TESTING FOR GENETIC MANIPULATION IN PLANTS (MOLECULAR METHODS OF PLANT ANALYSIS) pdf

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Testing for Genetic Manipulation in Plants (Molecular Methods of Plant Analysis) Softcover reprint of hardcover 1st ed. Edition by John Flex Jackson (Editor), Hans F. Linskens (Editor).

History of genetic engineering Many different discoveries and advancements lead to the development of genetic engineering. Human-directed genetic manipulation began with the domestication of plants and animals through artificial selection in about 12, BC. Hybridization was one way rapid changes in an organisms makeup could be introduced. Hybridization most likely first occurred when humans first grew similar, yet slightly different plants in close proximity. Frederick Sanger developed a method for sequencing DNA in , greatly increasing the genetic information available to researchers. After discovering the existence and properties of DNA , tools had to be developed that allowed it to be manipulated. Plasmids , discovered in , [6] became important tools for transferring information between cells and replicating DNA sequences. Polymerase chain reaction PCR , developed by Kary Mullis in , allowed small sections of DNA to be amplified replicated and aided identification and isolation of genetic material. Artificial competence was induced in Escherichia coli in by treating them with calcium chloride solution CaCl2. In the early s it was found that this bacteria inserted its DNA into plants using a Ti plasmid. This is driven by the goal for the resultant organism. In some cases only one or two genes are affected. For more complex objectives entire biosynthetic pathways involving multiple genes may be involved. Once found genes and other genetic information from a wide range of organisms can be inserted into bacteria for storage and modification, creating genetically modified bacteria in the process. Once a gene is isolated it can be stored inside the bacteria providing an unlimited supply for research. A simple screen involves randomly mutating DNA with chemicals or radiation and then selecting those that display the desired trait. For organisms where mutation is not practical, scientist instead look for individuals among the population who present the characteristic through naturally-occurring mutations. Processes that look at a phenotype and then try and identify the gene responsible are called forward genetics. The gene then needs to be mapped by comparing the inheritance of the phenotype with known genetic markers. Genes that are close together are likely to be inherited together. This approach involves targeting a specific gene with a mutation and then observing what phenotype develops. Conditional mutations are useful for identifying genes that are normally lethal if non-functional. Due to these insecticidal properties the bacteria was used as a biological insecticide , developed commercially in The cry proteins were discovered to provide the insecticidal activity in and by the s scientists had successfully cloned the gene that codes for this protein and expressed it in plants. Traditionally DNA was isolated from the cells of organisms. Once isolated, additional genetic elements are added to the gene to allow it to be expressed in the host organism and to aid selection. Extraction from cells[edit] Main article: The methods used vary depending on the type of cell. Once open, the DNA must be separated from the other cellular components. A ruptured cell contains proteins and other cell debris. This aqueous phase can be removed and further purified if necessary by repeating the phenol-chloroform steps. The nucleic acids can then be precipitated from the aqueous solution using ethanol or isopropanol. Any RNA can be removed by adding a ribonuclease that will degrade it. Many companies now sell kits that simplify the process. If the sequence is not known then a common method is to break the DNA up with a random digestion method. This is usually accomplished using restriction enzymes enzymes that cut DNA. A partial restriction digest cuts only some of the restriction sites, resulting in overlapping DNA fragment segments. The DNA fragments are put into individual plasmid vectors and grown inside bacteria. Once in the bacteria the plasmid is copied as the bacteria divides. To determine if a useful gene is present on a particular fragment the DNA library is screened for the desired phenotype. If the phenotype is detected then it is possible that the bacteria contains the target gene. If the gene does not have a detectable phenotype or a DNA library does not contain the correct gene, other methods must be used to isolate it. If the position of the gene can be determined using molecular markers then chromosome walking is one way to isolate the correct

