

## Genetic engineering: An additional tool for plant improvement

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Advances in gene transfer technologies have enabled the production of both monocot and dicot transgenic plants. With the biolistic method, genes can be transferred in recalcitrant crop plants and forest trees, independent of their genotype. Inexpensive methods for both stable and transient gene transfers - ultrasonication, direct DNA insertion during imbibition using somatic embryos, and silicon carbide fibres - have been developed. The frequency of *Agrobacterium*-mediated transformation rates of cloned genes can be enhanced in plant cells. The analysis of molecular markers (RFLPs, RAPDs, DNA fingerprints) can accomplish the characterization, gene mapping and identification and certification and patent protection of cultivars. With PCR, selective amplification of a specific DNA segment from a small amount of an organism's total DNA can be used to identify transgenic cultivars. The expression of a target gene can be inhibited with antisense RNA. So far, a limited number of genes have been identified and cloned with genetic engineering. With specific gene transfers, many goals such as biological control of insect pests and fungi, male sterility, virus resistance, improving seed protein, and production of transgenic plants as "bioreactors" can be accomplished. T-DNA mutagenesis may lead to learning more about the genetic control of plant development and morphogenesis, and isolation of useful mutants. Before genetic engineering becomes a reliable tool of plant breeding, more attention is needed to explore: (a) new plant genetic resources in order to identify and clone new genes, (b) fate of selective and scorable marker genes, and (c) field evaluation of transgenes in transgenic plants.

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### Introduction

Crop plants have been improved with traditional plant breeding methods utilizing natural variation within different genetic resources. The output of world food production has increased considerably with improved agronomic practices. However, a constant pressure still remains on plant breeders to

increase crop production due to ever increasing growth of human population.

Rapid advances have taken place in plant tissue culture and genetic engineering. With tissue culture we can select and produce plants with desirable traits such as high yield and herbicide and environmental stress resistance, both within a short period of time (JAIN and PEHU 1992). Successful genetic

engineering of plants requires: (a) gene isolation and identification, (b) insertion of isolated genes with selective marker genes into a cloning vector, (c) transformation of recipient plant cells, (d) regeneration of transgenic plants, and (e) testing inheritance and expression of the gene in transgenic plants. Since gene transfer technologies and selective and scorable marker genes are available, genetic transformation of monocot and dicot plants can be readily accomplished. Currently, the greatest constraint of genetic engineering on plant improvement is the availability of only a limited number of cloned genes for use in the improvement of the economic biological importance of crop plants. Until now, only a few genes which are of use in crop improvement have been cloned, characterized and transferred in plant cells. Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) techniques have become widely used for gene mapping and identification of agronomically-important crops (ELDRIDGE et al. 1992, HUBBARD et al. 1992, TANKSLEY et al. 1989). Furthermore, more useful isolated genes and their stable expression in transgenic plants will be needed for continued crop improvement.

In Finland, most of the crop production problems are related to the environmental stress - viruses, insect pests, fungal diseases, cold, day length, rainfall and drought. The environmental pollution is causing greenhouse effect and may have an adverse impact on global agriculture, including Finland. These problems may be addressed with genetic engineering.

### Gene transfer technologies

Progress in gene transfer technologies has revolutionized the introduction of exogenous DNA into recalcitrant plants such as maize, cotton, rice, soybean and forest trees to produce transgenic plants. CHRISTOU et al. (1991) suggested certain criteria for the development of a practical gene transformation system for any crop: (1) a cultivar or genotype-independent transformation system, (2)

the recovery of large number of transgenic plants for the evaluation of gene expression, and (3) minimizing tissue culture manipulations in order to avoid somaclonal variations.

