

bean) cultivars: Brittle Wax, Earliwax, Eastern Butterwax, Goldcoast Wax, Goldcrop Wax, Goldrush Wax, Moonrush Wax, Moongold Wax, Pencil Pod Black Wax, Pencil Pod Wax, Puregold Wax, Sunrise Wax, Tenderpod, and VC-1009.

**Susceptible yellow-podded, dry bean cultivar:** Yellow Eye.

Plants of yellow-podded cultivars showed local and systemic symptoms similar to those of the green-podded, but also developed necrotic spots and stem necrosis, often causing premature death.

**Inheritance of resistance to BBWV.** Plants of either parent reacted with local infection, but the susceptible 'CLRK' also developed a prominent foliar yellow mosaic, distortion, and plant stunting. Plants of  $F_1$  ('Sanilac' x 'CLRK') and  $F_1$  ('CLRK' x 'Sanilac') exhibited local lesions, but the virus failed to move systemically, revealing that resistance was dominant. The  $F_2$  populations of both crosses also responded with local lesions, but only about 25% were affected by prominent systemic mosaic and stunting, indicating that resistance was monogenic (Table 1). Confirmation of this mode of inheritance was obtained from progenies of reciprocal backcrosses. Plants of the backcross ('Sanilac' x 'CLRK') x 'Sanilac' failed to develop systemic symptoms, hence they were systemically resistant. Plants of the other backcross ('Sanilac' x 'CLRK') x 'CLRK' segregated in the ratio of one systemic resistant to one susceptible. The data presented in Table 1 indicate that resistance of BBWV in 'Sanilac' is conferred by a single dominant gene, to which the symbol *Bbw* (broad bean wilt) is assigned.

**Lack of seed transmission.** None of 471 'CLRK' plants derived from seeds of infected plants developed foliar symptoms, and ELISA was negative. Attempts to demonstrate seed transmission of BBWV in other species also have been unsuccessful (5, 10).

There are very few reports in the literature regarding the natural occurrence of BBWV in bean (1, 3). This can be attributed to the fact that symptoms caused by BBWV on naturally infected plants resemble those caused by well-known viruses (e.g., bean yellow mosaic, clover yellow vein, tobacco ring-spot, and others) that are equally destructive. However, BBWV can be effectively controlled, since a large number of bean lines are resistant, including leading commercial cultivars. This study confirmed field data that the majority of BBWV-susceptible cultivars were the yellow-podded or red kidney types. However, no direct relationship could be established between these types and BBWV susceptibility, because other cultivars of these types were resistant. Interestingly, yellow-podded cultivars developed by some seed companies were either all resistant or all susceptible to the virus.

In *P. vulgaris*, 26 genes had previously been found to confer resistance to viruses (4). Of these, nine are recessive whereas 17 are dominant or incompletely dominant. The newly characterized gene for resistance to BBWV is also dominant.

## Literature Cited

1. Lisa, V., G. Dellavalle, and S. Peressini. 1986. Broad bean wilt virus in fagiolo in Friuli-Venezia Giulia. Inform. Fitopat. 36:31-32.
2. Nawrocka, B.Z., C.I. Eckenrode, J.K. Uyemoto, and D.H. Young. 1975. Reflective mulches and foliar sprays for suppression of aphid-borne virus in lettuce. J. Econ. Ent. 68:694-698.
3. Provvidenti, R. 1983. Reaction of bean cultivars to broad bean wilt virus. Annu. Rpt. Bean Imp. Coop. 26:69-70.
4. Provvidenti, R. 1987. List of genes in *Phaseolus vulgaris* for resistance to viruses. Annu. Rpt. Bean Imp. Coop. 30:1-4.
5. Provvidenti, R. and H.C. Hoch. 1985. A foliar ringspot disease of *Thunbergia alata* caused by broad bean wilt virus. Plant Dis. 69:726.
6. Provvidenti, R., R.W. Robinson, and J.W. Shail. 1984. Incidence of broad bean wilt virus in lettuce in New York State and sources of resistance. HortScience 19:569-570.
7. Rist, D.L. and J.W. Lorbeer. 1988. Weed reservoirs of cucumber mosaic virus (CMV) and broad bean wilt virus (BBWV) and their association with CMV and BBWV diseases of commercial lettuce. Phytopathology 77:1618. (Abstr.)
8. Schroeder, W.T. and R. Provvidenti. 1970. A destructive blight of *Spinacia oleracea* incited by a strain of broad bean wilt virus. Phytopathology 60:1405-1406.
9. Stubbs, L.L. 1947. A destructive vascular wilt virus disease of broad bean (*Vicia faba* L.) in Victoria. J. Dept. Agr. Victoria 46:323-332.
10. Taylor, R.H. and L.L. Stubbs. 1972. Broad bean wilt virus. Description of Plant Viruses 81. Commonwealth Myc. Inst./Assoc. Applied Biol., Kew, Surrey, England.
11. Uyemoto, J.K., and R. Provvidenti. 1974. Isolation and identification of two serotypes of broad bean wilt virus. Phytopathology 64:1547-1548.