DNA fragment. If the gene expresses close homology to a known gene in another species, then it could be isolated by searching for genes in the library that closely match the known gene. Gel electrophoresis then sorts the fragments according to length. A marker with fragments of known lengths can be laid alongside the DNA to estimate the size of each band. The DNA band at the correct size should contain the gene, where it can be excised from the gel. Its effectiveness drops with larger genes and it has the potential to introduce errors into the sequence. It is possible to artificially synthesise genes. The gene can be modified at this stage for better expression or effectiveness. As well as the gene to be inserted most constructs contain a promoter and terminator region as well as a selectable marker gene. The promoter region initiates transcription of the gene and can be used to control the location and level of gene expression, while the terminator region ends transcription. A selectable marker, which in most cases confers antibiotic resistance to the organism it is expressed in, is used to determine which cells are transformed with the new gene. The constructs are made using recombinant DNA techniques, such as restriction digests , ligations and molecular cloning. Gene delivery Once the gene is constructed it must be stably integrated into the target organisms genome or exist as extrachromosomal DNA. There are a number of techniques available for inserting the gene into the host genome and they vary depending on the type of organism targeted. If the transgene is incorporated into somatic cells, the transgene can not be inherited. Transformation genetics Bacterial transformation involves moving a gene from one bacteria to another. It is integrated into the recipients plasmid. Transformation is the direct alteration of a cells genetic components by passing the genetic material through the cell membrane. Typically the cells are incubated in a solution containing divalent cations often calcium chloride under cold conditions, before being exposed to a heat pulse heat shock. Calcium chloride partially disrupts the cell membrane, which allows the recombinant DNA to enter the host cell. It is suggested that exposing the cells to divalent cations in cold condition may change or weaken the cell surface structure, making it more permeable to DNA. The heat-pulse is thought to create a thermal imbalance across the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall. Electroporation is another method of promoting competence. A gene gun uses biolistics to insert DNA into plant tissue A. The bacteria will attach to many of the plant cells exposed by the cuts. The plasmid T-DNA is integrated semi-randomly into the genome of the host cell. The only essential parts of the T-DNA are its two small 25 base pair border repeats, at least one of which is needed for plant transformation. An alternative method is agroinfiltration. This method can be used on plants that are not susceptible to Agrobacterium infection and also allows transformation of plant plastids. Plants cells can also be transformed using electroporation, which uses an electric shock to make the cell membrane permeable to plasmid DNA. Due to the damage caused to the cells and DNA the transformation efficiency of biolistics and electroporation is lower than agrobacterial transformation. Transfection Transformation has a different meaning in relation to animals, indicating progression to a cancerous state, so the process used to insert foreign DNA into animal cells is usually called transfection. Often these cells are stem cells that are used for gene therapy. Chemical based methods uses natural or synthetic compounds to form particles that facilitate the transfer of genes into cells. The solution, along with the DNA, is encaspulated by the cells and a small amount of DNA can be integrated into the genome. Other techniques include using electroporation and biolistics. When the pronuclei from the sperm head and egg are visible through the protoplasm the genetic material is injected into one of them. The oocyte is then implanted in the oviduct of a pseudopregnant animal. The gene is transfected into embryonic stem cells and then they are inserted into mouse blastocysts that are then implanted into foster mothers. The resulting offspring are chimeric , and further mating can produce mice fully transgenic with the gene of interest. Transduction genetics Transduction is the process by which foreign DNA is introduced into a cell by a virus or viral vector. The sequences that allow the virus to insert the genes into the host organism must be left intact. Popular virus vectors are developed from retroviruses or adenoviruses. Other viruses used as vectors include, lentiviruses , pox viruses and herpes viruses. The type of virus used will depend on the cells targeted and whether the DNA is to be altered permanently or temporarily. Regeneration[edit] As often only a single cell is transformed with

