Inducing Polyploidy in Magnolias

By August E. Kehr

The poisonous chemical colchicine has been used since 1937 to produce plants with doubled chromosome numbers. Until recently colchicine was used in human medicine for treatment of gout.

Colchicine is an alkaloid extracted from the seeds ands corms of *Colchicum autumnale* (meadow saffron or fall crocus). Extreme care must be taken in handling colchicine, especially to keep it out of one's eyes and to avoid swallowing even small amounts.

Colchicine is used in water solutions at concentrations from as low as 0.1 percent up to 1.00 percent. The water solution affects plant cells only during the dividing stages and has no noticeable effect on non-dividing cells. In normal cell divisions the chromosomes split lengthwise, and the halves move to opposite sides of the cell. A new cell wall forms between the two groups of chromosomes and thus two new cells are formed, each having the same chromosomes and of the same number as in the original cell.

If colchicine is absorbed into the dividing wall, the chromosomes split normally, but no cell wall forms between the two groups of chromosomes, and the resulting cell then contains both groups of chromosomes. As a consequence, the cell now has twice its original number of chromosomes. After the initial chromosome doubling, the cell will then divide normally, provided the chemical has diffused to other cells or if it has become too dilute to prevent cell wall development in later chromosome divisions within the cell. But if the concentration remains high, the cell eventually ceases further growth and is eventually killed.

One further fact is important in the process of successful induction of polyploids. Upward and outward growth of plants is governed by repeated divisions of a single cell called the apical cell in the shoot tips. If the apical cell is unaffected by chemical treatment, further upward and outward growth from the cell will likewise be unaffected, even though some of the secondary cells in the plant tissue may have become polyploidized. Therefore, unless the apical cell has been affected, the treatment will be unsuccessful. If the stem tip and its apical cell is pruned off the plant, secondary growth is forced to grow which may sometimes be affected.

In essence, therefore, two simultaneous conditions are critical for success: (1) the apical cell must be actively dividing at the time of treatment, and (2) the amount of chemical absorbed into the apical cell at the time of its division must be at a critical concentration level to prevent cell wall formation after one division, but not in such a high concentration as to prevent cell wall formation in a subsequent division.

These two conditions are difficult to achieve simultaneously; hence one should not attempt the experimental induction of polyploidy with colchicine unless one is willing to be exacting in methodology and patient enough to treat many plants in the expectation of having many failures.

Persistence and close observation of the plant growth after treatment are prerequisites to success. Dr. Haig Dermem, one of the pioneers in colchicine treatment of plants to induce doubling of chromosomes, critically examined his plants every day.

TREATING MAGNOLIAS

Colchicine Solution. Colchicine as marketed by many drug companies is purified as a yellow powder fully

soluble in water. One gram, costing about $30, will treat many thousands of plants. I usually make up a 1 percent stock solution by dissolving 100 milligrams (1/10 gram) in 100 cc. of distilled water. This stock solution will keep indefinitely in a refrigerator, especially if a crystal or two of moth flakes (para dichlorobenzene) is placed in the bottle to prevent mold growth. The crystals do not dissolve readily but float on top of the colchicine solution. emitting vapors that inhibit mold growth.

To make up the final solution, use 1 cc. of the stock solution to 19 cc. of water for a 0.25 percent colchicine solution. To this add about 5 drops of a non-phytotoxic wetting agent such as Santomerse, or any commercial spreader-sticker used to apply fungicides. Finally, add 5 drops of dimethyl sulfoxide (DMSO), which almost magically speeds up cell penetration and absorption manifold times.

WARNING: DMSO is a solvent obtained from wood processing. It has high penetrative powers, and is potentially so dangerous it has been outlawed for practically all medical uses, including treatment of arthritis, though it is still used illegally for that purpose by some persons. The use of DMSO in colchicine solution for doubling plant chromosomes is not illegal, but such use renders the normally dangerous and poisonous colchicine alkaloid even more potent and dangerous and extreme caution should be exercised in its use. By all means omit the use of DMSO if you are inclined to be careless, even if it does improve your chances of success.

