here is presented only as a reference of parameters that have been studied and have been found to affect the micrografting procedure.

Table 3. The effect of growth regulator pre-treatments on the grafting success of 'Star Ruby' grapefruit onto 3 rootstock cultivars.

Pre-treatment	Conc. (mg/l)	Percent successful grafts			
		'Troyer'	'Carrizo'	'Sacaton'	Mean ± S.D.
2,4-D	1	50	26.6	66 6	47.8 ± 16 4
2,4-D	10	75 4	73 3	78.5	75 7 ± 2.1
Kinetin	1	66 6	85.0	71 4	74 4 ± 7 8
Kinetin	10	33 3	56.6	33 3	41 1 ± 11 0
2,4-D + Kinetin	1 + 10	44 4	44.4	41 6	43.5 ± 1 3
2,4-D + Kinetin	10 + 1	61.6	55.5	50 0	55.7 ± 4 7
Water (control)	—	23 3	38.5	28 6	30 1 ± 6 3

from Edriss and Burger (1)

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PROSPECTS FOR GENETIC ENGINEERING IN PLANT PROPAGATION

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The rapidly evolving technology of genetic engineering is opening up exciting new possibilities for plant science and for plant propagation. Although, until now, practical applications of gene splicing techniques have lagged behind fundamental advances, several applications are now ripe for exploitation and it is these that I wish to address.

What is genetic engineering? Genetic engineering is less a discipline than a group of techniques applicable within many disciplines. These techniques are based upon an increasingly detailed understanding of the way DNA (deoxyribonucleic acid) is made, structured, read, regulated, processed, and translated into the metabolic machinery of life. Many of the tools of genetic engineering are, in fact, the very tools that cells use to manipulate their hereditary material and reproduce themselves. These tools include such things as plasmids, viruses, transposable elements, and restriction enzymes, all naturally occuring molecules that have been isolated, modified, and utilized by molecular biologists during the last two decades.

What are plasmids, viruses, transposable elements, and restriction enzymes? Very briefly, *plasmids* are extrachromosomal, usually circular, pieces of DNA, originally discovered in bacteria and since found in yeasts and mitochondria as well. Some plasmids have the ability to integrate into a chromosome of their host and later excise and move into other cells. In the process, the plasmids can move genes from one cell to another and alter their heritable characteristics. A bacterium, *Agrobacterium tumefasciens*, contains a plasmid that can spontaneously integrate into dicotyledonous host plants and insert bacterial genes that will be expressed by the plant.

Plant viruses are naturally occuring infective entities that consist of nucleic acid (RNA or DNA) often enclosed in a protein coat. The nucleic acid is the infective part and, as such, can move viral genes from one plant to another.

Transposable elements were originally described in maize but have since been found in bacteria, yeast, and *Drosophila* and, perhaps, in snapdragon as well. Transposable elements consist of short pieces of DNA that have the peculiar property of being able to insert into many parts of the host genome and affect the regulation of adjacent genes. Many transposable elements contain marker genes for antibiotic resistance and hence can be traced in and recovered from the host genome along with the adjacent genes whose regulation has been affected. In this way, selected genes can be located in a host plant or bacterium and in some cases those genes can be cloned (copied).

Restriction enzymes were discovered in bacteria and several hundred have now been found and purified for use. These enzymes recognize and cut given DNA sequences (usually 4 to 6 base pairs long) in such a way as to produce pieces of DNA with homologous or 'sticky' ends (Figure 1). Thus, any piece of DNA cut with a given restriction enzyme will have an end

that will match with and bind to the homologous end of any other piece of DNA cut with the same enzyme. The utility of this is obvious and far reaching. Selected pieces of DNA can be cut and then ligated (joined) to the ends of other pieces of DNA to make novel combinations of genes and gene parts. In addition, DNA molecules can be recognized by their number and location of restriction enzyme sites and changes in base sequence can result in changes in fragment size after cutting with restriction enzymes. Such enzymes are fundamental to genetic engineering and are a powerful tool for cutting and splicing DNA, the blueprint for life.