genetic material, the organism must be regenerated from that single cell. In plants this is accomplished through the use of tissue c ulture. If successful, the technique produces an adult plant that contains the transgene in every cell. All offspring from the first generation are heterozygous for the inserted gene and must be inbred to produce a homozygous specimen. Selectable markers are used to easily differentiate transformed from untransformed cells. Cells that have been successfully transformed with the DNA contain the marker gene, while those not transformed will not. By growing the cells in the presence of an antibiotic or chemical that selects or marks the cells expressing that gene, it is possible to separate modified from unmodified cells. Another screening method involves a DNA probe that sticks only to the inserted gene. These markers are usually present in the transgenic organism, although a number of strategies have been developed that can remove the selectable marker from the mature transgenic plant. Once confirmed methods that look for and measure the gene products RNA and protein are also used to assess gene expression, transcription, RNA processing patterns and expression and localization of protein product s. Gene targeting and Genome editing Traditional methods of genetic engineering generally inserts the new genetic material randomly within the host genome. This can impair or alter other genes within the organism.

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2: Biogenetic Services, Inc. - Plant, Animal and Human DNA Testing

Testing for Genetic Manipulation in Plants. Molecular Methods of Plant Analysis, Volume 22 Testing for Genetic Manipulation in Plants. Molecular Methods of Plant Analysis, Volume 22 Spangenberg, German This second multi-authored volume in the renamed series Modern Methods of Plant Analysis deals with methods of molecular biology applied to the analysis of genetically.

Marker-Assisted Selection Marker-assisted selection involves establishing a link between inheriting a desirable trait, such as milk yield, and segregating specific genetic markers that are coupled to that trait. Marker-assisted selection is important in animal breeding and selection strategies for studying complex traits governed by many genes Georges, The use of this method is expected to increase exponentially as genome-sequencing projects identify greater numbers of useful, segregated markers for economically important traits. Initially animals will be screened for genes that control simple traits that may be undesirable, such as horns in cattle or metabolic stress syndrome in pigs. In time, easily identifiable markers that accompany multiple genes controlling more complex traits, such as meat tenderness and taste, growth, offspring size, and disease resistance, will become available to improve animal health and production traits Dekkers and Hospital, Two notable examples can be found in sheep. One is the Booroola gene in which a single-nucleotide base change is responsible for the callipyge muscle hypertrophy phenotypeâ€”the only known example of polar over-dominance in a mammal Freking et al. Another is introgression of the Booroola gene into the Awassi and the Assaf dairy breeds Gootwine, Sequencing genomes of animals that are important to agriculture will identify genes that influence reproductive efficiency. For example, a growth-hormonereceptor variant on bovine chromosome 20 affects the yield and composition of milk, and is expected to increase milk production by kg per lactation and decrease milk fat from 4. Nontransgenic Methods of Animal Manipulation Biotechnology can be used to modify endocrine function of domestic animals and affect reproduction, lactation, and growth. For example, in pigs and rats Draghia-Akli et al. This biotechnology has the potential, by using specific hormones and growth factors during critical developmental periods, to enhance uterine capacity and to increase milk production. Page 35 Share Cite Suggested Citation: Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects. The National Academies Press. Wine, bread, and cheese are common examples of ancient foods, still popular today, that depend on microbial ingredients and activities. Endogenous populations of microbes, particularly bacteria and yeasts, are genetically varied enough to provide sufficiently different traits to allow the development of useful microbial strains through simple selection or induced mutation. Microorganisms play significant roles in food production. They serve primary and secondary roles in food fermentation and in food spoilage, and they can produce enzymes or other metabolites used in food production and processing. Fermentations can be initiated and conducted completely by the bacterial populations that are endogenous to the raw materials being fermented. However, it is more reliable in terms of uniformity and predictability to intentionally introduce starter cultures to initiate the fermentation and, in some instances, to perform the complete fermentation process. Most fermented products now are prepared this way in industrialized countries. The types of microorganisms that carry out food fermentations range from bacteria to molds and yeasts, but by far the most widely used organisms are lactic acid bacteria LAB and yeasts Sacchromyces cerevisiae. Traditional genetic modification methods that have been employedâ€”particularly for microbial starter culturesâ€”include selection, mutagenesis, conjugation, and protoplast fusion, the last of which is analogous to somatic hybridization in plant systems. Before molecular genetics was developed and applied to LAB, the most widely used genetic modification method was chemical- or ultra-violet-induced mutagenesis, followed by an enrichment or selection process for mutants with superior characteristics. A second traditional approach, conjugation, relies on natural methods of genetic exchange whereby DNA is transferred from one strain to another. A less common, but still useful, method has been to use protoplast fusion to facilitate recombination

