expression vector (Invitrogen) were selected using Zeocin (0.5 mg/ml). To release soluble 7BP anchored on the cell surface, we treated cells with thrombin (10 IU/ml) for 3 days under serum-free conditions. The cleaved 6-His– and FLAG epitope–tagged recombinant 7BP in serum-free conditioned media was purified by sequential nickel and anti-FLAG affinity chromatography. For antibody production, 5 mg of purified 7BP emulsified in Freund's adjuvant were injected into rabbits (Strategic BioSolutions Inc., Ramona, CA). Western blot analysis indicated that the antibody to 7BP does not recognize recombinant LGR8 proteins.

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Plant Biotechnology in China

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A survey of China's plant biotechnologists shows that China is developing the largest plant biotechnology capacity outside of North America. The list of genetically modified plant technologies in trials, including rice, wheat, potatoes, and peanuts, is impressive and differs from those being worked on in other countries. Poor farmers in China are cultivating more area of GM plants than are small farmers in any other developing country. A survey of agricultural producers in China demonstrates that *Bacillus thuringiensis* (Bt) cotton adoption increases production efficiency and improves farmer health.

Private life-science companies in the industrialized world perform most of the world's agricultural biotechnology research (*1*). Concerns have arisen in developing countries that their scientists and producers can only obtain genes and seeds from foreign companies and that biotechnology research does not focus on the crops that are important to the world's poor farmers. Recently, because of consumer resistance and governmental regulations affecting international trade in genetically modified products and the rising cost of commercializing new products, private research and development on plant biotechnology is declining, further jeopardizing the little private research that is done on developing country problems (*2*). In contrast, China is accelerating its investments in agricultural biotechnology research and is focusing on commodities that have been mostly ignored in the laboratories of industrialized countries. Small farmers in China have begun to aggressively adopt GM crops when permitted to do so.

The overall goal of this paper is to answer the questions: What is China doing in agricultural biotechnology research? Is China's public-sector–dominated investment strategy efficient? Can China be a source of plant biotechnology for its own farmers and for farmers in the rest of the world?

The first two sections of the paper document China's scientific achievements and research investments. In order to understand the input and output trends of China's plant biotechnology research, in 2000, a two-stage survey elicited information covering approximately 80% of the nation's plant biotechnology research laboratories in nine provinces and two municipalities. In the first stage, based on funding information from the Ministry of Science and Technology (MOST), a list of laboratories that potentially could have been involved in plant biotechnology research was created. Interviews with the research directors identified 35 institutes that conducted research (more than US$30,000) in tissue culture, genetic engineering, marker-assisted selection (MAS), diagnostic technology, microbiology, or other related areas. Twenty-nine institutes provided detailed information on their inputs and outputs for 1999, and 22 institutes provided historic data from 1986. The survey instrument, administered by Chinese Academy of Sciences and Chinese Academy of Agricultural Sciences research staff, contained sections on each institute's total revenues and expenditures, its personnel, investments in biotechnology facilities, and the status of its current and past experiments in the regulatory process. Details of the survey process and a copy of the survey instrument can be found on the *Science* Web site (*3*). The third and fourth sections analyze the economic, environmental, and health impacts of plant biotechnology research using data from a survey of 282 GM cotton farmers in North China.

Although China has spent the last 50 years building the most successful agricultural research system in the developing world—employing more than 70,000 scientists—research in modern plant biotechnology did not begin until the mid-1980s (*4*). Scientists now apply advanced biotechnology tools to the field of plant science, regularly working on the synthesis, isolation, and cloning of new genes and the transformations of plants with these genes. With the initiation of a research program on rice functional genomics in 1997, China's researchers began using AC/DS transposons and T-DNA insertion methods to create rice mutagenesis pools (*5*). Biotechnologists also have initiated functional genomics research for *Arabidopsis*. Our survey of China's laboratories identified over 50 plant species and more than 120 functional genes that scientists are using in plant genetic engineering, making China a global leader in the field.

China's scientists have generated an impressive array of new technologies. From 353 applications between 1996 and 2000, China's Office of Genetic Engineering Safety Administration approved 251 cases of GM plants, animals, and recombined microorganisms for field trials, environmental releases, or commercialization (Table 1, rows 1 and 2). Regulators approved 45 GM plant applications for field trials, 65 for environmental release, and 31 for commercialization (Table 1, rows 3 to 5).

