HortScience 26(1):62-64. 1991.

Pear in Vitro Propagation Using a Double-phase Culture System

R. Rodriguez and C. Díaz-Sala
Laboratorio de Fisiología Vegetal, Facultad de Biología, Universidad de Oviedo, Oviedo, Spain

L. Cuozzo and G. Ancora
Ente Nationale Delle Energie Alternative, Centro Ricerche Energia Cassacia, Rome, Italy

Additional index words. Pyrus communis, proliferation, elongation, rooting, cytokinins, auxins, gibberellins

Abstract. **Proliferation of *Pyrus communis* L. cv. Abate Fetel, Precoce Morettini, and Guyot was accomplished with a yield of 10 to 15 new shoots per explant. The in vitro procedure is based on the use of 6.7 μM BAP as an overlay on a modified MS medium. Rooting without callus formation was achieved by immersing the basal end in 5 μM IBA solution for 1 min. The possible inhibition of proliferation and plantlet regeneration by GA_3 and IBA is discussed. Chemical names used: 6-benzylaminopurine (BAP); indole-3-butyric acid (IBA); gibberellic acid (GA_3).**

Pear in vitro culture has already been reported (Chevreau et al., 1989; Duron, 1983; Marine, 1984; Shen and Mullins, 1984) and is a routine technique in several countries; however, propagation methods for 'Abate Fetel' are scarce (De Paoli, 1989) and we found no published reports for 'Precoce Morettini' and 'Guyot'. We have attempted to culture these cultivars (Estrada et al., 1987). To improve proliferation rates and shoot quality, we have studied the effects of several media using a double-phase liquid-agar solidified culture system (Viseur, 1987). The results are compared to those obtained using a solid medium, i.e., a single-phase culture system. Furthermore, the possible proliferation and plantlet regeneration inhibition caused by the application of GA_3, IBA, or their combination is discussed.

Branches (50 cm long) of mature field-grown trees were presterilized for 2 min in a solution of 8% (w/v) ferrous sulphate and 0.4% (w/v) captan, and their axillary buds were forced to grow by immersing the basal

Received for publication 31 Aug. 1989. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations,thispaper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Effect of single- and double-phase culture system on pear (cultivars Abate Fetel, Guyot, and Precoce Morettini) proliferation.[z] Results were taken after 40 days of culture in the presence of the plant growth regulators indicated in the medium code detailed in the text.

	Cultivars											
	Abate Fetel				Guyot				Precoce Morettini			
Medium	Multiple shoot induction (%)	Shoots/explant	Shoots (no.) ≥3 cm	Shoots (no.) <3 cm	Multiple shoot induction (%)	Shoots/explant	Shoots (no.) ≥3 cm	Shoots (no.) <3 cm	Multiple shoot induction (%)	Shoots/explant	Shoots (no.) ≥3 cm	Shoots (no.) <3 cm
MS2A/MS2A	90 e	4 d	3 c	1	95 d	7 f	4 e	3	95 d	10 g	4 d	6
MS2A	85 d	2 b	1 b	1	90 d	3 b	1 b	2	75 b	4 c	1 b	3
MS2 (control)	40 a	1 a	0 a	1	20 a	1 a	0 a	1	30 a	1 a	0 a	1
MS1A/MS1A	90 e	4 d	3 c	1	90 d	5 d	2 c	3	90 d	8 f	2 c	6
MS1A	70 c	3 c	1 b	2	70 b	3 b	1 b	2	80 c	3 b	1 b	2
MS1 (control)	40 a	1 a	0 a	1	20 a	1 a	0 a	1	30 a	1 a	0 a	1
MS2B/MS2B	85 d	5 e	4 d	1	95 d	10 g	5 f	5	95 d	15 i	12 h	3
MS2B	70 c	3 c	2 b	1	90 d	6 e	4 e	2	70 b	7 e	5 e	2
MS1B/MS1B	75 c	4 d	3 c	1	95 d	10 g	3 d	7	85 c	15 i	8 g	7
MS1B	60 b	3 c	2 b	1	90 d	5 d	2 c	3	70 b	4 c	2 c	2
MS2C/MS2C	85 d	12 h	10 f	2	90 d	7 f	3 d	4	90 d	12 h	6 f	6
MS2C	75 c	6 f	2 b	4	75 b	4 c	2 c	2	70 b	5 d	2 c	3
MS1C/MS1C	80 d	10 g	6 e	4	80 c	6 e	2 c	4	90 d	12 h	5 e	7
MS1C	80 d	5 e	2 b	3	75 b	3 b	1 b	2	70 b	3 b	0 a	3

[z]Values in a column followed by the same letter are not significantly different at $P = 0.05$, according to the Student-Newman-Keuls test.

ends of the branches in distilled water under controlled conditions (22 ± 3C, 16-hr photoperiod, 30 µmol·s^{-1}·m^{-2} cool-white fluorescent illumination). After 15 days, the initial explants (nodal shoot segments 3 to 4 cm 'long) bearing four axillary buds, were excised from the shoots that originated from the stimulated axillary buds. The explants were presterilized by immersion in 95% (v/v) ethanol for 5 min and surface-sterilized with 2.5% (w/v) sodium hypochlorite for 20 rein; afterwards, they were rinsed several times with sterile deionized water (Pérez et al., 1983).

