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Somaclonal Variation as a Tool for the Improvement of Perennial Fruit Crops

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Abstract

Somaclonal variation has proved useful in the improvement of certain agricultural crops, but its applicability to perennial fruit crops has not yet been demonstrated. At present, fruit crop improvement via somaclonal variation is limited by our ability to manipulate and regenerate popular clones in vitro. Rapid progress is being made in plant tissue culture techniques but little information is available on the full range of somaclonal variation that can be induced and on the various parameters that influence it. Even though our understanding of tissue culture-induced variation is limited, fruit crop breeders with amendable in vitro systems that desire to modify existing cultivars in small, discrete ways, may want to consider the use of somaclonal variation in their programs.

Introduction

Breeders of long-lived perennial crops are constantly seeking new techniques and methods for improving cultivars. Recent biotechnological advances now offer plant breeders new ways to manipulate and expand existing germplasm resources (10). Rapid progress is being made in the field of genetic transformation, and directed single gene incorporation is now possible. However, few genes of economic importance have been isolated.

Another method of creating genetic variability involves plant tissue culture to produce somaclonal variation. The term somaclonal variation is used to describe the phenotypic variation observed in plants regenerated after a passage through tissue culture (7). Using somaclonal variation, a plant scientist can exploit the tendency of rapidly dividing in vitro cell cultures to mutate and produce novel cell lines. Isolated mutant somaclones can then be differentiated into whole plants, with the horticultural qualities of the parent clone but will have been altered in a desired way, improving overall cultivar performance.

Phenotypic variability has been recognized for a long time in populations of regenerated plants (11). Somaclonal variation appears to result from both preexisting genetic variation present in the explant as well as from variation induced during the tissue culture phase (2). The variation may be stable, heritable, and genetic in nature or it may be unstable and epigenetic. Somaclonal variation can involve both single or multiple genes and can be due to alterations in DNA bases, genes, chromosomes, or even entire sets of chromosomes (13).

Plant scientists and breeders are beginning to characterize and utilize somaclonal variation. Although few thorough studies on the range of variants that can be generated have been completed, at least in some species, it is possible to generate economically important traits. Time and space limitations have restricted somaclonal variation studies in perennial crops but work done with citrus (17) and pineapple (21) indicate high levels of leaf polymorphism. One of the first crops in which somaclonal variation was incorporated into a breeding program was sugarcane, *Saccharum officinarum*. Variation for increased sucrose, yield, and disease resistance has been found in plants regenerated from tissue culture (4). Clones with resistance to several important pathogens of sugarcane have been isolated and tested for over 5 years with no apparent breakdown (13). Somaclonal variations in potato, *Solanum tubero-*

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sum, have shown variation in yield, tuber quality, plant uniformity, and disease resistance have all been reported (16). Tomato, *Lycopersicon esculentum*, somaclones have also been produced (2). Clones exhibiting jointless pedicels, altered fruit color, increased soluble solids, and disease resistance have been reported. In tobacco, *Nicotiana tabacum*, a large number of somaclones have been produced from a variety of callus sources. High levels of variation exist and many potentially useful variants have been observed that affect date of flowering, total vegetative yield, alkaloids, sugar, chlorophyll content, disease resistance, and male sterility (13). A tobacco breeding line resistant to hornworm has been released (9).

Somaclonal variation has had a major impact on rice, *Oryza sativa*, improvement. An impressive array of rice varieties, mostly dihaploids derived from microspores have been obtained (15). Currently more than 100,000 ha of these varieties are being grown in China. Some of the most useful mutations have involved plant height, heading date, salt tolerance, and the components of yield (13). Skirvin and Janick (17) developed and released an improved scented geranium, 'Velvet Rose,' following extensive screening of regenerated *Pelargonium spp.* somaclones.

Somaclonal variation, thus appears to be a source of useful variation in certain crops, but few detailed studies have been completed on parameters affecting somaclonal variation. The influence of plant genotype, explant tissue used for culture initiation, medium composition (the kinds and amounts of plant growth regulators, liquid vs. solid cultures, etc.), and method of regeneration (i.e. organogenesis vs. embryogenesis) all need to be more thoroughly investigated. However, certain species, genotypes and tissues do appear to be more subject to tissue culture induced variation, e.g., species which can tolerate high levels of chromosomal variation, including polyploids and asexually propagated crops in which sexual fertility is not important. The occurrence of somatic mutations in certain species and cultivars also might indicate a potential for using tissue culture induced variability for cultivar improvement.

