

# *Transgenic cereals: current status and future prospects*

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Transgenic cereals: current status and future prospects

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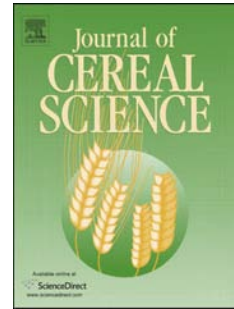
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- The current commercial status of GM cereal is described
- Research on input (agronomic characteristics) and output (grain quality etc) traits is reported
- Data from global field trials are summarised
- Research trends from examination of patent databases are reported
- Public perception and regulatory issues are discussed

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# 1 Transgenic cereals: current status and future prospects

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## 11 **Abstract**

12  
13  
14 This review summarises the history of transgenic (GM) cereals, principally maize, and then  
15 focuses on the scientific literature published in the last two years. It describes the production  
16 of GM cereals with modified traits, divided into input traits and output traits. The first  
17 category includes herbicide tolerance and insect resistance, and resistance to abiotic and  
18 biotic stresses; the second includes altered grains for starch, protein or nutrient quality, the  
19 use of cereals for the production of high value medical or other products, and the generation  
20 of plants with improved efficiency of biofuel production. Using data from field trial and  
21 patent databases the review considers the diversity of GM lines being tested for possible  
22 future development. It also summarises the dichotomy of response to GM products in various  
23 countries, describes the basis for the varied public acceptability of such products, and  
24 assesses the development of novel breeding techniques in the light of current GM regulatory  
25 procedures.

26

27 **Highlights**

28

29 *Keywords:* Genetically modified; Maize; Wheat; Barley

30

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50

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55

56

57            **6. Acceptance of GM crops**

58            *6.1 Regulatory aspects*

59            *6.2 Public perception*

60

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62

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64       **8.**

65       **1. Background**

66

67       On a global basis the cereals wheat, maize, rice, barley and sorghum are grown on almost 700  
68       million hectares and collectively they provide approximately 40% of the energy and protein  
69       components of the human diet (Table 1). They therefore represent a vital contribution to food  
70       security both at present and also in the future when population growth (Dunwell, 2013) and  
71       other social and economic trends will require an approximate doubling of food production by  
72       2050. Specific retrospective and prospective data for wheat yields, based on information from  
73       the Wheat initiative ([www.wheatinitiative.org](http://www.wheatinitiative.org)) are given in Table 2. In the words of the G20  
74       Agriculture vice-ministers and deputies report from 2012 “Increasing production and  
75       productivity on a sustainable basis in economic, social and environmental terms, while  
76       considering the diversity of agricultural conditions, is one of the most important challenges  
77       that the world faces today” (<http://www.g20.org/en>) . The UK Secretary of State for the  
78       Department for the Environment, Food and Rural Affairs made a major speech on 20<sup>th</sup> June  
79       2013 about the role of GM in the future of agriculture  
80       ([https://www.gov.uk/government/speeches/rt-hon-owen-paterson-mp-speech-to-rothamsted-](https://www.gov.uk/government/speeches/rt-hon-owen-paterson-mp-speech-to-rothamsted-research)  
81       research), and the European Academies Science Advisory Council has recently published a  
82       detailed report on the opportunities of using GM technologies in sustainable agriculture  
83       (EASAC, 2013).

84

85       Against the background of this need for increased agricultural production, this review will  
86       consider the history of genetically modified (GM) or transgenic cereals during the 30 year  
87       period since the production of the first GM plants in 1983, before discussing their present  
88       status and future potential. Information has been obtained not only from recent scientific

89 literature but also from analysis of regulatory databases for GM crops, and from the patent  
90 literature.

91

## 92 **2. Methods for production of GM plants**

93

94 The original method devised for the production of the first GM plants in 1983 depended on  
95 the use of the natural bacterial vector *Agrobacterium tumefaciens*. At that time it was  
96 assumed that this system could not be applied to cereal species and the emphasis for these  
97 crops was focussed on direct gene transfer methods, particularly the “gene-gun” or Biolistics  
98 technology. This technology was the first method successfully applied to maize. Since that  
99 time, significant improvements have been made to the *Agrobacterium* techniques, and these  
100 techniques can now also be applied to cereals. A recent summary of a diverse range of GM  
101 techniques is available in Dunwell and Wetten (2012).

102 These novel technologies include new methods for the design of constructs (Coussens et al.,  
103 2012; Karimi et al., 2013), that is the DNA sequences to be introduced and improved  
104 methods for DNA delivery. These latter methods include techniques for maize (Kirienko et  
105 al., 2012), wheat (Tamás-Nyitrai et al., 2012), rice (Duan et al., 2012b; Wakasa et al., 2012),  
106 barley (Holme et al., 2012a), triticale (Ziemienowicz et al., 2012), and tef (*Eragrostis tef*)  
107 (Gebre et al., 2013). There is also an improved understanding of the process of regeneration  
108 from plant cells in culture (Delporte et al., 2012), an important aspect of any system for high  
109 efficiency transformation.

110 Temporal and spatial stability of transgene expression, as well as well-defined transgene  
111 incorporation are additional features to be considered (Bregitzer and Brown, 2013; Kim and  
112 An, 2012). Likewise, it is of practical importance that GM lines can be rapidly identified,

113 both in the laboratory (Chen et al., 2012b; Han et al., 2013b; Hensel et al., 2012; Mieog et al.,  
114 2013; Xu et al., 2013a) and under field conditions.

115

116 Another objective in many GM research projects is the development of more efficient  
117 methods for the introduction of multiple genes. These include the construction of mini-  
118 chromosomes in rice (Xu et al., 2012a). Additionally, there has been significant progress with  
119 efforts to induce site-specific gene integration (Nandy et al., 2012; Kapusi et al., 2012) and to  
120 use GM techniques to suppress selected genes or gene families (Wang et al., 2013b). Some of  
121 these techniques are also associated with the new techniques described below in section 5.3.

122

123 Immediately following the description of GM plants of tobacco in 1983, the commercial  
124 focus became the development of GM maize, as this crop was already hybrid and annual  
125 sales of such high-value seed was an established part of the agricultural economy of the USA  
126 and elsewhere. In contrast, the other important cereals wheat and rice are self-pollinating  
127 crops and the value of seed sales is comparatively low, and any GM variety could in theory,  
128 if not in practice, be saved by the farmer for growth in subsequent years. For this reason,  
129 there have been several attempts to convert inbreeding species into hybrid crops either  
130 through the use of chemical hybridizing agents or via GM technology. One GM approach to  
131 the production of male sterility, a necessary component of any hybrid system (Feng et al.,  
132 2013), has recently been exemplified in wheat by expressing a barnase gene (Kempe et al.,  
133 2013).

134

135 In the summaries below, the specific traits incorporated into GM varieties will be divided into  
136 those that provide advantages to the farmer/grower, the so-called input traits and those that  
137 modify the characteristics of the harvested product, the so-called output traits.

138

139 **3. Input traits**

140

141 *3.1. Herbicide tolerance*

142

143 Prior to GM technology herbicides were classified into two categories, either selective, those  
144 that killed weeds and not crops, and non-selective, those that killed all plants. The  
145 development of selective herbicides, in particular, is a very difficult research challenge that  
146 requires an understanding of biochemical targets found only in weeds. Transgenic technology  
147 opened the possibility of converting non-selective compounds into selective ones, if a gene  
148 conferring resistance could be identified, isolated and then transferred into the crop of  
149 interest. The most obvious candidate for this strategy was glyphosate, a widely used selective  
150 herbicide marketed by Monsanto. Eventually, a bacterial resistance gene was identified and  
151 Monsanto subsequently acquired this technology, the means of introducing this gene into  
152 maize, and a company which owned elite maize inbred lines, the target for this technique.  
153 This company then had the significant commercial advantage of being able to sell both GM  
154 herbicide-tolerant (HT) varieties, and the herbicide in question. This combined approach  
155 became highly successful and provided the blueprint for many subsequent commercial  
156 programmes in maize and other crops. The second major herbicide resistant trait was that  
157 conferring tolerance to glufosinate. The commercial need for companies to be able to market  
158 both the herbicide and HT crops containing the gene conferring tolerance led to many  
159 conflicts associated with intellectual property rights (IPR) and many mergers and  
160 acquisitions. The process of consolidation of IPR began in earnest in August 1996 with  
161 AgrEvo's purchase of Plant Genetic Systems (PGS) for \$730 million, made when PGS's  
162 prior market capitalization was \$30 million. According to AgrEvo, \$700 million of the

163 purchase price was assigned to the valuation of the patent-protected trait technologies (ie  
164 glufosinate resistance gene) owned by PGS (Pila, 2009). In all such cases it is important to  
165 avoid any yield drag associated with the presence of the transgene (Darmency, 2013).

