emerge in the next season’s crop. Then the wheat is a weed which is known as a ‘volunteer’. If wheat volunteers are herbicide tolerant, they could become much more difficult for farmers to control. Wheat volunteers are a major problem because they can carry viral and fungal diseases over from one season to the next which may then spread to neighbouring fields. The persistence of GE volunteers could also add to gene flow, as the GE wheat could cross-pollinate wheat gown on the field in later seasons.

[*] *Neomycin is used in cases of drug resistant TB and in some veterinary medicines*

When considering the potential for Roundup Ready wheat to become a problem volunteer in Canada, it has been shown that wheat seed can persist in soil for at least five years and that spring wheat is grown, on average, twice in every five years.[55] This persistence rate is similar to that of oilseed rape, and although oilseed rape cross-pollination rates are higher that that of wheat, it is grown less frequently in rotations. The scientists from the University of Manitoba concluded that if Roundup Ready wheat was managed in the same was as herbicide tolerant oilseed rape that:
'a release of Roundup Ready wheat … would lead to perhaps an even more rapid and extensive uncontrolled spread of the Roundup Ready gene in the wheat genome of western Canada'.

Volunteer oilseed rape weeds that are tolerant to three herbicides (Liberty, Roundup and Clearfield), were first identified in Canada in1998, only 3 years after GE herbicide tolerant oilseed rape was first grown.[56,57] The emergence of herbicide tolerant oilseed rape volunteers in Canada is driving up the use of other, more toxic chemicals. Both 2,4D and paraquat (grammoxone) are being recommended by government agencies to control herbicide tolerant oilseed rape volunteers in Canada.[58]

Given the scale upon which wheat is grown globally, if herbicide tolerant volunteers were to become commonplace, the increase in use of damaging herbicides to control them could be enormous with serious implications for the environment. If farmers are unable or unwilling to control volunteers, diseases may spread more widely.

5.3 Herbicide toxicity

The use of GE herbicide tolerant wheat will inevitably involves the use of a herbicide in combination with the crop. If use of the herbicide increases, exposure of workers will increase and residues in the final product could increase consumer exposure. Glyphosate (Roundup) has relatively low acute toxicity to mammals as it acts on an enzyme system in plants which is not present in animals. However, preparations of glyphosate often include a surfactant which not only increases toxicity to fish and other aquatic species but can cause serious eye irritation and allergic reactions. There are some indications that chronic exposure to glyphosate can be harmful if administered at high doses over prolonged periods (Pesticide Action Network, 1996).

5.4 Indirect effects

As well as the possible effects on the environment that may arise through gene transfer, there may be other indirect effects as a result of the growing of GE crops. These may be associated with changed management practices that arise, or may result from knock on effects of the foreign gene product leading to impacts on agricultural biodiversity.

Management practices can have profound effects on agricultural biodiversity. The widespread use of herbicides in conventional farming has already been associated with declines in farmland birds and other species and there are fears that the increased use of broad spectrum herbicides with tolerant GE crops will result in the highly efficient removal of weeds and a consequent decrease in food supplies for invertebrates and birds. The use of Roundup Ready GE wheat could affect biodiversity because of the increased use of glyphosate, a broad spectrum weed killer. Although this is now a well recognised hazard that could arise from the increased use of glyphosate, there have been no studies conducted in relation to Roundup Ready wheat to determine the impact it may have. In Europe, under the revised regulations, (Directive 2001/18/EC) data on such indirect effects is now required before approval is given.

The other ways in which wheat is being genetically engineered could also have negative impacts on biodiversity although, as with gene flow, there is little or no research taking place to address such concerns. For example, the use of fungal resistant GE wheat could alter the normal plant/fungal interactions that take place.[59] The introduction of genes coding for compounds toxic to insects may lead to adverse effects on non-target species. When used in potatoes, the snow drop lectin gene which has been introduced into wheat to give insect resistance against grain aphid,[21] harmed beneficial insects (ladybirds) feeding on the pests (aphids) which had consumed the lectin. Female ladybird longevity was reduced and adverse effects on reproduction recorded.[60]

If resistance to environmental stress such as temperature or drought, allowed for expansion of the area of wheat grown into marginal lands or displaced other crops, there are likely to be environmental effects. The replacement of spring wheat by winter wheat varieties in the UK has, as already noted, cause serious adverse impacts on farmland biodiversity.[4] Once more, there appears to be no research such as modelling taking place to inform evaluations of possible impacts.

6. Conclusions

The growing of GE wheat could pose serious environmental and agricultural risks. Because wheat is gown on such a large scale across the world and the area is expected to expand, any adverse impacts could be enormous. However, there is very little research taking place which is addressing the potential environmental impacts. It is only in relation to possible hybridisation with an economically important weed of wheat in the USA that serious work is taking place. The initial findings show that the potential for a serious weed problem to emerge is very real. This would necessitate the use of other herbicides to control the weeds. A recent study by English Nature[61] revealed the widespread emergence of multiple herbicide resistant volunteer oilseed rape plants following the growing of GE oilseed rape in the Canadian prairies. As a result, known toxic chemicals such as 2,4-D are being used to control the new weeds. These problem weeds emerged only three years after GE oilseed rape was first grown in Canada illustrating how rapidly such situations may arise. In the Canadian situation, hybridisation with another species was not required so the emergence of herbicide tolerant jointed goatgrass weeds may be anticipated to take longer to become widespread. However, the finding of herbicide tolerant goatgrass in the year following an experimental trial is not encouraging.[43] While the problem of jointed goatgrass as a weed is currently restricted to the USA, the acquisition of herbicide tolerance or other stress resistance genes if these are used in GE wheat could lead to extensions to its range.

Jointed goatgrass is an introduced species in North America, but it and other related species could be at risk of gene introgression from GE wheat if they grow in close proximity. It is the Middle East countries - Iran, Iraq, Syria and Turkey - where wheat evolved that the risk will be greatest. The acquisition of genes giving resistance to disease or environmental stress could affect ecosystem balance. However, there is little or no research from which to evaluate the risks or to identify areas at greatest risk where wild and cultivated wheat are coincident.

The only type of GE wheat which is close to commercialisation in Roundup Ready – tolerant to Monsanto’s herbicide glyphosate. If commercialised, this would be added to the growing list of Roundup Ready crops and increase the use of glyphosate further which would also increase selection pressure for the emergence of glyphosate resistant weeds. There is already resistance to glyphosate occurring in the Australian populations of annual ryegrass and Malaysian populations of goosegrass.[62]

The further development of GE varieties is hindered by the technical problems which dog GE wheat. These are of more than academic interest because gene instability, silencing and rearrangements could have consequences for the performance of GE wheat, especially as it encounters different environments. It is unlikely that all potential performance problems will be identified during laboratory studies even though the most obvious will be. This raises questions for farmers about the reliability of GE wheat if they choose to use it. To maintain consumer choice and supply the demand for non-GM food in Europe and elsewhere farmers will also have to ensure separation of GE from non-GE wheat. To minimise gene flow from GE to non-GE or organic wheat will require separation distances in the order of 60 metres but further research on long-distance pollen movement is required

It is the vast scale upon which wheat is grown globally that means that the risks of GE wheat have to be taken seriously. Although gene flow is less likely than for more promiscuous species like oilseed rape, if GE wheat is grown commercially, it is inevitable at some stage. Currently there is no evidence that such are risks are being addressed in research programmes.

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