

EFFECT OF MEDIA AND MEDIA pH ON IN VITRO PROPAGATION OF 'NEMAGUARD' PEACH ROOTSTOCK*

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(Accepted for publication 30 March 1983)

ABSTRACT

Reeves, D.W., Horton, B.D. and Couvillon, G.A., 1983. Effect of media and media pH on in vitro propagation of 'Nemaguard' peach rootstock. *Scientia Hortic.*, 21: 353–357.

'Nemaguard' (*Prunus persica* L. Batsch × *Prunus davidiana* Carriere) peach rootstock explants grown in liquid vs. agar medium (modified Murashige and Skoog) had a mortality of 83 vs. 60% at the end of 8 weeks. Media pH (5.2 or 5.8) had no effect on mortality. However, the best survival, growth, and condition of explants occurred on agar medium at pH 5.8. With time cultures declined in vitro; of 180 initial explants, 10 shoots rooted and 4 plantlets survived the transfer to soil.

Keywords: in vitro; peach rootstock.

INTRODUCTION

There are many reports of successful micropropagation of *Prunus* spp. other than peach (Boxus and Quoirin, 1974; Mehra and Mehra, 1974; Zuccherelli, 1979; Rosati et al., 1980). Plum rootstocks are being produced commercially (Zuccherelli, 1979). Research with peach in vitro has produced infrequent and limited success. Peach explants have been established and shoot proliferation has been reported (Skirvin and Chu, 1977; Hammerschlag, 1982). Shoot tips of the peach rootstock GF 305 have been rooted in tubes with root-promoting medium (Nequerales and Jones, 1979). 'Harbrite' peach has been proliferated and rooted in vitro (Skirvin et al., 1981). Recently, 'Nemaguard' peach rootstock has been re-

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established in soil following in vitro procedures (Miller et al., 1982). No reports have indicated success with long-term sub-culturing of peach.

Our experience, as well as that of some other researchers, is that peach explants typically grow for 8–12 weeks and then decline and die (Nekrosova, 1964; Almehdi et al., 1982). The decline is characterized by chlorosis, leaf drop, and browning of tissue. A study dealing with *Cattleya* explants attributed similar symptoms of decline to the presence of phenolic compounds produced by the explant, and reported that medium pH affected explant survival (Ishii et al., 1979). Studies with *Prunus* varied media pH, before autoclaving, from 5 to 5.9 (Boxus and Quoirin, 1974; Zuccherelli, 1979; Rosati et al., 1980; Miller et al., 1982). Liquid medium has been reported to be beneficial in promoting growth and preventing decline of explants of peaches (Hammerschlag, 1982) and azaleas (Ma and Wang, 1977). The present study evaluates the effect of medium-type and pH on survival and growth of 'Nemaguard' peach explants.

MATERIALS AND METHODS

Shoot sections 1.5–2 cm long, possessing a single node, were cut from actively growing plants of 'Nemaguard' rootstock maintained in a greenhouse. Petioles were removed from nodal sections and the explants were placed in 200-ml flasks containing 1300 mg l⁻¹ each of chlortetracycline HCl, streptomycin sulfate and Captan [*cis*-N-(Trichloromethyl) thio]-4-cyclohexene-1,2 dicarboximide]. Two drops of phosphorus-free detergent were added as a surfactant. The nodal sections were shaken on a wrist-action shaker for 30 min, drained, and surface-sterilized in 10% commercial bleach (5.25% NaClO) and 0.01% Tween 20 (polyoxyethylene (20) sorbitan monolaurate) for 10 min, rinsed 3 times with sterile deionized water for 10 min each, and placed on modified half-strength Murashige and Skoog medium in petri dishes for 72 h before being transferred to tubes of treatment media.

The modified half-strength Murashige and Skoog medium was supplemented with (mg l⁻¹) 27.8 FeSO₄ · 7H₂O, 37.3 Na₂ · EDTA, 170 NaH₂PO₄ · H₂O, 100 i-inositol, 80 adenine sulfate, 30 000 sucrose, 1 benzyladenine (BA), 0.01 indole-3-butyric acid (IBA) and Staba vitamins (Skirvin and Chu, 1979). The pH was adjusted to 5.2 or 5.8 with 1 N NaOH before autoclaving and was not monitored after media preparation. Phytagar (Gibco Laboratories, 8175 Staley Rd., Grand Island, NY 14072, U.S.A.) was used at different concentrations (4 g l⁻¹ in pH 5.8 medium and 6 g l⁻¹ in pH 5.2 medium) to produce similar gel consistency in agar cultures. Both liquid and agar media were dispensed 20 ml per 25 × 150 mm tube. Filter-paper bridges were placed in cultures with liquid media. Clear plastic closures were used on all tubes. Agar cultures were placed on slanted racks, and liquid cultures were rotated at 1 r.p.m. on a roller drum. The filter paper facilitated the use of a maximum volume of media, which

washed over the explant without inundating it. All explants were grown at day/night temperatures of $26/20 \pm 2^\circ\text{C}$ under 16 h light supplied by F40T12 Sylvania Gro-lux Lifeline tubes at 50–65 microEinsteins $\text{m}^{-2} \text{s}^{-1}$.