166

167 At present most hybrid maize sold in the USA is resistant to one or more herbicides. The  
168 availability of such HT crops has provided the farmer with a variety of flexible options for  
169 weed control (Brookes and Barfoot, 2013a), despite some problems caused by the  
170 development of HT weeds, an issue that has stimulated the development of improved  
171 versions of glyphosate resistance genes and also of novel genes encoding resistance to other  
172 herbicides such as 2,4-D. In some regions, particularly in sub-Saharan Africa, HT maize has  
173 also provided a novel control strategy for hemi-parasitic weeds such as *Striga* (Ransom et al.,  
174 2012).

175

176 One novel finding in the area of HT crops is that showing the resistance of melatonin-rich  
177 GM rice plants to herbicide-induced oxidative stress (Park et al., 2013).

178

179 Monsanto also developed a glyphosate tolerant (Roundup Ready<sup>TM</sup>) version of wheat, and  
180 carried out successful field tests in the 1990s. Due to concerns about international trade of  
181 GM wheat, this project was suspended in 2005, although recently in April 2013 some HT  
182 wheat plants carrying the Monsanto CP4 gene for glyphosate tolerance have been discovered  
183 growing in a farm in Oregon; their origin is uncertain (Fox, 2013; Ledford, 2013).

184

185

186 *3.2 . Insect resistance*

187

188 The second target for GM development, together with herbicide tolerance, was insect  
189 resistance, specifically the potential that might be provided by the toxins found in the soil  
190 bacterium *Bacillus thuringiensis* (Bt). Various proteins from this bacterium were known to be  
191 toxic to a range of insects and had been used widely as sprays in agriculture and forestry  
192 since the 1950s. Improvements in molecular biology and microbiology during the 1980s  
193 meant that the genes encoding these proteins could now be isolated from various strains of  
194 the bacterium and introduced into crops. The first target was the corn borer (*Ostrinia*  
195 *nubilalis*), a lepidopteran pest of maize. Subsequently, other Bt genes were isolated; these  
196 provided resistance to other pests including the coleopteran species, corn root worm  
197 (*Diabrotica* spp.) (Narva et al., 2013). Present maize varieties sold in the USA have several  
198 Bt genes, usually combined with herbicide tolerance (Edgerton et al., 2012); in total there  
199 may be eight transgenes in a single variety. Recently the experience obtained from the first  
200 billion acres of Bt crops was reviewed (Tabasnik et al., 2013).

201

202 Such analysis has several aspects. One of the most important has been the need to prolong the  
203 life time of these GM varieties by avoiding the development of resistance in the target  
204 insects; the history of many insecticides suggests that resistance will eventually develop after  
205 prolonged application of any particular compound. Since the first GM products were  
206 marketed there has been advice on the need for refugia, areas of non-GM plants (Tabashnik  
207 and Gould 2012). This strategy reduces the incidence of insects carrying a mutant resistance  
208 gene in the homozygous state. As this refugia policy was not adopted by some farmers,  
209 resistant insects have indeed developed in recent years, and it is now suggested that at least  
210 five pests have developed such resistance (Tabasnik et al., 2013). Novel approaches to this  
211 issue include the combination of different Bt genes (Edwards et al., 2013), or genes with

212 different modes of action, and the adoption of seed mixes in which Bt and non-Bt seeds are  
213 combined (Carroll et al., 2013; Zukoff et al., 2012).

214

215 Another significant environmental concern is the possibility of non-target effects, that is the  
216 susceptibility of non-pest beneficial insects to the various insecticidal proteins. This is a key  
217 element of all regulatory applications for sale of such products. Recent studies of this topic  
218 include those on the effects of Bt rice on a generalist spider (Tian et al., 2012) and thrips  
219 (Akhtar et al., 2013), Bt maize on bees (Dai et al., 2012) and other arthropods (Alcantera  
220 2012; Comas et al., 2103), and the effect on aphids of GM wheat expressing a snowdrop  
221 lectin (Miao et al., 2011).

222

223 There have also been some unexpected beneficial side-effects of insect resistant crops. For  
224 example, Bt-expressing corn rootworm resistant maize has been shown to have improved  
225 nitrogen uptake and nitrogen use efficiency (Haegele and Below, 2013). These results may  
226 lead to improved agronomic practices (Bender et al., 2013). Similarly, increased microbial  
227 activity and nitrogen mineralization has also been shown in Bt maize (Velasco et al., 2013).  
228 This contrasts with the data of Cotta et al. (2013), Lupwayi and Blackshaw (2013) and  
229 Fließbach et al. (2013) who found no differences in the microbial communities from the  
230 rhizosphere of GM and non-GM maize, and particularly of Han et al. (2013a) who claim that  
231 Bt rice reduced the methane emission flux and the methanogenic archaeal and bacterial  
232 communities in paddy soils.

233

234 Other approaches to insect resistance include modification of the volatile emissions produced  
235 by a plant in order to deter pests or to attract beneficial insects. Such a study of GM maize  
236 expressing a terpene synthase gene showed that the costs of constitutive volatile production

237 outweighed its benefits (Robert et al., 2013). An alternative route is to use plant-derived  
238 double-stranded RNA to target the suppression of genes essential for insect survival. This  
239 method has been shown to be effective in inhibiting growth of the Western Corn Root Worm  
240 (*Diabrotica virgifera*) (Bachman et al., 2013; Bolognesi et al., 2012).

241

### 242 3.3. Pathogen tolerance

243

#### 244 3.3.1. Fungi

245 Although there are no commercial GM cereals with pathogen tolerance there has been a great  
246 deal of research on this subject, with promising results from both laboratory and field tests,  
247 particularly with wheat ([http://www.isaaa.org/resources/publications/pocketk/document/Doc-](http://www.isaaa.org/resources/publications/pocketk/document/Doc-Pocket%20K38.pdf)  
248 [Pocket%20K38.pdf](http://www.isaaa.org/resources/publications/pocketk/document/Doc-Pocket%20K38.pdf)). Wheat is affected by a number of fungal diseases such as stem rust  
249 (*Puccinia graminis*), *Septoria*, *Fusarium*, common bunt (*Tilletia tritici*) and take-all, caused  
250 by the fungus *Gaeumannomyces graminis*. Among these diseases, *Fusarium* is probably the  
251 most significant, causing crown rot and head blight that result in production of small and  
252 stunted grains or no grain at all. Some *Fusarium* strains also produce mycotoxins, compounds  
253 which when ingested by humans or animals may cause serious illness. These toxins, which  
254 are subject to regulation in the human food chain, can also inhibit the growth of yeast during  
255 the fermentation of cereal starch to produce bioethanol. For many years Syngenta worked on  
256 the development of a *Fusarium*-resistant wheat but this project was suspended in 2007, also  
257 after concerns about exports of GM wheat from the USA. Among the genes that have been  
258 shown to provide resistance to this fungus are a bovine lactoferrin gene (Han et al., 2012;  
259 Lakshman et al., 2013), an *Arabidopsis thaliana* *NPRI* (non-expressor of PR genes) gene  
260 (Gao et al., 2013), a polygalacturonase-inhibiting protein gene from *Phaseolus vulgaris*  
261 (PvPGIP) (Ferrari et al., 2012) (see also Janni et al., 2013), a lipid transfer gene from wheat



262 (Zhu et al., 2012b) and the antimicrobial peptides genes *MsrA2* and *IOR* (Badea et al., 2013).  
263 Results from this latter study showed that T3 generation GM plants had a 53% reduction in  
264 *Fusarium* damaged kernels, and some lines also had a 59% reduction in powdery mildew  
265 susceptibility compared with the non-GM control.

266

267 Other GM approaches to achieving mildew resistance in wheat include the use of virus-  
268 induced gene silencing (VIGS) of *Mlo* genes (Várallyay et al., 2012), alleles of the resistance  
269 locus *Pm3* in wheat, conferring race-specific resistance (Brunner et al., 2012). Related studies  
270 on this latter material showed that the mildew-resistant GM lines harboured bigger aphid  
271 populations (*Metopolophium dirhodum* and *Rhopalosiphum padi*) than the non-transgenic  
272 lines (von Burg et al., 2012). These results suggest that wheat plants that are protected from a  
273 particular pest (powdery mildew) became more favourable for another pest (aphids). Other  
274 evidence with the same material comes from a study of plots containing either monocultures  
275 or mixtures of two GM lines (Zeller et al., 2012). It was found that resistance to mildew  
276 increased with both GM richness (0, 1, or 2 *Pm3* transgenes with different resistance  
277 specificities per plot) and GM concentration (0%, 50%, or 100% of all plants in a plot with a  
278 *Pm3* transgene). Additional studies by Zeller et al. (2013) concluded that many genes  
279 providing resistance against fungal pathogens demonstrate a significant cost of resistance  
280 when expressed constitutively. Studies on powdery mildew in barley include one that  
281 examined the effect of modifying the expression of the HvNAC6 transcription factor (Chen et  
282 al., 2013).