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# An Improved Technique for Counting Chromosomes in Grapes

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**Additional index words.** cytology, fruit breeding, root tip squashes, *Vitis*

**Abstract.** Various procedures were compared to identify a technique that would produce clear countable chromosomes in grape (*Vitis* L.) root tip squashes. Harvesting roots at 1100 hr from greenhouse-grown plants, pretreating with 0.02 M 8-hydroxyquinoline at 18°C for 6 hr, fixing in Farmer's Fluid at 26° for 24 hr, hydrolyzing in 1 N HCl at 60° for 1 hr, and staining with an altered form of carbol fuchsin gave the best results.

The ability to count chromosomes has long been a valuable tool for plant breeders and cytogeneticists. This work can be difficult with grape (*Vitis* L.) since the chromosomes are extremely small, frequently having only a few cell divisions visible in a single root tip (14). Meiotic analysis is sometimes more successful (9) but requires plants at the flowering stage. A rapid counting technique, applicable to young seedlings, vegetative propagules, and possibly in vitro grown plantlets, would be very useful.

Root tips embedded in paraffin (4, 7) root tip squashes (17), actively growing shoot tips (11, 17), and the tips of young leaves (13) have been used for chromosome studies in *Vitis*. Aceto-carmin (1, 12, 17) and aceto-orcin (12, 13; H. Yamane, 1984; personal

communication) are the two most commonly used stains. These techniques are either difficult to use or require much care, precision, and time to execute properly.

This experiment was designed to study factors that influence the success of the root tip squashing procedure. This technique was chosen because of the relative ease of preparation of materials for chromosome counting. Various pretreatment, fixative, hydrolyzing agent, and stain combinations were compared, and the effect of time of day of root tip collection was examined.

Dormant hardwood cuttings of 'Cabernet Sauvignon' (*V. vinifera*) were planted in a greenhouse in Nov. 1986 to initiate root formation. The greenhouse was under a 24°/21°C day/night regime with metal halide supplemental light ( $\approx 154 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) supplied for 16 hr in each 24 hr. Before planting, the basal ends of the two bud cuttings were soaked in 1% hydrogen cyanamide for 15 min to overcome dormancy (10, 16). The cuttings were started in flats of moist Cornell soil mix A (2). Root tips were harvested 42 days after planting when each cut-