between two strains with superior but unique characteristics, producing a strain that possesses the desired characteristics of both parents. Protoplast fusion was classically used as a mapping method in bacteria and only recently has been used successfully to produce strains of LAB with desired characteristics Patnaik et al. It has, however, been successfully used for some time to generate yeast strains that produce a greater number of biochemical substrates for use in the fermentation process Pina et al. Given the number and combinations of desirable traits in starter culture organisms, producers have remained interested in developing improved starter cul- Page 36 Share Cite Suggested Citation: The traditional approach has been to identify endogenous strains with desirable traits by conducting many small-scale fermentations. This type of trial-and-error approach is far from practical because, while productive, low throughput is a limiting factor in the success rate. The second approach is to produce the desired traits in the laboratory using molecular genetic and genetic engineering techniques. With the burgeoning field of genomics and the public availability of hundreds of fully sequenced bacterial genomes, this approach has become highly attractive and efficient and is favored by industry. Its primary advantage is the precision with which starter culture strains can be engineered. The most common method used to introduce recombinant DNA into microorganisms is transformation, whereby DNA of interest is introduced directly into recipient cells by making them permeable using chemical agents, enzymes, or electroporation. The first method developed for LAB was plasmid protoplast fusion, in which recipient cells are stripped of walls and subsequently fused with polyethylene glycol, trapping the newly introduced DNA between the cells Kondo and McKay, Electroporation was developed for LAB during the late s and employs electrical currents to create pores in the cell envelope, allowing DNA from other sources to enter Luchansky et al. This method is probably the most widely used for research due to its simplicity. However, it lacks efficiency in many different species. Unlike transformation, transduction can be fraught with problems that cause deletions within the plasmid known as transductional shortening that are typically of undefined length. Microbial transformation is usually simpler and more efficient than transformation in higher organisms, and has been in use longer for the development of commercial strains. Academic research also has been able to scrutinize the molecular genetic effects of transformation in microbes to a much greater extent than it has in higher organisms. Principles gleaned from studies of microbes have proven instrumental in understanding analogous events in the molecular genetics of higher organisms. Transduction of a plasmid carrying the cohesive end region from Lactococcus lactis bacteriophage LC3. Appl Environ Microbiol Cold Spring Harbor Laboratory. Accessed November 4, Page 37 Share Cite Suggested Citation:

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3: Genetic engineering techniques - Wikipedia

Testing for Genetic Manipulation in Plants, Vol. in: Molecular Methods of Plant Analysis. P. Karlovsky; Article first published online: 6 FEB

