VOICE: I had the same problem with 'Crimson Pygmy' barberry cuttings, sticking them the same time you did. I took them early in July with ½ in. of last year's growth and found I could root a very high percentage with varying concentrations of hormone.

DALE MARONEK: Where did your roots form?

VOICE: Generally on the older .wood or the union of the two wood types.

JOERG LEISS: We also use old wood with excellent results in July.

SOMATIC MEIOSIS

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In order to obtain new genetic variability plant breeders have turned to wide hybridization. Wide hybridization is the process of obtaining progeny from crosses between distantly related species or genera. Wide hybridization could involve more classical approaches such as embryo culture (9) or some of the newer techniques of genetic engineering like electrically-induced cell fusion (13). In either case, a hybrid is produced which is likely to be intermediate in morphology between the two parents. Further breeding is generally required before a commercially valuable plant type is created.

In most instances wide-hybrids are sterile because of a lack of chromosome pairing during meiosis. This sterility can sometimes be corrected through chromosome doubling to create a type of polyploid called an amphidiploid. Amphidiploids, even though polyploid, behave like diploids in that only two of the potential four chromosomes in a set pair during meiosis (1).

Not all amphidiploids, however, are fertile. In the case of the interspecific hybrid *Lilium* 'Black Beauty' (*L. speciosum* × *L. henryi*), viable pollen was not produced until after the chromosomes were doubled. Five hundred and fifty pollinations with a very fertile amphidiploid *Lilium* 'White Henryi' (*L. henryi* × *L. leucanthum* var. *centifolium*) resulted in the pro-

duction of only five seeds which contained viable embryos. Potentially, 100,000 viable seeds could have been produced. (R.A. Griesbach, personal communication)

Plant breeders would greatly benefit from a procedure for inducing a meiotic-like process in mitotic or somatic cells. In this way, it might be possible to obtain progeny from sexually sterile hybrids. Meiosis, although quite complicated, is simple in principle and can be subdivided into two distinct events — recombination and segregation. During recombination, genetic material is exchanged between the pairs of homologous chromosomes of each set. After recombination, the two chromosomes separate or segregate into different gametes. Recombination and segregation result in new gene combinations which are not found within the two parents. For example, one parent could have large red flowers and the other parent small white flowers. Their offspring might all have large red flowers. Because of meiosis some of the progeny obtained by crossing the hybrids would be expected to have either small red flowers or large white flowers, two new gene combinations.

MITOTIC RECOMBINATION

It is possible to induce mitotic chromosomes to undergo recombination. Considerable information is known about this process in fungi (6). In fungal cells, the spontaneous frequency of mitotic recombination is about 1000-fold less than the meiotic process. Many drugs or chemicals which inhibit DNA synthesis (eg. mitomycin C, fluorodeoxyuridine, hydroxyurea, etc.) or chemical or physical processes which break the DNA (e.g. ultraviolet light, ethidium bromide, x-rays, etc.) have been found to greatly increase the frequency of mitotic recombination, to levels found within meiotic cells. Studies in fungi have indicated that there are major differences between mitotic and meiotic recombination. First, mitotic recombination occurs more frequently in regions near the chromosome's centromere. The opposite is true for meiotic recombination. Second, mitotic crossing-over appears to be initiated at random sites within the genome; while meiotic crossing-over is believed to be initiated at specific sites. Finally, mitotic recombination, unlike meiotic recombination, does not require DNA replication.

One measure of the frequency of mitotic recombination in higher eukaryotes is the occurrence of twin spots on differentiated tissue. The outcome of a mitotic recombinational event is the production of two different cell types. At the end of a developmental sequence (eg. leaf development) each of the two recombinant cells will have given rise to a distinctive

group of cells. The two cell groups will be spatially next to each other and will appear as a double or twin spot on a leaf or petal. As in fungi, the application of 10 μg/ml mitomycin C to germinating soybean seed can increase the frequency of twin spots or mitotic recombination five-fold over the spontaneous rate (12). The spontaneous frequency of mitotic recombination was 0.54 twin spots per leaf. When mitomycin C was applied to broadbean root tips both homologous and nonhomologous chromosome pairing was increased (1). Homologous chromosomes are the pair of chromosomes which are identical. On the average, 45% of the exchanges induced by mitomycin C involved homologous chromosomes. Data suggested that pairing was initiated in regions of heterochromatin or repetitive DNA which were not specific to any given chromosome type.