283

284 Other recent tests have described resistance to take-all in GM wheat lines expressing an  
285 R2R3-MYB gene from *Thinopyrum intermedium* (*TiMYB2R-1*) (Liu et al., 2013b) or a potato  
286 antimicrobial gene (Rong et al., 2013), to *Bipolaris sorokinia* by expression of the related

287 gene *TaPIMPI* (Zhang et al., 2012d), to *Penicillium* seed rot in lines expressing  
288 puroindolines (Kim et al., 2012), and to rust diseases by endogenous silencing of *Puccinia*  
289 pathogenicity genes (Panwar et al., 2013) and expression of the *Lr34* durable resistance gene  
290 (Risk et al., 2012, 2013) or TaRLP.1 (Jiang et al., 2013b). The recent discovery of the wheat  
291 *Sr35* gene that confers resistance to the Ug99 strain of rust (Saintenac et al., 2013) may also  
292 provide new GM strategies to combat this disease.

293

294 Related results from rice include resistance to rice blast (*Magnaporthe oryzae*) in lines  
295 expressing a chimeric receptor consisting of the rice chitin oligosaccharides binding protein  
296 (CEBiP) and the intracellular protein kinase region of *Xa21* (Kouzai et al., 2013). Similarly  
297 lines expressing the *WRKY30* gene showed improved resistance to rice blast and rice sheath  
298 blast (*Rhizoctonia solani*) (Peng et al., 2012), and lines expressing a bacterial  $\alpha$ -1,3-  
299 glucanase (AGL-rice) showed strong resistance not only to the two blast pathogens but also  
300 to the phylogenetically distant ascomycete *Cochlioborus miyabeanus* (Fujikawa et al., 2012).

301

302 In maize silencing of a putative cystatin gene (*CC9*) improved resistance to the biotrophic  
303 pathogen *Ustilago maydis* (van der Linde et al., 2012)

304

305 3.3.2. Bacteria

306

307 It has been shown recently that silencing of the dominant allele of rice bacterial blast  
308 resistance gene *Xa13* by using artificial microRNA technology generates plants highly  
309 resistant to this pathogen (Li et al., 2012a). These authors suggest that this approach may  
310 provide a paradigm that could be adapted to other recessive resistance genes. In an alternative  
311 approach, expression of *TaCPK2-A*, a calcium-dependent protein kinase gene that is required  
312 for wheat powdery mildew resistance has been shown to enhance bacterial blight resistance  
313 in transgenic rice Geng et al., 2013).

314

### 315 3.3.3. *Viruses*

316 Projects designed to improve virus resistance in cereals include expression of an artificial  
317 microRNA to provide resistance to wheat streak mosaic virus (Fahim et al., 2012), and of a  
318 dsRNA-specific endoribonuclease gene to provide resistance to maize rough dwarf disease  
319 (MRDD) (Cao et al., 2013). It has been reported that a wheat line with resistance to yellow  
320 mosaic virus is expected to be available in the market by 2015  
321 (<http://www.isaaa.org/resources/publications/pocketk/document/Doc-Pocket%20K38.pdf>).  
322 Related studies in rice include resistance to rice stripe disease (RSD) (caused by rice stripe  
323 virus, RSV) by expression of an RNAi construct containing the coat protein gene (CP) and  
324 disease specific protein gene (SP) sequences from RSV (Zhou et al., 2012b). A similar  
325 strategy was employed to improve resistance to the rice gall dwarf virus (RGDV) (Shimizu et  
326 al., 2012b) and rice grassy stunt virus (Shimizu et al., 2013).

327

328

### 329 3.4 *Abiotic stress*

330

331 Following the great commercial success of herbicide tolerant and insect resistant crops,  
332 research focus moved to the more difficult subject of tolerance to abiotic stress such as  
333 drought, salt tolerance and nitrogen and phosphate deficiency. The first commercial cereal  
334 product in this area is the Monsanto GM maize DroughtGard™ variety that expresses *cspB*,  
335 an RNA chaperone gene from *Bacillus subtilis* (Castiglioni et al., 2008). This gene, which  
336 increases yield under water-limited conditions, is also being incorporated into maize adapted  
337 to African conditions, as part of the WEMA project (Water Efficient Maize for Africa).  
338 There is a wide range of other approaches that are being tested at present in order to improve  
339 the growth of cereals under conditions of abiotic stress (Saint Pierre et al., 2012). For  
340 example, wheat over-expressing the 12-oxo-phytodienoic acid gene (*TaOPRI*) significantly  
341 enhanced the level of salinity tolerance (Dong et al., 2013). It is thought that this gene acts  
342 during episodes of abiotic stress response as a signaling compound associated with the  
343 regulation of the ABA-mediated signalling network. It is also reported that barley plants  
344 expressing the mitogen activated protein kinase HvMPK4 demonstrated improved tolerance  
345 to saline conditions (Abass and Morris, 2013).

346

347 Overexpression of a phytochrome-interacting factor-like protein, OsPIL1, in transgenic rice  
348 plants promoted internode elongation (Todaka et al., 2012). The data suggested that OsPIL1  
349 functions as a key regulatory factor of reduced plant height via cell wall-related genes in  
350 response to drought stress and may be useful in improving plant regrowth under such  
351 conditions.

352

353 GM rice overexpressing the transcription factor OsbZIP16 exhibited significantly improved  
354 drought resistance, which was positively correlated with the observed expression levels of  
355 OsbZIP16 (Chen et al., 2012a). Related data come from studies of GM rice overexpressing

356 Oshox22, which belongs to the homeodomain-leucine zipper (HD-Zip) family I of  
357 transcription factors (Zhang et al., 2012b). These authors conclude that Oshox22 affects ABA  
358 biosynthesis and regulates drought and salt responses through ABA-mediated signal  
359 transduction pathways. A number of similar results have been reported by overexpression of  
360 several diverse genes in GM rice. These include, *OrbHLH001*, a putative helix-loop-helix  
361 transcription factor, that confers salt tolerance (Chen et al., 2012a); ZFP182, a TFIIIA-type  
362 zinc finger protein, that significantly enhanced multiple abiotic stress tolerances, including  
363 salt, cold and drought tolerances (Huang et al., 2012); OsLEA3, a Late Embryogenesis  
364 Abundant protein, that showed significantly enhanced growth under saline conditions and  
365 was better able to recover after 20 days of drought (Duan and Cai, 2012); a DEAD-box  
366 helicase that improves growth in 200mM salt (Gill et al., 2013); and myo-inositol oxygenase  
367 (MIOX), (a unique monooxygenase that catalyzes the oxidation of myo-inositol to d-  
368 glucuronic acid) that improves drought tolerance by scavenging of reactive oxygenase  
369 species (Duan et al., 2012a). Studies on GM rice have also suggested that overexpression of a  
370 wheat gene encoding a salt-induced protein (TaSIP) (Du et al., 2013) and a sheepgrass gene  
371 (*LcSain1*) (Li et al., 2013e) may also be of benefit in enhancing salt tolerance. An equivalent  
372 investigation demonstrated that GM oats expressing the Arabidopsis *CBF3* gene exhibited  
373 improved growth and showed significant maintenance of leaf area, chlorophyll content,  
374 photosynthetic and transpiration rates, relative water content, as well as increased levels of  
375 proline and soluble sugars under high salt stress (Oraby et al., 2012). At a salinity stress level  
376 of 100mM, the GM plants showed a yield loss of 4-11% compared with >56% for the non-  
377 transgenic control. According to a recent report, field trials conducted in Australia in 2009  
378 (Table 3) showed that wheat lines expressing a salt tolerant gene *Nax2*) from *Triticum*  
379 *monococcum* produced 25% more yield than the control line in saline conditions  
380 <http://www.isaaa.org/resources/publications/pocketk/document/Doc-Pocket%20K38.pdf>).

381

382 In a similar study two wheat CBF transcription factors, TaCBF14 and TaCBF15, were  
383 transformed into spring barley, and analysis showed that transgenic lines were able to survive  
384 freezing temperatures several degrees lower than that which proved lethal for the wild-type  
385 spring barley (Soltész et al., 2013). Similar results with improved frost tolerance or other  
386 abiotic stress were achieved with GM barley expressing the rice transcription factor *Osmby4*  
387 (Soltész et al., 2011) or the wheat *TaDREB3* gene (Hackenberg et al., 2012; Kovalchuk et al.,  
388 2013).

