

Advances in plant biotechnology and its adoption in developing countries

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Developing countries are already benefiting and should continue to benefit significantly from advances in plant biotechnology. Insect-protected cotton containing a natural insecticide protein from *Bacillus thuringiensis* (Bt cotton) is providing millions of farmers with increased yields, reduced insecticide costs and fewer health risks. Many other useful plant biotechnology products that can benefit poor farmers and consumers are in the research and development pipelines of institutions in developing countries, and should soon reach farmers' fields.

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Abbreviations

Bt *Bacillus thuringiensis*
CMD Cassava mosaic disease
MAS marker-assisted selection
QTL quantitative trait locus

Introduction

Over the past half century, the genetic improvement of crops, combined with complementary agronomic practices, have benefited billions of poor people in developing countries. Asia's 'Green Revolution' achieved increases in crop productivity that were sufficient to lower the proportion of the population suffering from chronic hunger from 40% to 20% while the overall population more than doubled. In addition, by increasing yields on land already in production, hundreds of millions of hectares of tropical forests and other natural environments were saved from conversion to agriculture.

Yet, worldwide, roughly 800 million people remain chronically undernourished — consuming less than 2000 calories per day. Even more are malnourished

due to diets that are deficient in vitamins and mineral micronutrients. Many of the most seriously deprived live in rural areas where the Green Revolution technologies have not been adopted, primarily because of inadequate or unreliable rainfall and lack of irrigation [1].

In many developing countries, the majority of the population still produce most of their own food and depend on small-scale farming for their incomes and livelihoods. China and India each have more than 500 million people who depend on small-scale agriculture and Africa has more than 400 million. On the basis of these numbers alone, these countries stand to benefit most from further genetic improvements of crops using conventional plant breeding, as well as breeding supplemented by new biotechnologies that are derived from advances in plant molecular and cellular biology.

Realization of such benefits can already be seen with the adoption of transgenic crops. James [2^{*}] reports that more than 5.5 million farmers worldwide grew transgenic crops in 2001, including farmers in Argentina, China, South Africa, Mexico, Uruguay, Indonesia and, without government approval, Brazil. Of these, over 75% are small-scale cotton growers, mainly in China, who have readily adopted new cotton varieties that contain transgenes for insect resistance. The larger developing countries are investing substantial public resources in plant biotechnology research, with China's investment alone now more than US\$100 million year⁻¹ and projected to increase significantly [3]. Unlike the USA and Europe, where the public sector has developed many useful transgenic crops that are not being commercialized because of proprietary property and regulatory constraints [4^{*}], the public sector in China is delivering products. Most of the *Bacillus thuringiensis* (Bt) cotton varieties and all of the transgenic tobacco and tomato lines commercialized in China were developed by public research institutions [5]. And, it's not just China; promising transgenic lines of around twenty different crops produced by public research institutions in at least ten developing countries are now being officially field tested as they near national regulatory approval. As shown in Box 1, transgenes have been introduced into many important tropical crops. The majority of the transgenic lines have transgenes for traits, such as virus resistance, that can significantly benefit poor farmers who cannot afford more expensive disease control strategies and currently suffer significant crop losses.

Indeed, public research institutions in countries such as China, India and Brazil, which have both excellent

Box 1 Public research institutions in developing countries are conducting official field trials of more than 20 transgenic crops.

Countries conducting field trials

| | | |
|------------|--------|--------------|
| Argentina | Egypt | Philippines |
| Brazil | India | South Africa |
| China | Kenya | Thailand |
| Costa Rica | Mexico | |

Transgenic crops being tested

| | | |
|-------------|---------|--------------|
| Beans | Mustard | Squash |
| Cabbage | Papaya | Strawberry |
| Cauliflower | Peanut | Sugar Cane |
| Chili | Pepper | Sweet Potato |
| Cotton | Potato | Tobacco |
| Eucalyptus | Rape | Tomato |
| Maize | Rice | Wheat |
| Melon | Soybean | |

scientific capacity and greater ‘freedom-to-operate’, are likely to become the primary employers of plant biotechnology to deliver useful new varieties of tropical crops to farmers with limited purchasing power. The private sector is increasingly concentrating on only a handful of major crops and profitable markets. And, owing to proprietary property constraints, public sector institutions in industrialized countries find it increasingly difficult to commercialize products of plant biotechnology without corporate sponsors.