Breakthroughs on food crops that have received little attention elsewhere (>40% of

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the trials elsewhere in the world involve GM maize) demonstrate China's concern for food security (Table 2). Transgenic rice resistant to three of China's major rice pests—stem borer (using *Bt* and *CpTI* genes), planthopper, and bacterial leaf blight (using the *Xa21* gene)—have passed at least 2 years of environmental release trials. Researchers have moved GM wheat with barley yellow dwarf virus resistance to field trials. China's scientists are experimenting with GM potato and peanut.

The nation's public-dominated research system has given China's researchers a strong incentive to produce GM crops that increase yields and prevent pest outbreaks. In industrialized countries, 45% of field trials are for herbicide tolerance and improving product quality; only 19% are for insect resistance (*6*). In China, more than 90% of field trials target insect and disease resistance.

Unlike the rest of the world, in which most plant biotechnology research is financed privately, China's government funds almost all of its plant biotechnology research. MOST has increased plant biotechnology project funding in the sample institutes from $8 million in 1986 to $48 million in 1999 (Table 3) (*7*). After a number of adjustments (Table 3), China's total investment in plant biotechnology in 1999 was estimated to be $112 million.

Expenditures of this level demonstrate the seriousness of China's commitment to plant biotechnology. Government research administrators allocated about 9.2% of the national crop research budget to plant biotechnology in 1999, up from 1.2% in 1986. China's level far exceeds the 2 to 5% levels of other developing countries (*8*).

The developing world's other large biotechnology programs, in Brazil and India, fall short of China's. The Brazilian central agricultural research system, EMBRAPA, spends $2 million annually on genetic engineering (*9*). Foreign life-science firms in Brazil spend approximately $1 to 2 million on plant biotechnology research. The São Paulo research foundation, FAPESP, spends $5 to 10 million annually. The Indian government allocates $15 million (*10*). Even after adding the investment of private firms (an estimated US$10 million), plant biotechnology research expenditures in India are only around 20% of China's. Given these spending levels, China accounts for more than half of the developing world's expenditures on plant biotechnology.

Compared with the developed world, China's spending has been relatively small, less than 5% of total annual expenditures in industrialized countries, about $2 to $3 billion (*8*). Such an assessment changes, however, when comparing China to the public research spending of other countries and when considering its future plans. Globally, the public sector makes about 45% of plant biotechnology research expenditures. China currently accounts for more than 10% of this amount. In early 2001, China's officials announced plans to raise plant biotechnology research budgets by 400% before 2005. If achieved, China could account for nearly one-third of the world's public plant biotechnology spending.

China's agricultural biotechnology research staff has become one of the largest in the developing world (Table 3, rows 4 and 5). The number of scientists and professional staff rose from 740 in 1986 to 1,988 in 1999. A marked improvement also has occurred in the formal education and training of those engaged in biotechnology research (*11*).

In response to rising pesticide use and the emergence of a pesticide-resistant bollworm population in the late 1980s, China's scientists began research on GM cotton, launching the nation's most successful experience with GM crops. Starting with a gene isolated from the bacterium *Bacillus thuringiensis* (Bt), China's scientists modified the cotton plant using an artificially synthesized gene that was identified with sequencing techniques. Greenhouse testing began in the early 1990s. When area sown to cotton decreased because of pest losses in the mid-1990s, in 1997, the commercial use of GM cotton was approved. During the same year, Bt cotton varieties from publicly funded research institutes and from a Monsanto joint venture (with the U.S. seed company Delta and Pineland and the Hebei Provincial Seed Company) became available to farmers. Although officials had previously approved the commercial release of virus-resistant tomatoes and sweet peppers, as well as color-altered petunias, into circumscribed regions around certain cities, the release of Bt cotton began China's first large-scale commercial experience with a product of the nation's biotechnology research program. (In the early 1990s, virus-resistant tobacco variety had been commercialized before being removed from production because of pressure from an international tobacco importer.)

Response by China's poor farmers to the introduction of Bt cotton eliminates any doubt that GM crops can play a role in poor countries. From only 2000 hectares in 1997, Bt cotton's sown area grew to around 700,000 hectares in 2000 (*12*). By 2000, farmers planted Bt varieties on 20% of China's cotton acreage. The average farm size of the typical cotton farmer in the survey sample was less than 1 hectare (of which the cotton area was less than 0.5 hectare). Currently, Bt cotton in China is the world's most widespread transgenic crop program for small farmers.

