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Shoot Regeneration from Mature Cotyledons of Thin Shelled Almond. Cv. Waris

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ABSTRACT

Adventitious shoots were indirectly regenerated from the proximal ends of mature cotyledons of two week old invitro germinated seedlings of thin shelled almond (*Prunus dulcis*. Mill.) cv. Waris, when cultured on MS medium (half salt strength) (1962), fortified with BAP (2.5 µM). Further differentiation of the callus and shoot growth was arrested upon subculturing on same medium. Maximum elongation of these adventitious shoots was achieved on low BAP (0.5µM) concentration. Higher BAP (5 µM) and lower BAP (1 µM) concentrations resulted in very low percentage of differentiation (23% & 5% respectively), compared to 52.63% on BAP (2.5 µM).

In another trial massive, creamy white & nodular undifferentiated calli were produced when same explants were cultured under the combined influence of BAP (5 µM), NAA (10 µM) and CH (1%).

Keywords: Almond, cotyledons, adventitious shoots

Abbreviations: BAP- 6 Benzyle aminopurine phosphate; NAA-Napthelene acetic acid; CH- Casien hydrolysate; MS- Murashige & Skoog, MS(x1/2)- Murashige & Skoog (half salt strength); TDZ- Thidiazuron; IBA- Indole-3-butyric acid; Kn- Kinetin; IAA- Indole acetic acid; 2,4-D- 2,4, dichlorophenoxyacetic acid.

INTRODUCTION

Regeneration from mature stored cotyledons of almond represent a potential system for genetic transformation. For almond (*Prunus dulcis*. Mill.) efficient protocols have been developed for the production and selection of genetically transformed cells, but regeneration from mature explants has been poor and is the limiting step in the transformation process (Archilletti *et al*. 1995, Miguel & Oliveira, 1999). In other *Prunus* species like Apricot (Goffreda *et al*. 1995), Cherry (Lane & Cossio, 1986), Peach (Schneider *et al*. 1992) and

Plum (Mante *et al.*, 1989) regeneration efficiency has been improved by using juvenile explants from seedlings. But this approach would not maintain clonal integrity. Further juvenile explants are not the preferred tissue type for generating variation (Ainsely *et al.*, 2001). Keeping in view the recalcitrant nature of adult and somatic almond tissues to regenerate under *in vitro* conditions, an attempt has been made to develop an efficient shoot regeneration system from mature cotyledons of almond, which will help in genetic transformation and subsequent production of transgenic almonds for further studies.

MATERIAL AND METHODS

Authentic seeds of almond (*P. dulcis* Mill.) cv. Waris were obtained from the Biotechnology division of SKUAST, Shalimar, Srinagar. The seed kernels were soaked in 1% NaOCl for two nights. The soaked kernels were then pealed off at laminar airflow and then again sterilized with HgCl2 solution 0.2% for 20 min., followed by three rinses with glass double distilled water. The dried kernels were then inoculated on MS (x1/2) basal medium (1962). Cotyledons were then excised out from two weeks old germinated seedlings and cultured on MS (x1/2) medium supplemented with growth regulators and sucrose (3%). The pH was adjusted to 5.5 with 0.1 N HCl and 0.1 N NaOH, before gelling the medium with 0.8% difco-bacto agar. The medium after dispensing into vials was autoclaved at 15 lbs pressure for 15-20 minutes at 121°C. The cultures were incubated at a temperature of 25 ±5°C in cool and white fluorescent light (3000 lux) under 16hr light and 8 hr. dark regime.

RESULTS

The Morphogenetic response of mature almond cotyledons to various concentrations of growth regulators is summarized in Table 1.

Cotyledons from two week old germinated seedlings developed small creamy white callus from their proximal ends when cultured on MS (x1/2) + BAP (2.5μM) medium. These calli differentiated into 2-3 adventitious shoot buds on the same medium after 8 weeks without subculturing. (Fig.1a). Further growth of these shoot buds was arrested upon subculturing of the whole explant on the same medium. (Fig.1b), hence were subcultured alongwith their calli portions on low BAP concentration (0.5μM) where maximum shoot elongation upto 5 cm was achieved after 8 weeks (Fig.1c). Higher and lower concentrations of BAP (5μM & 1μM) resulted in extremely low percentage of shoot regeneration.

Table 1. Morphogenetic response of mature almond cotyledon to various concentrations of growth regulators.

Induction treatment	% cotyledons with buds	No. of buds/ cotyledon* X±S.E	shoots >5 mm after 8 wks. %
BAP (5 µM)	23.07	1.0±0.2	15.0
BAP (2.5 µM)	52.63	2.5±1.5	40.0
BAP(1.0 µM)	5.00	1.0±0.3	10.0
BAP (5 µM)+ NAA (10 µM)+ CH(1%)	Zero	0.0±0.0	0.0

*Data scored after 8 wks. of culture period

In another trial, the same explants produced huge, white, nodular and fast growing callus from their proximal ends after 8 weeks, without any differentiation into shoot or root buds even after subculturing on low BAP (0.5 µM) or (x1/2) basal medium. (Fig 1d).