Most gene transfers have been accomplished with *Agrobacterium tumefaciens* and *A. rhizogenes* with a wide range of dicotyledonous plants (FRALEY et al. 1986). Until recently, the monocotyledonous and particularly graminaceous crop species have been recalcitrant to gene transfer with *A. tumefaciens*. The demonstration of *Agrobacterium* T-DNA (AT-DNA) integration into genomic DNA of *Asparagus officinalis* (BYTEBIER et al. 1987), *Dioscorea bulbifera* (SCHAFER et al. 1987), *Oryza sativa* (RAINERI et al. 1990), *Triticum aestivum* (MOONEY et al. 1991) and *Zea mays* (GOULD et al. 1991) showed that monocot species could be transformed by *Agrobacterium*. The agroinfection (*Agrobacterium*-mediated transfer [A-MT] of viral sequences to plant cells) method was used to incorporate the cauliflower mosaic virus into the AT-DNA and to be inoculated onto *Brassica rapa* (GRIMSLEY et al. 1986). The development of viral symptoms in inoculated plants implied the presence of AT-DNA in cell nuclei based on replication of the virus in the host nucleus. The competence of susceptibility of plant cells to agroinfection is dependent upon meristem tissue of the shoot apex (GRIMSLEY et al. 1988) and the early developmental stages of immature embryos (SCHLÄPPI and HOHN 1992). Recently, A-MT has been used to introduce foreign genes into higher plant chloroplasts by site-specific homologous recombinations (VENKATESWARLU and NAZAR 1991).

Alternative methods such as electroporation, biolistics, liposomes etc. for genetic transformation have been developed (JAIN et al. 1988, POTRYKUS 1991). Electroporation is used for optimizing gene transfer efficiency and transient gene expression in plant protoplasts (FROMM et al. 1985, 1986) and for the uptake of various dyes and exogenous DNA in germinating pollen grains (MISHRA et al. 1987, ABDUL-BAKI et al. 1990). Since pollen is the natural carrier of DNA to the ovary in flower fertilization, transgenic pollen grains may be used to fertilize

ovaries *in vitro* and transgenic seeds can be obtained.

The biolistic delivery system allows the direct delivery of DNA into intact cells and tissues. In this method, DNA coated microprojectiles are accelerated to velocities that permit their penetration of cell walls and membranes without causing lethal damage to the tissues (SANFORD 1990, KLEIN et al. 1992). This system has facilitated gene transfer into many plant tissues (KLEIN et al. 1988), organelles (BOYNTON et al. 1988, BLOWERS et al. 1989), cell suspensions of *Hordeum vulgare* (MENDEL et al. 1989), embryogenic cell suspensions of *Triticum aestivum* (VASIL et al. 1991) and *Picea abies* (NEWTON et al. 1992), anther cultures (CREISSEN et al. 1990), and immature zygotic embryos (KARTHA et al. 1989) of *H. vulgare*, and *Oryza sativa* (CHRISTOU et al. 1991), higher plant chloroplasts (DANIEL et al. 1990, 1991, YE et al. 1990, SVAB et al. 1990), stem sections of cranberry (SERRES et al. 1992) and intact germinating embryos (LONSDALE et al. 1990). In addition to *P. abies* (NEWTON et al. 1992) transient expression of the GUS ( $\beta$ -glucuronidase) gene with biolistic technique has also been demonstrated in tissues of forest trees such as *P. mariana* (DUCHENCE and CHAREST 1991), *Pinus taeda* (STOMP et al. 1991) and *Liriodendron tulipifera* (WILDE et al. 1992). SANFORD (1992) has suggested that the critical parameters for the minimum variation and maximum efficiency of biolistic transformation, which are: (a) microprojectile parameters particle size, coating procedure, dry-down in the absence of free water and with low humidity; (b) biolistics parameters- pressure (velocity), distance to target, and trauma control; and (c) biological parameters- choice of target tissue, amount of exposed cell surface area, cellular state (i.e. healthy and mitotically active), and osmotic conditioning (reducing cellular turgor). SAUTTER et al. (1991) constructed a novel microprojectile accelerating system for efficient gene transfer into cells *in situ* that avoids binding of DNA to microprojectiles and keeps the DNA in solution.

JAIN (1986) has suggested the use of an ultraviolet laser beam for gene transfer through the drill-

ing of a hole in plant cell wall and injecting isolated foreign DNA into the cell. The laser beam technique can also be used in chromosome surgery for partial genome transfer. WEBER et al. (1989) demonstrated the incorporation of DNA into isolated chloroplasts of *B. napus* with UV-laser microbeam.

