

# Part II

# Agriculture

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**Chapters**  
**The Application of  
Genetics to Plants**

# Chapters

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# The Application of Genetics to Plants

## Perspective on plant breeding

As primitive people moved from hunting and gathering to farming, they learned to identify broad genetic traits, selecting and sowing seeds from plants that grew faster, produced larger fruit, or were more resistant to pests and diseases. Often, a single trait that appeared in one plant as a result of a mutation (see Tech. Note 1, p. 162.) was selected and bred to increase the trait's frequency in the total crop population.

Mendel's laws of trait segregation enabled breeders to predict the outcomes of hybridization and refinements in breeding methods. (See app. II-A.) Consequently, they achieved breeding objectives faster and with more precision, significantly increasing production. During the past 80 years classical applied genetics has been responsible for:

- e increased yields;
- m overcoming natural breeding barriers;
- e increased genetic diversity for specific uses;
- e expanded geographical limits where crops can be grown; and
- a improved plant quality.

Since the beginning of the 20th century, plant breeders have helped increase the productivity (see Tech. Note 2, p. 162.) of many important crops for food, feed, fiber, and pharmaceuticals by successfully developing cultivars (cultivated varieties) to fit specific environments and production practices. Some breeding objectives have met the needs of the local farmer, while other genetic improvements have been applied worldwide. The commercial development of hybrid corn in the 1920's and 1930's and of "green revolution" wheats in the 1950's and 1960's are but two examples of how plant breeding has affected the supply of food available to the world market. (See Tech. Note 3, p. 162.) A comparison of average yields per acre in

1930 and 1975 in table 24 gives a measure of the contribution of genetics.<sup>1</sup>

It is impossible to determine exactly to what degree applied genetics has directly contributed to increases in yield, because there have been simultaneous improvements in farm management, pest control, and cropping techniques using herbicides, irrigation, and fertilizers. Various estimates, however, indicate that applied genetics has accounted for as much as 50 percent of harvest increases in this century. The yield superiority of new varieties has been a major impetus to their adoption by farmers. Historically, the primary breeding objective has been to maintain and improve crop yields. Other

<sup>1</sup> F. Sprague, D. E. Alexander, and J. W. A. Breed "Plant Breeding and Genetic Engineering: A Perspective," *Biosciences*(1): 1980.

Table 24.—Average Yield per Acre of Major Crops in 1930 and 1975

	Average yield per acre			Percent increase
	1930	1975	Unit	
Wheat . . . . .	14.2	30.6	Bushels	115
Rye . . . . .	12.4	22.0	Bushels	77
Rice . . . . .	46.5	101.0	Bushels	117
Corn . . . . .	20.5	86.2	Bushels	320
Oats . . . . .	32.0	48.1	Bushels	
Barley . . . . .	23.8	44.0	Bushels	
Grain sorghum . . . . .	10.7	49.0	Bushels	
Cotton . . . . .	157.1	453.0	Pounds	188
Sugar beets . . . . .	11.9	19.3	Tons	62
Sugarcane . . . . .	15.5	37.4	Tons	141
Tobacco . . . . .	775.9	2,011.0	Pounds	159
Peanuts . . . . .	649.9	2,565.0	Pounds	295
Soybeans . . . . .	13.4	28.4	Bushels	112
Snap beans . . . . .	27.9	37.0	33	33
Potatoes . . . . .	61.0	251.0	129	129
Onions . . . . .	159.0	306.0	92	92
Tomatoes:				
Fresh market . . . . .	61.0	166.0	172	172
Processing . . . . .	4.3	22.1	Tons	413
Hops . . . . .	1,202.0	1,742.0	Pounds	45

SOURCE: U.S. Department of Agriculture, *USES: Conservation and Use* (Washington, D. C.: USDA, 1979).

breeding objectives are specific responses to the needs of local growers, to consumer demands, and to the requirements of the food processing firms and marketing systems.

Developing new varieties does the farmer little good unless they can be integrated profitably into the farming system either by increasing yields and the quality of crops or by keeping costs down. The three major goals of crop breeding are often interrelated. They are:

- to maintain or increase yields by selecting varieties for:
  - pest (disease) resistance;
  - drought resistance;
  - increased response to fertilizers; and
  - tolerance to adverse soil conditions.
- to increase the value of the yield by selecting varieties with such traits as:
  - increased oil content;
  - improved storage qualities;
  - improved milling and baking qualities; and
  - increased nutritional value, such as higher levels of proteins.
- to reduce production costs by selecting varieties that:
  - can be mechanically harvested, reducing labor requirements;
  - require fewer chemical protectants or fertilizers; and
  - can be used with minimum tillage systems, conserving fuel or labor by reducing the number of cultivation operations.

### ***The plant breeder's approach to commercialization of new varieties***

The commercialization of new varieties strongly depends on the genetic variability that can be selected and evaluated. A typical plant breeding system consists of six basic steps:

1. Selecting the crop to be bred.
2. Identifying the breeding goal.
3. choosing the methodological approach needed to reach that goal.
4. Exchanging genetic material by breeding.
5. Evaluating the resulting strain under field conditions, and correcting any deficiencies in meeting the breeding goal.

6. Producing the seed for distribution to the farmer.

The responsibilities for the different breeding phases are distributed but interactive. In the United States, responsibility for crop improvement through plant breeding is shared by the Federal and State governments, commercial firms, and foundations.<sup>2</sup> Although some specific genes have been identified for breeding programs, most improvements are due to gradual selection for favorable combinations of genes in superior lines. The ability to select promising lines is often more of an art (involving years of experience and intuition) than a science.

The plant breeder's approach is determined for the most part by the particular biological characteristics of the crop being bred—e.g., the breeder may choose to use a system of inbreeding or outbreeding, or the two in combination, as an approach to controlling and manipulating genetic variability. The choice is influenced by whether a particular plant in question naturally fertilizes itself or is fertilized by a neighboring plant. To a lesser degree, the breeding objectives influence the choice of methods and the sequence of breeding procedures.

Repeated cycles of self-fertilization reduce the heterozygosity in a plant, so that after numerous generations, the breeder has homozygous, pure lines that breed true. (See Tech. Note 4, p. 162.) Cross-fertilization, on the other hand, results in a new mixture of genes or increased genetic variability. Using these two approaches in combination produces a hybrid—several lines are inbred for homozygosity and then crossed to produce a parental line of enhanced genetic potential. More vigorous hybrids can be selected for further testing. The effects of hybrid vigor vary and include earlier germination, increased growth rate or size, and greater crop uniformity.

A second method for exchanging or adding genes is achieved through altering the number of chromosomes, or ploidy (see Tech. Note 5, p. 162.), of the plant. Since chromosomes are

<sup>2</sup>National Academy of Sciences, *Conservation of Resources: An Imperative*, Washington, D. C., 1978.

generally inherited in sets, plants whose ploidy is increased usually gain full sets of new chromosomes. over one-third of domesticated species are polyploids.<sup>3</sup> Generally, crop improvement due to increased ploidy corresponds to an overall enlargement in plant size; leaves can be broader and thicker with larger flowers, fruits, or seeds. A well-known example is the cultivated strawberry, which has four times more chromosomes than the wild type, and is much fleshier.

Another technique, called *backcrossing* can improve a commercially superior variety by lifting one or more desirable traits from an inferior one. Generally, this is accomplished by making a series of crosses from the inferior to the superior plant while selecting for the desired traits in each successive generation. Self-fertilizing the last backcrossed generation results in some progeny that are homozygous for the genes being transferred and that are identical with the superior variety in all other respects. Single gene resistance to plant pests and disease-causing agents has been successfully transferred through backcrossing.

### ***Major constraints on crop improvement***

Two of the many constraints on crop breeding are related to genetics.

*Many important traits are determined by several genes.*

The genetic bases for improvements in yield and other characteristics are not completely defined, mainly because most biological traits, such as plant height, are caused by the interaction of numerous genes. Although many—perhaps thousands—of genes contribute to quantitative traits, much variation can be explained by a few genes that have major impact on the observable appearance (phenotype)<sup>4</sup>—e.g., the height of some genetic dwarves in wheat can be doubled by a single gene. Many other genes contribute to the general health of the

plant (such as resistance to pests and diseases), although some of their contributions are small and difficult to assess. Favorable combinations of genes result in plants well-adapted to particular growing conditions and agronomic practices. With thousands of genes in a single plant contributing to overall fitness, the possible combinations are almost infinite.

Most poor combinations of genes are eliminated by selection of the best progeny; initially favorable combinations are preserved and improved. Literally millions of plants may be examined each year to find particularly favorable genotypes for development into new breeding stocks. Increasingly sophisticated field testing procedures, as well as advanced statistical analyses, are now used to evaluate the success of breeding efforts. Overall yield is still the most important criterion for success, although considerable care is taken to test stress tolerance, pest and disease resistances, mechanical harvestability, and consumer acceptability. Breeding programs with specialized goals often use rapid and accurate chemical procedures to screen lines and progeny for improvements.

Because the vigor of the plant depends on the interaction of many genes, it has been difficult to identify individual genes of physiological significance in whole plants. As a result, many important genes have not been mapped in major crop species. There is little doubt that breeders would select traits like photosynthetic efficiency (the ability to convert light to such organic compounds as carbohydrates) or mineral uptake if the genes could be identified and manipulated in the same ways that resistance is selected for pathogens.

*It is uncertain how much genetic variation for improvement exists.*

Although the world's germplasm resources have not been completely exploited, it has become more difficult for breeders to improve many of the highly developed varieties now in use—e.g., height reduction in wheat has made enormous contributions to its productivity, but further improvement on this basis seems to be limited.<sup>5</sup> A parallel condition in the potato crop

<sup>3</sup>W. J. C. Lawrence, *Plant Breeding* [London: Edward Arnold Ltd., 1968].

<sup>4</sup>See Thompson, Jr., "Analysis of Gene Number and Development in *Stachys* Systems," *in Svm Genetics Symposium*

<sup>5</sup>N. m. Jensen, "Limits to Growth in World Food Production," *Science*, 1978.

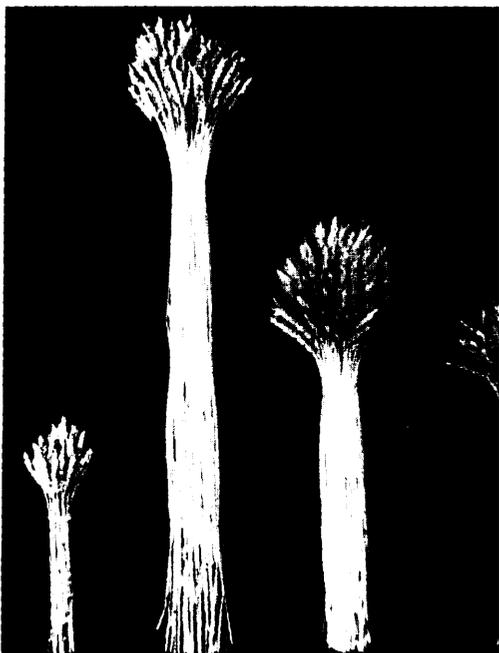


Photo credit: U.S. Department of Agriculture

Bundles of wheat showing variance in height

was recognized by the National Research Council's Committee on Genetic Vulnerability of Major Crops:<sup>6</sup>

If we bear in mind the fairly recent origin of modern potato varieties and that they are, for the most part, derived from the survivors of the late blight epidemics of the 1840's in Europe and North America, it seems likely that the genetic

<sup>6</sup>Academy of Sciences, of Sciences. *Genetic Vulnerability of Major Crops*. Washington, Wash 1972.

base was already somewhat narrow by the time modern potato breeding got under way. The five-fold increase in yield resulting from selection during the last 100 years of potato improvement has produced a group of varieties that are genetically similar and unlikely to respond to further selection for yield. In the long run response to selection for other characteristics is also likely to be limited.