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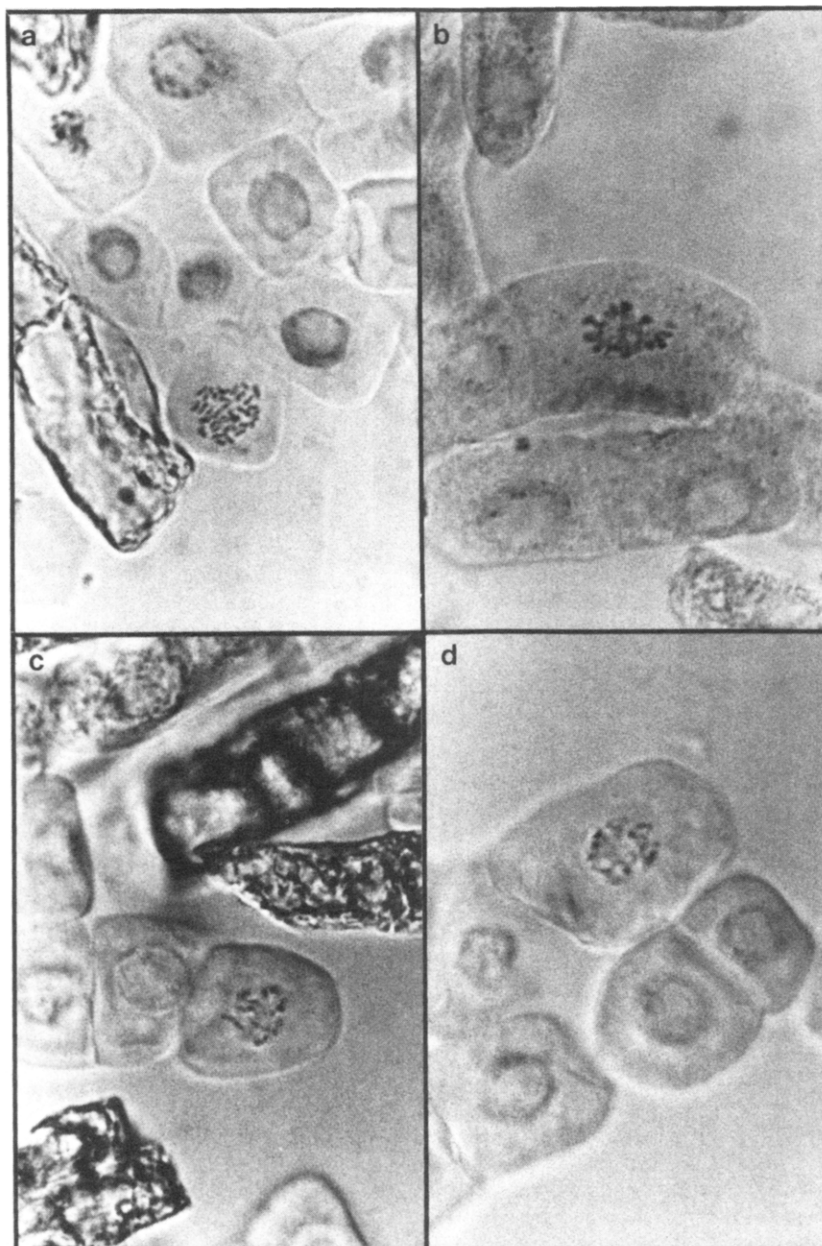


Fig. 1. Grape chromosomes, in root tips, photographed at 1250 $\times$ . Treatments are as follows; all harvested at 1100 HR. a. 8HQ 1 hr pretreatment, Farmer's fluid, HCl hydrolysis, altered carbol fuchsin stain; b. colchicine pretreatment, Farmer's fluid, HCl hydrolysis, altered carbol fuchsin stain; c. 8HQ 1 hr pretreatment, Farmer's fluid, HCl hydrolysis, aceto-orcein stain; d. 8HQ 1 hr pretreatment, Farmer's fluid, HCl hydrolysis, aceto-carmin stain.

ting had 2 to 3 fully expanded leaves and a well-developed root system.

At the time of harvest, six to eight root tips were excised and immediately placed in vials containing the pretreatment solutions. There were six vials per time-pretreatment-fixative combination. Following pretreatment, root tips were rinsed twice in distilled water and placed in fixative. The fixed root tips were rinsed once in 70% ethanol and put in fresh ethanol for storage at  $-18^{\circ}\text{C}$ . Three vials from each treatment were used in preliminary studies to test different stains and maceration techniques. The remaining vials were used to test the best combination of pretreatment, fixative, and time of harvest. The root tips were scored within 2 months of harvest. All examinations were done with an Olympus model BH-2 microscope at 1250 $\times$  magnification. The correct pressure

of the squash itself was especially important for success, with moderate downward and slight lateral pressure.

