Enzyme Biotechnology

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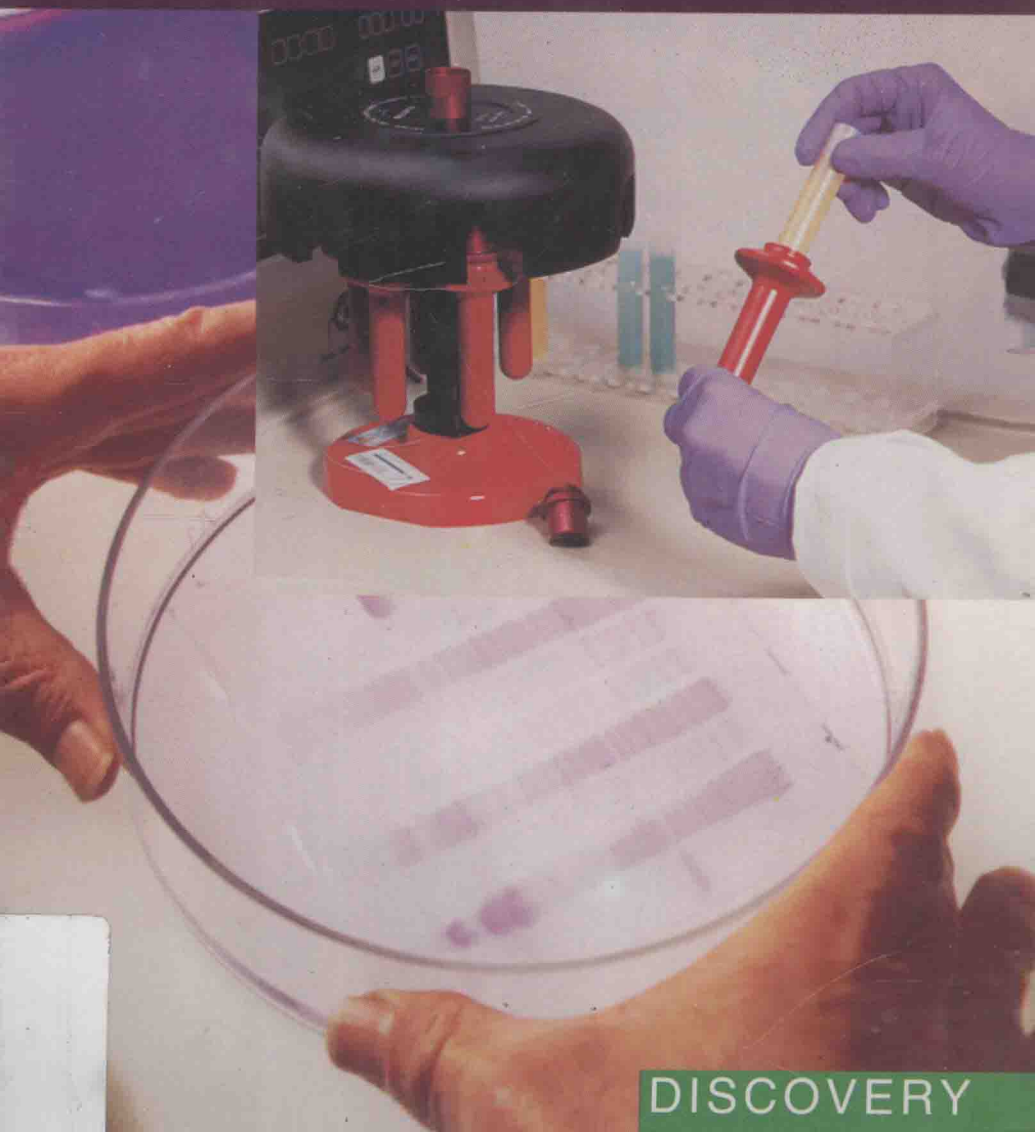

DISCOVERY

ENZYME BIOTECHNOLOGY

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Preface

Enzymes are the biological catalysts and are the basis of metabolism that keeps a cell or organism alive. This branch of science that deals with the study of enzymes has undergone remarkable development in the last three decades. These developments, along with recent widespread application of the newest biomedical technologies, have inbued optimism that new strategies can be developed for controlling the important diseases that for centuries have been the scourages of mankind. The present text offers a compredium of reviews of the most active areas of research in biochemistry, medicine and related fields of biological sciences. By intent, the chapters are not exhaustive reviews. Special attention has been given to improved and upto date methodologies and techniques which make this work indispensable for the biological research workers.