As these examples indicate, the level of genetic homogeneity of some crops may make selection for higher yields in general more difficult. Nevertheless, while the genetic basis for overall crop improvement is poorly understood, refinements in plant breeding techniques may increase the potential for greater efficiency in the transfer of genetic information for more precise selection methods, and as a new source of genetic variation.

Besides these two constraints, other pressures and limitations may also affect crop productivity; some are biological (see Tech. Note 6, p. 162.), requiring technological breakthroughs, while others are related to environmental, social, and political factors. (See Tech. Note 7, p. 162.)—e.g., it has been argued that the agricultural rate of growth is declining: In 1976, the U.S. Department of Agriculture (USDA) estimated that the *total-factor productivity* of U.S. agriculture increased by 2 percent per year from 1939 to 1960, but by only 0.9 percent from the period of 1960 to 1970.<sup>7</sup>

<sup>7</sup>U. S. Department of Agriculture, Economics, Statistics, and Cooperation Services, *Agricultural Productivity: The Limits*, Agriculture Information Bulletin 431, Washington, D. C., 1979.

## Genetic technologies as breeding tools

The new technologies may provide potentially useful tools, but they must be used in combination with classical plant breeding techniques to be effective. The technologies developed for classical plant breeding and those of the new genetics are not mutually exclusive, they are both tools for effectively manipulating genetic information through methods that have been adapted from genetic recombination observed in nature. Plant breeders have many techniques for artificially controlling pollina-

tion—some are capable of overcoming natural barriers such as incompatibility. Yet even though one new technology—protoplast fusion—allows breeders to overcome incompatibility, the new plant must still be selected, regenerated from single-cell culture, and evaluated under field conditions to ensure that the genetic change is stable and the attributes of the new variety meet commercial requirements. Evacuation is still the most expensive and time-consuming step.

### ***New genetic technologies for plant breeding***

The recent breakthroughs in genetic engineering permit the plant breeder to bypass the various natural breeding barriers that have limited control of the transfer of genetic information. While the new technologies do not necessarily offer the plant breeder the radical changes that recombinant DNA (rDNA) technology provides the microbiologist, they will, in theory, speedup and perfect the process of genetic refinement.

The new technologies fall into two categories: those involving genetic transformations through cell fusion, and those involving the insertion or modification of genetic information through the cloning (exactly copying) of DNA and DNA vectors (transfer DNA). Most genetic transformations require that enzymes digest the plant's impermeable cell wall, a process that leaves behind a cell without a wall, or a protoplast. Protoplasts can fuse with each other, as well as with other components of cells. In theory, their ability to do this permits a wider exchange of genetic information.

The approach exploiting the new technologies is usually a three-phase program.

- Phase 1. Isolated cells from a plant are established in tissue culture and kept alive.
- Phase 11. Genetic changes are engineered in those cells to alter the genetic makeup of the plant; and desired traits are selected at this stage, if possible.
- Phase 111. The regeneration of the altered single cells is initiated so that they grow into entire plants.

This approach contains similarities to the genetic manipulation of micro-organisms. However, there is one major conceptual difference. In micro-organisms, the changes made on the cellular level are the goals of the manipulation. With crops, changes made on the cellular level are meaningless unless they can be reproduced in the entire plant. Therefore, unless single cells in culture can be grown into mature plants that have the new, desired characteristics—a procedure which, at this time, has had limited success—the benefits of genetic engineering will

not be widespread. If the barriers can be overcome, the new technologies will offer a new way to control and direct the genetic characteristics of plants.

#### PHASE 1: TISSUE CULTURE TO CLONE PLANTS

Tissue culture involves growing cells from a plant in a culture or medium that will support them and keep them viable. It can be started at three different levels of biological organization: with plant organs (functional units such as leaves or roots);\* with tissues (functioning aggregates of one type of cell, such as epidermal cells (outermost layer) in a leaf; and with single cells. Tissue cultures by themselves offer specific benefits to plant breeders; just as fermentation is crucial to microbial genetic technologies, tissue culture is basic to the application of the other new genetic technologies for plants.

The idea of growing cells from higher plants or animals and then regenerating entire plants from these laboratory-grown cells is not new. However, a better scientific understanding now exists of what is needed to keep the plant parts alive.

In tissue culture, isolated single plant cells are typically induced to undergo repeated cell divisions in a broth or gel, the resulting amorphous cell clump is known as a callus. If culture conditions are readjusted when the callus appears, its cells can undergo further proliferation. As the resulting cells differentiate (become specialized), **they can grow into** the well-organized tissues and organs of a complete normal plant. The callus can be further subcultured, allowing mass propagation of a desired plant.

At this time, it is not uncommon to produce as many as a thousand plants from each gram of starting cells; 1 g of starting carrot callus routinely produces 500 plants. The ultimate goal of tissue culturing is to have these plantlets placed in regular soil so that they can grow and develop into fully functional mature plants. The complete cycle (from plant to cell to plant) permits production of plants on a far more massive scale, and in a far shorter period, than is possible by conventional means. (See table 25 for a

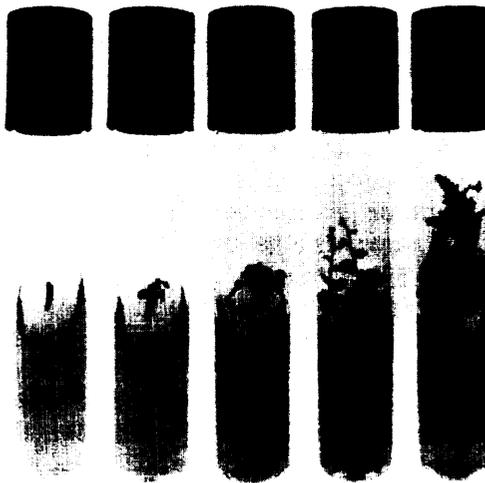
\*Also referred to as "organ" culture.



First stage in plant tissue culturing: inoculation of plant tissue



Figure 26.1



Shows the gradual development of the plant tissue on an agar medium

list of some plants propagated through tissue culture.)

Each of the four stages of the complete cycle—establishment in culture, organogenesis, plantlet amplification, and reestablishment in soil—requires precise biological environments that have to be determined on a species-by-spe-

cies basis. However, several commercial uses of tissue culture already exist. (See table 26.)

**Storage of Germplasm.**—Tissue culture can be used in the long-term storage of specialized germplasm, which involves freezing cells and types of shoots. The culture provides stable genetic material, reduces storage space, and decreases maintenance costs.

Carrot tissues have been frozen in liquid nitrogen, thawed 2 years later, and regenerated into normal plants. This technique has also proved successful with morning glories, sycamores, potatoes, and carnations. Generally, the technique is most useful for plant material that is vegetatively propagated, although if it can be generally applied it could become important for other agriculturally important crops.

**Production of pharmaceuticals and Other Chemicals From Plant Cells.**—Because plant cells in culture are similar to microorganisms in fermentation systems, they can be engineered to work as “factories” to produce

**Table 25.—Some Plants Propagated Through Tissue Culture for Production or Breeding**

Agriculture and horticulture	Poinsettia
Vegetable crops	Weeping fig
Asparagus	Rubber plant
Beet	Flowers
Brussels sprouts	African violet
Cauliflower	Anthruium
Eggplant	Chrysanthemum
Onion	Gerbera daisy
Spinach	Gloxinia
Sweet potato	Petunia
Tomato	Rose
Fruit and nut trees	Orchid
Almond	Ferns
Apple	Australian tree fern
Banana	Boston fern
Coffee	Maidenhair fern
Date	Rabbitsfoot fern
Grapefruit	Staghorn fern
Lemon	Sword fern
Olive	Bulbs
Orange	Lily
Peach	Daylily
Fruit and berries	Easter lily
Blackberry	Hyacinth
Grape	Pharmaceutical
Pineapple	Atropa
Strawberry	Ginseng
Foliage	Pyrethium
Silver vase	Silviculture (forestry)
Begonia	Douglas fir
Cryptanthus	Pine
Dieffenbachia	Quaking aspen
Dracaena	Redwood
Fiddleleaf	Rubber tree

SOURCE: Office of Technology Assessment.



**Table 26.—Representative List of Tissue Culture Programs of Commercial Significance in the United States**

Industry	Application	Economic benefits
Asparagus industry. . . . .	Rapid multiplication of seed stock	Improved productivity, earliness, and spear quality
Chemical and pharmaceutical.	Biosynthesis of chemicals	Reduced production costs
	Propagation of medicinal plants	High volumes of plants for planting
Citrus industry. . . . .	Virus elimination	Improved quality, high productivity
Coffee industry . . . . .	Disease resistance breeding	Disease resistance
Land reclamation. . . . .	Mass propagation	Availability of select clones of wild species for revegetation
Ornamental horticulture. . . . .	Mass propagation	Reduced costs of certain species
		Virus elimination of certain species
		Introduction of new selections
		Increased volumes of difficult selections
Pineapple industry . . . . .	Mass propagation	Improved quality in higher volumes
Strawberry industry. . . . .	Mass propagation	Rapid introduction of new strains

SOURCE: Office of Technology Assessment.

plant products or byproducts. In recent years, economic benefits have been achieved from the production of plant constituents through cell culture. Among those currently produced commercially are camptothecin (an alkaloid with antitumor and antileukemic activity), proteinase inhibitors (such as heparin), and antiviral substances. Flavorings, oils, other medicinal, and insecticides will also probably be extracted from the cells.

The vinca alkaloids—vincristine and vinblastine, for instance—are major chemotherapeutic agents in the treatment of leukemias and lymphomas. They are derived from the leaves of the Madagascar periwinkle (*Catharanthus roseus*). Over 2,000 kilograms (kg) of leaves are required for the production of every gram of vinca alkaloid at a cost of about \$250/g. Plant cells have recently been isolated from the periwinkle, immobilized, and placed in culture. This culture of cells not only continues to synthesize alkaloids at high rates, but even secretes the material directly into the culture medium instead of accumulating it within the cell, thus removing the need for extensive extraction procedures.

Similarly, cells from the Cowage velvetbean are currently being cultured in Japan as a source of L-Dopa, an important drug in the treatment of Parkinson's disease. Cells from the opium poppy synthesize both the plant's normal alkaloids in culture and, apparently, some alkaloids that have not as yet been found in extracts from the whole plant.

Another pharmaceutical, diosgenin, is the major raw material for the production of corticosteroids and sex steroids like the estrogens and progestins used in birth control pill. The large tuberous roots of its plant source, *Dioscorea*, are still collected for this purpose in the jungles of Central America, but its cells have been cultured in the laboratory.

