interactions also provides the first step in determining stability of sweet potato clones with much emphasis presently on high-yielding clones with wide adaptability.

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J. Amer. Soc. Hort. Sci. 112(3):583–587. 1987.

Histogenic Instability in Tissue Culture-proliferated Strawberry Plants

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Additional index words. *Fragaria vesca*, micropropagation, chimeras, variegation, meristem culture, somaclonal variation

Abstract. **A phenotypic and sexual analysis of *Fragaria vesca* 'Albo-Marginata' determined that the leaf variegation was of chimeral origin. Stable periclinal chimeras were established in vitro from runner tips. Plants were transferred to proliferation media containing 0.5 μM IBA, 0.3 μM GA_3, and BA at either 0, 1.3, 4.4, or 13.2 μM. Whereas the histogens of field-grown runner plants remained stable, more than 90% of the plantlets propagated in vitro varied from the original explants. Most variants were albino or were green, but some were mericlinal chimeras. Histological evidence indicated that many shoots were adventitious, arising from basal callus tissue or petioles. Chemical names used: 1*H*-indole-3-butanoic acid (IBA); gibberellic acid (GA_3); *N*-(phenylmethyl)-1*H*-purin-6-amine (BA).**

In dicots, the outer two cell layers or histogens (the LI and LII or "tunica") of the shoot apical meristem usually remain independent from each other and from the inner body (LIII or "corpus") (21). This development occurs because the planes of cell division in the tunica are anticlinal, with periclinal divisions being rare, and, if occurring, causing the occasional replacement of cell layers in the shoot apical meristem (23). A periclinal chimera possesses distinctly different genotypes in one or more complete layers of the shoot apical meristem. Chimeras can arise whenever a spontaneous or induced genetic change occurs in an apical initial cell in a histogen of a shoot apical meristem. They may become obvious, as with the appearance of patterned variegation (20), or with a change in surface features, such as thornlessness (5). However, not all genetic changes are expressed phenotypically. Undoubtedly, chimeras exist that appear identical to their nonchimeral "parent" but differ in subtle physiological and biochemical ways. Using tissue culture, Bush et al. (3) dissociated flower petals of the chimeral cultivars of 'Indianapolis' chrysanthemums into their component genotypes. They suggested that some of the regenerated variants that arose did so because of a rearrangement of chimeral cell layers that possessed genetic differences in addition to those for color.

The interaction of adjacent histogens composed of dissimilar

Received for publication 14 Aug. 1986. Publication no. 2826 of the Massachusetts Agricultural Experiment Station. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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genotypes can result in properties that differ from either homogeneous component of the chimera. This occurrence has been noted in interspecific chimeras (9) and in certain periclinal chimeras of carnation (15). Carnation petal mesophyll (LII), with a phenotypically unexpressed red genotype, influences the synthesis of pigments in the epidermal layer (LI) and results in unique petal pigmentation (15). Also, cytochimeras (those in which the ploidy levels between histogens differ) may not be evident macroscopically yet can occur spontaneously (6) or can be induced (18).

Since axillary buds have the same histogenic arrangement as the terminal shoot apical meristem (16), valuable periclinal chimeras are propagated asexually by stem or leaf-bud cuttings in order to maintain the histogenic integrity of the shoot apical meristem. Adventitious shoots are generally nonchimeral (2), or, on rare occasions, are chimeral, but can possess altered histogenic arrangements (10, 24). Since field-grown strawberry plants are propagated from runners (axillary shoots), some cultivars may exist or arise as periclinal chimeras, originating after a spontaneous mutation occurs in a single histogen of the shoot apical meristem of a seedling or runner plant. These cultivars would remain histogenically stable if propagated by runners. However, tissue culture propagation of strawberries is becoming increasingly common in Europe and North America. This research was initiated to determine the effect of conventional tissue culture propagation techniques on the stability of the histogens in the shoot apical meristem of tissue culture-proliferated strawberry plants.

