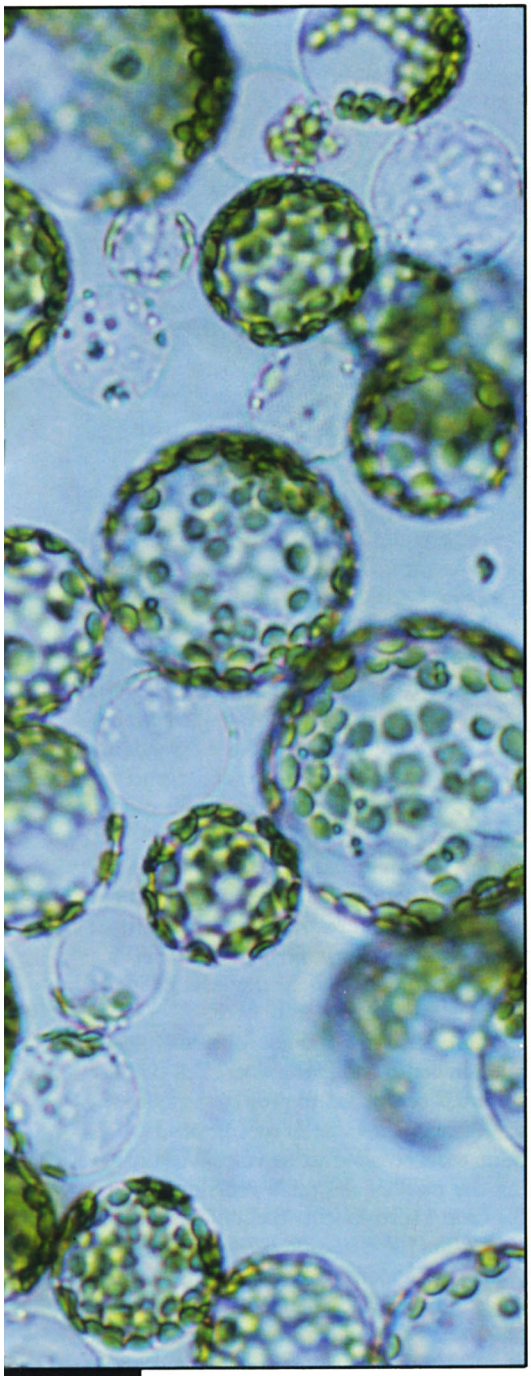

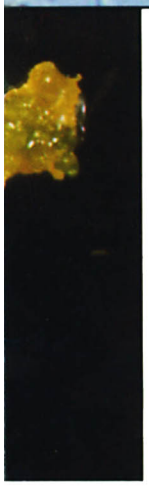

Above: Micrograph of freshly isolated lettuce leaf protoplasts containing bright green chloroplasts.

Left: Protoplast-derived calli giving rise to lettuce plants.

Above right: Lettuce plants ready for transplanting. Each plant originated from one protoplast.

into protoplast regeneration media, thus allowing the few resistant cells to grow into calli, while killing the millions of sensitive cells. In lettuce this technique has been used to select cells capable of surviving exposure to oxalic acid (the toxin produced by the lettuce drop organism) and ethylene gas (the chemical responsible for injury resulting in russet spotting). Selection pressures also can be applied to select for characteristics other than disease resistance. Cold and heat tolerance could be selected for, as well as tolerance to chemicals such as salts and herbicides.

We have used this selection pressure technique to identify the few lettuce protoclones capable of proliferating on media containing greatly reduced calcium levels. We hope that plants regenerated from these protoclones will be resistant to tipburn, a disease caused by a deficiency of calcium in the interior tissues of head lettuce.

Crops have been improved over the years by crossing plants with desirable traits, but the parent plants must be sexually compatible. The use of protoplast fusion can remove this restriction. Protoplasts from any two plants, regardless of species, can be fused. In many cases it has been possible to regenerate fused protoplasts into whole plants, resulting in "somatic hybrids" or asexual crosses. In theory, this technique opens up the potential "gene pool" of a plant to every other biological organism. In lettuce, this means that we can now tap the vast resources of wild lettuce species that contain desirable characteristics, such as genes for disease resistance, but that have previously been unavailable to breeders because of sexual incompatibility.

Crop improvement with this new methodology seems promising. Field testing and selection for desirable characteristics in head lettuce plants derived from protoplasts will be done for the first time in 1982.

Dean E. Engler, Research Assistant, and Raymond G. Grogan, Professor, Plant Pathology, U.C., Davis.

Regeneration of plants

Toshio Murashige

Reporting his pioneering experiments on plant cell culture to the German Academy of Science in 1902, G. Haberlandt predicted that someday "in this way one could successfully cultivate embryos from vegetative cells." Had IAA and kinetin been at his disposal, Haberlandt might have realized his prediction, and there is no telling how much further plant cell culture might be today.

Widespread success with plant cell cultures was made possible when the plant hormones auxin and cytokinin were discovered and when F. Skoog and C. O. Miller revealed in 1957 that regeneration of shoots and roots in cultured cells could be manipulated simply by varying the proportions of these hormones in the nutrient medium.

Genetic engineering of crop plants must usually begin with single cells or protoplasts as the objects of molecular manipulations. The effort must culminate with reconstituted plants. Regeneration of plants from isolated cells currently follows one of two pathways. In the first, plants are obtained through a sequence of shoot formation followed by rooting of the shoot. In the other, embryos—that is, structures with simultaneously differentiated shoots and roots—are initiated.

