Manipulating plants through single-cell techniques

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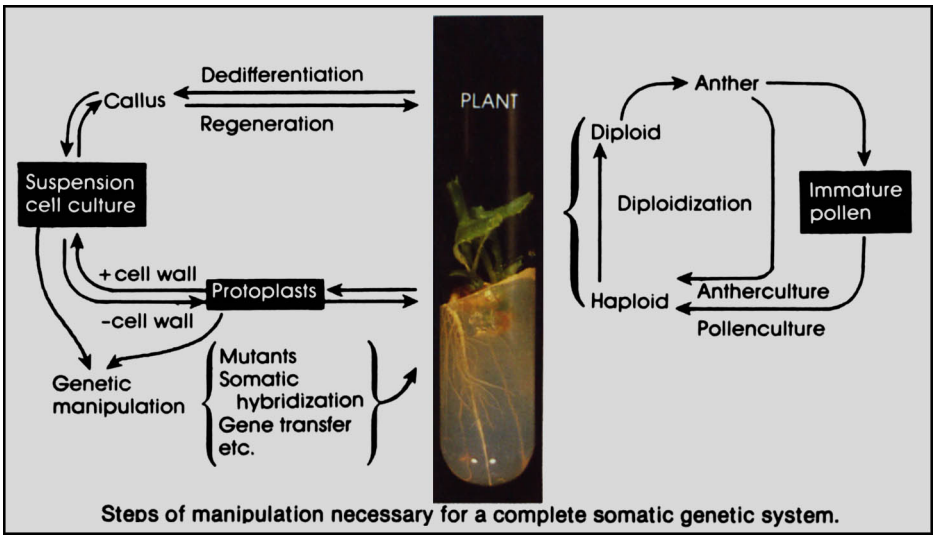
Tissue culture is a way of growing plants as isolated, undifferentiated cells. Such cells can divide indefinitely in culture and in many cases be induced to regenerate to whole plants, so that millions of clones can be produced from a single plant. The fact that plants can be propagated as single cells or clumps of cells presents a unique opportunity to apply the concepts and techniques of microbial genetics to plants. The objective of performing agricultural genetics in the laboratory is to generate novel phenotypes by single-cell manipulation and biochemical selection.

While conventional geneticists make sexual crosses at the whole plant level, somatic geneticists work with single cells grown in aseptic culture. Typically, small segments of a root or stem are stimulated to dedifferentiate and divide, generating a disorganized mass of cells, or callus. Callus can be placed in liquid shake culture to disperse the cell aggregates and grow as a fine suspension of cells.

Plant cells are normally surrounded by a rigid polysaccharide cell wall, which literally holds the plant together. Leaves and suspension culture cells can be treated with enzymes to dissolve the cell wall, liberating millions of naked cells—protoplasts. The protoplasts, under appropriate conditions, will replace the cell wall and divide again. At this point they can be maintained in tissue culture or regenerated to plants.

It can be seen from the diagram that all cultural steps are potentially reversible. Thus, suspension cells or protoplasts can be genetically altered, and whole plants regenerated. Genetic modification can be accomplished by changing the original or introducing foreign genetic material. Methods of somatic genetic manipulation include:

☐Mutant isolation. The capability of applying biochemical selection to a large, nearly homogenous population of cells grown in a dish allows efficient recovery of rare events like genetic mutants. In this manner, mutants of immediate application, such as herbicide-resistant strains, have been isolated. In other instances, mutants altered in biochemical pathways have been found. These are useful in studying the normal cellular processes and can serve as genetic markers for somatic hybridization and transformation experiments.



Steps of manipulation necessary for a complete somatic genetic system.

☐Somatic hybridization. The simplest way to combine genetic information of two cells is through fusion of their protoplasts. The resulting product is the sum of the two nuclear and cytoplasmic genomes. However, subsequent elimination of the genetic material from one or both parents often occurs. The somatic cross is a critical step in a somatic genetic system, and it has been successfully employed in complementation and dominance-recessiveness tests. Somatic crosses are useful in overcoming sexual incompatability between some related species. Although wide crosses often result in abnormal development and cytogenetic abnormalities in the hybrid, it is still possible to make somatic crosses wider than can be made by conventional means. Besides the nucleus, the cytoplasm also contains genetic information which is located in subcellular bodies—organelles, such as chloroplast and mitochondria. Usually the cytoplasm of pollens is not transferred to the eggs during fertilization; thus genetic exchange between organelles does not occur. On the other hand, cytoplasms in nuclei of both partners are mixed during protoplast fusion. This method enables the transfer of cytoplasmic traits such as male sterility from one plant species to another.