Materials and Methods

Plants of the variegated *Fragaria vesca* 'Albo-Marginata' L. (Fig. 1A) were obtained from Logee's greenhouses, Danielson, Conn. To determine if this cultivar is chimeral (i.e., contains genetically different histogens) or if its variegation is due to genetic expression, isolated plants were self-pollinated and the seedling population was analyzed. Microscopic examination of epidermal peels and free hand sections of leaf tissue were used to determine the phenotypic constitution of the derivatives of the histogens of the shoot apical meristem.

'Albo-Marginata' plants were established in vitro by sterilizing runner tips for 1 min in 0.5% sodium hypochlorite [10% (v/v) Clorox] plus 5 drops of surfactant (Tween 20) per 100 ml. Shoot apices ranging in size from 0.6 to 5.6 mm (mean, 3.2 mm) were excised and placed in 30-ml bottles containing 10 ml of medium. Medium was identical to that employed by Boxus (1), except that 40 $g \cdot liter^{-1}$ glucose was replaced by 30 $g \cdot liter^{-1}$ sucrose. Initially, the establishment medium contained no hormones. However, only seven of 194 shoot tips (3.6%) grew into plants. When medium containing 0.23 μM *N*-(2-furanylmethyl)-1*H*-purin-6-amine (kinetin) was used, the survival rate increased to 23.2%. This kinetin level allowed the establishment of plants without causing shoot multiplication. Eighteen weeks after shoot-tip excision, roots were removed, and well-developed shoots were placed one in each 120-ml wide mouth glass bottle containing 30 ml of sucrose-amended Boxus medium formulated with 0.49 μM IBA, 0.3 μM GA_3, and either 0, 1.3, 4.4, or 13.2 μM BA. There were five replications per treatment. After two 5-week subcultures, proliferated shoots were separated and transferred to rooting medium consisting of sucrose-amended Boxus medium plus 0.49 μM IBA. All cultures were maintained at 25°C in growth chambers equipped with cool-white fluorescent light (20 to 24 $\mu mol \cdot s^{-1} \cdot m^{-2}$) set on a 16-hr light : 8-hr dark cycle.

After shoots were rooted, data were collected by visually observing plantlets. Observations of epidermal peels were used to determine the phenotype of the LI. The LII genotype was determined easily by the presence of white or green leaf margins. The color of the inner mesophyll region determined the LIII genotype. In instances where the LII was green, transmitted light was used to determine if the underlying LIII was white (Fig. 1C). Plants were considered mericlinal chimeras if part of a leaf or a rosette of leaves was variegated while the remaining leaves were all white or all green (Fig. 1D).

To determine the origin of shoots, permanent slides of histological sections of shoot cultures were obtained using a standard dehydration series of ethanol and *tert*-butyl alcohol, paraffin embedding procedures, and safranin 0 and fast green staining solutions (0. Stein, personal communication).

Results and Discussion

All seedlings derived from self-pollinating 'Albo-Marginata' were albino, indicating that the LII, the histogen from which pollen and eggs of a dicot are derived normally (22), is genetically albino. Microscopic examination of epidermal peels verified the presence of green chloroplasts in the guard cells. Therefore, the LI of 'Albo-Marginata' is genetically green. Additional verification of the LI genotype arose when a single leaf on a field-grown plant exhibited a rare periclinal division of the LI on its margin (Fig. 1B). The inner leaf mesophyll, which is derived from the corpus (LIII) in dicots (22), is green on 'Albo-Marginata'. Therefore, the histogenic composition of the periclinal chimera 'Albo-Marginata' is GWG (Green-White-Green, LI, LII, and LIII, respectively).

The phenotype of the LI could not be determined reliably on plants grown or regenerated in vitro. Even the albino seedlings derived from self-fertilized 'Albo-Marginata' plants had light-green to green chloroplasts in their guard cells when they were grown in vitro. Tissue culture conditions appeared to allow normal chloroplast development in guard cells of plants possessing genetically defective chloroplasts. Therefore, on plants that were white, no determination of the LI phenotype could be made.