Fig. 1. Multiple shoot-bud development of 'Abate Fetel' pear explants after 40 days of double-phase culture on MS2 + 6.7 µM BAP.

For tissue culture, two MS (Murashige and Skoog, 1962) media were used: MS1 (MS salts plus 100 mg thiamine/liter) and MS2 (MS salts with half strength nitrates but double strength calcium chloride and magnesium sulphate plus (mg·liter^{-1}) 100 m-inositol, 1 nicotinic acid, 1 pyridoxine·HCl, and 2 ascorbic acid.

During the proliferation phase, MS1 and MS2 media were used with the following combinations of plant growth regulators (µM): MS1 + BAP 6.7, IBA 0.5, GA_3 0.3 (MS1A); MS2 + BAP 6.7, IBA 0.5, GA_3 0.3 MS2A); MS1 + BAP 6.7, IBA 0.5 (MS1B); MS2 + BAP 6.7, IBA 0.5 (MS2B); MS1 + BAP 6.7 (MS1C); MS2 + BAP 6.7 (MS2C).

Explants (20 to 30 per experiment) were grown in plastic containers (Magenta Co., Chicago) with 40 ml of culture medium (MS1 or MS2) plus 30 g sucrose and 7 g Difco Bacto-Agar/liter. The pH of the medium was 5.8 and it was autoclave at 1.2 kg·cm^{-2} for 15 min.

For double-phase culture, 10 ml of liquid medium (MS1-/MSl-, MS2-/MS2) was added after horizontally positioning the primary explants.

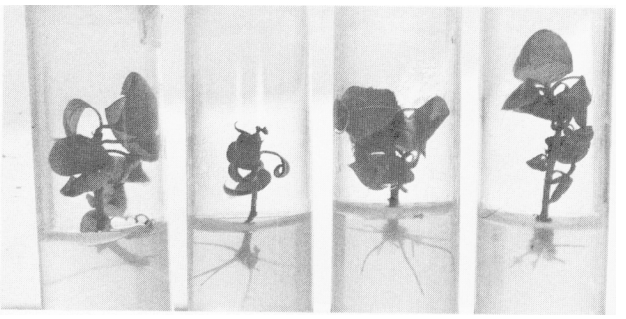

Fig. 2. 'Abate Fetel' root development after l-rein immersion in 5 µM IBA solution followed by 20 days on 1/2 MS2 basal media.

Rooting was accomplished by two methods: a) culture on solid MS2 medium plus IBA (30, 10,5 µM) or NAA (30, 10,5 µM) in darkness, followed by 10 to 15 days of culture on a half-strength solid hormone-free medium (MS2) under 16-hr photoperiod, and b) l-rein immersion in an IBA solution (5, -2.5, 0.5, 0.05 µM) followed by 20 days of culture on a half-strength solid hormone-free medium (MS2) with a 16-hr photoperiod.

Statistical analyses were performed using analysis of variance. The Student-Newman-Keuls test was used to separate differences among means at $P = 0.05$. A X^2 test was used to determine differences among frequencies.

After 40 days, there was a clear difference between the shoot number per explant obtained according to the culture system used (Table 1). Shoot multiplication was always higher when a liquid medium was used as an overlay.

'Guyot' gave the highest percentage of multiple shoot induction, followed by 'Precoce Morettini' and 'Abate Fetel'. Although shoot number per explant was not significantly different among cultivars, 'Guyot' and 'Precoce Morettini' behaved efficiently on MS2B and MS2C, whereas 'Abate Fetel' only responded on MS2C. From the results it seems to be clear that the presence of GA_3, IBA, or their combination inhibits BAP-induced responses.

In the presence of 6.7 µM BAP (MS1C, MS2C), in a double-phase culture system, 'Abate Fetel' proliferation (10 to 12 shoots per explant) was mainly due to the stimulation of axillary buds, which gave rise to simultaneous shoot-bud development and occasional multiple shoot buds (Fig. 1). Conversely, when 0.5 µM IBA was also present (MS1B, MS2B), the shoot number per explant. changed, and most of the new shoots resulted from branching. The combination of 0.3 µM GA_3 and 0.5 µM IBA in the presence of 6.7 µM BAP (MS1A, MS2A)