Liposomes (artificial lipid vesicles) have been used to deliver biologically active molecules into both animal and plant cells (JAIN et al. 1988, GAD et al. 1990, CABOCHE 1990). GAD et al. (1990) suggested several advantages of using liposomes as vectors for gene delivery into plant cells: (1) enhanced delivery of encapsulated DNA by membrane fusion, (2) prevention of damage of nucleic acids from nuclease activity, (3) targeting to specific cells, (4) delivery of intact small organelles, and (5) delivery into a variety of cell types by entry through plasmodesmata. The positively charged liposomes have been shown to deliver DNA into germinating pea pollen grains (AHOKAS 1987). The negatively charged liposomes have been demonstrated to adhere strongly to watermelon pollen grains and tubes and to fuse with pollen tube membrane (GAD et al. 1988). WANG et al. (1986) showed that pH-sensitive liposomes dramatically release their contents into cytoplasm of plant protoplasts at a pH less than or equal to 6.0. Recently, ZHU et al. (1990) transformed tobacco protoplasts with pH-sensitive liposomes and regenerated transgenic plants. In spite of several advantages of liposome-mediated gene transfer, still the main problem remains its low frequency of transformation rate of plant cells.

Another method sonication, has resulted in the transient expression of the CAT gene in protoplasts of *Nicotiana tabacum* and *Beta vulgaris* (JOERSBO and BRUNSTEDT 1990) and in GUS expression in leaf segments and regeneration of transgenic plants of *N. tabacum* (ZHANG et al. 1991). Another simple strategy for gene transfer is the insertion of exogenous DNA into embryo plant cells during imbibition using somatic embryos of *Medicago sativa* (SENARATNA et al. 1991). This method has been used to produce both transient and stable trans-

formations in *M. sativa*. Exogenous DNA has also been delivered into *Z. mays* and *N. tabacum* suspension culture cells with silicon carbide fibers to introduce GUS gene (KAEPLER et al. 1990). These techniques are inexpensive and relatively simple to use.

### Selectable marker genes

The introduction of new genes into crop plants always requires the introduction of selection marker gene (s). These are needed for the testing of gene expression and testing for favourable growth of transformed cells. Chimeric genes consisting of plant promoter sequences and bacterial antibiotic-resistance gene have mostly been used in the selection of transformed plant cells. The most commonly used selectable marker has been the neomycin phosphotransferase type II (NPT II) enzyme, originated from transposon Tn5. This enzyme detoxifies aminoglycoside compounds such as kanamycin (Kan) and G418 (SANGWAN and SANGWAN-NORREEL 1990). Among the visually scorable markers commonly used are the bacterial GUS and firefly luciferase genes (JAIN et al. 1988, SANGWAN and SANGWAN-NORREEL 1990). Similarly, other selectable markers that have been used are: bleomycin resistance; chloramphenicol acetyltransferase (CAT) which confers resistance-chloramphenicol; dehydrofolate reductase (DHFR)-methotrexate resistance; streptomycin phosphotransferase-streptomycin resistance; phosphinothricin acetyltransferase (bar gene)- phosphinothricin resistance; EPSP synthase (5, enolpruvylshikimate-3-phosphate synthase) (aro A gene)- glyphosate resistance; and gentamycin-3-N-acetyltransferase-gentamycin resistance (SANGWAN and SARGWAN-NORREEL 1990, UCHIMIYA et al. 1989). WALTERS et al. (1992) has used the selectable marker hygromycin phosphotransferase (HPT) gene for recovering transformed *Zea mays*. Most of the selective and scorable marker genes incorporate in plant chromosomes and rarely in organelles such as chloroplasts or mitochondria. However, DE BLOCK et al. (1985) demonstrated the insertion of the CAT gene into

the chloroplast genome.

The selectable and scorable marker genes and their encoded proteins remain in the transgenic plants and their food products. In the future, they will become integral components of crops and our diets. The major concern of regulatory committees and the individual consumers worldwide is the food and environmental safety of these markers. FLAVELL et al. (1992) has concluded that the NPT II marker gene and its product pose no risk to the mankind or to the environment. However, a case-by-case examination of each marker gene will be necessary before their broad use in agriculture will be permitted.

