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THE USE OF COLCHICINE IN PLANT BREEDING

by

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Within recent years there has been widespread interest among gardeners and amateur plant breeders concerning the use of colchicine (pronounced coal-chi-sene).

Many fantastic stories have been published and circulated as to the ultimate achievement of this so-called magic growth substance. It must be stated immediately that colchicine is not a growth substance at all. In fact, it often seriously holds back growth or even kills plant tissue. One should be a "doubting Thomas" when he hears predictions of giant flowers, vegetables and trees to result from a spraying with colchicine.

Colchicine is no growth elixir. Evidence is lacking of its effects on many plant species. However, the impression should not be made that it is of little economic value. In spite of the cold water that must be thrown on overenthusiastic popularizations of colchicine, the effect it has on doubling the number of chromosomes (carriers of hereditary traits in the cells) makes it unquestionably one of the most important genetic discoveries within the past decade.

What is Colchicine?

Colchicine is a powerful organic drug of unknown complex chemical structure. It is a narcotic alkaloid, chemically related to morphine and codeine. It is both potent and very poisonous. Unless administered in minute quantities it becomes a killing (lethal) drug. Even in low concentrations its immediate effect on growing tissue is to produce stunting and often extreme distortions.

Source: Colchicine is extracted from seeds or corms of Colchicum autumnale. This is a rather common wild, fall-flowering plant native to Europe and North Africa known as meadow saffron or fall crocus.

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Expense: The extracted colchicine material may be obtained in very small quantities in powder form (amorphous) from some chemical concerns and drugstores. The prevailing price is about $30.00 per ounce.

A small quantity of colchicine can be used to treat a large number of plants, seeds, or other types of materials if applied without undue waste. Where a dripping method is used the solution may be used more than once unless badly contaminated by mold or plant juices.

How Colchicine Works

Affects Cell Reproduction: Colchicine produces deep-seated changes in plants by doubling the number of chromosomes in a cell. The resulting condition is called polyploidy by scientists. The increased number of chromosomes usually brings about an increase in the size of each affected cell and causes various degrees of changes in their physiological functions.

In contrast to normal plants, those developed by colchicine treatment often show changes in height and width and thickness of branches, in size, shape, and texture of leaves, flowers, fruits and seeds, in fertility of flowers, and in response to environment. However, the exact degree of change produced when the chromosome number is doubled cannot be predicted. Certain spectacular changes referred to by some popular writers within recent years are not always to be expected.

Used on Dividing Cells: Colchicine in a water solution affects the plant cells if the drug comes into contact with them while they are dividing. (Cells divide to make more cells — they divide to multiply). It has no apparent effect on cells that are not dividing when the contact is made.

The Process of Cell Division: In normal cell division each chromosome rod splits lengthwise and the halves go to the opposite sides of the cell. There will be many chromosomes in each cell, and every one splits, with the halves migrating to opposite sides of the cells.

A new cell wall forms between the two masses of chromosomes and thus two cells are formed from each dividing cell. Each new cell has the same number of chromosomes as the cell that divided. The dividing cells are referred to as mother cells, and the newly formed ones as daughter cells (see fig. 1).

Colchicine Interferes: If colchicine is present in the dividing cell, the split chromosomes do NOT migrate to the opposite sides of the cell and the cell does not divide into daughter cells as it normally would. Thus from the colchicine treatment there results a cell with twice as many chromosomes in its nucleus as the same cell had previous to treatment. (The chromosomes are in only the nucleus of each cell). (See fig. 2.)

After such initial chromosome doubling, the normal process is for the colchicine to diffuse away from the plant tissues or to become so diluted as to be ineffective. Then the nuclei in the cells with the double chromosome number can divide normally to form new daughter cells. Then, however, the daughter cells have double the number of chromosomes of the cells of the same species before the colchicine treatment, and something new has been created.

Occurs in Nature, Too: The doubling of chromosomes occurs continually and accidentally in nature. It accounts for many plant variations. Very often, however, the doubling is a detriment to the plant.

Technique of Using Colchicine

Generally, colchicine is used in water solution in concentrations from as low as 0.01 per cent up to 2.00* per cent. The assistance of a druggist should be obtained in preparing the desired percentages and quantities of colchicine unless one is technically familiar with these details.

