Micropropagation of difficult-to-root white spruce using tiny segments from genetically improved seeds aims for more wood formation in a prolonged juvenile phase, and efficient nitrogen use on poor soil.

Improving woody crops

Don J. Durzan

Genetic engineering and cell and tissue culture have already begun to influence the breeding and vegetative propagation of superior rootstocks and woody perennial trees for efficient forestry systems and urban plantings. In our laboratory, hard-to-root biomass species such as Douglas-fir, white spruce, and jack pine have been cloned through micropropagation. The American elm has been propagated from cell suspension cultures. With similar methods being used for fruit and nut trees, valuable rootstocks of *Prunus* and *Pistacia* species are at the point of being cloned and modified to capture the maximum genetic variation available. Currently, a considerably smaller proportion is obtained through conventional selection and breeding.

Problems with woody species based on their large size, age, complex natural products, and elusive reproductive processes are being bypassed with invigorated tissues, which may double the genetic gains affecting productivity. Gains being sought through selection, propagation, and engineering include resistance to insects and disease, production of pathogen-free stocks, rapid growth of wood-producing tall trees, and inhibited wood production in small fruit trees to foster precocious fruiting and convenient mechanical harvesting. In our cloning experiments, we are searching for trees less dependent on nitrogenous fertilizers, more responsive to cultural practices, and able to grow in poor and saline soils, on steep slopes, and in dry and harsh climates.

Geneticist Bill Libby with his prized 14-year-old coast redwood clones at the Russell Reservation test plantation near Berkeley.

Some, but not yet all, of the superior trees can now be propagated independently of the constraints of natural climatic cycles. Cherry, almond, and pistachio trees and tissues are being reduced to cells so that multiple copies of each variety are available on a massive scale for performance tests in many environments. This technique permits us to estimate the total available genetic variation and to sort out interactions between genetics and environment. Early screening to certify new varieties for quality and trueness to type is becoming more efficient. However, problems with the control of growth and maturity of tissues still limit our approaches to tree improvement. Nevertheless, members of the Department of Pomology have started to collect and preserve valuable varieties as tissue cultures for our Germplasm Repository to be opened in 1982.

Now that cell suspensions of many woody species can be established, application of recombinant DNA technologies to protoplasts is being evaluated. We are exploring how to fuse protoplasts, induce phase changes, and improve upon the biosynthetic potential of cells especially for the products of photosynthesis and the building blocks of proteins. Efforts are under way to scale-up low molecular weight transformations and the conversion of biomass using immobilized cell and enzyme systems.

Don J. Durzan, Chairman, Pomology, U.C., Davis.

Cloning coast redwoods

William J. Libby

In a redwood breeding program, time is a problem. Between germination (or planting) and harvest as a renewable source of wood, a redwood must survive and grow in a minimally managed environment for three to eight decades. Trees in park and amenity plantings may be expected to grow for centuries, or even millenia. Redwood foresters thus, and properly, tend to be conservative.

The first step in redwood breeding has been to identify families or populations of trees that are well adapted to particular sites. This is based on the reasonable assumption that, having evolved on a site, they are adapted to it. Site adaptation is important not only in California, in the native range of the redwood, *Sequoia sempervirens*, but also in places like France and New Zealand, where the redwood is gaining importance as an exotic plantation tree.

In most years, in the central part of the redwood's native range, demand for seedlings far exceeds the supply of local-origin seed. One solution to this problem is to clone redwoods native to the plantation region. We have four options with this approach. The first is to find young native seedlings that have successfully established themselves and to bring cuttings from them into the greenhouse. Since the trees are juvenile, their cuttings root easily, and a small collection of known-origin clones can be expanded to thousands of young trees in two or three years.

A second option is to take cuttings of outstanding mature trees. Such cuttings are difficult to root, and they frequently grow in branch form for many years when they do root. Such rooted cuttings planted together should produce a large amount of seed, which could be used to establish new stands. However, there are still problems in obtaining abundant seed production in such seed-orchards, and the site-adaptation of the open-pollinated offspring from a multi-parent seed-orchard is uncertain.

As a third option, outstanding parent trees are crossed in specific combinations, and their juvenile seedling offspring are cloned and tested. We are looking not only for adaptation to various sites but also for clones for-

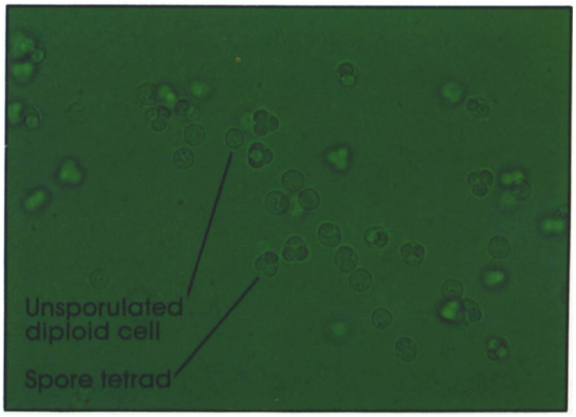
tuitously combining many of the best traits of their parents. Again, time is a problem. It takes time for characteristics of the bole, branches, and wood to develop to a point where evaluation is appropriate. Each year, some clones are disqualified, while the rest are used with increasing confidence.