The in vitro proliferation of strawberry plants under the influence of BA led to significant changes in the histogenic composition of regenerated plants (Table 1). In the absence of BA, there was only a slight increase in plant number, and all plants were GWG. These plants presumably arose from axillary buds. However, as little as 1.3 μM BA resulted in 86.9% of the recovered plantlets differing histogenically from the original GWG explant (Table 1). The highest percentage of variants were homogeneous, being either all-green or all-white (Table 2). Since periclinal chimeras other than the original GWG type were not recovered (Table 2), it is unlikely that any of the ''white'' variants were periclinal chimeras of the GWW type. In addition, no new periclinal arrangements were recovered.

Homogenous variants could have arisen as adventitious shoots. Strawberry plants can be formed in vitro from adventitious meristems. Anthers (14, 17), meristem callus (13), petioles (as seen in our lab), and leaves (R.H. Zimmerman, personal communication) have given rise to whole plants in vitro.

Histological sections (Fig. 2) of shoot cultures indicated that at least some of the new shoots were not of axillary origin. The adventitious shoots arose from the basal callus regions of the shoot culture as well as from petiole tissue. Some shoots arose from vitrified leaf and petiole tissue (Fig. 2B). Such adventitious shoots would not be chimeral if they arose from single

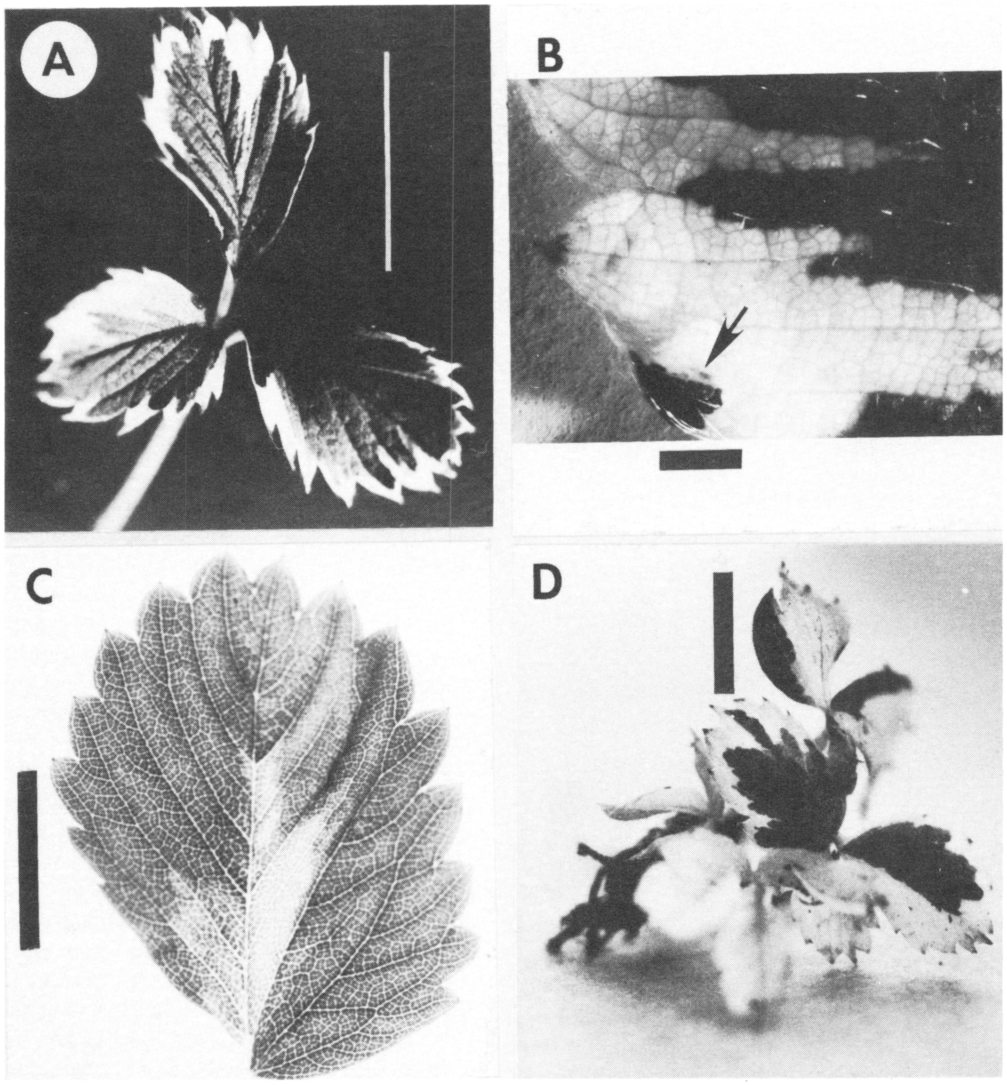

Fig. 1. **(A)** Typical leaf of *Fragaria vesca* 'Albo-Marginata'. Bar = 2.5 cm. **(B)** Green island (arrow) on leaf margin of 'Albo-Marginata'. The island is due to the expression of the genetically green epidermis (LI) now displaced into an otherwise albino mesophyll (LII). The cells express their genetic potential for chlorophyll production. Bar = 0.2 cm. **(C)** Green-Green-White leaflet taken from a mericlinal variant. Lighter inner region is the albino inner mesophyll (LIII) surrounded by green outer mesophyll cells (LII). Photograph taken with transmitted light. Bar = 1.0 cm. **(D)** Mericlinal strawberry chimera. Half of the plant is albino. Bar = 0.5 cm.

Table 1. Percentage of tissue culture-propagated shoots differing in histogenic composition from the original explant as influenced by BA level in media. There were five shoots per treatment.

