Transgenic plants: some current issues

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Transgenic plants have already been produced in about 60 plant species. A number of these transgenic crop plants, carrying genes for traits of economic importance have either been released or are ready to be released for commercial cultivation. With the success of these transgenic plants, the following three issues are being widely discussed; (a) biosafety of growing these transgenic plants; (b) transgene silencing or inactivation induced due to homology between multiple copies of transgenes/endogenous genes and (c) intellectual property rights (IPRs) involved in commercializing these transgenic plants, particularly the broad patents. These three issues have been discussed in this article in some detail, using suitable examples and citing relevant literature on these issues published in recent years.

TRANSGENIC plants have now been produced in a large number of plant species (~ 60)[1], and their production in most crop species has now become routine. Although initially these were produced using some reporter genes demonstrating the feasibility of the technology, later a number of individual genes for desirable traits of economic importance were incorporated into many crop plants. In other cases, promoter sequences have been used, which allow enhanced/reduced and/or tissue-specific expression of the adjoining genes according to requirement. In some other cases, antisense RNA genes have been introduced to inhibit the expression of some existing genes in a desirable manner. All these approaches led to the development of transgenic crop plants of commercial value, many of them already field tested (over 1000 field tests with transgenic crops have already been conducted[2]) and a few of them even released for commercial cultivation during 1994–96 (*NBIAP News Reports*, 1995, 1996). 'Flavr Savr' and 'Endless Summer' tomatoes, 'Freedom II' squash, 'high-lauric' rapeseed (canola) and 'Roundup Ready' soybean are some examples of crops that are already being commercially grown in developed countries. During 1995, in USA, full registration was also granted to genetically engineered insect resistant potato (NewLeaf), corn (Maximizer) and cotton (BollGard), each containing *Bt* toxin gene derived from *Bacillus thuringiensis* and producing an insecticide, thus falling in EPA's definition of plant pesticides[3–5]. The pace at which the commercial success of these transgenic crops has been achieved, though remarkable, has already raised several questions, which are being widely debated. The following three issues, which are currently receiving the attention of research workers around the world, will be briefly discussed in this article with a view to drawing the attention of readers to the limitation, which these transgenic crops are facing before they reach the marketplace: (i) biosafety of growing transgenic crops, and of using their products as food; (ii) homology-dependent gene silencing or transgene inactivation in transgenic plants and (iii) intellectual property rights (IPRs) involved in the release of transgenic crops to the farmers for commercial cultivation.

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Biosafety of transgenic plants

In most developed countries and to some extent even in the developing countries, there are agencies which will examine whether or not a particular transgenic plant is safe to be field tested or marketed. In this connection, the Third International Symposium on the biosafety results of field tests of genetically modified plants and microorganisms ('1994 Biosafety Symposium') held in November 1994 at Monterey, California is relevant (see ref. 12). (Fourth International Biosafety Symposium will be held in China during July 1996.) The key topics for discussion at this symposium included the following: (i) potential of GMOs (genetically modified organisms) to become weeds; (ii) opportunities for gene flow from a GMO to another organism; (iii) impacts of growing GMOs on biodiversity and (iv) the capacity of pest and pathogens to adapt to the cultivation of GMOs. At this symposium, it was generally accepted that the GMOs would not differ from products of traditional plant breeding with respect to the above ecological risks, even though no organized field tests seem to have been conducted to advance our general understanding of the field level ecology of agricultural systems[6].

Environmental impact of growing transgenic plants

At the end of the '1994 Biosafety Symposium', it was realized that tests covering thousands of acres of land, or commercialization of several transgenic crops will actually be needed to assess the risk of gene transfer from transgenic plants to other plants and to measure the true environmental impact of growing transgenic

crops[7]. In this connection, for microorganisms, it is extremely difficult to ensure that genetically engineered organisms are not inadvertently introduced into the external environment during a field trial. But in case of transgenic crop plants, wind and bee-mediated pollen dissemination are the most likely means of transfer of a transgene into adjacent plants. The methods for risk assessment of transgenic plants have recently been discussed in some detail[8,9].

