# Tissue culture and subsequent field evaluation of strawberry

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## Summary

Strawberry plants of cv. Redlands Crimson, free of strawberry mild yellow edge virus (MYEV), have been produced by apical tip culture on a modified Adams (1972) medium. Explants from six other cultivars were grown on this medium and considerable variation in growth response was observed. No shoot growth was obtained on this medium with explants of cv. Phenomenal, however, growth was initiated on a modified Lee and De Fossard (1975) medium. Low media pH (4.2) inhibited shoot growth. Yield of six clones of cv. Redlands Crimson, free of MYEV and one virus infected clone were monitored for a fruiting season. Removal of MYEV had no effect on fruit quality, but uninfected clones had a 13% yield advantage of marketable fruit over the whole season. This increase was shown to occur during the two fruiting flushes and was a result of more vigorous plants having the capacity to produce more fruit. Commercial plantings of clones free of MYEV over five seasons have been characterised by high early yields.

## INTRODUCTION

Queensland's main commercial strawberry (*Fragaria×ananassa* Duch.) cultivar is Redlands Crimson. This cultivar is adapted to subtropical winter production, has high quality fruit and good agronomic characteristics, but at the time of its first release cv. Redlands Crimson carried mild yellow edge virus (MYEV), (Greber 1979). Fulton (1977) emphasised the need for virus eradication before release of any new cultivars.

Mellor and Fitzpatrick (1961) reported that MYEV is not readily inactivated by heat therapy. Heat-stable viruses have been eliminated from strawberry cultivars by apical tip culture of plants which have been subjected to heat treatment. Belkengren and Miller (1962) reported the elimination of strawberry latent A virus. McGrew (1965) removed yellow edge, crinkle and vein banding viruses. Mullin et al. (1974) reported elimination of mild yellow edge, mottle and pallidosis from cv. Fresno. MYEV alone has never been shown to reduce yield in strawberries. Barritt and Loo (1973), working with cvv. Northwest and Hood, stated that MYEV did not affect runnering, vigour or yield. Comparing MYEV free and infected clones of cv. Northwest, Lawrence and Miller (1968) found no significant difference in the number of crowns or flowers produced but they did not look at fruit set. Swartz et al. (1981) studied field performance and phenotypic stability of tissue culture propagated strawberries which had been initiated from meristematic tips and then multiplied in culture. The effects of tissue culture on field performance were increased vigour and increased apical bud activity. This resulted in a greater number of crowns per unit area (grown as a matted row) and greater number of flower trusses per crown. Similar results have been reported by Bedard and Garneau (1985), and increased susceptibility to two root-rot diseases with tissue cultured plants has been reported by Shoemaker et al. (1985).

A programme was undertaken to free cv. Redlands Crimson of MYEV via apical tip culture of heat treated plants. Field plantings were subsequently assessed for fruit quality and yield. This paper also reports on apical tip culture of seven other cultivars, and variations in their response to culture media.

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## MATERIALS AND METHODS

### Tissue culture of eight cultivars

Cvv. Redlands Crimson, Phenomenal, Naratoga, Torrey and breeding lines DK6, MT2, MT4 and TE4, are well or moderately well adapted to winter growing conditions in Queensland. Plants of these cultivars were heat treated for 30 days at 38°C with continuous light, and then transferred to a small glasshouse where temperatures ranged from 10°C to 35°C and relative humidity was low. To enhance runner production, natural light was augmented with incandescent bulbs to give continuous light. Runners produced from these plants were allowed no contact with soil or water.

Apical tips runners were disinfested in sodium hypochlorite solution (1.0% available chlorine) for 10 min, followed by two rinses in sterile water. Twelve shoot tips of each cultivar with two to four leaf primordia attached were removed and cultured on 10 mL of medium in 30 mL polycarbonate tubes with screw caps. Initially, explants of all cultivars were placed on filter paper bridges on a modified Adams (1972) medium (Table 1).

