sion percentage ranging from 70 to 80%. However, the highest leaf abscission (80 to 82%) occurred at pH 5 and 6 when 50 mM buffers were used with the abscission percentage decreasing considerably as pH increased, reaching a minimum at pH 8. It appears that ethylene is lost too rapidly in solutions above pH 6 to effectively cause abscission.

Discussion

The property of CGA 13586 as a releaser of ethylene results from its great reactivity with water, especially at high pH levels. The initial rate of ethylene evolution is much higher than that from ethephon. The rate of evolution per mole at a pH near 6 was found to be about 50 × as great as the rate from ethephon, the latter based on data of Warner and Leopold (5); the ratio is still higher at pH values above or below 6.

Hydroxyl ions are consumed and chloride ions are released when CGA 13586 reacts with water (4). This leads to acidification of the solution as the reaction proceeds and a consequent decrease in the rate of the reaction. Any excess HCl remaining during evaporation of a spray solution may be expected to volatilize and be lost along with the water.

CGA 13586 induces greater fruit abscission in olives but lower leaf abscission than ethephon or cycloheximide (2). The causes of this difference, as studied by ethylene evolution from fruits and leaves is not clear (Fig. 2). Initial rates of ethylene release from both fruits and leaves sprayed with CGA 13586 are much higher than those from fruits or leaves sprayed with ethephon but the ethylene output from plant parts treated with CGA 13586 decreased rapidly and is lower than from those treated with ethephon after 3 to 5 days. It is possible the prolonged presence of ethylene following ethephon sprays is more effective in causing leaf abscission than the shorter duration of high ethylene resulting from CGA 13586 with a much lower rate of ethylene production (2). Ethylene produced inside the tissue (as is very probably the case for the injury-stimulated ethylene from CHI) is probably effective in promoting abscission. Ethylene-releasing substances, such as CGA 13586 and ethephon, on the other hand, may release a large proportion of the ethylene at the tissue surface where much of it is lost to the atmosphere.

CGA 13586 shows promise as a suitable material for use in mechanical harvesting procedures with California table olive cultivars, since the ethylene released causes a marked reduction in fruit removal force and significant increases in fruit removal percentages following tree shaking. Some leaf removal occurred in the tests reported here but it was not excessive.

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Tissue Culture-induced Variation in Scented *Pelargonium* spp.[1]

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Additional index words. plant breeding, scented geranium, chimera, polyploidy

Abstract. In vitro **techniques were developed to regenerate plantlets (calliclones) from callus of scented geraniums (*Pelargonium* spp.). Calliclones were compared to plants derived from stem, root, and petiole cuttings of 5 cultivars. Plants from stem cuttings of all cultivars were uniform and identical to the parental clone. Plants from root and petiole cuttings were more variable with the amount of variation dependent upon cultivar. High variability was associated with calliclones. Aberrant types included changes in plant and organ size, leaf and flower morphology, essential oil constituents, fasciation, pubesence, and anthocyanin pigmentation. Calliclone variation was dependent upon clone and age of callus. Variability in calliclones was due to segregation of chimeral tissue, euploid changes, and heritable changes which may involve individual chromosomal aberrations or simple gene mutations. Variability of calliclones might be exploited for improvement of vegetatively propagated crops especially highly polyploid, sterile lines.**

Crop improvement is based on the creation of variable populations from which superior genotypes are isolated. Traditional plant breeding is dependent upon the sexual cycle, i.e., variation in the population is released and stored through the processes of meiosis and fertilization. Experience has shown that crop improvement utilizing this system may be remarkably efficient at first, but genetic advances become increasingly difficult with time. Certain unique combinations seem difficult to improve.

Dependence on the sexual system may impose a restraint to genetic improvement, especially severe for polyploid, vegetatively-propagated crops where the economic portion is not the seed. Crops cultured for their vegetative organs might be rendered more efficient by the complete elimination of the sexual apparatus, mainly as a conservation of biological function and energy. Further, the sexual system is difficult to control. Mild changes cannot be obtained except by backcross procedures which are time-consuming and unsuitable to many genetic systems. Changes obtained by mutagenic agents are random and the resulting plants are often chimeral. Desirable changes accompanied by a defective sexual system usually become lost or unavailable. Thus, genetic improvement is not directly available for completely sterile plants; the only variations that occur in these types are due to selection within spontaneous mutations (sports). Further, in polyploids, genetic gain is slow and

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efficiency of traditional breeding techniques is low.

Variability has been an ubiquitious feature in tissue culture (5, 8, 11, 15, 17) and has been thought a handicap to many physiological and genetic studies. Exploiting cellular variations through tissue culture may provide a new means for plant improvement. This can be achieved by imposing selection on variable populations of cells in culture that may arise naturally or be induced by mutagenic agents with the eventual derivation of superior sporophytes from a single cell.

The objective of this study was to investigate the variability associated with tissue culture of various clones of geranium in order to determine the feasibility of such a system to improve vegetatively-propagated crop plants.

Materials and Methods

Techniques for producing plants from callus culture (calliclones) were modified from Pillai and Hildebrandt (10). Disinfection was best obtained by soaking explants in 0.525% sodium hypochlorite (10% Clorox) for 20 min followed by two 5 min washes in sterile distilled water. Callus production was induced in the dark at 25°C on Murashige and Skoog (MS) medium (9) supplemented with NAA (1 ppm), kinetin (5 ppm), adenine (40 ppm), and vitamin supplement obtained from E. J. Staba, Univ. of Minnesota. Amounts per liter = cyanocobalmin (1.5 μg), folic acid (0.5 mg), riboflavin (0.5 mg), biotin (1 mg), choline chloride (1 mg), Ca pantothenate (1 mg), thiamine HC1 (1 mg), nicotinamide (2 mg), and pyridoxine HC1 (2 mg). Although there were differences between cultivars, shootlet production was best with Pillai and Hildebrandt's (10) modification of MS medium supplemented with NAA (0.1 ppm) and kinetin (10 ppm) under diffuse light (16 hr day) at about 25°C. Rooting occurred when young shootlets were transplanted to White's medium (16) without hormones but supplemented with iron (37.3 mg/liter Na_2EDTA and 27.8 mg/liter $FeSO_4 \cdot 7H_2O$). Calliclones grew rapidly in the greenhouse.

Variability of calliclones was compared with plants derived from stem, root, and petiole cuttings from the same parental source. Stem sections placed in sand or peat-perlite mix rooted within 2 weeks under mist. Root suckers appeared from the surface of pots or bench especially when roots attached to the plant were exposed to light (Fig. 1). Petiole cuttings rooted in sand or a peat-perlite mix (Fig. 2) and shoots were induced on the callus mass that formed on the cut end of the petiole.

Fig. 1. 'Rober's Lemon Rose' (parental *lobed*) with a *dentated* root sucker. 'Rober's Lemon Rose' is obviously a periclinal chimera.

Fig. 2. Shoot forming from petiole cutting of 'Rober's Lemon Rose' showing *dentated* foliage from *lobed* phenotype.

