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|  |  | **PLANT GENETIC ENGINEERING (TRANSFORMATION)** |  |  |
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|  | *Plant genetic engineering* refers to the transfer of foreign DNA which codes for specific genetic information, from a donor species into a recipient plant species by means of a bacterial plasmid, virus, or other vector. The procedure is also referred to as *transformation*. For the plant breeder, plant genetic engineering has the potential for transferring a desirable foreign gene from a wide range of sources, including non-plant genetic material, into an economic crop species without sexual hybridization. In many respects, plant genetic engineering (transformation) is comparable to the backcross method of breeding in which desirable genes are transferred to a recipient genotype by a succession of crosses. The molecular biologist inserts a segment of DNA that codes for a desirable trait into the plant genotype where it replicates and is expressed in the new plant genotype. The difference is that the plant breeder can employ the backcross only among species that are cross-fertile, whereas the molecular biologist is not limited to obtaining the DNA from a donor plant species that is cross-fertile with the recipient plant species. |  |
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| Somatic cell hybridization. (A) Plants cells from parent species. (B) Plant protoplasts from parent species after cell walls have been removed. (C) Suspension culture of protoplasts from parent species.(D) Hybrid protoplasts following fusion. (E) Hybrid plantlet regenerated from fused protoplasts. Fusion of protoplasts and regeneration of plants would permit hybrids to be obtained between unrelated species that do not produce seeds following cross-pollination. |

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|  | The crop species that nave been *genetically transformed* with foreign DNA includes corn, alfalfa, orchardgrass, potato (Fig. 8.11), cauliflower, soybean (Fig. 8.12), lettuce, sunflower, tall fescue, carrot, canola, white clover, cotton (Fig. 8.13), tomato, and others; the list continues to grow. Currently, few genetically transformed cultivars have been released. In part, this is due to the rigorous testing procedures mandated before organisms, genetically altered through molecular techniques, can be released. |  |
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| Fig. 8.11.Potato plant on right was genetically transformed with a gene fromBacillus thuringiensis var. tenebrionis which gives resistance to the Colorado potato beetle. The potato on the left does not have the gene for resistance to the Colorado potato beetle and was severly damaged by the feeding larvae. |

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|  | ***Genetic Transformation*** |  |
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|  | A procedure for transformation through recombinant DNA techniques developed with prokaryotic cells in microorganisms was described by S.N. Cohen in 1975. In the bacterial |  |
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| Fig. 8.12.Transgenic soybean on the left was not damaged by the herbicide glyphosate. Nontransgenic soybean on the right does not contain the gene for resistance to glyphosate and was destroyed after application of the herbicide |
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| Fig. 8.13.Transgenic cotton on the right with the Bt gene from Bacillus thuringiensis var. kurstaki which gives resistance to Lepidopteran insects. Boll on left is nontransgenic and is susceptible to feeding by Lepidopteran insects. |