In studies conducted on potatoes in New Zealand[10], and on oil-seed rape (called canola in USA/Canada) in UK[11], no evidence was available to show that transgenic plants could pass on their foreign genes to other species. However, in crops like sunflower, sorghum and sugarbeets, it was agreed in the '1994 Biosafety Symposium', that the transfer of genes is highly probable[11,12]. In view of this, there is a consensus that once a transgenic plant is out in the field, one should assume that transgene will escape, which necessitates an assessment of the level of risk involved in such an escape. In such an assessment, if hazardous risk (like that of the production of weed species) is predicted on the basis of nature of the transgene(s) and the plant species involved, field trials should not be allowed/conducted[13].

Another important class of transgenic plants that need to be assessed for biosafety are virus-resistant transgenic plants carrying a segment of virus genome. Evidence has been made available to suggest that the transgenic RNA released due to the expression of foreign gene in such transgenic plants may undergo RNA–RNA recombination, when infected by another virus, thus making the production of a dangerous new virus possible[14]. This situation, however, does not differ from the possibility of recombination between two virus genomes due to mixed infection of a common host plant under ordinary conditions.

It is also argued that field tests generally should not be conducted in regions representing centres of origin/diversity, because these centres are mainly located in developing countries, where governments and citizens may be attracted by the benefits and may ignore the risks[13]. For instance, in China, ignoring the risks involved, 2300 hectares of land has been placed under transgenic crops and another 50,000 to 60,000 hectares of land has been placed under soybean crop inoculated with genetically engineered *Rhizobium*[15]. In India, however, no transgenic crops have so far been released for cultivation, but revised guidelines for field trials, handling and risk assessment of transgenic plants were recently issued[16] (the first guidelines were issued in 1990).

Safety of selectable markers in transgenic crops used as food

Marker genes (selectable and scoreable markers) like *nptII*, normally present in transgenic plants, have also been examined for safety[17]. It has been shown in several studies that the enzyme *nptII*, has no adverse effects[18,19]. It has also been estimated that extensive planting of transgenic plants with *nptII* gene (assuming that the gene will be transferred from plants to microbes), will not cause an increase of more than 0.0001 per cent in the frequency of kanamycin-resistant bacteria. Similarly, if *nptII* is transferred through pollen to related plants, the latter will not be any different than the transgenic plant itself. Keeping the above in mind, we can assume that transgenic plants with selectable marker genes like *nptII* are safe to be used for large-scale cultivation (for a review, see ref. 17).

Should transgenic food be labelled for consumers

There have also been discussions in several countries on whether or not it should be mandatory to label the genetically engineered food[20,21]. No such mandatory labeling has been considered necessary in USA, unless the transgenic food is significantly different in composition or is potentially allergenic. For instance, if a tomato has a significantly reduced content of vitamin C, this alteration should be disclosed[20]. Similarly, tomato having a gene from peanut should disclose this (because peanut contains known allergens) unless transgenic tomato was shown not to induce allergic reactions in people known to be sensitive to peanut. In this connection, the example of transgenic canola carrying a gene encoding a 'Brazil nut protein' can be of interest, because Brazil nut protein being allergenic[22], these transgenic canolas were considered to have no commercial utility.

Gene-silencing or transgene inactivation in transgenic plants

During 1989 to 1991, a series of reports were published which established that the presence of multiple copies of a transgene in a plant nucleus can lead to silencing of some or all copies of the gene including the endogenous gene having homology with the transgene (for reviews, see refs 23–27). It has also been established that in all such cases of transgene silencing, loss of expression of the transgenes is not due to loss of these genes but due to their inactivation. The following are the variations in the phenomenon of transgene silencing.

Inactivation of introduced T-DNA or a novel transgene

T-DNA transferred from *Agrobacterium tumefaciens* whenever inactivated, was associated with the presence of multiple copies and hypermethylation of the introduced

T-DNA[28,29]. Similarly when multiple copies of a novel transgene are inserted, such a transgene is likely to be inactivated; the best example is that of an *AI* gene introduced into white flowering mutant of *Petunia hybrida*, where insertion of a single copy gave coloured flowers but insertion of multiple copies gave white flowers[30]. The transinactivation was associated with methylation of sites within 35S promoter or in the transgene[31,32]. Several other examples of such inactivation of transgenes are listed in Table 1 and the systems proposed to avoid this inactivation are listed in Table 2.