Propagule variability. Plant types were grouped on the basis of gross morphological changes. Three leaf measurements were obtained from each plant (Fig. 3) to derive 3 parameters: *leaf size* (length × leaf width), *leaf shape* (length/leaf width), and *leaf form* (leaf width/waist width). All measurements were obtained from the 4th, 5th, and 6th fully expanded leaf from the apex of each plant.

Ploidy was estimated by measurements of stomates, nuclei, pollen grains, and direct chromosome counts of root tips. Stomatal size was determined by averaging 10 measurements

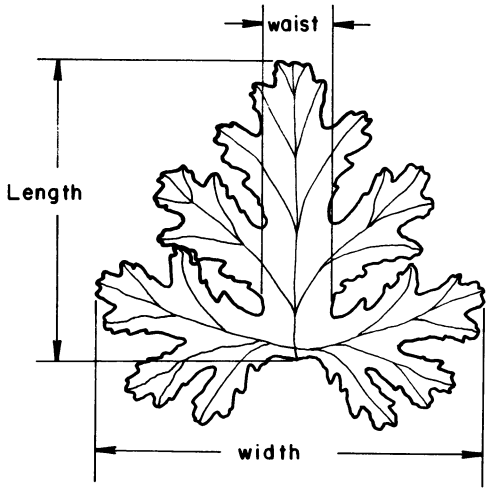

Fig. 3. The three leaf measurements taken from each plant.

from the lower epidermis of the 4th fully expanded leaf of each plant. Nuclear size in histogenic layers was determined on sectioned stem apices stained with safranin and fast green. Pollen viability was determined after aceto-carmine staining and size from the diameter of at least 10 grains. Chromosome counts were made from root tips pretreated with paradichlorobenzene, fixed in 1 acetic acid:3 alcohol, and softened in 1 N HCl for 10 min prior to staining with aceto-carmine. Observations and measurements subsequently made on specific clones included phyllotaxy, anthocyanin pigmentation, variegation, pubescence, gland size, leaf hair length, insect infestation, and flower morphology. Essential oil determinations were made after extracting epidermal glandular oils from 1 g of leaf tissue by grinding in a tissue homogenizer with 3 ml of distilled water and then shaking the liquid with 1 ml of petroleum ether. The two immiscible solutions formed an emulsion which was separated by centrifugation (2000 rpm for 10 min). The ether phase was characterized with a Hewlett-Packard 402 high efficiency gas chromatograph.

Results

There were large differences in the ability of *Pelargonium* species and cultivars to produce calliclones. None of 10 cultivars of *P. hortorum* Bailey, formed plantlets. However, *P. domesticum* Bailey cv. Country Girl and 5 of the 6 scented geraniums ['Rober's Lemon Rose' (*P. graveolens* Thunb.), 'Clorinda' (*P. domesticum* x *P. denticulatum* Poir.), 'Attar of Rose' (*P. capitatum* (L.) L'Heritier ex. Aiton), 'Old Fashioned Rose' (*P. graveolens* Thunb.), 'Snowflake' (*P. adcifolium*), and 'Lemon' (*P. crispum* L'Her.)] produced calliclones readily.

'Rober's Lemon Rose'

'Rober's Lemon Rose' (RLR) originated as a seedling about 1950 by Mr. Ernest Rober (Personal communication: Howard Wilson, Wilson Brothers Nursery, Inc., Roachdale, Indiana; Fred Bode, Southern California Geranium Gardens, Encintas, California.) The plant is semi-erect; leaves are asymmetrical with finger-like, non-dentated lobes and is rose-scented with a secondary lemony fragrance; flowers have small twisted petals, 2-parted stigma, reduced anthers and are ♀ and ♂ sterile.

Plants derived from stem cuttings were uniform but those from root cuttings, petiole cuttings, and calliclones (with a single exception) were morphologically distinct from the parental clones herein designated *lobed* (Table 1, 2). The propagules segregated into 6 morphological types (Fig. 4). Plants from root cuttings were all *dentated.* Plants from petiole cuttings were either *dentated* (94%) or *hairy* (6%). The 166 calliclones were either *lobed* (0.6%), *dentated* (83.7%), *hairy* (12.6%), *dwarf* (1.8%), *flat leaf* (0.6%), or *hairy dwarf* (0.6%).

Observations made on other propagule characteristics are summarized in Table 3. Variation in phyllotaxy was observed in some calliclones with a change from 2/5 to 1/2 preceding flowering. Subsequent studies, however, indicated that this change was common in RLR and all of its propagules. A similar phyllotaxy change was reported by Abo El-Nil and

Table 1. Phenotypes and leaf measurements of propagules of the parental clone (*lobed*) of 'Rober's Lemon Rose' scented geranium.

Propagule *Phenotype*	n	Leaf measurements: Size (mm^2) (lxw)	Shape (l/w)	Form (w/waist)	Stomate size (μ)
Stem cuttings					
Lobed	15	36.2 ab[z]	0.69 cd	10.4 c	18.0 b
Root cuttings					
Dentated	29	58.1 b	0.61 a	6.8 b	16.9 a
Petiole cuttings					
Dentated	29	39.9 ab	0.64 ab	6.4 b	17.5 b
Hairy	2	35.6 ab	0.66 ab	4.0 a	23.0 ef
Calliclones					
Lobed	1	34.9 ab	0.68 bcd	12.1 d	16.7 a
Dentated	139	53.5 b	0.65 ab	6.6 b	18.4 c
Hairy	21	58.7 b	0.64 ab	4.4 a	24.7 f
Hairy dwarf	1	38.9 ab	0.65 abc	3.7 a	21.7 e
Dwarf	3	16.3 a	0.74 e	3.6 a	19.2 d
Flat leaf	1	60.6 b	0.73 de	4.0 a	19.0 cd

[z]Mean separation within columns by LSD, 5% level.

Table 2. No. of propagules significantly different from either *lobed* stem cuttings or *dentated* root cuttings of 'Rober's Lemon Rose' scented geranium.

Propagule *Phenotype*	n	No. plants significantly different (5%): Leaf size	Leaf shape	Leaf form	Stomate size	Total plants
		from *lobed* stem cuttings				
Root cuttings						
Dentated	29	10	8	1	0	16
Leaf cuttings						
Dentated	29	0	1	1	0	2
Hairy	2	0	0	2	2	2
Calliclones						
Lobed	1	0	0	0	0	0
Dentated	139	19	0	5	5	29
Hairy	21	2	0	17	21	21
Hairy dwarf	1	0	0	1	1	1
Dwarf	3	0	0	3	0	3
Flat leaf	1	0	0	1	0	1
		from *dentated* root cuttings				
Petiole cuttings						
Dentated	29	0	0	0	0	0
Hairy	2	0	0	0	2	2
Calliclones						
Lobed	1	0	0	1	0	1
Dentated	139	1	0	4	5	10
Hairy	21	1	0	0	21	21
Hairy dwarf	1	0	0	0	1	1
Dwarf	3	1	1	0	0	2
Flat leaf	1	0	0	0	0	0