MITOTIC SEGREGATION

One way of inducing mitotic segregation is through chromosome elimination or reduction. If one doubles the chromosomes number and then reduces it back to the original level, the outcome is the same as segregation. There are several means of increasing chromosome elimination in somatic tissues; p-fluorophenylalanine was one of the first drugs which was used for this purpose (5). Black currant cuttings treated with this drug showed that about 25% of the newly formed roots had cells which contained reduced chromosome numbers. In diploid fungi, culture medium supplemented with low levels of griseofulvin (eg. 20 μg/ml) could induce both chromosome reduction and chromosome recombination (4,7). The chromosome loss was complete but gradual in that it could take up to four weeks before stable clones were produced. About 93% of the clones were haploid; the other 7% were diploid mitotic recombinants.

Griseofulvin has been applied to cultured, diploid alfalfa cells (11). Low doses (150 μg/ml for two cell cycles) caused both an increase and decrease in chromosome number. About 30% of the cells had a chromosome number below the diploid level and about 60% had a chromosome number above the diploid level. Griseofulvin treatment appeared to induce chromosome loss by producing abnormally shaped cells which had extensive cytoplasmic extrusions and irregular cell plates during mitosis (8). Griseofulvin also acted as a potent, mitotic, arresting agent blocking cells at mitotic metaphase. The combination of these effects resulted in the production of minicells containing varying numbers of chromosomes.

Griseofulvin has also been applied to cultured cells from a petunia somatic hybrid (3). In this case, whole plants have

been regenerated from the treated cells. About 9% of the regenerated plants were affected by the drug. Of these 9%, 47% had a reduced chromosome number. Many of the affected regenerates initially expressed a very high degree of both leaf and flower variegation which disappeared over time. For example, one regenerate started out producing magenta flowers having over 500 white sectors per flower. After six months, this plant was stably producing solid white flowers. Approximately 0.1% of the sectors initially produced by this plant were twin spots or due to somatic recombination. The practical outcome of griseofulvin treatment was the production of fertile plants which had reduced chromosome numbers and new gene combinations for flower color!

CONCLUSION

It appears that there are several different ways in which an artificial process that leads to genetic recombination and segregation can be induced in somatic cells. A combination of several approaches might work the best. The major difference between the meiotic process and the mitotic process is that the meiotic process is very highly ordered and excecuted while the mitotic process appears to be more random.

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CLONAL PROPAGATION OF PERENNIAL PLANTS FROM FLOWERS BY TISSUE CULTURE[1]

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Abstract. Several herbaceous and woody perennial plants have been clonally propagated by tissue culture using the flowers as explants. Even tetraploid plants regenerated from callus from flowers seem to be genetically stable. Flowers from several *Rhododendron* species and cultivars regenerated plants on Anderson's medium. The epigenetic change from maturity to juvenility may take place in some flower tissues before the formation of an embryo.

The propagation of perennial plants by tissue culture, particularly woody plants, has lagged behind the propagation of herbaceous annual and tropical plants. There are several reasons for this. First, obtaining a sterile explant can be a problem. Then, the problem that propagators have long recognized as loss of juvenility is magnified when we propagate perennial plants by tissue culture. Finally, there is the worry that genetic or even epigenetic changes will cause the plants to vary from the clone of interest. The following discussion will cover some of the advantages of using the flower parts of perennial plants as an explant source for tissue culture propagation in relation to these problems.

We should first examine some of the anatomical similarities and differences between vegetative and flowering growth of the terminal meristem. This phenomenon is covered extensively in Esau's classic anatomy textbook (6) and Gemmell's monograph (8). The vegetative meristem initiates stem and leaves in a very ordered pattern by growth protuberences at regular intervals on the flanks of the meristem. In the axils of

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