Table 1. Modified Adams (1972) medium used for single shoot and root growth, and modified Lee and De Fossard (1975) medium for shoot proliferation

| Compound | Media and component concentration | |
|---|---|---|
| | Adams (1972) | Lee and De Fossard (1975) |
| Sucrose | 87.71m mole/L | 87.71m mole/L |
| $NH_4NO_3$ | 20.60 " | 10.00 " |
| $KNO_3$ | 18.80 " | 10.00 " |
| $NaH_2PO_4$ | 0 " | 1.00 " |
| $CaCl_2.2H_2O$ | 3.00 " | 2.00 " |
| $MgSO_4.7H_2O$ | 1.50 " | 1.50 " |
| $KH_2PO_4$ | 1.25 " | 0 " |
| Fe (sequestrene) 6% Fe W/W | 0.11 " | 0 " |
| NaFeEDTA | 0 μ mole/L | 50.00 μ mole/L |
| $MnSO_4.4H_2O$ | 100.0 " | 50.00 " |
| $H_3BO_3$ | 100.0 " | 50.00 " |
| $ZnSO_4.7H_2O$ | 36.80 " | 20.00 " |
| KI | 5.00 " | 2.50 " |
| $(NH_4)_6Mo_7O_{24}.4H_2O$ | 1.30 " | 0 " |
| $Na_2MoO_4$ | 0 " | 0.10 " |
| $CuSO_4.5H_2O$ | 0.10 " | 0.10 " |
| $CoCl_2.6H_2O$ | 0.10 " | 0.50 " |
| Thiamine hydrochloride | 2.97 " | 2.00 " |
| Pyridoxine hydrochloride | 4.88 " | 3.00 " |
| Nicotinic acid | 40.70 " | 20.00 " |
| Riboflavin | 1.33 " | 0 " |
| Calcium pantothenate | 10.50 " | 0 " |
| Folic acid | 0.22 " | 0 " |
| Biotin | 0.04 " | 0 " |
| Choline chloride | 7.16 " | 0 " |
| para-Amino-benzoic acid | 0.36 " | 0 " |
| Inositol | 55.50 " | 300.00 " |
| Indole-3-butyric acid | 4.92 " | 0 " |
| Indole acetic acid | 0 " | 2.00 " |
| 6-Benzyl amino purine | 0.44 " | 0 " |
| Kinetin | 0 " | 5.00 " |
| Agar | 0 " | 8.00 g/L |

Sequestrene was used in place of NaFe EDTA, and ammonium molybdate was substituted for sodium molybdate. As cv. Phenomenal failed to respond to this medium, explants were subsequently grown on a modified Lee and De Fossard (1975) medium. Variations included the use of NaFe EDTA in place of $FeSO_4$, $Na_2$ EDTA and $Na_2SO_4$; omission of all growth factors except inositol, nicotinic acid, pyridoxine HCl and thiamine HCl; use of only one auxin–IAA; and one cytokinin–kinetin, (Table 1). The pH of all media was

adjusted to 6.0 with 0.2 M potassium hydroxide, prior to autoclaving at 121°C for 15 min. Cultures were incubated at 25°C with a photoperiod of 18 h light and 6 h darkness. After 5 to 6 weeks in culture, the plantlets were transferred to pots containing 1:1:1 steam sterilised peat:sand:soil mix and grown in an aphid proof screenhouse.

The plants were individually indexed by leaf grafting to seedlings of *Fragaria vesca* L. and plants of the UC4 clone (Frazier 1974) four times over the period of a year or until MYEV infection was confirmed. All leaf grafts were made to young vigorous indicator plants using two grafts per plant. Indicators were maintained at 20°C for best symptom development.

### Evaluation of MYEV free and infected clones of cv. Redlands Crimson

Six virus free clones (produced by tissue culture) and one virus infected clone were grown to compare yield, fruit quality, and incidence of berry diseases.

The MYEV infected clone of Redlands Crimson was derived from progeny of plants previously subjected to heat therapy which had failed to remove MYEV permanently. Infection was demonstrated by leaf-graft indexing (Bringhurst and Voth 1956) on a line of *Fragaria vesca* L. derived from the clone used by Stubbs (1957) and Frazier's UC1 clone where the old leaf symptom is diagnostic for MYEV. They were also indexed on the UC4 and UC5 clones (Frazier 1974) which produce a severe necrotic reaction after 3 to 4 weeks with MYEV infection. Aphid transmission tests with *Chaetosiphon fragaefolii* (Cock.), sap transfers to herbacious indicators and grafts to *Fragaria virginiana* (Duch.) clones did not show any other viruses to be present.