Other plant products, from flavorings and oils to insecticides, industrial organic chemicals, and sweeteners, are also beginning to be derived from plants in cell-cultures. Glycyrrhiza, the nonnutritive sweetener of licorice, has been produced in cultures of *Glycyrrhiza glabra*, and

anthraquinones, which are used as dye bases, accumulate in copious amounts over several weeks in cultures of the mulberry, *Morinda citrifolia*.

#### PHASE II: ENGINEERING CHANGES TO ALTER GENETIC MAKEUP; SELECTING DESIRED TRAITS

The second phase of the cycle involves the genetic manipulation of cells in tissue culture, followed by the selection of desired traits. Tissue culturing, in combination with the new genetic tools, could allow the insertion of new genetic information directly into plant cells. Several approaches to exchanging genetic information through new engineering technologies exist:

- culturing plant sex cells and embryos;
- protoplasm fusion; and
- transfer by DNA clones and foreign vectors.

These are then followed by:

- . screening for desired traits.

Culturing Plant Cells and Embryos.—Culturing the plant's sex cells—the egg from the ovary and the pollen from the anther (pollen-secreting organ)—can increase the efficiency of creating pure plant lines for breeding. Since sex cells contain only a single set of unpaired chromosomes per cell, plantlets derived from them also contain only a single set. Thus, any genetic change will become apparent in the regenerated plant, because a second paired gene cannot mask its effect. Large numbers of haploid plants (cells contain half the normal number of chromosomes) have been produced for more than 20 species. Simple treatment with the chemical, colchicine, can usually induce them to duplicate their genomes (haploid set of chromosomes)—resulting in fully normal, diploid plants. The only major crop that has been bred by this technique is the asparagus.<sup>8</sup>

If the remaining technical barriers can be overcome, the technique can be used to enhance the selection of elite trees and to create hybrids of important crops. Although still

<sup>8</sup>J. G. and J. G. Torrey, "Cytod in Cultured Cells and Tissues," and HortSci 1977.

primarily experimental, successful plant sex-cell cultures have been achieved for a variety of important cultivars, including rice, tobacco, wheat, barley, oats, sorghum, and tomato. However, because the technique can lead to bizarre unstable chromosomal arrangements, it has had few applications.

Embryo cultures have been used to germinate, *in vitro*, those embryos that might not otherwise survive because of basic incompatibilities, especially when plants from different genera are crossed. Embryos may function as starting material in tissue culture systems requiring juvenile material. They are being used to speed up germination in such species as oil palms, which take up to 2 years to germinate under natural conditions.

**Protoplasm Fusion.**—In protoplasm fusion, either two entire protoplasts are brought together, or a single protoplasm is joined to cell components—or organelles—from a second protoplasm. When the components are mixed under the right conditions, they fuse to form a single hybrid cell. The hybrids can be induced to proliferate and to regenerate cell walls. The functional plant cell that results may often be cultured further and regenerated into an entire plant—one that contains a combination of genetic material from both starting plant cell progenitors. When protoplasts are induced to fuse, they can, in theory, exchange genetic information without the restriction of natural breeding barriers. At present, protoplasm fusion still has many limitations, mainly due to the instability of chromosome pairing.

Organelles are small, specialized components within the cell, such as chloroplasts and mitochondria. Some organelles, called plastids, carry their own autonomously replicating genes, as a result, they may hold promise for gene transfer and for carrying new genetic information into protoplasts in cultures, or possibly for influencing the functions of genes in the cell nucleus. (See Tech. Note 8, p. 163.)

The feasibility of protoplasm fusion has been borne out in recent work with tobacco—a plant that seems particularly amenable to manipulation in culture. An albino mutant of *Nicotiana*

*tabacum* was fused with a variety of a sexually incompatible *Nicotiana* species. The resultant hybrids were easily recognized by their intermediate light green color. They have now been regenerated into adult plants, and are currently being used as a promising source of hornworm resistance in tobacco plants.

**Transfer by DNA Clones and Foreign Vectors.**—Recombinant DNA technology makes possible the selection and production of more copies (amplification) of specific DNA segments. Several basic approaches exist. In the “shotgun” approach, the whole plant genome is cut by one or more of the commercially available restriction enzymes. The DNA to be transferred is then attached to a plasmid or phage, which carries genetic information into the plant cell.—E.g., a gene coding for a protein (zein) that is a major component of corn seeds has been spliced into plasmids and cloned in microorganisms. It is hoped that the zein-gene sequence can be modified through this approach to increase the nutritional quality of corn protein before it is reintroduced into the corn plant.

Foreign vectors are nonplant materials (viruses and bacterial plasmids) that can be used to transfer DNA into higher plant cells. Transformation through foreign vectors might improve plant varieties or, by amplifying the desired DNA sequence, make it easier to recover a cell product from culture. In addition, methods have been discovered that eliminate the foreign DNA from the transformed mixture, leaving only the desired gene in the transformed plant. The most promising vector so far seems to be the tumor-inducing (Ti) plasmid carried by *Agrobacterium tumefaciens*. This bacterium causes tumorous growths around the root crowns of plants. It infects one major group of plants—the dicots (such as peas and beans), so-called because their germinating seeds initially sprout double leaves. Its virulence is due to the Ti plasmid, which, when it is transferred to plant cells, induces tumors. Once inside the cell, a smaller segment of the Ti plasmid, called T-DNA, is actually incorporated into the recipient plant cell’s chromosomes. It is carried in this form, replicating right along with the rest of the

chromosomal DNA as plant cell proliferation proceeds. Researchers have been wondering whether new genetic material for plant improvement can be inserted into the T-DNA region and carried into plant cell chromosomes in functional form.

Adding foreign genetic material to the T-DNA region has proved successful in several experiments. Furthermore, it has been found that one type of plant tumor cell that contains mutagenized T-DNA can be regenerated into a complete plant. This new discovery supports the use of the *Agrobacterium* system as a model for the introduction of foreign genes into the single cells of higher plants.

Many unanswered questions remain before *Agrobacterium* becomes a useful vector for plant breeding. Considerable controversy exists about exactly where the Ti plasmid integrates into the host plant chromosomes; some insertions might disrupt plant genes required for growth. In addition, these transformations may not be genetically stable in recipient plants; there is evidence that the progeny of Ti-plasmid-containing plants do not retain copies of the Ti sequence. Finally, *Agrobacterium* does not readily infect monocots (a second group of plants), which limits its use for major grain crops.

Another promising vector is the cauliflower mosaic virus (CaMV). Since none of the known plant DNA viruses has ever been found in plant nuclear DNA, CaMV may be used as a vector for introducing genetic information into plant cytoplasm. Although studies of the structural organization, transcription, and translation of the CaMV are being undertaken, information available today suggests that the system needs further evaluation before it can be considered an alternative to the *Agrobacterium* system.

Although work remains to be done on Ti-plasmid and CaMV genetic mechanisms, these systems have enormous potential. Most immediately, they offer ways of examining basic mechanisms of differentiation and genetic regulation and of delineating the organization of the genome within the higher plant cell. If this can be accomplished, the systems may provide a way of incorporating complex genetic traits into whole plants in stable and lasting form.

**Screening for Desired Traits.**—The benefits of any genetic alteration will be realized only if they are combined with an adequate system of selection to recover the desired traits. In some cases, selection pressures can be useful in recovery. <sup>8</sup>The toxin from plant pathogens, for example, can help to identify disease resistance in plants by killing those that are not resistant. So far, this method has been limited to identifying toxins excreted by bacteria or fungi and their analog; after sugarcane calluses were exposed to toxins of leaf blight, the resistant lines that survived were then used to develop new commercial varieties. In theory, however, it is possible to select for many important traits. Tissue culture breeding for resistance to salts, herbicides, high or low temperatures, drought, and new varieties that are more responsive to fertilizers is currently under study.

Five basic problems must be overcome before any selected trait can be considered beneficial (see figure 28):

- the trait itself must be identified;
- a selection scheme must be found to identify cells with altered properties;
- the properties must prove to be due to genetic changes;
- cells with altered properties must confer similar properties on the whole plant; and
- the alteration must not adversely affect such commercially important characteristics as yield.

While initial screens involving are easier to carry out than screening tests involving entire plants, tolerance at the cellular level must be confirmed by inoculations of the mature plants with the actual pathogen under field conditions.

### PHASE III: REGENERATING WHOLE PLANTS FROM CELLS IN TISSUE CULTURE

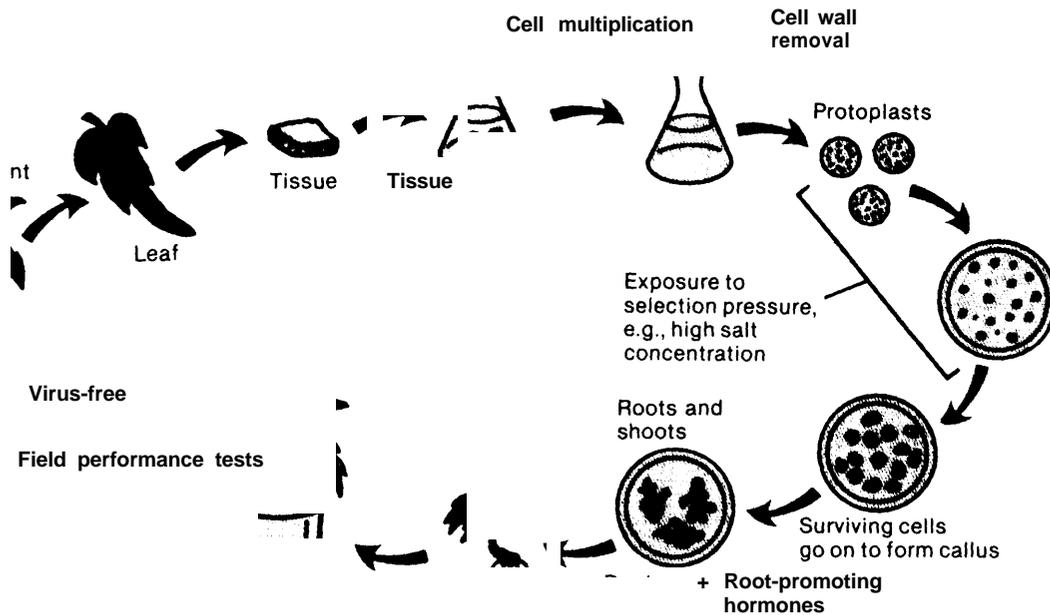
New methods are being developed to:

- increase the speed with which crops are multiplied through mass propagation, and
- create and maintain disease-free plants.

**Mass Propagation.**—The greatest single use of tissue culture systems to date has been for mass propagation, to establish selected

<sup>8</sup>J. F. Shepard, D. Bidney, and E. Shahin, "Potato Protoplasts in Crop Improvement," *Science* 208:17, 1980.

Figure 28.—The Process of Plant Regeneration From Single Cells in Culture



The process of plant propagation from single cells in culture can produce plants with selected characteristics. These selections must be tested in the field to evaluate their performance.

SOURCE: Office of Technology Assessment.