Mark all containers carefully and store them where they cannot be reached by children or uninformed persons. The author knows of no one using dilute solutions of colchicine who has ever suffered harm, and he would like to see that good record maintained by magnoliaphiles.

Treating Germinating Seedlings. Magnolia seedlings may be treated when the cotyledons (or first leaf-like structures) are fully expanded and the first true leaves are just beginning to appear. One drop of the colchicine solution is placed in the center of the cotyledons, completely covering the very young true leaves. These treatments must be made under controlled conditions, as in a greenhouse, or a cold frame where the humidity is kept as high as possible. The solution is absorbed at the most effective concentration if it can be kept from drying out. As it dries out, concentration of the colchicine increases and becomes more toxic to the dividing cells. In addition, the longer the solution is kept in liquid form at the effective concentration, the more apt the apical cell is to be affected and the chromosomes of that particular cell to be doubled.

A single treatment, if effective, usually will result in almost complete cessation of growth of the seedling for a period of one to two months. Those plants in which growth continues unabated at a normal pace (an indication that the apical cell is not affected) should be given additional treatments as necessary.

When growth resumes in about 6-8 weeks, affected plants will show such symptons as abnormal formulations of or crinkling of leaves, or unusual growth of some type. Some of the plants with such symptoms will resume normal growth within a month, and these may be considered as only possible candidates for further observation as potential doubled forms. Other seedlings may require two to three months to recommence growth and hence make almost no growth the first season. These are more likely candidates.

Finally, there will be seedlings that make no further growth the first year beyond the cotyledon stage and at the end of the season remain as small stubs of plants with no cotyledons and no true leaves. As long as the plants in this last group remain viable and have viable buds, they are excellent

candidates for the polyploid condition. If this latter group can be kept over winter, their growth the following spring will be extremely slow but will eventually speed up as the season progresses. Growth of the doubled forms will produce leaves with lower length-width ratios than the undoubled forms. Also many doubled plants, though not all, will have a rough appearing leaf surface (see photos).

Larger Plants. In treatment of larger plants, it is far more difficult to achieve success than when working with seedlings. This is so because the apical cell is buried deep inside many unfolding leaves and is more difficult to reach with the proper concentration of colchicine. To date the author does not believe he has produced a successful polyploid by this method, though he has hopes for a plant of M. × 'Betty,' a sterile triploid. If the chromosome number in this plant could be doubled, the expected result would be a fertile hexaploid.

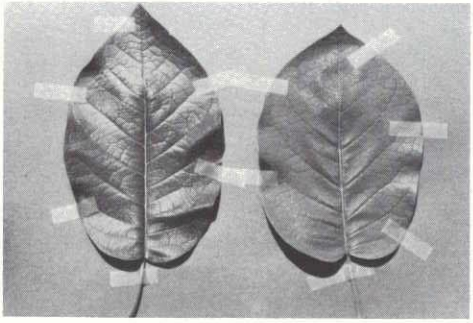
Dormant plants that are to be treated should be grown in pots and placed in a greenhouse or other lighted structure where the environment can be controlled. Treatment with 0.25 percent colchicine should begin when the buds have swollen but have not yet broken the sheath in which they are enclosed. The top half of the bud is cut off and the colchicine solution dripped into the lower half. This process is continued as long as the solution is infiltrating the lower half of the bud. The treated buds are then labeled with a tag or plastic ribbon for future identification. Treatment of the buds should be repeated daily for at least 10 days. Do not attempt to treat flower buds, only vegetative buds.

The potted plants may be planted in the field and the treated buds observed frequently for any symptoms of changed growth patterns.

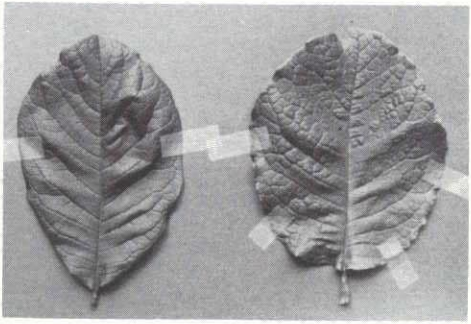
If the terminal growth is normal and rapid, it's an indication that the solution did not penetrate sufficiently to affect the apical bud. In such cases the terminal growth should be pruned off to force growth in some of the lower side buds, which also are good candidates for creating the desired doubled condition but will not grow unless forced to do so by removal of the faster-growing, undoubled terminal shoots.