To make the work more comprehensive and informative, the author has consulted many authoritative books, research journals, abstracts, monographs etc. He is grateful to all those great scholars whose work are cited or substantially reproduced.

There can be no claim to originality except in the manner of treatment and much of the information has been obtained from the books and scientific journals available in the different libraries.

The author expresses his thanks to his friends and colleagues whose continue inspirations have initiated him to bring out this book.

The author expresses his gratitude to Mr. Wasan and staff of M/s Discovery Publishing House for their whole hearted co-operation in the publication of this book.

In the mean time, the author will remain sincerely responsible for any shortcomings of the book and be grateful to the readers for their suggestions and constructive criticism for the continuous betterment of the book. He takes this opportunity to appeal to the readers to send their suggestions straightaway to his Publisher.

Author

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Introduction

Technological advances in biotechnology, including genetic engineering, have enabled transfer of genetic traits both within species and between entirely different plant and animal species. Currently, biotechnology techniques are being used in various fields, including agriculture, veterinary medicine, pharmaceutical development, forestry, energy conservation, and waste treatment. These techniques, if applied responsibly, have the potential to increase productivity in crops and livestock, control pests, produce new food and fiber crops, and develop effective medicines,. Potential environmental and forestry through improved nutrient availability in crops and livestock, use of fewer artificial inputs (e.g., synthetic nitrogen fertilizers, insecticides, and fungicides), and more cost effective and environmentally friendly waste management practices, such as bioremediation.

If realized, these improvements will help to protect ecological systems by reducing habitat degradation. In addition, some of the biotechnology techniques should improve the economics of agricultural and forestry production systems. Although genetic engineering can be expected to provide major benefits to agriculture and the environment, risks with the use of this technology should also be recognized. In this chapter, we assess the environmental, health, and socioeconomic benefits and risks of biotechnology, including genetic engineering, in agricultural systems.

DISEASE-RESISTANT CROPS

Resistance against crop disease in plants, caused by viruses, bacteria and fungi is now being explored through biotechnology and genetic engineering techniques as a way to reduce the loss of crops. Because viruses in the field cannot easily be treated, the production of genetically engineered, virus-resistant crops is agronomically significant. In addition, few antibacterial chemicals are available to control bacterial diseases. It has been estimated that viruses, bacteria, and fungi are collectively responsible for significant crop losses estimated at 12%, or nine hundred million tons, of preharvest yield worldwide.

More than 350 field tests of genetically engineered disease-resistant plants have been approved in the United States since 1987, and the majority of these have been created to produce disease-resistant, genetically-engineered crops impervious to viral infections. Success in engineering virus resistance in tobacco, alfalfa, potato, cucumber (*Cucumis sativus*), melon (*Cucumis melo*), alfalfa, and tomato plants have been reported by respectively.

Field trials with tobacco containing the gene from the mosaic virus for the production of the coat protein have shown that resistance can be transgenically induced. For example, in China, field trials of tobacco that contains the tobacco mosaic virus and tomatoes with cucumber mosaic virus are under way. Efforts are also being aimed at rice because of its importance as a staple crop in this region. In Japan, a method for producing fertile transgenic rice plants using an electroporation system has been developed. Transgenic rice plants expressing the rice stripe virus–coat protein (RSV–CP) have been developed to fight the rice stripe virus, one of the major viruses of rice plants in Japan, Korea, China, and Taiwan.

The findings of a 3-year biosafety study of ecological risks have demonstrated that expressing the introduced gene (RSV–CP) in a japonica rice variety (Kinuhikari) resulted in transgenic rice plants that did not: (1) affect morphological and ecological traits with the exception of some somaclonal variations, (2) hybridize with closely grown rice plants, (3) exhibit the tendency to become

weeds, (4) produce any detectable toxic substances, and (5) have any observable effects on subsequent cultivation, microorgansims in soil insects in florae, or on surrounding plants. However, these results will need to be followed up with longer-term biosafety assessment in the future.