The treatments were as follows:

A. Time of day: T1, 0800 HR; T2, 1100 HR; T3, 1400 HR.

B. Pretreatment: P1, none; P2, 0.02 M aqueous 8-hydroxyquinoline,  $18^{\circ}\text{C}$ , 1 hr (6); and P3, identically treated, but 6 hr; P4, 0.2% aqueous colchicine,  $18^{\circ}$ , 90 min (6).

C. Fixative: F1, 1 chloroform : 1 glacial acetic acid : 2 95% ethanol,  $26^{\circ}\text{C}$ , 24 hr (Carnoy's Fluid) (15); F2, 1 glacial acetic acid : 2 95% ethanol,  $26^{\circ}$ , 24 hr (Farmer's Fluid) (5).

D. Maceration: M1, manual chopping with scalpel; M2, heating in 1 N HCl,  $60^{\circ}\text{C}$ , 1 hr (6); M3, heating gently over a flame in 1 N HCl : aceto-orcein (1:10) (18).

E. Stain: S1, aceto-orcein (5); S2, aceto-

carmin (a slightly rusty needle was used in preparation) (5); S3, carbol fuchsin (8); S4, modified carbol fuchsin (3); S5, altered carbol fuchsin, a 1:1 (v/v) mixture of S3 and S4.

Standard carbol fuchsin is prepared by dissolving 3 g of basic fuchsin in 100 ml of 70% ethanol, and mixing 10 ml of this solution with 90 ml of 5% phenol. The final stain is made by mixing 45 ml of this stock solution with 6 ml of glacial acetic acid and 6 ml of 37% formaldehyde (8). The modified carbol fuchsin stain is prepared by mixing 5 ml of standard carbol fuchsin stain in 95 ml of 45% acetic acid and adding 1.8 g of sorbitol (3).

Various staining and maceration techniques were screened initially to determine their general effect on the preserved root tips. Following this preliminary screening, the best

maceration-stain combination was tested on all of the time-pretreatment-fixative combinations.

**Stain.** Use of aceto-orcein resulted in much extranuclear staining, particularly of cytoplasm, with only moderately intense chromosomal staining (Fig. 1C). Aceto-carmine failed to stain any part of the cell distinctly, and the chromosomes were only faintly visible under the best combination of other treatments (Fig. 1D).

In preliminary tests, standard carbol fuchsin stained cellular material too intensely while the modified form failed to color reliably the chromosomes. The altered form of carbol fuchsin (S5) stained chromosomes with great intensity while leaving the rest of the cellular material unstained (Fig. 1A), and therefore was selected for further testing.

**Maceration.** Manual maceration of the tissue generally produced poor squashes. There was insufficient breakdown of the tissue to liberate single cells or small clumps. Carbol fuchsin particularly had a strong affinity for the cut cellular surfaces, staining small pieces entirely purple and the margins of the larger pieces intensely. Aceto-orcein also stained the cut surface but not as darkly. With manual maceration, very few chromosomes were visible and those that were observed were poorly defined.

Hydrolysis using a 10:1 mixture of aceto-orcein and 1 N HCl, followed by gentle heating over a flame produced maceration that was better than manual chopping but inferior to heating in HCl alone. The tissue macerated in this manner was unevenly hydrolyzed and unevenly stained, with heat causing some distortion in cell shape. The aceto-orcein stained darkly on the cut surfaces and did not stain the chromosomes well.

Hydrolysis in 1 N HCl for 1 hr at 60°C produced superior maceration. The cells remained intact and undistorted in shape while the middle lamella softened and dissolved. Good single cell layer spreads were then possible with fine stain infiltration.

The combination of hydrolysis in 1 N HCl at 60°C for 1 hr, followed by direct staining with altered carbol fuchsin produced superior results. This combination was then tested further (Table 1).