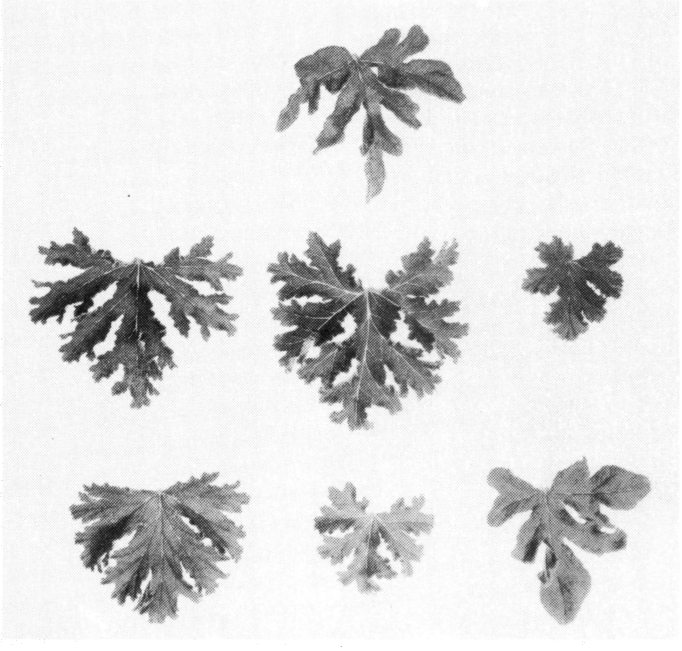

Fig. 4. Leaf phenotypes of 'Rober's Lemon Rose' and its calliclones: (Top to bottom, left to right) parental *lobed; dentated, hairy, dwarf* calliclones; *flat leaf, hairy dwarf, lobed* calliclones.

Table 3. Comparison of phenotypes derived from propagules of 'Rober's Lemon Rose' scented geranium.

Variable	Parental (*lobed*)	Calliclones					
		Lobed	*Dentated*	*Hairy*	*Hairy dwarf*	*Dwarf*	*Flat leaf*
Chromosome number	72	72	72	144	–	72	72 (?)
Stomate length (μ)	18.0	16.7	18.4	24.7	21.7	19.2	19.0
Flower							
Relative size	normal	normal	normal	large	–	normal	normal
No. stigma parts	2	2	5	5	–	5	5
Pollen							
Volume (μ^3 x 1000)	363	371	296	742	–	280	180
Viability (%)	0	0	0	56	–	0	0
Hair length (mm)	.53	.49	.54	.66	.69	.47	.51
Gland size (μ)							
height	27	–	30	34	–	27	25
width	14	–	15	16	–	14	16
No. major peaks by GLC	3	3	3	3	–	3	2
Plant ht (cm)	–	–	92	97	54	60	70

Hildebrandt (1) in 'Lady Ester' garden geranium. The morphology and origin of the calliclone phenotypes follow.

Dentated. This is a normal sized plant but leaves are symmetrical and highly dentated. Flowers are similar in size and are sterile as *lobed* but petals are smooth with a 5-parted stigma, typical for scented geraniums (Fig. 5).

The occurrence of *dentated* from root and petiole cuttings indicated that RLR is a periclinal chimera with the *dentated* character present in internal tissues (Fig. 1 and 2). Using the *lobed* stem cuttings as standard, 165 of the 166 calliclones were phenotypically different; using *dentated* root cuttings as standard reduced the variant phenotypes to only 27 of the 166 calliclones. However, 10 of the *dentated* calliclones had leaf measurements significantly different from the root cutting means (Table 2).

Hairy and Hairy Dwarf. Hairy plants, although originally selected on the basis of thicker, silvery-green leaves, resembled *dentated* in most characters. They were distinguishable from *dentated* by large stomates, larger size of leaves, hairs, glands, and pollen. (The single *hairy dwarf* resembled *hairy* but had smaller leaves with a change in leaf form value.) These gigas characters suggested that *hairy* was a polyploid of the *dentated* form of RLR. Subsequent root tip chromosome counts of RLR confirmed *P. graveolens* as 2n = 72 (6) and the polyploid nature of *hairy*, 2n = 144.

The flowers of *hairy* resembled *dentated* in form with a 5-parted stigma but had distinctly larger petals (Table 3, Fig. 5). However, the anthers borne on extended filaments were bright orange that dehisced normally with abundant pollen. Of the pollen, 56% stained normally with aceto-carmine as compared to 0% for all other forms of RLR. Selfed flowers have produced seed and a small percentage are viable.

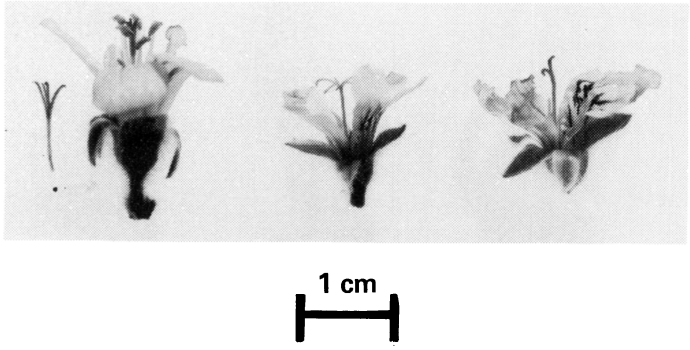

Fig. 5. Flower morphology of 'Rober's Lemon Rose' (right to left): parental *lobed* with 2-parted stigma and irregular petals, *dentated* with 5-parted stigma, and *hairy* with 5-parted stigma and prominant extended anthers containing stainable pollen.

The sterile nature and high chromosome count of both the parental *lobed* and *dentated* forms suggests that RLR is a sterile interspecific hybrid. Thus, polyploidy might be expected to increase fertility. Tamai and co-workers (12, 13) have shown that artificially-produced tetraploids of sterile clones of *P. roseum* (2n = 77) and *P. denticulatum* (2n = 88) exhibited similar gigas characteristics of pollen, stomata, hairs, and glands with fertility partially restored.

The *hairy* type arose from petiole cuttings of RLR with a frequency of 6% and from calliclones with a frequency of 9%. One explanation is that the *lobed* form of RLR is a cytochimera for 2n and 4n tissue. To confirm this, nuclear size was determined in histogenic layers of the apical meristem of clones of *lobed, dentated, dwarf,* and *hairy* using paraffin sections. The nuclei size in all histogenic layers of each clone were of similar magnitude indicating that none of these clones were cytochimeral. However, the nuclear volume of the *hairy* plant was almost twice that of the others ($356\mu^3$ vs. $195\mu^3$).

These results indicated that *hairy* and, no doubt, *hairy dwarf* are due to spontaneous chromosome doubling. This doubling occurred in petiole cuttings and calliclones but not from root suckers. Both calliclones and leaf cuttings involve extensive callus proliferation and spontaneous doubling probably occurs at this stage.

Flat leaf. The *flat leaf* had round leaves that tended to lie in a single plane. The plants were dwarfed and appeared to be unusually insect free. One cutting of the original *flat leaf* flowered. Flowers have a 5-parted stigma, and reduced anthers as does *dentated* but petals do not enlarge normally at anthesis. Pollen size, however, was much smaller than any other type (Table 3). This phenotype is suggestive of chromosome loss.