389

390 Encouraging data have also been produced from studies of GM rice overexpressing OsNAC9,  
391 a member of the rice NAC domain family (Redillas et al., 2012). Root-specific (RCc3) and  
392 constitutive (GOS2) promoters were used to overexpress OsNAC9 and field evaluations over  
393 two seasons showed that grain yields of the RCc3:OsNAC9 and the GOS2:OsNAC9 plants  
394 were increased by 13%-18% and 13%-32% under normal conditions, respectively. Under  
395 drought conditions, RCc3:OsNAC9 plants showed an increased grain yield of 28%-72%.  
396 Both transgenic lines exhibited altered root architecture involving an enlarged stele and  
397 aerenchyma. One approach to the identification of genes that might confer improved drought  
398 tolerance in wheat involves use of the VIGS technique (Manmathan et al., 2013).

399

400 Studies on improving crop growth under conditions of nutritional limitation include results  
401 from the overexpression of *Theilungiella halophila* H<sup>+</sup>-pyrophosphatase gene in maize (Pei et  
402 al., 2012). Under phosphate sufficient conditions, GM plants showed more vigorous root  
403 growth than the wild type, and under phosphate deficit stress they also developed more robust  
404 root systems. This advantage improved phosphate uptake, and the GM plants subsequently  
405 accumulated more phosphorus. In an associated study it was found that overexpression of the

406 phosphate transporter *Pht1* promoted phosphate uptake in GM rice (Sun et al., 2012). A  
407 similar project concerns the use of the phosphate starvation response regulator *Ta-PHR1* to  
408 increase yield in wheat (Wang et al., 2013a).

409

410 One of the most ambitious of plans to improve growth under conditions of nitrogen  
411 deficiency is the project to engineer nitrogen fixation into cereals. For example, the Bill &  
412 Melinda Gates Foundation is funding the ENSA (Engineering Nitrogen Symbiosis for Africa)  
413 project (<https://www.ensa.ac.uk/news/page/3>).

414

415 In addition to the problems of reduced growth under conditions of nutrient deficiency, the  
416 ions of certain metals inhibit normal development. One example is the inhibitory effect of  
417 excess aluminium in acid soils, and this was the subject of a recent genetic study on the root  
418 hairs of wheat (Delhaize et al., 2012). An alternative approach is represented by a study of  
419 the multidrug and toxic compound extrusion (*TaMATE1B*) gene in wheat (Tovkach et al.,  
420 2013) and in wheat and barley (Zhou et al., 2013). One approach to improving growth in  
421 alkaline soils is demonstrated by results from GM rice expressing the barley iron-  
422 phytosiderophore transporter (*HvYS1*). This gene enables barley plants to take up iron from  
423 alkaline soils, and the GM rice plants grown in alkaline soil exhibited enhanced growth, yield  
424 and iron concentration in leaves compared to the wild type plants which were severely  
425 stunted (Gómez-Galera et al., 2012).

426

427 Other related recent studies include one on GM rice in which overexpression of a protein  
428 disulphide isomerase-like protein from the thermophilic archaea *Methanothermobacter*  
429 *thermoautotrophicum* enhances tolerance to mercury (Chen et al., 2012d) and one that

430 demonstrated the role of the Zn/Cd transporter OSHMA2 in cadmium accumulation in rice  
431 (Takahashi et al., 2012).

432

### 433 3.5 Yield traits

434

435 The obvious aim of all the agronomic traits mentioned to date is to increase or to stabilise  
436 yield under field conditions (Shi et al., 2013). There are also future new opportunities to  
437 improve the underlying physiological performance of the plant itself. One recent example of  
438 this is investigation in rice of the major grain length QTL, qGL3, which encodes a putative  
439 protein phosphatase with a Kelch-like repeat domain (OsPPKL1). It was found that a rare  
440 allele of this gene, qgl3 leads to a long grain phenotype, and transgenic studies confirmed that  
441 OsPPKL1 and OsPPKL3 function as negative regulators of grain length, whereas OsPPKL2  
442 as a positive regulator (Zhang et al., 2012c). Grain size in rice can also be increased by  
443 overexpression of a *TIFY* gene, *TIFY11b* (Hakata et al., 2012), whereas grain number in this  
444 crop can be increased by expression of the zinc finger transcription factor DROUGHT AND  
445 SALT TOLERANCE (DST), which itself regulates the expression of a cytokinin oxidase  
446 *Gn1a/OsCKX2* (*Grain number 1a/Cytokinin oxidase 2*) (Li et al., 2013c). Corresponding  
447 transgenic research in wheat has identified the role of TaGW2-A, a functional E3 RING  
448 ubiquitin ligase, in regulating grain size (Bednarek et al., 2012).

449

450 An important quality trait related to yield is the problem of post harvest sprouting. Among the  
451 GM approaches to overcoming this problem is the use of an antisense version of the *trx s*  
452 (*thioredoxin s*) gene from *Phalaris coerulescens* to reduce the endogenous *trx h* gene in  
453 wheat (Guo et al., 2011).

454



455 Amongst the most radical of research efforts are attempts to introduce the C4 photosynthetic  
456 trait, as found in maize, into C3 cereals such as rice. This is the subject of many programmes  
457 (see C4rice.irri.org). One recent report in this area is the finding that expression of the maize  
458 phosphoenolpyruvate carboxylase gene in wheat increases the rate of photosynthesis in the  
459 GM plants to 31.95  $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ , some 26% greater than the rate in untransformed control  
460 plants (Hu et al., 2012c). It was also found recently that constitutive expression of the rice  
461 gene OsTLP27 under the control of the CaMV 35S promoter resulted in increased pigment  
462 content and enhanced photochemical efficiency in terms of the values of maximal  
463 photochemical efficiency of photosystem II (PSII) ( $F(v)/F(m)$ ), effective quantum yield of  
464 PSII ( $\Phi\text{PSII}$ ), electron transport rate (ETR) and photochemical quenching (qP) (Hu et al.,  
465 2012a).

466

467 Of course, in any studies of GM cereals, as with other crops, it is always important to  
468 examine the whole plant performance, including the photosynthetic efficiency, in order to  
469 identify any non-intended effects (Sun et al., 2013).

470

## 471 **4 Output traits**

472

### 473 *4.1. Modified grain quality*

474

#### 475 *4.1.1. Nutrition*

476

477 Transgenic technologies provide a large variety of opportunities to modify the nutritional  
478 components in cereal crops (Bhullar and Gruissem, 2013; Demont and Stein, 2013; Morell,  
479 2012; Pérez-Massot et al., 2013; Rawat et al., 2013). These include modified proteins

480 (Wenefrida et al., 2013), carbohydrate, oils, and other minor compounds and these will be  
481 considered in turn.

482 Among the first reported GM lines of wheat were ones with modified subunits of the high  
483 molecular weight glutenin protein that confers good breadmaking quality. Recent reports in  
484 this area include the generation of GM wheat with enhancement in the concentration of high-  
485 molecular-weight glutenin subunit 1Dy10 and associated benefit in sponge and dough baking  
486 of wheat flour blends (Graybosch et al., 2013). It is also reported that such improved baking  
487 quality can be achieved without the need for selectable marker genes (Qin et al., 2013), and  
488 that coexpression of high molecular weight glutenin subunit 1Ax1 and puroindoline improves  
489 dough mixing properties in durum wheat (*Triticum turgidum* L. ssp. *durum*) (Li et al.,  
490 2012b). Similarly it is reported that GM methods can be used to reduce the expression of  $\gamma$ -  
491 gliadins and thereby potentially improve the dough mixing and bread making properties of  
492 wheat flour (Gil-Humanes et al., 2012). As part of related projects it has been shown that the  
493 starch characteristics of GM wheat overexpressing the Dx5 high molecular weight glutenin  
494 subunit are substantially equivalent to those in nonmodified wheat (Beckles et al., 2012), and  
495 that isolation of enriched gluten fractions from lines modified to overproduce HMW glutenin  
496 subunits Dx5 and/or Dy10 may require modified separation technologies (Robertson et al.,  
497 2013). Studies on the GM modification of such subunits may also lead to the production of  
498 novel proteins encoded by altered versions of either the transforming or endogenous genes  
499 (Blechl and Vensel, 2013). A relevant similar study is that on transgenic rice seed expressing  
500 the wheat HMW subunit (Oszvald et al., 2013). Another aspect of this type of study that has  
501 importance in any future regulatory submission is the determination of potential changes in  
502 the allergenicity of the GM material (Lupi et al., 2013).

