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Micropropagation of two French prune cultivars (*Prunus domestica* L.)

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SUMMARY

Phloroglucinol (1,3,5-trihydroxybenzene, PG, 162 mg l⁻¹) was an essential component of media (Murashige-Skoog (MS) containing benzyladenine, 1.0 mg l⁻¹ and γ -indolebutyric acid (IBA), 0.1 mg l⁻¹) for establishment and growth of proliferating shoot cultures of "Commune d'Agen" and "d'Ente 707" prunes. Addition of PG (162 mg l⁻¹) to the medium ((MS) containing indole-3-acetic acid (IAA) or α -naphthaleneacetic acid (NAA) or IBA, each at 1.0 mg l⁻¹) was also required for formation of adventitious roots *in vitro* by microcuttings. Cultures (23 \pm 2 °C) did not grow if the photoperiod exceeded 12 h. Microcuttings formed roots only after their parent shoot cultures had been conditioned by 4 subcultures, each of one month. Thereafter, rooting capacity increased with subculture. Up to 90 % of microcuttings from subculture 7 formed roots when cultured on media containing both IAA (1.0 mg l⁻¹) and PG (162 mg l⁻¹). Establishment of own-rooted plants under glasshouse conditions was more difficult with "Commune d'Agen" (20 %) than with "d'Ente 707" (70 %). Micropropagation of these prune cultivars is discussed in relation to plant propagation and plant improvement.

Additional key words : *Micropropagation, French prune, d'Ente 707, d'Agen, adventitious roots in vitro, phloroglucinol.*

RÉSUMÉ

Micropropagation de deux variétés françaises de prunier (Prunus domestica L).

Le phloroglucinol (1,3,5-hydroxybenzène ; PG ; 162 mg l⁻¹) est un constituant indispensable du milieu de culture (M.S. plus benzyladénine 1.0 mg l⁻¹ et acide γ -indole butyrique (AIB) 0,1 mg l⁻¹) pour l'établissement et la prolifération de bourgeons axillaires des variétés « Commune d'Agen » et « d'Ente 707 ». L'addition de PG (162 mg l⁻¹) au milieu (M.S. complété soit par l'AIB, 1,0 mg l⁻¹ soit par l'acide indole acétique (AIA) 1,0 mg l⁻¹ soit par l'acide α -naphtalène acétique (ANA) 1,0 mg l⁻¹) est aussi nécessaire pour la formation *in vitro* de racines adventives à partir de microboutures. Les cultures (placées à 23 \pm 2 °C) ne se développent pas si la photopériode excède 12 h. Les microboutures forment des racines seulement après un conditionnement des tiges-mères par 4 subcultures d'un mois chacune. Ensuite, le rendement d'enracinement augmente avec les subcultures ; après 7 subcultures, la fréquence des boutures formant des racines sur milieu M.S. plus PG 162 mg l⁻¹ et AIA 1,0 mg l⁻¹ peut atteindre 90 p. 100. L'établissement en serre des vitroplants enracinés est plus difficile avec la variété « Commune d'Agen » (20 p. 100) qu'avec « d'Ente 707 » (70 p. 100). La micropropagation de ces variétés de prunier est replacée dans le contexte général de la multiplication et de l'amélioration des plantes.

Mots-clés additionnels : *Micropropagation, prunier français, d'Ente 707, d'Agen, racines adventives in vitro, phloroglucinol.*

I. INTRODUCTION

Most of the commercially important cultivars of pome and stone fruits are difficult to root from cuttings and are usually propagated by budding onto clonal or seedling rootstocks. With several scion cultivars of apples (LANE, 1978 ; JONES *et al.*, 1979 ; ZIMMERMANN & BROOME, 1980 ; SRISKANDARAJAH & MULLINS, 1981), pears (SINGHA, 1980) and various *Prunus* (TABACHNIK & KESTER, 1977 ; JONES & HOPGOOD, 1979 ; CHANCEL *et al.*, 1980) own-rooted

trees have been produced by micropropagation *in vitro*. In this procedures buds are cultured on cytokinin-enriched media to induce the outgrowth of many axillary shoots. These shoots are then made into microcuttings and are stimulated to form adventitious roots by culture on media containing auxins. The present report concerns the regeneration of own-rooted plants of two cultivars of French prune, d'Agen and the d'Ente (I.N.R.A. selection 707), plants which are known to be especially difficult to propagate from cuttings.

Special attention was given to two factors: (i) effects of phloroglucinol, a phenolic additive which promotes root formation in microcuttings of some fruit plants (JONES & HATFIELD, 1976) but not others (ZIMMERMANN & BROOME, 1980) and (ii) effects of photoperiod and prolonged subculture, treatments which promote root formation *in vitro* in difficult-to-root cultivars of apple (SRISKANDARAJAH *et al.*, 1982).