Additional pruning should be done to encourage or force growth in any treated side shoot which has any appearance of abnormal leaf growth. Such abnormal growth may disappear in subsequent leaves, but one should continue to watch for symptoms of doubling in the normal growth — the wider, rounder leaves with heavier textures.

Of course, if you are fortunate enough to have a good microscope, you can detect doubled cells in such plants by microscopic techniques. A 500-600X power microscope will suffice



Magnolia stellata *diploid (left) and polyploid (right). Note differences in length-width ratios.*



Magnolia sieboldii *diploid (left) and polyploid (right) exhibit same differences.*

for such determinations, made by comparing treated with untreated shoots. Doubled leaf cells have twice the volume of undoubled cells and are about 20 percent wider in diameter.

Tagged buds and subsequent growth should be observed closely for at least two growing seasons. Growth arising from treated buds should be compared to untreated parts carefully to distinguish the slightest difference. Those with any noticeable differences are likely candidates to watch until flowering. At flowering time — which, of course, might be several seasons away — pollen can be examined under a microscope and the ploidy determined easily and accurately. The pollen cells, or grains, contain twice the volume of the undoubled pollen cells of the same plant or species or hybrid and have a diameter 1¼ times that of the undoubled types.

RESULTS TO DATE

The author at present has four good size plants (two to four feet tall) of *M. stellata* and two plants of *M. sieboldii* that apparently are doubled and are believed to be tetraploids. As indicated in the foregoing, the leaves on these plants have a lower ratio of length to width than the untreated sister plants.

Leaves, as many as feasible, were carefully measured in August 1984 and length-width ratios of several magnolias were determined as follows:

	Avg. length-width ratio of leaves
Supposed stellata tetraploids:	
No. 1	1.42
No. 2	1.51
No. 3	1.50
No. 4	1.58
Stellata untreated:	
No. 1	1.71
No. 2	1.75
No. 3	1.80
Stellata 'Centennial'	175
Supposed sieboldii tetraploids:	
No. 1	1.48
No. 2	1.44
Sieboldii untreated:	
No. 1	1.85
No. 2	1.73
No. 3	1.68
No. 4	1.88

The author had not previously recognized *M. stellata* plant No. 4 as possibly tetraploid until the leaf measurements were taken. Of course it may not be conclusively considered a tetraploid because it had a leaf length-width ratio somewhat higher than the other three supposed tetraploids of that species and of the two plants of doubled *M. sieboldii*.

Smaller seedlings of other plants that show initially encouraging symptoms of polyploidy include *M. acuminata*, *M. macrophylla*, *M. virginiana*, *M. hypoleuca*, *M.* (species unknown, but probably *dawsoniana)*, and *M. sprengeri* 'Diva.' The last is normally a hexaploid with 114 chromosomes. If it later can be verified that 'Diva' is truly doubled, it would mean a total of 228 chromosomes, or 12 fold (duodecaploid)—an awesome number of chromosomes, and an awesome word!

Magnolia Sources

From time to time I receive inquiries from individuals and landscaping firms seeking local sources of particular Magnolia species or cultivars. Therefore, I would like to appeal to those who are retail or wholesale nurserymen to send me lists of the magnolias you stock, so I can refer these inquiries to the proper sources. On the lists, please indicate the sizes available, whether you are a wholesaler or retailer, and whether you ship, Overseas sources are desired as well as those in North America, since requests are being received from all over the world. Even If you offer only common cultivars of magnolia such as *M.* × *soulangiana*, let me hear from you since it is often the more common cultivars that I receive inquiries about. —Charles E. Tubesing, Secretary-Treasurer, AMS, 9280 #3 Road, Richmond, B.C., Canada V7A 1V9.