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DWARFING PLANTS THROUGH APPLIED MOLECULAR BIOLOGY

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ABSTRACT

By applying techniques of molecular biology, scientists can genetically transform plants to reduce their size. Reduction in size is possible through the incorporation and expression of the rolC gene. The rolC gene was isolated from the Agrobacterium rhizogenes that causes the 'hairy root' disease in plants. The product of the rolC gene metabolizes the inactive form of cytokinin in the plant cell to an active form. The increased cellular concentration of the active form of cytokinin causes a shorter internodal length and a reduced apical dominance which increases branching. Fertile transgenic plants have been obtained with a 25% reduction in height. The development of dwarf or semidwarf fruit trees through the incorporation of the rolC gene would reduce costs associated with pruning, pest control and harvesting.

INTRODUCTION

Various ways of controlling plant growth and size have developed over time. The size of a plant is controlled naturally by its environment. In arid regions, some plants modify their growth rate and mature size in order to survive in the stressful environment. However, using environmental stress to control plant size also limits potential production from the plant.

Pruning is the most common method applied to control plant size and shape. In tea production, the harvest of the growing shoot

prunes the plant and causes it to develop into a dense shrub. Grapes are trained on trellises and pruned to control fruit production and limit plant size. Systems are being developed to train fruit trees for mechanical harvest. Vigorous pruning is required to maintain tree size and shape to accommodate a mechanical harvester and yet maintain a substantial yield. The cost and expertise needed in pruning is a major input.

Plant breeders have selected for and developed dwarf plants. The development of dwarf wheat by Nobel Laureate Norman Borlaug ushered in the "Green Revolution." The use of dwarf grain cultivars has led to major increases in harvestable yield. In vegetable crops, bush-type cultivars have been developed for most vining species. Shorter bananas have increased resistance to lodging caused by strong winds and are now commonly grown. A drawback in breeding dwarf plants, especially fruit trees, are the years it takes to develop a dwarf cultivar.

Grafting can be used to control the size of the mature tree. However, dwarfing rootstocks have been extensively developed and tested only for apples.

Plant growth regulators have been routinely used on nursery and floriculture crops to control plant height. These compounds have had limited use on food production. Cycocel has been used on rice to control height and reduce lodging. Bonzi has been used to control vegetative growth and promote flowering in mangoes. A disadvantage is that the plant growth regulator must be absorbed into the cells and tissues of the plant and may accumulate in the developing fruit.

All of the above mentioned systems for controlling plant growth are limited to a certain species or are costly to the fruit grower. Applying molecular biology techniques to develop dwarf fruit trees is now on the horizon. First, a brief review is needed of the genetics involved:

DNA is a double stranded helix located in the plant cell nucleus. The two strands of the helix are connected by the chemical bases adenine, guanine, thymine and cytosine, where adenine pairs with thymine and guanine pairs with cytosine. A gene is a segment of DNA that has a specific sequence of these chemical bases. During protein biosynthesis, special enzymes open the DNA at the gene. A copy of the single DNA strand for the gene sequence is made, messenger RNA (mRNA), in which uracil replaces thymine as a base. The mRNA moves out of the nucleus and into the cytoplasm where each three letter base

sequence is translated into one amino acid. Chains of amino acids make up the protein that compose all living things.

Recombinant DNA Technology

In recombinant DNA technology, certain bacteria such as Escherichia coli contain plasmids, an independent small circular piece of DNA that carries only a few genes. Plasmids are easily isolated from E. coli and digested with restriction enzymes to open the plasmid for genetic modification. The same restriction enzymes are used to cut out and isolate a gene of interest from its source DNA i.e. plant, animal or prokaryote. The isolated gene and the opened plasmid are mixed together. The cut ends of both the plasmid and the new gene are sticky so they will attach to each other and recombine to form a new loop containing the inserted gene.

