

SECOND EDITION

Edited by
C. Neal Stewart, Jr.

Plant Biotechnology and Genetics

Principles, Techniques,
and Applications



WILEY

PLANT BIOTECHNOLOGY AND GENETICS

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To the next generation of pioneers.

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FOREWORD

An international (but widely unnoticed) race took place in the mid-1970s to understand how *Agrobacterium tumefaciens* caused plant cells to grow rapidly into a gall that produced its favorite substrates—called “opines.” Belgian, German, Australian, French, and US groups were at the forefront of different aspects of the puzzle. By 1977, it was clear that gene transfer from the bacterium to its plant host was the secret, and that the genes from the bacterium were functioning to alter characteristics of the plant cells. Participants in the race as well as observers began to speculate that we might exploit the capability of this cunning bacterium in order to get plants to produce our favorite substrates. Small startup companies and multinational corporations took notice and began to work with *Agrobacterium* and other means of gene transfer to plants. One by one the problems were dealt with, and each step in the use of *Agrobacterium* for the genetic engineering of a tobacco plant was demonstrated.

As I look back to those early experiments, I see that we have come a long way since the birth of plant biotechnology, which most of us who served as midwives would date from the Miami Winter Symposium of January 1983. The infant technology was weak and wobbly, but its viability and vitality were already clear. Its growth and development were foreseeable although not predictable in detail. I thought that the difficult part was behind us, and now (as I used to predict at the end of my lectures) the main challenge would be thinking of what genes we might use to bring about desired changes in crop plants. Unseen at that early date were the interesting problems, some technical and some of other kinds, to be encountered and overcome.

To my surprise, one of the biggest challenges turned out to be tobacco, which worked so well that it made us cocky. Tobacco was the guinea pig of the plant kingdom in 1983. This plant has an uncanny ability to reproduce a new plant from (almost) any of its cells. We practiced our gene transfer experiments on tobacco cells with impunity, and we could coax transgenic plants to develop from almost any cell into which *Agrobacterium* had transferred our experimental gene. This ease of regeneration of tobacco did not prepare us for the real world, whose principal food crops (unlike tobacco) were monocots—corn, wheat, rice, sorghum, and millet—to which the technology would ultimately need to be applied. Regeneration of these monocot plants from certain rare cells would be needed, and gene transfer to those very cells must be achieved. This process took years of research, and solutions were unique for each plant. In addition, much of the work was performed in small or large biotech companies, which sought to block competitors by applying for patent protection on methods they developed. Thus, still other methods had to be developed if licensing was not an option.

Another challenge we faced was bringing about expression of the “transgenes” we introduced into the plant cell. We optimistically supposed that any transgene, if given a plant gene promoter, would function in plants. After all, in 1983 the first gene everyone tried, the one coding for neomycin phosphotransferase II, had worked beautifully! The gene encoding a *Bacillus thuringiensis* insecticidal protein (nicknamed Bt, among other things, in the lab) was to teach us humility. Considerable ingenuity was needed to figure out why the Bt gene refused to express properly in the plant, and what to do about it. In the end, we learned to avoid many problems by using an artificial copy of this Bt gene constructed from plant-preferred codons. Although we thought of the genetic code as universal, as a practical matter, correct and fluent gene translation turned out to require, where a choice of codons was provided, that we use the plant’s favorites.

An entirely new problem was how to determine product safety. Once the transgenic plant was performing properly, how should it be tested for any unforeseen properties that might conceivably make it harmful, toxic, allergenic, weedy (i.e., a pest in subsequent crops grown in the field), or disagreeable in any other way one could imagine? Ultimately, as they gained experience with these new products, regulatory agencies developed protocols for testing transgenic plants. The transgene must be stable, the plant must produce no new material that looks like an allergen, and the plant must have (at least) the original nutritional value expected of that food. In essence, it must be the same familiar plant you start with except for the (predicted) new trait encoded by the transgene. And of course the protein encoded by the transgene must be safe—for consumption by humans or animals if it is food or feed, and by nontarget organisms in the environment likely to encounter it. Plants made by traditional plant breeding using “wide crossing” to bring in a desired gene from a distant (weedy or progenitor) relative are more likely to have unexpected properties than are transgenic plants. That is because unwanted and unknown genes will always be linked to the desirable trait sought in the wide cross.