Insect-resistant Bt cotton leads the way

Transgenic cotton varieties containing insect-resistance genes derived from the insecticidal microbe *Bacillus thuringiensis* are now being grown commercially in China, South Africa, Mexico, Argentina, Indonesia, and India. Pray *et al.* [6,7**] have followed the adoption of Bt cotton in China, which began in 1997. By 2001, 3.5 million farmers, growing on average 0.42 hectares, planted 1.5 million hectares of Bt cotton. This equates to roughly 31% of the area planted to cotton in China. The rapid spread of Bt cotton was driven by the farmers’ demand for a technology that increases yield, reduces insecticide use and costs, reduces insecticide poisonings and requires less labor. Initial yield increases were in the 5–10% range and modest increases continued over time, suggesting that farmers are learning to manage Bt varieties better. There is no indication that insect pests are becoming resistant to Bt cotton.

The use of insecticides in China has reduced substantially due to the use of Bt cotton. The use of formulated insecticide fell by 20 000 tons in 1999 and 78 000 tons in 2001, the latter being roughly a quarter of all of the insecticide sprayed in China before the adoption of Bt cotton. Cost savings for farmers are now beginning to push down the price of cotton, so consumers will also benefit. Bt technology is being used increasingly in China as a component of integrated pest management strategies.

Table 1

Costs and benefits of Bt cotton production on the Makhathini Flats, South Africa 1999 – 2000.

| | Conventional cotton | Bt cotton |
|--|---------------------|-----------|
| Yield (kg ha ⁻¹) | 261 | 471 |
| Value of output (Rand [R] ha ⁻¹) | 568 | 905 |
| Seed cost (R ha ⁻¹) | 91 | 197 |
| Insecticide cost (R ha ⁻¹) | 116 | 72 |
| Gross margin (R ha ⁻¹) | 361 | 638 |

Source [9].

As documented by Fang *et al.* [8], much of the Bt cotton grown in China was originally developed by the Beijing-based Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences. This government-sponsored research institute has obtained patent, plant variety and trademark protection in China for its insect-resistant Bt cotton. The original transgenic lines were sub-licensed to provincial seed companies and the transgenes were backcrossed into more than 22 well-adapted local varieties. Sub-licenses also have been issued to Chinese companies for the commercialization of Chinese Bt cotton technology in Vietnam, Cambodia, Thailand and India.

In South Africa, Bt cotton is being grown by both large-scale and small-scale farmers, with small holders who farm intensively gaining the most from the technology [9,10]. The greatest benefit for all farmers is a significant reduction in insecticide costs. Table 1 summarizes the direct costs and benefits of Bt-cotton production for those farming 1–3 hectares of rain-fed cotton in the Makhathini Flats region of Northern Kwa-Zulu Natal during the 1999–2000 growing season. These farmers, half of whom are women, received a 77% higher return from Bt cotton than from conventional varieties and also benefited through a significant reduction in the number of necessary insecticide applications, which are labor intensive and dangerous for smallholders.

In the states of Coahuila and Durango in north central Mexico, Traxler *et al.* [11] credit Bt cotton with solving a pest infestation problem caused largely by pink bollworm and tobacco budworm that threatened an industry of small-holder (2–10 hectares) and medium-sized (30–120 hectares) producers. The Bt cotton was deployed as part of the government’s integrated cotton pest management program. Bt varieties were grown on 96% of the area sown to cotton and reduced insecticide use by 80%. Pest levels declined to a new low and reached an equilibrium with beneficial insects. Farmers in this region are now reluctant to use insecticides for fear of upsetting the new equilibrium. For 1997 and 1998 combined, an estimated economic surplus of US\$6 million was generated by Bt cotton in Coahuila and Durango, with 86% accrued to farmers and 14% to seed suppliers.

Bt-cotton varieties, provided through Monsanto alliances, were recently introduced to smallholders in India and Indonesia. In India, cotton is responsible for more than half of total insecticide use, yet yield losses from insects are still 50–60%. On-farm field trials of Bt cotton in years with high bollworm pressure gave gains of 80% over yields provided by conventional varieties. Indian farmers are naturally anxious to access Bt-cotton seed, and the technology is expected to spread rapidly and cover 25% of the Indian cotton area by 2005 (M Qaim, personal communication).

Insect-resistant Bt cereals

Bt maize is being grown commercially in Argentina and South Africa as well as in North America and Europe. In South Africa, Bt-maize production includes Bt-white-maize grown by smallholders for their own consumption. Bt maize has been field tested and is nearing approval in China, Brazil, Egypt and the Philippines. A publicly produced Bt maize is soon to be field tested in Kenya [12,13].

