RESEARCH PAPER New Biotechnology • Volume 27, Number 5 • November 2010

Needs for and environmental risks from transgenic crops in the developing world

Jonathan Gressel

Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel

The developing world has many unique constraints to crop production and, lacking inputs, they are best overcome if solutions are seed borne. Classical breeding cannot overcome many of these constraints because the species have attained a 'genetic glass ceiling', the genes are not available within the species. Transgenics can supply the genes, but typically not as 'hand me down genes' from the developed world because of the unique problems: mainly parasitic weeds, and weedy rice, stem borers and post-harvest insects, viral diseases, tropical mycotoxins, anti-feedants, toxic heavy metals and mineral deficiencies. Public sector involvement is imperative for genetically engineering against these constraints, as the private biotechnology sector does not see the developing world as a viable market in most instances. Rice, sorghum, barley, wheat and millets have related weeds, and in certain cases, transgenic gene containment and/or mitigation is necessary to prevent establishment of transgenes in the weedy relatives.

Contents

Introduction

The least developed areas of the world are dependent on a small number of crops for caloric input: S.E. Asia depends predominantly on rice; Africa on maize, sorghum or cassava; with strong

---

E-mail address: jonathan.gressel@weizmann.ac.il.

1871-6784/$ - see front matter doi:10.1016/j.nbt.2010.05.015

regional predominance of single crops. The world as a whole is not much less diverse with 80% of human and livestock calories coming from but four crops. This lack of crop biodiversity is frightening considering what a single new disease or other constraint might do to these four, wheat, rice, maize and soybeans.

The globalisation of a few crops is actually due to the greater genetic diversity within these few crops allowing them to be cultivated in many areas. Indigenous or other crops do not have the same genetic potential to spread and overcome constraints; if the necessary genes are lacking, no amount of traditional or sophisticated breeding can cause them to come forth; each crop has its own 'genetic glass ceiling' [1], which can only be breached by bringing the needed genes from wherever they might occur by genetic engineering. Even the four major crops have their own genetic glass ceilings, as demonstrated by the phenomenal success of engineering herbicide resistance or insect resistance into their genomes, allowing cost/environmentally friendly control of weeds and insects. The Irish potato famine could have been obviated and present extensive fungicide use can be replaced by transgenes conferring blight resistance that have been generated [2], but not commercialised.

A number of constraints to developing world agriculture are described below as examples of problems that have not been solved by breeding owing to lack of endogenous genes, with possible biotechnological solutions. One major constraint – the lack of pro-vitamin A from grain crops, is discussed in a separate paper in this issue. Many of these solutions will have to be developed by the public sector, as there is not enough interest by the large, major crop, developed world focused, multinationals. They will then have to be commercialised by public–local private sector cooperation. Only the agronomic constraints are discussed below, not the infrastructural problems that must be solved by politicians and cannot be solved by biologists and genetic engineers.

While all transgenic crops released so far are clearly devoid of environmental risks, and do bear environmental benefits [3], there are instances where there can be agro-environmental risks. These risks are limited to those cases where a crop has a weedy relative that could become more competitive should the transgene introgress (cross into) the weed. This is especially risky with herbicide resistance in rice, sorghum, wheat, barley and sunflowers, which do have such pernicious weedy, interbreeding relatives. Still, there are genetic engineering solutions to limit the gene flow and to mitigate it by preventing the weeds from being competitive.

Major weed problems requiring genetic engineering solutions

While there are many weeds that require control, most can be controlled by herbicides, whose use is becoming universal, except in Africa where manual (usually 'femanual') control predominates. Two such major weed problems not amenable to present herbicides or manual control nor to breeding, are described below.

Weedy rice in rice

Rice culture is rapidly being transformed from back-breaking, labour-intensive hand transplanting to direct seeding into the paddy. Hand transplanting gave rice a month head start over weeds. Most weeds in direct seeded rice can be controlled by herbicides, except one, a weedy form of rice that has evolved in farmers' fields to a form that spills its seeds before crop harvest ('shatters') and is taller than rice, and often the few seeds that remain and contaminate the crops seeds have an undesirable telltale red colour. The weedy rice problem is a major constraint where direct seeding started first: the Americas, Europe and now Thailand, Vietnam, Malaysia and elsewhere [4,5].