Runners from six MYEV free clones of cv. Redlands Crimson were taken from the glasshouse and field planted at Redlands Horticultural Research Station in April. These plants, as well as the MYEV infected clone, were allowed to develop, flower, fruit and produce runners as the season progressed through to 17 March of the following year. At this stage runners were planted 40 cm apart in an off-set pattern in double rows on raised beds at 1.5 m centres. The plants were mulched with aluminium coated black polythene, and wood shavings between the beds. A randomised complete block design containing 20 plants per plot with 5 replications was used.

Harvests were commenced of fruit more than three quarters full coloured on 2 June and continued twice weekly until 4 October. Fruit were graded into perfect (fruit greater than 5 g were considered marketable), imperfect (lacking complete seed development or split and hollow at the apical end), and diseased types. To monitor differences which occurred between clones at fruiting flushes, data for analayses were grouped into 6 three weekly periods.

The aphicide demeton-S-methyl was applied at regular intervals to prevent colonisation and spread of aphids. After the final harvest, a random sample of both tissue culture propagated plants and the virus infected control were indexed to UC4 indicator plants to test for virus.

### RESULTS AND DISCUSSION

After dissection, the normal pattern of growth was for cultured explants to enlarge and produce unifoliate leaves within the first two weeks and to produce roots wihin the next two weeks. Some cultures produced a small amount of callus which subsequently produced two or three shoots.

In preliminary experiments with a media pH of 4.2, most explants died. When the pH was adjusted to 6.0 before autoclaving, the percentage of explants developing into plants was greatly improved (Table 2).This is consistent with the findings of Mullin *et al.* (1974) who indicated that a pH less than 4.5 was lethal to strawberries. Another noteworthy finding in preliminary experimentation with cv. Redlands Crimson was that disinfestation could be achieved by treatment of apical tips of runners, with outer leaves removed, for

15 s in sodium hypochlorite (0.25% w/v available chlorine).This suggests that the growth of runners in a clean, low humidity environment prior to dissection contributed much towards the disinfestation of the apices.

**Table 2. Percentage of apical tips which produced plants out of 12 replications for eight strawberry cultivars when cultured a modified Adams (1972) medium, Table 1**

| Cultivar | Cultures free from contamination (No.) | Plants produced (No.) | Cultures which produced plants (%) |
|---|---|---|---|
| Phenomenal | 12 | 0 | 0 |
| Torrey | 12 | 1 | 8 |
| Naratoga | 12 | 5 | 41 |
| DK6 | 9 | 4 | 44 |
| TE4 | 10 | 6 | 60 |
| MT2 | 11 | 8 | 72 |
| MT11 | 11 | 8 | 72 |
| Redlands Crimson | 12 | 9 | 75 |

The percentage of apical tips which grew into plants on the modified Adams (1972) medium, varied between cultivars (Table 2), with no explants of cv. Phenomenal surviving and a very poor response for cv. Torrey. A number of attempts to grow cv. Phenomenal in experiments comparing filter paper bridges and agar based media, and using modifications of Adams (1972) medium were not successful. Subsequently, shoot tips of cv. Phenomenal were cultured on a modified Lee and De Fossard (1975) medium (Table 1). On this medium, eight out of eleven explants proliferated callus then produced multiple buds. After three weeks these shoots were transferred and grew on either of the defined media (Table 1).

Of the nine plants of cv. Redlands Crimson indexed for virus, six were virus free. Virus free plants of other clones were recovered.

In the field experiment there were no significant differences between clones, in the percentages of fruit which were diseased or imperfect. Berries from all clones were similar in colour and texture. Thus there was no effect of MYEV removal or tissue culture on these characteristics.

