

several U.S. selections, many cultivars were used as female trees and those most often used are presented in Table 1. In 564 crosses involving 9 cultivars and 16 selections, a total of 122,874 pollinations produced 69,432 seed. This is an overall ratio of 1 seed per 2 pollinations. This ratio ranged from 2 seed for each pollination in 'Kieffer' to only 1 seed per 10 pollinations for 'Dawn'. Between these two extremes, 21 of the 25 seed parents produced from 1 seed/10 flowers to 1 seed/flower (Table 2). The most productive of these were 'Magness', 'Maxine', U.S. 309, U.S. 386, U.S. 446, and U.S. 56111-20.

The 'Magness' pear tree has small, unattractive, male-sterile flowers and comes into bearing very slowly. Artificial pollinations have resulted in 24 percent of the flowers being fertilized in nearly 9000 pollinations (2). The data in Table 1 on other male sterile parents show that there is no apparent correlation between male sterility, fruit set and seed production.

A viable breeding program can best be maintained when 2,000-4,000 seedlings are planted in the field each year. Thus, the production of about 7,000 seeds per year appears quite satisfactory if the seedlings are not inoculated and screened for fire blight

**Table 2. Grouping of 25 Seed Parents into 4 Categories based on Fruit Set and Seed Production.**

Number of seed parents	Number of fruit/flower	Number of seed/flower	
3	0.04-0.07	1.5-3.1	0.0-0.15
11	0.09-0.12	3.1-4.7	0.15-0.5
10	0.09-0.25	3.2-6.9	0.5-1.0
1	>0.25	>6.9	>1.0

resistance. During the past 5 years, most seedling populations in our breeding program have been screened with the mass-inoculation technique (3). When seedlings are screened for one or more diseases or insects, one must expect an 85-90% mortality. Therefore, if the screened seedlings represent about 10% of the seedlings produced, our aim is to make approximately 30,000 pollinations per year.

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## Fruit Improvement Through Single Cell Culture<sup>1</sup>

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Many fruit cultivars, although widely grown and popular with the public, are found to be deficient for certain characteristics such as disease and insect resistance, pigmentation, time of harvest, fruit set, nutritive value, storage ability, etc. Cultivar improvement for such characters has traditionally been approached from

two directions: 1) using the sexual system to produce variable populations from which improved forms of the original cultivar can be selected, and 2) the use of mutagens (both chemical and physical) on clonal plants to produce mutants which will represent an improved form of the parental plant.

<sup>1</sup>This paper is primarily a discussion of the plant breeding ramifications of a review paper by the author covering all areas of variation associated with plant cell culture (8).

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The sexual approach has been remarkably efficient, but as unique cultivars have been developed, the public's conception of particular fruits may become so prejudiced that later cultivars must resemble the older standard type or face consumer rejection. For example, the 'Bartlett' pear, introduced about 1770, is still the standard of quality in pear production although it is extremely sensitive to fireblight and, hence, is unsuited for culture in the humid portions of the world (including much of the United States).

Due to heterozygosity, polyploidization, and outcrossing, most fruit crops do not breed true from seed. Hybridization of fruit crops may yield improved plant types, some of which may superficially resemble the parental cultivar, but the likelihood of developing a clone sexually that is identical to the parental cultivar with only a single improvement (e.g., 'Jonathan' apple with fireblight resistance) is essentially nil. The sexual system imposes an even more severe restraint to genetic improvement for fruit crops which are partially or completely sterile (e.g., 'Thompson Seedless' grapes).

The use of mutagens on the parental cultivar is, in theory, a good procedure to obtain improved forms of a clone, but in practice has usually proved to be less than satisfactory. The problem is that when mutations are induced in a multicellular organism, the mutated cells are immediately placed in competition with all of the non-mutated cells which surround it. The mutated cell may develop into a mass of cells and perhaps into a cell layer provided that the mutation does not reduce the division rate or limit the cell's ability to grow outward and express its own phenotype. In most cases the mutated cell's ability to duplicate is so reduced that only a limited number of mutated plants and plant parts are observed.

In addition, because most mutations occur in rapidly dividing cellular layers at the apices of plants (the histogenic layers), often only a single layer is affected. If the mutation occurs in the epidermal ( $L_I$ ) layer, for instance, then the mutation may be expressed directly, but if it occurs in the internal layers ( $L_{II}$ ,  $L_{III}$ ), mericlinal or periclinal chimeras of various complexity may arise. If the chimera is mericlinal a segment of the plant may exhibit the mutant character either directly or with adventitious bud formation. If the Chimera is periclinal ("hand-in-glove"), the mutation may be completely masked within the epidermal layer.

Inability to either recognize or separate fruit crop chimeras into pure types has limited the usefulness of mutation induction to improve particular clones. This unfortunate situation could be avoided by growing shoots or even complete plants from single cells. When a mutation occurs in a single cell, the resulting individual will be either completely normal or completely mutant in genotype. In addition, with plants derived from single cells, the number of mutated plants produced should be considerably higher and the range of mutation types wider. It therefore seems to be of utmost importance, in fruit crops (as well as other vegetatively propagated plants) to look for or to develop methods by which shoots or plants can be obtained from single cells.

