Lesson 1:
Traditional Plant Breeding

Traditional plant breeding technologies are described in this lesson. Basic breeding practices include natural crossbreeding, selective breeding, and hybridization. Modern plant biotechnology is founded in plant breeding practices that are thousands of years old.

Natural Crossbreeding

Natural crossbreeding is the name for the reproductive process in which two plant varieties, which have different genotypes, sexually reproduce without human intervention. Natural crossbreeding allows the random mixing of genes to occur within a species. Desirable and undesirable traits are combined within the plant species. Plants that receive more vigorous genes generally grow and reproduce better than plants that do not. Weaker plants will therefore very gradually diminish in number as stronger plants dominate. This gradual improvement of the species can be accelerated through selective breeding practices.

Selective Breeding

Selective breeding is the process of identifying plants with desirable traits and causing them to reproduce. Plants are selectively bred mainly for two reasons: to increase the production of the useful parts of the plant or to increase the ability of a plant to withstand harsh environments, disease, or plant pests. Selective breeding can be done asexually or sexually. Many horticulture crops are reproduced asexually. Most field crops have traditionally been reproduced by sexual breeding methods, but more recently the asexual method of plant tissue culture has been used. Sexual breeding methods include inbreeding and hybridization. Inbreeding is the crossing of closely related plants to cause their offspring's traits to become more homozygous. Hybridization occurs when two inbred plants that are genetically different are crossed to produce plants that are superior to both the parent plants.

Advantages and Disadvantages of Selective Breeding

Selective breeding has two advantages. It allows the plant breeder to increase the occurrence of desired plant traits; typically, the most important trait is crop yield. Selective breeding also helps to make the performance of a crop more predictable since the selected plants are multiplied by controlling their pollination to produce a more uniform crop of seeds. This seed crop is sold to farmers who produce the final product.

Selective breeding also has some disadvantages. While the occurrence of desired traits increases, the occurrence of undesirable traits may also increase. As the selected plants are bred, the plants become more homozygous for both desired and undesired traits. A second disadvantage is that the genetic diversity of a crop species decreases as more similar plants are selected. Some native varieties of crops have been lost due to the extensive use of fast-growing high-yield varieties that crowd out the plants that grow more slowly. Another disadvantage of selective breeding is that the uniformity of the crop plants can increase insect problems. Insects that like a certain crop can multiply quickly when the crop is more uniform and cause a greater amount of crop damage.

Hybrids

Hybrids are plants produced by crossing two inbred lines of plants that are greatly different genetically. Before a hybrid is produced, inbreeding is used to develop a consistent plant phenotype. Breeders force a plant to self-pollinate and then force its offspring to do the same. They repeat this process five to seven times so that the plants will consistently express the same phenotype.

Breeders use three common methods to produce a hybrid seed. The first method is called a single cross. When inbred plant Z is crossed with inbred plant Y, the result is a single cross hybrid, ZY. The second method of hybridization is called a three-way cross. A three-way cross is made by taking a single cross ZY and crossing it with another unrelated inbred plant X to produce a three-way cross hybrid, ZYX. The third type of hybridization is the double cross. In a double cross, breeders make two single crosses, and then they cross the two single cross hybrids.

A hybrid generally displays more vigorous growth than both of its parents. This extra vigor in its growth is called hybrid vigor, or heterosis. However, hybrid plants usually either are sterile or

produce offspring that do not perform well and are inferior to the hybrid. Extensive work must continually be done to supply hybrid seed to producers.

Summary

Breeders have replaced natural crossbreeding of plants with selective breeding and hybridization to produce superior plants. These crop development methods have laid the foundation for the modern methods of plant development--plant tissue culture and genetic manipulation.

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Lesson 2: Plant Tissue Culture

The horticulture industry regularly uses a biotechnology technique called plant tissue culture as an effective method of propagating horticultural crops such as ferns and orchids. Plant tissue culture is also being used to grow whole plants from genetically modified plant cells. This lesson will introduce and explain the process of plant tissue culture. The steps in tissue culturing and the stages of tissue culture growth will also be examined.

Plant Tissue Culture

Plant tissue culture is an asexual method of reproduction. The plants produced will be exact clones of a single parent plant. Plant tissue culture involves selecting a piece of a parent plant and placing it in a sterile artificial media where it grows into a new plant.