Cumulative yield data for the whole season shows that MYEV free clones produced an increase in yield of 10% over the MYEV infected clone for ungraded fruit and 13% for marketable fruit (Table 3).The main commercial yield advantage was early in the season when yields are low and prices are high on local markets. During the first six weeks of production, Clones 2 and 5 provided yields of 0.87 and 0.88 kg marketable fruit per plot compared with ($P<0.01$) 0.41 kg in Clone 1 (MYEV infected). From the yield data within each harvest period (Table 3), it can be seen that significant yield increases occurred in periods one and four. These periods represent the beginning of the first and second fruiting flushes which are characteristic for strawberries when grown in this environment. From visual observations it was apparent that MYEV free plants grew more vigorously prior to fruiting and were capable of producing higher yields. The significant differences between MYEV free and infected clones in percentages of unmarketable fruit (Table 4), show that some of the yield advantage in period four is due to better fruit size development, however, most of the yield advantage was via the production of higher numbers of berries. During period four, Clone 2 produced 300 fruit per plot compared to 175 in Clone 1 (MYEV infected).

Swartz *et al.* (1981) suggest that increased vigour in the field may be related to forced proliferation *in vitro*. However, in our experiment plants were in culture for a period of six weeks on low hormone concentrations, and subsequently grown for a year before field

evaluation. Thus the increased yields appear due to MYEV removal. Differences were observed in yield between the tissue cultured clones. Although callus culture is recognised for production of variation in plants (Larkin and Scowcroft 1981; Skirvin 1978; Yurgalevitch *et al.* 1985), apical tip culture is normally considered to be free from resultant offtypes. This result could be explained by the tendency of some explants to produce callus before shoots, however, the amount of callus produced was minimal.

**Table 3. Fruit yield per plot (20 plants) within each harvest period for fruit greater than 5 g**

| Clone* | Yield (kg) | | | | | | | s.e.m. |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Harvest period | | | | | | | | |
| 2 Jun to 24 Jun | 0.19*a*† | 0.50*b* | 0.48*b* | 0.36*b* | 0.55*b* | 0.33*b* | 0.18*a* | 0.058 |
| 24 Jun to 13 Jul | 0.21 | 0.37 | 0.33 | 0.28 | 0.32 | 0.25 | 0.18 | 0.051 |
| 15 Jul to 2 Aug | 0.75 | 0.87 | 0.91 | 0.71 | 0.66 | 0.90 | 1.05 | 0.091 |
| 5 Aug to 25 Aug | 1.53*a* | 2.77*b* | 2.63*b* | 2.13*c* | 2.43*bc* | 2.46*bc* | 2.59*bc* | 0.125 |
| 28 Aug to 13 Sep | 2.73 | 2.59 | 2.59 | 2.52 | 2.70 | 2.70 | 2.56 | 0.118 |
| 16 Sep to 4 Oct | 2.94 | 2.74 | 2.94 | 3.28 | 3.03 | 2.75 | 2.72 | 0.156 |
| Total | 8.36*a* | 9.85*b* | 9.87*b* | 9.27*ab* | 9.07*b* | 9.39*b* | 9.30*ab* | 0.247 |

* Strawberry clones cv. Redlands Crimson; 1 MYEV infected; 2 to 7 MYEV free.

† Within rows numbers followed by no letter, or a letter in common are not signficantly different from each other, (*P*<0.01).

**Table 4. The proportion of fruit less than 5 g as a per cent of the total number of fruit in each harvest period**

| Clone | Yield (kg) | | | | | | | s.e.m. |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Harvest period | | | | | | | | |
| 2 Jun to 24 Jun | 3.2† | 2.4 | 3.2 | 2.6 | 4.1 | 2.4 | 8.3 | 1.558 |
| 24 Jun to 13 Jul | 20.4 | 29.2 | 32.3 | 31.7 | 26.5 | 21.8 | 19.9 | 3.786 |
| 15 Jul to 2 Aug | 18.4 | 14.1 | 10.2 | 14.4 | 17.4 | 7.5 | 9.8 | 2.615 |
| 5 Aug to 23 Aug | 29.8*a* | 20.8*b* | 22.1*b* | 22.3*b* | 20.5*b* | 23.2*b* | 22.0*b* | 1.590 |
| 28 Aug to 13 Sep | 40.1*c* | 45.4*cd* | 46.7*d* | 43.5*cd* | 46.6*d* | 43.6*cd* | 43.8*cd* | 1.416 |
| 16 Sep to 4 Oct | 45.7 | 46.5 | 41.8 | 39.8 | 42.1 | 40.6 | 39.2 | 2.157 |
| Total | 39.2 | 37.3 | 36.3 | 35.4 | 37.2 | 35.1 | 34.3 | 1.053 |

* Strawberry clones cv. Redlands Crimson; 1 MYEV infected; 2 to 7 MYEV free.