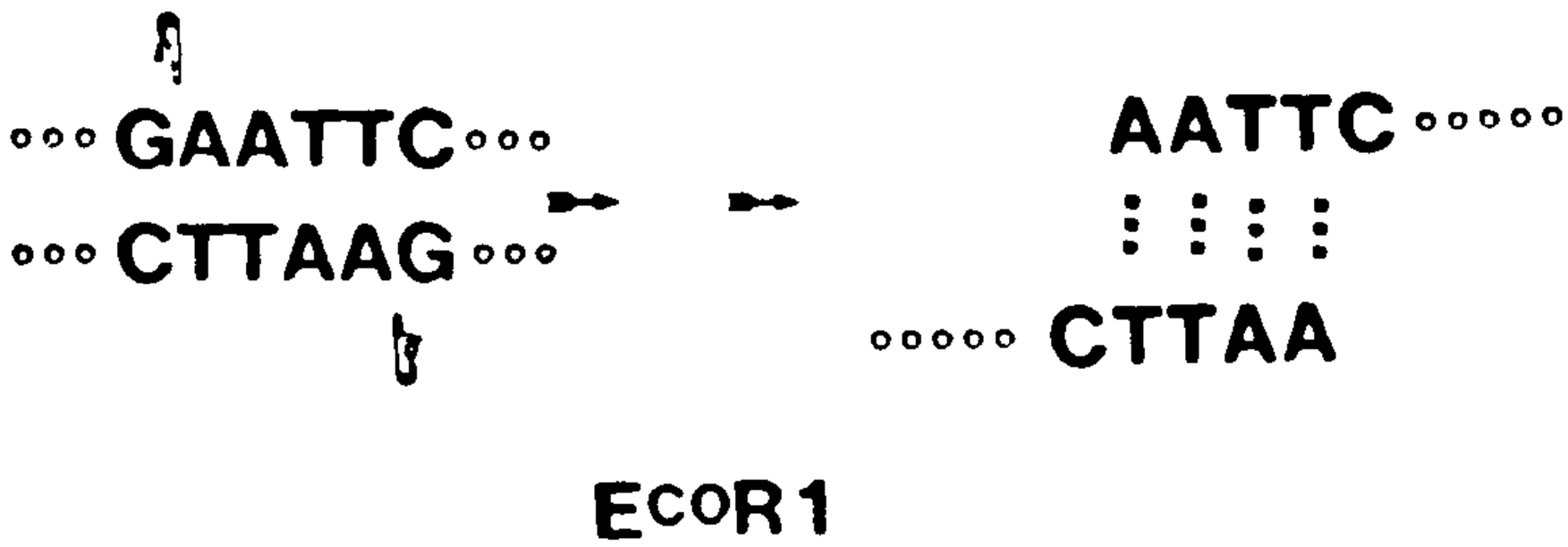

Figure 1. The restriction enzyme EcoRl cuts the double stranded DNA molecule at the two sites indicated by the pointing fingers It only does so after recognizing the six-base sequence at left After cutting, two DNA strands with the complementary sequences shown on right can be joined together as shown by solid dotted lines Hollow dotted lines indicate unspecified DNA base sequences

How can plant propagators benefit from these new tools? Specific genes can be turned on and off by transposable elements. Such elements insert in the genome and often prevent the reading of an adjacent gene. One must then select a plant with a transposable element in the gene of choice. For example, in maize, transposable elements were first recognized by their ability to alter pigment formation in corn kernels. These elements move in and out of the pigment gene, turning it off and on resulting in a color mosaic that we call Indian corn. Such an element, if stabilized and transferred to other plants could be used to rapidly select for rich color variation that could otherwise only be secured through years of breeding. If the elements recently identified in snapdragon turn out to be true transposable elements, we may already have a system for selecting color variants in an ornamental plant.

The Ti (tumor inducing) plasmid of the bacterium *Agrobacterium tumefasciens* (cause of crown gall disease) has some

unique features that make it particularly useful. Upon plant infection by the bacterium, the plasmid integrates into a plant chromosome and causes the plant to read and express bacterial genes. Among others, this plasmid carries genes for the production of the plant hormones, auxin and cytokinin, and these genes are expressed in the plant. It is the additional production of these bacterially coded plant hormones that is largely responsible for the plant tumors that are characteristic of infection by this plant pathogen. But the genetic engineers have gone to work and selected strains of *Agrobacterium* that code for high auxin production and low cytokinin production or low auxin and high cytokinin. The result is bacteria that will induce either root formation or shoot formation rather than the disorganized cell proliferation that results in a tumor. In fact, a naturally occurring species of related bacterium, *Agrobacterium rhizogenes*, causes a root proliferation known as hairy-root disease. Preliminary work has already begun at the University of California at Davis to use the "rooty" bacterial strains to induce rooting in woody perennial plants. The utility of such a method of rooting would be great.