Advantages and Disadvantages of Plant Tissue Culture

One of the main reasons that plant tissue culture was developed was that some plants are very difficult to propagate commercially. Tissue culturing allows plant growers to mass propagate clones of a highly desirable plant. They can produce thousands of plants from a few pieces of one plant. Another advantage of plant tissue culture is that pathogen-free plants can be produced since certain portions of a plant do not contain viruses that are found in the rest of the plant. A third advantage is the conservation of time, because plants can be propagated from tissue culture at any time during the year. A fourth advantage is the conservation of growing space. Tissue culture plants are divided multiple times and begin as very small plants that grow slightly more slowly than normal. The number of plants traditionally grown on one acre can therefore be grown on shelves in 50 to 60 square feet of space.

With the advantages come some disadvantages. Plant tissue culture is an expensive method of plant propagation requiring an extensive amount of sophisticated equipment and facilities. In addition, plant tissue cultures are susceptible to contamination by microorganisms. Tissue cultures are destroyed if contamination occurs. A third disadvantage is that commercial tissue culture requires skilled workers, which adds to the total cost of producing the plants.

Plant Tissue Culture Equipment

The equipment and supply needs for plant tissue culture can be broken down into three separate areas--preparation, transfer, and growth. Several types of equipment and supplies are needed when preparing to do a tissue culture. A refrigerator is needed to store the chemicals for the growing media. The pH of the growing media must be checked with a pH meter. A scale or balance is required to measure the quantities of the media ingredients. The ingredients are warmed on a heating plate. Finally, an autoclave is required to sterilize the media and the equipment used in transferring the plants. When transfer is carried out, a fume or air flow hood is used to reduce the movement of air (which may contain microorganisms) in the work area. The technician holds the plant tissue with a sterile forceps and uses a scalpel to divide the plant for culturing. Test tubes or petri dishes hold the growing media and the plant tissue after transfer. For some types of tissue culture, a dissecting microscope is used to help in the selection of the tissue for culturing. When the process is completed, the tissue cultures are put in a growth chamber, a room that controls exposure to heat and light.

Steps in Plant Tissue Culture

The first step in plant tissue culture is media preparation. The initial growing media varies slightly in composition depending on the plant species. However, it generally contains plant nutrients, mineral salts with vitamins, plant hormones that regulate growth, pure water, sugar, and agar (if a semi-solid media is needed).

The second step in the process is the selection and collection of the explant. The explant is the portion of the parent plant that will be used to grow new plants. Depending on the plant being propagated, a shoot tip, bud, section of a leaf with veins, node, or bud scale may be chosen. Different plants grow better from different types of explants. The explant is nearly always selected from rapidly growing tissue since this tissue is best able to produce the new plant. It is also important that healthy and disease-free parent tissue be used.

After the explant has been chosen, the next step is cleaning the explant. It must be disinfected. If the explant is woody tissue, alcohol is used as a disinfectant; for most other plant tissues, a 10 percent bleach solution is used. The explant is soaked in the bleach solution for only ten minutes. If it is soaked longer than ten minutes, damage to the plant tissue can occur. Often a drop or two of detergent is added to the bleach solution as a wetting agent to help the disinfectant apply to surfaces more effectively. After it is soaked in the disinfectant, the explant is rinsed at least three times in pure water to remove any remaining bleach solution.

The fourth step involves transferring the explant to the growing media. The collected plant tissues are trimmed, and some types of tissues can be divided for growing more plants. A dissecting microscope is sometimes needed if the explant is very small. The explant is then transferred to the growing media. This procedure must take place in a sterile environment.

Stages of Tissue Culture Growth

The first stage of tissue culture growth is called the initiation and establishment stage. This stage lasts about four to six weeks. A callus composed of rapidly dividing cells forms and grows in response to the wounding or cutting of the plant tissue. A shoot or immature stem begins to grow during this first stage.

The second stage of tissue culture growth is the proliferation or multiplication stage. It lasts one to three months. During this stage, the shoot multiplies into many shoots. These new shoots can be divided to increase the number of plants produced. A slightly different growing media is used in this stage.