Solutions to weedy rice: All herbicides that control weedy rice also kill rice. They are botanically the same interbreeding species, and thus have the same metabolism. Only countries that heavily subsidise rice cultivation can use expensive machinery to transplant rice seedlings, and workers are unwilling/unavailable to return to transplanting, even in some of the poorest countries.

Developing herbicide resistant rice has been a proven solution. A mutant rice was found that was resistant to the acetolactate synthase (ALS-AHAS) inhibiting herbicides and has been widely commercialised as a solution [6]. Additionally, genetic engineers have developed rice resistant to the herbicides glyphosate [7] and glufosinate [8], and both kill the weedy rice.

Even though rice is 'cleistogamous', pollinating itself before the flowers open, there is some pollen transfer, and the ALS resistance gene has spread rapidly into weedy rice, wherever used [4]. In some places the herbicide resistant rice was withdrawn, and the transgenic herbicide resistant rice was not released, as it was demonstrated in the laboratory that there would be gene flow [9].

This gene flow can be mitigated, as described in a later section.

Parasitic weeds

Root parasitic weeds (*Orobanche* spp.; *Striga* spp.) are widespread. *Orobanche* spp. (broomrapes) attack grain legumes, vegetable crops and sunflower especially around the Mediterranean basin into Eastern Europe. The only solution to broomrapes was to fumigate the infested soil with the now banned methyl bromide, and was affordable only for expensive 'truck' crops. *Striga* (witchweed) species attack maize, sorghum, millet and grain legumes throughout much of sub-Sahara Africa, and are a major reason for the low productivity in these areas, where they have a 20–100% yield reduction in any given season [10,11].

These root parasites attach only to host crop roots, waiting for a host root to pass nearby, stimulating germination and attachment. They do most of their damage while still underground. When the flower stalk emerges late in season, it is a sign to the farmer that the crop has been devastated. Pulling up stalks by hand does not help this year's crop, and actually can damage the crop root system, but does prevent each stalk from dropping tens of thousands of tiny seeds back into the soil. Some herbicides can control the emerging stalks but few farmers can afford to spray when they know that this season's crop is partially or fully lost.

Solutions to parasitic weeds: Some crop rotations, sanitation, hand roguing to prevent spread can reduce the problem. One intercrop, *Desmodium* can prevent *Striga* development in limited geographical areas in Africa, and its foliage is excellent cattle/goat fodder [12].

There has been considerable success with breeding sorghum for *Striga* resistance, but it requires a complicated combination of separate recessive genes each on different chromosomes, controlling partial prevention of secretion of germination stimulation, partial inhibition of attachment structures and then attachment, and partial inhibition of penetration of the crop vascular system [13]. This has been successful enough to rightly cause the superbreeder Gebisa Ejeta to be awarded the 2009 World Food Prize for this work. Sorghum co-evolved with *Striga* in Africa and thus possessed these modicums of resistance that could be combined. Maize was introduced to Africa and breeding for resistance was less successful, although periodically there are publications claiming some resistance, claims that later seem to vanish.

Biotechnology has been and can be helpful. Transgenic herbicide resistance is a simple workable solution, well demonstrated in the laboratory for *Orobanche* [5,14–17]. It has not made it to the field, except for transgenic glyphosate resistant maize, released in South Africa, where there is no *Striga*. A tissue culture derived, herbicide resistant maize mutation has been crossed into African maize varieties and hybrids and has been released [17–19]. It has a novel cost-saving technology advance; instead of spraying the herbicide over the whole field, the herbicide is applied to the seeds before planting [18], requiring over 90% less herbicide than that required when sprayed. A similar mutation was found in sorghum and is being readied for commercialisation [20].