Flat leaf, unlike the other aberrant types, had a somewhat different odor from RLR. Gas chromatographic analysis was made of the parental *lobed* and the first 51 calliclones obtained from tissue culture which included the parental *lobed* (1 plant), *dentated* (45 plants), *hairy* (1 plant), *dwarf* (3 plants), and *flat leaf* (1 plant).

The *lobed* clone produced 3 major peaks: I (t_R = 0.13), II (t_R = 0.24), and III (t_R = 0.34) and a number of variable secondary peaks (Fig. 6). Peaks II and III were tentatively identified as α-terpineol and geraniol on the basis of retention time (4). Peak I was not identified but had a retention time near that of menthone (a mixture of dl-menthone and dl-isomenthone) which is a major constituent of *P. roseum* x *P. denticulatum* hybrids (14).

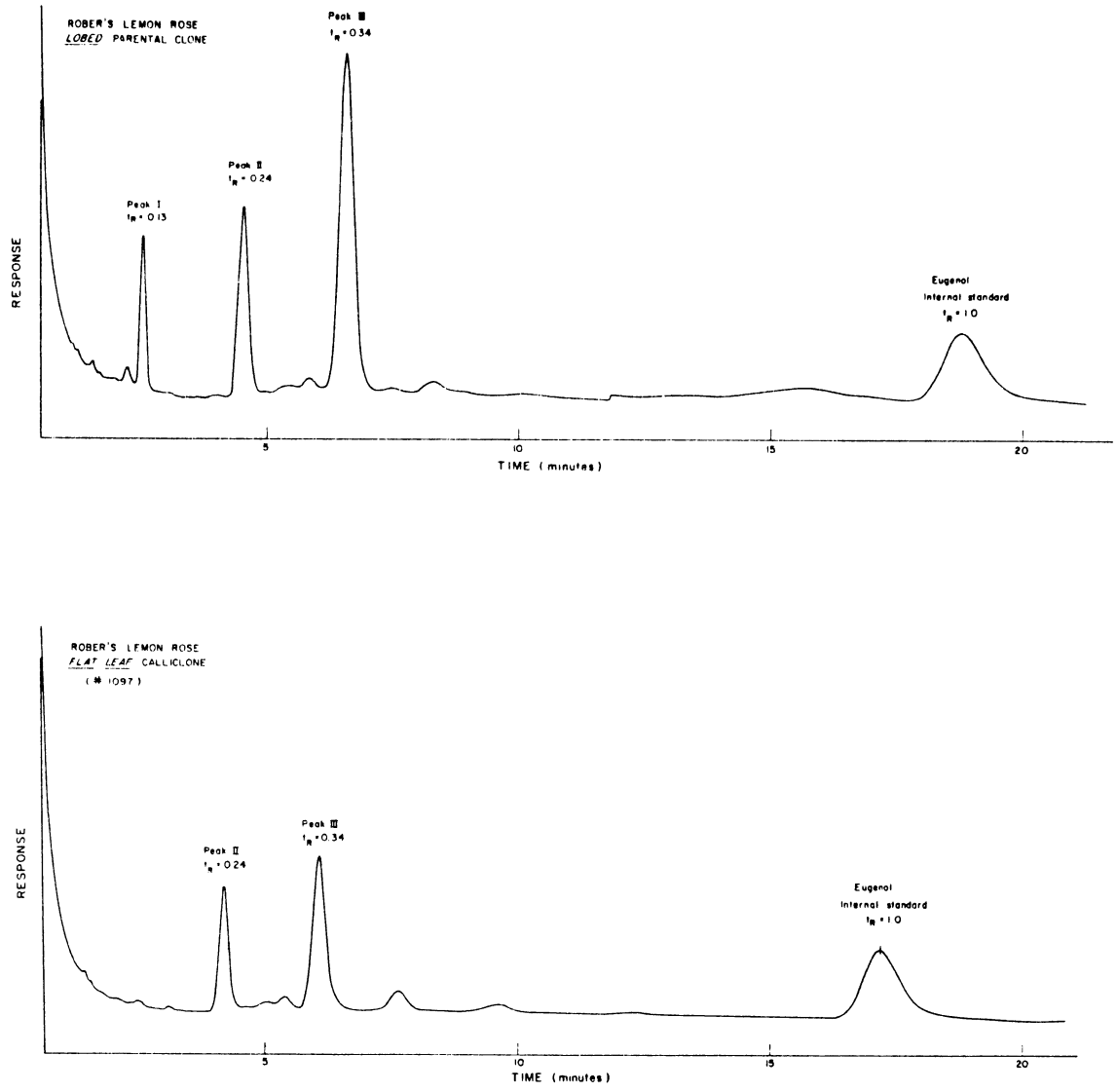

Fig. 6. The essential oil peaks of 'Rober's Lemon Rose' scented geranium compared to its *flat leaf* calliclone.

All of the calliclones except for *flat leaf* were identical to the parental phenotype with regard to these 3 major peaks. The *flat leaf* clone consistently lacked Peak I. Our conclusion is that this lack of a major essential oil peak represents a stable heritable change.

Dwarf. Dwarf plants had significantly smaller leaves than the other types; leaves were also roundish with a wide waist. Serveral cuttings of one of the *dwarf* calliclones flowered. Floral morphology resembled *dentated* with symmetrical petals, 5-parted stigma, and undeveloped anthers. Pollen size was smaller than that of *lobed* but larger than *flat leaf. Dwarf* did not appear in petiole or root cuttings and appears to represent a sudden heritable change.

Lobed. The single *lobed* calliclone resembled the parent clone for leaf and flower morphology and stomate length. This calliclone may have been derived from a bud on the stem tissue explant, may represent an isolate from the cells of the epidermis (L_I), or may be chimeral arising from cells of 2 or more layers but may or may not be identical to the parental clone. Subsequent calliclones from this selection gave only *dentated* and *hairy* phenotypes (Table 4); the *lobed* phenotype did not reappear. This proves that the *lobed* calliclone was perichimeral as the parental clone. The true origin remains unknown; the possibility is strong that it arose from an axillary bud.

Calliclones stability. A 2nd cycle of calliclones was produced to determine the stability of the aberrant phenotypes produced from tissue culture. Calliclones derived directly from the parental clone were designated the C-1 generation, calliclones derived from tissue culture of C-1 individuals were designated the C-2. C-2 segregation derived from *lobed, dentated, hairy, dwarf,* and *flat leaf* are compared to C-1 segregation in Table 4.

Of 27 C-2 clones derived from the single C-1 *lobed*, 2[illegible]

Table 4. Distribution of phenotypes in the C-1 and C-2 generations of tissue culture of 'Rober's Lemon Rose' scented geranium.

Phenotype	C-1 generation (from parental *lobed*) n-166	C-2 generation, from: *Lobed* n=27	*Dentated* n=148	*Hairy* n=20	*Dwarf* n=14	*Flat leaf* n=4
Lobed	1	0	0	0	0	0
Dentated	139	25	135	3	0	0
Hairy	21	2	13	17	0	0
Hairy dwarf	1	0	0	0	0	0
Dwarf	3	0	0	0	14	0
Flat leaf	1	0	0	0	0	4

were *dentated* and 2 were *hairy;* none were *lobed.* This predominance of *dentated* resembles the parental *lobed* segregation from root and petiole cuttings and calliclones. The similarity between segregation patterns confirms that the C-1 *lobed* is a periclinal chimera and is probably identical to the parental clone.