A total of 180 explants, 5 per experimental unit, were randomized in a factorial design with unequal replications, i.e. 10 replications of agar and 8 replications of liquid media. Explants were transferred to fresh media at 28-day intervals. Mortality counts, and growth and condition ratings, using a scale of 0–9, were made twice at monthly intervals. A growth rating of 0 represented no bud-break and a rating of 9 represented the most growth. Most growth was defined as overall quantity of new tissue, regardless of type, i.e. a combination of shoot elongation, bud and leaf formation. A condition rating of 0 represented dead explants and a rating of 9 represented no chlorosis or tissue browning. Differences in mortality were tested by χ^2 . Contaminated cultures and buds that did not break at the end of 1 month were excluded from the analysis of data on growth and condition. Ratings of surviving explants were subjected to analysis of variance and mean separation using Fisher's protected LSD.

RESULTS AND DISCUSSION

There were no differences in growth or condition of explants after 28 days; however, mortality was lower on agar than in liquid medium (Table I). There was no difference in mortality due to pH, but liquid pH 5.2 had

TABLE I

χ^2 analyses of mortality of 'Nemaguard' peach explants cultured in agar or liquid at pH 5.8 or 5.2. Values within a comparison and followed by a different letter are significantly different at the $P < 0.05$ level

Comparison	Mortality (%)	
	1 month	2 months
Agar	4.5 a	59.7 a
Liquid	20.0 b	82.5 b
pH 5.2	13.8 a	69.6 a
pH 5.8	10.2 a	71.5 a
Agar pH 5.2	0.0 a	56.4 a
Liquid pH 5.2	27.5 b	82.5 b
Agar pH 5.8	8.3 a	62.5 a
Liquid pH 5.8	12.5 a	82.5 a
Agar pH 5.2	0.0 a	56.4 a
Agar pH 5.8	8.3 a	62.5 a
Liquid pH 5.2	27.5 a	82.5 a
Liquid pH 5.8	12.5 a	82.5 a

a higher mortality rate than did agar medium. Mortality was higher in liquid than in agar after 2 months. Mortality rate was not affected by pH, but was increased by liquid pH 5.2 (Table I).

There were no differences in growth or condition due to media, but the interaction between pH and media at the end of 2 months was significant (Table II). Growth and condition of surviving explants on agar were better at pH 5.8, but surviving explants in liquid were better at pH 5.2. In this experiment, effects of pH may be confounded with agar concentrations. Preliminary experiments, however, indicated that the narrow range of agar concentration used in this experiment would have influenced plant response less than would varying the gel consistency of the media. Growth of peach has been reported to be greater on liquid than on agar media in explants cultured for 3 weeks, but survival was <50% in 3 of 11 cultivars tested (Hammerschlag, 1982). Although leaves expanded more in liquid medium than agar medium, our results showed no differences in either growth or condition of explants cultured for 8 weeks in liquid and agar media; however, liquid medium accelerated decline and mortality.

The least mortality was obtained on agar medium, and the best growth was obtained on agar medium at pH 5.8. A gradual decline of cultures was evident, but an average of 5 shoots per surviving explant was obtained from cultures on agar after 2 months. These shoots were excised and placed on pH 5.8 agar medium with BA reduced to 0.1 mg l⁻¹ to allow elongation. Three weeks later, shoots 2 cm or longer were transferred to rooting-medium (pH 5.8 agar), which was identical to proliferation media except that the BA and adenine sulfate were omitted and IBA was increased to 2 mg l⁻¹. The surviving 'Nemaguard' explants in liquid culture grew well, but due to their growth pattern, i.e. good leaf formation and expansion but poor shoot formation, they provided no shoots suitable for transfer to rooting-medium. Within 21 days, a total of 10 shoots had rooted well enough to transfer to potting-soil. These were transferred to a greenhouse,

TABLE II

Growth and condition of surviving 'Nemaguard' peach explants cultured in agar or liquid media at pH 5.8 or 5.2 for 2 months. Means are from 7–18 explants. Mean separation within columns by Fishers protected LSD 0.05. Values within a column and followed by a different letter are significantly different at the $P < 0.05$ level

Medium	pH	Rating	
		Growth	Condition
Agar	5.8	7.1 a	7.5 a
	5.2	5.5 b	5.7 bc
Liquid	5.8	5.7 ab	4.6 c
	5.2	6.4 ab	6.9 ab

enclosed in a plastic tent, and subjected to intermittent mist (15 s every 30 min) for 24 h/day. Frequency of misting was gradually decreased as plants became acclimatized. Four plants survived and were transferred to clay pots.

CONCLUSION

The decline of peach cultures *in vitro* places a severe handicap on researchers in that it makes it difficult to accumulate and maintain quantities of cultures necessary to evaluate factors that affect propagation. This decline usually fragments the experimental design, making it difficult statistically to separate treatment effects, particularly on shoot proliferation and rooting. For this reason, evidence of means to lessen decline and mortality is important. Since most researchers working with peach *in vitro* use Murashige and Skoog salts or some modification of it, our data suggest that the best peach explant growth with the least decline and mortality can be obtained on agar medium at pH 5.8. Further work is needed to determine whether this conclusion remains valid over a wide range of peach cultivars.

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