Once the needed sorghum genes are isolated and cloned, they could be transformed in a single, dominantly inherited construct containing a group of clustered genes, which would be a very effective strategy. Such resistance could easily be backcrossed into local varieties and land races preserving crop biodiversity, because it is inherited as a single dominant gene and not four separate recessive genes. Perhaps the resistance genes from sorghum, once isolated, could be stacked with those responsible for *Desmodium* allelochemical production, along with resistance genes being found in cowpea [21] and rice [22], all into minichromosomes [23] or into the genome at one locus. It would be very hard for the parasitic weeds to overcome such resistance and many crop species could be engineered with the same gene cluster.

Other approaches are also beginning to work [24] after initial reports of failure [25]. RNAi constructs encoding genes that suppress parasite-only metabolic pathways have been engineered into the crop. The present constructs only lower the number of emerging *Orobanche* attachments on the transgenic tomatoes where this was tested [24], but presumably with different gene configurations and promoters, it will soon be possible to use this technology effectively.

What risks might resistance to parasites have? The parasite-affected crops with interbreeding weeds are rice, sorghum, sunflower and carrots. What parasite-resistance traits might confer a fitness advantage on the weeds? Clearly herbicide resistance would – but only where herbicides are widely used, and are sprayed. Little herbicide is actually used in Africa, and only when herbicide is used would there be an advantage. If the crop seed alone is treated with herbicide, only resistant weeds within less than 15 cm of a crop would be affected by the herbicide [26]. The rest would not, and there would be no advantage to the resistance genes. Those developing the non-transgenic herbicide resistant sorghum use the above as excuses why they do not fear gene flow. Conversely, one cannot prevent the mutant gene from moving, as a similar mutant moved in rice. There are ways to preclude such movement or mitigate its effects with transgenics, as discussed in a later section.

Insect constraints to crop production requiring biotech interventions

Stem borers

Lepidopterous insects are major problems on grain crops in the developed and developing world [27]. Besides the damage to yield by their feeding, winds easily knock over the larvae-hollowed stems, causing breakage before harvest. These insects are also vectors of disease causing fungi, including those *Fusarium* species that secrete fumonisins, that cause oesophageal cancer in humans and other syndromes in livestock.

These and other lepidopteran insects have been controlled by spraying organophosphate or other insecticides in the developed world, or as small pinches of granular insecticide by bare hands or as drops of liquid formulations with a medicine dropper into the leaf whorl of maize in Africa. Organic agriculture has used sprays of dried *Bacillus thuringiensis* (Bt) bacteria to control these insects. Genetic engineers isolated the gene encoding the active toxin in Bt and inserted the gene directly into the crop, obviating the need for the middleman. Such maize and cotton is widely grown in the developed world (the Americas, Spain, China, India and South Africa) [3], and maize varieties are being developed by the public sector for other areas of sub-Saharan Africa. The problem here though is one typical of how the developing world is given 'hand me down genes' that do not always fit. The strains of Bt genes that have been given to Africa are appropriate for the European corn borer and not for the African corn borer. The strains thus far developed have not been compared with the more limited approach to stem borers (and *Striga*) of co-cultivation with *Desmodium* [12]. Synthetic Bt strains that should be far more effective in Africa are not being used [28], probably because they would require undergoing new regulatory procedures. Various Bt genes are needed in other crops as well.

It is not clear whether such Bt genes in sorghum (for example) would confer a selective advantage to weedy sorghum should it move.

Grain weevils and moths

Post-harvest insects are especially bad pests, especially in humid tropical countries where grain cannot be properly dried and where closed storage facilities are lacking, especially on the farm. Not only do the insects wreak havoc on the grain, leaving it part-eaten and full of larvae, but also they are vectors for the *Aspergillus* species that produce aflatoxins [27]. These mycotoxins prevent liver adsorption of food at low doses, increase the risk of hepatitis, liver cancer at higher chronic doses and can cause rapid death at acute doses.

There is little published effort on finding transgenes that can prevent attack by these pests in cereal grains but there has been a modicum of success with legumes [29].

There should be Bt genes that deal with these pests, but such efforts to screen for them are unknown. When companies possessing huge libraries of Bt strains were canvassed in the past, they said they had no interest in making their libraries available for post-harvest insect control in the developing world [27].