503

504 In addition to efforts to modify baking and bread-making quality there have also been  
505 projects to modify the particular amino acid profile of cereals, in particular to increase the  
506 levels of lysine. GM approaches in this area have included the expression of the *sb401* gene,  
507 which encodes a lysine-rich protein, in GM maize; this leads to increased levels of lysine and  
508 total protein in the seeds (Tang et al., 2013) (see also Wang et al., 2013c). A three generation  
509 rat feeding trial of GM rice with increased levels of lysine has shown no adverse effects  
510 (Zhou et al., 2012a). In a related study, expression of a bacterial serine acetyltransferase  
511 (EcSAT) in rice lead to significantly higher levels of both soluble and protein-bound  
512 methionine, isoleucine, cysteine, and glutathione (Nguyen et al., 2012).

513

514 Alongside the many projects that are designed to modify protein quantity and quality in  
515 cereals are several that focus on aspects of starch synthesis (Blennow et al., 2013). These  
516 include GM rice lines produced by introducing a cDNA for *starch synthase IIa* (*SSIIa*) from  
517 an indica cultivar (*SSIIa* (I), coding for active *SSIIa*) into an isoamylase1 (*ISA1*)-deficient  
518 mutant (*isa1*) that was derived from a japonica cultivar (bearing inactive *SSIIa* proteins). The  
519 storage  $\alpha$ -glucan of these GM lines was shown to have altered solubility and crystallinity  
520 (Fujita et al., 2012). Many of these projects are designed to produce products with improved  
521 health benefits. For example, using a chimeric RNAi hairpin Carciofi et al. (2012a)  
522 simultaneously suppressed all genes coding for starch branching enzymes (SBE I, SBE IIa,  
523 SBE IIb) in barley, resulting in production of amylose-only starch granules in the endosperm.  
524 The authors claim that this is the first time that pure amylose has been generated with high  
525 yield in a living organism, and the resulting lines with so-called “resistant starch” would have  
526 potential in reducing the glycaemic index of diets. Such improvements may be of particular  
527 value to diabetics and this has been shown experimentally in a study in which a high-amylose  
528 GM rice, produced by inhibition of two isoforms of the starch branching enzyme, improved

529 indices of animal health in normal and diabetic rats (Zhu et al., 2012). It was observed in a  
530 similar study on GM durum wheat, in which the gene encoding one isoform of SBE was  
531 silenced, that various protein differences were present in the endosperm of the transgenics  
532 (Sestili et al., 2013). Rapid testing of constructs for use in such studies may be achieved by  
533 using transgenic callus, rather than mature seed; this system has been developed first in  
534 barley (Carciofi et al., 2012b).

535

536 GM triticale lines expressing one or both of the *sucrose-sucrose 1-fructosyltransferase (1-*  
537 *SST)* gene from rye and or the *sucrose-fructan 6-fructosyltransferase (6-SFT)* gene from  
538 wheat accumulated 50% less starch and 10-20 times more fructan, particularly 6-kestose, in  
539 the dry seed compared to the untransformed control (Diedhiou et al., 2012). This is one of the  
540 first reports of GM cereals with production of fructans (Kooiker et al., 2013) in seeds.

541

542 An alternative route to the alteration of starch content was demonstrated by a study on GM  
543 maize expressing the potato gene *StSUS* that encodes an isoform of sucrose synthase. Seeds  
544 from these transgenic plants accumulated 10-15% more starch at the mature stage, and  
545 contained a higher amylose/amylopectin balance than the WT control seeds (Li et al., 2013a).  
546 Possibly the most complex of these studies on maize was that in which the expression of six  
547 genes was modified; this led to a 2.8-7.7% increase in endosperm starch and a 37.8-43.7%  
548 increase in the proportion of amylose (Jiang et al., 2013a). Additionally there was a 20.1-  
549 34.7% increase in 1000-grain weight and a 13.9-19.05% increase in ear weight. Other  
550 associated studies include the effect of the granule-bound starch synthase (GBSS), (known as  
551 waxy protein), on the amylose content of GM durum wheat (Sestili et al., 2012).

552

553 Among other investigations of starch biosynthetic pathway is that on the maize *shrunk-2*  
554 (*Sh2*) gene, which encodes the large subunit of the rate-limiting starch biosynthetic enzyme,  
555 ADP-glucose pyrophosphorylase (Tuncel and Okita, 2013). Expression in maize of a  
556 transgenic form of this enzyme with enhanced heat stability and reduced phosphate inhibition  
557 was shown to increase yield up to 64% (Hannah et al., 2012). The extent of this yield increase  
558 was found to be dependent on temperatures during the first 4 days post pollination, and the  
559 authors also demonstrated that the transgene acts in the maternal tissue to increase seed  
560 number, and thus yield.

561

562 Suppression of the *CSLF6* gene in wheat has been shown to reduce the level of glucan and  
563 provides an opportunity to improve the level of dietary fibre (Nemeth et al., 2010), and  
564 similar suppression of glucosyl transferase genes decreases the arabinoxylan content  
565 (Lovegrove et al., 2013).

566

567 GM wheat and barley with a range of modified grain traits are among the list of lines that  
568 have been tested in the field in Australia (Table 3).

569

570 In the area of lipid research it has been shown that the levels of oleic acid (Zaplin et al., 2013)  
571 and  $\alpha$ -linolenic acid (Liu et al., 2012) in rice seed can be increased by manipulation of  
572 various fatty acid desaturase (FAD) genes.

573

574 Another significant area relates to vitamin and mineral content, particularly iron, with studies  
575 on rice and maize summarised in Table 4. The classic example of vitamin increase is the  
576 generation of “Golden Rice” (Potrykus, 2012) with higher levels of provitamin A, a  
577 compound deficient in many subsistence diets based on rice. Such deficiency may lead to

578 juvenile blindness and even death. Other recent results on modifying vitamin levels in rice  
579 include expression of *Arabidopsis thaliana*  $\rho$ -hydroxyphenylpyruvate dioxygenase (HPPD),  
580 which catalyzes the first committed step in vitamin E biosynthesis (Farré et al., 2012, 2013)  
581 and *Arabidopsis*  $\gamma$ -tocopherol methyltransferase ( $\gamma$ -TMT) (Zhang et al., 2013a), which  
582 catalyzes the final step in this pathway. In a related study, Chaudhary and Khurana (2013)  
583 produced GM wheat overexpressing the endogenous *HPPD* gene and observed a 2.4 fold  
584 increase in the level of tocochromomanol, one of an important group of plastidic lipophilic  
585 antioxidants, which may have significant benefits in the human diet.

586

587 Results relating to iron and zinc accumulation in GM wheat expressing a ferritin gene have  
588 been discussed recently by Neal et al. (2013). In addition to increases in the levels of vitamins  
589 and minerals, GM techniques have also been used recently to improve the content of  
590 beneficial compounds such as flavonoids (Ogo et al., 2013) and sakuranetin, a flavonoid  
591 phytoalexin (Shimizu et al., 2012a) in rice. Related research demonstrating the effects of  
592 purple, anthocyanin-containing, wheat on extending the lifespan of nematodes (Chen et al.,  
593 2013b) may be developed through GM technology.

594

#### 595 *4.2 Enzymes, diagnostics and vaccines*

596 Probably the first commercial plant –derived industrial enzyme was trypsin, produced in  
597 maize kernels and marketed by Sigma (Product Code T3449) under the brand name  
598 TrypZean<sup>®</sup>. This company also markets maize-derived recombinant avidin (Product Code  
599 A8706). As summarised recently (Xu et al., 2012b) other recombinant products produced  
600 from corn included  $\beta$ -glucuronidase, aprotinin and a range of degradative enzymes (also see

601 biofuel section below). There have been significant environmental concerns expressed in the  
602 USA with some of these plant derived products.

603

604 Among the most significant of GM maize products are those expressing the phytase enzyme.  
605 Such products are designed to overcome the problem caused by phytate, a phosphorus  
606 containing compound that is present in maize grain but one in which the phosphate is  
607 unavailable to monogastric animals such as poultry and pigs and therefore causes pollution  
608 from their waste. Maize expressing a phytase gene from *Aspergillus niger* is the first GM  
609 maize to receive a biosafety certificate in China (Chen et al., 2013a) (see also Xia et al.,  
610 2012). An alternative approach is to use RNAi techniques to downregulate the *myo*-inositol-  
611 3-phosphate synthase (*MIPS*) gene that catalyzes the first step of phytic acid biosynthesis in  
612 rice (Ali et al., 2013), or to employ cisgenic methods (Holme et al., 2012b). The value of  
613 such low-phytate maize products has been recently confirmed in feeding trials with poultry  
614 (Gao et al., 2012; Ma et al., 2013; Wang et al., 2013e) and pigs (Li et al., 2013d). A similar  
615 benefit may derive from GM maize expressing a fungal  $\beta$ -mannanase from *Bispora* (Xu et al.,  
616 2013b).