Farmers are receiving the greatest benefit from Bt cotton's reduced pesticide need. Bt cotton farmers reduced pesticide use by an average of 13 sprayings (49.9 kg) per hectare per season (Table 4). This reduced costs by $762 per hectare per season. Farmers also significantly reduced labor for pest control. After holding the incidence of pests, pesticide price, and farmer's age and education constant, regression analysis finds that Bt cotton adopters use significantly less pesticides

Table 1. Agricultural biotechnology testing in China, 1997 to July 2000 and to July 1999 where figures are not available (NA). Total products include plants, microorganisms, animals. Source: Authors' survey.

Tested and approved	1997	1998	1999	July 2000	Total
Total products	57	68	126	102	353
Submitted					
Approved	46	52	94	59	251
Approvals for plants					
Field trials	29	8	28	NA	45
Environmental release	6	9	30	NA	65
Commercialization	4	2	24	1	31

Table 2. Genetically modified plants (commercialized and in trials) in China, 1999. BADH, betaine aldehyde dehydrogenase; BYDV, barley yellow dwarf virus. Source: Authors' survey.

Crop	Introduced trait
1. Cotton	Insect resistance*
	Disease resistance
2. Rice	Insect resistance
	Disease resistance
	Herbicide resistance
	Salt tolerance (BADH)
3. Wheat	BYDV resistance
	Quality improvement
4. Maize	Insect resistance (Bt)
	Quality improvement
5. Soybean	Herbicide resistance
6. Potato	Disease resistance
	Quality improvement
7. Rape seed	Disease resistance
8. Peanut	Virus resistance
9. Tobacco	Insect resistance
10. Cabbage	Virus resistance
11. Tomato	Virus resistance*
	Shelf-life altered*
	Cold tolerance
12. Melon	Virus resistance
13. Sweet pepper	Virus resistance*
14. Chili	Virus resistance
15. Petunia	Colored altered*
16. Papaya	Virus resistance

*Approved for commercialization; others waiting for commercialization or environmental release.

when pesticide use is measured by the number of sprayings, the quantity of pesticide used, or total cost (*13*).

The decrease in pesticide use has increased production efficiency. Although yields and the price of Bt and non-Bt varieties were the same, the costs savings and reduction in labor enjoyed by Bt cotton users reduced the cost of producing a kilogram of cotton by 28%, from $2.23 to $1.61 (Table 4). Multivariate production efficiency analysis demonstrates that the results are statistically valid (*14*).

China's experience with Bt cotton demonstrates the direct and indirect benefits of its investment in plant biotechnology research and product development. According to our research, the total benefits from the adoption of Bt cotton in 1999 were $334 million (*15*). Ignoring the benefits created by foreign life-science firms, the benefits from the main variety created and extended by one of China's publicly funded research institutes were $197 million. Farmers captured most of the benefits, because government procurement prevented cotton prices from declining (which would have shifted some of the benefits to consumers). Hence, the social benefits from research on one crop, cotton, in only the second year of its adoption were enough to fund all of the government's crop biotechnology research in 1999. As Bt cotton spreads, the social benefits from this crop will easily pay for all China's past biotech expenditures on all crops.

The survey also showed that farmers reduced use of toxic pesticides, organophosphates and organochlorines, by more than 80% and that this reduction appears to have improved farmer health. The survey asked farmers if they had suffered from headaches, nausea, skin pain, or digestive problems after applying pesticides. If the answer was "yes," it was registered as an incidence of "poisoning." Only 4.7% of Bt cotton growers reported poisonings; 11% of the farmers using both Bt and unaltered varieties reported poisonings; whereas 22% of those using only non-Bt varieties reported poisonings.

Although China is still struggling with issues of consumer safety and acceptance, many competing factors are putting pressures on policy makers to decide whether or not continuing commercializing transgenic crops. The demand of producers (for productivity-enhancing technology) and consumers (for cost savings), the current size and rate of increase of research investments, and past success in developing technologies suggest that products from China's plant biotechnology industry will one day become widespread inside China. China also could become an exporter of biotechnology research methods and commodities as opportunities for contract research. The sales of genes, markers, and other tools as well as exporting GM varieties, are expanding in both industrialized and developing countries. Globally, China has several advantages; it has many well-trained scientists, a low-cost research environment, and large collections of germ plasm.