Diseases where the breeders have not found resistance

There has been little effort to find transgenes that confer resistance to fungal diseases, even with the threat of new wheat rust strains appearing out of Africa. This is less the case with viral diseases where one can use the viral genes either to produce coat protein in the plant, precluding viral growth, or using anti-sensing or RNAi constructs against viral genes transformed into the crop. There has been some success with this approach with cassava [30] and maize streak [31] viruses.

Mobilisation of minerals/prevention of mineral uptake

Many of the inputs that seem inexpensive in the developed world, are expensive in the developing world; fertiliser costs 4–6 times more in real terms and far more relative to farmers' income. Many soils contain considerable amounts of phosphate, which alas is unavailable to plant roots. Various transgenes are being tested that 'mobilise' soil phosphorus rendering it available to plants [32]. Similar genes are needed to mobilise iron, especially for crops cultivated in high pH soils. Conversely, some minerals are at toxic levels in many soils and gene systems are needed that can exclude them from the edible portions of plants. Minerals that need to be excluded include aluminium, arsenate and cadmium. This is an active research area [33].

Transgenes to deal with toxins/anti-feedants

Diets are often monotonous and bad in the developing world. Some foods are fine if mixed into a diet, but are basically poisonous if they are the sole or major source of nutrition. As discussed earlier, poor control of insects not only lowers yield, but also vectors pathogens that release mycotoxins. A few examples of such problems are described below to provide a feeling of how genetic engineering can overcome such problems. The reader should note carefully that most of these problems do not have a sufficient market value to justify involvement of private biotechnology companies (except to sell seed) whereas the public health aspect justifies public sector involvement.

Phosphorus fertiliser is expensive and resources are being rapidly depleted. Much of the phosphate in the plant ends up as a polymer, phytic acid, which cannot be degraded by monogastric animals, necessitating addition of phosphorus to feed. Phytic acid binds iron and zinc rendering them unavailable to monogastric animals (including humans), engendering dietary deficiencies despite the presence of these minerals. Genes preventing the biosynthesis of phytic acid in seeds can be transformed into crops, and/or a gene encoding phytase, which degrades phytic acid can be transformed into crops [34]. Either way one gets adequate dietary phosphorus, iron and zinc without added cost.

Pearl millet, when a major component of the diet, causes goitre because it contains vitexin, which inhibits thyroxin production [35]. The genes encoding vitexin biosynthesis are known [36], and using antisense technology can be suppressed, preventing this problem [1]. Grasspeas contain a compound causing a syndrome known as lathyrism [1]. Suppressing this transgenically will be harder than dealing with vitexin, as the genes encoding the pathway have yet to be isolated.

Soybeans contain allergens that cause severe diarrhoea in infants ingesting soy based milk. This can be crucial for mothers unable to afford more expensive cow's milk in poor countries. The genes that have been isolated can be used to suppress the production of the major allergen [37]. Hopefully the day will arrive when infant formula will bear the label 'contains only soybeans genetically engineered to contain no allergens – does not contain allergenic native soybean products'. This reduced allergy soybean will also allow the use of more soy meal in feed pellets for aquaculture, reducing the need for fish meal.

Genes that encode enzymes degrading mycotoxins have been isolated [38], but have not been deployed in crops for fear of public reaction. It is sad that it is perceived that the public prefers liver damage, cancer and so on over genetically engineered products. Those who mould public perception by misinformation and disinformation should have second thoughts about their ethics.

Wasted feed in biofuel crops proposed for the developing world

Much is being made of the efforts to attain fuel sufficiency by cultivating oilseed crops such as castor bean and *Jatropha* (common name: vomit nut) [39]. These related species produce related toxins, ricin and curcin, respectively, among the most potent toxins known. The literature about them uses understatement in saying that the residual material after oil extraction is 'inedible' (when it is poisonous). The high protein meal is to be used as 'manure' without any environmental impact studies to see what the long lasting poisons do to soil biota. To throw away what could be excellent animal feed seems scandalous in areas of the world where there is little animal protein in the diet. The genes encoding both curcin and ricin are known, which would allow facile genetic engineering suppression of toxin production [39]. Should these undomesticated species be cultivated without dealing with the toxins as well as other issues such as seed shatter, non uniform ripening, need for hand picking and other traits, which render these hard to cultivate?