Photo credit: Plant Resources Institute

Multiplying shoots of jojoba plant in tissue culture on a petri dish. These plants may potentially be selected for higher oil content

which stock of or plant

Cu as st aspari carri recei way, thou

which it multiplies and the reduced costs of stock plant maintenance. A tissue culture stock of only 2 square feet (ft<sup>2</sup>) can produce 20,000 plants per month.<sup>10</sup>

Currently, mass production of such cultivars as strawberries (see Tech. Note 9, p. 163.), asparagus, oil palms, and pineapples is being carried out through plant tissue cultures.<sup>11</sup> Very recently, alfalfa was propagated in the same way, giving rise to over 200,000 plants, several thousand of which are currently being tested in field trials. Also, 1,300 oil palms, selected for high yield and disease resistance, are being tested in Malaysia.<sup>12</sup> Other crops not produced by this method but for which cell culture is an important source of breeding variation include

culture because of the increased speed with sources of improved seed or cutting material. (See table 26.) In some cases, producing plants by other means is simply not economically competitive. A classic example is the Boston fern, which, while it is easy to propagate from runner tips, is commercially propagated through tissue

<sup>10</sup> Late. "Propagation (Propagation of Ornamental) Tissue Culture," in *Plant Cell, Tissue, and Organ Culture*, P. S. Bajaj (ed.), Springer-Verlag, New York, 1977, p. 1977.

<sup>11</sup> T. Current St "Current Status of Plant Cell and Cultures," 202:127-147, 1977.

<sup>12</sup> "The Second Green Revolution," special report, *Business Week*, 1, 25, 1980.

beets, brussels sprout, cauliflower, tomatoes, citrus fruits, and bananas. Various horticultural plants—such as chrysanthemums, carnations, African violets, foliage plants, and ferns—are also being produced by in vitro techniques.

Accelerating propagation and selection in culture is especially compelling for economically important forest species for which traditional breeding approaches take a century or more. Trees that reach maturity within 5 years require approximately 50 years to achieve a useful homozygous strain for further breeding. Species such as the sequoia, which do not flower until they are 15 to 20 years old, require between 1 and 2 centuries before traits are stabilized and preliminary field trials are eval-

uated. Thus, tissue culture production of trees has become an area of considerable interest. Already, 2,500 tissue-cultured redwoods have been grown under field conditions for comparison with regular, sexually produced seedlings. (See app. II-B.) Loblolly pine and Douglas fir are also being cultured; the number of trees that can be grown from cells in 100 liters (l) of media in 3 months are enough to reforest roughly 120,000 acres of land at a 12 x 12 ft spacing.<sup>13</sup> To date, 3,000 tissue-cultured Douglas firs have actually been planted in natural soil conditions. (See figure 29.)

J. [13] "Progress and Promise in Forest Genetics," in Proceedings, 50th Anniversary Symposium Paper, *Science and Technology... The Cutting Edge* (Appleton, Wis: Institute of Paper Chemistry, 1980).

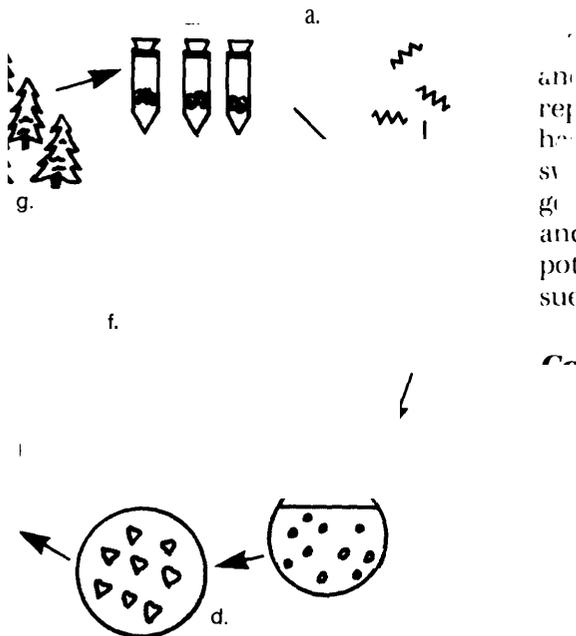


A plantlet of loblolly pine grown in Weyerhaeuser Co.'s tissue culture laboratory. The next step in this procedure is to transfer the plantlet from its sterile and humid environment to the soil



you will find it is a forest.

Figure 29.—A Model for Genetic Engineering of Forest Trees



- Selection of genetic material from germplasm bank
- Insertion of selected genes into protoplasts
- Regeneration of cells from protoplasts and multiplication of cell clones
- Mass production of embryos from cells
- Encapsulation to form 'seeds'
- Field germination of 'seeds'
- Forests of new trees

SOURCE: Office of Technology Assessment.

**Creation and Maintenance of Disease-Free Plants.**—Cultivars maintained through standard asexual propagation over long periods often pick up viruses or other harmful pathogens, which while they might not necessarily kill the plants, may cause less healthy growth. A plant's true economic potential may be reached only if these pathogens are removed—a task which culturing of a plant's meristem (growing point) and subsequent heat therapy can perform. Not all plants produced through these methods are virus-free, so screening cells for viruses must be done to ensure a pathogen-free plant. In horticultural species, the advantages of

virus-free stock often appear as larger flowers, more vigorous growth, and improved foliage quality.

Today, virus-free fruit plants are maintained and distributed from both private and public repositories. Work of commercial importance has been done with such plants as strawberries, sweet potatoes, citrus, freesias, irises, rhubarbs, gooseberries, lilies, hops, gladiolus, geraniums, and chrysanthemums. <sup>14</sup> Over 134 virus-free potato cultures have also been developed by tissue culture. <sup>15</sup>

### Constraints on the new genetic technologies

Although genetic information has been transferred by vectors and protoplasm fusion, no DNA transformations of commercial value have yet been performed. The constraints on the successful application of molecular genetic technologies are both technical and institutional.

#### TECHNICAL CONSTRAINTS

Molecular engineering has been impeded by a lack of understanding about which genes would be useful for plant breeding purposes, as well as by insufficient knowledge about cytogenetics. In addition, the available tools—vectors and mutants—and methods for transforming plant cells using purified DNA are still limited.

Cells carrying traits important to crop productivity must be identified after they have been genetically altered. Even if selection for an identified trait is successful, it must be demonstrated that cells with altered properties confer similar properties on tissues, organs, and ultimately on the whole plant, and that the genetic change does not adversely affect yield or other desired characteristics. Finally, only limited success has been achieved in regenerating whole plants from individual cells. While the list of plant species that can be regenerated from tissue culture has increased over the last 5 years, it includes mostly vegetables, fruit and

K. Iizato, K. Tanaka, M. Hayashi and H. Sam and H. etior "Production of Physiologically Active Substances from Suspension Cultures," H. J. Tissue (ed.), *Tissue Plant Science* (New York: Academic Press, 1974).  
op. cit. op.

nut trees, flowers, and foliage crops. Some of the most important crops—like wheat, oats, and barley—have yet to be regenerated. In addition, cells that form calluses in culture cannot always be coaxed into forming embryos, which must precede the formation of leaves, shoots, and roots. Technical breakthroughs have come on a species-by-species basis; key technical discoveries are not often applicable to all plants. And even when the new technologies succeed in transferring genetic information, the changes can be unstable.

The hope that protoplasm fusion would open extensive avenues for gene transfer between distantly related plant species has diminished with the observation of this instability. However, if whole chromosomes or chromosome fragments could be transferred in plants where sexual hybridization is presently impossible, the possibilities would be enormous.

#### INSTITUTIONAL CONSTRAINTS

Institutional constraints on molecular genetics include those in funding, in regulation, in manpower, and in industry.

Federal funding for plant molecular genetics in agriculture has come from the National Science Foundation (NSF) and from USDA. Research support in USDA is channeled primarily through the flexible Competitive Grants Program (fiscal year 1980 budget of \$15 million) for the support of new research directions in plant biology. The panel on genetic mechanisms (annual budget less than \$4 million) is of particular significance for developing new genetic technologies. The panel's charter specifically seeks proposals on novel genetic technologies. The remaining three panels concerned with plants—nitrogen, photosynthesis, and stress—also support projects to define the molecular basis of fundamental plant properties. The success of the USDA Competitive Grants Program is hard to assess after just 2 years of operation; however, its budget over the past 2 years has severely limited expansion of the program into new areas of research.

Some private institutions<sup>16</sup> argue that the

<sup>16</sup>V. A. present and Future Trends in the Trends in C. Vol. H, Working Papers, King Papers, Imp. Genetics, NTIS, 1981.

Competitive Grants Program is shifting support from ongoing USDA programs to new genetics research programs that are not aimed at the important problems facing agriculture today. There is no opposition to supporting the molecular approaches as long as they do not come at the expense of traditional breeding programs, and as long as both molecular biologists and classical geneticists working with major crop plants are assured of enough support to foster research groups of sufficient size.

At present, funds from nine programs at the NSF—primarily in the Directorate for Biologic, Behavioral, and Social Sciences—support plant research. The total support for the plant sciences may be as high as \$25 million, of which only about \$1 million is designated specifically for molecular genetics.

*The regulation of the release of genetically altered plants into the environment* has not had much effect to date. As of November 1980, only one application [which requested exception from the NIH Guidelines (see ch. 11) to release rDNA-treated corn into the environment] has been filed with the Office of Recombinant DNA Activities (ORDA). Whether regulation will produce major obstacles is difficult to predict at present. It is also unclear whether restrictions will be placed on other genetic activities, such as protoplasm fusion. Currently, at least one other nation (New Zealand) includes such restrictions in its guidelines. It is not clear how much the uncertainty of possible ecological disruption and the attendant liability concerns from intentional release of genetically engineered plants has prevented the industrial sector from moving toward commercial application of the new technology.

*Only a few universities have expertise* in both plant and molecular biology. In addition, only a few scientists work with modern molecular techniques related to whole plant problems. As a result, a business firm could easily develop a capability exceeding that at any individual U.S. university. However, building industrial laboratories and hiring from the universities could easily deplete the expertise at the university level. With the recent investment activity in bioengineering firms, this trend has already

begun; in the long-run it could have serious consequences for the quality of university research.

Despite these constraints, progress in overcoming the difficulties is continuing. At the prestigious 1980 Gordon Conference, where scientists meet to exchange ideas and recent findings, plant molecular biology was added to the list of meetings for the first time. In addition, four other recent meetings have concentrated on plant molecular biology.<sup>17</sup> Up to 50 percent of the participants at these meetings came from nonplant-oriented disciplines searching for future research topics. This influx of investigators from other fields can be expected to enrich the variety of approaches used to solve the problems of the plant breeder.

<sup>17</sup>Organization and Expression in Plants, 1979, in Edinburgh held in Edinburgh, Scotland, July 1979; *Genetic Engineering of Symbiotic Nitrogen Conservation of Nitrogen*, June 80, Vol 2, 1980, Tahoe City, California; *Biologists Look at Green Plants*, 1979, *Syn Annual Symposium*, 2, 1980, West Germ; *West and Fourth and Fourth Symposium Symposium on Nitrogen*, Dec. 1-5, 1979, Dec. 1-5, 1980, Canberra,

Finally, as a general rule, tradeoffs arise in the use of the new technologies that may interfere with their application. It is impossible to get something for nothing from nature—e.g., in nitrogen fixation the symbiotic relationship between plant and micro-organism requires energy from the plant; screening for plants that can produce and transfer the end products of photosynthesis to the nodules in the root more efficiently may reduce inorganic nitrogen requirements but may also reduce the overall yield. This was the case for the high lysine varieties of corn. (See Tech. Note 10, p. 163.) Farmers in the United States tended to avoid them because improving the protein quality reduced the yield, an unacceptable tradeoff at the market price. Thus, unless the genetic innovation fits the requirements of the total agricultural industry, potentials for crop improvement may not be realized.