Transinactivation of independent transgenes

Sometimes more than one independent transgenes are inserted during an exercise of pyramiding of genes. Such transgenes may be transinactivated, if there is sequence homology either in the structural transgene or in its associated promoter[33–36]. In a report of transgenic tobacco carrying two transgenes (*nptII* and *nos*), it was shown that the loss of kanamycin resistance (conferred by *nptII*) was associated with methylation of CpG residues in its nopaline synthase promoter sequence and that this inactivation was more common in plants that were heterozygous[37].

Transinactivation can also occur between two alleles of a transgene giving different phenotypes. In petunia, when a white variant 17W (isolated from a transgenic petunia plant) was combined with a red form 17R by crossing, the progeny did not have the expected red flowers, but had predominantly white flowers. It was shown in this case that inactivation of *AI* gene in 17W was associated with hypermethylation of 35S promoter and that in the heterozygous state this allele transinactivated the allele derived from 17R genotype[38]. This example of transgene silencing may be compared with allelic interactions (e.g. paramutation at *R* locus in corn) leading to silenced state of endogenous genes[39,40].

Co-suppression of a transgene and its homologous endogenous gene

Introduction of extra copies of an endogenous gene to boost its expression may also often result in the coordinate silencing not only of the introduced transgene but also of the endogenous gene. This phenomenon is often described as co-suppression, which was first described in *Petunia*, when a chalcone synthase gene (*chs*) involved in a floral pigment biosynthesis was introduced in a deep violet flowering line of *Petunia hybrida*. Surprisingly, up to 42% of transgenic plants had white or variegated flowers[41,42]. It was shown in this case that co-suppression was due to a post-transcriptional event[43,44], and was independent of promoter, which was necessary for transcription. Co-suppression has now been reported for many different transgenes[45–47], and a single transgene can cause co-suppression of two or more endogenous genes[48,49].

In summary, the transinactivation or co-suppression of genes is dependent upon sequence homology between genes or their promoters, can be epigenitically reversible, and is sometimes more effective in hemizygous than in homozygous condition. The inactivation may involve inhibition of transcription or degradation of mRNA, may be under developmental control and does not necessarily occur with all the copies of a transgene.

Transgenic plants and intellectual property rights

The laws governing patents and other kinds of IPRs differ in different countries and are still in the process of being finalized in several countries including those which are the major players in the field of agricultural biotechnology and are now also members of World

Table 1. Examples of transgene inactivation in plants (modified from ref. 25)

Engineered plant	Transgene and source	Transgene product	Transgene phenotype	Mechanism of silencing*
Petunia hybrida	*AI* (*Zea mays*)	Dihydroflavonol 4-reductase	Novel flower pigmentation	Transgene promoter methylation
Petunia hybrida	*chs* (*P. hybrida*)	Chalcone synthase	Novel flower pigmentation	Co-suppression of ectopic and endogenous genes (post-transcriptional; no methylation)
Nicotiana tabacum	*T-DNA* (*A. tumefaciens*)	Not applicable	Tumour formation	Methylation of T-DNA
Nicotiana tabacum	*nos* (*A. tumefaciens*)	Nopaline synthase	Nopaline biosynthesis	Transgene promoter methylation
Nicotiana tabacum	*nptII* (*E. coli*)	Neomycin phosphotransferase	Antibiotic resistance	Reversible over generations
Nicotiana tabacum	TEV-CP (Tobacco etch virus)	Coat protein	Virus resistance	Post-transcriptional
Medicago sativa	*bar* (*Streptomyces hygroscopicus*)	Phosphinothrycin acetyl transferase (PAT)	Herbicide resistance	Transgene inactivation affected by environment
Oriza sativa	*nptII* (*E. coli*)	Neomycin phosphotransferase	Antibiotic resistance	Transgene inactivation, amplification and loss

*For detailed references, see text and consult ref. 25.

Table 2. Development of systems to avoid transgene inactivation (modified from ref. 25)

Probable cause of inactivation	System to avoid inactivation
Multiple transgene integration	Selection for single copy insertion or development of methods to ensure single copy insertion
Transgene methylation	Use of demethylation sequences
Detrimental effect of sequences adjacent to transgene integration site	Engineer transgenes as distinct structural domains using MAR (matrix attachment regions)
Co-suppression and/or over-expression effects	a) Selection/screening for stable transgene expression b) Development of site-specific recombination systems

For detailed references, see text and consult ref. 25.