The final problem—one still unsolved in many parts of the world—is that the transgenic plant, once certified safe and functional, must be accepted by consumers. Here, I speak as an aging but fond midwife looking at this adolescent technology that I helped to birth. I find that we are now facing a new kind of challenge, one on which all of the science discussed here seems to have surprisingly little impact.

Many consumers oppose transgenic plants as something either dangerous or unethical, possibly both. These opponents are not likely to inform themselves about plant biotechnology by reading materials such as you will find assembled between the covers of this book. But many are at least curious about this unknown thing that they oppose. I hope that many of you who read this book will become informed advocates of plant biotechnology. Talk to the curious. Replace suspicion, where you can, with information. Replace doubt with evidence. I do not think, however, that in order to spread trust, it is necessary to teach everyone about this technology. People are busy. They will not expend the time and energy to inform themselves in depth. I think that you only need to convince people that *you* have studied this subject in detail, that you have read this book, that you harbor no bias, and that you think that it is safe and natural, as I believe you will.

I have invested most of my career in developing and exploiting the technology for putting new genes into plants. My greatest hope is to see wide—at least wider—acceptance of transgenic plants by consumers during my lifetime. Transgene integration by plants is a natural phenomenon, so much so that we are still trying to figure out exactly how Mother Nature does it. *Agrobacterium* was a microbial genetic engineer long before I began studying DNA. Plant biotechnology has already made significant and positive environmental contributions, as you will discover in the very first chapter of this book. It has the potential to be a powerful new tool for plant breeders, one that they will surely need in facing the challenges of rapid climate change, flood and drought, global warming, as well as the new pests and diseases that these changes may bring. The years ahead promise to be very challenging and interesting. I think that this book will serve you readers well as you prepare for your various roles in meeting those challenges. Enjoy your travels through these chapters and beyond, and I sincerely hope that your journey may turn out to be as interesting and rewarding as mine has been.

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PREFACE

I vividly recall having a series of conversations back in the mid-1990s with “older” plant biotechnologists. These were the seasoned veterans who’d been on the cutting edge of figuring out how to make transgenic plants and how they might partially solve some critical problems in agriculture. They had been through the long days, weeks, months, and years of making genetically engineered commercial crops a reality as the middle of that decade saw the first commercial products hit the market. These scientists had worked out the basic science on how to produce recombinant DNA; genetically engineer the novel DNA sequences into plant cells; and then recover, for the first time, genetically engineered crops. They had witnessed challenge after challenge in the lab. They’d plodded through failures—many failures—and then, finally, success! After the promising transgenic crop lines had been produced, then came the arduous process of plant breeding, which was needed to move the useful traits into agronomic varieties that farmers would want to grow. Then came the field testing, seed production, and then...let’s not forget about all the regulatory approvals. Each step was like those taken by a toddler. It was all new ground. The difference between walking and falling down was measured in millimeters. And the baby put one foot in front of the other, often with great pauses to regain balance. Finally, the faithful day would arrive when the genetically engineered seed would be planted and bear fruit in farmers’ fields. And there we were.

It wasn’t a shock in the mid-1990s when these scientists expressed to me their feelings that went something like, “all the really fun stuff has already been done.” I was still a pretty young scientist at the time, and so who was I to question their insights? These insights from giants who stood on the shoulders of giants? So, in these awestruck moments, I asked polite questions, listened to their stories, and like a fawning fan I would muster an occasional “cool!” To be honest, their words and attitudes took a little wind out of my sails after I went back to my own little lab and office. From their perspective, indeed, the big challenges of moving those first molecules from idea to seed could never be matched again. But still, I thought about the future of the field and plodded along with my own ideas and research. I wanted to make the world a better place and believed that we could innovate with plant biotechnology—even, maybe, despite the assertion that all the coolest and most fun stuff had already been done. So I thought.