The third stage is the pretransplant stage. It lasts about three weeks. Roots begin to grow. A slightly different growing media is used. The plants begin to photosynthesize and require more light. Near the end of this stage, the young plants are stressed slightly in a process called hardening off, which involves exposing the young plants to conditions outside the sterile container in which they grow.

The fourth stage of plant tissue culture growth is called the transplanting stage. The growing plants are put into pots and moved to a shady, humid greenhouse. After a several weeks, the plants are again hardened off and then moved to a regular greenhouse where they will receive full sun and less humidity.

The Use of Tissue Culture in Genetic Engineering

When a single plant cell or group of cells is genetically modified, tissue culture is often used to rapidly grow a set of plants. These plants are genetically identical to the plant cell or cells used to create them. A second use of plant tissue culture is for the screening of a large number of plants for certain characteristics. If a more drought-tolerant plant is desired, then hundreds of tissue cultures can be taken from a wide range of plant varieties and mutations to find this characteristic. The tissue cultures from the plants are grown and exposed to drought conditions. Those plants that show promise are grown to full size. This screening allows researchers to focus on plant varieties that might contain desirable genetic traits.

Summary

Tissue culture has become an important tool for plant breeders and researchers. It allows breeders to quickly produce large numbers of mature clones of a parent plant. Researchers are able to genetically modify a plant cell or group of cells and then grow these modified cells into a mature plant. However, plant tissue culture is an expensive process and can fail if the cultures become contaminated. Tissue culturing involves four major steps: media preparation, choosing the explant, cleaning the explant, and transferring the explant to the growth media. Growth also occurs in four stages: initiation and establishment, proliferation, pretransplant, and transplant.

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Lesson 3:
Genetically Modified Plants

The era of genetic engineering in plant agriculture began with the approval of the first genetically modified food crop in 1994. Since then several genetically modified plants have been developed and approved for use. This lesson will examine how these modified plants were developed and how they function.

Developing Genetically Modified Plants

The first step in the development of a genetically modified plant is to find, isolate, and clone the gene or genes that cause the expression of the desired trait in an organism. Often the gene expressing a desired trait has not been identified or located in the DNA of that organism. The process of finding the needed gene can be very difficult. It is easier, however, if researchers have made a genome map of the particular organism, since they will have identified markers that will narrow the search considerably.

The second step involves the selection of a means of genetic transfer. A bacterium or virus can be used as a vector, the vehicle for moving the desired gene into the plant cells. The desired gene and a marker gene are inserted into the bacterium or virus, and the microorganism is placed in contact with the plant cells to be modified. The bacteria or virus infects the cells and transfers the desired gene to the plant cells.

A gene gun can also be used to transfer a desired gene into a plant cell. The desired gene and a marker gene are inserted into plasmid; the plasmid is then placed on the surface of very small (1 mm in diameter), heavy metal pellets. These pellets, usually made of gold, are shot into the plant cells with the use of a small high-pressure gun. High pressure is needed to penetrate the plant's cell wall.

A third method of gene transfer involves the use of chemicals to weaken or dissolve the cell wall. The desired gene could then physically be placed in the cell. After the gene is incorporated, the plant cell is stimulated to repair the cell wall.

The third step in developing genetically modified plants is the selection of the plant cells that incorporate the desired gene into their DNA. The plant cells that contain the desired gene will also contain the marker gene, which is designed to be easily identifiable. These cells will be grown into mature plants through tissue culturing.

How Herbicide-Tolerant Plants Function

Many herbicides kill plants by chemically blocking a metabolic pathway. A metabolic pathway is a series of chemical reactions that are necessary for the survival of a plant. Herbicide-tolerant plants can bypass the blocked portion of the metabolic pathway. This ability comes from genes that produce certain enzymes that provide a different chemical route around the blocked portion. An example of a herbicide-tolerant crop is Roundup Ready™ soybeans from Monsanto. These plants can tolerate glyphosate, the active ingredient in the herbicide. Glyphosate blocks a section of the metabolic pathway, which kills most types of plants. Researchers discovered that a common bacteria found in soil contains a gene that resists glyphosate. They inserted this gene into soybean plants. It produces an enzyme that provides a chemical path around the blocked portion. With this new gene, the soybean plants are not noticeably affected by Roundup™ or any glyphosate herbicide.