BA (μM)	No. plants identical to original explant	No. variants	Variants (%)
0	8	0	0
1.3	20	133	86.9
4.4	14	296	95.5
13.2	8	124	93.9

cells or groups of similar cells, i.e., derivatives of a single histogen of the shoot apical meristem.

Homogeneous variants also could have arisen as axillary shoots from homogeneous sectors of mericlinal variants or as axillary shoots from previously formed homogeneous variants. The mericlinal chimeras that were recovered could have arisen as adventitious shoots of multicellular origin. The occurrence of such chimeras is well-documented (12). However, most of the mericlinal shoots maintained a GWG sector. A shift in the position of the rapidly dividing apical initial cells of the shoot apical meristem could have caused a lateral or periclinal displacement of some initial cells in a particular histogen. This phenomenon has been noticed on periclinal chimeras growing in vivo (23). Displacement may be encouraged by the high rates of meristematic cell division caused by the imposed hormonal regimes. The appearance of clustered meristematic apices easily could disrupt the usual anticlinal cell-division planes in the outer layers of the shoot apical meristem.

The instability of histogens in vitro is in sharp contrast to the stability of field-grown 'Albo-Marginata' plants. Axillary shoots of strawberries appear to possess histogenically stable shoot apical meristem initials. Runner-produced 'Albo-Marginata' plants have yielded only one variant (homogeneous green) in 10 years of field production (R.M. Logee, personal communication). The only form of variation that we have noticed in greenhouse-grown plants is the occasional production of an all-green or all-white

Table 2. Mean (± SE) and histogenic composition of tissue culture-proliferated strawberry plants as influenced by the BA level in the proliferation media

	No. plants								
	Normal	Variants[z]							
μM BA	GWG	?WW[y]	GGG	GWW	GGW	WGG	WGW	WWG	Mericlinal
0	1.6 ± 0.3	0	0	0	0	0	0	0	0
1.3	4.0 ± 1.3	6.2 ± 5.0	17.2 ± 6.7	0	0	0	0	0	3.2 ± 1.3
4.4	2.8 ± 0.7	18.4 ± 7.3	36.2 ± 12.6	0	0	0	0	0	4.6 ± 2.6
13.2	1.6 ± 1.3	4.8 ± 1.1	15.4 ± 4.6	0	0	0	0	0	4.6 ± 1.8

[z]With the exception of lethal "white" variants, data were collected after establishment in soil in a greenhouse. The original explants are included in the values.
[y]The "?" refers to the uncertain phenotype of the LI.