**Time of day.** The time of sampling appeared to influence how many cells are in active cell division. While the 0800 HR collection produced good results, harvesting at 1100 HR yielded the greatest number and clarity of metaphase plates (Table 1). Collections made at 1400 HR had markedly fewer countable divisions than either of the earlier sampling times. Root tips harvested at 1100 HR exhibited about three times as many countable mitotic divisions as those from the 1400 HR sample.

**Pretreatment.** The effect of pretreatment was noticeable, but not as great as anticipated. The 8-hydroxyquinoline (8HQ) treatments produced the most condensed and readily visible chromosomes (Fig. 1A). The 6-hr treatment with 8HQ appeared slightly superior to the 1-hr treatment (Table 1). One of the useful effects of the 8HQ pretreatment

Table 1. A comparison of time of day, pretreatment, and fixative combinations on root tip mitotic divisions in 'Cabernet Sauvignon.'

Treatment <sup>a</sup>	Visible chromosomes <sup>b</sup>	Staining intensity <sup>c</sup>	Nuclear sphericity <sup>d</sup>	Overall rating <sup>e</sup>
T1-P1-F1	+	+	+	5
T1-P1-F2	+	+	++	5
T1-P2-F1	++	+++	++	3
T1-P2-F2	+	+	++	5
T1-P3-F1	++	+++	++	2
T1-P3-F2	+++	++	+++	2
T1-P4-F1	++	+	+	4
T1-P4-F2	+	+	+	5
T2-P1-F1	++	++	+	3
T2-P1-F2	+	+	+	5
T2-P2-F1	+	++	+++	4
T2-P2-F2	+++	++	++	1
T2-P3-F1	++	+	++	1
T2-P3-F2	+++	++	++	1
T2-P4-F1	++	++	++	1
T2-P4-F2	++	++	+	2
T3-P1-F1	+	++	++	4
T3-P1-F2	+	+	++	4
T3-P2-F1	+	+	+++	4
T3-P2-F2	+	++	+++	4
T3-P4-F1 <sup>a</sup>	+	+	+	5
T3-P4-F2	+	+	+	5

<sup>a</sup>Treatments are as follows: time of sampling, T1 = 0800 HR, T2 = 1100 HR, T3 = 1400 HR; pretreatment, P1 = none, P2 = 8HQ, 1 hr, P3 = 8HQ, 6 hr, P4 = colchicine; fixative, F1 = Carnoy's, F2 = Farmer's

<sup>b</sup>Visible chromosomes = the relative clarity and frequency of countable chromosomes in metaphase. + = poor, ++ = moderate, +++ = good.

<sup>c</sup>Staining intensity = stain specificity for chromosome material and lack of extraneous staining

<sup>d</sup>Nuclear sphericity = a three-dimensional spherical effect of the nucleus, causing it to be highly visible.

<sup>e</sup>Overall rating = a relative rating combining the above three criteria, with 1 = best and 5 = worst

<sup>a</sup>P3 was not tested at T3.

was enhanced visibility and sphericity of the nuclei when a Nomarski differential interference contrast attachment was used on the microscope. This characteristic accelerated the scanning for mitotic divisions because the dividing and nondividing nuclei were more easily distinguished. The concentration of 8HQ used in this study, 0.02 M, is 10 times more concentrated than that reportedly used by other researchers (6, 15; H. Yamane, 1984; personal communication). Surprisingly, the treatment with colchicine produced little improvement in clarity of chromosomes over that of the control (Fig. 1B). The colchicine pretreatment also induced much less nuclear sphericity than did the 8HQ.

**Fixative.** The two fixatives were very similar in their effect. However, with roots harvested as 1100 HR, when they were most actively dividing, the chromosomes were slightly easier to count when the tissue had been fixed in the Farmer's fluid fixative (Table 1).