☐Transformation. In this method, cells or protoplasts are treated or injected with DNA-containing material to transfer the encoded genetic information to the recipient cell. The foreign DNA could potentially be isolated chromosomes, DNA enclosed in membrane vesicles, organelles, specific gene(s) cloned in vectors, or even naked nuclear DNA (see vector section). The DNA is usually introduced via the uptake into protoplast or fusion between the organelle or vesicle membranes and that of the recipient protoplast. Protoplasts are usually used in these experiments to avoid problems of penetration through the cell wall, although the development of microinjection and other novel techniques might overcome this limitation.

The major advantage of transformation over protoplast fusion is that far smaller amounts of DNA are transferred, even single genes. It is considered less likely to result in abnormal products than the mixing of two entire genomes. The problem is that the tech-

nologies are only at the developmental stage and, unlike fusion, are not yet routine.

The manipulations described are eminently feasible and in some cases have been successfully accomplished. However, we are only at the beginning of somatic genetic manipulation of crops, primarily because many important crop plants behave poorly in culture. For example, corn protoplasts usually cannot divide; soybean callus does not regenerate to plants. Also, too little is known about the fundamental processes of plant development and gene regulation. Once a foreign gene is introduced into a plant, one faces the next stage of problems: will the gene express in the appropriate organ; will it cause side-effects to weaken the plant? These questions must be addressed and solved before somatic methods can be employed to produce better crops.

Several aspects of tissue culture are currently being applied to agriculture. For example, the orchid industry now relies almost exclusively on tissue culture to propagate orchids that are difficult to breed. Also, tissue culture multiplication can often be used to eliminate virus contamination in seed stock.

Vegetative propagation by tissue culture from a single plant might be expected to yield identical plants, because all cells would be of identical genetic constitution, barring very rare mutational events. However, quite striking variability has been found in regenerated plants. This variation may offer a new source of valuable genetic traits for plant breeding.

Another application of tissue culture is in the increasing use of haploid plants. Germ cells, usually the immature pollens either enclosed in (another culture) or isolated from the anther (pollen culture) are cultured to produce new plants. Since these germ cells are haploid, the derived callus or plants are also haploid—having only half the chromosome number of the diploid parent. Such plants can be treated with colchicine and made diploid again. During the processes, the plant becomes homozygous at all loci. Plant breeders often have to self-pollinate strains for many generations to produce "true-breeding, pure" lines. The anther or pollen culture provides a quick way to produce homozygous lines in a single step. Finally, somatic genetic systems based on haploid cultures have the advantage of allowing isolation of recessive mutants.

We believe we can look forward to tissue culture making a modest contribution to plant breeding and agriculture in the next few years. This contribution might be expected to increase radically as the capability of manipulating crops in culture improves, gene transfer and cloning technologies develop, and the knowledge of plant growth and development increases.

Protoplast regeneration

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Plant cells without walls (protoplasts) can be isolated from leaves by a process of enzymatically digesting away the middle lamellae between cells and the cell walls. Tremendously large numbers of protoplasts can be isolated from a single leaf; yields are typically two to four million protoplasts per gram of leaf tissue. Development of techniques and procedures causing isolated protoplasts to reform their walls, proliferate, and regenerate into whole plants is essential for the utilization of the new genetic technology.

In recent months we have succeeded in developing culture media and the methodology for regeneration of lettuce protoplasts into whole plants. When isolated lettuce protoplasts are maintained in the right conditions, they can be induced to reform their walls and divide to form unorganized clumps of cells (P-calli). These P-calli are transferred to media with the proper balance and concentration of plant hormones and other ingredients to induce the formation of shoots. The shoots then are transferred to media for further growth and eventual root production. These regenerated plants then can be transplanted into a greenhouse for seed production and, finally, the progeny are evaluated and selected for desirable characteristics in the field.

One would expect all plants regenerated from a single lettuce leaf to be identical, since their production involves no sexual process. Observations of regenerates, however, reveals the astonishing result that many of them are different from the source plant and from each other. Other researchers have found a similar frequency of variation in potatoes, which has proved to be stable over many generations. Although the reasons for genetic variation among regenerates are not fully understood, it may be possible to obtain desirable improvements in horticultural characteristics, such as enhanced green color, uniform maturity, and resistance to diseases, because of this inherent variability of protoplast regenerates.

The millions of protoplasts that can be cultured in a single petri dish can be subjected to specific selection pressures that will eliminate all but the very few tolerant protoplasts. For example, many plant-disease-causing organisms produce toxins that can be incorporated