Trade Organization (WTO). The overwhelming majority of patent claims in the area of plant biotechnology during the last 10 years actually originated in these industrialized countries (including Europe, USA, Canada, Japan, Australia, New Zealand and Israel) which account for nearly 100% of plant utility patents. The South is virtually unrepresented, despite the fact that much of the patented germplasm originated there[50]. However, the most important country in the field of patenting plants is USA, where the following three separate systems are available for protecting intellectual property rights for plant material: (i) 'The 1930 Plant Patent Act' (covering only asexually reproduced plants); (ii) 'The 1970 Plant Variety Protection Act or PVPA' (US version of 'plant breeders' rights'), which was amended in 1994 to broaden its scope to include all material harvested from patented variety and to limit the farmer's and breeder's exemptions and (iii) 'The 1985 Utility (Industrial) Plant Patents' (covering all kinds of plants, just like industrial patents). Using the above systems, at least four kinds of patents are available in USA in the area of plant biotechnology: (a) patents for innovations (e.g. procedures for hybrid rice production), (b) patents under PVPA (e.g. inbreds and hybrids of maize), (c) utility patents covering a wide group of plants (see below), and (d) patents on research tools (e.g. DNA and protein sequences used for a variety of purposes). Among the above, the utility patent is considered to be the most powerful protection of IPRs for plants and related subjects[50]. These patents are not restricted to individual cultivars, but a single patent may cover several varieties, an entire genus or species, genes/proteins or technology and processes[50]. However, often no questions of infringement are raised for many of the patented technologies, processes and genes for specific traits, used for the production of transgenic plants, until a transgenic plant is ready to be marketed. At the time of marketing, however, one needs to ensure that no patent or other IPR has been infringed, so that licences may have to be obtained for patented technologies/processes or for specific genes used for the production of the concerned transgenic plant. In view of this, following the marketing of transgenic plants, legal battles have recently been witnessed in USA and Europe, where products of agricultural biotechnology are reaching the market at a fast pace.

Broad patents in agricultural biotechnology

One area of IPRs related to agricultural biotechnology, which has received maximum attention in recent years is the broad nature of several patents awarded to some biotechnology companies (Table 3). These broad patents have been opposed by many NGOs and have been considered as a threat to food security, because this will allow a monopoly of a single corporation to dictate terms and conditions for future plant breeding research[50,51].

The most widely discussed case in agricultural biotechnology is the broad patent for transgenic cotton (covering all kinds of transgenic cotton) issued in October 1992 to Agracetus (Middleton, WI in USA), a subsidiary of WR Grace and Co.[52,53]. A similar patent for transgenic cotton was granted to Agracetus in India also in 1991. A patent for herbicide-resistant transgenic soybean was also issued to Agracetus in Europe and applications by Agracetus for patenting this soybean have been made in USA and Canada also. For transgenic cotton, Agracetus was initially awarded the product by process claim in April 1991, but later keeping in view the Scripps vs Genentech case, a broad product claim was granted on 27 October 1992. (Scripps had a patent for a purified clotting factor made from blood; later Genentech produced it using genetic engineering, which was challenged for infringement of Scripp's patent; court ruled that Genentech should obtain a licence from Scripps; see ref. 54.) Several other patents granted to Agracetus are listed in Table 4.

A biotech subsidiary (Biosem) of the seed industry giant, 'Goupe Limagrain' (France) holds a patent on virtually all transgenic *Cucumis melo* (melons, muskmelons and cantaloupes) produced using *Agrobacterium tumefaciens* system. DNA Plant Technology (USA) has patented all transgenic pepper plants (genus *Capsicum*) and transgenic garden pea plants. Calgene Inc. (USA) claims ownership of all genetically engineered plants in the *Brassica* family (*Brassica* includes rapeseed, broccoli, cauliflower, cabbage and brussels sprouts) and Escagenetics holds a patent on all transgenic coffee (*C. arabica*) plants[50] (Table 3).