II. MATERIALS AND METHODS

Three-year-old container-grown mother trees of the common d'Agen prune and of the I.N.R.A. selection, d'Ente 707 (*Prunus domestica* L.), were provided by the Fruit Variety Foundation, Biological and Chemical Research Institute, NSW Department of Agriculture, Rydalmere, NSW. The mother trees were grown in the glasshouse and were pruned repeatedly to provide supplies of leafy shoots. The un lignified portions of these shoots were made into single node cuttings and the explants were surface sterilized by standard procedures. The basal medium for culture establishment was that of MURASHIGE & SKOOG (1962, MS-medium). Additives included benzyladenine (BA, 1.0 mg l⁻¹), γ -indolebutyric acid (IBA, 0.1 mg l⁻¹) and phloroglucinol (PG, 162 mg l⁻¹).

The medium contained sucrose (3 % w/v) and was solidified with agar (0.8 % w/v). The pH was adjusted to 5.5 before autoclaving. Cultures were grown at 23 \pm 2 °C and illuminated (12, 16 or 24 h) by cool white fluorescent lamps (Osram QS). The irradiance at the level of the explants was 7-15 Wm⁻². Subcultures were made at 4-weekly intervals. Axillary shoots from the initial cultures were made into microcuttings (20 mm) which were either subcultured to produce further generations of axillary shoots or treated to induce roots. For rooting the microcuttings were grown either on filter paper bridges in test tubes (25 mm) with half-strength MS-medium containing IBA (0.2-3.0 mg l⁻¹) and PG (162 mg l⁻¹) or with an agar-based medium (0.8 % w/v) supplemented with mixtures of PG (162 mg l⁻¹) and one of the following auxins (1.0 mg l⁻¹): IBA, indole-3-acetic acid (IAA), or α -naphthaleneacetic acid (NAA). For establishment of plants under glasshouse conditions, the rooted microcuttings were planted into propagation tubes (80 \times 25 mm) containing a pasteurized mixture of peat and perlite (1 : 3). The propagation tubes were kept initially in trays covered by transparent polyurethane film so as to maintain a high humidity. These plants, which were grown under the same conditions of light and temperature as for micropropagation, were ready for transfer to normal glasshouse conditions after 3 weeks.

III. RESULTS

A. Shoot growth : requirement for phloroglucinol (PG)

With all treatments, bud burst occurred after one week but elongation of the main shoot and production of axillary shoots occurred only in media containing both BA and PG. In the absence of PG the initial

explants produced a rosette of leaves (fig. 1A) which subsequently turned brown and died. In subcultures, as in the initial culture, the emergence and elongation of leafy shoots was dependent upon the presence of phloroglucinol.

B. Shoot growth : effects of culture environment and subculture

Cultures grew weakly and became chlorotic in long photoperiods (16 h) or under continuous illumination. The optimum photoperiod for shoot growth was 12 h. Vigorous growth was restored when chlorotic plants grown under long-day conditions were transferred to a 12 h photoperiod. Cultures incubated at 23 \pm 2 °C produced greater numbers of axillary shoots than those incubated at 26 \pm 2 °C. In most cultures a single extension shoot was produced by the initial explant. Production of axillary shoots commenced at the 2nd subculture and rose to a maximum after 3 or 4 subcultures. There was considerable variation among explants in total numbers of axillary shoots and means varied from 6-9 (table 1, fig. 1B).

C. Formation of adventitious roots by microcuttings and plant establishment

Microcuttings from the initial explants and from the first 4 subcultures could not be made to form adventitious roots. Root formation first occurred in material from the 5th subculture, i.e. after 6 months of culture *in vitro* (fig. 1C). In subsequent subcultures there was a progressive increase in the percentage of microcuttings which formed roots (table 2). At the 10th subculture, and thereafter, it was observed that explants produced adventitious roots while growing in the shoot proliferation medium in the presence of BA (1.0 mg l⁻¹).

Adventitious roots were formed only when microcuttings from the 5th and subsequent subcultures were grown on agar-based medium (half-strength MS) containing PG. Microcuttings did not form roots in the absence of PG or if grown on filter-paper bridges with liquid medium. The addition of an auxin, IBA, IAA or NAA (1.0 mg l⁻¹), to cultures containing PG caused a marked increase in the proportion of microcuttings which formed roots and in the numbers of roots per microcutting (table 2). When supplied at a higher concentration (3 mg l⁻¹) each of these auxins promoted

TABLE 1

Production of axillary shoots in vitro by explants of d'Ente prune. Production in vitro de tiges axillaires à partir d'explants de la variété de prunier d'Ente.

Subculture No. (*)	No. Explants	Mean Shoots/Explant (**)
2	30	2.7 \pm 0.9
3	30	5.9 \pm 1.9
4	30	9.6 \pm 6.4
5	30	7.6 \pm 3.4
6	30	7.3 \pm 2.1

(*) Duration of each subculture : one month.