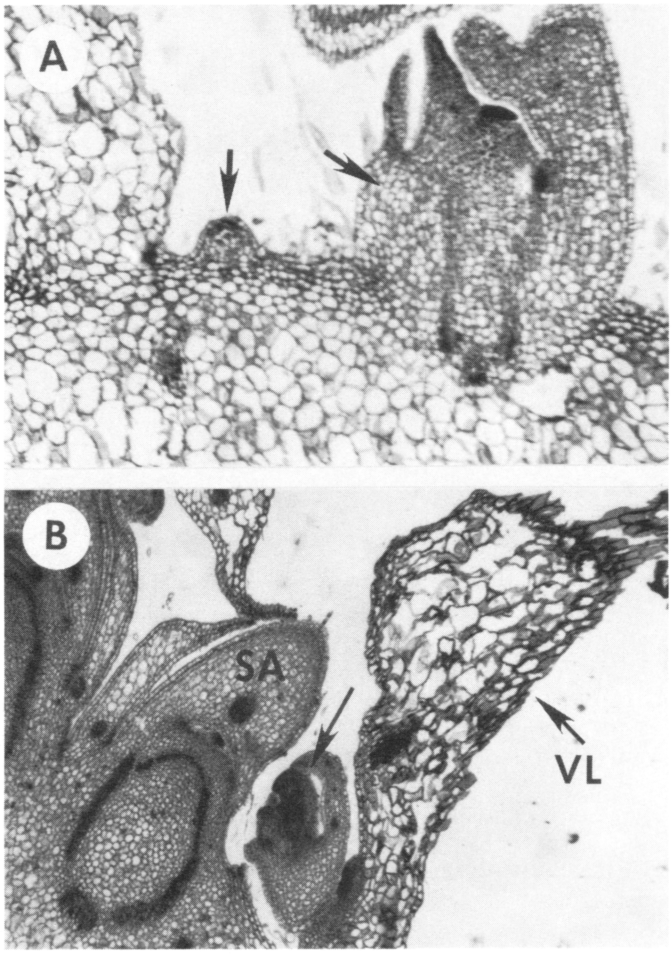

Fig. 2. Histological sections of strawberry shoot cultures 1 month after a serial subculture on medium containing 13.2 μM BA. (**A**) Adventitious shoots (arrows) arising from basal callus. (**B**) Adventitious shoot (arrow) arising from a vitreous leaf (VL). SA = shoot apex of the shoot that gave rise to the vitreous leaf with its adventitious shoot.

leaflet on an otherwise GWG trifoliate leaf. This variation apparently occurs during leaf development and not in the shoot apical meristem since runners produced from these plants continue to produce stable GWG periclinal chimeras. In addition, meristem (shoot) tips established in culture always produced stable GWG plants on media not conducive to shoot multiplication.

Mechnical fragmentation of shoot apices has led to changes in histogenic stability of plants regenerated from the chimeral grape cultivar 'Meunier' (19). This study agreed with ours in that field-grown chimeral plants rarely produced shoots that are nonchimeral, while tissue-cultured plants produced numerous variants. Similar results were also obtained with chimeral thornless blackberries, where both chimeral and homogeneous plants were recovered in vitro (11). In vitro separation of chimeras also has been demonstrated in ornamentals (4, 7, 8).

Tissue culture techniques appear to be the most rapid means of separating chimeral strawberry plants into their component genotypes. When using mutation breeding techniques, chimeras frequently arise because all of the apical initials of existing meristems are not mutated. Propagation of such plants by tissue culture would allow for the recovery of homogeneous mutants.

The histogenic instability of tissue culture-proliferated strawberry plants will cause variants only if the original meristem is chimeral or cytochimeral. Our results offer an explanation for the occurrence of two distinctly different populations arising from an apparently homogeneous explant. In future studies, serious consideration must be given to the possibility that seemingly induced variation can be caused by histogenic instabilities in chimeral explants and not by genetic instabilities resulting from tissue culture techniques.

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