Results show that a combination of harvesting at 1100 HR, pretreating in 8-hydroxyquinoline for 6 hr, fixing in Farmer's fluid, hydrolyzing 1 N HCl at 60°C for 1 hr, and staining with altered carbol fuchsin maximized the number of clear countable mitotic divisions in grape root tips. Under our conditions, 10 to 20 countable divisions per root tip are usually visible using this combination of treatments.

#### Literature Cited

1. Alley, C.J. 1957. Cytogenetics of *Vitis*. II.

Chromosomes behavior and the fertility of some autotetraploid derivatives of *Vitis vinifera* L. J. Hered. 48:194-202.

2. Boodley, J.W. and R. Sheldrake Jr. 1982. Cornell peat lite mixes for commercial plant growing. Cornell Univ. Coop. Ext., Ithaca, N.Y. Info. Bul. 43.
3. Carr, D.H. and J.E. Walker. 1961. Carbol fuchsin as a stain for human chromosomes. Stain Technol. 36:233-236.
4. Chadha, T.R. and S.K. Mukherjee. 1975. Growth and brittleness in shoots of some induced tetraploids in grapes as related to anatomical characteristics. Indian Agr. 19:333-340.
5. Darlington, C.D. and L.F. LaCour. 1942. The handling of chromosomes. Allen and Unwin, London, p. 116.
6. Dyer, A.F. 1979. Investigating chromosomes. Wiley, New York. p. 5, 7.
7. Einset, J. and C. Pratt. 1954. "Giant" sports of grapes. Proc. Amer. Soc. Hort. Sci. 63:251-256.
8. Gamborg, O.L. and L.Z. Wetter (eds.). 1975. Plant tissue culture methods. Natl. Res. Council of Canada. Prairie Res. Lab. Saskatoon, Sask. Canada p. 60-61.
9. Goldy, R.G. 1985. Relationship of morphological development to meiotic occurrence in various small fruit. HortScience 20:255-256.
10. Iwasaki, K. and R.J. Weaver. 1977. Effects of chilling, calcium cyanamide, and bud scale removal on bud break, rooting, and inhibitor content of buds of 'Zinfandel' grape (*Vitis vinifera* L.). J. Amer. Soc. Hort. Sci. 102:584-587.
11. Jelenkovic, G. and H.P. Olmo. 1968. Cytogenetics of *Vitis*. III. Partially fertile F1 diploid hybrids between *V. vinifera* L. x *V. rotundifolia* Michx. Vitis 7:281-293.

12. Lelakis, P. 1956. On a rapid method of control of the success of induction of polyploidy in woody plants. *Le Progrès Agricole et Viticole* 145:27-28. (In French)
13. Omura, M. and R. Akihama. 1980. Cellular variation in chromosome number induced by *p*-Fluorophenylalanine in grape seedlings. *HortScience* 15:751-752.
14. Saurer, W. and A.J. Antcliff. 1969. Polyploid mutants in grapes. *HortScience* 4:226-227.
15. Sharma, A.K. and A. Sharma. 1965. Chromosome techniques: Theory and practice. Butterworth, Washington, D.C. p. 58-59.
16. Shulman, Y., G. Nir, L. Fanberstein, and S. Lavie. 1983. The effect of cyanamide on the release from dormancy of grapevine buds. *Scientia Hort.* 19:97-104.
17. Thompson, M.M. and H.P. Olmo. 1963. Cytological studies of cytochimeric and tetraploid grapes. *Amer. J. Bot.* 50:901-906.

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# A Dominant Gene for Thornlessness Obtained from the Chimeraal Thornless Loganberry

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**Additional index words.** Tayberry, blackberry, *Rubus* hybrids, micropropagation, tissue culture, in vitro

**Abstract.** The thornless loganberry is a periclinal chimera in which a mutation for thornlessness apparently is confined to the outer plant layer (L1). Genetic thornless plants were obtained from this clone in Italy by selfing micropropagated plants and in New Zealand by histogenic layer manipulation. In both instances the middle plant layer (L2) apparently had been displaced by the L1 layer, so that the mutant character was transmitted by the gametes. The inheritance of thornlessness in progenies derived from this material has now been studied in Italy, New Zealand, and Scotland. In crosses, the segregation ratios did not fit closely to those expected if thornlessness were conferred by a dominant gene, largely, because thornless segregates were frequently in excess when the thornless parent was female and tended to be deficient when the latter was male. In selfings, the ratios were closer to 2:1 than to 3:1. However, the segregation of a single dominant gene for thornlessness is the most likely explanation of the results and it is proposed that the gene be designated *Sf<sub>L</sub>*. The homozygous dominant genotypes probably had a lower viability than the heterozygous ones.