## Impacts on generating new varieties

Progress in the manipulation of gene expression in eukaryotic (nucleus-containing) cells, which include the cells of higher plants, has been enormous. Most of the new methodologies have been derived from fruit flies and mammalian tissue culture lines; but many should be directly applicable to studies with plant genes. There has been great progress in isolating specific RNA from plants, in cloning plant DNA, and in understanding more about the organization of plant genomes. Techniques are available for manipulating organs, tissues, cells, or protoplasts in culture; for selecting markers; for regenerating plants; and for testing the genetic basis of novel traits. So far however, these techniques are routine only in a few species. Perfecting procedures for regenerating single cells into whole plants is a prerequisite for the success of many of the novel genetic technologies. In addition, work is progressing on viruses, the Ti plasmid of *Agrobacterium*, and engineered cloning vehicles for introducing DNA into plants in a directed fashion. There

have been few demonstrations in which the inheritance of a new trait was maintained over several sexual generations in the whole plant.

Because new varieties have to be tested under different environmental conditions once the problems of plant regeneration are overcome, it is difficult to assess the specific impacts of the new technologies.—E. g., it is impossible to determine at this time whether technical and biological barriers will ever be overcome for regenerating wheat from protoplasts. Nevertheless, the impact of genetics on the structure of American agriculture can be discussed with some degree of confidence.

Genetic engineering can affect not only what crops can be grown, but where and how those crops are cultivated. Although it is a variable in production, it usually acts in conjunction with other biological and mechanical innovations, whose deployment is governed by social, economic, and political factors.

### Examples of new genetic approaches

The ways in which the new genetic approaches could aid modern agriculture are described in the following two examples:

#### SELECTION OF PLANTS FOR METABOLIC EFFICIENCY

Because terrestrial plants are immobile, they live and die according to the dictates of the soil and weather conditions in which they are planted; any environmental stress can greatly reduce their yield. The major soil stresses faced by plants include insufficient soil nutrients and water or toxic excesses of minerals and salts. The total land area with these conditions approaches 4 billion hectares (ha), or about 30 percent of the land area of the Earth.

Traditionally, through the use of fertilizers, lime, drainage, or freshwater irrigation, environments have been manipulated to suit the plant. Modern genetic technologies might make it easier to modify the plant to suit the environment.

Many micro-organisms and some higher plants can tolerate salt levels equal to or greater than those of sea water. While salt tolerance has been achieved in some varieties of plants, the classical breeding process is arduous and limited. If the genes can be identified, the possibility of actually transferring those for salt tolerance into plants makes the adaptation of plants to high salt, semiarid regions with high mineral toxicities or deficiencies a more feasible prospect. In the future, selecting among tissue cultures for metabolic efficiency could become important. Tissue culture systems could be used to select cell lines for resistance to salts and for responsiveness to low-nutrient levels or less fertilizer. However, too little is known about the biochemistry and physiology of plants to allow a more directed approach at this time. Chances for success would be increased with a better understanding of plant cell biology.

Such techniques could be applied to agricultural programs in less developed countries, where, commonly, supplies of fertilizers and lime are scarce, the potential for irrigation is small, and adequate support for technological innovation is limited. In addition, the United States itself contains marginal land that could be exploited for forest products and biomass. The semiarid lands of the Southwest, impoverished land in the Lake States, and reclaimed mining lands could become cost-effective areas for production.

#### NITROGEN FIXATION

It has been known since the early 1800's that biological fixation of nitrogen is important to soil fertility. In fixation, micro-organisms, such as the bacterium *Rhizobium*, transform atmospheric nitrogen into a form that plants can use. In some cases—e.g., with legumes this process occurs through a symbiotic relationship between the micro-organism and the plant in specialized nodules on the plant roots. Unfortunately, the major cereal crops such as

wheat, corn, rice, and forage grasses do not have the capacity to fix atmospheric nitrogen, thus are largely dependent on chemically produced nitrogen fertilizers. Because of these crops, it has been estimated that the world demand for nitrogen fertilizers will grow from 51.4 million metric tonnes (1979 estimate) to 144 million to 180 million tonnes by the year 2000.<sup>1</sup> Therefore, geneticists are looking into the possibility that the genes for nitrogen fixation present in certain bacteria (called "nif genes") can be transferred to the major crops.

Laboratory investigation has focused on the molecular biology of nitrogen fixation in the free living bacterium, *Klebsiella pneumoniae*. A cluster of 15 nif genes has been successfully cloned onto bacterial plasmids using rDNA technology. These clones are being used to study the molecular regulation of nif gene expression and the physical organization of the nif genes on the *Klebsiella* chromosome. In addition they have aided the search for nitrogen fixation genes in other bacteria.

It is thought that a self-sufficient package of nitrogen-fixing genes evolved during the course of plant adaptation, and that this unit has been transferred in a functional form to a variety of different bacterial species, including *Klebsiella* and *Rhizobium*. If the right DNA vector can be found, the nif genes might be transferred from bacteria to plants. The chloroplasts, the cauliflower mosaic virus, and the *Agrobacterium* Ti-plasmid are being investigated as possible vectors.

The way that *Agrobacteria*, in particular, infect cells is similar to the way *Rhizobia* infect plants and form nitrogen-fixing nodules. In both cases, the physical attachment between bacterium and plant tissue is necessary for successful infection. In the case of *Agrobacteria*, tumors form when a segment of the Ti-plasmid is inserted into the nuclear genome of the plant cell. Scientists do not yet know exactly how a segment of the rhizobial genome is transferred into the root tissue to induce the formation of nodules; nevertheless, it is hoped that *Agrobacteria* will act as vectors for the introduction and expression of foreign genes into plant cells, just as *Rhizobia* do naturally.

Other researchers have been investigating the requirements for getting nif genes to express themselves in plants. Nif genes from *Klebsiella* have already been transferred into common yeast, an organism that can be grown in environments without oxygen. Unfortunately, the presence of oxygen destroys a major enzyme for nitrogen fixation and severely limits the potential applications in higher plants. Nevertheless, it is hoped that nif gene expression in yeast will be applicable to higher plants.

An approach that does not involve genetic engineering, uses improved *Rhizobia* strains that are symbiotic with soybeans. Through selection, *Rhizobia* mutants are being found that out-perform the original wild strains. Further

<sup>1</sup>U. S. "Biological Nitrogen + Nitrogen." *Supporting Papers: World Food and a Food a Study* (Washington, D. C.: National Academy of Sciences, 1977).

testing is needed to determine whether the improvement can be maintained in field trials, where the improved strains must compete against wild-type *Rhizobia* already present in the soil.

Another way to improve nitrogen fixation is to select plants that have more efficient symbiotic relationships with nitrogen-fixing organisms. Since the biological process requires a large amount of energy from the plant, it may be possible to select for plants that are more efficient in producing, and then to transfer the end products of photosynthesis to the nodules in the roots. Also existing nitrogen-fixing bacterial strains that can interact with crop plants which do not ordinarily fix nitrogen could be searched for or developed.

Reducing the amount of chemically fixed nitrogen fertilizer—and the cost of the natural gas previously used in the chemical process—would be the largest benefit of successfully fixing nitrogen in crops. Environmental benefits, from the smaller amount of fertilizer runoff into water systems, would accrue as well. But is it difficult to predict when these will become reality. Experts in the field disagree: some feel the breakthrough is imminent; others feel that it might take several decades to achieve.

The refinements in breeding methods provided by the new technologies may allow major crops to be bred more and more for specialized uses—as feed for specific animals, perhaps, or to conform to special processing requirements. In addition, since the populations in less developed countries suffer more often from major nutritional deficiencies than those in industrialized countries, a specific export market of cereal grains for human consumption, like wheat with higher protein levels, may be developed.

But genetic methods are only the tools and catalysts for the changes in how society produces its food; financial pressures and Federal regulation will continue to direct their course. E.g., the automation of tissue culture systems will decrease the labor needed to direct plant propagation and drastically reduce the cost per plantlet to a level competitive with seed prices for many crops. While such breakthroughs may increase the commercial applications of many technologies, the effects of a displaced labor force and cheaper and more efficient plants are hard to predict.

Although it is difficult to make economic projections, there are several areas where genetic technologies will clearly have an impact if the predicted breakthroughs occur:

- Batch culture of plant cells in automated systems will be enhanced by the ability to engineer and select strains that produce larger quantities of plant substances, such as pharmaceutical drugs.
- The technologies will allow development of elite tree lines that will greatly increase yield, both through breeding programs similar to those used for agricultural crops and by overcoming breeding barriers and lengthy breeding cycles. Refined methods of selection and hybridization will increase the potential of short-rotation forestry, which can provide cellulosic substrates for such products as ethanol or methanol.
- The biological efficiency of many economically important crops will increase. Advances will depend on the ability of the techniques to select for whole plant characteristics, such as photosynthetic soil and nutrient efficiency.<sup>19</sup>
- Besides narrowing breeding goals, the techniques will increase the potential for faster improvement of underexploited plants with promising economic value.

For such advances to occur, genetic factors must be selected from superior germplasm, the genetic contributions must be integrated into improved cultural practices, and the improved varieties must be efficiently propagated for distribution.

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For the soybean and tomato crops, the research area for improved biological efficiency received the highest allotment of funds in fiscal year 1978. Total funding was \$12.9 million for soybeans and \$2.1 million for tomatoes. The second largest category to be funded was control of diseases and nematodes of soybeans at \$5.1 million and for tomato at \$1.6 million.

## Genetic variability, crop vulnerability, and storage of germplasm

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Successful plant breeding is based on the amount of genetic diversity available for the insertion of new genes into plants. Hence, it is essential to have an adequate scientific understanding of how much genetic erosion has taken place and how much germplasm is needed. Neither of these questions can be satisfactorily answered today.

### ***The amount of genetic erosion that has taken place***

Most genetic diversity is being lost because of the displacement of vegetation in areas outside the United States. The demand for increased agricultural production is a principal pressure causing deforestation of tropical latitudes (see Tech. Note 11, p. 163), zones that contain extensive genetic diversity for both plants and animals.

It has been estimated that several hundred plant species become extinct every year and that thousands of indigenous crop varieties (wild types) have already been lost. However, it is difficult to measure this loss, not only because resources are on foreign soil but because erosion must be examined on a species-by-species basis. In theory, an adequate evaluation would require knowledge of both the quantity of diversity within a species and the breadth of that diversity; this process has in practice, just begun. What is known is that the lost material cannot be replaced.

### ***The amount of germplasm needed***

Germplasm is needed as a resource for improving characteristics of plants and as a means for guaranteeing supplies of known plant derivatives and potential new ones. Even if plant breeders adequately understand the amount of germplasm presently needed, it is dif-

ficult to predict future needs. Because pests and pathogens are constantly mutating, there is always the possibility that some resistance will be broken down. Even though genetic diversity can reduce the severity of economic loss, an epidemic might require the introduction of a new resistant variety. In addition, other pressures will determine which crops will be grown for food, fiber, fuel, and pharmaceuticals, and how they will be cultivated; genetic diversity will be fundamental to these innovations.