The gene of interest is the rolC gene. The rolC gene was isolated from Agrobacterium rhizogenes which causes the hairy root disease in plants. The rolC gene product is an enzyme that metabolizes cytokinin glucosides to produce the active form of the cytokinin. The increased cytokinin level results in decreased cell elongation and apical dominance. The rolC gene is inserted into a plasmid vector between genes for NPTII and GUS. NPTII, neomycin phosphotransferase II, provides antibiotic resistance to kanamycin and GUS, B-glucuronidase, provides a colorimetric assay to indicate gene incorporation. This rolC plasmid is put back into E. coli where batch cultures can be grown and large quantities of the modified plasmid can be isolated for use in plant transformation systems.

Agrobacterium Mediated Gene Insertion

Agrobacterium tumefaciens has the ability to infect and transfer plasmid DNA into plants which is incorporated into the plant chromosomes resulting in tumorous galls to form. Disarmed A. tumefaciens have had the tumorous gall forming genes removed but still contain the genes needed for DNA insertion into the plant chromosomes. The rolC plasmid is put into disarmed A. tumefaciens and can be used to genetically transform In vitro-grown plant material.

Tobacco leaf tissue is selected and cocultivated for 48 hours with an overnight suspension culture of A. tumefaciens containing the

rolC plasmid. The leaf tissue is rinsed in antibiotic solutions and placed on regeneration medium that also contains antibiotics: timentin to control bacterial growth and kanamycin to select for transgenic cells. Over time, shoot meristems form from the transgenic cells and develop into plants. The transgenic plants are established in the greenhouse to observe their phenotype. Transgenic tobacco plants have smaller leaves, are at least 25% shorter, flower earlier and form axillary branches. To verify gene incorporation, flowers are self-pollinated and the seeds grown on kanamycin-containing medium. Nontransgenic seeds will germinate but not develop past the cotyledon stage and become white in the presence of kanamycin, while transgenic seedlings are green and develop normally. The ratio of the number of kanamycin-resistant to kanamycin-susceptible seedlings (living: dead), is used to determine gene insertion on different plant chromosomes (Table 1). A 3:1 ratio indicates gene insertion on one chromosome and a 15:1 ratio indicates gene insertion on two chromosomes.

Microprojectile Gene Insertion

Another system used to insert DNA into plant tissues is through the use of microprojectiles. Gold or tungsten micro-particles, 1 micron in size, have plasmid DNA precipitated onto them. The DNA-coated microparticles are bombarded at in vitro-grown plant tissues by using a sudden burst of gas pressure. The randomly scattered microprojectiles penetrate into plant cells and release the attached DNA. Plant tissues are then grown on regeneration medium containing a selection component.

Pollen Electroporation

A recent technology being applied to plant systems is pollen electroporation which incorporates a slight manipulation of the plant's normal reproductive cycle. Pollen is collected and germinated to a pollen tube length of 1 mm. The germinated pollen is mixed with DNA and electroporated which causes holes to form in the callose wall of the pollen tube and allows the DNA to move into the pollen tube. The electroporated pollen is applied to the floral stigma and the developing seeds are screened for transgenic plants. This technique is applicable to

plants with extended pollen viability and large seed set. All the lab work required for pollen electroporation can be accomplished in an afternoon.

CONCLUSION

These plant transformation systems can be used to insert the rolC gene into tropical fruits and ornamentals. Development of rolC dwarfed fruit trees would benefit both large scale and back yard growers by 1) reducing pruning costs; 2) allowing the use of smaller equipment; 3) requiring application of less spray material; 4) lowering harvest cost; 5) facilitating easier crop management; 6) labor force prefers smaller trees.

Table 1. Evaluation of F1 seedlings, from self pollinated rolC transgenic tobacco plants after 21 days on 100 mg/l kanamycin.

<u>Transgenic Plant</u>	<u>Seed CaDsule</u>	<u>Total Seed</u>		<u>Dead</u>	<u>Ratio</u>	<u>χ^2</u>
		<u>Germinating</u>	<u>Alive</u>			
1	1	100	71	29	3:1	
1	2	101	75	26	3:1	*
1	3	102	74	28	3:1	
2	1	88	85	3	15:1	
2	2	94	87	7	15:1	*
2	3	105	103	2	15:1	