Male sterility is a trait that may be amendable to transfer into commercial crops to facilitate production of hybrid seed. Male sterility in maize has been found to be located on the mitochondrial genome and may be amendable to cloning on a mitochondrial plasmid and transfer into the mitochondria of other plants. Other types of male germicide genes may exist in other plants and could be of great use.

Plant disease resistance has been little studied on a molecular level but some progress has recently been made in understanding the molecular genetics of plant-pathogen interactions. The ability of plants to express hypersensitive resistance to disease-causing microorganisms results from the interaction of genes in the plant with genes in the pathogen. This interaction was first clearly expressed by Flor (1) and was based on the pattern of inheritance of resistance genes in a host and avirulence genes in the pathogen. Thus, disease resistance is expressed when resistance genes in the plant interact with avirulence genes in the pathogen. Workers at the University of California at Berkeley have recently cloned an avirulence gene from a bacterial plant pathogen and are currently well positioned to study the analagous resistance gene in the plant (3). Such resistance genes, if cloned, would be invaluable in producing disease resistant plant lines without the necessity of generations of backcrosses to incorporate the genes into a new genetic background.

Looking even further into the future, recent work opens

the possibility of constructing artificial chromosomes for insertion into higher organisms. Small artificial chromosomes have already been constructed and put into yeast where they have behaved stably through mitosis and meiosis (2,4). Artificial chromosomes in plants would make possible the insertion and stable replication of large pieces of foreign DNA and the creation of wholly new organisms. Though there are certainly many problems to work out and years of research ahead, the prospects for advances in the genetic modification of plants are quite exciting. With the proper attention and research, plants could be modified to make propagation easier, faster, cheaper, and more reliable through genetically programmed disease resistance, rootability, shoot production, and interesting new genetically programmed variants of successful cultivars.

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VOICE: Are all viruses removed from the very young tissues of the apical meristem in meristem culture?

DAVID BURGER: My understanding is, and it is not a complete understanding, that much of the way viruses get into tissues is through vascular systems, and in the apical meristems in the very young cells of the shoot apex there is not a well-defined vascular system. So that if viruses were to get into the apex they would have to do it by diffusion and that is quite slow, as opposed to being transported with the vascular stream. So my understanding is that most shoot apical meristems do not contain viruses for this reason. If it is the case, quite often heat treatment can be used ahead of time before micrografting techniques are used. This way their differential growth is being used so that the plant outgrows the virus.

HARRY LAGERSTEDT: I have three quick questions on micrografting of citrus. *One*, why do you remove the cotyledons? *Two*, why do you remove the root system? and *Three*, what is the time interval between grafting and putting the grafts into containers?

DAVID BURGER: In answer to the first question, in citrus if the cotyledons are attached then the axillary bud in that cotyledon actually tends to grow faster than if it's removed. Also, once the micrograft has been performed you are almost always back into the culture tube on a weekly basis, if not on a daily basis, removing adventitious buds that are forming, and so it is just a matter whether that axillary bud does elongate — you are going to remove the cotyledons anyhow. It's just a matter of ease.

The answer to the second question is very similar in that after the micrograft is performed it is a very difficult technique to get that completed micrograft into the culture tube. You want to have the root as short as possible so that it goes easily into the culture vessel. We shorten it to 2 cm. below the cotyledons basically because that is a very straight portion of the root system and it slides easily into the culture tube.

Lastly, the length of time between micrografting and placing the graft into the containers is quite variable. It can be anywhere from a few weeks to a couple of months. It depends on the success or the ability of the person doing the micrografting, the cultivars that are used, and the conditions that occur.

DON DILLON: Why go through all the trouble of grafting? Why don't you just put a set of roots on your meristem?

DAVID BURGER: You mean take the apical meristem and have it develop adventitious roots. That's a very good alternative and it would be very viable. Unfortunately, many woody plants and many plants in general are difficult to meristem. Orchids have been quite successful and many other plants have been as well. There are others that have been quite resistant to being amenable to this technique. I feel that by doing a graft we avoid the problem of the plant not being able to form entire plants on its own — then we will provide the roots for the shoot tip. But it is a very good alternative and you are absolutely right — that for plants that will form roots from an apical meristem, that is definitely the way to go.

DON DILLON: Now, have you tried it on citrus and found it did not work?

DAVID BURGER: No, I have not tried it and I am unaware of people that have meristemmed citrus. I think that if it had been successful it would probably be used more.