Eleven different C-1 *dentated* clones produced a total of 148 C-2 clones. Of these 135 were *dentated* and 13 were *hairy* (Table 4).

Of 20 C-2 calliclones produced from 2 *hairy* clones, all except 3 maintained their large stomate size. The 3 C-2 clones from *hairy* which had normal stomate size were *dentated* in phenotype, and represent spontaneous reduction in chromosome number ("haploidy").

Of the 14 C-2 clones derived from *dwarf,* all resembled the C-1 *dwarfs* in leaf shape and form; leaf and plant size was reduced but there was considerable variation. Apparently dwarf represents a morphologically aberrant type isolated from tissue culture, although phenotypic expression is still variable. Further generations of callus culture from these C-2 types must be analyzed to determine whether this character can be stabilized.

Four C-2 clones were produced from the single C-1 *flat leaf* and all were phenotypically *flat leaf.* The insect resistant phenotype of the original *flat leaf* was maintained in the C-2. The C-2 *flat leaf* calliclones were analyzed for their essential oils and all lacked Peak I. The stabilization of *flat leaf* in tissue culture is evidence that this plant type represents a heritable change. The small leaf phenotype of the *flat leaf* originally led to some confusion with the *dwarf* which were somewhat variable (particularly in the C-2). Analysis of the essential oils of the *dwarf* (C-1 and C-2) showed no segregation for the 3 major peaks and clearly separated the *dwarf* from the *flat leaf* phenotypes.

To determine if variability within a single phenotype changed from C-0 to C-1 to C-2 an analysis of *dentated* phenotypes was made. The C-0 generation consisted of *dentated* plants derived from root and petiole cuttings of the parental RLR; *dentated* C-1 clones were derived from the parental *lobed;* and C-2 *dentated* were derived from C-1 *dentated. Hairy* segregates were removed from analysis of each generation.

The means and variances of the 4 leaf characters of *dentated* phenotypes of the C-0, C-1, and C-2 generations are presented in Table 5. There were no significant differences between the variance of root and petiole cuttings within the C-0. The variance of the C-1 was significantly higher than C-0 petiole cuttings for leaf size and significantly higher than C-0 petiole cuttings for leaf form. The variance of the C-2 was significantly less than the C-1 for both of these characters. Differences were also detected within C-2 families for leaf and stomatal size.

The greater variability of C-1 as compared to plants derived from root or petiole cuttings suggests cellular variability of the parental clones; C-1 clones may be derived from various cellular layers as compared to root or petiole cuttings. The reduction in variability from the C-1 to C-2 generations suggests stabilization of cell types.

'Clorinda'

'Clorinda', a hybrid of *P. denticulatum* (scented) and *P. domesticum* (regal) (18) dates back at least 50 years (2). The plant has an unpleasant fragrance, but large showy flowers. Leaves of the *parental* clone are lobed with a characteristic "wide waist" (Fig. 3, 7).

Plants derived from stem cuttings were compared to those from root cuttings and calliclones (Table 6 and 7). Calliclones were obtained from a single explant which was subcultured

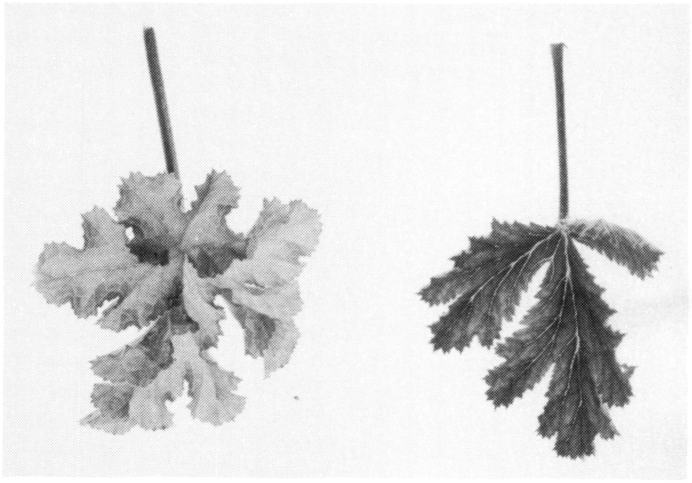

Fig. 7. Leaf morphology of 'Clorinda' scented geranium. (Left) *parental,* (right) *narrow leaf.*

Table 5. Means and variances of leaf characters of C-0, C-1, and C-2 *dentated* calliclones of 'Rober's Lemon Rose' scented geranium.

Generation Propagule	n	Leaf size (mm^2) (lxw) $\bar{X}$	Leaf size (mm^2) (lxw) $\bar{X}s^2$	Leaf shape (l/w) $\bar{X}$	Leaf shape (l/w) $\bar{X}s^2$	Leaf form (w/waist) $\bar{X}$	Leaf form (w/waist) $\bar{X}s^2$	Stomate size (μ) $\bar{X}$	Stomate size (μ) $\bar{X}s^2$
C-0 generation									
Root cuttings	28	57.3 d[z]	3.4 a	0.61 ab	0.02 ns	6.8 d	0.68 bc	16.9 a	0.97 ab
Petiole cuttings	30	38.2 ab	3.3 a	0.62 a	0.02 ns	6.2 e	0.54 ab	17.6 a	0.89 a
C-1 generation									
Calliclones	139	53.5 cd	8.0 c	0.65 cde	0.02 ns	6.6 e	0.69 c	13.4 b	0.91 a
C-2 generation	160	53.0 cd	6.7 b	0.66 e	0.02 ns	5.5 bc	0.51 a	18.8 c	0.93 ab
Within C–2 generations									
1076	8	63.8 de	6.2 abc	0.64 bcd	0.02	5.7 cd	0.61	18.6 bc	1.18 b
1077	2	51.4 bcd	4.3 abc	0.65 bcde	0.03	5.6 bcd	0.30	18.0 b	1.08 ab
1078	7	36.4 a	3.9 ab	0.62 ab	0.01	4.8 ab	0.48	19.0 c	0.93 ab
1081	17	51.7 cd	8.0 c	0.66 e	0.02	5.3 bc	0.38	18.2 b	0.89 a
1087	27	47.9 bc	5.5 abc	0.64 bcd	0.02	5.7 cd	0.59	18.4 bc	0.91 a
1090	19	53.2 cd	6.1 abc	0.64 bc	0.03	6.2 d	0.72	18.8 c	0.87 a
1092	15	66.0 e	8.3 c	0.65 de	0.03	4.3 a	0.34	20.3 d	0.91 a
1095	6	55.0 cd	7.6 bc	0.63 bc	0.02	5.8 cd	0.32	18.8 c	1.08 ab
1100	15	63.0 de	8.8 c	0.66 e	0.02	5.4 bc	0.44	18.0 c	0.81 a
1103	9	42.9 ab	7.8 bc	0.66 e	0.02	5.5 bc	0.55	18.2 b	0.87 a
1111	11	43.6 ab	3.5 a	0.68 f	0.02	5.2 bc	0.38	18.2 b	1.01 ab

[z]Mean separation within column within group by LSD, 5% level.