When we fast-forward about 10 years later, I thought it would be a fun project to put together a plant biotech textbook to support the course I’d offered to teach. The product of all the fun would be what became the first edition of the title in your hands. As that book came together, I sometimes thought about what I’d been told by these sages. The content of the text in the book, it seemed, mostly consisted of the tried and true technologies that were used in making those first engineered plants. There were also stories told of the glory days by scientists who penned their “Life boxes” in the book. After a while, however, I noticed that the first edition was starting to be somewhat dated itself. There were now new DNA sequencing technologies. There were new analytical techniques. New genome editing tools and synthetic biology tools had been invented and it was clear they would have an impact on plants. Computers had also changed what could be done and the speed tasks could be performed. So I embarked on updating the book and the second edition took shape.

Sometime in the last year or so, while working on the book, it really started to hit me, and has since pounded me like a John Henry sledgehammer on railroad spikes: those good old days were not the best days of plant biotechnology after all. The best and most fun stuff has not been done yet.

Yes, of course, a baby only learns to walk once, but now plant biotechnologists could sprint. It became clear that genome editing tools could allow biotechnologists to reconfigure existing genes in plants in ways never imagined by the early pioneers of biotechnology. Recently, a chromosome has been totally synthesized and installed into yeast—how long would it be before whole new entire pathways could be installed into plants to enable them to do things not even thought possible in the good old days? I have become convinced that the most intriguing and exciting days in plant biology and biotechnology are to be ushered in as computationally enabled genetics matures and becomes widely utilized. Crop productivity will continue to be improved using new innovations. Increased yield will feed more people with more nutritious food. And the readers of this book will be the ones to usher in the next wave of innovation. That is best and most fun part for me right now—making the future reality.

The second edition contains all updated chapters and new chapters in systems and synthetic biology. The “Life box” profiles of the plant biologists and biotechnologists who have made a difference in the field have been updated and the number of scientists who are profiled has been expanded. The lecture slides for open access to instructors and students remain at <http://plantsciences.utk.edu/pbg/>, and these are updated each time I teach the class. Feel free to offer any suggestions or slides of your own that I could use to update this resource.

I’m very grateful to the chapter authors and Life Box authors—both carried over from the first edition of the book—and the new ones. Thanks to my lab crew for their patience during the preparation of the book. I’m particularly indebted to Jennifer Hinds at the University of Tennessee. Jennifer did so much work on the book, I can’t begin make a list of her contributions. This much is certain: without Jennifer, there would be no second edition of the book. Thanks, Jennifer! You’re awesome!!

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June 21, 2015

The Impact of Biotechnology on Plant Agriculture

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1.0. CHAPTER SUMMARY AND OBJECTIVES

1.0.1. Summary

Since the first stably transgenic plant produced in the early 1980s and the first commercialized transgenic plant in 1994, biotechnology has revolutionized plant agriculture. In the United States, between 80 and 90% of the maize (corn), soybean, cotton, and canola crops are transgenic for insect resistance, herbicide resistance, or both. Biotechnology has been the most rapidly adopted technology in the history of agriculture and continues to expand in much of the developed and developing world.

1.0.2. Discussion Questions

1. What biotechnology crops are grown and where?
2. Why do farmers use biotech crops?
3. How has the adoption of plant biotechnology impacted the environment?

1.1. INTRODUCTION

The technology of genetic modification (GM, also stands for “genetically modified”), which consists of genetic engineering and also known as genetic transformation, has now been utilized globally on a widespread commercial basis for 18 years; and by 2012, 17.3 million farmers in 28 countries had planted 160 million hectares of crops using this technology. These milestones provide an opportunity to critically assess the impact of this technology on global agriculture. This chapter therefore examines specific global socioeconomic impacts on farm income and environmental impacts with respect to pesticide usage and greenhouse gas (GHG) emissions of the technology. Further details can be found in Brookes and Barfoot (2014a, b).

