The Colchicine Story

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Excitement ran high in the middle of this century after the discovery that colchicine could be used to double the number of chromosomes in higher plants (Blakeslee and Avery, 1937; Eigsti, 1938). The lead article in the Oct. 1953 issue of the U.S. Dept. of Agriculture's (USDA's) *Agricultural Research* proclaimed "Man made polyploids can save a million years..." and "...we no longer have to wait an eon for an accident of nature to break through the stone walls that scientists have reached in some phases of breeding. Polyploidy, artificially induced with colchicine, may provide in one season just the plant material a breeder needs ..." (USDA, 1953).

The excitement came from the discovery that in most plant species a doubling of the chromosome number translated into larger cell sizes and subsequently larger plant parts. Several useful prospects were envisioned, including 1) direct increases in plant and fruit size, 2) production of seedless fruits and vegetables by generating triploid hybrids, and 3) the generation of bridge species by inducing polyploidy in the related diploid species of polyploid crops (Darrow, 1959; Eigsti and Dustin, 1955). Some even predicted that colchicine could be used to produce new crops by restoring fertility in otherwise infertile F_1 hybrids.

COLCHIPLOIDY

In the late 1930s to early 1950s, colchicine was used to double the chromosome numbers of numerous crop species (Blakeslee and Avery, 1937; Eigsti, 1938, 1992). Dermen of the USDA led the charge by inducing polyploidy in apples (Dermen, 1952; USDA, 1956), cranberries (Dermen, 1944), grapes (Dermen, 1954; USDA, 1955), peaches (Dermen, 1947a), pears (Dermen, 1947b), and strawberries (Dermen and Darrow, 1938). Kihara (1951) developed seedless watermelons about the same time as Tereda and Masuda (Eigsti, 1992). Chromosome numbers in several flowers were doubled, including marigolds, pinks, snapdragons, petunias (Nebel and Ruddle, 1938), delphinium (Mehlquist et al., 1943), and lilies (Emsweller, 1947). The ploidy levels of lettuce (Thompson and Kosar, 1939) and chili peppers (Pal and Ramanujan, 1939) were successfully doubled along with the agronomic crops cotton (Beasley, 1940), potato (Johnston, 1939), rye (Myers, 1939), sugar beet (Rasmussen and Levan, 1939), wheat (Sears, 1939), and tobacco (Smith, 1939). By 1979, the chromosome numbers of well over 150 plant species had been doubled using colchicine (Dewey, 1979).

Unfortunately, amid all this promise, only a small number of artificially induced crops were ever released and few ever dominated world markets. Ledyard Stebbins (1956) proved very prophetic when he suggested at the 1954 Brookhaven Symposium on Plant Breeding that sugar beets, clover, rye, grapes, watermelons, and various ornamentals had the highest direct potential as artificial autopolyploids. Since the 1950s, the only successful colchicine-doubled crops have been 1) triploid sugar beets, which were most popular in Europe in the 1970s; 2) tetraploid clovers, which play a minor role in Europe today; 3) triploid seedless watermelons, which are very popular in Israel and Japan, and have ≈10% of the U.S. market; 4) tetraploid rye, which is widely grown in Eastern Europe; 5) the rye x wheat hybrid triticale, which is now an important cereal crop across the world (Villareal et al., 1990); and 6) a number of flower crops, including snapdragons, marigolds, zinnias, impatiens, lilies, delphinium, and daylily (Dewey, 1979; Griesbach, pers. comm.; Sparnaaij, 1979). A colchiploid grape was released, but was never a commercial success (Einset and Pratt, 1975).

Most induced polyploids have failed due to reduced yield and/or erratic bearing (Dewey, 1979). In most cases, artificially doubled genomes suffer meiotic irregularities. This is less of a problem in flower, root, and leaf crops, but such reduced fertility can greatly hamper seed production and fruit development. Other problems that have arisen are inappropriate changes in architecture, slowed development rates, and poor adaptation (Bennett, 1972; Levin, 1983; Van't Hoff and Sparrow, 1963). Changes in the surface : volume ratios of cells through nuclear enlargement appear to have cascading physiological and morphological effects as membrane sites become limiting and cellular concentrations change (Hancock, 1992). Even among the most successful colchiploid crops, considerable breeding improvement had to be made on the raw polyploids before they were released. This has been the case in a number of floricultural crops, including delphinium, daylily, and lily (R. Griesbach, pers. comm.).

