SYNTHESIZING PLANT CHIMERAS AS A SOURCE OF NEW PHENOTYPES[1]

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A chimera is a plant possessing two or more genetically distinct tissues. In horticulturally important species, chimeras are manifested frequently by useful or ornamental characteristics. For example, some thornless blackberry cultivars contain epidermal tissue which has genes for "thornlessness", whereas the inner tissue layers can give rise to thorny branches (3). Chimeras for flower or bract color exist in carnations, mums, and poinsettias (9,10,11). In addition, leaf variegation can be caused by green and albino cell genotypes coexisting in a single shoot (4). While variegation is obvious in the leaves, its actual origin is from the cells which give rise to the leaves, namely the "apical initials" of the shoot tip or shoot apical meristem. Most higher plants have a structured shoot tip which contains two or three cell layers, each with a set of apical initials. These layers generally remain independent from each other as the apex continues to produce cells. The layers have been termed the LI (the outermost), the LII (the middle), and the LIII (the innermost). When the cells of a given layer are genetically different from those in the other cell layers of the shoot tip, the plant is known as a periclinal chimera. Other less stable chimeral arrangements can exist, but because of their instability they have not become horticulturally important. It is important to realize that the term "variegation" is not synonymous with "chimera" and that variegation can be caused by factors other than chimerism (4,12). For example, coleus plants, which are popular because they have variegated leaves, *are not chimeras. The entire plant is composed* of cells with the same genetic makeup. However, the genes "tell" the plant the position on the leaf where various pigments should be produced or destroyed. One analgous case in animals is the panda bear. Although the animal is composed of one genotype, dark pigments are produced only in certain body regions. If it were possible to regenerate panda bears from "black cells" and from "white cells", the resulting animals would still appear identical to the original "variegated" panda bear. If, in theory, the bear was actually a chimera, cells from its white body parts would yield white panda bears, and

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cells from its black parts would yield black panda bears.

With few exceptions, this "regeneration test" holds true for periclinal chimeras in plants. If small tissue pieces excised from the differently colored regions of a variegated leaf are tissue-cultured, and if the regenerated plants still have the same pattern of variegation, the chances are good that the plant is not a periclinal chimera, i.e., it does not possess a unique homogeneous genotype in each cell layer. If, however, the white portions of a leaf regenerate white plants and if the green portions regenerate green plants, then the plant is most likely a chimera, and the tissue culture process has separated the chimera into its component cell types. Such a separation is usually undesirable, because it is the chimeral nature of a plant which gives it its unique appearance. Therefore, propagation of chimeras via adventitious shoots rarely result in chimeral "offspring". Fortunately, axillary buds do maintain the periclinal arrangement which exists in the terminal shoot tip. Therefore, plant chimeras can be propagated by stem cuttings, leaf-bud cuttings, grafting, or budding.

Most horticulturally important chimeras have arisen spontaneously after a mutation has occurred in one or more cell layers in the shoot tip. Since spontaneous mutations rarely produce desired changes, it would be useful to develop techniques for synthesizing chimeral shoot apical meristems from two known cell types. For example, if the disease resistance of a particular plant was known to be due to epidermal characteristics, it would be beneficial for this epidermis to be "transferred" to less resistant cultivars. It would be possible to create new flower colors if the epidermis of a red-flowering cultivar was "transferred" to a yellow-flowering cultivar.

From a historical perspective, experimental techniques for synthesizing chimeras have been developed in the Solanaceae, where chimeral shoots, i.e. graft chimeras, were forced from the graft unions of grafts between tomato and nightshade. The grafting technique is limited because it is only useful for graft compatible combinations and for species which will form adventitious shoots from a graft-union. The technology of plant tissue culture has yielded a new tool for manipulating chimeral plants *in vitro*. In many species, whole plants can be regenerated from callus cultures or from cell suspensions if the appropriate media are used. Since chimeras can be regenerated from callus tissue that forms at the graft union, perhaps they can also arise *in vitro* from callus cultures composed of two genetically different cell types. This hypothesis has been tested experimentally by several researchers, but the results have been disappointing (1,5). There are, however, a few reports that such a system is feasible (2,7).