1.2. CULTIVATION OF BIOTECHNOLOGY (GM) CROPS

Although the first commercial GM crops were planted in 1994 (tomatoes), 1996 was the first year in which a significant area of crops containing GM traits were planted (1.66 million hectares). Since then, there has been a dramatic increase in plantings, and by 2012 the global planted area reached over 160.4 million hectares.

Almost all of the global GM crop area derives from soybean, maize (corn), cotton, and canola (Fig. 1.1). In 2012, GM soybean accounted for the largest share (49%) of total GM crop cultivation, followed by maize (32%), cotton (14%), and canola (5%). In terms of the share of total global plantings to these four crops accounted for by GM crops, GM traits accounted for a majority of soybean grown (73%) in 2012 (i.e., non-GM soybean accounted for 27% of global soybean acreage in 2012). For the other three main crops, the GM shares in 2012 of total crop production were 29% for maize, 59% for cotton, and 26% for canola (i.e., the majority of global plantings of maize and canola continued to be non-GM in 2012). The trend in plantings of GM crops (by crop) from 1996 to 2012 is shown in Figure 1.2. In terms of the type of biotechnology trait planted, Figure 1.3 shows that GM herbicide-tolerant soybeans dominate, accounting for 38% of the total, followed by insect-resistant (largely Bt) maize, herbicide-tolerant maize, and insect-resistant cotton with respective shares of 26, 19, and 11%. It is worth noting that the total number of plantings by trait produces a higher global planted area (209.2 million hectares) than the global area by crop (160.4 million hectares) because of the planting of some crops containing the stacked traits of herbicide tolerance and insect resistance (e.g., a single plant with two biotech traits).

In total, GM herbicide-tolerant (GM HT) crops account for 63%, and GM insect-resistant (GM IR) crops account for 37% of global plantings. Finally, looking at where biotech crops have been grown, the United States had the largest share of global GM crop plantings in 2012