Use Weak Solution: The colchicine solution at the beginning and during the time of treatment should be at room temperature. With small seedlings or fast growing herb plants, it is best that the treatment be made with as weak a solution as may be effective, as early in the active growth of the plant as possible, and for a short period of time. Application of a strong solution or a prolonged treatment may prevent growth of the treated material or even kill it.

Reach Right Cells: Success in the treatment of growing material is based on the principle that the chemical should reach the actively dividing and growing cells; therefore, the manner of treatment depends upon the type of material to be treated. A few of the methods which may be modified to suit your particular material and inclinations are as follows:

Seed treatment

Seeds of many varieties of plants may be (and have been) soaked in solutions ranging from 0.20 per cent to 1.60 per cent for one to 10 days before planting. Soaking of seeds should be done in a very shallow container so that the seeds are not deprived of oxygen.

Seed treatment methods should be used only for seed that germinate quickly (2 - 4 days). With slow germinating seed, the treatment should be deferred until the seed commences active germination. Then the treatment is fundamentally the same as with seedlings.

Seedling treatment

Freshly-germinated seeds are kept immersed in about 0.20 per cent colchicine solution in a shallow container or placed on filter paper, blotting paper or other absorbent paper spread out in shallow dishes. The absorbent paper must be thoroughly wet with the solution from 3 to 24 hours, depending upon how rapidly the seedlings are growing and how bulky they are.

If the material is suitable, the young seedlings may be treated as follows: the root ends are placed on a strip of absorbent cotton, thoroughly wet with water, and then rolled into a bundle; the cotton covers the root ends and forms a plug that will fit loosely into a small vial. The bundle of plants is then placed in the vial with only the stem ends under or at least in contact with the water solution of colchicine (see fig. 4).

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*1/10 gram in 100 cc. water = .01%
2 grams in 100 cc. water = 2.0%

This is a preferred method inasmuch as the root system is kept moist and practically unaffected by the chemical, thus preventing some of the mortality that usually follows treatment of whole seedlings. When seeds are germinated in the dark the resulting seedlings, being longer than normal, are often more suitable for this type of treatment.

Treatment of bulbs and corms

When treating bulbs a somewhat modified technique must be followed in order to reach the growing tip with the colchicine solution. The terminal growing points in bulbs and corms are buried within a mass of scales or other storage tissues. Some popular bulbs, like tulips, narcissus, bulb iris, and onions are difficult to treat with colchicine. In these and similar cases, colchicine may be introduced into the growing regions by a hypodermic needle (see fig. 3). In gladiolus, penetration of the colchicine solution into small cormels* has been achieved in a partial vacuum.

Lily bulb scales may be detached and immediately immersed in a 0.20 per cent solution for about two hours; then planted with the tips exposed.

Treatment of growing shoots and buds

Tips of rapidly-growing shoots or entire growing buds may be treated by brushing or dripping a drop or more of the colchicine solution (0.50 - 1.00%) over the exposed tips once or several times. The tips are exposed by cutting off the small young leaves surrounding the growing point. If the plant is flexible one may bend it over and immerse the growing tip directly for a number of hours in a vessel containing a solution of colchicine (see fig. 5).

Another modification involves affixing a wad of non-absorbent cotton to the growing point and keeping it moistened with colchicine solution (see fig. 6).

The addition of a wetting agent (Santomerse**) to the colchicine solution makes a better contact between the solution and the dividing cells at the apex. One satisfactory method is to add 2 to 6 drops of 10% Santomerse to each 10 cc. (cubic centimeters) of solution.

Caution — Care in Handling Colchicine: Since colchicine is such a powerful drug, caution should be exercised in handling the material and the solutions. Care should be taken to avoid rubbing it in the eyes which may have dangerous consequences. The hands should be washed after contact with the chemical to prevent possible skin irritation.

Recognizing Results

Careful scrutiny of treated material is essential in recognizing affected areas. Polyploidy caused by colchicine can often be recognized in leaves that are longer or broader than normal, or in changes in part of a leaf. Often a half, one side of the midrib, is larger than the other part and heavier in texture. Such leaves should be looked for in parts of the treated material that develop from the

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*Cormels are modified stems that resemble bulbs such as crocus and gladiolus.

