SOMACLONAL VARIATION

The harvest of new carrot varieties, created by somaclonal variation.

Harvest of an agronomic anomaly

By JULIE ANN MILLER

A surprising source of genetic variation is being used to make a carrot that is crisper, crunchier and sweeter for snacks and a tomato that is juicier and tastier for the consumer market, as well as a tomato that has a lower water content for commercial processing. Called somaclonal (or gametoclonal) variation, the technique takes advantage of spontaneous genetic changes that occur in plant cells grown in laboratory tissue culture. The method's practitioners expect it to provide a quicker path to many breeding goals than either traditional methods or current work with recombinant DNA. Some new strains of wheat, carrot, tomato, celery and other crops created via somaclonal variation are already undergoing field tests.

An irony lies behind the method. Both scientists and science fiction writers envisioned that cloning — the production of many organisms from a single cell grown in tissue culture — would produce row upon row of genetically identical plants and animals. And, in fact, cloning is already providing genetic carbon copies of strawberry, asparagus and oil palm plants for commercial use. But the most important use of plant cloning may turn out to be its unexpected production of *variants* useful for the breeding of new crop strains.

For example, tomatoes altered by somaclonal variation are being studied at DNA Plant Technology Corp. in Cinnaminson, N.J. Among 230 regenerated plants, 13 discrete mutations in genes of the nucleus have been identified, in work funded by the Campbell Soup Co. of Camden, N.J.

Yellow and orange tomatoes have been produced by somaclonal variation.

These variants include recessive mutations for male sterility, mottled leaf appearance, altered flower and fruit color, a lethal chlorophyll deficiency and a yellowing of the leaves (called virescence). One mutation, called jointless pedicel, is desirable for mechanical harvesting because it gives a harvested fruit with no stem attached. The New Jersey group has also found dominant mutations controlling fruit ripening and growth characteristics.

To create variants, researchers place plant tissue in a container of culture medium, a mixture of substances supporting cell growth. The tissue soon loses the specific characteristics that make it, for instance, a leaf or a stem, and these "de-differentiated" cells grow into an unorganized mass, called callus. After a period of proliferation, the callus is moved into other culture media that encourage the cells to differentiate so that the callus regenerates into full plants.

Candidates for new tomato varieties are regenerated from cells in tissue culture.

In some cases, plants regenerated from cells thought to be genetically identical turn out to be strikingly dissimilar. They often differ in height, color, shape, disease resistance, yield and maturation characteristics. Reported almost 20 years ago, this variability was initially regarded as a frustrating and embarrassing artifact of tissue culture, unworthy of further scientific scrutiny. But in 1981, P.J. Larkin and W.R. Scowcroft of the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Canberra, Australia, reported that such variability is widespread and proposed that it could be a new source of traits for plant improvement.

"Plant cell culture has provided a new and exciting option for obtaining increased genetic variability relatively rapidly and without sophisticated technology," Larkin and Scowcroft wrote in THEORETICAL AND APPLIED GENETICS (Vol. 60, p. 197-214, 1981).

It may be especially valuable for limited improvements of an existing crop plant, because new traits can appear without disturbing the array of desirable traits that are already present. In contrast, traditional plant breeding shuffles all the traits of the plants that are bred.

Somaclonal variation has been observed in more than 30 species, including most important crops. There are examples in wheat, maize, celery, tomato, banana, oil palm, sugarcane, potato, oats, rice, lettuce, tobacco, carrot, barley and alfalfa. It has produced many genetic changes not previously observed. In wheat, several thousand lines created by somaclonal variation have been selected for further study. "We can reach targets not available by other methods," W.J. Peacock of CSIRO said in West Berlin at the recent Dahlem conference on biotechnology.

Variation occurs both in traits under simple genetic control, such as wheat grain color, and in those under polygenic control, such as plant height. There are even claims of changes in characteristics as complex as yield.

The changes observed in regenerated plants can be inherited by the progeny, generation after generation. Most frequently the pattern of inheritance indicates a recessive gene, but dominant and codominant mutations also appear.

As somaclonal variation is being incorporated into breeding programs, the mechanisms underlying it still remain obscure. "We need to look at the variants at the cell level," says Peacock. "My guess is that there is a whole range of mutations. The question is whether there is something unique about them."

Scowcroft, Peacock and their colleagues are analyzing a variety of somaclonal mutants. Some of the variants represent changes of a single nucleotide in a DNA molecule; others arise from more massive chromosomal abnormalities including the trading of segments between

After somaclonal variation, wheat varies in height (left) and head shape (right). In photo at left, plants on the ends were the source of tissue for plants in between.

chromosomes, the loss or the duplication of a section of a chromosome and the loss of entire chromosomes. Sometimes more than a dozen varied characteristics appear in a single regenerated plant. There is an intriguing possibility that at least some somaclonal variation is due to the movement of transposable elements within the chromosomes.