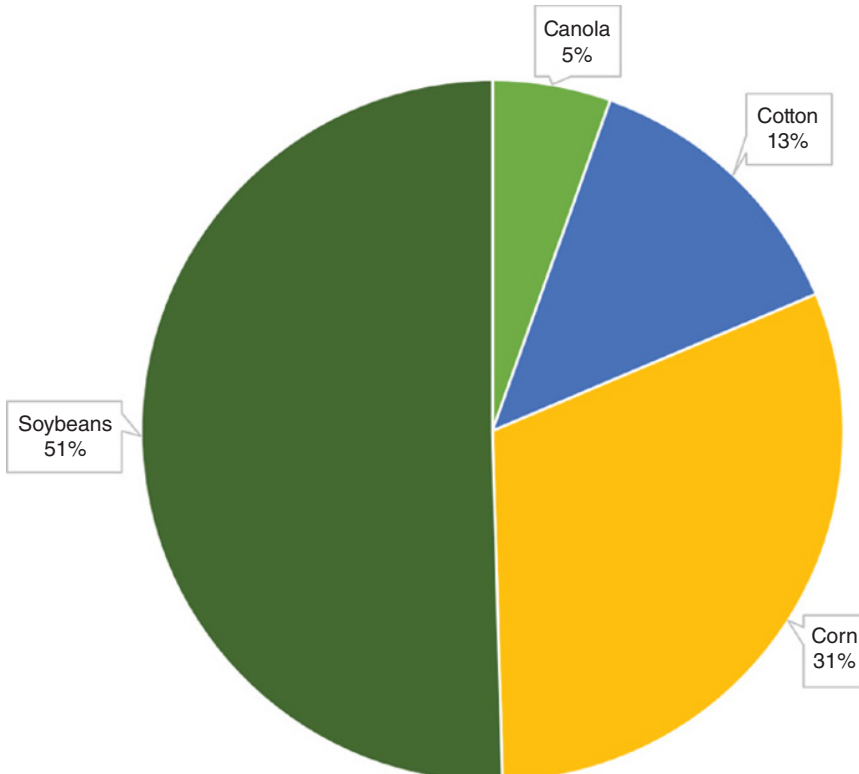


Figure 1.1. Global GM crop plantings in 2012 by crop (base area: 160.4 million hectare). (Sources: ISAAA, Canola Council of Canada, CropLife Canada, USDA, CSIRO, ArgenBio.)

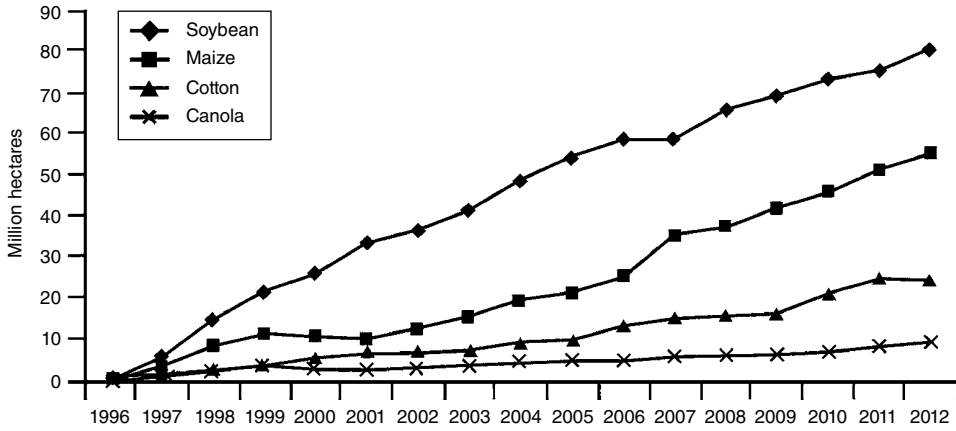


Figure 1.2. Global GM crop plantings by crop 1996–2012. (Sources: ISAAA, Canola Council of Canada, CropLife Canada, USDA, CSIRO, ArgenBio.)