How Insect-Resistant Plants Function

A common soil bacterium called *Bacillus thuringiensis (Bt)* was first identified in 1911. Different strains of the bacteria produce a protein that kills some types of insects. When ingested it dissolves the wall of the insect's gut, which causes the insect to be unable to eat and eventually to die. *Bt* was registered as a biopesticide in 1961. However, the pesticide produced from the bacteria had several drawbacks; it was expensive, had to be eaten by insects to work, broke down in sunlight, and was easily washed away by rain. These factors have limited the use of *Bt*-derived pesticides.

Each *Bt* strain kills a specific type of insect. The genes that cause the production of the protein in specific *Bt* strains have been isolated and transferred to several crop plants. For example, Monsanto has developed potatoes, corn, and cotton plants that incorporate *Bt* to protect them against particular insects.

The uses of the *Bt* gene are limited because not all insects are affected by one of the strains of *Bt*. In addition, some people are concerned that insects will become tolerant of the protein produced by the *Bt* gene. If this happens, crops incorporating *Bt* will lose their advantage.

How Disease-Resistant Plants Function

Researchers have developed virus-resistant plants by inserting a small portion of DNA from the virus into the DNA of the plant. This modification gives the plant an immunity to the disease. The Freedom II™ yellow crookneck squash developed by Asgrow is an example of a virus-resistant plant. It resists two types of the mosaic virus.

The development of bacteria- and fungus-resistant plants has not been as easy or as fast as the development of virus-resistant plants. Current research in this area centers on trying to enhance the plant's natural immune response to attacks by bacteria and fungi. Some plants seem able to withstand them better than others. Scientists are trying to find out how these plants work so that they can genetically modify crop plants to have this ability. Bacteria- and fungus-resistant plants are not currently available, nor are they expected to be for several years.

The Effect of Biotechnology on Food Quality and Processing

Biotechnology has been used to enhance food quality and food processing. One of the first genetically engineered plants was the FlavrSavr™ tomato. The tomato was developed by Calgene, a subsidiary of Monsanto, to have a vine-ripened taste and a longer shelf life. Genes were inserted into the tomato plant to delay the softening of the tomato by causing the production of an enzyme that slows the breakdown of pectin in the tomato. Pectin prevents the tomato from getting soft and rotting. Four other companies have gained approval for similar genetically modified tomatoes.

Genetically modified canola and corn plants have also been developed. Both corn and canola plants have been genetically modified to produce grains that are higher in oil content and have a modified oil composition. This means that the levels of saturated and unsaturated oil from these plants have been changed to meet different uses. An example of this type of product is a canola plant from Calgene named Laurical® that produces seeds high in lauric acid. The oil they produce can be used in processing many food products.

Monsanto is working on high-starch potatoes. These potatoes are higher in starch and lower in water content than unmodified potatoes. When the new potatoes are sliced into chips or french fries and deep fried, they will absorb less oil. They will therefore have a lower fat content.

Summary

The genetic engineering of plants has yielded better quality foods and plants that are herbicide tolerant, insect resistant, and disease resistant. The development of these plants involves the location, isolation, cloning, and transferral of the desired genes.

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Lesson 4: Emerging Applications of Plant Biotechnology

The field of plant biotechnology is one of the most rapidly developing areas of biotechnology. The pace of growth is due to several factors, but the most important reasons are the potential for high profits and the relative simplicity of plant manipulation through recombinant DNA technology. As plant biotechnology continues to advance, the list of products developed through its application will grow. Some of these products are examined in this lesson. Biofuels and biopolymers, for example, are two of the most promising products currently under development. The needs of the plant products industry will influence the future of plant biotechnology.

Biofuels

Biofuels are combustible substances derived from organic sources. Nearly all biofuels are plant-derived. Several types of biofuels exist. Alcohol-based fuels are made by fermenting plant materials. Gasohol is one example; it is a fuel composed of 10 percent alcohol and 90 percent gasoline. Gasohol with a 10 percent ethanol (a type of alcohol) blend is available to motorists in many gas stations across the country. Normally, the ethanol blend is slightly higher in price than the regular petroleum fuel. Researchers are searching for plants that can be modified to produce ethanol more economically.