A series of nutrient formulations is usually required by either path. In the method of separate shoot- and root-forming steps, a critical cell mass, or callus, is prerequisite to any organ formation. An auxin and sometimes also a cytokinin must be provided for callus development. When transferred to a medium containing a relatively high level of cytokinin and a low level of auxin, the callus differentiates shoots. Shoots of suitable size are separated and recultured in still another medium, one lacking cytokinin but containing some auxin, to generate roots. Additional supplements, such as adenine and tyrosine, may enhance the shoot initiation step. Similarly, rooting may be improved by reducing the salts and by including cofactors, such as phloroglucinol and caffeic acid.

In the embryo method, the cells are first stimulated to divide and induced, or otherwise prepared for eventual development into embryos. The induced cells are then allowed to proceed with embryo formation. The medium for the cell division and induction phase often contains an auxin, such as 2, 4-D; a high level of nitrogen, preferably NH_4^+; and sufficient potassium. For the embryo devel-

Herb Quick

Date palms were successfully regenerated from cell tissue culture by manipulation of the make-up of the growth medium.

opment phase, the auxin is excluded from the medium.

Aeration is important for embryo or organ development. Anaerobic conditions cause alcohol accumulation and can lead to intoxication of the cells and diminution of their capacity for organized development.

Embryos can be produced in darkness, but shoot and roots are more likely to develop in illuminated cultures. Gro Lux and Cool-White fluorescent lamps that emit high levels of blue and red light have been most effective. Low light intensities, about 100 foot-candles as encountered in household illumination, are preferable during the shoot and root differentiation steps. However, just before transplanting to soil, plants from tissue should be hardened by exposure for a two- to three-week period to a higher light intensity, near 1,000 foot-candles. Plant regeneration of some species is controlled by photoperiodism, like their flowering behavior. Thus, for example, kalanchoe cells regenerate better under short days, and walnuts proliferate shoots more readily under long days.

The temperature in which cells are incubated can also be important to plant regeneration. Differences in day and night optima, or thermoperiodism, must not be ignored when working with plants that differ in origin from tropical to temperate and alpine habitats.

All plant cells probably have totipotential, or the capacity to reproduce entire plants. Nevertheless, the ease of expression of that potential differs among plant species and varieties, as well as among cells within the same plant. Some cells are more responsive than others to manipulations that result in embryo or organ formation. J. G. Torrey has identified the manipulatable cells by the term meristemoid. In appearance, meristemoids resemble very closely cells of apical meristems or of embryos as found in seeds. Indeed, meristemoids are more often isolatable from apical meristems and young embryos. Non-meristemoid cells sometimes differentiate into meristemoids *in vitro*—for example, tobacco stem pith, carrot root phloem, potato leaf mesophyll, and citrus nucellus. Unfortunately, the basis for this differentiation remains unclear.

Meanwhile, the chances of obtaining plants in cell cultures can be increased by observing a few key relationships. Cells of plants that are in the juvenile phase of development, that is, not yet competent to flower, are more regenerative than those of adult plants. Even among juvenile sources, the younger the plant or organ, generally the higher their tendency to contain regenerative cells. Seasonal climatic requirements of the donor plant or organ must be satisfied before cell culture: it must be chilled, grown under appropriate photoperiod, and the like. As a rule, highly regenerative cell lines can be derived from a poorly regenerating tissue by repeatedly selecting for the trait during the course of subcultures.

Molecular biology may someday furnish methods that would enable selective control of gene repression and derepression. Perhaps, then, it will be possible to achieve expression of totipotential of any plant cell, whether meristemoid or nonmeristemoid and regardless of species or variety.

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Somaclonal variation

Thomas J. Orton

Successful application of *in vitro* cell and tissue culture technology to crop improvement hinges on the ability to regenerate plants of known genetic constitution. For example, when using cell or tissue culture as a means of cloning, or amplifying numbers of plants for field or seed production, it is essential that regenerated "copy" plants be genetically similar or identical to the original. Alternatively, when using this approach to develop a new improved variety, a selection scheme would be devised that would theoretically find only cells with altered genotypes at loci whose function bears on a desired character, but which were genetically identical to the original tissue donor in all other respects. However, some of the earliest research papers in this area have documented the existence of spontaneous genetic variability in both cultured cells and corresponding regenerated plants. A useful label, "somaclonal" variation, has recently been advanced for this phenomenon—"soma," occurring in somatic tissues as opposed to sexual progeny, and "clonal," expressed as differences among and within clones.

The cumulative evidence from over 400 scientific papers shows that somaclonal variation in cultured cells is more the rule than the exception. The most common observations are of changes in the number and structure of chromosomes, the subcellular organelles that carry individual genes. Evidence suggests that these chromosomal changes increase with culture age and are antagonistic to the regeneration process. Regenerated plants with altered chromosomal changes often show changes in leaf shape and color, growth rate and habit, and sexual fertility. Such changes are sometimes seen in regenerated plants with apparently normal chromosome constitution, implying that somaclonal variation may extend to the level of individual genes.

Somaclonal variation is obviously highly undesirable in situations where cell or tissue culture is being used to preserve genetic identity. A limited number of specific observations point to its possible use as a means of expanding the pool of desirable genetic variability for crop improvement. Examples include altered plant habit and flower type in chrysanthemum and increased yield and disease resistance in regenerated plants of sugarcane and potato as compared with the original tissue donor. Unfortunately, the phenomenon is ill-understood, and we are presently unable to direct its manifestations in any way.

Research conducted recently at Davis has shed some new light on somaclonal variation. Using celery as a model organism, we have shown that variation occurs at the level of the single gene as well as the chromosome, although the precise nature of the lesions has not yet been pinpointed. Certain of these chromosomal and single gene changes are transmittable to regenerated plants, and they behave sexually in a predictable fashion. Some populations of regenerated plants show chromosomal abnormalities but little or no accompanying visible alteration, perhaps because of observed mixtures of normal with