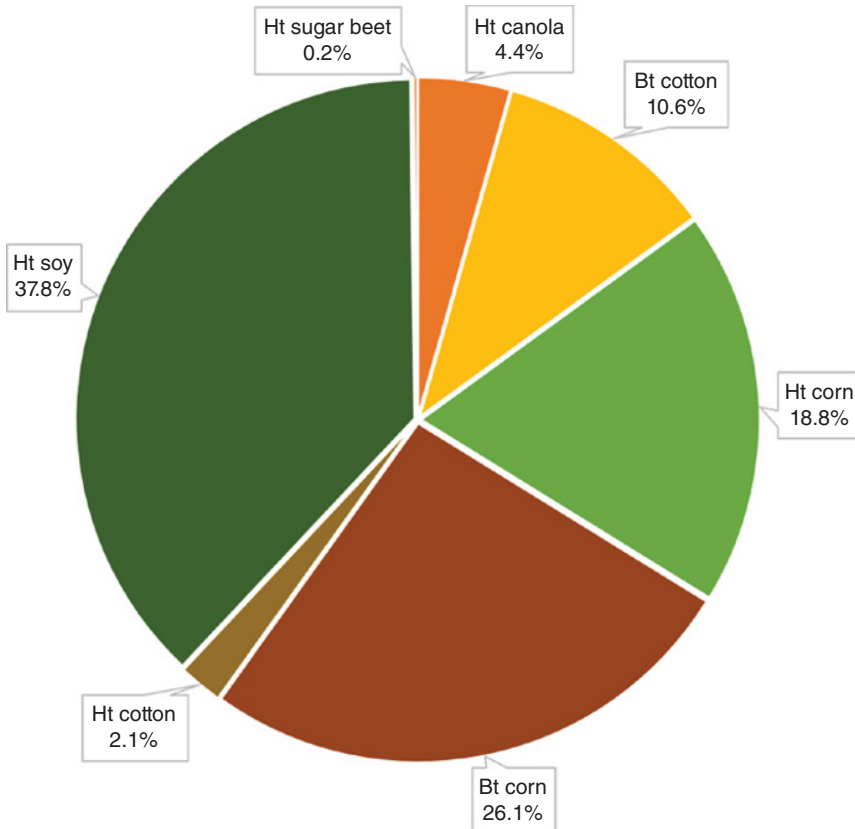


Figure 1.3. Global GM crop plantings by main trait and crop: 2012. (Sources: Various, including ISAAA, Canola Council of Canada, CropLife Canada, USDA, CSIRO, ArgenBio.)

(40%: 64.1 million hectares), followed by Brazil (37.2 million hectares: 23% of the global total) and Argentina (14%: 23.1 million hectares). The other main countries planting GM crops in 2012 were India, Canada, and China (Fig. 1.4). In 2012, there were also additional GM crop plantings of papaya (395 hectares), squash (2000 hectares), alfalfa (425,000 hectares), and sugar

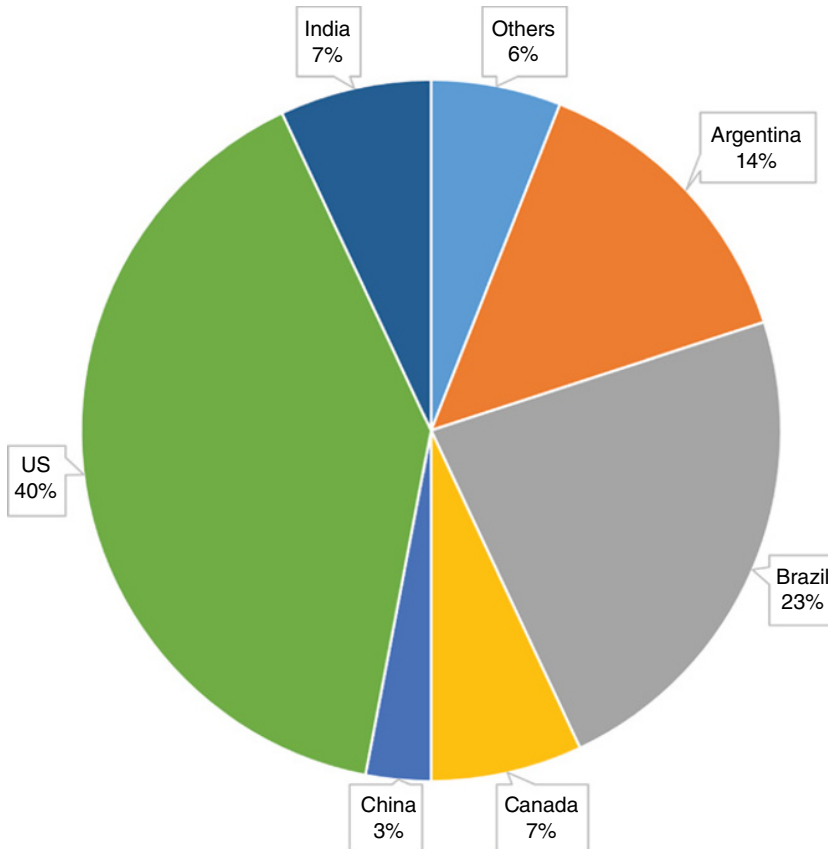


Figure 1.4. Global GM crop plantings 2012 by country. (Sources: ISAAA, Canola Council of Canada, CropLife Canada, USDA, CSIRO, ArgenBio.)

beet (490,000 hectares) in the United States, of papaya (5000 hectares) in China and of sugar beet (13,500 hectares) in Canada.