Environmental biosafety considerations – gene flow

There has been a considerable amount of dissemination of disinformation on how transgenes might wander from crops and introgress into unrelated species (horizontal gene flow) as well as into related wild species [1]. These are specious claims; horizontal gene flow among unrelated species is very common among bacteria. While known in evolutionary time, it is virtually unknown in human time in higher species. Because both the number of pollen grains drops off exponentially with distance and pollen vitality also drops off with time, it is likely that only small amounts of pollen will go from a crop to a wild species in nature. This rare pollen will compete with native pollen. As the crop and wild species are different, the crop pollen would have to overcome species recognition barriers and even if successful, the hybrid would either be sterile or unfit to compete with the wild type. Survival of the fittest is fierce in plants where hundreds of seeds compete to replace a parent plant. Thus, transgenic crops crossing with wild relatives is not much more of a problem than non-transgenic crops crossing with wild relatives.

That does not mean that there can be no problems from gene flow; as discussed above with non-transgenic herbicide resistant rice there is indeed a problem. The problem is not of gene flow to a distant relative in the wild habitat, but to a weedy form of the crop, adjacent to it in the agro-ecosystem [40]. It is here where solutions to gene flow are needed, as described with non-transgenic rice. Fascinatingly, there are no regulatory restrictions to cultivating non-transgenic herbicide resistant rice owing to gene flow issues, but these issues are considered when, instead of a transgene, a mutant gene is used. With the transgene, there are ways to prevent/delay gene flow; no such failsafe mechanisms exist with the mutant genes.

There are two ways to deal with gene flow; before it occurs (containment) and after it occurs (mitigation), as described below.

Containing gene flow

Many methods have been proposed but only some have been tested [1]. These include:

- Engineering the transgene onto the chloroplast genome instead of the nuclear genome. If there is maternal inheritance of chloroplasts, pollen from the crop cannot transfer the trait. This is incorrect about 0.4% of the time, as that amount of pollen transfers the chloroplast genome. More importantly, the proponents ignored that the related weed could pollinate the crop giving an identical hybrid, but with the transgene. The related weed can be the recurrent pollen parent transferring the trait into the weed.
- Use GURTs. Genetic Use Restriction Technologies (more expressively called 'Terminator Genes') are technologies that allow a crop to be cultivated for a single season, and progeny from outcrosses would also die. Still, the transgene could flow in the fields used to produce seed (<1% of agricultural fields). Whether GURTS are 100% suicidal is unclear, as they have not been deployed.
- Single generation transformations. Various attenuated plant viruses can be used as vectors to introduce transgenes into crops. Some of these viruses are not transmitted by seed or pollen, so the transgene DNA cannot be disseminated to weedy relatives of the crop. The technology is presently cumbersome and has not been commercially deployed.

Mitigation of transgene flow

Mitigation is based on co-inheritance of the transgene of choice, which may provide a selective advantage to a weedy relative, with a mitigating transgene that renders the weedy recipient unfit to compete with cohorts [40]. This mitigator gene can encode a trait that is either neutral or beneficial to the crop. These two (or more) transgenes are transformed into the crop in a tandem construct (are covalently bound to each other) and thus will be inherited together, and will very rarely segregate from each other. Wherever the gene of choice goes, so goes the unfit mitigator [41].

Typical mitigating genes are:

- Anti-shattering genes, which prevent seeds from falling to the ground, resulting in their being harvested.
- Dwarfing genes – increase yield of crops (e.g. the first green revolution) but render weeds non-competitive.
- Uniform germination genes – desired in a crop but prevent weeds from having a 'hedge'; if they all come up together they can be exterminated together.
- Susceptibility to a herbicide not used in the current season with the transgenic crop, but used in the following season [4].

Other genes can be used for special purposes, such as non-bolting (no premature flowering) with various root crops, or sterility for vegetatively propagated crops such as potato.