Even if genetic needs can be adequately identified, there is disagreement about how much germplasm to collect. In the past, its collection has been guided by differences in morphology (form and structure), which have not often been directly correlated to breeding objectives. Furthermore, the extent to which the new genetic technologies will affect genetic variability, vulnerability, or the storage of germplasm, has not been determined. (See app. II-A.)

In addition to its uses in plant improvement, germplasm can provide both old and new products. Recent interest in growing guayule as a source of hydrocarbons (for rubber, energy materials, etc.) has focused attention on plants that may possibly be underutilized. It has been found that past collections of guayule germplasm have not been adequately maintained, making current genetic improvements more difficult. In addition, half of the world's medicinal compounds are obtained from plants; maintaining as many varieties as possible would ensure the availability of compounds known to be useful, as well as new, and as yet undiscovered compounds—e.g., the quinine drugs used in the treatment of malaria were originally obtained from the Cinchona plant. A USDA collection of superior germplasm established in 1940 in Guatemala was not maintained. As a

quence, difficulties arose during the Vietnam War when the new antimalarial drugs became less effective on resistant strains of the parasite and natural quinines were once again used.

An important distinction exists between preserving genetic resources in situ and preserving germplasm stored in repositories. Although genetic loss can occur at each location, evolution will continue only in natural ecosystems. With better storage techniques, seed loss and genetic “drift” can be kept to a minimum. Nevertheless, species extinction in situ will continue.

### ***The National Germplasm System***

USDA has been responsible for collecting and cataloging seed (mostly from agriculturally important plants) since 1898. Yet it is important to realize that other Federal agencies also have responsibilities for gene resource management. (See table 27.) Over the past century, over 440,000 plant introductions from more than 150 expeditions to centers of crop diversity have been cataloged.

The expeditions were needed because the United States is gene poor. The economically im-

**Table 27.—Gene Resource Responsibilities of Federal Agencies**

Agency	Type of ecosystems under Federal ownership/control	Responsibilities
U.S. Department of Agriculture Animal & Plant Health Inspection Service . . . . .	—	Controls insect and disease problems of commercially valuable animals and plants.
Forest Service . . . . .	Forestlands and rangelands (U.S. National Forest)	Manages forest land and rangeland living resources for production.
Science & Education Administration . . . . .	—	Develops animal breeds, crop varieties, and microbial strains.
Soil Conservation Service . . . . .	—	Manages a system for conserving crop gene resources. Develops plant varieties suitable for reducing soil erosion and other problems.
Department of Commerce National Oceanic & Atmospheric Administration . . . . .	Oceans—between 3 and 200 miles off the U.S. coasts	Manages marine fisheries.
Department of Energy . . . . .	—	Develops new energy sources from biomass.
Department of Health & Human Services National Institutes of Health . . . . .	—	Utilizes animals, plants, and micro-organisms in medical research.
Department of the Interior Bureau of Land Management . . . . .	Forest lands, rangelands, and deserts	Manages forest, range, and desert living resources for production.
Fish & Wildlife Service . . . . .	Broad range of habitats, including oceans up to 3 miles off U.S. coasts	Manages game animals, including fish, birds, and mammals.
National Park Service . . . . .	Forest lands, rangelands, and deserts (U.S. National Parks)	Conserves forestland, rangeland, and desert-living resources.
Department of State . . . . .	—	Concerned with international relations regarding gene resources.
Agency for International Development . . . . .	—	Assists in the development of industries in other countries including their agriculture, forestry, and fisheries.
Environmental Protection Agency . . . . .	—	Regulates and monitors pollution.
National Science Foundation . . . . .	—	Provides funding for genetic stock collections and for research related to gene resource conservation.

SOURCE: David . . . . . National . . . . . for Gene Resource Conservation.

portant food plants indigenous to the continental United States are limited to the sunflower, cranberry, blueberry, strawberry, and pecan. The centers of genetic diversity, found mostly in tropical latitudes around the world, are believed to be the areas where progenitors of major crop plants originated. Today, they contain genetic diversity that can be used for plant improvement.

It is difficult to estimate the financial return from the germplasm that has been collected, but its impact on the breeding system has been substantial. A wild melon collected in India, for instance, was the source of resistance to powdery mildew and prevented the destruction of California melons. A seemingly useless wheat strain from Turkey—thin-stalked, highly susceptible to red rust, and with poor milling properties—was the source of genetic resistance to stripe rust when it became a problem in the Pacific Northwest. Similarly, a Peruvian species contributed “ripe rot” resistance to American pepper plants, while a Korean cucumber strain provided high-yield production of hybrid cucumber seed for U.S. farmers. And a gene for resistance to Northern corn blight transferred to Corn Belt hybrids has resulted in an estimated savings of 30 to 50 bushels (bu) per acre, with a seasonal value in excess of \$200 million.<sup>20</sup> (See table 28.)

The effort to store and evaluate this collected germplasm was promoted by the Agricultural Marketing Act of 1946, which authorized regional and interregional plant introduction stations (National Seed Storage Centers) run cooperatively by both Federal and State Governments. The federally controlled National Seed Storage Laboratory in Fort Collins, Colo., was established in 1958 to provide permanent storage for seed. In the 1970's, it was recognized that the system should include clonal material for vegetatively propagated crops, which cannot be stored as seed. Although their storage requires more space than comparable seed stor-

**Table 28.—Estimated Economic Rates of Return From Germplasm Accessions**

1. A plant introduction of wheat from Turkey was found to have resistance to all known races of common and dwarf bunts, resistance to stripe rust and flag smut, plus field resistance to powdery and snow mold. It has contributed to many commercial varieties, with estimated annual benefits of \$50 million.
2. The highly successful variety of short-strawed wheat, 'Gaines' has in its lineage three plant introductions that contributed to the genes for the short stature and for resistance to several diseases. During the 3 years, 1964-66, about 60 percent of the wheat grown in the State of Washington was with the variety 'Gaines'. Increased production with this variety averaged slightly over 13 million bu or \$17.5 million per year in the 3-year period.
3. Two soybean introductions from Nanking and China were used for large-scale production, because they are well-adapted to a wide range of soil conditions. All major soybean varieties now grown in the Southern United States contain genes from one or both of these introductions. Farm gate value of soybean crop in the South exceeded \$2 billion in 1974.
4. Two varieties of white, seedless grapes resulted from crosses of two plant introductions. These varieties ripen 2 weeks ahead of 'Thompson Seedless'. Benefits to the California grape industry estimated to be more than \$5 million annually.

SOURCE: U.S. Department of Agriculture, Agricultural Research Service. —  
 SOURCE: U.S. Department of Agriculture, Agricultural Research Service. —  
 Maintenance, Evaluation, and Documentation of Plant Introductions, National Research Program No. 20160 (Washington, D. C., U.S. Government Printing Office, 1976).

age, 12 new repositories for fruit and nut crops as well as for other important crops, from hops to mint, were proposed by the National Germplasm Committee as additions to the National Germplasm System (see Tech. Note 12, p. 163). (The development of tissue culture storage methods may reduce storage costs for these proposed repositories.)

The National Germplasm System is a vital link in ensuring that germplasm now existing will still be available in the future. However, the present system was challenged after the Southern corn blight epidemic of 1970. Many scientists questioned whether it was large enough and broad enough in its present form to provide the genetic resources that might be needed.

The devastating effects of the corn blight of 1970 actually led to the coining of the term crop vulnerability. During the epidemic, as much as 15 percent of the entire yield was lost. Some fields lost their whole crop, and entire sections of some Southern States lost 50 percent of their

<sup>20</sup> U.S. Department of Agriculture, Agricultural Research Service, *Introduction, Maintenance, Evaluation, and Documentation of Plant Introductions*, National Research Program No. 20160 (Washington, D. C., U.S. Government Printing Office, 1976).

corn. Epidemics like this one are, of course, not new. In the 19th century, the phylloxera disease of grapes almost destroyed the wine industry of France, coffee rust disrupted the economy of Ceylon, and the potato famine triggered extensive local starvation in Ireland and mass emigration to North America. In 1916, the red rust destroyed 2 million bu of wheat in the United States and an additional million in Canada. Further epidemics of wheat rust occurred in 1935 and 1953. The corn blight epidemic in the United States stimulated a study that led to the publication of a report on the "Genetic Vulnerability of Major Crops".<sup>21</sup> It contained two central findings: that vulnerability stems from genetic uniformity, and that some American crops are, on this basis, highly vulnerable. (See table 29.)

However, genetic variability, is only a hedge against vulnerability. It does not guarantee that an epidemic will be avoided. In addition, pathogens from abroad can become serious problems when they are introduced into new environments. As clearly stated in the study, a triangular relationship exists between host, pathogen, and environment, and the coincidence of their interaction dictates the severity of disease.

<sup>21</sup> National Academy of Sciences, *Genetic Vulnerability of Major Crops*, Washington, D. C., 1972.

### The basis for genetic uniformity

Crop uniformity results most often from societal decisions on how to produce food. The structure of agriculture is extremely sensitive to changes in the market. Some of the basic factors influencing uniformity are:

- on the consumer's demand for high-quality produce;
- on the food processing industry's demand for harvest uniformity;
- on the farmer's demand for the "best" variety that offers high yields and meets the needs of a mechanized farm system; and
- on the increased world demand for food, which is related to both economic and population growth.

New varieties of crops are bred all the time, but several can dominate agricultural production—e.g., Norman Borlaug and his colleagues in Mexico pioneered the "green revolution" by developing high-yielding varieties (HYV) of wheat that required less daylight to mature and possessed stiffer straw and shorter stems. Since the new varieties (see Tech. Note 13, p. 163) gave excellent yields in response to applications of fertilizer, pesticides, and irrigation, the innovation was subsequently introduced into countries like India and Pakistan. When a single

Table 29.—Acreage and Farm Value of Major U.S. Crops and Extent to Which Small Numbers of Varieties Dominate Crop Average (1969 figures)

Crop	Acreage (millions)	Value (millions of dollars)	Total varieties	Major varieties	Acreage (percent)
Bean, dry . . . . .	1.4	143	25	2	60
Bean, snap. . . . .	0.3	99	70	3	76
Cotton . . . . .	11.2	1,200	50	3	53
Corn . . . . .	66.3	5,200	197 <sup>a</sup>	6 <sup>b</sup>	71
Millet . . . . .	2.0	?		3	100
Peanut . . . . .	1.4	312	15	9	95
Peas . . . . .	0.4	80	50	2	96
Potato . . . . .	1.4	616	82	4	72
Rice . . . . .	1.8	449	14	4	65
Sorghum . . . . .	16.8	795	?	?	?
Soybean . . . . .	42.4	2,500	62	6	58
Sugar beet . . . . .		367	16	2	42
Sweet potato . . . . .	0.13	63	48		69
Wheat . . . . .	44.3	1,800	269	9	50

<sup>a</sup> seeds, forage, and public. <sup>b</sup> were of major ed in t lines used in breeding the major varieties of corn, so the of var number tcher higher.