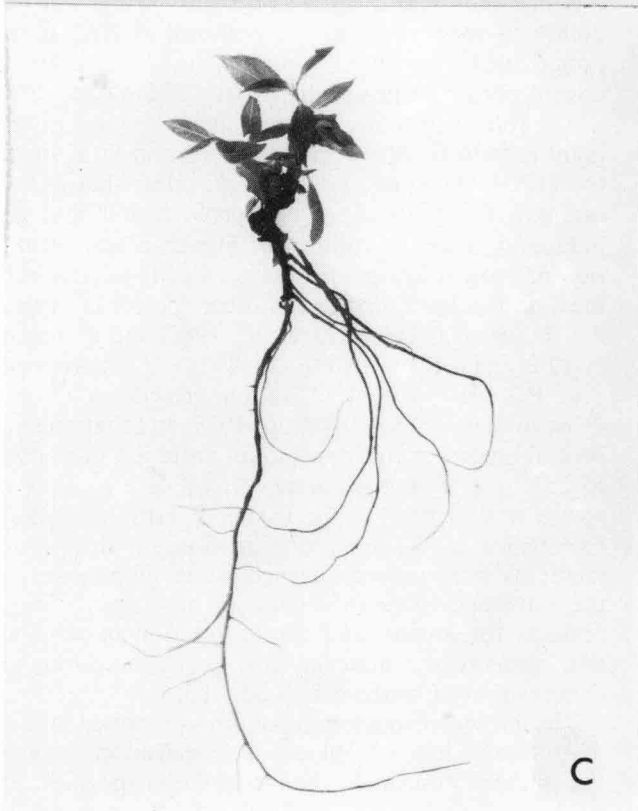
(**) Recorded after one month.



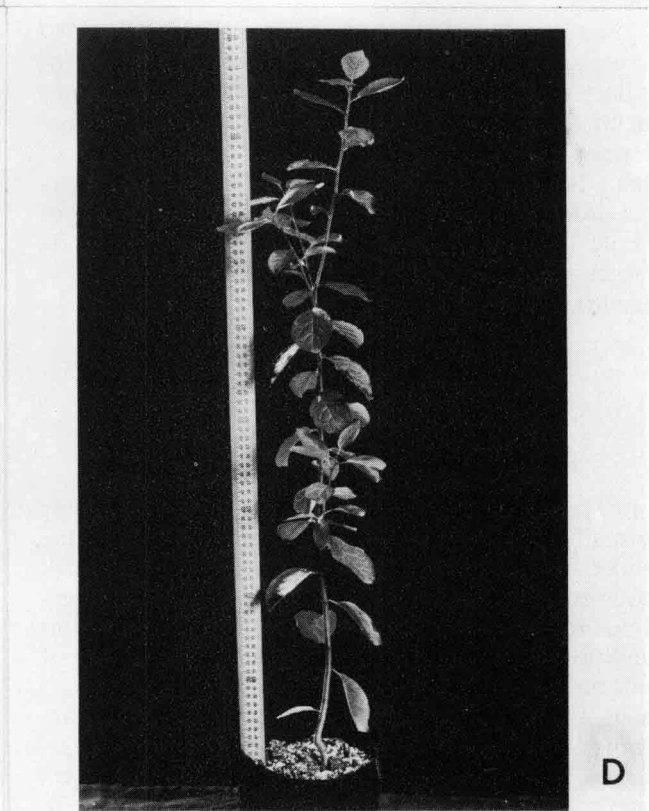
A



B



C



D

Figure 1A
Nodal explant of d'Ente prune cultured in absence of PG. Explants produced only a rosette of leaves ($\times 4,68$).

Explant provenant de bourgeon axillaire de prunier d'Ente, cultivé en absence de PG. L'explant produit uniquement une rosette de feuilles ($\times 4,68$).

Figure 1B
Proliferation of axillary shoots by a subcultured explant of d'Ente prune ($\times 1,7$).

Prolifération de bourgeons axillaires par subcultures d'un explant de prunier d'Ente ($\times 1,7$).

Figure 1C
Rooted microcutting of d'Ente prune ($\times 0,71$).
Microbouture enracinée de prunier d'Ente ($\times 0,71$).

Figure 1D
Own-rooted tree of d'Ente prune produced by micropropagation (Scale = 1 m). Photographed 2 months after removal of rooted microcutting from the culture vessel.

Prunier d'Ente obtenu par micropropagation (échelle = 1 m), photographié 2 mois après le transfert de la microbouture enracinée.