**"Santomerse" is a chemical preparation used as an emulsifying agent and prepared by the Monsanto Chemical Company, St. Louis, Mo.

main growing point after the treatment or from axillary buds (buds in axils) that were present in the treated regions. Polyploid branches may be grown from buds in the axils (angles between branches or leaves and the stems from which the branches or leaves arise) of the changed leaves by cutting off the part above these leaves.

May Need Microscope: Some changes often can be recognized only by careful observation, involving the use of a microscope or a high-magnifying hand lens. Changes caused by colchicine may be so inconspicuous that they at times escape casual observation. In interpreting results from the use of colchicine it is highly desirable, and sometimes necessary, to: know the growth habits and cultural requirements of the experimental plant; have expert knowledge of plant structure; have some knowledge of the principles of genetics and cytology; have experience in recognizing and evaluting induced changes; have training in the use of the microscope.

A high-magnifying hand lens in some cases may make it possible to see a change in size and distribution of stomates (small openings in the leaf) in comparison with stomates of a normal leaf. Changes in superficial structures such as hairs and glands that are of epidermal origin may also be detected.

Points to Remember

It must be emphasized that desirable changes in many plants are not to be expected, since many are already natural polyploids and further doubling of the chromosomes may only result in inferior plants. Also, it cannot be emphasized too strongly that colchicine treatment frequently has been unsuccessful, especially with woody plants and other material where penetration of the chemical into the growing region is difficult. Furthermore, the doubled chromosome forms, if produced, are not always improvements over the normal type. Workers at the Oregon Experiment Station are using colchicine in experimental work and should be consulted regarding specific questions not covered in this report.

Conclusions

The use of colchicine for doubling the chromosome number of plant species has opened a large reservoir of possibilities in plant breeding work. However, it offers no simple magic road to the production of spectacular new horticultural varieties of genuine merit.

The amateur plant breeder may expect results in keeping with the time and detail he wishes to give the subject.

REFERENCES

For a more extended discussion of artificially induced polyploidy and information on the results obtained, reference should be made to the following list:

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3. The Use of Colchicine in Plant Breeding, by Haig Dermen, U.S.D.A., Bureau of Plant Industry, Beltsville, Maryland. 1942.

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2. S. B. Penick Co., 50 Church Street, New York 7, New York.