1.3. WHY FARMERS USE BIOTECH CROPS

The primary driver of adoption among farmers (both large commercial and small-scale subsistence) has been the positive impact on farm income. The adoption of biotechnology has had a very positive impact on farm income derived mainly from a combination of enhanced productivity and efficiency gains (Table 1.1). In 2012, the direct global farm income benefit from GM crops was \$18.8 billion. This is equivalent to having added 5.6% to the value of global production of the four main crops of soybean, maize, canola, and cotton, a substantial impact. Since 1996, worldwide farm incomes have increased by \$116.6 billion, directly because of the adoption of GM crop technology.

The largest gains in farm income in 2012 have arisen in the maize sector, largely from yield gains. The \$6.7 billion additional income generated by GM IR maize in 2012 has been equivalent to adding 6.6% to the value of the crop in the GM crop-growing countries, or adding the equivalent of 3% to the \$226 billion value of the global maize crop in 2012. Cumulatively since 1996, GM IR technology has added \$32.3 billion to the income of global maize farmers.

Substantial gains have also arisen in the cotton sector through a combination of higher yields and lower costs. In 2012, cotton farm income levels in the GM-adopting countries increased by

TABLE 1.1. Global Farm Income Benefits from Growing GM Crops 1996–2012 (Million US \$)

Trait	Increase in farm income 2012	Increase in farm income 1996–2012	Farm income benefit in 2012 as percentage of total value of production of these crops in GM adopting countries	Farm income benefit in 2012 as percentage of total value of global production of crop
GM herbicide-tolerant soybeans	4,797.9	37,008.6	4.4	4.0
GM herbicide-tolerant maize	1,197.9	5,414.7	1.2	0.5
GM herbicide-tolerant cotton	147.2	1,371.6	0.4	0.3
GM herbicide-tolerant canola	481.0	3,664.4	4.9	1.3
GM insect-resistant maize	6,727.8	32,317.2	6.6	3.0
GM insect-resistant cotton	5,331.3	36,317.2	13.1	11.2
Others	86.3	496.7	N/A	N/A
Total	18,769.4	116,590.4	6.8	5.6

Notes: All values are nominal. Others=Virus resistant papaya and squash and herbicide-tolerant sugar beet. Totals for the value shares exclude “other crops” (i.e., relate to the four main crops of soybeans, maize, canola, and cotton). Farm income calculations are net farm income changes after inclusion of impacts on yield, crop quality, and key variable costs of production (e.g., payment of seed premia, impact on crop protection expenditure). N/A=not applicable.

\$5.5 billion; and since 1996, the sector has benefited from an additional \$37.7 billion. The 2012 income gains are equivalent to adding 13.5% to the value of the cotton crop in these countries, or 11.5% to the \$47 billion value of total global cotton production. This is a substantial increase in value-added terms for two new cotton seed technologies.

Significant increases to farm incomes have also resulted in the soybean and canola sectors. The GM HT technology in soybeans has boosted farm incomes by \$4.8 billion in 2012, and since 1996 has delivered over \$37 billion of extra farm income. In the canola sector (largely North American) an additional \$3.66 billion has been generated (1996–2012).

Overall, the economic gains derived from planting GM crops have been of two main types: (a) increased yields (associated mostly with GM IR technology) and (b) reduced costs of production derived from less expenditure on crop protection (insecticides and herbicides) products and fuel.

Table 1.2 summarizes farm income impacts in key GM-adopting countries highlighting the important farm income benefit arising from GM HT soybeans in South America (Argentina, Bolivia, Brazil, Paraguay, and Uruguay), GM IR cotton in China and India, and a range of GM cultivars in the United States. It also illustrates the growing level of farm income benefits being obtained in South Africa, the Philippines, Mexico, and Colombia from planting GM crops.