In order to develop a reliable procedure for the synthesis of plant chimeras, a model system was tested using two species in the genus *Nicotiana*. The species chosen were *N. tabacum* and *N. glauca*. These species are ideal for studying chimeral synthesis because they are graft compatible, can be easily regenerated from cell culture, and contain the necessary morphological markers, e.g. leaf color, leaf shape, flower color, leaf pubescence, etc., for early and easy identification of periclinal chimeral arrangements.

Two techniques were used to determine if interspecific chimeras could be synthesized from *N. glauca* and *N. tabacum*. The first consisted of reciprocally splice-grafting the two species. After the graft union had healed and scion growth had occurred, the scion was pruned and trimmed away, leaving a thin layer of scion cells above the graft union. Several auxin-lanolin, paste treatments were applied to encourage callus formation. While the auxin encouraged callus formation, it reduced the number of adventitious shoots which arose from the region of the graft-union. Maximum shoot production from the graft union was encouraged by the removal of all axillary buds and adventitious shoots which arose from regions other than the graft union. A few of the shoots produced were chimeras and clearly displayed leaves which were composed of cells of both species. These shoots were propagated by stem cuttings, and eventually two types of periclinal chimeras were obtained. With repeated pruning and asexual propagation, two shoots spontaneously arose and had different chimeral arrangements in their apices. A total of four of the six possible periclinal chimeras now exist. The flowers of these different chimeras are unique and possess some characteristics, e.g. colors, previously nonexistent in the genus *Nicotiana* (6) (Figure 1). Leaf shapes were also unique. Casual observations indicate that certain chimeral arrangements may be resistant to mites and to *Alternaria*, a fungal pathogen which can infect tobacco leaves.

The second technique utilized tissue culture methods and consisted of mixing callus cultures of both species (8). The "mosaic" callus masses were allowed to grow together on callus-induction medium until they appeared to be a single piece of tissue. This tissue was placed on shoot-induction medium. It was hoped that some of the organizing shoots would be chimeral. However, only nonchimeral shoots of each species were recovered. Possibly, the process of shoot formation from a graft union differs from that in cultured callus.

Recently, experiments were conducted to determine if adventitious shoots derived from chimeral leaf tissue would be chimeral or nonchimeral. Leaf discs were removed from the periclinal chimeras and placed on a medium which encour-

