REGENERATION OF APPLE PLANTS FROM SHOOT MERISTEM-TIPS

W. DAVID LANE

Agriculture Canada Research Station, Summerland, B.C. V0H 1ZO (Canada)

(Received March 16th, 1978)
(Revision received June 6th, 1978)
(Accepted June 6th, 1978)

SUMMARY

Plants of apple (Malus domestica Borkh.) were regenerated from proliferating meristem-tips grown on nutrient medium. Only benzyladenine (BA), at an optimum of $5 \times 10^{-6}$ M, was required for initial growth and development of meristem-tip explants which produced proliferating shoot cultures in high frequency. Naphthaleneacetic acid (NAA) at $10^{-5}$ M was used to initiate roots. Plantlets were then transferred to a growth regulator free medium where roots developed fully before potting. Temperatures below 28°C and high salt concentration decreased rooting efficiency.

INTRODUCTION

Meristem-tip culture has recently become an important technique, particularly with vegetatively-propagated species. Very rapid regeneration rates have been achieved with a number of different species [1,2] and the technique is particularly valuable for rapid clonal multiplication [3]. It is also used for producing virus-free plants [4], international exchange of disease-free plants [5] as well as such plant breeding functions as germplasm storage [6] and maintaining, in healthy condition, special breeding lines [7].

Apples are usually multiplied by grafting the fruiting cultivar onto a rootstock. Regeneration from meristem tips promises to be a quicker and cheaper method. Self-rooted trees from culture would also avoid graft incompatibilities and diseases introduced during propagation.

Apple has been cultured previously but shoot multiplication was not achieved and rooting was either low in frequency or did not occur [8,9]. Jones [10] reported better results culturing shoot tips (1–2 cm long) of rootstock clones.

Abbreviations: BA, benzyladenine; GA₃, gibberellic acid; IBA, indole-3-butyric acid; NAA naphthaleneacetic acid.
but presented little quantitative data. The present paper describes the pro-
cedure for regenerating apple plants from proliferating meristem-tips.

MATERIALS AND METHODS

*Malus domestica* Borkh. 'McIntosh' seedlings were used in all experiments; in addition 'McIntosh' (MacSpur®) and 'Delicious' (Harrold Red) were often included. Shoots were collected from both greenhouse and field grown trees, the outer tissue cut away, and the tip dipped in 70% ethanol (5 s) then blotted and dissected. Meristem-tips (approx. 0.5 mm wide) containing 3--4 leaf primordia were carefully dissected from the shoot tip using a scalpel (Brad Parker No. 11 blade), a Spencer stereo microscope (× 25) and a laminar flow, sterile air cabinet.

Immediately after dissection tips were placed in 2 × 15 cm glass test-tubes containing Murashige and Skoog medium [11] with 2% sucrose plus various growth regulators. The medium was solidified with Difco Bacto Agar (0.7%) purified by washing thoroughly in distilled water. Indole-3-butyric acid (IBA), NAA, BA, and gibberellic acid (GA₃) were obtained from Sigma Chemical Co. Sterilization was by heating to 121°C for 15 min after adjusting the pH to 5.3.

The test-tubes containing the meristem-tips were closed with metal caps, sealed with Parafilm® and incubated in a growth chamber illuminated with cool white fluorescent lamps adjusted for a 16 h light period and 28°C day, 22°C night temperature. Light intensity was 1500 lux at test tube level. Rooting experiments were in other similar growth chambers with a light period of 18 h and an intensity of 800 lux.

Experiments investigating initial growth and rooting consisted of 10 replications, those investigating multiplication, 5. All experiments were repeated at least twice. Multiplication and rooting experiments were initiated with single shoots (1--4 cm long) obtained from multiplying cultures.

RESULTS

*Initiation and Multiplication*

The first transfer to fresh medium was after 6 weeks growth, by which time a rosette of 6--10 new leaves had developed. Two months later actively multiplying cultures with 30--50 shoots were well established. Subculturing was then initiated and repeated at 1 month intervals for 8 months without a decrease in shoot production. Multiplying shoots developed from axillary buds.