Table 3. Plant biotechnology research budget and research staff in China, 1986–99. Data for expenditures by sample institutes and estimates for all China are from 22 research institutes. Estimates for total plant biotechnology investments in China were calculated by taking the sum of investment in core, project, equipment, and other expenditure categories for 22 institutes (totaled in row 1) and multiplying by a factor of 1.30 (in 1986) to 1.41 (in 1999), which adjusts for the fact (based on the ratio of staff) that the historic data were missing from the seven institutes (of the 29 institutes that provided data for 1999) and multiplying by a factor of 1.20, which adjusts for the fact that the surveyed sample only covered 80% of all institutes doing plant biotechnology work. This number (total project and staff funding) was multiplied by 1.25 to account for the fact that China's research expenditure figures do not include charges for facilities. A final adjustment (5% before 1995 and 10% in 1995 and 1999) accounts for private sector research [including that from foreign sources (*18*)]. To go from our survey number of 92.8 million yuan in project expenditures in 1999 to our total estimate of research investments of $112 million, one multiplies 92.8 by 1.41 × 1.2 × 1.25 × 1.10/8.25 × 4.29333. Details of the survey and a copy of the survey instrument can be found on the *Science* Web site (*3*). Figures in the table were converted from Chinese yuan by dividing by the official exchange rate (8.25 in 1999) and multiplying by the price pooling program (PPP) multiplier [4.29333 (*18*)]. Source: Authors' survey.

	1986	1990	1995	1999
Budget				
Expenditures by sample institutes (PPP million US dollars)	8	14	17	48
Estimates for all China (PPP million US dollars)	17	31	40	112
Plant biotech as percent of total public agricultural research budget (%)	1.2	2.7	3.1	6.4/9.2*
Staff (number of scientists)				
Total staff working full time on plant biotech for sample institutes	641	808	968	1205
Estimates for total staff working full time on plant biotech for all China	740	1067	1447	1988

*The number 9.2 is plant biotechnology as a percentage of the total public plant research budget.

Table 4. Yields, costs, and pesticide use by cotton varieties in the sampled households, 1999. U.S. dollars are converted from yuan at 8.25 exchange rate and to PPP terms by multiplying by 4.2933. Source: Authors' survey of cotton producers.

Variety of cotton	Yield (kg/ha)	Total production costs per kg cotton (US$/kg)	Pesticide use per hectare		
			Number of applications	Quantity (kg)	Cost (US dollars)
With Bt	3371	1.61	6.6	11.8	136
Without Bt	3186	2.23	19.8	60.7	762

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using only the officially reported area by the main foreign variety (100,000 hectares) and main Chinese variety (120,000 hectares) and using the variety-specific yields (3440 kg/ha for the foreign variety; 3500 kg/ha for the main Chinese variety) and the variety-specific cost savings per kilogram (0.8 yuan or $0.416 for the foreign variety; 0.9 yuan or $0.468 for the main Chinese variety). In PPP terms, the benefit of the Chinese variety is 120,000 ha × 3500 kg/ha × 0.468 $/kg = $197 million. If the total estimated cotton area (700,000 hectares) or the average savings per kilogram [1.18 yuan/kg or 0.62 $/kg (Table 4)] were used, estimated benefits would be higher. Some benefits are due to labor savings, which may not be immediately of value to the farmer. In the longer run labor savings is important for increasing productivity.

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Complete Development of Mosquito Phases of the Malaria Parasite in Vitro

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Methods for reproducible in vitro development of the mosquito stages of malaria parasites to produce infective sporozoites have been elusive for over 40 years. We have cultured gametocytes of *Plasmodium berghei* through to infectious sporozoites with efficiencies similar to those recorded in vivo and without the need for salivary gland invasion. Oocysts developed extracellularly in a system whose essential elements include co-cultured *Drosophila* S2 cells, basement membrane matrix, and insect tissue culture medium. Sporozoite production required the presence of para-aminobenzoic acid. The entire life cycle of *P. berghei*, a useful model malaria parasite, can now be achieved in vitro.