A fourth option is now becoming possible. Tissues from outstanding mature trees may be cultured in nutrient medium, becoming undifferentiated masses of cells (callus). Fragments of the callus can be induced to differentiate into small plants resembling seedlings, and when these have become large enough, juvenile cuttings can be taken from them. An important aspect of this method is manipulation of the developmental, or maturation, state of a clone. Early promising results in rejuvenating redwood clones have become available from the tissue-culture laboratories of Professor E. Ball and Professor T. Murashige, at the University of California's Santa Cruz, Irvine, and Riverside campuses, and from France.

There is one report of a hybrid between coast redwood and giant sequoia. We and others have not been able to repeat it by normal controlled-pollination crosses. Interspecific hybrids with giant sequoia or other species may someday be created by cell fusion in culture, followed by recovery and cloning of the hybrid plants.

As a general principle, the more the newly selected redwoods are like previously tested trees, the quicker they can be used for large-scale reforestation. Conversely, if the new trees are radically different genetically, they must properly be tested in many conditions for many decades before their widespread use is appropriate. Some new techniques will be immediately valuable, for example, in rejuvenating tested trees or clones, or rapidly expanding clones of known families. But the great promise of the new techniques for producing radically new trees must be tempered by the need to be sure those trees can survive and perform well, for many decades, in the varied and uncertain environments of our forests.

William J. Libby, Professor, Genetics, U.C., Berkeley.



Microphotograph of sporulated wine yeast strain shows unsporulated diploid vegetative cells and sporulated cells, which contain four ascospores. The diploid cells have two sets of chromosomes in each nucleus; the ascospores have only one. The spores result from meiotic divisions similar to those occurring in nearly all higher plants and animals.

Genetic alteration of yeast

Richard Snow

Yeast is one of the major industrial microorganisms, used in the brewing, baking, and wine industries. Most improvements in wine making have resulted from better grape varieties (such as Ruby Cabernet developed at University of California, Davis) or from improvements in fermentation practices. Not much attention has been given to planned improvement of the other organism on which the wine industry is based, the wine yeast. Yeast has many favorable characteristics making it one of the best organisms to use for basic genetics research, and as a result, our genetic understanding of it has reached an extremely high level.

Yeast is classified scientifically with the fungi, the same group to which the common mushroom and many plant disease organisms belong. Baker's, brewer's, and wine yeasts belong to the same species, *Saccharomyces cerevisiae*. Surveys of many wine yeast strains have been made for characteristics of interest to the wine maker, and in every case they have uncovered a great deal of variability, indicating considerable genetic heterogeneity that could be exploited by appropriate breeding programs.

The standard breeding method of crossing strains and selecting desirable recombinant progeny can be applied to yeast, just as it can to most other agriculturally important plants and animals. But one of the most attractive features of yeast is that the new techniques of transformation with foreign DNA and protoplast fusion also can be applied. These techniques open the door to the use of genetic engineering methods in yeast improvement. In my laboratory we are working on one such project, the introduction of a gene from a bacterial strain into a wine yeast strain.

This gene codes for the structure of an enzyme that converts malic acid (the principal grape acid) into lactic acid—the process of malolactic fermentation. This conversion is of importance in preventing wine spoilage and in cases where the grape must is too acid. To cause malolactic fermentation, wine makers either hold the must under conditions that encourage the bacteria naturally present to multiply, or they inoculate with a starter culture of the desired bacteria. It would be desirable to have a yeast strain that could carry out both the malolactic and the alcoholic fermentations at the same time.

We have been able to isolate the malolactic gene from a species of lactic acid bacteria by cloning it on a plasmid. A plasmid carrying the gene has been put into the bacterium *Escherichia coli* (a widely used genetic organism) and into a laboratory yeast strain. In both cases, the recipient organisms acquired the malolactic function, indicating that the gene is working in its new hosts. At present its expression in yeast is too low to be of use to wine makers, but we expect to be able to increase its activity greatly.

The selective introduction of genes from one organism into another will be important in future plant breeding, and in many cases will circumvent laborious (or impossible) breeding programs. The malolactic case is one example: yeast and bacteria cannot be crossed directly. What we learn about increasing the expression of our bacterial gene in yeast should also have direct application to many other cases of agricultural importance.

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