SOURCE: National Academy of Sciences, *Genetic Vulnerability of Major Crops*, Washington, D. C., 1972.

variety dominates the planting of a crop, there is some loss of genetic variability, the resulting uniformity causes crop vulnerability—and the displacement of indigenous varieties—a real problem.

The rate of adoption of HYVs levels off below 100 percent in most countries, mainly because of the numerous factors affecting supply and demand:<sup>22</sup>

- supply factors:
  - the present HYVs are not suitable for all soil and climatic conditions;
  - they require seeds and inputs (such as fertilizers, water, and pesticides) that are either unavailable or not fully utilized by every farmer; and
  - in some regions, a strong demand still exists for the longer straw of traditional varieties.
- demand factors:
  - consumers may not prefer the HYVs over traditional food varieties;
  - Government price policies may not encourage the production of HYVs.

For these and other reasons, countries already using a great deal of HYVs will continue to adopt them more slowly.

### ***Six factors affecting adequate management of genetic resources***

#### ***1. Estimating the potential value of genetic resources is difficult.***

Of the world's estimated 300,000 species of higher plants, only about 1 percent have been screened for their use in meeting the diverse demands for food, animal feed, fiber, and pharmaceuticals.<sup>23</sup> Genetic resources not yet collected or evaluated are valuable until proven otherwise, and the efforts to conserve, collect, and evaluate plant resources should reflect this assumption. This point of view was strongly re-

<sup>22</sup>D. G. Dalrymple, *Development and Spread of High-Yielding Varieties of Wheat and Rice in the Less Developed Nations*, 6th ed. (Washington D. C.: U.S. Department of Agriculture, Office of International Cooperation and Development in cooperation with U.S. Agency for International Development, 1978).

<sup>23</sup>N. Myers, "[conserving Our Global stock,]" *Environment* 21(9):25, 1979.

flected in a 1978 recommendation by the National Plant Genetic Resources Board. It's recommendation was that four major areas of genetic storage—collection, maintenance, evaluation, and distribution—be viewed as a "continuum that sets up a gene flow from source to end use".<sup>24</sup>

#### ***z. The management of genetic resources is complex and costly.***

The question of how much germplasm to collect is difficult and strongly influenced by cost. Thus far, only a fraction of the available diversity has been collected. A better scientific understanding of the genetic makeup and previous breeding history of major crops will help determine just how much germplasm should be collected. Efforts to give priorities for collection<sup>25</sup> have been hindered by the scientific gaps in knowledge about what is presently stored worldwide. And while attempts have been made to estimate the economic return from introduction of specific plants (see table 28), the degree to which agricultural production and stability are dependent on genetic variability has not been adequately analyzed.

Evaluation of genetic characteristics must be conducted at different ecological sites by multidisciplinary teams. The data obtained will only be useful if adequately assessed and made available to the breeding community (see Tech. Note 14, p. 163).

Germplasm must be adequately maintained to assure viability, "working stocks" must be made available to the breeding community. The primary objective of storing germplasm is to make the genetic information available to breeders and researchers.

#### ***3. How much plant diversity can be lost without disrupting the ecological balances of natural and agricultural systems is not known.***

<sup>24</sup>Report to the Secretary of Agriculture, by the Assistant Secretary for Conservation, Research, and Education based on the deliberations and recommendations, National Plant Genetic Resources Board, July 1978.

<sup>25</sup>Secretariat, International Board for Plant Genetic Resources, *Annual Report 1978*, Rome; Consultative Group on International Agricultural Research, 1979.

The arguments parallel those previously discussed in Congress for protection of endangered species (see Tech. Note 15, p. 163). The last decade has shown that modes of production and development can severely affect the ecological balance of complex ecosystems. What is not known is how much species disruption can take place before the quality of life is also affected.

4. *The extent to which the new genetic technologies will affect genetic variability, germplasm storage methodologies, and crop vulnerability has not been determined.*

The new genetic technologies could either increase or decrease crop vulnerability. In theory, they could be useful in developing early warning systems for vulnerability by screening for inherent weaknesses in major crop resistance. However, the relationship between the genetic characteristics of plant varieties and their pests and pathogens is not understood (see Tech. Note 16, p. 164).

The new technologies may also enhance the prospects of using variability, creating new sources of genetic diversity and storing genetic material by:

- increasing variability during cell regeneration,
- incorporating new combinations of genetic information during cell fusion,
- changing the ploidy level of plants, and
- introducing foreign (nonplant) material and distantly related plant material by means of rDNA.

With the potential benefits, however, come risks. Because genetic changes during the development of new varieties are often cumulative, and because superior varieties are often used extensively, the new technologies could increase both the degree of genetic uniformity and the rate at which improved varieties displace indigenous crop types. Furthermore, it has not been determined how overcoming natural breeding barriers by cell fusion or rDNA will affect a crop's susceptibility to pests and diseases.

5. *Because pests and pathogens are constantly mutating plant resistance can be broken down, requiring the introduction of new varieties.*

Historically, success and failure in breeding programs are linked to pests and pathogens overcoming resistance. Hence, plant breeders try to keep one step ahead of mutations or changes in pest and pathogen populations; a plant variety usually lasts only 5 to 15 years on the market. There is some evidence that pathogens are becoming more virulent and aggressive—which could increase the rate of infection, enhancing the potential for an epidemic (see Tech. Note 17, p. 164).

6. *Other economic and social pressures affect the use of genetic resources.*

The Plant Variety Protection Act has been criticized for being a primary cause of planting uniform varieties, loss of germplasm, and conglomerate acquisition of seed companies. In its opponents' view, such ownership rights provide a strong incentive for seed companies to encourage farmers to buy "superior" varieties that can be protected, instead of indigenous varieties that cannot. They also make plant breeding so lucrative that the ownership of seed companies, is being concentrated in multinational corporations—e.g., opponents claim that 79 percent of the U.S. patents on beans have been issued to four companies and that almost 50 once-independent seed companies have been acquired by The Upjohn Co., ITT, and others.<sup>26</sup> One concern raised about such ownership is that some of these companies also make fertilizer and pesticides and have no incentive to breed for pest resistance or nitrogen-fixation. For the above reasons, one public interest group has concluded:<sup>27</sup>

[t]hanks to the patent laws, the bulk of the world's food supply is now owned and developed by a handful of corporations which alone, without any public input, determine which strains are used and how.

Numerous arguments have been advanced against the above position. Planting of a single variety, for instance, is claimed to be a function of the normal desires of farmers to purchase the best available seed, especially in the com-

<sup>26</sup> R. *et al.*, *Seed* (London: International Coalition for Action, 1979).  
<sup>27</sup> Curiae, *et al.*, *100 S. Ct. 100* (1980), p. 9.

petitive environment in which they operate. Moreover, hybrid varieties (such as corn), are not covered by the plant protection laws; yet they comprise about 90 percent of the seed trade.

As for the loss of varieties by vegetation displacement, statutory protection has been too recent to counter a phenomenon that has occurred over a 30- to 40-year period, and available evidence indicates that some crops are actually becoming more diverse. Since most major food crops are sexually produced, they have only been subject to protection since 1970 when the Plant Variety Protection Act was passed; the first certificates under that Act were not even issued until 1972. Moreover, at least in the case of wheat, as many new varieties were developed in the 7 years after the passage of the Plant Variety Protection Act as in the previous 17.<sup>28</sup>

It is clear that large corporations have been acquiring seed companies. However, the con-

nection between this trend and the plant variety protection laws is disputed. One explanation is that the takeovers are part of the general take-over movement that has involved all parts of the economy during the past decade. Since the passage of the 1970 Act, the number of seed companies, especially soybean, wheat, and cereal grains, has increased.<sup>29</sup> While there were six companies working with soybean breeding prior to 1970, there are 25 at this time.<sup>30</sup>

Thus, to date, although no conclusive connection has been demonstrated between the two plant protection laws and the loss of genetic diversity, the use of uniform varieties, or the claims of increasing concentration in the plant breeding industry; the question is still controversial and these complex problems are still unresolved.

96.1115, 96th Cong., 2d Sess., H. R. 2844, H. R. note 35 (Statement of Harold Loden, Executive Director of the American Seed Trade Association).

for the American Seed Manufacturers' Association v. Amicus Curiae, 100 S. Ct. 2204 (1980), p. 26.

## Summary

The science and structure of agriculture are not static. The technical and industrial revolutions and the population explosion have all contributed to agricultural trends that influence the impacts of the new technologies. Several factors affect U.S. agriculture in particular:

To some degree, the United States depends on germplasm from sources abroad, which are, for the most part, located in less developed countries; furthermore, the amount of germplasm from these areas that should be collected has not been determined.

Genetic diversity in areas abroad is being lost. The pressures of urbanization, industrial development, and the demands for more efficient, more intensive agricultural production are forcing the disappearance of biological natural resources in which the supply of germplasm is maintained.

- This lost genetic diversity is irreplaceable.
- The world's major food crops are becoming more vulnerable as a result of genetic uniformity.

The solutions—examining the risks and evaluating the tradeoffs—are not limited to securing and storing varieties of seed in manmade repositories; genetic evolution—one of the keys to genetic diversity and a continuous supply of new germplasm—cannot take place on storage shelves. Until specific gaps in man's understanding of plant genetics are filled, and until the breeding community is able to identify, collect, and evaluate sources of genetic diversity, it is essential that natural resources providing germplasm be preserved.

## Issues and Options—Plants

**ISSUE:** Should an assessment be conducted to determine how much plant germplasm needs to be maintained?

An understanding of how much germplasm should be protected and maintained would make the management of genetic resources simpler. But no complete answers exist; nobody knows how much diversity is being lost by vegetation displacement in areas mostly outside the United States.

### OPTIONS:

*A. Congress could commission a study on how much genetic variability is needed or desirable to meet present and future needs.*

A comprehensive evaluation of the National Germplasm System's needs in collecting, evaluating, maintaining, and distributing genetic resources for plant breeding and research could serve as a baseline for further assessment. This evaluation would require extensive cooperation among the Federal, State, and private components linked to the National Germplasm System.

*B. Congress could commission a study on the need for international cooperation to manage and preserve genetic resources both in natural ecosystems and in repositories.*

This investigation could include an evaluation of the rate at which genetic diversity is being lost from natural and agricultural systems, and an estimate of the effects this loss will have. Until such information is at hand, Congress could:

- Instruct the Department of State to have its delegations to the United Nations Educational, Scientific, and Cultural Organization (UNESCO) and United Nations Environmental Program (UNEP) encourage efforts to establish biosphere reserves and other protected natural areas in less developed countries, especially those within the tropical latitudes. These reserves would serve as a source for continued natural mutation and variation.

- Instruct the Agency for International Development (AID) to place high priority on, and accelerate its activities in, assisting less developed countries to establish biosphere reserves and other protected natural areas, providing for their protection, and support associate research and training.
- Instruct the International Bank for Reconstruction and Development (World Bank) to give high priority to providing loans to those less developed countries that wish to establish biosphere reserves and other protected natural areas as well as to promote activities related to biosphere reserve preservation, and the research and management of these areas and resources.
- Make a one-time special contribution to UNESCO to accelerate the establishment of biosphere reserves.