GENETIC BRIDGES

Colchicine has proven to be most useful in producing genetic bridges in agronomic and floricultural crops. Some of the most notable successes have come in cotton, forage grasses, potatoes, oats, tobacco, wheat (Dewey, 1979), lily, and impatiens (R. Griesbach, pers. comm.; Sparnaaij, 1979). In many of these crops, the use of induced polyploids or their progeny has been almost routine in breeding programs. Vegetable and fruit crops have also been artificially doubled as potential bridges, but very few genes have found their way into cultivars from these colchiploids. Induced polyploidy has been employed widely in only *Brassica* (McNaughton, 1995), *Rubus* (Ourecky, 1975), and *Ribes* (Keep, 1975).

SUMMARY

There may be several reasons why artificial polyploidy has been used less in horticultural than agronomic species. First, the long period of time to flowering has made the incorporation of induced polyploids into cultivars of most woody perennials a long and tedious process. Second, many of the diploid progenitors of our horticultural crops produce significant numbers of $2n$ gametes, making direct crosses possible without the need for colchicine-induced doubling. Perhaps more importantly, many horticultural breeding efforts are relatively young, are based on natural polyploids, and useful genes are still held in sufficient abundance in the native progenitor species that there is no need to use exotic sources.

Induced polyploidy did not achieve its initial promise of directly producing larger fruits and vegetables, although it has been successfully employed to increase flower size and regularity in floricultural crops. Its use to produce seedlessness in fruit crops also met with only modest success. Colchicine has proven to be most useful as a means of producing bridge species in agronomic and floricultural crops. Colchicine did provide the impetus for key discoveries on the dynamics of chromosomal inheritance and the evolutionary relationships of many crop taxa, and especially in human chromosome cytology, but its direct horticultural importance has been limited outside the floricultural industry.

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Commercial Micropropagation

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The origins of tissue culture derive from the pioneering research of Gottlieb Haberlandt (1902) who first attempted to culture isolated plant cells. Clonal propagation via tissue culture, i.e., micropropagation, as a concept was first presented to the scientific community in 1960 (Morel, 1960). The necessary tools that made micropropagation a possibility, such as the development of media and an understanding of growth regulators, have been available only since the late 1950s, and it was not until the early 1960s that a generalized culture medium was established. The history of tissue culture can be gleaned from the introductions to classic papers in this area by Joseph Arditti, A.D. Krikorian, Peter Carlson, Roberta Smith, and J.H. Gould (Janick, 1989). The actual establishment of commercial micropropagation as an industry became a reality during the 1970s and 1980s. Thus, the micropropagation industry is only 15 to 20 years old (Jones and Sluis, 1991; Zimmerman and Jones, 1991), and retrospectives on commercial micropropagation are few and far between.

Has commercial micropropagation delivered all that it promised? No. Must the entire micropropagation community be defensive because a few early proponents prophesied that micropropagation would be the answer to many (if not all) problems associated with conventional propagation? Probably yes.

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Like many areas of research, endeavors such as micropropagation that initially appear to be simple transform themselves into a Pandora's box full of questions. Even today much is not fully understood about micropropagation, and although general guidelines for micropropagation have been established, each plant species is unique. Despite these problems, there are a large number of species being micropropagated on a commercial scale throughout the world (see Henley, 1992).

The advantages associated with micropropagation include year-round production of clonally identical, pest-free plants. Micropropagation offers the possibility of rapid clonal production of superior plants or lines for commercial sale where demand is high and supply is low or for establishment of plantings for special uses in a compressed time frame (Murashige and Huang, 1987).

My objective is to present data supporting the viewpoint that commercial micropropagation is alive and well and has been and will continue to be fashionably correct, much like the classical white Oxford button-down shirt. I will review commercial micropropagation and the worldwide distribution of laboratories, with specific emphasis on the industry in the United States. I also will cover barriers and stumbling blocks, both scientific and business-related, facing commercial micropropagation.

DISTRIBUTION AND PRODUCTION

Surveys conducted in the late 1980s reported 248 commercial micropropagation laboratories in Western Europe (with 15% produc-