Table 6. Phenotypes and leaf measurements of propagules derived from 'Clorinda' scented geranium.

Propagule *Phenotype*	n	Leaf measurements: Size (mm^2) (l x w)	Shape (l/w)	Form (w/waist)	Stomate size (μ)
Stem cuttings					
Parental	14	28.8 b[z]	0.61 a	3.6 a	18.0 b
Root cuttings					
Parental	17	72.1 d	0.63 ab	3.7 a	17.8 ab
Calliclones					
Parental	16	34.4 c	0.63 ab	3.1 a	17.8 ab
Narrow waist	25	35.7 c	0.64 bc	8.1 b	17.6 a
Dwarf narrow waist	9	18.3 ab	0.69 c	5.1 a	20.3 c
Miniature	4	–	–	–	24.5 e
Flat leaf dwarf	1	17.7 ab	0.71 c	3.3 a	23.5 d
Hairless	1	7.8 a	0.64 abc	2.9 a	22.6 d

[z]Mean separation within columns by LSD, 5% level.

Table 7. No. of propagules significantly different from *symmetrical* stem cuttings of 'Clorinda' scented geranium.

Propagule *Phenotype*	n	No. plants significantly different (5%): Leaf size	Leaf shape	Leaf form	Stomate size	Total plants
Root cuttings						
Parental	17	15	1	0	0	15
Calliclone						
Parental	16	2	0	0	0	2
Narrow waist	25	2	0	16	0	17
Dwarf narrow waist	9	0	3	2	4	7
Miniature	4	–	–	–	4	4
Flat leaf dwarf	1	1	0	0	1	1
Hairless	1	1	0	0	1	1
Calliclone root cuttings						
Parental	4	4	0	0	0	4
Narrow waist	11	6	5	3	0	10

7 times with shootlets removed in transfer 2–7 (Table 8).

Plants derived from stem cuttings of 'Clorinda' were uniform. Variation in plants from root cuttings was largely due to an increase in leaf size, the "large leaf syndrome." Variation in calliclones was the highest of the scented geranium cultivars tested. As subculturing increased there appeared to be an increase in the frequency of aberrant types. The ability to root on White's medium was lost completely in the 4th and 7th transfer.

The 56 calliclones were classified into 5 distinct phenotypes: *parental* (16 plants), *narrow waist* (25 plants), (Fig. 7), *dwarf narrow waist* (9 plants), *miniature* (4 plants), *flat leaf dwarf* (1 plant) and *hairless* (1 plant).

Parental was normal with regard to leaf morphology and plant size. However, 2 plants exhibited significantly larger leaves as did many of the plants derived from root cuttings.

Narrow waist was characterized by leaves with an extremely small "waist" and a normal sized plant; stomate size was normal. This leaf phenotype appeared in subculture generations but never appeared in plants derived from root or stem cuttings. Root cuttings from *narrow waist* maintained that phenotype. However, *narrow waist* phenotype was characterized by instability. This included dwarf plant (see below), color changes, stem fasciation, and phyllotaxy changes from the normal 2/5.

Dwarf narrow waist, a combination of abnormal leaf on a small plant, had larger stomates. One plant of this phenotype lacked anthocyanin pigmentation on the stem and petiole.

Table 8. Segregation of calliclone phenotypes and various plant characters as a function of the number of transfers in tissue culture of 'Clorinda' scented geranium.

Propagule	No. of plants: Transfer no. 2	3	4	5	6	7
Parental	3	10	–	3	0	–
Narrow waist	2	0	–	23	0	–
Dwarf narrow waist	0	0	–	7	1	–
Dwarf anthocyaninless	0	0	–	1	–	–
Miniature	0	0	–	4	0	–
Flat leaf dwarf	0	0	–	1	0	–
Hairless	0	1	–	0	0	–
Total calliclones	5	11	10[z]	39	1	56[z]

[z]Lack of rooting on White medium.

Miniature were very small plants (less than 10 cm tall after a year of growth) which appeared only in the 5th transfer. Plants were difficult to maintain and stomates were measured only on 2 of the 4 plants, both of which were large (23.9 and 25.4μ vs. 18.0μ for stem cuttings). Miniature plants (and other large stomate forms) are presumed to be polyploid based on studies with RLR.

Flat leaf dwarf was a small plant with large leaves, each oriented in a single plane. It appeared in the 5th subculture.

Hairless had small leaves without pubescence and large stomates on a normal sized plant. It appeared in the 3rd subculture.

'Snowflake'

'Snowflake' (*P. adcifolium*) is a lemon-scented geranium named for its foliage which contains small areas of white flecks throughout the green leaf. This pattern is unstable. Some plants become grossly chimeral with the flecking pattern replaced by various sized sectors of green or albino tissue. Stem and root cuttings from green clones stabilize as green. In this study we compared plants derived from stem, root, and petiole cuttings with calliclones, all derived from the flecked 'Snowflake'.

Stem cuttings and the 2 petiole cuttings of 50 that produced shoots continued the flecked pattern but plants derived from root cuttings and calliclones segregated as shown in Table 9. In the sectoral chimeras, measurements were made separately on *green, fleck,* or *albino* sectors.

Most of the variability in the 'Snowflake' propagules appeared to be due to the instability of the flecked condition. The patterns of variegation transmission are explainable if we assume that there are 3 types of cells: unstable green, stable green, and stable albino. The unstable green produced unstable green, stable green, and stable albino with the flecking pattern due to islands of albino cells among the green. Previous investigations have shown that plants derived from petiole and root cuttings of geranium are presumably derived from single cells (3, 8) as is common among a number of other plant families such as Liliaceae, Begoniaceae, Compositae, and Gesneriaceae. Thus, segregation depends upon which of the 3 cells (unstable green, stable green, or stable albino) is involved. If the cell is unstable green the phenotype will be *fleck;* if the cell is stable green or albino the resulting clone is *green* or *albino.* The chimeras may have originated from 2 or more cells or from subsequent segregation into the stable forms. The numbers of propagules are too small to explain the preponderance of all green suckers from *fleck* root cuttings (14 *green* out of 20) vs. the preponderance of *fleck* calliclones from *fleck* callus (1 *green* out of 9). Petiole cuttings might be expected to segregate but only 2 formed shoots, both of which were *fleck.*

Table 9. Phenotypes and leaf measurements of various propagules derived from 'Snowflake' (*fleck*) scented geranium.

Propagule *Phenotype*	n	Leaf measurements: size (mm^2) (l x w)	Shape (l/w)	Form (w/waist)	Stomate (μ)
Stem cuttings					
fleck	2	67.8	0.66	1.4	19.9
Petiole cuttings					
fleck	2	167.5*	0.65	1.8	19.9
Root cuttings					
fleck	4	91.4	0.66	1.8	21.2
green	14	93.4	0.64	1.7	19.2
fleck-albino chimera	2	61.7	0.65	1.9	19.2
Calliclones					
fleck	5	67.8	0.64	1.6	21.0
fleck (large leaf)	1	144.4*	0.64	1.6	20.1
green	1	91.2	0.65	1.5	20.1
fleck-green chimera	1				
fleck		74.4	0.68	1.4	21.2
green		59.4	0.64	1.5	20.1
fleck-albino chimera	1				
fleck		45.7	0.74*	1.6	20.3
albino		45.7	0.74*	1.6	19.0

*Means followed by an asterick are significantly different from other means within columns.