### Enhancement of genetic transformation rates

The efficient transfer of cloned genes into plants is a prerequisite to the understanding of the regulation of gene expression. The mechanisms that govern uptake and integration of foreign DNA into plant cells are unknown. GIETL et al. (1987) suggested that single-stranded-DNA-binding proteins are involved in the production of T-DNA intermediates or that they have a carrier or protective function during T-DNA transfer with *Agrobacterium*. The direct microinjection of exogenous DNA into the nucleus of plant cells has enhanced the frequency of transformation. This has led to the assumption that the nuclear membrane might be a substantial barrier for the import and incorporation of foreign DNA in plant cells (MEYER et al. 1985). Genetic transformation rates can also be affected by physical and chemical parameters, the type and variety of experimental plant species, and the efficiency of integration of heterologous plasmid DNA via illegitimate recombination (KÖHLER et al. 1990). There is no clear correlation exists between speed of initiation of cell division and the rate of transformation (TYAGI et al. 1989).

In A-MT, the "vir" (virulence) region of the Ti plasmid is essential for T-DNA transfer, and it is induced by specific wound substances from plants (SCHÄFER et al. 1987). The frequency of A-MT can

be increased with acetosyringone (naturally occurring wound response phenolics, which activates the "vir" region in *A. tumefaciens*) in *Arabidopsis* (SHEIKHOLESLAM and WEEKS 1987) and *Atropa belladonna* (MATHEWS et al. 1990). In *Solanum tuberosum*, addition of silver thiosulphate along with acetosyringone improved the transformation efficiency (HIGGINS et al. 1992). RODENBERG et al. (1989) reported several-fold higher stable transformants with single-stranded DNA rather than the number obtained with the corresponding double-stranded DNA, suggesting that it might be single-stranded DNA that is transferred from *Agrobacterium* to plant cells. Irradiation of *B. nigra*, *N. tabacum*, *Petunia hybrida* and *Vigna aconitifolia* protoplasts treated with plasmid-DNA and PEG enhanced transformation rates 6-7 folds (KÖHLER et al. 1989, 1990). The increased integration rates of plasmid sequences into plant genome may in part be governed by DNA repair mechanisms in transformed colonies, derived from irradiated protoplasts.

Bombardment of plant tissues with microprojectiles prior to *Agrobacterium*-mediated transformation resulted in 100 times more transformants with tobacco leaves than when similar leaves were subjected to the standard particle gun transformation protocol (BIDNEY et al. 1992). Similarly, bombardment of sunflower apical meristems with microprojectiles prior to *Agrobacterium* treatment produced more cell transformants than *Agrobacterium* alone (BIDNEY et al. 1992). Bombardment of target tissue prior to the *Agrobacterium*-mediated transformation generates microwounds in the tissue and results in enhanced transformation frequency.

An increase in the transient gene expression has also been demonstrated by: (a) exon 1 and intron 1 of the shrunken 1 gene in *H. vulgare*, *Z. mays* and *O. sativa* protoplasts (MAAS et al. 1991); (b) treatment with cell wall inhibitor, 2,6-dichlorobenzonitrile (DB) in *N. tabacum* and *B. napus* (CHAPEL and GLIMELIUS 1990, BINNS 1991); (c) pretreating *P. abies* embryogenic cell cultures with abscisic acid (ABA) for 3 days before biolistic bombardment (NEWTON et al. 1992); and (d) single-stranded

DNA as a vehicle for transformation (RODENBERG et al. 1989).

### T-DNA mutagenesis

The *Agrobacterium*-mediated transformation involves the stable integration of *Agrobacterium* T-DNA into DNA of plant chromosomes. The integration of T-DNA within the active endogenous plant gene will often inactivate that gene which may in turn cause phenotypic mutations. The site of insertion in a phenotypic mutant can be mapped genetically (using markers contained within the T-DNA) and the sequences for the inactivated endogenous gene isolated by virtue of their proximity to the inserted T-DNA can be determined. Hopefully, the function for the endogenous gene can also be ascribed (KNIGHT 1992). VAN LIJSEBETTENS (1991) reported the genetic mapping of a gene involved in leaf morphology of *A. thaliana*. With a similar approach, KOMARI (1990) demonstrated phenotypically the production of abnormal tobacco plants with double flowers, wavy stems and bent leaf-edges. Results such as these may eventually provide information on genetic control of plant development and morphogenesis.