Another important example of a broad patent is the patent no. 5,380,831 granted in January 1995 to Mycogen (a California-based Biotech company). This patent covered 'any method of modifying *Bacillus thuringiensis* (*Bt*) genes to make them resemble plant genes', and, therefore, is extremely broad and covers all insect-resistant crop plants using modified *Bt* genes. Mycogen holds

Table 3. Some broad patents for plants, traits and technology or processes (modified from RAFI, July–August 1995)

Patent holder	Patent no.	Subject of patent
Plant patents		
W. R. Grace and Co.	5,519,135	All transgenic cotton (revoked, but still effective till appeals are exhausted)
	EPO 0301749	All transgenic soybean (opposition filed by RAFI, other NGOs and seed corporations)
Calgene Inc.	5,188,958	All transgenic plants of Brassica family (*Agrobacterium* mediated only)
Escagenetics Corp.	5,334,529	All transgenic *C. arabica* (coffee)
Biosem, subsidiary of Limagrain (France)	5,422,259	All transgenic *Cucumis melo*, modified using *A. tumefaciens*
DNA Plant Technology (DNAP)	5,262,316	All transgenic pepper (genus *Capsicum*)
Fresh World (subsidiary of DNAP)	5,286,635	All transgenic pea (*Pisum sativum*)
Plants with specific traits patented		
Dekalb Genetic Corp.	5,258,300	All transgenic plants with increased lysine content
Lubrizol Corp.	4,627,192	All sunflower plants/seeds with increased oleic fatty acid (> 80%)
Pioneer Hi-Bred	5,276,264	Sunflower products with low level of saturated fatty acids
Lucky Biotech Corp. and Univ. of California	5,234,834	Fruits/seeds/vegetables of transgenic plants with supersweet thaumatin/monellin genes
Plant Genetic Systems	5,254,799	All transgenic plants with *Bt* gene (using *Agrobacterium*)
Upjohn Co	5,349,128	All transgenic plants from Cucurbitaceae and Solanaceae, carrying 'cucumber mosaic virus coat protein gene'
Technology/processes		
Enzo Biochem Inc.	?	'Antisense' technology
Mycogen Corp.	5,380,831	Process used to synthesize *Bt* genes

this patent not only in USA, but also in Australia, New Zealand, South Africa, Taiwan and Europe. The patent is also pending in the following countries: Argentina, Canada, China, Japan, Korea, Russia, Ukraine.

Apart from the above, many other food and industrial crops are the subject of sweeping patent claims. This trend has been considered morally unacceptable and fundamentally inequitable[50]. These sweeping patent claims extending to all plants engineered to express a specific gene or to exhibit a particular trait demonstrate that the intellectual property system in these cases is out of control. We think that, such a system of protection, if allowed, will work well only for a handful of industrial corporations, who will have a monopoly control over even those crops, that feed and sustain humankind. Eventually they will also have a legal right to determine the future of high-tech research for the entire segment of agriculture and plant breeding.

The broad patent for transgenic cotton granted to Agracetus has been challenged, and in US the Patent and Trademark Office (PTO) re-examined the issue and revoked the patent in December 1994. In India also, ICAR and the Department of Biotechnology (Govt of India) were successful in getting this patent revoked in 1994. However, Agracetus has an opportunity to appeal against the reversal decision on their broad patent and final outcome will be known only in due course of time[52].

Transgenic plants have also been covered by patents rather indirectly through patenting technologies, DNA sequences or expressed genes in seeds, etc. The PTO's definition of a 'transgenic plant patent' includes only claims on entire plants that have been altered with foreign DNA. However, many patented DNA sequences extend to plants that contain the patented DNA sequence[55]. For instance, Calgene's patent # 5,420,034 on *Brassica* (producing valuable oils), though issued to cover the seed with specific promoters, will automatically extend to the transgenic plants[56].

Limitations to commercialize transgenic plants

The ability of a company to commercialize a genetically engineered product is also limited by a large number of technologies and/or raw material used for developing a product. Some of the patented technologies include

Table 4. Some of the patents granted to Agracetus

Technology	Patent	Date
Agrobacterium tumefaciens mediated transformation of cotton	US patent no. 5,004,863	2 April 1991
Gene gun method for genetic engineering soybean	US patent no. 5,015,580	April 1991
'Accell' = FE gene gun	US patent no. 5,120,657	June 1992
All transgenic cotton	US patent no. 5,159,135 (also granted patent in India)	October 1992
All transgenic soybean	Europe patent no. 0,301,749,B1	March 1994

transformation methods and gene silencing technique (e.g. anti-sense technology). Similarly, promoter sequences and selectable markers/scoreable markers, trait specific genes (e.g. herbicide resistance, insect resistance, etc.) and the germplasm are also needed for production of transgenic plants. Many of these technologies and raw material have been patented and the ownership of several others is uncertain. The products developed using these patented technologies and/or raw material cannot be used or sold without a licence, and in some cases these may not even be available on a licence basis, if exclusive licence has been issued to one company. Cross-licensing and joint ventures between patent holders for key technologies can be other solutions. The problem of IPRs associated with commercialization of transgenic crops will be illustrated using three examples.