The "large leaf syndrome" appeared in the 2 *fleck* petiole cuttings and a single *fleck* calliclone. In addition, a change in leaf shape appeared in a *fleck* albino sectoral chimera.

'Attar of Rose'

'Attar of Rose' (*P. capitatum*), the oldest of the cultivars used in these studies, was introduced into England in the late 1600's (18). It is a trailing growth habit and is rose-scented. Flowering is profuse during most of the summer months.

A total of 65 calliclones were produced and compared to plants derived from stem and root cuttings (Tables 10 and 11). Ten of the 65 calliclones were obviously variant. Of these 8 were smaller plant types classified as *miniature* (1 plant), *dwarf* (6 plants), and *small* (1 plant); 2 other types were based on leaf morphology: *thick leaf* (1 plant) and *round leaf* (1 plant). The *thick leaf* plant had very large stomates (24.3 vs. 18.6μ for the stem cuttings) indicating polyploidy; this plant produced some stainable pollen and may be partially male fertile.

About half of the calliclones classified as *parental* by inspection proved to be aberrant from analysis of the leaf characters; most of these were due to a change in leaf form with 25 of the 55 *parental* clones having narrower "waists" than the stem or root cuttings. Other significant changes in this group included increased leaf size (2 of 55 plants) and changes in stomate size (7 plants).

Table 10. Phenotypes and leaf measurements of propagules derived from 'Attar of Rose' scented geranium.

Propagule *Phenotype*	n	Leaf measurements: Size (mm^2) (l x w)	Shape (l/w)	Form (w/waist)	Stomate size (μ)
Stem cuttings					
Parental	5	32.3 d[z]	0.67 a	3.1 c	18.6 b
Root cuttings					
Parental	6	45.0 e	0.67 a	3.1 c	17.1 a
Calliclones					
Parental	55	28.3 c	0.73 b	2.3 b	19.2 bc
Miniature	1	8.3 a	0.87 c	1.6 a	19.8 bc
Dwarf	6	20.3 b	0.76 b	2.1 a	20.1 c
Small	1	16.9 ab	0.76 b	2.1 ab	19.0 bc
Thick leaf	1	30.0 cd	0.67 a	2.1 ab	24.3 d
Round leaf	1	8.4 a	0.98 d	1.7 a	19.9 bc

[z]Mean separation within columns by LSD, 5% level.

Table 11. No. of propagules significantly different from stem cuttings of 'Attar of Rose' scented geranium.

Propagule *Phenotype*	n	No. plants significantly different (5%): Leaf size	Leaf shape	Leaf form	Stomate size	Total plants
Root cuttings						
Parental	6	0	0	0	0	0
Calliclones						
Parental	55	2	0	25	7	28
Miniature	1	1	1	1	0	1
Dwarf	6	2	1	5	2	5
Small	1	0	0	1	0	1
Thick leaf	1	0	0	1	1	1
Round leaf	1	1	1	1	0	1

Plants derived from root cuttings exhibited a larger average leaf size than plants derived from stem cuttings or calliclones. However, the extreme variability in calliclones of 'Attar of Rose' is not explainable on the assumption that this cultivar is a complex chimera because root cuttings showed no variant types. Apparently this clone is unstable in tissue culture.

'Old Fashioned Rose'

'Old Fashioned Rose' (*P. graveolens*), introduced into England about 1774 (18), is semi-erect and rose-scented; it flowers only rarely. Propagules derived from stem cuttings, root cuttings, and calliclones are compared in Tables 12 and 13. Although petiole cuttings rooted easily, none formed shoots.

One calliclone of the 57 produced was classified by inspection as a dwarf variant. However, analysis of leaf morphological characters indicated 4 plants with significantly reduced leaf

Table 12. Phenotypes and leaf measurements of propagules derived from 'Old Fashioned Rose' scented geranium.

Propagule *Phenotype*	n	Leaf measurements: Size (mm^2) (l x w)	Shape (l/w)	Form (w/waist)	Stomate size (μ)
Stem cuttings					
Parental	17	59.0 b[z]	0.68 b	9.6 a	18.2 bc
Root cuttings					
Parental	15	67.8 b	0.64 a	9.9 a	17.8 ab
Calliclones					
Parental	56	43.7 a	0.69 b	8.5 a	18.2 c
Dwarf (987)	1	51.1 ab	0.64 ab	10.3 a	17.3 a

[z]Mean separation within columns by LSD, 5% level.

Table 13. No. of propagules significantly different from the stem cuttings of 'Old Fashioned Rose' scented geranium.

Propagule *Phenotype*	n	No. plants significantly different (5%): Leaf size	Leaf shape	Leaf form	Stomate size	Total plants
Root cuttings						
Parental	15	0	0	0	0	0
Calliclones						
Parental	56	4	1	0	1	6
Dwarf (987)	1	0	0	0	0	0

size; 1 plant with a change in leaf shape; and 1 plant with significantly smaller stomates. There was no significant variability associated with plants derived from root cuttings. 'Old Fashioned Rose' appears to be relatively stable in tissue culture and variation is minimal in comparison to the other cultivars.

Discussion

Source of variation in tissue culture. Plants derived from stem cuttings of all scented geranium cultivars were extremely uniform. Apparently the apical meristem of buds maintains the integrity of the histogenic layers and retains stability, even in periclinal chimeras such as RLR. Plants derived from root and petiole cuttings were less stable with the amount of variation dependent upon cultivar. In RLR, a periclinal chimera, root and petiole cuttings segregated distinctly different morphological types than stem cuttings.

Instability of root and petiole cuttings as compared to stem cuttings may be explained either as a result of inherent cell variation with the asexually-propagated clones or induced variation brought about by the release of authority imposed by the apical meristems. Evidence that adventitious shoots of geranium are derived from single cells (3, 7) suggests that some variability obtained from root and petiole cuttings represent cell variability in a complex chimera. The appearance of polyploids from both petiole cuttings and calliclones in RLR suggest that the formation of shoots from petiole callus represents an "*in vivo*" system analogous to that found in *in vitro* tissue-culture.

Variability associated with calliclones was greater than from either stem, root, or petiole cuttings in all clones. Calliclone variation was most dependent upon clone and this effect appeared independent of their age. Calliclone variability in some old clones was minimal ('Old Fashioned Rose' introduced in 1774) while others ('Attar of Rose', 1690) was very high; 'Rober's Lemon Rose' (about 1950) also showed high variability. Calliclones of unstable genotypes ('Snowflake') maintained this instability. There was evidence that calliclone variability increased with subculture of the callus ('Clorinda').