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|  | model, plasmids of the bacterial species *Escherichia coli* are used as vectors. Plasmids are circular, extrachromosomal DNA molecules that replicate independently of the bacterial chromosome. The plasmid molecules are isolated from the bacterial cells and digested with an enzyme, *restriction endonuclease*, that cleaves the molecule at a specific site. A small segment of foreign DNA carrying the desired genetic information is inserted between the broken ends of the plasmid molecule, and another enzyme is used to reform the circle. The vector is then reintroduced into *an E. coli* cell. In addition to the foreign DNA segment, the plasmid vector carries a replicator gene so that it will be reproduced in the *E. coli* cell, and a marker gene so that an *E. coli* cell containing a plasmid vector may be identified and isolated. |  |
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|  | The first genetically transformed plants were developed in the early 1980s. One of the first genes transferred to plants was a gene from bacteria called *neo* that codes for antibiotic resistance. When the antibiotic resistant gene *neo* was introduced into plant cells, the genetically transformed cells were easily identified because they grew in the presence of the antibiotic, kanamycin or G-418. This transfer was mediated with the bacterial pathogen *Agrobacterium tumefaciens* which is able to transfer a piece of its DNA (T-DNA) into the DNA of the plant resulting in a new, genetically transformed plant cell. *Agrobacterium rhizogenes* is another bacteria used in transformation but its use is not as frequent as *Agrobacterium tumefaciens*. |  |
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|  | *Agrobacterium tumefaciens* is a pathogenic soil bacteria which causes tumors, called crown galls, in dicotyledonous plants. *Agrobacterium tumefaciens* infects plants by transferring T- |  |
|  | DNA of the Ti-plasmid into plant cells and the T-DNA becomes incorporated into the plant's DNA, hence causing the crown gall disease. The galls or tumors are developed because the T-DNA from the bacteria has genes which regulate the biosynthesis of the plant hormones indoleacetic acid (IAA) and cytokinin. After plants become infected with *A. tumefaciens*, abnormal levels of IAA and cytokinin cause anomalous growth and tumor formation. Mutants of *A. tumefaciens* have been developed in which the T-DNA does not produce IAA or cytokinin. Foreign genes are incorporated into these non-hormonal producing *A. tumefaciens* strains as part of the T-DNA. As a result, the modified *A. tumefaciens* strains serve as a vehicle to introduce the foreign genes into plants. This process now makes it possible to genetically engineer specific crops plants. Utilization of *A. tumefaciens* as a vector in genetic transformation has the limitation that most monocotyledonous species, which include the major cereals, are not easily infected by *A. tumefaciens*. |  |
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|  | Adaptation of the bacterial model for foreign gene transfer to crop plants requires these steps: |  |
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|  | * Introduction of the foreign gene into the T-DNA of the bacteria,
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|  | * Introduction of the bacteria containing the foreign gene into cells of host plants,
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|  | * Integration of the foreign gene into the genome of the host cell,
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|  | * Expression of the foreign gene in the regenerated crop plant, and
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|  | * Transmission of the foreign gene and its expression through normal sexual processes to plants in succeeding generations in seed reproduced species, or through normal asexual propagation in vegetatively reproduced species.
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|  | A simplified model for plant genetic engineering, using a bacterial plasmid as a vector, is illustrated in Fig. 8.14. The *foreign DNA strands* and the *DNA strands of the cloning vehicle*, or bacterial *vector*, are cleaved with a restriction enzyme. In this model, the double strands of DNA are broken, leaving complementary nucleotides. If a fragment of the foreign DNA becomes inserted into a break in the plasmid DNA, the ends of the circular plasmid strands are joined and the break is annealed with the enzyme *DNA ligase*. The formidable step is to obtain insertion of the foreign DNA segment into the plant genome where it will be replicated and expressed. Insertion of the DNA segment into a plant cell or protoplast where it will be replicated requires a suitable vector, but efficient vectors by which this may be routinely accomplished for many crop species have been difficult to identify. |  |
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|  | Because many crop plants cannot be genetically transformed by bacterial mediated procedures, other techniques that involve direct DNA uptake by cells or protoplasts have been developed. These techniques include incubation in polyethylene glycol, insertion of DNA using a particle gun, and electroporation (electric shock). Polyethylene glycol and electroporation are used to genetically transform protoplasts. Protoplasts are incubated with the DNA or genes of interest under controlled laboratory conditions. The polyethylene glycol, or an electrical shock, facilitates uptake of the foreign DNA and its incorporation into the protoplast DNA. Once this step is accomplished, it is necessary to regenerate the plant which is often complicated. If protoplasts can't be developed in a particular plant species, DNA coated onto tungsten or gold particles can be projected into target cells using a particle gun. Again, the plant needs to be regenerated, a process that is generally easier in dicots as compared to monocots. But in the later cases, using the particle gun, regeneration is from cells rather than protoplasts which is often easier. |  |
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|  | Routine utilization of plant genetic engineering techniques in plant breeding will require the availability of efficient transformation systems, and detailed information on the location, |  |
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| Fig. 8.14.Model for cleaving foreign DNA and a bacterial plasmid with restriction endonuclease. The foreign DNA is transferred to and inserted in a plant chromosome by means of a bacterial plasmid vector. |
|  | structure, and function of the foreign gene to be transformed. Most major plant-breeding problems involve quantitative characters controlled by polygenes and will not be solved by transfer of small, isolated DNA segments or single genes. Developing molecular biological techniques are being used to study quantitative traits at the DNA level. A major contribution from recombinant DNA research may be a better understanding of basic plant genetics and a more complete knowledge of the plant genome, from which plant breeding will benefit. |  |
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