In terms of the division of the economic benefits, it is interesting to note that farmers in developing countries derived in 2012 (46.2%) relative to farmers in developed countries (Table 1.3). The vast majority of these income gains for developing country farmers have been from GM IR cotton and GM HT soybean.¹

¹The author acknowledges that the classification of different countries into “developing” or “developed” status affects the distribution of benefits between these two categories of country. The definition used here is consistent with the definition used by others, including the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) (see the review by James (2012)].

TABLE 1.2. GM Crop Farm Income Benefits During 1996–2012 in Selected Countries (Million US \$)

	GM HT soybeans	GM HT maize	GM HT cotton	GM HT canola	GM IR maize	GM IR cotton	Total
United States	16,668.7	3752.3	975.8	268.3	26,375.9	4,046.7	52,087.7
Argentina	13,738.5	766.7	107.0	N/A	495.2	456.4	15,563.8
Brazil	4,825.6	703.4	92.5	N/A	2,761.7	13.3	8,396.5
Paraguay	828	N/A	N/A	N/A	N/A	N/A	828.0
Canada	358	81.3	N/A	3368.8	1,042.9	N/A	4,851.0
South Africa	9.1	4.1	3.2	N/A	1,100.6	34.2	1,151.2
China	N/A	N/A	N/A	N/A	N/A	15,270.4	15,270.4
India	N/A	N/A	N/A	N/A	N/A	14,557.1	14,557.1
Australia	N/A	N/A	78.6	27.3	N/A	659.6	765.5
Mexico	5.0	N/A	96.4	N/A	N/A	136.6	238.0
Philippines	N/A	104.7	N/A	N/A	273.6	N/A	378.3
Romania	44.6	N/A	N/A	N/A	N/A	N/A	44.6
Uruguay	103.8	N/A	N/A	N/A	17.6	N/A	121.4
Spain	N/A	N/A	N/A	N/A	176.3	N/A	176.3
Other EU	N/A	N/A	N/A	N/A	18.8	N/A	18.8
Colombia	N/A	1.7	18.1	N/A	47.4	15.4	826.6
Bolivia	432.2	N/A	N/A	N/A	N/A	N/A	432.2
Burma	N/A	N/A	N/A	N/A	N/A	215.4	215.4
Pakistan	N/A	N/A	N/A	N/A	N/A	725.1	725.1
Burkina Faso	N/A	N/A	N/A	N/A	N/A	186.9	186.9
Honduras	N/A	N/A	N/A	N/A	6.9	N/A	6.9

Notes: All values are nominal. Farm income calculations are net farm income changes after inclusion of impacts on yield, crop quality, and key variable costs of production (e.g., payment of seed premia, impact on crop protection expenditure). N/A=not applicable. US total figure also includes \$491 million for other crops/traits (not included in the table). Also not included in the table is \$5.5 million extra farm income from GM HT sugar beet in Canada.

TABLE 1.3. GM Crop Farm Income Benefits, 2012: Developing Versus Developed Countries (Million US \$)

	Developed	Developing
GM HT soybeans	2,955.4	1842.5
GM HT maize	654.0	543.9
GM HT cotton	71.4	75.8
GM HT canola	481.0	0
GM IR maize	5,327.5	1400.3
GM IR cotton	530.7	4800.7
GM virus-resistant papaya and squash and GM HT sugar beet	86.3	0
Total	10,106.3	8663.2

Note: Developing countries=All countries in South America, Mexico, Honduras, Burkina Faso, India, China, the Philippines, and South Africa.

Examination of the cost farmers pay for accessing GM technology relative to the total gains derived shows that across the four main GM crops, the total cost was equal to about 23% of the total farm income gains (Table 1.4). For farmers in developing countries, the total cost is equal to about 21% of total farm income gains, while for farmers in developed countries the cost is about 25% of the total farm income gain. Although circumstances vary between countries, the higher share of total technology gains accounted for by farm income gains in developing countries, relative to the farm income share in developed countries, reflects factors such as weaker provision and enforcement of intellectual property rights in developing countries and the higher average