Concluding remarks

There are often ample alternative inputs to transgenic crops, and a great biodiversity of affordable food in the developed word. Such luxuries are not as widespread in the developing world. Thus, it is that transgenics have much more to offer the developing world than the developed. In some developing world crops, it will be necessary to insert failsafe mechanisms to mitigate gene flow from crops to weeds. The public sector will have to perform the product development needed in most instances, as the multi-national private sector does not understand the market or its needs. In the developed world, transgenics have not substantially increased yields, but have reduced inputs and thus increased profitability. In the developing world where the inputs were too costly, transgenic crops can vastly increase yields by inexpensively providing the input in the seed. It is not hard to double yields, when they are a third of the world average.

References

1 Gressel, J. (2008) *Genetic Glass Ceilings: Transgenics for Crop Biodiversity*. Johns Hopkins University Press

2 Kramer, L.C. *et al*. (2009) Correlation between transcript abundance of the RB gene and the level of the RB-mediated late blight resistance in potato. *Mol. Plant Microbe Interact*. 22, 447–455

3 Brookes, G. and Barfoot, P. (2008) Global impact of biotech crops: socio-economic and environmental effects, 1996–2006. *AgBioForum* 11, 21–28

4 Gressel, J. and Valverde, B.E. (2009) A strategy to provide long-term control of weedy rice while mitigating herbicide resistance transgene flow, and its potential use for other crops with related weeds. *Pest Manag. Sci*. 65, 723–731

5 Gressel, J. and Valverde, B.E. (2009) The other, ignored HIV—highly invasive vegetation. *Food Security* 1, 463–478

6 Sanchez Olguin, E.R. *et al*. (2009) Assessment of gene flow from a herbicide-resistant indica rice (*Oryza sativa* L.) to the Costa Rican weedy rice (*Oryza sativa*) in Tropical America: factors affecting hybridization rates and characterization of F-1 hybrids. *Transgenic Res*. 18, 633–647

7 see: http://www.isb.vt.edu/cfdocs/fieldtests3.cfm

8 Messeguer, J. *et al*. (2001) Field assessments of gene flow from transgenic to cultivated rice (*Oryza sativa* L.) using a herbicide resistance gene as tracer marker. *Theor. Appl. Genet*. 103, 1151–1159

9 Gealy, D.R. (2005) Gene movement between rice (*Oryza sativa*) and weedy rice (*Oryza sativa*): a U.S. temperate rice perspective. In *Crop Ferality and Volunteerism* (Gressel, J., ed.), CRC Press

10 Ejeta, G. and Gressel, J., eds) (2007) *Integrating New Technologies for Striga Control—Towards Ending the Witch Hunt*, World Scientific

11 Parker, C. (2009) Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Manag. Sci*. 65, 453–459

12 Khan, Z.R. *et al*. (2008) On-farm evaluation of the 'push-pull' technology for the control of stemborers and striga weed on maize in western Kenya. *Field Crops Res*. 106, 224–233

13 Ejeta, G. *et al*. (2007) Dissecting a complex trait to simpler components for effective breeding of sorghum with a high level of *Striga* resistance. In *Integrating New Technologies for Striga Control—Towards Ending the Witch Hunt* (Ejeta, G. and Gressel, J., eds), pp. 87–98, World Scientific

14 Joel, D.M. *et al*. (1995) Transgenic crops against parasites. *Nature* 374, 220–221

15 Surov, T. *et al*. (1998) Generation of transgenic asulam-resistant potatoes to facilitate eradication of parasitic broomrapes (*Orobanche* spp.) with the *sul* gene as the selectable marker. *Theor. Appl. Genet*. 96, 132–137

16 Aviv, D. *et al*. (2002) Transformation of carrots with mutant acetolactate synthase for *Orobanche* (broomrape) control. *Pest Manag. Sci*. 58, 1187–1193

17 Gressel, J. (2009) Crops with target-site herbicide resistance for *Orobanche* and *Striga* control. *Pest Manag. Sci*. 65, 560–565

18 Kanampiu, F.K. *et al*. (2003) Multi-site, multi-season field tests demonstrate that herbicide seed-coating herbicide-resistance maize controls *Striga* spp. and increases yields in several African countries. *Crop Prot*. 22, 697–706