Plant oil-based fuels, or biodiesels, are made from seeds with a high oil content. Most of these fuels are the result of the addition of methanol (wood alcohol) to the plant oil and the removal of a sticky substance called glycerine. Soybean and rapeseed oils are most commonly used in the production of biodiesel. Biodiesel can be used as a fuel by itself, or it can be blended with petroleum diesel fuel. It is a cleaner-burning fuel that produces less wear on an engine than petroleum diesel. The challenge facing those seeking to increase the use of biodiesel is its cost, which is currently nearly four times the cost of petroleum diesel. Scientists are looking for ways to engineer plants to produce larger quantities of oil and to require less extensive processing to produce biodiesel.

Another type of biofuel is biogas. Methane gas is a byproduct derived from the anaerobic (oxygen-free) digestion of plant materials and/or animal waste by microorganisms. Many people in India and China use small-scale methane production chambers to supply fuel for cooking and lighting. Although methane gas has some limitations as a fuel because it cannot be easily transported or compressed into a liquid, researchers are examining the possibility of developing plants that would produce crop residue that is more useful for methane production. Microorganisms are also being examined to see if they can be modified to better digest crop residues and produce more methane.

Biopolymers

Biopolymers are complex chemical compounds produced by living things. Biopolymers from genetically engineered plants may be useful in a variety of industries. Several different groups of biopolymers are used for a variety of applications.

Carbohydrates are one group of biopolymers. All plants produce carbohydrates, but not all plants are a good source of food-grade carbohydrates. Modified corn starch is one of the plant carbohydrates most widely used in foods because it is a cheap source of starch. However, it breaks down when heated in a microwave oven. Researchers are looking at ways that the corn plant can be modified to produce a starch that can be heated. Another important carbohydrate molecule is sugar. Scientists are researching the possibility of genetically altering potato plants so that their leaves will have a high sugar content. The plants could then be used as a source of sugar.

Fatty acids are a second type of biopolymer. Researchers are working on modifying corn and canola to produce oils with an altered level of fatty acids. They may contain a high level of either saturated or unsaturated fatty acids, depending on which is needed for a given application.

Another kind of biopolymer is high-value pharmaceutical proteins. Two examples of human health products that are proteins are insulin and blood plasma. Plants provide the potential for such proteins to be produced at a lower cost than currently available.

A fourth group of biopolymers is industrial enzymes. Enzymes are needed for brewing to aid in fermentation, in the paper industry to process and bleach paper, and in the livestock industry as a feed additive to aid in digestion in animals. These types of enzymes are needed in large quantities, and scientists are trying to modify plants to provide these enzymes at a low price.

Another type of biopolymer being researched is bioplastics. Plants naturally have a very small amount of the chemical components of plastic in their tissue. Scientists are attempting to develop plants with tissue that contains a much higher level of these components. Researchers have already engineered plants to increase these levels; the chemicals make up 4 percent of the modified plants. If this percentage can be increased, the world will have an expanded source of biodegradable plastics.

Plant Traits Desired by Producers

The ability to genetically engineer plants has caused plant breeders and researchers to stop asking what traits are available in a plant species and to start asking what traits are needed or desired by producers and which plant can best be modified to fulfill this need. As genes for more and more traits are discovered, the possible genetic combinations grow as well. Researchers are focusing on several major areas. One of these areas is that of environmentally tolerant plants, including the development of plants that are drought-tolerant, frost-tolerant, and salt-tolerant. The development of better forestry products is another area of plant research. Traits desired in this area include stronger wood, fire-resistant wood, and trees that grow more quickly. A third area of research involves the development of food products with an improved taste. Products being investigated include sweet corn and peas that stay sweet longer and naturally decaffeinated coffee. Research into fiber crops is being conducted as well. Scientists are trying to produce naturally colored and fade-resistant cotton. Naturally blue cotton that resists fading would create a revolution in the denim industry.

Summary

The rapidly expanding field of plant biotechnology is the focus of a great deal of interest. Biofuels and biopolymers are two examples of the emerging applications of plant biotechnology. Scientists are examining many plants, animals, and microorganisms as a part of their attempts to enhance the traits of crop plants and increase their usefulness.