### Technologies for gene identification

#### Restriction fragment length polymorphism (RFLP)

In the past, plant breeders have developed new cultivars by selecting plants with desirable phenotypes without the use of molecular markers and without the understanding of how genes control characteristics of economic interest. Now, to aid the breeders, RFLP linkage maps have been constructed for many major crop plants such as *Lycopersicon esculentum* (BERNATZKY and TANKSLEY 1986), *Solanum tuberosum* (BONIERABLE et al. 1988, JACOBS et al. 1990), *Secale cereale* (WANG et al. 1991), *Z. mays* (BURR et al. 1988), *Lactuca sativa* (LANDRY et al. 1987), *T. aestivum* (KAM-MORGAN and GILL 1989), and *O. sativa* (MCCOUCH et al. 1988). These RFLP

linkage maps and markers are helpful for selecting and locating desirable genes such as disease resistance (SARFATTI et al. 1991, BENTOLILA et al. 1991; YU et al. 1991, PARAN et al. 1991, MELCHINGER 1990), root knot nematode resistance (KLEIN-LANKHORST et al. 1991, MESSEGUER et al. 1991, POWELL et al. 1991), insect resistance (NIENHUIS et al. 1987), soluble solids content (OSBORN et al. 1987), characterization of somaclonal variation (MULLER et al. 1990), molecular genetic variability (KOCHERT et al. 1991), determination of quantitative trait loci underlying tolerance to low-phosphorous stress (REITER et al. 1991), cultivar identification, certification and patent protection (HUBBARD et al. 1992) and applications to forest genetics and tree improvement (NEALE and WILLIAMS 1991). ALY et al. (1992) demonstrated the genetic origin of walnut somatic embryos using RFLP and isozymes analysis. So far, RFLP maps have been constructed for mostly diploid crop plant species. RFLP mapping of polyploids such as sugarcane, *Fragaria x ananassa* etc. have lagged behind. WU et al. (1992) proposed a general method for mapping polyploids based on the segregation (in 1:1 ratio) of single-dose restriction fragments (SDRFS) in gametes of heterozygous plants.

#### Random amplified polymorphic DNA (RAPD)

WILLIAMS et al. (1990) has described a new technique random amplified polymorphic DNA (RAPD), which can be used for genetic mapping and gene identification. Genetic maps, consisting of RAPD markers can be obtained more efficiently and with greater density than with RFLP or targeted PCR-based methods. XU et al. (1992) identified somatic hybrids between *Solanum tuberosum* and *S. brevidens* with RAPD markers in a very short time when compared to RFLP markers. For preliminary screening of somatic hybrids, this method could detect hybridity of fusion products at either the callus level or at a juvenile stage of growth. RAPD markers have been used for mapping traits in segregating populations, disease resistance and analysis of molecular taxonomy

(RAFALSKI et al. 1991).

#### Polymerase chain reaction (PCR)

The polymerase chain reaction is an enzymatic method for generating millions of identical copies of relatively short DNA molecule in a few hours without the need for conventional cloning techniques. Since its introduction by SAIKI et al. (1985), amplification of DNA by PCR has been used for many applications in biology and medicine. With PCR, specific sequences have been detected in transformed plant tissues of *N. tabacum*, *N. rustica* (HAMILL et al. 1990 b, 1991) and *Z. mays* (GOULD et al. 1991). WILLIAMS et al. (1990) generated RAPDs with PCR-amplified DNA sequences in total genomic DNA. The characterization of different allelic forms and the detailed study of genetic polymorphisms can be done by direct cloning of genomic sequences amplified by PCR. The use of PCR revealed inter- and intra-specific genetic polymorphism in *T. aestivum* (D'OVIDIO et al. 1990), cloning and nucleotide sequence of a PCR amplified DNA fragment corresponding to a gamma gliadin gene from *Triticum turgidum* (D'OVIDIO et al. 1991), genome-specific markers in *Brassica* (QUIROS et al. 1991), nucleotide sequence of PCR-amplified osmotin cDNA from salt-adapted *Nicotiana* cell suspension cultures (KUMAR and SPENCER 1992), DNA markers linked to nematode resistance in *Beta vulgaris* (JUNG et al. 1992) and detection of transcripts homologous to the self-incompatibility gene in *Brassica* anthers (GUILLUY et al. 1991). Furthermore, PCR-amplification, combined with custom-synthesized oligodeoxy-ribo-nucleotide (oligo) primers, can be used to ease subsequent genetic engineering steps such as translational fusions (SLIGHTOM 1991). The gene (cp) encoding the coat protein of cucumber mosaic virus was PCR-engineered for expression in *Nicotiana* (NAMBA et al. 1991). With inverse PCR, T-DNA copy number have been estimated in young transgenic *Nicotiana* plants within 10 weeks of transformation with *A. tumefaciens* (DOES et al. 1991). Recently, JONGEDIJK et al. (1992) used PCR and

