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Adventitious shoot regeneration from leaf tissue of three pear (*Pyrus sp.*) cultivars in vitro

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ABSTRACT

To develop an adventitious regeneration system for pear cultivars, several experiments were conducted with 2 cultivars of Pyrus communis L. ('Seckel' and 'Louise Bonne') and one cultivar of P. bretschneideri Rehd. ('Crystal Pear'). Half-leaves, taken from shoots proliferating on Lepoivre medium, were plated in petri-dishes on medium supplemented with various combinations of cytokinins and auxins. Cultures of the above cultivars had been established from mature trees. Among the growth regulators tested, thidiazuron (TDZ), combined with naphthaleneacetic acid (NAA), was the most efficient for stimulation of adventitious shoots. The optimum level of TDZ was about 3 uM; shoot regeneration was observed over a wide range of TDZ and NAA concentrations (0.5 to 5 uM and 2.5 to 13 um, respectively). Among different macronutrient compositions, 1/2 and 1/4 Murashige and Skoog were the most effective. Sucrose concentrations (10 to 50 g L-1) had a linear significant effect on shoot regeneration of 'Crystal Pear'.

ABBREVIATIONS

BAP: 6-benzylaminopurine; IBA: indole-3-butyric acid; NAA: a-naphthaleneacetic acid; NoA: 2-naphthoxyacetic acid; 2,4-D: 2,4-dichlorophenoxyacetic acid; TDZ: thidiazuron (N-phenyl-N'-1,2,3-thidiazol-5-ylurea); MS: Murashige and Skoog (1962) macroelements; L: Lepoivre (Quoirin and Lepoivre, 1977) macroelements.

INTRODUCTION

Adventitious regeneration occurs after cells from organized tissues dedifferentiate and then reorganize by meristem or somatic embryo formation, either directly or with a callus intermediary. This process is a very important step for the induction of variability through parasexual methods: somaclonal variation, induced mutagenesis and transformation.

Although adventitious organogenesis has been achieved in several woody fruit species (Hutchinson and Zimmerman, 1987) most regenerations have been from juvenile tissues of seedling origin. Direct improvement of fruit cultivars requires the control of regeneration from mature tissues. Promising results have been obtained from apple leaf discs (James et al., 1984) and stem internodes (Jones et al., 1984; Evaldsson, 1985) as well as from Prunus root callus (Druart, 1980; James et al., 1987).

In vitro growth of Pear (Pyrus) species has been reviewed (Singha, 1986). P. communis and P. pyrifolia cultivars have been micropropagated (Singha, 1986), and stored for a long period of time (Wanas et al., 1986). Somatic embryogenesis has been reported from seedling explants (Mehra and Jaidka, 1979; 1985) and plantlets have been regenerated from protoplast cultures of a wild pear (Ochatt and Caso, 1986). Protoplasts have been isolated from 'Williams' pear, but regeneration has not been reported (Revilla et al., 1987).

The purpose of this study was to develop an efficient technique to regenerate adventitious shoots from leaf tissue of several pear cultivars. This is the first step in the development of an in vitro mutagenesis program.

MATERIAL AND METHODS

This study was conducted with 2 cultivars of P. communis ('Seckel' and 'Louise Bonne') and one cultivar of P. bretschneideri ('Crystal Pear').

Shoot cultures, which had been established from mature trees, were maintained on Lepoivre proliferation medium (Quoirin and Lepoivre, 1977), supplemented with BAP (4.4 uM), IBA (0.5) uM, GA_3 (0.3 uM), 30 g l^{-1} sucrose and 6 g l^{-1} Difco Bacto agar (Fig. 1). To prevent the growth of internal bacteria on the medium, 10 mg l^{-1} gentamycin sulfate was added prior to autoclaving. The antibiotic was necessary in all experiements. The cultures were grown at 22° to 24°C, under cool white fluorescent tubes (40 $Em^{-2}s^{-1}$), with a 16/8 h light/ dark photoperiod, and transferred to fresh medium every 4 weeks.

For regeneration experiments, leaves were excised from the proliferating shoots, cut in halves perpendicularly to the mid rib, and randomly plated in 100x15 mm petri dishes, with the abaxial leaf surface in contact with the medium. Except in experiments 2 and 3, Lepoivre proliferation medium with 30 g l^{-1} sucrose, 8 g l^{-1} agar, and 10 mg l^{-1} gentamycin sulfate was used with various growth regulator combinations. In all experiments, the cultures were incubated in the dark for 25 days, then transferred to a corresponding auxin-free medium in ambient light. All data were collected weekly after cultures were moved from the dark.

After shoots were regenerated on an explant (Fig. 2), they spontaneously began to proliferate on the regeneration medium. The rapidly proliferating shoots often masked further differentiation. For

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