Field and greenhouse tests of Bt rice produced by public institutions have demonstrated the effectiveness of Bt technology in controlling rice pests in China [14,15], India [16], and Pakistan [17]. Unlike chemical insecticides, Bt rice effectively controls Lepidopteran pests without inducing the emergence of other rice pests, such as brown plant hopper [18**]. As a component of integrated pest management systems, Bt rice has the potential to increase yields and greatly reduce insecticide use in Asia.

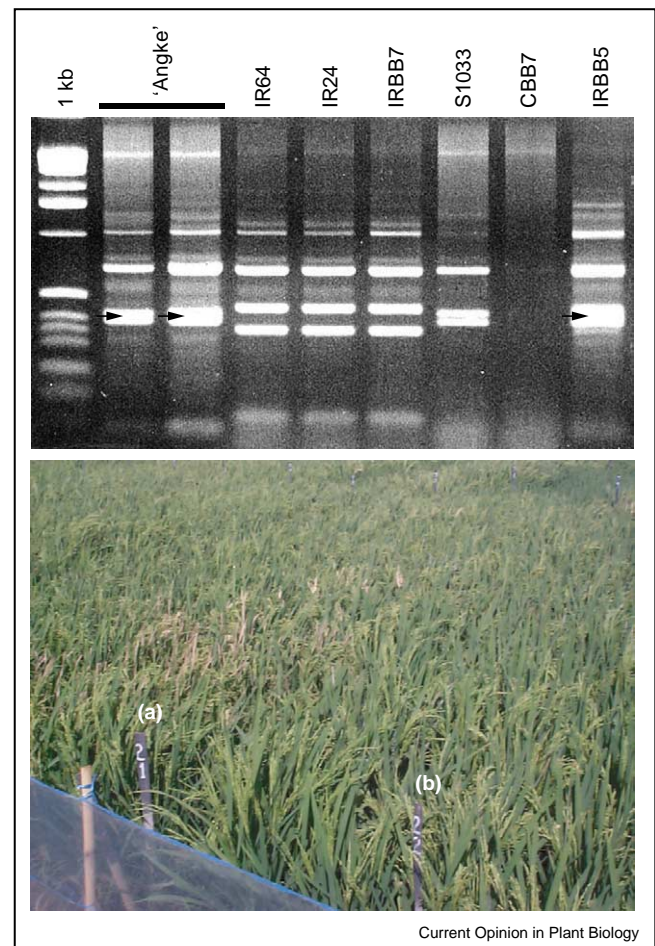
Disease resistance

Several field tests of transgenic crops containing genes for bacterial and fungal disease resistance are under way in developing countries and results have been promising so far [19–21]. However, none of these crops has yet been commercialized. Rather, the first biotechnology-derived disease resistant lines to be commercialized have resulted from pyramiding natural resistance genes via marker-assisted selection (MAS).

In January 2002, the government of Indonesia released two new rice varieties, 'Angke' and 'Conde', which were derived by disease resistance breeding augmented with polymerase chain reaction (PCR)-based MAS to pyramid bacterial blight resistance genes into commercially adapted varieties [22]. These new varieties are the product of more than ten years of international collaborative research efforts that led to a better understanding of population genetics and genome structure of the rice pathogen 'bacterial blight' (*Xanthomonas oryzae*) [23] and to an increasing inventory of bacterial blight resistance genes from the genomes of *Oryza sativa* L. and near relatives such as *Oryza minuta* [24**].

Figure 1 illustrates the selective addition of a resistance gene, *Xa5*, into the IR64 genetic background to augment

Figure 1



(Lower) Photograph illustrating the susceptibility of (a) the IR64 breeding line before the bacterial blight resistance gene *xa5* was introgressed into it by marker-assisted selection, creating (b) the new blight resistant rice variety 'Angke'. The photograph was taken at the Research Institute for Rice, Sukamandi, West Java, Indonesia. The research is a collaborative effort of the Asian Rice Biotechnology Network (ARBN). Photo courtesy of M Bustamam, Indonesian Agriculture Biotechnology and Genetic Resources Research Institute, Indonesia, and M Bernardo, International Rice Research Institute (IRRI) and ARBN, Philippines. (Upper) Photograph of a gel showing the banding patterns of: lane 1, a 1-kb marker; lanes 2 and 3, the *xa5*-linked marker RG556 from 'Angke' the new resistant variety; lane 4, IR64 (the recurrent parent); lanes 5–8, negative controls; and lane 9, IRBB5 (the positive control and donor breeding line). The arrows denote the presence of the *xa5* gene marker. Photo courtesy of CM Vera Cruz and H Leung, IRRI/ARBN.

the *Xa4* resistance gene already present in the IR64 breeding line. Similar work, also focusing on pyramiding bacterial blight resistance genes using MAS, has advanced to field trials in northern India [25], eastern India [26] and in China's hybrid rice breeding program [27].