inverse PCR-based identification of transgenic *S. tuberosum* plants from original cultivars. Several recent articles on PCR have been published (KRAWETZ 1989, ERLICH et al. 1991, GIBBS 1991, ROSENTHAL 1992).

### DNA fingerprint analysis

In genomic DNA of various species, minisatellite and microsatellite DNA sequences have been found to detect several loci consisting of tandem repeats of a short nucleotide sequence (10-60 base pairs). Analysis of these sequences yields very high levels of polymorphism. This is due to tandem repeats, presumably resulting from unequal mitotic or meiotic exchanges or by DNA slippage during replication. At a given locus, numerous alleles differing in the number of repeats, may thus occur. Probes have been found that hybridize to fragments from several variable loci simultaneously, producing an autoradiograph with a complex fragment pattern, called a DNA "fingerprint" (TZURI et al. 1991). These fragments are inherited in a Mendelian fashion, and they therefore provide a technique suitable for genetic analysis. DNA fingerprints are highly individual-specific (JEFFERY et al. 1985). They are applicable in: (a) genetic variation studies, (b) forensic and ecological studies, (c) in breeding programme and in population genetics, and (d) in the analysis and characterization of a plant genome (WEISING and KAHL 1990). TZURI et al. (1991) identified cultivars of rose, gerbera and carnation ornamental plants, and studied genetic variability in *Salix* (VAHALA et al. 1991), *Malus*, *Prunus*, *Rubus* (NYBOM 1990, NYBOM et al. 1990, NYBOM and SCHAAL 1990), indicating the use of DNA fingerprinting for genotype identification.

### Antisense RNA

Regulation of gene expression in plant cells is helpful in the understanding the function of gene products. The expression of target genes can effectively be inhibited in a number of different organisms by antisense genes which acts by synthesizing anti-

sense RNA which forms a duplex with the target mRNA. This mRNA is then either inaccessible for nuclear processing, is rapidly degraded by RNases or is blocked for translation (van der KROL et al. 1990). ECKER and DAVIS (1986) reported that the transient expression of the CAT gene was inhibited when an antisense CAT gene was simultaneously introduced into protoplasts. A high ratio of antisense to sense RNA was necessary for effective inhibition of gene expression. ROTHSTEIN et al. (1987) observed a stable and heritable inhibition of nopaline synthase (nos) expression in *Nicotiana* plants transformed with sense and antisense constructs of the nos gene. While investigating the effect of nos antisense sequences from different regions of the gene on nos expression, SANDLER et al. (1988) showed that inhibition of nos expression varied with the region of nos antisense used. DELAUNEY et al. (1988) demonstrated that fusion of an antisense CAT gene to a hygromycin resistance gene inhibited CAT expression while the antisense CAT alone was ineffective. Expression of antisense RNA in transgenic plants has been effective in reducing polygalacturonase activity (SMITH et al. 1988, 1990), chalcone synthase expression for inhibiting flower pigmentation (VAN DER KROL et al. 1990), GUS (ROBERT et al. 1990), organ-specific modulation of gene expression (CANNON et al. 1990), carotenoid biosynthesis (BIRD et al. 1991), decreased ribulose-1,5-bisphosphate carboxylase-oxygenase activity in transgenic *Nicotiana* plants (QUICK et al. 1991, STITT et al. 1991), and ethylene synthesis (HAMILTON et al. 1990). Resistance to specific viruses by expression of a negative-sense coat protein gene transcript have been shown for the potato virus X (PVX) (HEMENWAY et al. 1988) or cauliflower mosaic virus (CMV) (CUOZZO et al. 1988) and the potato leafroll virus (PLRV) (KAWCHUK et al. 1991). In all cases, the protection occurred, but only at low virus inoculum levels.