There are several methods which might be useful in the production of large numbers of plants of single-cell origin, some of these include: 1) tissue culture (8); 2) production of adventitious shoots from internodal portions of plants (2, 3); 3) leaf-petiole cuttings (1); and, 4) root sucker formation (9). The propensity of certain fruit crops to produce adventitious shoots from either stem or root portions (e.g., brambles and apple rootstocks) may be useful to produce large numbers

of pure-type individuals which vary only slightly from the parental clone. The intra-clonal variation sought in such studies may either be induced by mutagens or appear spontaneously.

Since a clone is ultimately derived from the union of a single egg and sperm, one would expect that all cells of a clone are genetically identical and therefore single cells of a clone which are induced to yield whole plants should exhibit extreme uniformity. Such is not the case, and variability, **not uniformity**, seems to be the rule (4, 5, 6, 7, 8, 10, 11, 12) with nearly all plants cultured in tissue culture exhibiting some form of variation such as: A) Physical and morphological changes in undifferentiated callus (habituation, biochemical sensitivity and requirements, change in growth habit, modification of cellular constituents); B) Variability in organogenesis; and C) Changes manifested in differentiated plants (growth rate, growth form, flower morphology, leaf morphology, increases or decreases in production of certain biochemicals, phyllotaxy, partial disease resistance, etc.).

Variation of this nature has been thoroughly documented for many crops (8). The utilization of such tissue culture derived intra-clonal variants for fruit crop improvement is presently blocked by a few technical barriers which include: 1) the routine production of contaminant-free cultures from mature fruit cultivars; 2) the development of suitable media for callus proliferation; 3) development of optimal media for plantlet production from single cells; and 4) production of independent plants from callus in quantity.

The natural variability associated with callus-derived clones (calliclones) represents a pool upon which selection can be imposed. The amount of variability that can be expected will vary with the clone. This system of exploiting natural variation seems

especially applicable to old cultivars which would be expected to have accumulated large numbers of mutant cells that may have stabilized into chimeras of various complexity.

Variability of callus and calliclones may also be increased by aging and repeated transfer. Other techniques useful to increase the percentage of variability will, of course, include the use of mutagenic agents in the callus proliferation stage and selection applied to single cell clones for stress conditions and/or ability to resist or utilize specific metabolites.

The association of polyploidy with tissue culture may be utilized as a technique to obtain either increased or reduced chromosome number. However, affinity of cells to double in culture appears to be a disadvantage in most cases and a screening technique may be necessary to eliminate this type of change.

There are a number of immediate and potential situations in which such variation could be exploited. These include: 1) old vegetatively-propagated adapted clones (e.g., 'Bartlett' pear, 'Concord' grape, 'Russet Burbank' potato, and clonal rootstocks such as the Malling series); 2) seedless or apomictic lines (e.g., 'Thompson Seedless' grape, 'Washington Navel' orange, and certain brambles, and Kentucky bluegrass); and 3) sterile or non-flowering lines (e.g., certain sweet potatoes).

The ability to obtain whole plants from single cells of fruit cultivars will hold special interest for the plant breeder, and will undoubtedly provide an additional method to increase intra-clonal variation. Its most important use will be to obtain mild changes in unique, highly-adapted clones or to obtain variability in clones in which the sexual apparatus is disturbed. This system will probably never completely replace conventional breeding methods for most crops, but

it does appear to offer significant benefits in fruit improvement.

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## 'Brandywine' — A New Purple Raspberry Cultivar

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'Brandywine', formerly tested as NY 905, is a new purple raspberry cultivar released by the New York State Agricultural Experiment Station, Geneva, N.Y. This cultivar is recommended for home gardens and commercial trial because of its superior vigor, productivity, large fruit size and good processing qualities compared to the cultivars shown in Table 1.

'Brandywine' was selected from a cross between New York 631 (a purple-fruited raspberry) and 'Hilton' (a red raspberry) (Fig. 1). Seven selections were made in 1966 from the original population of 172 seedlings. A second test planting was established in 1967 where it has consistently given good performance. It has been tested in New York, Ohio, Massachusetts, Wisconsin and parts of Pennsylvania

where it is rapidly becoming the most promising purple cultivar tested.

Canes are erect and vigorous (up to 2-3 m in length and 12-25 mm in diameter), seldom bending under the weight of the fruit (Table 1). Basal fruiting laterals are profuse and highly productive, frequently touching the soil so that a straw mulch should be used to prevent soil from splashing on the fruit. The plants do not sucker but form well-defined hills of 6-10 canes. In most plantings 'Brandywine' canes are pruned like red raspberries but some modification of cultural practices may be made to take advantage of the vigorous growth, for example, pruning at a desired height to stimulate branching. 'Brandywine' is propagated by tip-layering. Because of the erect growth habit, layers should be made in late

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