In Africa, Cassava mosaic disease (CMD) is the most widespread and damaging disease of cassava. Epidemics of CMD can reduce yields by 100%, and losses of 20–90%

are common throughout Africa. Farmers cannot afford to use insecticides to control the white fly vector of CMD, so host plant resistance is the best means of control. A good source of resistance was first detected in third generation backcross progeny from an interspecific cross between cultivated cassava and the wild relative *Manihot glaziovii* [28].

Recently, a simple sequence repeat (SSR) marker and a restriction fragment length polymorphism (RFLP) marker linked to a novel dominant gene that confers resistance to CMD were identified in cassava using bulk segregant analysis [29^{*}]. The SSR and RFLP markers were calculated to be 8 cM and 9 cM, respectively, from the gene. The gene was detected in the Nigerian variety 'TME3', and was shown to be qualitative in nature and stable across environments. This result should enable the marker-assisted selection of CMD-resistant cassava genotypes in Africa and in Latin America. CMD has not yet been noted in Latin America, but the vector has recently been found and local varieties are highly susceptible.

Striga control

Striga, or 'witchweed', compromises several species (most notably *Striga hermonthica* and *Striga asiatica*) of parasitic weeds that attack maize, sorghum, millet, rice and cowpea throughout most of sub-Saharan Africa [30]. Estimates of the economic losses caused by *Striga* reach as high US\$7 billion annually [30].

Maize is particularly sensitive to parasitism by *Striga* but, unfortunately, researchers have not yet found significant genetic resistance to *Striga* within the genome of cultivated maize. At present, genetic research is focused on introgressing resistance or tolerance traits that are found in the relatives of maize, teosinte and *Tripsacum*. Meanwhile, a promising seed-based strategy has been developed as an alternative means of controlling *Striga*. This strategy is based on resistance to the herbicide imazapyr that was first discovered in 1991 through *in vitro* selection [31].

Kanampiu *et al.* [32] bred imazapyr resistance into maize varieties that were adapted to East African conditions. They then coated the seed of these varieties with a magnesium salt of imazapyr before planting them in soil that was infested with viable *Striga* seed. As shown in Figure 2, during the three months after planting, almost no *Striga* parasitized plants grew from seed treated with 0.3 mg imazapyr. The few *Striga* plants that did emerge did not produce flowers and died soon after emergence. In contrast, untreated maize plots suffered from high *Striga* emergence. These results have since been confirmed on farmers' fields (Figure 3), where the cost of applying herbicide to the seed was just US\$4 ha⁻¹ [33^{**}]. The development of this method, combined with agronomic control strategies, may provide effective control of *Striga* in Africa.

Figure 2



Effect of imazapyr-resistant maize seed coated with 3 mg imazapyr per seed on emergence of *Striga hermonthica* in fields artificially infested with *Striga* seed.

Drought tolerance

Many of the world's poorest people farm in areas with inadequate or unreliable rainfall. Furthermore, agricultural sources of fresh water are decreasing in quantity and quality throughout the world. This is true not only for cereal grain farmers who depend on rainfall for crop production but increasingly for farmers in irrigated areas, which are falling into the 'poorly' or 'partially' irrigated category. Thus, there is a need to achieve greater drought tolerance while not reducing the yield potential of crops in years when abundant water resources are available.

The use of transgenics to provide enhanced drought tolerance is still experimental, but progress is being made. Research on dehydration tolerance has established the bases of at least four signal transduction pathways that function under conditions of abiotic stress and has identified binding elements that bring about stress-inducible gene expression [34–36]. Wu *et al.* [37,38] expressed several 'candidate genes' for abiotic stress tolerance in rice that clearly increased the accumulation of biomass under water deficit. The obvious next step is to investigate the impact of candidate-gene constructs, which are driven by promoters that are induced by water stress, by measuring the growth and agronomic yield of whole plants on a field scale in well-controlled 'managed stress environments'. Hopefully, this can be done by a consortium of plant molecular biologists, integrative plant biologists and agronomists working together in a country, such as China, that is committed to developing drought-tolerant varieties.