The next chapter Chapter 3 presents a detailed analysis of the likelihood for these methods to result in unintentional compositional changes. These changes, along with natural evolutionary changes, have resulted in common food species that are now genetically different from their ancestors. Advantageous outcomes of these genetic modifications include increased food production, reliability, and yields; enhanced taste and nutritional value; and decreased losses due to various biotic and abiotic stresses, such as fungal and bacterial pathogens. These objectives continue to motivate modern breeders and food scientists, who have designed newer genetic modification methods for identifying, selecting, and analyzing individual organisms that possess genetically enhanced features. For plant species, it can take up to 12 years to develop, evaluate, and release a new variety of crop in accordance with international requirements, which specify that any new variety must meet at least three criteria: While advances in modification methods hold the potential for reducing the time it takes to bring new foods to the marketplace, an important benefit of a long evaluation period is that it provides opportunities for greater assurance that deleterious features will be identified and potentially harmful new varieties can be eliminated before commercial release. As discussed more fully in Chapter 5 , it is both prudent and preferable to identify potentially hazardous products before they are made commercially available, and with few exceptions standard plant breeding practices have been very successful in doing so. The others are eaten or discarded. The seeds from the superior plants are sown to produce a new generation of plants, all or most of which will carry and express the desired traits. Over a period of several years, these plants or their seeds are saved and replanted, which increases the population of superior plants and shifts the genetic population so that it is dominated by the superior genotype. This very old method of breeding has been enhanced with modern technology. An example of modern methods of simple selection is marker-assisted selection, which uses molecular analysis to detect plants likely to express desired features, such as disease resistance to one or more specific pathogens in a population. Often traits considered beneficial to breeders are detrimental to the plant from the standpoint of environmental fitness. For example, the reduction of unpalatable chemicals in a plant makes it more appealing to human consumers but may also attract more feeding by insects and other pests, making it less likely to survive in an unmanaged environment. As a result, cultivated crop varieties rarely establish populations in the wild when they escape from the farm. Crossing Crossing occurs when a plant breeder takes pollen from one plant and brushes it onto the pistil of a sexually compatible plant, producing a hybrid that carries genes from both parents. When the hybrid progeny reaches flowering maturity, it also may be used as a parent. Plant breeders usually want to combine the useful features of two plants. For example, they might add a disease-resistance gene from one plant to another that is high-yielding but disease-susceptible, while leaving behind any undesirable genetic traits of the disease-resistant plant, such as poor fertility and seed yield, susceptibility to insects or other diseases, or the production of antinutritional metabolites. Because of the random nature of recombining genes and traits in crossed plants, breeders usually have to make hundreds or thousands of hybrid progeny to create and identify those few that possess useful features with a minimum of undesirable features. For example, the majority of progeny may show the desired disease resistance, but unwanted genetic features of the disease-resistant parent may also be present in some. Interspecies Crossing Interspecies crossing can take place through various means. Closely related species, such as cultivated oat Avena sativa and its weedy relative wild oat Avena fatua , may cross-pollinate for exchange of genetic information, although this is not generally the case. Genes from one species also can naturally integrate into the genomes of more distant relatives under certain conditions. Some food plants can carry genes that originate in different species, transferred both by nature and by human intervention. For example, common wheat varieties carry genes from rye. A common potato, Solanum