3. Eimer and Amend, 633-635 Greenwich St., New York, New York.

4. Mallinckrodt Chemical Works, New York, New York.

5. Merck and Company, Inc., Rahway, New Jersey.

6. Inland Alkaloid Co., Tipton, Indiana.

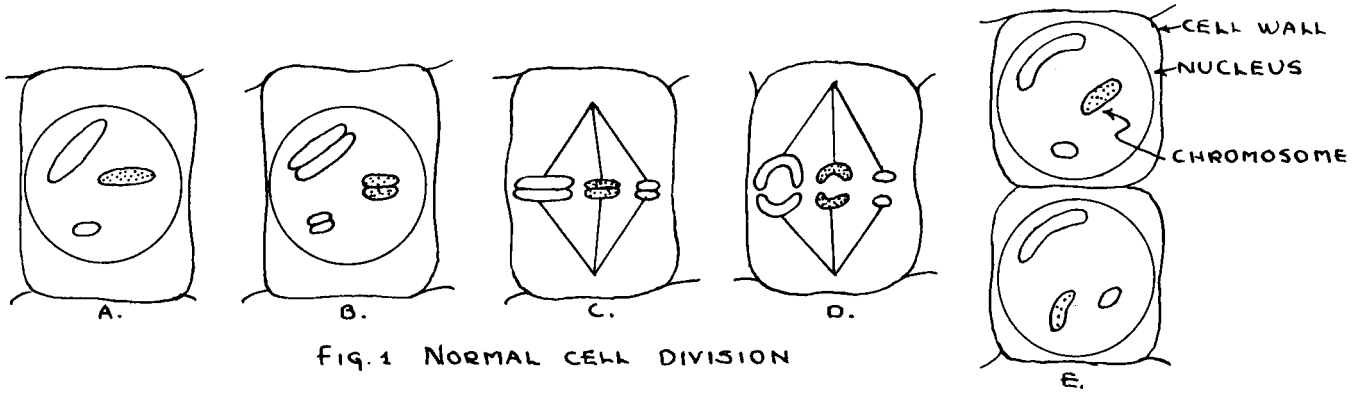

FIG. 1 NORMAL CELL DIVISION

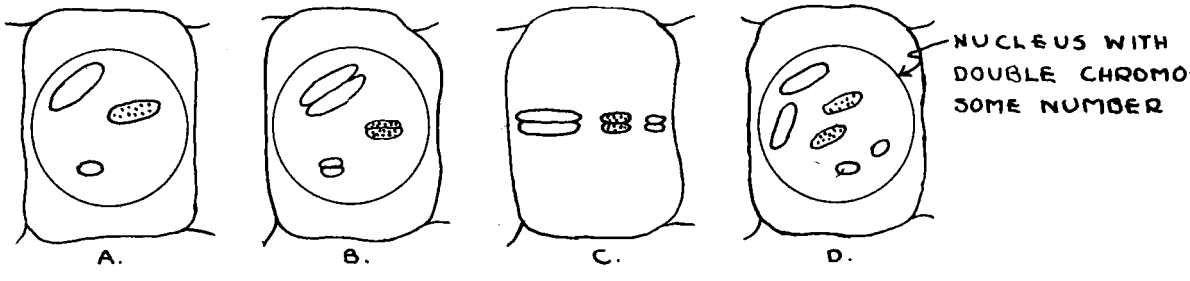

FIG. 2 CELL DIVISION WITH COLCHICINE TREATMENT

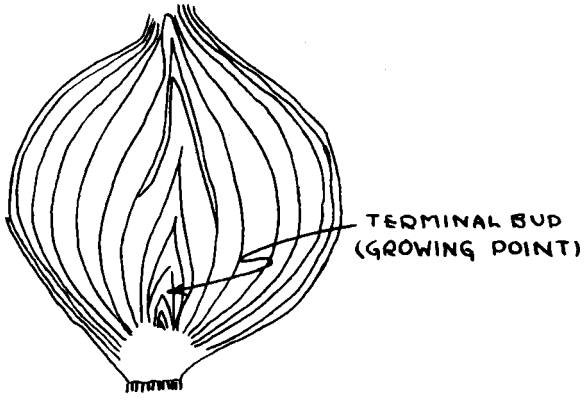

FIG. 3 ONION BULB

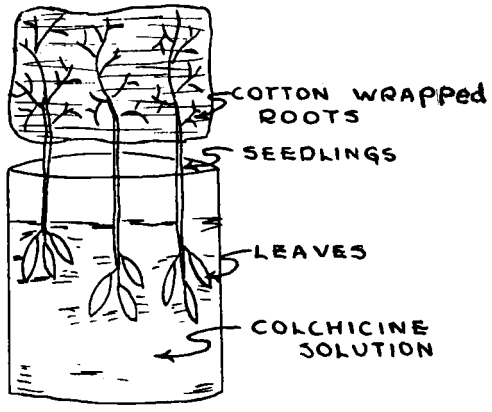

FIG. 4 SEEDLINGS INVERTED IN GLASS VIAL OF COLCHICINE

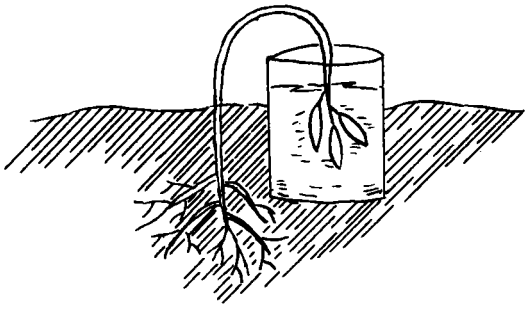

FIG. 5 PLANT TIP IMMERSED IN COLCHICINE

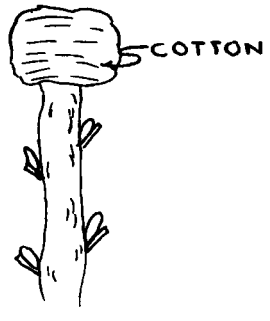

FIG. 6 NON-ABSORBENT COTTON AFFIXED TO GROWING POINT