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Strategies for Developing Horticulturally Useful Genes: Introduction to the Colloquium

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The first transgenic plants were produced more than 10 years ago (Fraley et al., 1983; Herrera-Estrella et al., 1983; Otten et al., 1981). Since then, numerous plants have been engineered to contain genes that enhance disease resistance and herbicide tolerance; delay ripening; and alter either flower color, morphogenesis, or oil quality (Owens, 1995). These advances were made possible by groundbreaking research that determined that: 1) crown gall disease is caused by the transfer of plasmid DNA of *Agrobacterium tumefaciens* (Akiyoshi et al., 1984; Chilton et al., 1977; Schroder et al., 1983; Thomashow et al., 1986); 2) the transferred genes responsible for tumor formation could be removed and replaced by other genes (Zambryski et al., 1983); 3) infection with disarmed strains of *Agrobacterium* allowed regeneration of morphologically normal plants that contained the substituted gene (DeBlock et al., 1984; Zambryski et al., 1983); and 4) the transferred genes are inherited in a Mendelian fashion (DeBlock et al., 1984; Horsch et al., 1984). Equally important were experiments that led to the development of artificial delivery systems that made transformation of monocot plants possible (Fromm et al., 1985; Klein et al., 1988).

The first transgenic plants contained prokaryotic marker genes. More recently, genes derived from plants and other organisms have been introduced into plants, resulting in measurable plant improve-

ment. Recently, efforts have focused on isolating agronomically important plant genes and on improving gene design to maximize expression in plants.

The participants of this colloquium have focused on strategies for developing horticulturally useful genes based on our understanding of the recent advances in gene identification, isolation, regulation, and plant transformation. The first paper presents an overview of gene availability, identification, and regulation, as well as the current status of crop improvement through genetic engineering. Subsequent papers focus on specific genes useful for the improvement of horticultural crops. Emphasis has been placed on genes for disease resistance, phenotype modification through hormonal control (cytokinins and ethylene), and color modification.

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Overview of Gene Availability, Identification, and Regulation

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In 1981, the first transgenic plant capable of Mendelian transmission of an introduced gene was reported (Otten et al., 1981). In 1994, barely more than a dozen years later, the first genetically engineered major crop, bromoxynil-resistant cotton (*Gossypium hirsutum* L.), was deregulated by the U.S. Dept. of Agriculture for commercial production. Shortly thereafter, the first genetically engineered food product, the slow-softening Flavr-Savr tomato (*Lycopersicon esculentum* Mill.), was similarly approved for marketing. These historic milestones foretell a future in which gene transfer will be used increasingly for the introduction of important agronomic traits into crops. In planning for this future, it is useful to consider what genes are currently available for transfer, how new genes may be identified, and what elements of gene design affect expression of the transgene.

GENE AVAILABILITY

Table 1 lists more than three dozen genes that, while not exhaustive, indicate the range of genes that have been used to produce phenotypes of potential agronomic importance.

Crop quality or yield

The introduction of antisense genes for specific enzymes has been used to impart such diverse phenotypes as slow-softening tomatoes, which permit longer vine-ripening while retaining good shipping qualities (Hamilton et al., 1990; Oeller et al., 1991; Sheehy et al., 1988; Smith et al., 1988), and highly saturated canola (*Brassica napus* L.) oil, which provides a noncatalytically hydrogenated substitute for margarine (Knutzon et al., 1992; also see Ohlrogge, 1994). The use of sense constructs of other genes have increased the level of polyunsaturated fat in canola oil (Arondel et al., 1992; Okuley et al., 1994), produced sweeter tomatoes (Penarrubia et al., 1992), and altered photosynthate partitioning—owing to diminished regulation of transgene enzyme products in heterologous hosts (Micallef et al., 1993; Stark et al., 1992). Male-sterile plants, useful for hybrid seed production (Mariana et al., 1990), and novel flower colors (Meyer et al., 1992) also have been produced with sense constructs.

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Specialty products

Genes from various sources have been used to produce specialty products. Poly- β -hydroxybutyrate, a thermoplastic feedstock (Poirier et al., 1992), and cyclodextrin, a complexing agent (Oakes et al., 1991), have been synthesized by plants carrying engineered bacterial genes. Engineered human genes have been used to cause production of human serum albumin (Sijmons et al., 1990) and the endorphin leu-enkephalin (Vandekerckhove et al., 1989) in plants. Likewise, a plant lauroyl-ACP thioesterase gene placed under control of a highly active promoter was used to substantially shift the oil content of canola oil to lauric acid, an ingredient of soaps and cosmetics (see Ohlrogge, 1994; Voelker et al., 1992).

Environmental stress tolerance

Several transgenes were used to enhance tolerance to environmental stress. Production of the osmolyte mannitol imparted salt tolerance to tobacco (*Nicotiana tabacum* L.) (Tarczynski et al., 1993), while expression of a glycerol-3-phosphate acyltransferase gene from cold-tolerant *Arabidopsis thaliana* (L.) Heynh. increased the proportion of *cis*-unsaturated fatty acids in membrane phosphatidylglycerol of cold-sensitive tobacco—thereby increasing its tolerance to chilling (Murata et al., 1992). Freezing tolerance was enhanced by transgenes for either fish antifreeze protein (Hightower et al., 1991) or superoxide dismutase (McKersie et al., 1993; Van Camp et al., 1994). The latter enzyme destroys active-oxygen species generated in plants by freeze-thaw cycles or by certain herbicides, and confers herbicide and ozone tolerance, as well.

Herbicide tolerance

Engineered resistance to rapidly degraded herbicides—part of a strategy for environmentally safer weed control—has been achieved in two ways: by introducing bacterial genes that either degrade or detoxify the chemicals (2,4-dichlorophenoxy)acetic acid (2,4-D monooxygenase) (Streber and Willmitzer, 1989) and (bromoxynil-specific nitrilase) (Stalker et al., 1988), respectively, or, alternatively, by introducing a gene encoding a structurally altered target enzyme, 5-enolpyruvylshikimate-3-P (EPSP) synthase (Comai et al., 1985) or acetolactate synthase, that no longer binds the herbicide (Haughn et al., 1987).