TABLE 1.1 : PLANTS GENETICALLY ENGINEERED FOR VIRUS RESISTANCE THAT HAVE BEEN APPROVED FOR FIELD TESTS

Crop	Disease(s)	Research organization
Alfalfa	Alfalfa mosaic virus, Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV)	Pioneer Hi-Bred
Barley	Barley yellow dwaft virus (BYDV)	USDA
Beets	Beet necrotic yellow vein virus	Betaseed
Cantelope and squash	CMV, papaya ringspot virus (PRV) Zucchini yellow mosaic virus (ZYMV), Waltermelon mosaic virus II (WMVII)	Upjohn
	CMV	Harris Morna Seed
	ZYMV	Michigan State University
	ZYMV	Rogers NK Seed
	Soybean mosaic virus (SMV)	Cornell University
	SMV, CMV	New York State Experiment Station
Corn	Maize dwarf mosaic virus (MDMV) Maize chlorotic mottle virus (MCMV, Maize chlorotic dwarf virus (MCDV)	Pioneer Hi-Bred
	MDMV	Northup King
	MDMV	EdKelb
	MDMV	Rogers NK Seed
Cucumbers	CMV	New York State Experiment Station
Lettuce	Tomato spotted wilt virus (TSWV)	Upjohn
Papayas	PRV	University of Howaii
Peanuts	TSWV	Agracetus
Plum Trees	PRV, plum pox virus	USDA
Potatoes	Potato leaf roll virus (PLRV), Potato virus X (PVX), Potato virus Y (PVY)	Monsanto
	PLRV, PVY, late blight of potatoes	Frito-Lay

Table 1.1 Contd.

Crop	Disease(s)	Research organization
Potatoes	PLRV	Calgene
	PLRV, PRY	University of Idaho
	PLRV, PVY	North Carolina State University
Soybeans	SMV	Pioneer Hi-Bred
Tobacco	ALMV, tobacco etch virus (TEV), Tobacco vein motting virus	
	TEV, PVY	University of Florida
	TEV, PVY	North Carolina State University
	TMV	Oklahoma State University
	TEV	USDA
Tomatoes	TMV	Monsanto
	CMV, tomato yellow leafcurl virus	
	TMV,ToMV	Upjohn
	ToMV	Rogers NK Seed
	CMV	PetoSeed
	CMV	Asgrow
	CMV	Harris Moran Seeds
	CMV	New York State Experiment Station
	CMV	USDA

In the United States, squash and the papaya are two of the more recent models of crop engineering for virus resistance. In 1994, the genetically engineered,virus-resistant squash developed by Asgrow seed company for resistance to zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus II (WMV II) was one of the first genetically engineered crops commercialized in the United States. Researchers have also developed two genetically engineered papaya lines by utilizing rDNA techniques to isolate and clone a papaya ringspot virus (PRV) that encodes for the production of the viral coat protein. Papaya, one of the three largest crops in Hawaii, has been decimated in recent years by PVR. Hawaiian papaya growers believe these two lines of genetically engineered, disease resistant papaya could save the $45 million Hawaiian papaya industry from extinction. However, it should be noted that, although the mild strain of PRV displayed excellent resistance to PRV isolates from Hawaii, it showed only moderate to no protection to isolates from different geographic regions (e.g.Bahamas, Mexico, China, Brazil and Australia).

ENGINEERING BACTERIAL AND FUNGI RESISTANCE IN CROPS

Harms (1992), who has reviewed recent developments in the production of resistance to fungal and bacterial diseases via genetic engineering points out that, although there has been much research on how to incorporate such resistance into crop plants, few improvements have been made in this area. Chen and Gu (1993) have described efforts that are being made to combat bacterial blight, which can reduce rice yields by as much as 10%, through genetic engineering.

Developing disease-resistant crops should also receive high priority secondary to the large amounts of fungicides that are currently applied to fruit and vegetable crops. Aspelin et al. (1994) reported that in 1993 131 million pounds of pesticidal active ingredient was applied at a cost of $584 million. Fungicides are sometimes harmful to beneficial insects and toxic to earthworms and many other beneficial soil biota. The number and activity of these soil biota are important in maintaining soil fertility over time because they recycle nutrients in organic matter and aid in water percolation and soil aeration. Furthermore, fungicides rank highest for carcinogenicity of all pesticides applied to agriculture and account for approximately 70% of human health problems associated with pesticide use.