While the mechanisms remain puzzling, the timing of somaclonal mutation has been established. Much of the variation arises during the period of tissue culture, rather than as a result of any unmasking of variation present in the parent plant. "Tissue culture is a severe perturbation to regular development," Peacock says.

However mysterious the basis of somaclonal variation, it is already being put to work. "This new tool provides scientists with the ability to develop new breeding lines for the generation of improved varieties for food and industrial products from existing varieties within a reasonable time frame," said scientists at DNA Plant Technology Corp. (DNAP) in an article last year in FOOD TECHNOLOGY (Vol. 38, p. 112-119). Although some scientists believe the spectrum of variants obtained by somaclonal variation will be limited, David Evans, DNAP's vice president of corporate research, says, "Any trait we have looked for, we have found.

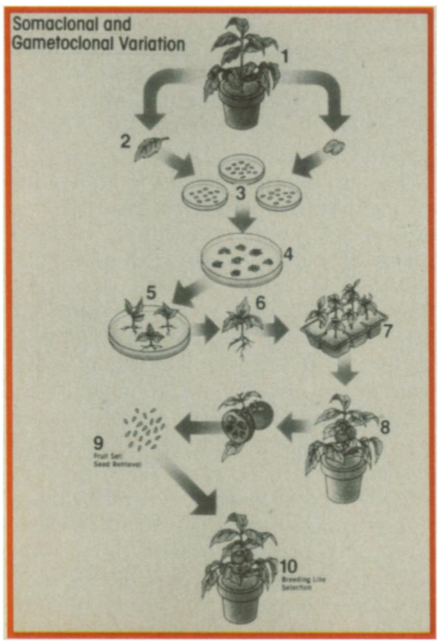
"The challenge has been to design the appropriate breeding program," Evans said at a recent meeting on biotechnology and agriculture in Beltsville, Md. DNAP researchers have concentrated on tomato improvement via somaclonal variation, although they also are applying the technique to carrots, celery, peppers and rice.

The best-analyzed tomato variant is the tangerine-virescent character, a single-gene recessive trait that results in orange flowers and tomatoes and in young leaves that are yellow but then turn green. This mutant was found to be a new form, or allele, of a previously recognized gene whose chromosonal position is known. The allele discovered by traditional plant genetics is called tangerine because of its orange fruit. From crossing the two "tangerine" plants, Evans and William R. Sharp conclude that the gene locus contains two elements that mutate independently — one controlling chlorophyll synthesis (influencing leaf color) and one controlling carotenoid pigment synthesis (influencing fruit and flower color).

Somaclonal variation can produce mutations in genes of chloroplasts and mitochondria, organelles in the cell cytoplasm. Such mutations have not been employed by traditional plant breeders. The chloroplast variants described so far include an increased leaf size, darkened leaf color or reduced fruit set.

A variation on the somaclonal variation technique is gametoclonal variation. The techniques differ in that the starting material for gametoclonal variation is the reproductive material, such as plant anthers, rather than body tissue. Consequently, the regenerated plants have only one copy of each chromosome (are haploid), rather than two copies (diploid).

Gametoclonal variation is useful to geneticists because a recessive gene mutation is not masked by a normal gene. To get plants for breeding, however, the chromosome number must be doubled, for example with a chemical called colchicine. Preliminary results suggest that the spectrum of mutations obtained with gametoclonal variation is different from that obtained by somaclonal variation. In addition, this technique applied to a hybrid of two closely related breeding lines is useful for combining traits already available.



Field trial of tomatoes recovered from somaclonal variation research. New varieties include tomatoes of increased fruit size and pigmentation.

A major limitation of these techniques is that the plants must be regenerated from cells in tissue culture. This regeneration so far has been achieved for about 100 species of plants, and further progress is anticipated. Another limitation is that some of the mutations are not stable but disappear in subsequent generations. For plants that reproduce sexually, this problem can be largely circumvented by employing only those variations that are present in the progeny of the regenerated plants, rather than the larger number that may be present in the regenerated plant.

For crops such as potatoes and sugarcane that are propagated primarily through vegetative, rather than sexual, processes, problems have arisen from unstable mutations generated by somaclonal variation. But in these crops the traditional breeding methods are difficult, if not impossible, to apply.

The DNAP scientists are optimistic. "It is expected," they write, "that during the next few years somaclonal variation will allow breeders to introduce new useful characteristics into all the major plant varieties, thereby developing new, improved varieties in a shorter period of time." □

This is the second of a series of articles on current developments in biotechnology.