DISCUSSION

The main objective of this study is to develop an efficient protocol for complete regeneration of almond seedlings through callus differentiation from mature cotyledon explants.

Regeneration from adult explants of almond has been found very poor compared to juvenile explants of seedlings, which regenerated into complete plantlets indirectly (Mehra & Mehra, 1974; Miguel & Oliveira, 1999). Cotyledons have often been reported to be regenerative organs in tissue culture of stone fruits (Mehra & Mehra, 1974; Mante *et al.*, 1989; Hammerschalag, 1985). Ainsley (2001) reported 100% adventitious regeneration from immature almond (Carmel) cotyledons in presence of TDZ (10 µM). Mehra & Mehra (1974) has reported 5% differentiation of mature almond cotyledons into complete plantlets in presence of NAA (5 µM)+ Kn (1 µM)+ CH (1%). Miguel & Oliveira (1999) reported indirect shoot regeneration from mature leaf explants in presence of TDZ (6.6 µM)+ IAA (0.28 µM)+ 2,4-D (0.04 µM). In our studies 50% adventitious shoot regeneration was

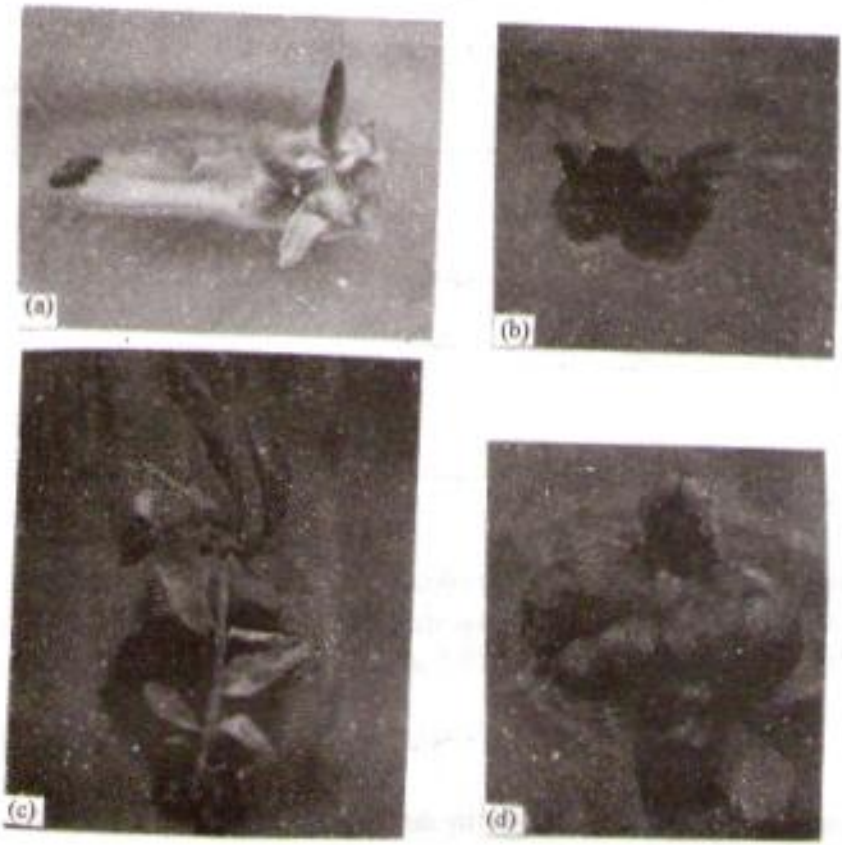

Fig 1 (a-d) Morphogenetic response of almond cotyledon to different growth regulators.

a) Shoot bud regeneration from almond cotyledon on MS (x1/2)+BAP (2.5 µM) (after 8 weeks).

b) Subcultured explant of Fig. 1a showing arrested adventitious bud growth on MS (x1/2)+BAP (2.5 µM) (after 8 weeks).

c) Shoot elongation on MS (x1/2)+BAP (0.5 µM) (after 8 weeks).

d) Massive nodular white callus on MS(x1/2)+ BAP(5µM)+ NAA (10 µM)+CH(1%) (after 8 weeks).

achieved from proximal ends of mature almond cotyledons in presence of BAP (2.5 µM) alone. These results are also in contrast to the observations recorded in other *Prunus* species as peach (Mante *et al*., 1989), cherry (Hokanson *et al*., 2000), apricot (Pieterse, 1989) and peach (Pooler and Scorza, 1995), where shoot regeneration was achieved under the combined influence of IBA or NAA and BAP or TDZ.

In the present studies massive undifferentiated calli were produced on the combination of BAP (5 µM) + NAA (10 µM) + CH (1%), which is in line with studies of Antonelli (1991), Miguel and Oliviera (1999) and Ainsley *et al*. (2001) in almond. However, these observations contradict the published reports of Mehra & Mehra (1974), Hisajima (1992) and Antonelli (1991).

The present findings are significant for future tissue culture and genetic transformation research with almond and may also assist in understanding the mechanism that controls the regeneration process in this plant.

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