tuberosum, can cross with relatives of other species, such as S. Chromosome engineering is the term given to nonrecombinant deoxyribonucleic acid rDNA cytogenetic manipulations, in which portions of chromosomes from near or distant species are recombined through a natural process called chromosomal translocation. Sears , pioneered the human exploitation of this process, which proved valuable for transferring traits that were otherwise unattainable, such as pest or disease resistance, into crop species. However, because transferring large segments of chromosomes also transferred a number of neutral or detrimental genes, the utility of this technique was limited. Recent refinements allow plant breeders to restrict the transferred genetic material, focusing more on the gene of interest Lukaszewski, As a result, chromosome engineering is becoming more competitive with rDNA technology in its ability to transfer relatively small pieces of DNA. Several crop species, such as corn, soybean, rice, barley, and potato, have been improved using chromosome engineering Gupta and Tsuchiya, Embryo Rescue Sometimes human technical intervention is required to complete an interspecies gene transfer. Some plants will cross-pollinate and the resulting fertilized hybrid embryo develops but is unable to mature and sprout. Modern plant breeders work around this problem by pollinating naturally and then removing the plant embryo before it stops growing, placing it in a tissue-culture environment where it can complete its development. Such embryo rescue is not considered genetic engineering, and it is not commonly used to derive new varieties directly, but it is used instead as an intermediary step in transferring genes from distant, sexually incompatible relatives through intermediate, partially compatible relatives of both the donor and recipient species. Somatic Hybridization Recent advances in tissue-culture technologies have provided new opportunities for recombining genes from different plant sources. In somatic hybridization, a process also known as cell fusion, cells growing in a culture medium are stripped of their protective walls, usually using pectinase, cellulase, and hemicellulase enzymes. These stripped cells, called protoplasts, are pooled from different sources and, through the use of varied techniques such as electrical shock, are fused with one another. When two protoplasts fuse, the resulting somatic hybrid contains the genetic material from both plant sources. This method overcomes physical barriers to pollen-based hybridization, but not basic chromosomal incompatibilities. If the somatic hybrid is compatible and healthy, it may grow a new cell wall, begin mitotic divisions, and ultimately grow into a hybrid plant that carries genetic features of both parents. While protoplast fusions are easily accomplished, as almost all plants and animals have cells suitable for this process, relatively few are capable of regenerating a whole organism, and fewer still are capable of sexual reproduction. This non-genetic engineering technique is not common in plant breeding as the resulting range of successful, fertile hybrids has not extended much beyond what is possible using other conventional technologies. Somaclonal Variation Somaclonal variation is the name given to spontaneous mutations that occur when plant cells are grown in vitro. For many years plants regenerated from tis-sue culture sometimes had novel features. It was not until the s that two Australian scientists thought this phenomenon might provide a new source of genetic variability, and that some of the variant plants might carry attributes of value to plant breeders Larkin and Scowcroft, Through the s plant breeders around the world grew plants in vitro and scored regenerants for potentially valuable variants in a range of different crops. New varieties of several crops, such as flax, were developed and commercially released Rowland et al. Molecular analyses of these new varieties were not required by regulators at that time, nor were they conducted by developers to ascertain the nature of the underlying genetic changes driving the variant features. Somaclonal variation is still used by some breeders, particularly in developing countries, but this non-genetic engineering technique has largely been supplanted by more predictable genetic engineering technologies. Induced Chemical and X-ray Mutagenesis Mutation breeding involves exposing plants or seeds to mutagenic agents e. The breeder can adjust the dose of the mutagen so that it is enough to result in some mutations, but not enough to be lethal. Typically a large number of plants or seeds are mutagenized, grown to reproductive maturity, and progeny are derived. The progeny are assessed for phenotypic expression of potentially valuable new traits. As with somaclonal variation, the vast majority of mutations resulting from this technique are deleterious, and only chance determines if any genetic changes useful to humans will appear. Other than through varying the dosage, there