### Transgenic plants as bioreactors

Plants have traditionally been the source of food and raw materials for many industries. For pharma-

ceutical industries, plant cell and callus cultures could be exploited for the production of secondary metabolites. With the advent of plant genetic engineering, plants could be used as a vehicle for the production of new compounds, and the overproduction, or conversion of compounds naturally occurring in the host plant (PEN et al. 1992). There are several reports on the expression of commercially useful heterologous proteins and peptides in plants such as Human serum albumin (HSA) protein (SIJMONS et al. 1990), alpha-amylase from *B. licheniformis* (PEN et al. 1992), antibiotics (HIATT et al. 1989, DURING et al. 1990), interferon (DEZOETEN et al. 1989) and enkephalins (VANDEKERCKHOVE et al. 1989). The success of producing proteins in plants will depend upon the expression level of the protein and the cost of production when compared with other established protocols. Alpha-amylase is a bulk enzyme used for industrial applications, but not in the medical field where purity requirements are not very stringent. For such proteins, transgenic plants could be used as ideal bioreactors for mass production at low costs.

### Cloned genes and their expression

Until now, only single, plant genes have been cloned, characterized and transferred into plant cells. These are herbicide resistance (COMAI et al. 1985, SHAH et al. 1986, STALKER et al. 1988; STREBER and WILLMITZER 1989, D'HALLUIN et al. 1992), isopentyl transferase (SMIGOCCI and OWENS 1988), insect resistance from *B. thuringiensis* (VAECK et al. 1987, DELANNAY et al. 1989, PERLAK et al. 1990, CHENG et al. 1992), insect pest resistance due to cowpea trypsin inhibitors coding for proteinase inhibitors (HILDER et al. 1987, 1989), fungal protection (LOGEMANN et al. 1992), virus coat protein (BEACHY et al. 1990, POWELL et al. 1986, 1989, 1990, KAWCHUK et al. 1990, KALLERHOFF et al. 1990, EHLERS et al. 1991, GIELEN et al. 1991), pollen development (MARIANI et al. 1990), phaseolin gene (CHEE et al. 1986, SENGUPTA-GOPALAN et al. 1985), methionine-rich seed protein from Brazil nut (ALTENBACH et al. 1992), yeast ornithine de-

carboxylase gene for increasing plant secondary product nicotine (HAMILL et al. 1990 a), chalcone synthase gene (DOERNER et al. 1990) and cold resistance (KURKELA and FRANCK 1990). There is still an uphill task of identifying the sets of genes that regulate important agronomic traits in food crops such as yield, grain moisture content at harvest, tillering, and increase in number of spikelets.

Biocontrol of insect pests in plants can be accomplished with genetically engineered 'biopesticides'. Baculoviruses provide an alternative to chemicals for controlling insect pests. They can also be applied by spraying. STEWART et al. (1991) constructed a baculovirus insecticide containing an insect-specific neurotoxin from the venom of the North African (Algerian) scorpion, *Androctonus australis*. In a similar report, a toxin (TxP-I) gene from the female mite (*Pyemotes tritici*) venom which causes muscle-contraction paralysis when injected into insects. It was cloned, sequenced and expressed in baculovirus (TOMALISKI and MILLER 1991). Larvae infected with this recombinant baculovirus became paralysed during infection, thus reflecting an effective insect biocontrol method.

### Conclusions

Genetic engineering has an enormous potential for plant improvement by introducing foreign genes in cells or tissues of both monocot and dicot plants. Transient and stable gene transfer in plant tissues and cells should be independent of genotype. The regulation of gene expression can be studied efficiently by enhancement of frequency gene transfer. With antisense RNA, the expression of a target gene can be inhibited such as flower pigmentation, virus resistance, carotenoid biosynthesis.