For over a century, a major objective of malaria control programs has been to block parasite transmission by mosquitoes. Such approaches would clearly benefit from a better understanding of parasite development within the vector, initiated when gametocytes are taken up in a blood meal. Fertilization of macrogametes within the mosquito midgut produces zygotes that transform into motile and invasive ookinetes. These penetrate and traverse the midgut epithelium and become sessile vegetative oocysts lying beneath the midgut basement lamina, each potentially producing 2 to 8000 sporozoites. Knowledge of the mosquito-related factors regulating these processes is improving (*1*–*3*), but it is difficult to determine the specific and separate effects of these factors in vivo. Early events associated with midgut invasion have recently been studied in vitro with the use of midgut preparations (*4*–*6*) or co-cultured mosquito cells (*7*), but these systems do not sustain long-term development or simulate oocyst interaction with the basal lamina and do not permit investigation of sporozoite differentiation.

Fertilization and ookinete development can be achieved in vitro for many malaria parasite species, including *Plasmodium berghei*, a parasite of rodents (*8*, *9*). These culture systems have facilitated the study of ookinete molecules that may be targeted by antibodies induced by transmission-blocking vaccines or drugs (*10*, *11*). After many pioneering attempts (*12*, *13*), it is only recently that in vitro transformation of *Plasmodium gallinaceum* and *Plasmodium falciparum* ookinetes into oocysts and sporozoites has been achieved, but the numbers of oocysts produced are low and, more importantly, the infectivity of these sporozoites has not been demonstrated (*14*, *15*). Here we confirm the need for a basement membrane–like substrate such as Matrigel, which may mimic the basal lamina of the mosquito midgut epithelium. In addition, co-culture with *Drosophila melanogaster* S2 cells is necessary for development, although the role of these insect cells is unclear.

We have based our work on the previously described *P. gallinaceum* culture system (*15*) and, where appropriate, substituted conditions that more nearly mimicked the mosquito environment or provided factors known to enhance oocyst growth in vivo. Thus, a culture system has been developed that consistently supports the transformation of large numbers of *P. berghei* ookinetes to extracellular oocysts and the production of infective sporozoites with efficiencies approaching those seen in vivo.

Plasmodium berghei ANKA (clone 2.34) ookinetes were produced in vitro (*8*, *9*) and cultured to produce oocysts in eight-chamber slides (*16*). Previously, cultures of other malaria species used supplemented RPMI 1640 (*15*), a mammalian medium traditionally used to culture ookinetes. A comparison of oocysts growing extracellularly in RPMI 1640 and Schneider's medium (*17*), whose composition mirrors the high aminoacidaemia of mosquito hemolymph (*18*), demonstrated that Schneider's medium significantly improved oocyst yield [multiple analysis of variance (MANOVA) over time: $F_{3,66} = 3.06$, $P = 0.03$ (*19*)]. Therefore, a classic insect medium, Schneider's medium, was used in all subsequent investigations. Nutrition of oocysts may be better supported by this medium, or Schneider's medium may be more suitable for the co-cultured insect cells because growth of *Drosophila* S2 cells is retarded in RPMI (*20*).

Extracellular oocyst development did not occur if chambers were not initially coated with Matrigel. Many ookinetes burrowed into the Matrigel matrix within hours and, within 1 to 2 days, transformed into oocysts within and on the surface of the matrix. Parasites not firmly attached to the matrix were probably removed during the repeated medium changes, which may account, in part, for the decline over time in oocyst number recovered from each chamber (*19*). We have previously observed that *P. berghei* ookinetes attach to plastic wells coated with the basal lamina components laminin, collagen IV, or fibronectin. Some ookinete-oocyst transformation occurs when bound to a lami-

Table 1. Summary of optimum culture conditions for sporogonic stages of *P. berghei*. *Drosophila melanogaster* S2 cells were incubated at 19° to 20°C in air on a layer of Matrigel in a ratio of 10:1 with ookinetes (*36*).

Oocyst culture medium (pH 7)	per 100 ml
Schneider's medium	83.48 ml
Fetal bovine serum, heat-inactivated	15 ml
$NaHCO_3$	23.8 mM
Hypoxanthine	36.7 mM
Lipoprotein and cholesterol	200 μl
PABA	44 nM
Penicillin	10,000 U
Streptomycin	10 mg
Gentamicin	20 mg

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