617

618 Although no GM lines in this category have yet been approved for commercialisation, there  
619 has been considerable activity, over many years, in the area of plant-derived vaccines and  
620 other potential pharmaceutical products. This summary describes some of the recent activity  
621 in this 'pharming' area. The justification for such research lies in the assumed economic  
622 benefit that might derive from using plants rather than other expression systems (eg animal  
623 cells or bacteria) for production of high-value, bioactive compounds. Cereals, principally rice  
624 (Greenham and Altosaar, 2012; Takaiwa, 2013), maize, and barley (Magnusdottir et al.,  
625 2013) (<http://www.orfgenetics.com/>) have become the crops of choice, as proteins can be

626 expressed at high levels in the seed and stored for extended periods without significant  
627 deterioration. Additionally, seed-derived antigens provide the possibility of oral delivery as  
628 an alternative to injection; this method may be of particular relevance in the area of  
629 veterinary medicine. Recent examples include the induction of a protective immune response  
630 to rabies virus in sheep after oral immunization with GM maize kernels that express the  
631 rabies virus glycoprotein (Loza-Rubio et al., 2012), and the proven immunogenicity of foot-  
632 and-mouth disease virus structural polyprotein P1 (Wang et al., 2012) and MOMP protein  
633 (Zhang et al., 2013a) expressed in GM rice, and the porcine reproductive and respiratory  
634 syndrome virus (PRRSV) expressed in GM maize (Hu et al., 2012b). Other similar examples  
635 are the demonstration of immunogenicity of a neutralizing epitope from porcine epidemic  
636 diarrhoea virus (PEDV) fused to an M cell-targeting ligand fusion protein and expressed in  
637 GM rice (Huy et al., 2012) and the successful production of the hepatitis B surface antigen  
638 (HBsAG) in maize (Hayden et al., 2012a,b). This latter study represents the first description  
639 of a commercially feasible oral subunit vaccine production system for a major human disease,  
640 though there has also been much publicity given to the potential of maize as a production  
641 system for an HIV neutralizing monoclonal antibody (Sabalza et al., 2012).

642

643 Recently it was confirmed that rice-derived recombinant human serum transferrin (hTF)  
644 represents a safe and animal-free alternative to human plasma-derived hTF for bioprocessing  
645 and biopharmaceutical applications (Zhang et al., 2012).

646

647 Another area of related research is that on allergens. For example, GM rice seeds have been  
648 used for the production of a recombinant hypoallergenic birch pollen allergen Bet v 1 (Wang  
649 et al., 2013d), and a hypoallergenic Der f 2 (Yang et al., 2012a) and Der p 1 (Saeki et al.,  
650 2012, 2013) derivatives of the House Dust Mite (HDM) allergen from *Dermatophagoides*



651 *pteronysinus*. These products may be useful in allergen-specific immunotherapy. Similarly,  
652 human interleukin IL-10 (hIL-10), a therapeutic treatment candidate for inflammatory allergy  
653 and autoimmune diseases, has been produced in rice seed and effectively delivered directly to  
654 gut-associated lymphoreticular tissue (GALT) via bio-encapsulation (Yang et al., 2012b).  
655 Related research is being conducted on the similar molecule hIL-7 (Kudo et al., 2013). Rice  
656 is also the production system for human alpha-antitrypsin (AAT), a compound used as  
657 therapy of individuals with mutations in the *AAT* gene (Zhang et al., 2013b).

658

#### 659 *4.3 Biofuels*

660

661 To date the only GM cereal with a biofuel-related trait that has been commercialised is  
662 Enogen™, a maize hybrid expressing a thermostable alpha amylase for efficient starch  
663 hydrolysis and higher bioethanol yields. Details of this Syngenta product, which was  
664 approved by the USDA on 12<sup>th</sup> February 2011, are available at  
665 (<http://www.syngenta.com/country/us/en/enogen/Pages/Home.aspx> and  
666 [http://www.syngenta.com/country/us/en/agriculture/seeds/corn/enogen/stewardship/Documen  
667 ts/June%2014th,%202011/Enogen%20Overview.pdf](http://www.syngenta.com/country/us/en/agriculture/seeds/corn/enogen/stewardship/Documents/June%2014th,%202011/Enogen%20Overview.pdf)). It is stated that ethanol throughput  
668 during fermentation with this product is increased by 5.2% and the financial benefit is  
669 between 8-15 US cents per gallon. A news item from 12<sup>th</sup> June 2013  
670 ([http://www.agprofessional.com/news/Syngenta-footprint-for-Enogen-corn-grows-to-11-  
671 ethanol-plants-211053531.html](http://www.agprofessional.com/news/Syngenta-footprint-for-Enogen-corn-grows-to-11-ethanol-plants-211053531.html)) states that a total of 11 ethanol plants in the US have now  
672 signed agreements to use this product; such plants pay the farmer an average premium of 40  
673 cents per bushel for Enogen™ corn. Present research in Syngenta and elsewhere is also  
674 focussed on the potential for the production of recombinant cell-wall degrading enzymes in  
675 GM plants, in order to avoid the significant cost of adding exogenous enzymes during the

676 production of fermentable sugars from biomass (Sainz, 2009). As part of this strategic goal,  
677 Syngenta have signed research agreements which include those with Diversa in 2007, and  
678 Verenum (now owners of Diversa) and Protéus in 2009.

679 Other relevant recent studies in this area include the production of:- bacterial  
680 amylopullulanase in maize grain (Nahampun et al., 2013); thermostable xylanase in maize  
681 stover (Shen et al., 2012); glycoside hydrolases (Brunecky et al., 2012); and an *Acidothermus*  
682 *cellulolyticus* endoglucanase in transgenic rice seeds (Zhang et al., 2012a). Additionally,  
683 down regulation of the enzyme cinnamyl alcohol dehydrogenase in maize has been shown to  
684 produce a higher amount of biomass and a higher level of cellulosic ethanol in assays  
685 (Fornalé et al., 2012). It is hoped that these various approaches will lead to significant  
686 improvements in the efficiency of biofuel production and thereby reduce the conflict between  
687 the demands for food and fuel (Zhang, 2013).

688

## 689 **5 Pipeline of future products**

690

### 691 *5.1 Field trials*

692

693 One simple method to assess the direction of future research on GM cereals in both  
694 commercial and non-commercial programmes is to examine the various public databases that  
695 summarise the applications for field testing. Such information is available from the regulatory  
696 authorities in the various jurisdictions around the world. Data for the USA are available at  
697 <http://www.isb.vt.edu/search-release-data.aspx> and can be summarised as follows:-

698 **Maize:** A total of 8294 applications have been submitted in the period from 1996 to date  
699 (latest 14<sup>th</sup> June 2013). Many of these are from commercial companies and understandably  
700 have limited details of the genes being tested because of Confidential Business Information  
701 (CBI) restrictions. However, among the most recent application from a non-commercial  
702 institution is one from the Cold Spring Harbor Laboratory that lists a total of 78 genes to be  
703 tested.

704 **Wheat:** A total of 510 applications for have been submitted in the period from 1996 to date  
705 (latest 22<sup>nd</sup> April 2013). The traits for trial in the 13 applications for 2013 include:- Nitrogen  
706 use efficiency (Arcadia); Fusarium resistance (Uni. Minnesota); nitrogen metabolism,  
707 drought/heat tolerance, water use efficiency, yield increase, modified flowering time, altered  
708 oil content, fungal tolerance, insect resistance, herbicide tolerance (Monsanto); increased  
709 carbohydrate, improved grain processing (Uni. Nebraska); herbicide tolerance (and other CBI  
710 traits) (Pioneer); and CBI traits (Biogemma); breadmaking quality (USDA).

711 **Barley:** a total of 109 applications were submitted in the period from 1994 to 2013 (latest  
712 15<sup>th</sup> May 2013). The traits for trial in the 6 applications for 2012 include:- starch quality  
713 (USDA); nitrogen utilisation efficiency (Arcadia); Fusarium resistance (USDA); and  
714 Rhizoctonia resistance (Washington State University).

715 Data for the EU are available at [http://gmoinfo.jrc.ec.europa.eu/gmp\\_browse.aspx](http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx) and are  
716 summarised in Table 5. This list is relatively short and does not include many of the  
717 commercial trials of maize. Among the interesting trials is that testing wheat designed to have  
718 reduced levels of epitopes linked to celiac disease, and that designed to deter aphids by  
719 expression of an alarm pheromone.