Such measures for in situ preservation and management are necessary for long-term maintenance of genetic diversity. Future needs are difficult to predict; and the resources, once lost are irreplaceable.

*C. Congress could commission a study on how to develop an early warning system to recognize potential vulnerability of crops.*

A followup study to the 1972 National Academy of Science's report on major crop vulnerability could be commissioned. Where high genetic uniformity still exists, proposals could be suggested to overcome it. In addition, the avenues by which private seed companies could be encouraged to increase the levels of genetic diversity could be investigated. The study could also consider to what extent the crossing of natural breeding barriers as a consequence of the new genetic technologies will increase the risks of crop vulnerability.

**ISSUE:** What are the most appropriate approaches for overcoming the various technical constraints that limit the success of molecular genetics for plant improvement?

Although genetic information has been transferred by vectors and protoplasm fusion, DNA transformations of commercial value have not yet been performed. Molecular engineering has been impeded by the lack of vectors that can transfer novel genetic material into plants, by insufficient knowledge about which genes would be useful for breeding purposes, and by a lack of understanding of the incompatibility of chromosomes from diverse sources. Another impediment has been the lack of researchers from a variety of disciplines.

#### OPTIONS:

- A. *Increase the level of funding for plant molecular genetics through:*
1. *the National Science Foundation (NSF) and*
  2. *the Competitive Grants Program of the U.S. Department of Agriculture (USDA).*
- B. *Establish research units devoted to plant molecular genetics under the auspices of the National Institutes of Health (NIH), with emphasis*

*on potential pharmaceuticals derived from plants.*

- C. *Establish an institute for plant molecular genetics under the Science and Education Administration at USDA that would include multidisciplinary teams to consider both basic research questions and direct applications of the technology to commercial needs and practices.*

The discoveries of molecular plant genetics will be used in conjunction with traditional breeding programs. Therefore, each of the three options would require additional appropriations for agricultural research. Existing funding structures could be used for all three, but institutional reorganization would be required for options B and C. The main argument for increasing USDA funding is that it is the lead agency for agricultural research, for increasing NSF and NIH funding, that they currently have the greatest expertise in molecular techniques. Option C emphasizes the importance of the *interdisciplinary* needs of this research.

## Technical notes

1. A recent example of such a mutation was the opaque-2 gene in corn, which was responsible for increasing the corn's content of the amino acid lysine.
2. There is disagreement about what is meant by productivity and how it is measured. Statistical field data can be expressed in various ways—e.g., output per man-hour, crop yield per unit area, or output per unit of total inputs used in production. A productivity measurement is a relationship among physical units of production. It differs from measurements of efficiency, which relate to economic and social values.
3. Nevertheless, some parts of the world continue to lack adequate supplies of food. A recent study by the Presidential Commission on World Hunger<sup>31</sup> estimates that "at least one out of every eight men, women, and children on earth suffers malnutrition severe enough to shorten life, stunt physical growth, and dull mental ability."
4. In theory, pure lines produce only identical *gametes*, which makes them true breeders. Successive crossbreeding will result in a mixture of gametes with varying combinations of genes at a given locus on homologous chromosomes.
5. Normally, chromosomes are inherited in sets. The more frequent diploid state consists of two sets in each plant. Because chromosome pairs are homologous (have the same linear gene sequence), cells must maintain a degree of genetic integrity between chromosome pairs during cell division. Therefore, increases in ploidy involve entire sets of chromosomes—diploid (2-set) is manipulated to triploid (3-set) or even to tetraploid (4-set).
6. The estimated theoretical limit to efficiency of photosynthesis during the growth cycle is 8.7 percent. However, the record U.S. State average (116 bu/acre, Illinois, 1975) for corn, having a high photosynthetic rate in comparison to other major crops, approaches only 1 percent efficiency.<sup>32</sup> Since a major limiting step in plant productivity lies in this efficiency for the photosynthetic process, there is potential for plant breeding strategies to improve the efficiency of photosynthesis of many other important crops. This would have a tremendous impact on agricultural productivity.
7. It is difficult to separate social values from the economic structures affecting the productivity of American agriculture. Social pressures and decisions are complex

<sup>31</sup>Report of the Presidential Commission on World Hunger, *World Hunger: The Challenge Ahead*, Washington, D. C., March 1980.

<sup>32</sup>Office of Technology Assessment, *Office of Technology Assessment, Congress for Biological Resources: Technical Analysis of Agricultural Productivity*, U.S. Government Printing Office, (1980).

and integrated—e.g., conflicts between developing maximum productivity and environmental concerns are typified by the removal of effective pesticides from the market. Applications of existing or new technologies may be screened by the public for acceptable environmental impact. Conflict also exists between higher productivity and higher nutritive content in food, since selection for one often hurts the other.

8. A critical photosynthetic enzyme (ribulose biphosphate carbonylase) is formed from information supplied by different genes located independently in the chloroplast (a plastid) and the nucleus of the cell. It is composed of two separate protein chains that must link together within the chloroplast. The larger of these chains is coded for by a gene in the chloroplast—and it is this gene that has been recently isolated and cloned. The smaller subunit, however, derives from the plant nucleus itself. This cooperation between the nucleus and the chloroplast to produce the functional expression of a gene is an interesting phenomenon. Because it exists, the genetics of the cell could be manipulated so that cytoplasmically introduced genes can influence nuclear gene functions. Perhaps most importantly at this stage, plastid genes are prime candidates to clarify the basic molecular genetic mechanisms in higher plants.
9. The advantages to using mass propagation techniques for strawberry plants are that those produced from tissue culture are virus-free, and a plantlet produced in tissue culture can produce more shoots or runners for transplanting.  
The disadvantages are that during the first year the fruit tends to be smaller and, therefore, less commercially acceptable; the plants from tissue culture may have trouble adapting to soil conditions, which can affect their vigor, especially during the first growing season; and the price per plantlet ready for planting from tissue culture systems may be more expensive than commercial prices for rooted shoots or runners bought in bulk.
10. Wheat protein is deficient in several amino acids, including lysine. Considerable attention has been devoted in the past 5 to 10 years to improving the nutritional properties of wheat. Thousands of lines have been screened for high protein, with good success, and high lysine genes with poor success. Some high protein varieties have been developed, but adoption by the farmer has been mediocre at best, partly because of reduced yield levels. There are some "exceptions;—e.g., the Variety "Plainsman V" has maintained both high protein and yield levels, which indicates that there is no consistent relationship between low protein and high yields in some varieties.
11. Some 42 percent of the total land area in the tropics, consisting of 1.9 billion hectares, contains significant forest cover. It is difficult to measure precisely the amount of permanent forest cover that is being lost; however, it has been estimated that 40 percent of "closed" forest (having a continuous closed canopy) has already been lost, with 1 to 2 percent cleared annually.

If the highest predicted rate of loss continues, half of the remaining closed forest area will be lost by the year 2000.<sup>33</sup> The significance of this loss is expressed by Norman Myers in his report, *Conversion of Tropical Moist Forests*, prepared for the Committee on Research Priorities in Tropical Biology of the National Academy of Science's National Research Council: "Extrapolation of figures from well-known groups of organisms suggest that there are usually twice as many species in the tropics as temperate regions. If two-thirds of the tropical species occur in TMF (tropical moist forests), a reasonable extrapolation from known relationships, then the species of the TMF should amount to some 40 to so percent of the planet's stock of species—or somewhere between 2 million and 5 million species altogether. In other words, nearly half of all species on Earth are apparently contained in a biome that comprises only 6 percent of the globe's land surface. Probably no more than 300,000 of these species—no more than 15 percent and possibly much less—have ever been given a Latin name, and most are totally unknown."<sup>34</sup>

12. In 1975, the Committee estimated that \$4 million would be necessary for capital costs of each repository, with recurring annual expenses of \$1.4 million for salaries and operations. USDA has allocated \$1.16 million for its share of the construction costs for the first facility to be constructed at the Oregon State University in Corvallis.
13. High yielding varieties (HYVs) can be defined as potentially high-yielding, usually semidwarf (shorter than conventional), types that have been developed in national research programs worldwide. Wheat varieties were developed by the International Maize and Wheat Improvement Center and rice varieties by international Rice Research Institute. Many improved varieties of major crops of conventional height are not currently considered HYV types, but they have often been incorporated into HYV breeding. HYVs, because of biological and management factors, rarely reach their full harvest potential.
14. Although the National Germplasm System successfully handles some 500,000 units to meet annual germplasm requests, many accessions—like the 35,000 to 40,000 wheat accessions stored at the Plant Genetics and Germplasm Institute at Beltsville, Md.—have yet to be examined. Furthermore, the varieties released for sale by the seed companies are not presently evaluated for their comparative genetic differences.
15. For comparison, the National Germplasm System functions on less than \$10 million annually, whereas the Endangered Species Program had a fiscal year 1980 budget of over \$23 million. The funds allocated to the En-

<sup>33</sup> The President's U.S. Interagency Task Force on the Forests, *Tropical For Tropical Forests: A Policy, and Program for the State Department* publication, publication No. 87-01, D.C., May, 1987, p. 11.  
<sup>34</sup> *Conversion of Tropical Moist Forests*, prepared for the Committee on Research Priorities in Tropical Biology of the National Research Council, National Academy of Sciences, Washington, D.C., 1975, p. 11.

dangered Species Program are used for such activities as listing endangered species, purchasing habitats for protection, and law enforcement.

16. The uses of pest-resistant wheat and corn cultivars on a large scale for both diseases and insects are classic success stories of host-plant resistance. However, recent trends in the Great Plains Wheat Belt are disturbing. The acreage of Hessian fly-resistant wheats in Kansas and Nebraska has decreased from about 66 percent in 1973 to about 42 percent in 1977. Hessian fly infestations have increased where susceptible cultivars have been planted. In South Dakota in 1978, in an area not normally heavily infested, an estimated 1.25 million acres of spring wheat were infested resulting in losses of \$25 million to \$50 million. An even greater decrease in resistant wheat acreage is expected in the next 2 to 5 years as a result of releases of cultivars that have improved agronomic traits and disease resistance but that are susceptible to the Hessian fly. Insect resistance has not been a significant component of commercial breeding programs.<sup>35</sup>

<sup>35</sup>Office of Technology Assessment, U.S. Congress, *Wheat: A Century of Progress* (vol. 2), Washington, D. C.: U.S. Government Printing Office, 1979, p. 106.

17. Expressed in genetic terms, cases exist '(where the introduction of novel sources of major gene resistance into commercial cultivars of crop plants has resulted in an increase in their frequency of corresponding virulence genes in the pathogen' )." This has been reported in Australia with wheat stem rust, barley powdery mildew, tomato leaf mold, and lettuce downy mildew. Evidence suggests that there is considerable gene flow in the various pathogen populations-e.g., asexual transfer can quickly alter the frequency of virulence genes. Furthermore, pressures brought about in the evolutionary process have developed such a high degree of complexity in both resistance and virulence mechanisms, that breeding approaches, especially those only using single gene resistance, can be easily overcome.

<sup>36</sup>R. C. Slattock, E. C. Janssen, E. W. H. Reid, and J. A. J. H. van der Plig, "Interpretation of the Frequencies of Host Specific Phenotypes in U.S. Populations of *Phytophthora blight* in North Wales," *Ann. Wale. Agr. Appl.* 1977.