Currently many research groups worldwide are attempting to demonstrate the success of MAS in breeding for

Figure 3



Effect of imazapyr-resistant maize seed coated with 3 mg imazapyr per seed on emergence of *Striga hermonthica* in farmer's field infested with *Striga* seed. Plants grown from coated seed are in the background and control plants in the foreground. (Photo courtesy of D Friesen.)

drought tolerance in various cereal crops. A key challenge faced by these groups is determination of genomic regions (i.e. quantitative trait loci [QTLs]) that enhance performance across varying combinations of water-stress conditions, plant growth stages and environments. Ribaut *et al.* [39••] examined the genetic control of the drought tolerance that has been successfully introduced into maize varieties in southern Africa [40]. They focused on the molecular-genetic dissection of component traits that are associated with this tolerance, and identified QTLs that are associated with components of yield of crops under drought stress.

Rice is a hydrophyte, and the enhancement of the rice root system to better extract available soil water during water-deficit periods is a straightforward target for the improvement of drought tolerance. Several studies have focused on augmenting the root system of the broadly adapted rice variety IR64 for greater root depth derived from the upland variety Azucena. Near isogenic lines and specific QTL markers were produced that show promise for the manipulation of genetic potential for root-system depth [41•]. Zhang *et al.* [42•] also identified and tagged QTLs for morphological and physiological traits thought to be relevant to drought tolerance in rice.

Recent approaches for improving drought tolerance in pearl millet have focused on the development of QTL

molecular markers for drought tolerance during the vulnerable flowering and grain-filling stages [43]. One QTL, which explained 23% of yield under water deficits, was common across environments and has been integrated into pearl millet breeding programs using markers. In sorghum, drought that occurs after flowering is particularly detrimental to yields and the 'stay green' trait (i.e. lack of green leaf senescence) has been associated with greater drought tolerance [44]. Sanchez *et al.* [45•] review the mapping of 'stay green' QTLs for drought tolerance, reporting that four QTLs are consistently associated with the 'stay green' trait in field experiments and explain 53% of the phenotypic variation.

Enhanced human nutrition

Despite progress with supplementation and fortification programs, there is compelling evidence that persistent deficiencies of iron, zinc, iodine and vitamins remain a major cause of numerous human health problems in developing countries [46]. For example, a recent analysis indicates that 127 million pre-school children still suffer from vitamin A deficiencies, leading to blindness and early death [47•]. Now, through advances in plant biotechnology there are new opportunities to complement supplementation by including enhanced human nutrition — along with higher yields, reduced losses and greater tolerance of adverse growing conditions — as an important objective when developing crop varieties. Moreover,

an effective system for the dissemination of such crop varieties is already in place and has the potential to provide the 'difficult-to-reach' rural poor with nutritionally enhanced staple foods [48,49].

Beyer *et al.* [50**] reported further advances in the development of 'Golden Rice': transgenic lines that are engineered to synthesize provitamin A (β -carotene) in the rice endosperm. Mannose has been used as a selective agent so that the new lines contain no antibiotic resistance genes [51]. β -carotene synthesis was achieved by adding only two genes, daffodil phytoene synthase (*psy*) and bacterial phytoene desaturase (*crtI*), with endosperm-specific promoters. These new 'clean' lines are being sent to collaborating breeding programs in Asia where they will be crossed with local varieties that are well adapted to the regions where vitamin A deficiency is still prevalent.

Conclusions

From a human welfare standpoint, the greatest benefits of plant biotechnology will surely be derived from the adoption of improved crop varieties in the developing countries of the world where billions of people still depend on agriculture for their livelihoods. There are already more farmers growing and benefiting from Bt cotton in China than there are farmers in the USA.

Fortunately, larger developing countries such as China, India and Brazil are building real capacity to generate plant biotechnologies, to incorporate these new tools into their national crop improvement programs, and to produce new crop varieties on the basis of farmers needs as well as profit potential. It will be important for these countries to put in place intellectual property and regulatory policies that will assure safety and encourage the private sector to develop and market new crop varieties. At the same time, they must enable and empower their research institutions in the public sector to continue producing and delivering products that are targeted to the needs of poor farmers and of consumers who will never be well served by the 'for-profit' sector.

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