720

721 Data from Australia are available at  
722 <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>. A summary is given in  
723 Table 3, which identifies trials of wheat and barley with modified grain traits and with  
724 various genes providing tolerance to abiotic stress. More complete detail may be obtained  
725 from the application dossiers published by the various regulatory authorities.

726

## 727 *5.2 Patents*

728

729 In any consideration of future trends it is of great value to assess the patent literature, as this  
730 provides a summary of those novel technologies that are the subject of research activity,  
731 particularly in commercial companies who will publish information in patent applications  
732 prior to it emerging in the conventional scientific literature. The most recent overall review of  
733 this area is that of Dunwell (2010) who includes a discussion of IPR relevant to the research  
734 scientist and to those interested in international development, globalization, and sociological  
735 and ethical aspects of the public- and private-sector relationships. Data on patent application  
736 and granted patents are available in many publically accessible databases, with the most  
737 complete being that at <http://www.patentlens.net/>. The extent of patent activity in the area of  
738 GM cereals is exemplified by the selection of recent US patents (Table 6a) and patent  
739 applications (Table 6b). The subject matter of these patents, taken from a short period of  
740 time, covers all the major themes discussed in this review. It is always necessary to point out  
741 the commercial reality that few, if any, of the patents and applications in these lists will ever  
742 produce a financial profit. The most common reasons for this lack of success are unexpected  
743 additional costs of development or failure of the underlying science during the transfer from  
744 laboratory to field scale.

745

746 *5.3 New Breeding Techniques*

747

748 It is more than twenty years ago that the various GM regulatory legislations were enacted. For  
749 example, the first iteration of the EU Directive that controls the Deliberate Release of  
750 genetically modified organisms (GMOs) into the environment was adopted in 1990. The  
751 foundation of this approach was to define an organism based on how it was made and the nature  
752 of the resulting alterations to its genetic material. However, since that time a number of reports,  
753 including the last review of the current 2001/18 Directive (EPEC, 2011), have highlighted  
754 concerns about the clarity of the definition of a GMO when applying it to organisms produced  
755 by particular new methodologies. These new breeding techniques (NBTs) include:  
756 cisgenesis/intragenesis; site directed mutagenesis; genome editing using zinc finger nucleases,  
757 TALENs (Wendt et al., 2013), CRISPRs (Shan et al., 2013) and other similar systems (Li et al.,  
758 2013b; Nekrasov et al., 2013); RNA dependent DNA methylation (and other epigenetic  
759 methods) (Higo et al., 2012), and reverse breeding. Reports that have considered these NBTs in  
760 more detail include that from an EU Commission Working Group on 'New Techniques', a  
761 series of papers by the Dutch committee COGEM (COGEM, 2006, 2009, 2010) and an  
762 Austrian report (Brüller et al., 2012). A report from the EU Joint Research Centre also provides  
763 useful background on the subject (Lusser et al., 2011). In principle, these techniques can be  
764 applied to any crop, including cereals. For example, there is much support in certain areas for  
765 the concept of cisgenesis, whereby the DNA introduced into recipient crop comes from a  
766 sexually compatible relative, and this method has been used to produce low-phytate barley  
767 (Holme et al., 2013). In some of these methods, although molecular gene transfer techniques  
768 are used to generate the new line, there is no transgene present in the final product. Example of  
769 this involve techniques for the modification of recombination or the rapid generation of mutants

770 by suppressing the activity of DNA repair systems (Xu et al., 2012c) or generating transposon  
771 induced chromosomal rearrangements (Yu et al., 2012).

772

773 Such problems of enforcement and uncertainty about whether or not new methods fall within  
774 the existing legislation (Pauwels et al., 2013) has led many to argue in favour of a so-called  
775 “phenotype” (or “product”) based (EASAC, 2013) or “process-agnostic” system (Ammann,  
776 2013).

777

778

## 779 **6 Acceptance of GM crops**

780

781 The commercial exploitation of GM crops varies greatly across the globe with a clear  
782 dichotomy between the position in North and South America, where such crops are grown  
783 widely, to Europe where there is little GM agriculture, though large imports of GM material  
784 for animal feed (Fresco, 2013; Masip et al., 2013). The foundation for this difference lies in a  
785 complex mixture of political, social and economic considerations. Within Europe it has been  
786 argued by some that the present regulatory impasse, whereby it has not proved possible for  
787 the 29 EU states to achieve political consensus for approval of GM crops for cultivation,  
788 should be bypassed by allowing states to determine their own policy. However, others  
789 consider this to a retrogressive approach that would lead to dangerous inconsistencies in the  
790 regulatory approach (Biszko, 2012).

791

### 792 *6.1 Regulatory aspects*

793

794 Before any GM product can reach the market it must receive approval from the relevant  
795 regulatory authority in the appropriate legislative area. The two most important aspects of  
796 such a process are food and feed safety and the potential for harm to human health and the  
797 environment (Romeis et al., 2013). There is great deal of published information on these  
798 topics (eg <http://www.efsa.europa.eu/en/panels/gmo.htm>) and it will not be repeated here, but  
799 some of the recent information on compositional analysis has been summarised by Herman  
800 and Price (2013), Kitta (2013) and Privalle et al. (2013). Other specific recent data include  
801 information on transcriptome changes in maize expressing a phytase gene (Rao et al., 2013),  
802 tests for possible changes in allergens in GM maize (Fonseca et al., 2012) and a proteomic  
803 study on GM rice (Gong et al., 2102). Animal feeding tests (Buzoianu et al., 2013) are also a  
804 required part of any regulatory process, though the outcome of some such tests has recently  
805 provoked further controversy about GM safety (Arjó et al., 2013; Fresco, 2013) .

806

807 As regards possible environmental effects, a large-scale analysis has shown convincing  
808 evidence that one consequence of the global cultivation of GM crops has been a significant  
809 reduction both in the amount of pesticide sprayed (~8-9%) and in the release of greenhouse  
810 gas emissions from the cropping area (Brookes and Barfoot, 2013b).

811

812 Other environmental issues with all GM crops include possible transgene spread to wild  
813 relatives (Chandler and Dunwell, 2008). Among the important variables in this context is the  
814 relative fitness of the crop-weed hybrid and this is the subject of a recent study that examined  
815 GM insect resistant rice (Yang et al., 2012c). Recent studies on GM wheat include  
816 assessment of the impact of any GM pollen transfer either within or between crops (Loureiro  
817 et al., 2012; Foetzki et al., 2012; Rieben et al., 2011). There is also discussion about the  
818 possible persistence of feral populations of GM crops (Raybould et al., 2013).

819

820 An interesting additional aspect relates to the possible effect of GM crops on the soil  
821 microflora. This is the subject of one study on rice in which the expression of phenylalanine  
822 ammonia-lyase was inhibited by RNAi methods (Fang et al., 2013). It was concluded that the  
823 GM rice had less rhizospheric bacterial diversity than the non-GM control.

824

### 825 *6.2 Public perception*

826 This is a very complex area and there have been many published surveys on consumer  
827 attitudes to GM. Some of these surveys are international in scope (Frewer et al., 2013)  
828 whereas others examine attitudes in specific regions such as Europe (Cecciolli and Hixon,  
829 2012; Gaskell et al., 2011), Switzerland (Speiser et al., 2013), Spain (Costa-Font and Gil,  
830 2012; Rodríguez-Entrena and Sayadi, 2013) and Japan (Ishiyama et al., 2012). Among issues  
831 considered in such surveys are questions relating to basic knowledge of science (Mielby et  
832 al., 2013), ethics (Du, 2012; Gregorowius et al., 2012), human rights (Srivatava, 2013),  
833 effects on the developing world (Jacobsen and Myhr, 2013; Okeno et al., 2012), the need for  
834 choice (Mather et al., 2012), labelling (Benny, 2012), and coexistence with organic  
835 agriculture (Areal et al., 2012).

836

## 837 **7 Conclusions**

838

839 It remains to be seen whether the prospects and opportunities (Chen and Lin, 2013; Dunwell,  
840 2011) described above will be translated into successful GM products in the future and  
841 whether GM technologies are compatible with sustainable (Bruce, 2012; Hansson and  
842 Joelsson, 2012) and biodiverse (Jacobsen et al., 2013) agriculture.



