All Variegated Plants Are Not Chimeras

Norris *et al.* (*1*) presented data that "unequivocally support the idea of multilayer development of adventitious shoots from chimeral African violet leaf tissue" and concluded therefore that "adventitious shoots are of multicellular origin." Their conclusions, based on the recovery of variegated adventitious shoots regenerated from variegated leaves cultured in vitro, rely on the assumption that the plants used in the experiment were periclinal chimeras having different genotypes in the different histogens of the shoot apical meristem. Most seedlings of variegated African violets (for example, cv. Tommie Lou) are variegated (*2*). Periclinal chimeras would never yield variegated offspring, but rather all green or all white seedlings, depending on the genotype of the histogen that produces the eggs (the second layer of the tunica in most dicots) (*3*). Since seedlings are of single-cell origin, it is most likely that variegation pattern in African violets is due to genetic expression, as in many *Coleus* varieties (*4*), and not to genetic differences between leaf cells. Therefore, variegated adventitious shoots can be of single-cell origin.

Critical observations of leaf variegation patterns will clearly show which are due to genetic expression and which are caused by contributions in leaf ontogeny of independent and genetically different apical layers of a periclinal chimera. The cultivars used by Norris *et al.* were not periclinal chimeras. We observed free-hand sections of cv. Tommie Lou leaves at a magnification of ×10. Some leaves with green tissue in the blade had no green tissue in the petiole. Dark green areas had green palisade cells [of LII origin (*5*)], but the subepidermal cells on the abaxial side (also of LII origin) were not green. Green areas were often isolated inside white areas, and white spots appeared in green areas. There was no evidence of the independent and continuous cell lineages derived from the layers in the shoot apex, which are clearly seen in true periclinal chimeras (*6*).

Norris *et al.* regenerated more than 1000 cv. Tommie Lou plants, all of which had variegation patterns identical to the mother leaf. Even if the leaves were from periclinal chimeras, one should expect the organizing meristems of adventitious shoots to have various histogenic combinations (for example, GWG, WGW, WGG, GWW, and GGW) (*7*). In actuality, their results give unequivocal evidence that the same genetic information controlling the pattern of leaf variegation is in all cells in all layers of the leaf. Sunblade and Meyer (*8*) reported the same results as Norris *et al.* when regenerating cv. Tommie Lou in vitro, but their conclusion (namely, that genetic expression is responsible) agrees with ours. We believe, therefore, that Norris *et al.* neither proved nor disproved multicellular origin of adventitious shoots in African violets.

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References and Notes

1. R. Norris, R. H. Smith, K. C. Vaughn, *Science* **220**, 75 (1983).
2. F. Tinari, personal communication.
3. R. N. Stewart and L. G. Burk, *Am. J. Bot.* **57**, 1010 (1970).
4. D. C. Rife and C. L. Boye, *Genetics* **25**, 132 (1940).
5. LII refers to the second layer of the tunica in the shoot apical meristem.
6. R. N. Stewart, P. Semeniuk, H. Dermen, *Am. J. Bot.* **61**, 54 (1974).
7. G and W refer to green and white layers of leaf cells arising from the three layers of the dicotyledon apex. For example, GWG indicates the combination green, white, and green for the outer layer of the tunica, the second layer of the tunica, and the corpus, respectively.
8. T. M. Sunblade and M. M. Meyer, *HortScience* **17**, 532 (1982).

13 May 1983; accepted 20 October 1983

A plant chimera is an individual organism or part thereof, composed of two or more genotypically different tissues. Our report (*1*) focused on the idea that there is a multilayer development of adventitious shoots from chimeral African violet leaf tissue. This concept was supported by the large numbers of duplicated variegated African violets obtained from tissue culture by means of a direct sequence (without callus) of shoot development. In a subsequent study (*2*), growth regulator combinations that promoted callus resulted in a wide range of variegation patterns, as well as in albino plants from the white callus regions and green plants from the green callus regions. This critical observation indicates that the plants used in the study were, in fact, chimeras. If the plants were variegated as a result of pattern genes, there would have been no sorting out into pure green or pure white clones, but only more variegated progeny.

Variegated African violets used in our studies show an inheritance profile that is different from the nucleus-controlled variegation in *Coleus* (*3*). Maternal, non-Mendelian inheritance of African violet variegation has been established (*4*). If the variegated plant (cv. Tommie Lou) is the maternal parent, most seedlings are variegated. However, the reciprocal cross on a maternal green parent results in green seedlings. The reduced numbers of seedlings in the maternal variegated cross as compared with the reciprocal cross probably indicates embryonic lethals (unpublished data). These inheritance data agree with a plastome mutation and not with a "false chimera" from differential gene expression. The stability in inheritance and propagation of the hybrids of cv. Tommie Lou may be best interpreted as having a stable "mixed" heteroplastidic LII layer (the second layer of the tunica) with a dominant plastome mutant, as Vaughn and Wilson found in a chimeral *Hosta* mutant (*5*).

Our histological analysis of these chimeras did not always follow the desired, easily interpreted, independent and continous cell lineages derived from the layers of the shoot apex. Rather, it appeared that histogenic interactions were present, so that mutant tissue next to green had more normal plastids while normal tissue next to mutant had more abnormal plastids (*6*). Similar observations have been made on other chimeral forms (*7*). Although these findings complicate analysis by simple light microscopy of free-hand sections, they do not nullify the conclusion that these plants are chimeras. Indeed, intertissue interactions may be among the factors that stabilize chimeral associations.

Our conclusion is that histological, inheritance, and tissue culture regeneration data strongly support these cultivars as true chimeras and indicate that their duplication required a multilayer origin.

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References and Notes

1. R. Norris, R. H. Smith, K. C. Vaughn, *Science* **220**, 75 (1983).
2. R. H. Smith and R. E. Norris, *HortScience* **18**, 436 (1983).
3. C. L. Boye and D. C. Rife, *J. Hered.* **29**, 55 (1938).
4. O. Heeckt, *Afr. Violet Mag.* **13**, 38 (1960); L. Lyons, personal communication.
5. K. C. Vaughn and K. G. Wilson, *J. Hered.* **71**, 203 (1980).
6. R. E. Norris, thesis, Texas A&M University (1981).
7. K. C. Vaughn, *HortScience*, in press.

17 November 1983