Critics of the application of biotechnology to crop improvement often state that for many crops adequate variation already exists, and that efficient methods for utilizing this variation are needed. Unfortunately, the genetic diversity of many plant species is rapidly being eroded and the amount of perennial fruit germplasm that can be maintained is limited by cost, space, and preservation techniques. Methods of generating horticulturally important variability are a concern of some fruit breeders now and may be so for breeders of other crops in the future. Even when desired traits are available, if they are not present in closely related, well adapted types, it may be difficult to incorporate them directly into useful cultivars.

There are certain aspects of perennial fruit crops that make them particularly well suited to the improvement via somal clonal variation. First, backcrossing and recurrent selection are often impractical with perennial fruit crops because of high levels of inbreeding depression and long generation times. Additionally, there may be strong market pressures for a particular, uniform fruit type, or disease pressure on a popular but susceptible cultivar, that would favor the incorporation of one or a few desirable genes into an already widely accepted cultivar.

Limitations in the application of somaclonal variation to perennial crop improvement also exist. Induced mutations are generally regressive in nature and are thus masked in the heterozygous condition. If the genetic integrity of a cultivar is to be main-

tained, the fruit breeder is limited to the variation appearing in the primary regenerated plants, which would be only a subset of the total variation induced. Also, it is generally impossible to select for horticulturally important traits in vitro or even to screen for them at the seedling stage. Therefore, to detect useful mutations, it may be necessary to regenerate large numbers of plants and grow them to maturity. Currently many fruit crop researchers are limited by the inability to regenerate plants in vitro however, rapid progress is being made in this field. To date, researchers have successfully regenerated in vitro the following major perennial fruit crops, grape (6), banana and plantain (5), citrus (19), apple (22), blueberry (18), pineapple (14), papaya (8), date palm (20), peach (3) and mango (unpublished, Dewald and Litz).

In conclusion, although somaclonal variation probably will not be the panacea for modern perennial fruit breeding, it is another procedure for generating variability and currently may be the most promising method for producing small genetic changes in fruit cultivars. Somaclonal variation should not be viewed as a procedure to displace conventional plant breeding, but rather a process that allows breeding to proceed at a more rapid pace. Fruit breeders of crops with amenable tissue culture systems where slight cultivar modification is a desirable objective or where conventional breeding progress has been slow (such as citrus and banana) may want to consider the use of somaconal variation in their programs.

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A New Fruit Variant in Peach

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Numerous single-gene mutations affecting both vegetative and reproductive plant parts have been described in peach [Prunus persica (L.) Batsch] (1). This report describes a novel fruit mutation with a hard, shell-like exocarp.

The mutant phenotype was discovered as a whole tree mutation on grafted trees of NCX 2612 [('J.H. Hale' x 'Prairie Dawn') x 'Redskin']. The original seedling of NCX 2612 had normal fruit and possessed sufficient fruit size and quality to justify propagation for inclusion in advanced breeding trials. Four of 12 trees propagated by T-budding showed the mutant phenotype. Mature mutant fruit are approximately 4 cm in diameter and have a hard, shell-like exocarp, approximately 1 mm thick. At maturity, the exocarp and mesocarp are dry, and fruit persist on the tree indefinitely. The pith-like mesocarp is 7 to 10 mm deep. Mutant fruit look similar to pear fruit with acute boron deficiency symptoms (2). Nutrient analysis of fruit and vegetative tissues from normal and mutant trees revealed normal boron levels in all tissues in both tree types. Although speculative, the basis for the mutation may be boron related. Initial fruit set is generally quite low, and considerable fruit drop occurs at the initiation of pit hardening. Flowers are showy, and pollen abundant. Pollen germination on artificial media has ranged from 50 to 80%. Mutant fruit are indistinguishable from normal peach fruit during the first 3 to 4 weeks of development. Approximately 4 weeks after bloom, the suture becomes pronounced, fruit change to a lighter green than normal fruit, and pubescence begins to drop (Fig. 1). Cracking of the exocarp and profuse gumming from these cracks follow shortly (Fig. 2). Cross-sectional cuts through the mid-section of the fruit at this time reveal numerous, randomly distributed small (0.2 to 0.5 mm) gum ducts in the mesocarp. The mesocarp is sticky to the touch. Seeds from fruit that reach maturity are viable and exhibit normal germination. Vegetatively, the mutant phenotype is identical to normal peaches except for the presence of intense red pigmentation streaks on shoots of current season's growth. Fourteen open-pollinated seed-

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