843

844 **References**

845

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Table 1. Global area, production, yield and contribution to the human diet for major cereal crops

	2010 (FAOSTAT)					2009 (FAOSTAT)			
	Area		Production		Yield	Energy		Protein	
	Mha	%	MT	%	Tonnes/ha	kcal/ capita/d	%	g/ capita/d	%
Wheat	217	32	651	27	3.0	532	18.8	16.2	20.4
Maize	162	24	844	35	5.2	141	5.0	3.4	2.3
Rice	154	23	672	28	4.4	536	18.9	10.1	12.7
Barley	48	7	123	5	2.6	7	0.2	0.2	0.3
Sorghum	41	6	56	2	1.4	32	1.1	1.0	1.3
Total	683	100	2432	100	3.6	1248	44	30.9	38.6

Adapted from Wheat Initiative (2013)

Table 2. Evolution of wheat yield over 10-year periods since 1960 (FAO) and projected needs for 2050

Period	Mean area harvested/yr (Mha)	Mean production/yr (Mt)	Mean production increase/yr (%)	Mean yield (t/ha)	Mean yield increase/yr (%)
1961-1970	213	278		1.3	
1971-1980	225	388	3.9	1.7	3.2
1981-1990	229	509	3.1	2.2	2.9
1991-2000	220	571	1.2	2.6	1.7
2001-2010	216	622	0.9	2.9	1.1
2050 (target)	220	1045	1.7	4.75	1.6

Adapted from Wheat Initiative (2013)

Table 3. Field trials of GM wheat and barley in Australia: Applications and licences for Dealings involving Intentional Release (DIR) into the environment

Number	Organisation	Description	Crop(s)	Trait	Date
DIR117	CSIRO	grain composition, nutrient utilisation	wheat, barley	nutrition, yield	Mar 2013
DIR112	CSIRO	grain composition, nutrient utilisation	wheat, barley	nutrition, yield	Mar 2012
DIR111	CSIRO	grain composition, nutrient utilisation	wheat, barley	yield, disease, stress	Feb 2012
DIR102	Uni. Adelaide	abiotic stress	wheat, barley	yield, stress	Jun 2010
DIR100	CSIRO	drought, heat	wheat	yield, stress	Jun 2010
DIR099	CSIRO	grain composition, nutrient utilisation	wheat, barley	nutrition, yield	Mar 2013
DIR094	CSIRO	nutrient utilisation	wheat, barley	yield	Jul 2009
DIR093	CSIRO	grain starch	wheat, barley	nutrition	Jun 2009

DIR092	CSIRO	grain composition	wheat	nutrition, processing	May 2009
DIR080	Vict. Dept. Prim. Indust.	drought	wheat	abiotic stress	Jun 2008
DIR077	Uni. Adelaide	stress, glucan	wheat, barley	stress, nutrition	Jun 2008
DIR071	Vict. Dept. Prim. Indust.	drought	wheat	abiotic stress	Jun 2007
DIR061	Grain Biotech	salt tolerance	wheat	stress tolerance	Withdrawn
DIR054	CSIRO	grain starch	wheat	nutrition	Apr 2005
DIR054	Grain Biotech	salt tolerance	wheat	stress tolerance	Apr 2005

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Summary of data from the Office of the Gene Regulator. Available at:-

<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>

Table 4. Transgenic cereals with enhanced content of vitamins and minerals

Nutrient	Species	Genes used	Total increase (fold increase over WT)	References
Vitamin A	Maize	<i>PacrtB, PacrtI</i>	33.6 µg/g DW (34)	Aluru et al., 2008
	Maize	<i>Zmpsy1, PacrtI, PcrW, Gglycb</i>	146.7 µg/g DW (133)	Zhu et al., 2008
	Maize	<i>Zmpsy1, PacrtI</i>	163.2 µg/g DW (112)	Naqvi et al., 2009
	Wheat	<i>Zmpsy1, PacrtI</i>	4.96 µg/g DW (10.8)	Cong et al., 2009
	Rice	<i>Nppsy1, EucrtI</i>	1.6 µg/g	Ye et al., 2000
	Rice	<i>Zmppsy1, EucrtI</i>	37 µg/g (23)	Paine et al., 2005
	Vitamin C	Maize	<i>Osdhar</i>	110 µg/g DW (6)
Vitamin E	Rice	<i>HPPD</i>		Farré et al., 2012
		$\gamma$ -TMT		Zhang et al., 2013a
Folic acid	Rice	<i>Atgtpchi, Atadcs</i>	38.3 nmol/g (100)	Storozhenko et al., 2007
Iron	Rice	<i>Osnas2</i>	19 µg/g DW in polished seeds (4.2)	Johnson et al., 2011
	Rice	<i>Gm ferritin, Af phytase, Osnas1</i>	7 µg/g DW in polished seeds (4–6.3)	Wirth et al., 2009
	Rice	Activation tagging of <i>Osnas3</i>	32 µg/g DW in dehusked seeds (2.9)	Lee et al., 2009



	Maize	<i>Gm ferritin</i> and <i>Af phytase</i>	30 µg/g DW in whole seed (2)	Drakakaki et al., 2005
	Rice	<i>Ferritin</i>	7 µg/g DW in polished seed (6)	Masuda et al., 2012, 2013
Zinc	Rice	Activation tagging of <i>Osnas2</i>	40–45 µg/g DW in polished seeds (2.9)	Lee et al., 2011
	Rice	<i>Osnas2</i>	52–76 µg/g DW in polished seeds (2.2)	Johnson et al., 2011
	Rice	<i>Gm ferritin</i> , <i>Af phytase</i> , <i>Osnas1</i>	35 µg/g DW in polished seeds (1.6)	Wirth et al., 2009

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Data adapted from Pérez-Massot et al. (2012) and other sources.

Table 5. Summary of selected field trials of GM cereals in the EU

Number	State	Date	Institution	Subject
B/ES/13/19	Spain	May 2013	INIA	Bt maize
B/ES/13/20	Spain	May 2013	CSIC	Wheat with low content of celiac-toxic epitopes
B/ES/13/15	Spain	March 2013	Limagrain	Bt, HR maize
B/ES/13/16	Spain	March 2013	Uni. Lleida	High vitamin maize
B/DK/12/01	Denmark	April 2012	Univ. Aarhus	Cisgenic barley with improved phytase activity
B/SE/12/484	Sweden	Feb 2012	Swedish Univ. Agric. Sci.	Barley with improved nitrogen use efficiency
B/GB/11/R8/01	UK	Oct 2011	Rothamsted	Wheat producing aphid alarm
B/PL/11/02-10	Poland	Sept 2011	Plant Breed. Acclim. Instit.	Transgenic Triticale
B/CZ/11/2	Czech	Mar 2011	Instit. Exper. Botany	Barley with phytase
B/IS/09/01	Iceland	Apr 2009	ORF Genetics	Transgenic barley, comparison

of processing quality

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Available from JRC database ([http://gmoinfo.jrc.ec.europa.eu/gmp\\_browse.aspx](http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx))

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Table 6. Summary of selected USA granted patents (a) and patent applications (b) relating to GM cereals; data from 2013. Data are from the USPTO

(<http://www.uspto.gov/patents/process/search/index.jsp>).

(a)

Number	Date	Inventor	Subject
8,440,886	14 May	Lundquist et al.	Transgenic maize
8,440,881	14 May	Park et al.	Genes for yield
8,431,775	30 April	Hegstad et al.	<i>knotted1</i> gene
8,431,402	30 April	Vasudevan et al.	Sorghum regeneration
8,426,704	23 April	Hirel et al.	Glutamine synthetase
8,426,677	23 April	Yu et al.	GA20 oxidase
8,426,676	23 April	Oswald et al.	Pyruvate kinases
8,420,893	16 April	Gordon-Kamm et al.	AP2 domain transcript. factor
8,415,526	9 April	McGonigle	Artificial microRNAs
8,404,933	26 March	Chen et al.	Herbicide resistance gene
8,404,930	26 March	Wu et al.	Monocot transformation
8,404,929`	26 March	Gruis et al.	Reducing gene expression

(b)

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20130133111	23 May	Lyznik et al.	MAPKKK genes to improve yield
20130133101	23 May	Rodiuc et al	Phytosulfokines and pathogen resistance
20130125266	16 May	Hiei et al.	Agrobacterium, barley transformation
20130125264	16 May	Frankard et al.	Genes for yield
20130125258	16 May	Emmanuel et al.	Genes for yield
20130117894	9 May	Frohberg et al.	Starch synthase
20130117888	9 May	Sanz Molinero et al.	Genes for yield
20130116124	9 May	Fernandez et al.	Bacterial volatiles and starch
20130111634	2 May	Kurek et al.	Artificial microRNAs
20130111632	2 May	Champion et al.	Jasmonic acid
20130111620	2 May	D'Halluin et al.	Meganucleases
20130111618	2 May	Mankin et al.	Herbicide tolerance

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