Variability in calliclones included both gross changes (e.g., leaf morphology, loss of anthocyanin coloration, loss of pubescence, dwarf growing habit, changes in essential oil constituents, flower morphology) as well as quantitative changes that were observed from leaf measurements. These changes were attributed to segregation of chimeral tissue (RLR), polyploid changes (RLR, 'Attar of Rose', and 'Clorinda'), and unknown heritable changes possibly individual chromosome aberrations or gene mutations. A "large leaf syndrome" found in plants derived from root and petiole cuttings was also observed. This character (as well as phyllotaxy changes) may either be due to growth phase changes, such as reversion to the juvenile stage, or may represent possible elimination of virus. The polyploid changes associated with calliclones included chromosome doubling, as well as reduction (haploidy) of calliclone polyploids (RLR).

The stabilization of some of the aberrant calliclone phenotypes in a second cycle of tissue culture indicated a fixation of genetic changes. The decrease in variability from calliclone cycles suggests cellular variability associated with the original clones.

Feasibility of intraclonal plant improvement. The present study suggests a model system for future work in intraclonal plant improvement especially adapted to highly polyploid, sterile, asexually propagated clones. The first step is the development of suitable tissue-culture techniques. These involve:

1) Production of contaminant-free callus cultures.
2) Development of suitable medium for callus proliferation.
3) Development of media for shoot regeneration and rooting.
4) Production of independent plantlets (calliclones) in quantity.

Variability associated with calliclones derived from tissue culture is a pool on which selection can be imposed. The amount of variability that can be expected will vary with the clone. This system seems especially applicable to old cultivars which would be expected to have accumulated large numbers of mutant cells that may have stabilized into chimeras of various complexity, but our results suggests that some relatively young clones may show high calliclone variability.

Variability of callus and calliclones may increase by aging and repeated subculturing. Other techniques useful to increase the percentage of variability should be:

1) Use of mutagenic agents in the callus proliferation stage.
2) Selection applied to single cell clones for stress conditions and/or ability to resist or utilize specific metabolites.

The association of polyploidy with tissue culture may be utilized as a technique to obtain either increased or reduced chromosome number. However, the affinity of clones to double in culture appears to be a disadvantage in most cases and a screening technique may be necessary to eliminate this type of mutation. Simple stomate measurements were found useful to detect such chromosomal changes.

There are a number of immediate and potential situations in which calliclone variation could be exploited. These include:

1) Old vegetatively-propagated highly adapted clones (e.g., 'Bartlett' pear, 'Concord' grape, 'Russet Burbank' potato, and clonal root stocks such as the Malling series);
2) Seedless or apomictic lines (e.g., 'Thompson Seedless' grape, 'Kentucky' bluegrass, 'Washington Navel' orange);
3) Sterile or non-flowering lines (e.g., many scented geraniums and sweet potato clones).

Our study suggests that tissue culture techniques may have special uses for the plant breeder, and should provide an additional method to increase intraclonal variation. Its use will be important as a method to obtain mild changes in unique, highly-adapted, clones or to obtain variability in clones in which the sexual apparatus is disturbed. There are special problems and the system is not intended to replace conventional breediing methods for most crops. Nevertheless, the approach is encouraging and may provide significant benefits for crop improvement, especially useful for vegetative crops with high chromosome numbers for which traditional breeding systems have been found wanting.

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Growth and Fruiting Responses of Vigorous Apple Branches to Pruning and Branch Orientation Treatments[1]

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Abstract. **Vigorous branches of apple (*Malus domestica* Borkh. cv. Delicious) were headed-back into previous season's growth (1-year-old wood). Increased pruning severity (removal of a greater fraction of 1-year-old wood) resulted in increased shoot growth from 1- and 2-year-old branch segments. Fruitfulness decreased as pruning severity increased. Heading-back altered the distribution of growth between 1- and 2-year-old segments of horizontal and downward-oriented branches but resulted in a total increase in growth only in vertically-oriented branches.**

Excessive vigor and low fruitfulness characterize many 'Delicious' apple plantings in New York. These problems are aggravated by the very upright growth habit of young trees of this cultivar. Failure to prune and train these trees leads to unsatisfactory tree structure, delayed production and finally, shading with loss of fruiting wood. Pruning vigorous trees to control canopy shape often leads to an increase in vegetative growth.

Few reports in the literature discuss the growth responses of vigorous apple branches to removal of specific quantities of wood. Gardner (6) made extensive studies of growth responses in 1-year-old shoots to various degrees of heading-back pruning. As pruning severity increased, there were decreases in the no. of shoots, the total new shoot growth, and the no. of spurs developing from the 1-year-old shoot. The average length of the new shoots was constant. Maggs (9) headed-back 1-year-old rooted stool-bed shoots of M 25 and found that equal or decreased no. of new shoots grew from the stool shoots as pruning severity increased. When the no. of developing shoots was not restricted by disbudding shortly after bud-break, the total length of the new growth was the same for the various degrees of heading-back. In a similar experiment with several levels of purning carried out on 3-year-old trees, Knight (8) found an increased amount of new growth up to the point where 2/3 of the 2-year-old wood was removed from each branch. These reports provide no information on the effect of pruning on fruitfulness or growth responses of branch sections of different ages. Much previous work was done while caustic fungicide sprays were in use and/or with cultivars inherently less vigorous than 'Delicious'.

Dermine and Monin (4) and Jonkers (7) found no effects on shoot growth or flower-bud formation as a result of bending branches away from the vertical. Gardner (5) reported either no effects of branch bending on growth and spur development or an increased shoot growth from bent shoots, depending on the cultivar. Mika (12) reported that 1-year-old laterals had less vegetative growth than controls when bent but also showed less flower-bud formation. Mullins (13) grew entire grafted 1-year-old trees in a vertical, horizontal or inverted orientation. He found different patterns of growth and flower-bud formation in response to orientation, but total growth and total flower-buds formed were similar among treatments. Wareing and Nasr (19) showed reduced growth and increased flower-bud formation in potted M 26 trees grown horizontally. In a 10-year field trial with 'Cox's Orange Pippin'/M 25 trees, Preston (15) reported that trees pruned to promote a spreading habit produced about 25% more fruit per acre than trees pruned to an upright habit, although the weight of prunings from each treatment was equivalent. Much of the variation in results discussed above is due to differences in cultivar and tree vigor. This paper describes effects of specific pruning cuts on vegetative regrowth and fruiting of excessively vigorous upright branches of 'Delicious' apple. The effects of pruning and branch orientation on vegetative growth are also described.

Materials and Methods

In March, 1970, and March, 1971, the following pruning treatments were made on separate groups of 8-year-old (9 years old in 1971) 'Delicious'/M 7 trees: 1) check; 2) terminal bud only removed; 3) 1/3 of previous season's terminal growth removed; 4) 2/3 of previous season's terminal growth removed; 5) all of previous season's terminal growth removed. Each treatment was applied to every branch of an entire tree and single-tree plots were replicated in a randomized complete-block design. The no. and length of shoots developing from 1- and 2-year-old sections of 10 upright branches per tree were measured after 1 growing season and the data averaged for each tree. In 1971 blossom clusters and fruits were counted on the 1970, 1969 and 1968 branch sections of 10 replicate

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