One way to reduce crop losses to fungi and the external application of fungicides is to introduce genes that encode proteins with antifungal properties into crop plants. Several genes have been identified so far in fungi, bacteria, and plants that are effective for the engineering of resistance to fungi based on their ability to produce enzymes, such as chitinase, that attack the cell wall of fungi. Transgenic tobacco plants with a chitinase gene from beans produced elevated levels of chitinase in roots and leaves compared with control plants in greenhouse experiments. Both experimental and control plants were grown in soil inoculated with the fungal pathogen *Rhizoctonia solani*. A positive association was also found between the level of chitinase expressed in the experimental plants and survival. Broglie et al. (1993) and Lin et al. (1995) also reported some success has been achieved with engineering

resistance to the stem pathogen (*Rhizoctonia solani*) in oilseed rape or canola (*Brassica napus*) and rice, respectively. The engineering of resistance to the fugnus *Fusarium oxysporum* in the tomato has also been successful.

Creation of New Weeds

In terms of risks, it has been proposed that large-scale cultivation of plants expressing viral and bacterial genes could lead to novel ecological risks. The most significant ecological risk would be gene transfer via pollination from cultivated crops to wild relatives. For example, it has been postulated that the virus-resistant squash (*Cucurbita pepo*), which is native to the southern United States, where it is an agricultural weed. If the virus-resistance genes were to spread, newly disease-resistant wild squash could become a hardier, more abundant weed. Moreover, because the United States is the origin for squash, changes in the genetic make-up of wild squash could lessen its value to squash breeders.

Another area of concern is the production of virus-resistant sugar beets, which is likely to result in exchange of genes between cultivated and wild populations of beets (*Beta vulgaris L*) because production areas contain wild or weed beet populations, or both, separated by only a few kilometers. A genetic exchange could take place owing to wind pollination, biotic pollination, or the common *gynoniecy* of wild beets. A genetic introgression from seed beet to wild beet popluations has already been observed in Europe.

Viruses that Infect New Hosts

Some plant pathologists also hypothesize that development of virus-resistant crops may allow viruses to infect new hosts through transencapsidation. This may be especially important for certain viruses (e.g., luteoviruses) for which possible heterologus encapsidation other viral RNAs with the expressed coat protein is known to occur naturally. With other viruses, such as the PRV, risk of heteroencapsidation is thought to be minimal because the papaya itself is infected by very few viruses.

Creation of New Viruses

Virus-resistant crops may also lead to the creation of new viruses through an exchange of genetic material or recombination

between RNA virus genomes. Recombination between RNA virus genomes requires infection of the same host cell with two or more viruses. Several authors have pointed out that recombination may also occur in genetically engineered plants expressing viral sequences on infection with a single virus and that large-scale cultivation of such plants may lead to increased possibilities of combinations. It has recently been shown that an RNA transcribed from a transgene can recombine with an infecting virus to produce highly virulent new viruses.

An overall strategy of risk assessment utilizing an incremental approach entails: (1) identifying potential hazards, (2) determining frequency of recombination between homologous but nonidentical sequences, and (3) determining whether such recombinants can have already demonstrated that, even though a particular pseudorecombinant (resulting from pseudorecombination or the situation in which gene components of one virus are exchanged with the proteins of another coat) had enhanced fitness relative to either of the other original strains.

HERBICIDE-RESISTANT CROPS (HRCs)

At the moment at least 4 engineered crops for target herbicide resistance are on the market, and 13 among the key crops in world production have been extensively tested in field trials. In addition, some crops (e.g., corn) are being engineered to contain both herbicide (Glyphosate) and insecticide-resistance (Bt δ-endotoxin). The potential benefits and risks of herbicide-resistant crops (GRCs) are discussed in this section.

Possible Reduced Use of Herbicides

Proponents have argued that reduction of herbicides adopted for HRC crops occurs primarily because these "new" herbicides are needed in lower doses (if compared for instance with atrazine, 2,4-D, and *alachlor*) and are applied later in crops, post-emergence. However, higher resistance of the crop to the target herbicide would, in practice, suggest to the farmer to adopt a higher rate than advised to ensure that all weeds are burned in one tractor trip with the targeted broad spectrum herbicide.

Improved Integrated Pest Management (IPM)

Integrated pest management IPM could benefit from some HRCs if alternative non-chemical methods were applied first to control weeds and the target herbicide were used later, only when and where the threshold of weeds is surpassed, in postemergence. If HRCs could be implemented in IPM programs without underestimating the induction of weed resistance and adopting all the available nonchemical alternatives to manage weed control, this technology would be a step toward more sustainable agriculture. However, in practice, insufficient work of extension outreach and appropriate protocols promoted by the producers could only lead to a further link of the farmland to the producers and their marketing policies aimed at increasing their sales of the targeted herbicides aside to their HRCs seeds.