DNA Plant Technology's (DNAP's) 'Endless Summer' tomato

'Endless Summer' was due to be released by DNAP in USA during the summer of 1995 (ref. 57). They are facing difficulties, because they used the following patented technologies/sequences; anti-sense technology called 'TRANSWITCH', *Agrobacterium tumefaciens*-mediated transformation technology, the CaMV35S promoter, selectable markers like *KanR* or *nptII* with its promoter and terminator and the ACC synthase gene. DNAP is making efforts to overcome the difficulties due to patented technologies used by them[57]. In collaboration with DuPont, DNAP is in the process of substituting a proprietary fruit-specific promoter to drive expression of the TRANSWITCH construct and also substituting an *ALS* (= acetolactase synthase gene providing resistance against several herbicides) based selectable marker system (*ALS* gene patented by Dupont + patented promoter and terminator) for *KanR* or *nptII*. DNAP is also trying a patent for *ALS* promoter. For the other remaining technologies, licences are the obvious solution. The new technology will be used for the new second generation version of the 'Endless Summer tomato' to be released later in 1996. This will allow DNAP the complete 'freedom to operate'.

Mycogen's and Ciba Seeds' insect-resistant 'Maximizer' hybrid corn

In the year 1996, 'Mycogen' and 'Ciba Seeds' are expected to be the first two companies to commercialize *Bt* insect-resistant seed corn, which has shown very effective protection from the 'European corn borer'[57]. The IPR position is complicated, because a number of companies are involved in a number of patented technology pieces and a number of other patent filings are pending for decisions. Mycogen's strategy to become the first company to commercialize insect-resistant corn and to create for itself 'freedom to operate' involves the following two phases: The *first phase* involved cross-licencing and collaboration with 'Ciba Seeds' during 1993. 'Ciba Seeds' got access to Mycogen's technologies for producing transgenics using synthetic *Bt* and Mycogen got access to the first generation transformed corn plants produced by 'Ciba Seeds'. In the *second phase*, Mycogen is creating 'freedom to operate' for second generation *Bt* seed corn products using a proprietary transformation system developed, a number of patented T-DNA promoters to replace CaMV35S and a *bar* gene licensed directly from Hoechst (earlier it was obtained through Ciba Seeds).

Calgene's (Davis, California) 'Flavr Savr' tomato

After marketing 'Flavr Savr' tomato in 1994, Calgene had to face a patent infringement suit with Enzo Biochem Inc. of New York – the company which owns the rights to the 'antisense gene modification process' used by Calgene to produce 'Flavr Savr'[58]. Enzo Biochem alleges that Calgene infringes on its patent for altering gene, and Calgene claims that Enzo's patent is invalid, because, according to them, a Calgene researcher was the first to demonstrate how the process works.

Conclusions

During 1996 and in the following years, many transgenic crop plants will reach the farmers for cultivation, after the necessary approval from agencies, which oversee whether or not it is safe to grow these transgenic plants either for field tests or for commercial cultivation. However, the issues involved in biosafety of these transgenic plants will keep on being discussed in biosafety symposia/seminars regularly organized in dif-

ferent parts of the world. This will result in formulation of guidelines which will be followed, but will not prohibit either the production or the commercial utilization of these transgenic plants. New strategies for the production of transgenic plants will also be developed to deal with the problem of homology dependent-transgene silencing or transinactivation, so that this problem will be overcome rather than discourage the production of more transgenic plants. However, the major problem will be to deal with intellectual property rights, particularly the broad patents, which will be overzealously procured in view of the heavy investments made or being made and in view of the involvement of private companies, which may often be concerned with the financial returns rather than the revolution in crop productivity expected from the use of transgenic plants. However, these problems will be sorted out in the coming years, so that the transgenic plants will supplement (not replace) the conventional plant breeding in a big way in the twenty first century.

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