† Within rows numbers followed by no letter, or a letter in common are not significantly different from each other, (*P*<0.01).

Virus-free plants of cv. Redlands Crimson have been released through a controlled runner scheme over the past five years and now provide the basis for production in southeast Queensland. Commercial plantings of these clones have been characterised by high early yields of berries.

# References

Adams, A. N. (1972), An improved medium for strawberry meristem culture, *Journal of Horticultural Science* **47**, 263–64.

Barritt, B. H. and Loo, H. Y. S. (1973), Effects of mottle, crinkle and mild yellow edge viruses on growth and yield of Hood and Northwest strawberries, *Canadian Journal of Plant Science* **53** (3), 605–607.

Bedard, R. and Garneau, A. (1985), Field evaluation of tissue-cultured strawberry plants, *HortScience* **20** (3), 44.

Belkengren, R. O. and Miller, P. W. (1962), Culture of apical meristems of *Fragaria vesca* strawberry plants as a method of excluding latent A virus, *Plant Disease Reporter* **46**, 118–21.

Bringhurst, R. S. and Voth, V. (1956), Strawberry virus transmission by grafting excised leaves, *Plant Disease Reporter* **40**, 590–600.

Frazier, N. W. (1974), Six new strawberry indicator clones evaluated for the detection and diagnosis of twelve graft-transmissible diseases, *Plant Disease Reporter* **58**, 28–31.

Fulton, J. P. (1977), Strawberry viruses, *HortScience* **12**, 470–71.

Greber, R. S. (1979), Virus diseases of Queensland strawberries and the epidemiological effects of the strawberry runner approval scheme, *Queensland Journal of Agricultural and Animal Sciences* **36**, 93–103.

Larkin, P. J. and Scowcroft, W. R. (1981), Somaclonal variation–a novel source of variability from cell cultures for plant improvement, *Theoretical and Applied Genetics* **60**, 197–214.

Lawrence, F. J. and Miller, P. W. (1968), Effects of virus infection on the strawberry cultivar Northwest, *Plant Disease Reporter* **52**, 811–13.

Lee, E. C. M. and De Fossard, R. A. (1975), Regeneration of strawberry plants from tissue cultures, *Combined Proceedings of the International Plant Propagators' Society* **25**, 277–85.

McGrew, J. R. (1965), Eradication of latent C virus in the Suwannee variety of strawberry by heat plus excised runner tip culture, *Phytopathology* **55**, 480–81.

Mellor, F. C. and Fitzpatrick, R. E. (1961), Strawbery viruses, *Canadian Plant Disease Survey* **41**, 218–55.

Mullin, R. H., Smith S. H., Frazier, N. W., Schlegel, D. E. and McGall, S. R. (1974), Meristem culture frees strawberries of mild yellow edge, pallidosis and mottle diseases, *Phytopathology* **64**, 1425–29.

Shoemaker, N. P., Swartz, H. J. and Galetta, G. J. (1985), Cultivar dependent variation in pathogen resistance due to tissue culture–propagation of strawberries, *HortScience* **20**, 253–54.

Skirvin, R. M. (1978), Natural and induced variation in tissue culture, *Euphytica* **27**, 241–66.

Stubbs, L. L. (1957), A comparison of three low-persistence viruses of the strawberry, *Australian Journal of Agricultural Research* **8**, 652–58.

Swartz, H. J., Galletta, G. J. and Zimmerman, R. H. (1981), Field performance and phenotypic stability of tissue culture–propagated strawberries, *Journal of the American Society for Horticultural Science* **106**, 667–73.

Yurgalevitch, C. M., Janes, H. W. and Chin, C. (1985), Somaclonal variation in *Fragaria, HortScience* **20** (3), 585.

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