TABLE 1.2 : HERBICIDE-RESISTANT CROPS (HRCS) APPROVED FOR FIELD TESTS

Crop	Herbicide	Research organization
Alfalfa	Glyphosate	Northrup King
Barley	Glufosinate/Bialaphos	USDA
Canola (oilseed rape)	Glufosinate/Bialaphos	University of Idaho
		Hoechst–Roussel/AgrEvo
	Glyphosate	InterMountain Canola
		Monasnto
Corn	Glufosinate/Bialaphos	Hoechst–Roussel/AgrEvo
		ICI
		Upjohn
		Cargill
		DeKalb
		Holdens
		Pioneer Hi-Bred
		Asgrow
		Great Llkes Hybrids
		Ciba–Geigy
		Genetic Enterprises
	Glyphosate	Monsanto
		EdKalb
	Sulfonylurea	Pioneer Hi-Bred

Table 1.2 Contd.

Crop	Herbicide	Research organization
		Du Pont
	Imidazolinone	American Cyanamid
Cotton	Glyphosate	Monsanto
		Daiyland Seeds
		Northrup King
	Bromoxynil	Calene
	Monsanto	
	Rhone Poulence	
	Sulfonylurea	Du Pont
		Delta and Pine Land
	Imidazolinone	Phytogen
Peanuts	Glufosinate/Bealaphos	University of Florida
Potatoes	Bromoxynil	University of Idaho
		USDA
	2,4-D	USDA
	Glyphosate	Monsanto
	Imidazolinone	American Cyanamid
Rice	Glufosinate/Bialaphos	Louisiana State University
Soybeans	Glyphosate	Monsanto
		Upjohn
		Pioneer Hi-Bred
		Northrup King
		Agri-Pro
	Glufosinate/Bialaphos	Upjohn
		Hoechst/AgrEvo
	Sulfonylurea	Du Pont
Sugar beets	Glufosinate/Bialaphos	Hoechst–Roussel
	Glyphosate	American Crystal Sugar
Tobacco	Sulfonylurea	American Cyanmid
Tomatoes	Glyphosate	Monsanto
	Glufosinate/Bialaphos	Canners Seed
Wheat	Glufosinate/Bialaphos	AgrEvo

Benefits to Developing Countries

Although the majority of HRCs currently on the market and under development belong to key crops in Western agriculture, a few innovations have been proposed that would help developing countries. For example, HRCs have been proposed for improved control of parasitic flowering seeds such as *Orobanche* and *Stringa*, both which can severely reduce grain yields. The HRCs would permit more effective herbicide action against the soil

parasitic weed without damaging the target crop. Trials on boomrape have demonstrated that the engineered plants, can overproduce at a rate at least double the reproduction of the control plants. However, the authors observed that this technology can only be used with weeds that do not have the potential to interbreed with wild relatives that could themselves become weeds. For example, in northern African countries, most crops such as sorghum, wheat, and canola (oilseed rape) have their wild relatives nearby, which therefore increases the risk that genes from the herbicide-resistant crop varieties can be transferred to wild relatives. The same gene escape risks are possible for tomato, corn, and potato in South America.

POTENTIAL RISKS

The risk that herbicide-resistant genes from a transgenic crop variety can be transferred via pollination into weedy relatives has been demonstrated for canola (oilseed rape) and sugar beet. Mikkelsen et al. (1996) and Brown and Brown (1996) have shown that herbicide-resistant genes from a transgenic canola move quickly into wild relative weedy populations. Boudry et al. (1994) also noted consistent gene flow between the cultivated sugar beets and weed beet populations.

Repeated use of the same herbicide in the same area creates problems of plant resistance to the target herbicide. This concern has consistent bases in the recent history of herbicides. For instance, if glyphosate from an actual few million hectares of crops were allowed to associate with HRC crops, the resulting acreage could be around 70 million ha, and pressure on weeds to evolve resulting acreage could be around 70 million ha, and pressure on weeds to evolve resistant biotypes could be pronounced. Sulfonylureas and imidazolinones, to be targeted in HRC crops, are particularly prone to rapid evolution of resistant weeds and have already resulted in several cases of resistance. Extensive adoption of HRCs will increase the acreage and surface treated, thereby exacerbating the resistance problems.

Environmental Risks

Even if less environmentally persistent than previous herbicides