Explants from rapidly growing greenhouse trees initially grew more rapidly than meristem tips from field trees, however, growth rates soon equalized. Explants from dormant trees grew well, even if chilling requirements had not been full satisfied, but meristem-tips obtained from larger buds near the branch terminus grew better than smaller basal buds. The pattern of development of cultivars was the same, although there were small differences in rates of growth, in vitro grown shoots resembling those grown in vivo, but lacking trichomes.
Contamination of new cultures was less than 5% and 80% of explants were established as multiplying shoot cultures (Fig. 1).

The optimum concentration of BA for shoot multiplication was $5 \times 10^{-6}$ M (Fig. 2). If no growth regulators were in the medium, growth slowed and eventually stopped; at $10^{-5}$ M, BA was toxic and the cultures died. Suboptimal concentrations of BA resulted in shoot multiplication but at a slower rate.

The influence of GA$_3$ and NAA in combination with $5 \times 10^{-6}$ M BA was also determined (Fig. 3). When GA$_3$ and NAA were combined with BA inhibition occurred, NAA having the greatest effect.

Fig. 1. A: Shoot multiplication in medium containing BA ($5 \times 10^{-6}$). $\times 1$; B: Root initials on shoot from medium containing NAA ($10^{-4}$M); C: Plantlet with roots which developed after transfer to growth regulator-free medium; D: Plant in soil.
**Root Initiation and Development**

Rooting consisted of root initiation and root development phases. NAA at $10^{-5}$ M in a medium containing half the normal concentration of salts was best for induction of root initials. After 4 weeks incubation many root initials but little callus had formed. Full root development was inhibited by this concentration of NAA so plantlets were transferred to a growth regulator free medium for 3 weeks.

Temperature during root initiation was very important to rooting success (Fig. 4), the optimum being 28°C. At lower temperatures shoots first accumulated anthocyanins then became chlorotic. Rooting efficiency also decreased if IBA was substituted for NAA or if the salt concentration was standard. Reducing the sucrose concentration below 2% resulted in greener and healthier appearing shoots but root initiation decreased in proportion to the sucrose reduction. Sucrose concentrations higher than 5.2% also resulted in a sharp reduction of rooting.

Rooted plants were transplanted from culture tubes to pots containing sand: perlite, 1 : 1. Humidity was gradually lowered and the plants then transferred to the greenhouse.

**DISCUSSION**

These experiments show the important role of BA in the growth and development of apple meristem-tip cultures. Axillary bud development, as a response to BA, is known to vary with species and concentration [12], the optimum of $5 \times 10^{-6}$ M shown here being similar to that of rose [13] also a woody species and related to apple. The inhibition of shoot multiplication by NAA in the present experiments appears similar to previously reported inhibitory effects.
of auxin on other species [1,13]. This suggests that apple cultures with their abundance of shoot tips apparently have sufficient endogenous auxin for growth; GA\(_3\) also appears adequate as it too was slightly inhibitory when added to the medium.

Explants from terminal buds were established in culture with greater frequency than basal ones. Zieslin [14] has examined the effect of bud position on growth rate, by pruning to direct bud growth, and found a similar response; basal buds grew least and growth increased with successive bud position. The smaller size of meristem-tip explants from basal buds compared to terminal ones could account for their poorer growth both in vitro and in vivo. Growth of meristem-tips from dormant buds which had not satisfied their chilling requirements was, unexpectedly, normal. Dormant buds normally will not grow because of growth inhibitors and the restricted movement of oxygen and other gases to the meristem [15]. It appears that bud scale removal during dissection and the free exchange of gases to the exposed meristem-tips in culture released them from dormancy allowing growth.

High auxin levels sometime interfere with rooting because excess callus growth results [16]. Little callus growth occurred with apple enabling the use of a high auxin concentration to rapidly induce root initials. Rooting efficiency was improved if salt concentration was reduced but it was also important to maintain normal sucrose levels indicating the importance of nutrients as well as auxins for rooting.

REFERENCES