is no means to control the effects of the mutagen or to target particular genes or traits. The mutagenic effects appear to be random throughout the genome and, even if a useful mutation occurs in a particular plant, deleterious mutations also will likely occur. Once a useful mutation is identified, breeders work to reduce the deleterious mutations or other undesirable features of the mutated plant. Nevertheless, crops derived from mutation breeding still are likely to carry DNA alterations beyond the specific mutation that provided the superior trait. Induced-mutation crops in most countries including the United States are not regulated for food or environmental safety, and breeders generally do not conduct molecular genetic analyses on such crops to characterize the mutations or determine their extent. Consequently, it is almost certain that mutations other than those resulting in identified useful traits also occur and may not be obvious, remaining uncharacterized with unknown effects. In the United States, crop varieties ranging from wheat to grapefruit have been mutated since the technique was first used in the s. There are no records of the molecular characterizations of these mutant crops and, in most cases, no records to retrace their subsequent use. Cell Selection Several commercial crop varieties have been developed using cell selection, including varieties of soybeans Sebastian and Chaleff, , canola Swanson et al. The cells are then excised and grown in culture. Initially the population is genetically homogeneous, but changes can occur spontaneously as in somaclonal variation or be induced using mutagenic agents. Cells with a desired phenotypic variation may be selected and regenerated into a whole plant. For example, adding a suitable amount of the appropriate herbicide to the culture medium may identify cells expressing a novel variant phenotype of herbicide resistance. In theory, all of the normal, susceptible cells will succumb to the herbicide, but a newly resistant cell will survive and perhaps even continue to grow. An herbicide-resistant cell and its derived progeny cell line thus can be selected and regenerated into a whole plant, which is then tested to ensure that the phenotypic trait is stable and results from a heritable genetic alteration. In practice, many factors influence the success of the selection procedure, and the desired trait must have a biochemical basis that lends itself to selection in vitro and at a cellular level. Breeders cannot select for increased yield in cell cultures because the cellular mechanism for this trait is not known. The advantage of cell selection over conventional breeding is the ability to inexpensively screen large numbers of cells in a petri dish in a short time instead of breeding a similar number of plants in an expensive, large field trial conducted over an entire growing season. Like somaclonal variation, cell selection has largely been superceded by recombinant technologies because of their greater precision, higher rates of success, and fewer undocumented mutations. Genetic Engineering As noted in Chapter 1 , this report defines genetic engineering specifically as one type of genetic modification that involves an intended targeted change in a plant or animal gene sequence to effect a specific result through the use of rDNA technology. A variety of genetic engineering techniques are described in the following text. Microbial Vectors Agrobacterium tumefaciens is a naturally occurring soil microbe best known for causing crown gall disease on susceptible plant species. It is an unusual pathogen because when it infects a host, it transfers a portion of its own DNA into the plant cell. The transferred DNA is stably integrated into the plant DNA, and the plant then reads and expresses the transferred genes as if they were its own. The transferred genes direct the production of several substances that mediate the development of a crown gall. Among these substances is one or more unusual nonprotein amino acids, called opines. Opines are translocated throughout the plant, so food developed from crown gall-infected plants will carry these opines. In the early s strains of Agrobacterium were developed that lacked the disease-causing genes but maintained the ability to attach to susceptible plant cells and transfer DNA. By substituting the DNA of interest for the crown gall disease-causing DNA, scientists derived new strains of Agrobacterium that deliver and stably integrate specific new genetic material into the cells of target plant species. If the transformed cell then is regenerated into a whole fertile plant, all cells in the progeny also carry and may express the inserted genes. Agrobacterium is a naturally occurring genetic engineering agent and is responsible for the majority of GE plants in commercial production. This is a crude but effective physical method of DNA delivery, especially in species such as corn, rice, and other cereal grains, which Agrobacterium does not naturally transform. Many GE plants in commercial production were initially transformed using microprojectile

delivery. Electroporation In electroporation, plant protoplasts take up macromolecules from their surrounding fluid, facilitated by an electrical impulse. Cells growing in a culture medium are stripped of their protective walls, resulting in protoplasts. Supplying known DNA to the protoplast culture medium and then applying the electrical pulse temporarily destabilizes the cell membrane, allowing the DNA to enter the cell. Transformed cells can then regenerate their cell walls and grow to whole, fertile transgenic plants. Electroporation is limited by the poor efficiency of most plant species to regenerate from protoplasts. Microinjection DNA can be injected directly into anchored cells. Some proportion of these cells will survive and integrate the injected DNA. However, the process is labor intensive and inefficient compared with other methods. Barbara McClintock first described such transposable elements in corn plants during the s Cold Spring Harbor Laboratory,

4: MOLECULAR-GENETIC ANALYSIS OF PLANT CYTOCHROME PDEPENDENT MONOOXYGENASES

Molecular Methods of Plant Analysis Concept of the Series The powerful recombinant DNA technology and related developments have had an enormous impact on molecular biology. Any treatment of plant analyÂ- sis must make use of these new methods. Developments have been so fast and the methods so powerful that the editors of Modern Methods of Plant AnalyÂ- sis have now decided to rename the series Molecular Methods of Plant AnalyÂ- sis.

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