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Lesson 5: The Impact of Plant Biotechnology

Many biotechnology products recently released on the commercial agricultural market have been genetically modified plants. *Bt* corn, *Bt* cotton, Roundup Ready™ soybeans, and modified canola are a few examples. The effect of these products and of plant biotechnology is only beginning to be realized. This lesson will explore the impact that plant biotechnology is having on the creation of jobs, economics, human health, and society.

Career Opportunities in Plant Biotechnology

Plant biotechnology offers many of the same career opportunities as those listed in Unit 5 for animal biotechnology. General career areas in biotechnology are research and development, quality control, clinical research, manufacturing and production, regulatory affairs, information systems, marketing and sales, and administration. However, some career positions are unique to the field of plant biotechnology. Plant scientists, greenhouse managers, and tissue culture technicians are a few examples. A bachelor of science degree in agronomy or biochemistry provides a good starting point for a career in plant biotechnology.

Economic Factors Affecting Producers

The true test of any technology is its feasibility and profitability. Developments in plant biotechnology must be financially beneficial for producers if the products are to succeed. Whether it is insect-resistant corn or herbicide-tolerant soybeans, the plant crop developed must be able to increase producer profits. The producer needs to be confident that the price charged for these genetically modified seeds will be recovered, along with a greater profit than that obtained with traditional plant crops.

Modified crops present both benefits and drawbacks for producers. Most of the genetically altered crops currently in use have been modified to resist insects, disease, or herbicides. These crops lower input costs by reducing the amount of chemicals needed to grow them. However, the seed for genetically modified crops is generally higher in price. In addition, several modified crops that producers are beginning to use yield a seed with a modified composition that must be kept separate from unmodified crops when they are harvested, transported, stored, and processed to avoid using them for the wrong application. Crops with a modified composition also must be sold to specific processors. The advantage to these specialized crops is that they are often sold at a premium price, which should offset the costs of handling them and the limited market. These additional risks to the producer require that the modified crop have a higher profit potential. If the profit potential is great enough, producers will readily accept these new crops.

Consumer Health and Safety Concerns

The public has shown much more confidence in the safety of plant biotechnology than in animal biotechnology. Consumers have some health concerns about genetically modified foods. Questions about the healthiness of modified foods arise with foods in which the composition of the edible portion of the plant has changed. The FDA has helped to ease some of these concerns by requiring that foods in which the composition of the food has changed be labeled. Some consumers also worry that a plant altered for an industrial purpose will wind up in the food supply; for example, corn genetically modified for ethanol production could be mixed with other corn for corn meal without anyone knowing. Such safety and health concerns held by consumers must be responsibly addressed if plant biotechnology products are to succeed.

The Global Social Impacts of Plant Biotechnology

Both positive and negative global impacts are associated with the introduction of plant biotechnologies. Some transgenic plants can greatly benefit developing countries, such as the sweet potato resistant to the feathery mottle virus (FMV) developed with support from Monsanto and the U.S. Agency for International Development

(USAID). This FMV-resistant sweet potato could nearly double the potato harvest in Africa, where sweet potatoes are grown as a staple crop, and farmers could provide more food for their families. The development of environmentally tolerant plants could help reduce the risk of famine caused by drought or flooding in some countries. Even more exciting is the possibility of developing edible plant vaccines, which would allow millions of poor people to receive vaccines.

However, the development of some genetically modified crops can destroy the profitability of agricultural cash crops that may be vital to a country's economy. For example, the new genetically modified canola seed has the same fatty acid content as many of the tropical oils, such as palm and coconut oils. Producers in the United States and Canada could raise a crop of canola cheaper than these oils could be imported. Many countries have depended on tropical oils for most of their national income, and their economies could be severely hurt if the modified canola is grown. Also, if potatoes, tobacco, and other plants are genetically modified to produce a high amount of sugar in their leaves, the price for sugar from sugar cane will drop, causing problems for growers worldwide. These changes have both an economic and social impact on the countries affected. Will the net effect of plant biotechnology be positive for most countries? Only time will reveal the answer to this question.

Summary

The field of plant biotechnology is growing quickly. Many different types of career opportunities exist in this field. As more genetically modified plants are developed and offered to producers, the ability of producers to measure the economic risks and profit potential of these crops will be vitally important. Consumer concerns about the safety and healthiness of foods made from modified plants must be addressed. The global social impact of each modified plant must also be examined to help minimize negative results.

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