19 De Groote, H. *et al.* (2008) The potential of a herbicide resistant maize technology for *Striga* control in Africa. *Agric. Syst.* 97, 83–94

20 Tuinstra, M.R. *et al.* (2009) Efficacy of herbicide seed treatments for controlling *Striga* infestation of sorghum. *Crop Sci.* 49, 923–929

21 Timko, M.P. and Li, J. (2009) Gene-for-gene resistance in *Striga*–cowpea associations. *Science* 325, 1094

22 Swarbick, P.J. *et al.* (2009) A major QTL for resistance of rice to the parasitic plant *Striga hermonthica* is not dependent on genetic background. *Pest Manag. Sci.* 65, 528–532

23 Ananiev, E.V. *et al.* (2009) Artificial chromosome formation in maize (*Zea mays* L.). *Chromosoma* 118, 157–177

24 Aly, R. *et al.* (2009) Gene silencing of mannose 6-phosphate reductase in the parasitic weed *Orobanche aegyptiaca* through the production of homologous dsRNA sequences in the host plant. *Plant Biotechnol. J.* 7, 487–498

25 de Framond, A. *et al.* (2007) Effects of *Striga* parasitism of transgenic maize armed with RNAi constructs targetting essential *S. asiatica* genes. In *Integrating New Technologies for Striga Control: Ending the Witch-hunt* (Ejeta, G. and Gressel, J., eds), World Scientific

26 Kanampiu, F.K. *et al.* (2002) Imazapyr and pyrithiobac movement in soil and from maize seed coats controls *Striga* while allowing legume intercropping. *Crop Prot.* 21, 611–619

27 Gressel, J. *et al.* (2004) Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. *Crop Prot.* 23, 661–668

28 Avisar, D. *et al.* (2009) The *Bacillus thuringiensis* delta-endotoxin Cry1C as a potential bioinsecticide in plants. *Plant Sci.* 176, 315–324

29 De Sousa-Majer, M.J. *et al.* (2007) Bean alpha-amylase inhibitors in transgenic peas inhibit development of pea weevil larvae. *J. Econ. Entomol.* 100, 1416–1422

30 Vanderschuren, H. *et al.* (2009) Dose-dependent RNAi-mediated geminivirus resistance in the tropical root crop cassava. *Plant Mol. Biol.* 70, 265–272

31 Shepherd, D.N. *et al.* (2009) Transgenic strategies for developing crops resistant to geminiviruses. *Plant Sci.* 176, 1–11

32 Roemer, W. (2006) New findings on phosphate uptake in plants—a literature survey. *Arch. Agronomy Soil Sci.* 52 ISSN 0365-0340 (print)|1476-3567 (electronic)

33 Mench, M. *et al.* (2009) Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. *Environ. Sci. Pollut. Res.* 16, 876–900

34 Raboy, V. (2009) Approaches and challenges to engineering seed phytate and total phosphorus. *Plant Sci.* 177, 281–296

35 Elnour, A. *et al.* (2000) Endemic goiter with iodine sufficiency: a possible role for the consumption of pearl millet in the etiology of endemic goiter. *Am. J. Clin. Nutr.* 71, 59–66

36 Gebhardt, Y. *et al.* (2005) Molecular evolution of flavonoid dioxygenases in the family Apiaceae. *Phytochemistry* 66, 1273–1284

37 Herman, E.M. (2008) Mitigation of soybean allergy by development of low allergen content seeds. *Food Contam.: Mycotoxins Food Allergens* 1001, 431–445

38 Kimura, M. *et al.* (2006) Molecular biology and biotechnology for reduction of *Fusarium* mycotoxin contamination. *Pesticide Biochem. Physiol.* 86, 117–123

39 Gressel, J. (2008) Transgenics are imperative for biofuel crops. *Plant Sci.* 174, 246–263

40 Gressel, J. (1999) Tandem constructs: preventing the rise of superweeds. *Trends Biotechnol.* 17, 361–366

41 Al-Ahmad, H. *et al.* (2006) Mitigation of establishment of *Brassica napus* transgenes in volunteers using a tandem construct containing a selectively unfit gene. *Plant Biotechnol. J.* 4, 7–21