TABLE 2

Adventitious root formation in vitro in the d'Ente prune : effects of subculture and auxins on rooting of microcuttings grown on media containing phloroglucinol ().*

Formation in vitro de racines adventives chez le prunier d'Ente : Effets des subcultures et des auxines sur l'enracinement de microboutures cultivées en milieu contenant du phloroglucinol ().*

Origin of microcutting : Subculture No. (**)	Control % Rooted (***) Mean No. roots (***)		IBA (1.0 mg l ⁻¹) % Rooted Mean No. roots		NAA (1.0 mg l ⁻¹) % Rooted Mean No. roots		IAA (1.0 mg l ⁻¹) % Rooted Mean No. roots	
1-4	0	0	0	0	0	0	0	0
5	16	1.6 ± 0.8	53	1.8 ± 1.0	30	1.8 ± 0.8	72	2.3 ± 1.2
6	10	1.3 ± 0.7	60	2.1 ± 0.9	45	2.3 ± 0.7	82	2.4 ± 0.7
7	40	1.2 ± 0.9	75	2.5 ± 0.9	60	4.0 ± 0.8	90	2.7 ± 0.8

(*) 162 mg l⁻¹; (**) Duration of each subculture : one month; (***) Recorded after one month.

the outgrowth of callus from the bases of the microcuttings but no roots were formed. In auxin-treated (1.0 mg l⁻¹) material, as in the controls, there was a progressive increase in the percentage of rooted microcuttings with increasing numbers of subcultures. With IAA, for example, no roots were formed during the first 4 subcultures. Thereafter, the percentage of rooted microcuttings rose to 90 at the 7th subculture (table 2).

The responses of explants of d'Agen and d'Ente 707 were essentially similar with respect to production of axillary shoots and to formation of adventitious roots but there was a pronounced and consistent difference between the cultivars in the ease of establishment of plants from rooted microcuttings (fig. 1D). The success rate in establishing container-grown plants of d'Ente 707 was approximately 70 % but the common d'Agen was much more difficult to establish (20 % success).

IV. DISCUSSION

In French prune, as in some other species which are normally difficult to root from cuttings, aseptic culture can be used to condition the plant material so that it acquires an enhanced capacity for formation of adventitious root primordia. In the present experiments this conditioning process involved prolonged subculture of buds on a cytokinin-enriched medium (BA, 1.0 mg l⁻¹) so as to produce successive generations of axillary shoots. These shoots, when made into microcuttings, showed a progressive increase in their ability to form roots with increasing numbers of subcultures (table 2). A similar response has been found in the micropropagation of apple cultivars (SRISKANDARAJAH *et al.*, 1982). Here, the effect of long-term subculture was to cause a progressive reduction in the endogenous levels of abscisic acid (SRISKANDARAJAH, 1983) and gibberellinlike compounds (TAKENO *et al.*, 1982), substances which are known to inhibit the formation of adventitious root primordia when applied exogenously. Similar changes in endogenous hormones may lead to the enhancement of root formation in French prune but this has yet to be investigated.

Research with apple cultivars has shown that improved rooting of microcuttings occurs primarily with material which has been subcultured under continuous illumination (SRISKANDARAJAH *et al.*, 1982). Cultures of French prune, however, could not be grown with photoperiods in excess of 12 h and the conditioning process which leads to root formation in prunes seems to be less affected by light. A notable property of conditioned shoots of d'Agen and d'Ente prune was their ability to form roots in the presence of BA, a compound which normally inhibits the formation of adventitious root primordia (SMITH & THORPE, 1975).

The role of phenolic compounds in the regulation of plant growth *in vitro* is controversial and little understood (POESSEL *et al.*, 1982) but it is clear that PG was essential for both the shoot proliferation and root induction phases in cultures of French prune. Promotion of growth *in vitro* by inclusion of phenolics in the medium has been reported in other species of *Prunus*, e.g. *P. persica* (CHANCEL *et al.*, 1980) and *P. insititia* cv "Pixy" (JONES & HOPGOOD, 1979). It is noteworthy that PG was without effect on growth *in vitro* of *P. avium* (JONES & HOPGOOD, 1978) and that there are several reports in the literature of variation in response to PG by differing genotypes of apple e.g. ZIMMERMANN & BROOME (1980), JAMES & THURBON (1981). Experience in the micropropagation of fruit crops, including work with the French prune, emphasises that the individual species and cultivars have specific requirements for growth and root formation *in vitro* and that general recommendations for procedures and composition of media are ill-advised.

The micropropagation procedures described here are of potential interest for plant propagation provided that there is no genetic change in the propagules. The fidelity of reproduction of the genotypes concerned has yet to be tested by field experiment and it would be premature to conclude that micropropagation is suitable for clonal multiplication of d'Agen and d'Ente prunes. Of equal interest is the possibility that micropropagation, involving prolonged subcultures with BA and PG, leads to horticulturally-useful variation within prune cultivars. This possibility is being investigated.

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