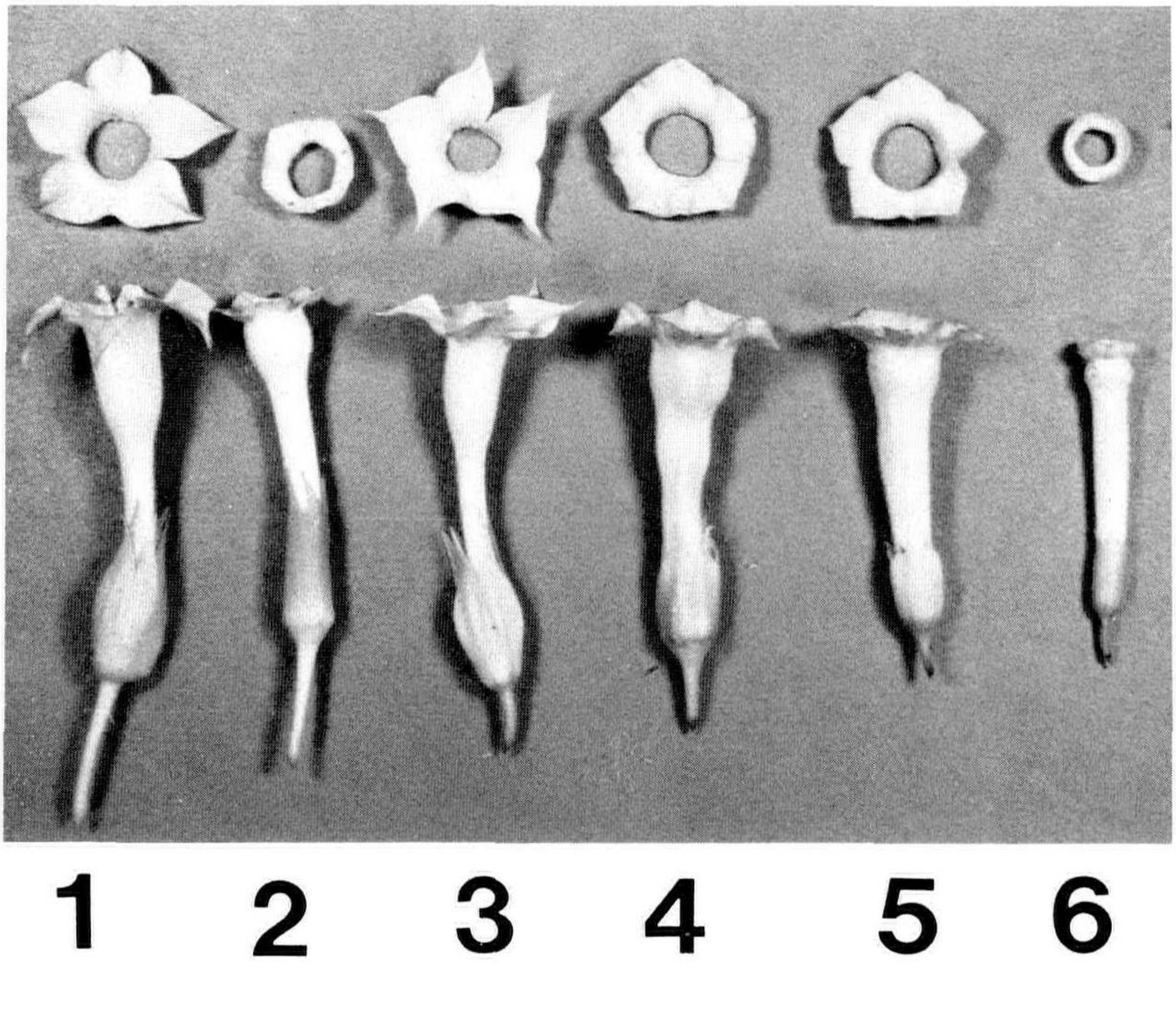

Figure 1. Flowers of *Nicotiana tabacum*, *N. glauca*, and four interspecific chimeras. 1 = Flower from *N. tabacum* with a pink corolla limb, off-white corolla tube. 2 = Flower from G-T-T chimera (actually a *N. tabacum* flower covered by an *N. glauca* epidermis). The flower is bright yellow and the corolla limb is not sharply lobed. 3 = Flower from T-T-G chimera. The flower is pink with an off-white corolla tube and yellow veins. 4 = Flowers from T-G-T chimera. Corolla tube is yellow, limb is bronze. 5 = Flowers from T-G-G chimera (actually and *N. glauca* flower with a *N. tabacum* epidermis). The flower is similar to #4 but the tube is not distorted. 6 = Flower from *N. glauca*, all yellow but not deeply lobed.

aged slight callus formation at the cut edges before the production of shoots. It was hoped that a regeneration scheme such as this would allow shoot formation to proceed quickly, thereby reducing the amount of "unmixing". "Unmixing" is known to occur when chimeral callus is allowed to grow in an undifferentiated state. Large colonies of identical cells will form (1), and this occurrence reduces the chance of recovering shoots organized from two unlike cells. After shoot regeneration, 51 of the 658 shoots recovered were chimeras. In addition, the chimeras did not always have the same appearance, i.e. periclinal arrangement, as the leaf disc from which they originated. These results are encouraging because they indicate that it

is possible to regenerate chimeras from adventitious shoots arising from tissue cultures, and that new types of chimeras can be recovered. As shown in Figure 1, each type of chimera can result in a unique phenotype. These new chimeras can be propagated by stem or leaf-bud cuttings and will rarely produce nonchimeral shoots.

CONCLUSIONS

The unique nature of many chimeral plants can make them valuable additions to horticulture. While plant breeding remains the strongest force in the development of new cultivars, the synthesis of chimeras may result in phenotypes which cannot result following conventional breeding. Yet, a greater understanding of shoot formation *in vitro* must exist before synthesis of chimeras in tissue culture will significantly contribute to the addition of valuable chimeral cultivars.

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