The molecular markers - RFLP's, RAPD's, DNA-fingerprints - can be of practical value to plant breeders especially in characterization for agronomic and morphological traits, certification and patent protection, and cultivar identification. Therefore, an interaction between molecular biologists and plant breeders will be desirable.

Transgenic plants may be developed to use as



bioreactors for bulk production of commercial grade products. The biological control of insect pests and fungi need to be addressed using genetic engineering. Such results would reduce the use of chemical pesticides and fungicides. Many single plant genes have been transferred in monocot and dicot plants and yet more task of identifying genes that regulate agronomically important traits in food crops lies ahead. Genetic control of plant development and morphogenesis require attention for better plant production. The emerging new technologies may not efficiently solve all the problems. Therefore, they should be selectively used depending on the nature of the problems. More attention is needed for field evaluation of transgenes, and the fate of selective and scorable marker genes in transgenic crop plants. The major contributions of genetic engineering in crop improvement would ultimately make it a new reliable tool for plant improvement.

The agronomical problems in Finland are somewhat different than the problems in warmer

climatic countries. The crop plants are mainly damaged due to environmental stress - viruses, insect pests, diseases, cold, day length, rainfall and drought - in Finland. Therefore, agriculture biotechnology research needs direction to tackle these problems and also to improve tillering, grain filling, nitrogen fixation in fodder crops, fodder quality - improving seed proteins. The global environmental pollution is causing greenhouse effect. The adaptation of crop plants to these environmental changes may be slow and thereby may result in crop damage and ultimately reduction in yield. Transgenic plants - resistant to environmental pollution stress - may be developed. In cereals, attention is needed: (a) to prevent dormancy breakdown due to rains which results in poor end-use quality, and (b) to study the mechanism of hull formation in naked oats. Naked oats have high feeding value.

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## SELOSTUS

### Geenien muuntelu, lisäkeino kasvinparannukseen

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Geeninsiirtoteknologiassa tapahtunut edistys on tehnyt mahdolliseksi yksi- ja kaksisirkkaisten siirtogeenisten kasvien tuotannon. Biolistisella menetelmällä voidaan siirtää genejä vaikeasti käsiteltäviin viljelykasveihin ja metsäpuihin riippumatta niiden genotyypistä. Pysyvää ja lyhytaikaista geenien siirtoa varten on kehitetty halvempia menetelmiä: ultraäänimetodi, suora DNA-siirto liotusvaiheessa käyttäen kuivia somaattisia alkioita ja silikoni karbiidikuidut. *Agrobacterium* -välitteistä kloonattujen geenien siirtoa kasvisoluihin voidaan tehostaa. Molekyyli-merkkien - RFLP't, RAPD't ja DNA-sormenjälki - analysointi voi täydentää viljelykasvien kuvailua, geenikartoitusta ja tunnistamista, takuutodistuksen antoa sekä patenttien suojaamista. PCR:n avulla voidaan valikoidusti monistaa spesifinen DNA-segmentti ottamalla vain pieni määrä organismin koko DNA:sta ja tunnistaa siirtogeeninen viljelykasvi. Kohteena olevan geenin toiminta voidaan estää

antisense RNA'lla.

Toistaiseksi geeniteknologian avulla on tunnistettu ja kloonattu rajoitettu määrä genejä. Spesifillä geeninsiirrolla päästään moniin päämääriin: tuhohyönteisten ja sienien biologiseen torjuntaan, koiras-steriiliyteen, viruskestävyyteen, siemenproteiinien parantamiseen ja siirtogeenisten kasvien käyttöön bioreaktoreina. T-DNA mutageneesin avulla voidaan saada tietoa kasvin kehityksen ja morfogeneesin geneettisestä säätelystä sekä eristää hyödyllisiä mutanteja.

Ennen kuin geeniteknologia voi olla luotettava keino jalostaa kasveja, olisi kiinnitettävä enemmän huomiota kasvien geneettisten varojen selvittämiseen, jotta voitaisiin löytää ja kloonata uusia genejä. Samoin olisi selvitettävä selektiivisten ja raportoivien merkigeenien vaikutuksia kasveissa ja tutkittava kenttäoloissa siirtogenejä ja siirtogeenisiä kasveja.