Thornlessness is one of the most important characteristics required by the industry for blackberry and *Rubus* hybrids. For hybrids at the tetraploid level, the recessive gene *s*, which originated in *Rubus rusticanus* var. *inermis* from 'Merton Thornless' blackberry (9), has been used successfully to breed such tetraploid cultivars as 'Thornfree' (10) and 'Black Satin'; however, for breeding at higher

ploidy levels, it is more difficult to use a recessive gene and breeders have sought a dominant gene for thornlessness. Gene *Sf* from 'Austin Thornless' blackberry (6) has been used extensively, but no cultivars carrying it have yet been obtained, possibly because this gene is associated with undesirable plant traits.

Thornless mutants of several *Rubus* cultivars have proved to be periclinal chimeras in which the thornlessness is confined to the outer layer of the plant (L1), and does not contribute to gamete development. The mutants' sexual progenies are therefore all thorny. McPheeters and Skirvin (7) obtained genetic thornless material by histogenic layer manipulation of tetraploid chimeraal 'Thornless Evergreen' blackberry. Others (1) showed that thornlessness in this material was conferred by a dominant gene, which they designated *S<sub>TE</sub>*. Rosati et al. (8) identified a small pro-

portion of genetic thornless plants among a large number obtained by micropropagation of hexaploid chimeraal 'Thornless Logan'. Hall et al. (2) obtained similar plants by histogenic layer manipulation of the same chimeraal thornless hybrid.

Hall's group also (3) showed that their nonchimeraal variant of 'Thornless Logan' had potential as a cultivar, because it had no associated undesirable traits and cropped similarly to the chimeraal form. They, therefore, released it under the name 'Lincoln Logan'. Inbred thornless derivatives of the genetic thornless plants obtained by Rosati et al. (8) showed variable merit, but one of them (No. 4.6.4.4) (i.e., seedling n. 4 in the progeny of the plant 4.6.4) was comparable to the loganberry. These two sources of genetic thornlessness from the loganberry are therefore valuable for breeding *Rubus* cultivars at the hexaploid level. It is particularly desirable to introduce thornlessness from this source into the 'Tayberry', which is a loganberry-type hybrid superior in many traits (5), as well as to improved hexaploid blackberries. We report here some early results of our study of the inheritance of thornlessness in such material.

Crosses with 'Lincoln Logan' were made at Lincoln, New Zealand and crosses involving the Italian source of thornlessness from loganberry were made at Bologna, Italy. Crosses with the 'Lincoln Logan' x 'Tayberry' hybrid were also made at Dundee. The seeds obtained from the crosses were shared among the three centers of Bologna (B), Dundee (D), and Lincoln (L), (Tables 1 and 2) where they were germinated and the segregants classified as thorny or thornless at the fourth true-leaf stage. The segregation data were analysed by  $\chi^2$  to test whether they supported the hypothesis that thornlessness was determined by a single dominant gene or by two dominant complementary genes.

A self of the original chimeraal clone of 'Thornless Loganberry' gave 109 thorny and no thornless offspring, thereby supporting the nontransmissible nature of the thornless character in this clone.

None of the data for the segregation of thornlessness in the material derived from 'Lincoln Logan' showed a close fit to the expected ratio (Table 1). When 'Lincoln Logan' was used as the male parent, there was a large deficiency of thornless segregates (27%) while crosses in the reciprocal direction gave a small but significant excess of thornless types (57%). With both directions of crosses there was significant heterogeneity in the proportion of thorny segregates

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