Micropropagation of Almond

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ABSTRACT

Shoot tip explants of *Prunus dulcis* (Miller). D. A. Webb Kagzzi excised from *in vitro* germinated seedlings were used as primary explants and grown on MS medium (1962) supplemented with BAP (4.4 μM). The effect of explant length with or without apical bud on the formation of multiple adventitious shoots were studied. Explants (1 cm long) without apical bud produced maximum number of adventitious shoots than smaller explants (0.5 cm) with or without apical bud under influence of same BAP concentration. Shoot proliferation and multiplication continued upto five subcultures. Shoot elongation was attempted at low BAP concentration in isolated shoots after subculturing on MS + BAP (0.44 μM) + GA_3 (0.37 μM) + NAA (0.53 μM). Finally these elongated shoots were cultured on rooting medium. Root initiation was achieved on MS (half strength) + IBA (2.5 μM), followed by its subculturing on hormone free medium for root elongation. In case IBA (2.5 μM) was replaced by IAA (10μM), initiation as well as elongation were achieved on the same medium.

Keywords: Almond, shoot apex, micropropagation, rooting.

Abbreviations: BAP- 6-Benzyl amino purine, IBA - Indole –3 butyric acid; IAA- Indole-3 – acetic acid; MS - Murashige and Skoog (full strength); MS (x ½) - Murashige and Skoog (half salt strength)

INTRODUCTION

Almond *Prunus dulcis* (Miller). D. A. Webb Var. Kagzzi, belongs to family rosaceae and is taxonomically related to other stone fruits like peach, plum, cherry and apricots. Almond kernels are highly energetic, delicious, cholestrol free, rich in fats, proteins, carbohydrates and minerals in addition to dietary fibre.

Almond is difficult to propagate by cuttings because of poor rooting capacity. The usual method of propagation is by nursery budding (Hartman *et al*.,1997) which is cumbersome. Being one of noble nuts and cash crops of Kashmir valley quick propagation of this superior variety is essential for improving the economy and status of our fruit industry. Thus a need arises to

switch over to new techniques of propagation. The technique of *in vitro* culture which has gained worldwide importance has been put to exploitation by a number of workers in propagation of important trees. (Aneja and Atal, 1969; Winton, 1970, 71 and Mehra and Mehra, 1974). This technique is considered superior to conventional methods of propagation because of quick propagation rate of plants in relatively shorter period, irrespective of the season. Tissue culture studies of almond have received less attention as compared to other stone fruits. Although some work has been reported on almonds (Mehra and Mehra 1974; Tabachnik and Kester, 1977; Kester *et al.*, 1986), till date no such studies have been carried out in J & K state. An attempt was therefore made to initiate work for quick propagation of almond using tissue culture technique which have proved successful.

MATERIAL AND METHODS

Authentic seeds of *Prunus dulcis* var. Kagzzi were collected from Koil aerodrome orchard (District Pulwama) in autumn. The seed kernels were chilled for 40 days followed by soaking in filtered water for 24 hrs. The testa (seed coat) of the soaked kernels was peeled off under laminar air flow and the white kernels were then sterilized with $HgCl_2$ (0.2%) solution for 7-10 minutes, followed by rinsing three times with autoclaved double distilled water. The sterile kernels were then inoculated on MS (x ½) (1962) basal medium and about 5 cm long seedlings were produced within 10 days.

Shoot tip explants (0.5 cm and 1 cm long) with and without apical bud from these *in vitro* born seedlings were then aseptically excised and inoculated on MS (1962) medium augmented with 3% sucrose and different growth adjuvants. The pH was adjusted to 5.5 with 0.1N HCl and 0.1 N NaOH before gelling the medium with 0.8 % difco bacto agar. The medium after dispensing into vials was finally autoclaved for 15-20 min. at 15 lbs. pressure and 121°C temperature.

After inoculation of the explants, cultures were incubated at a temperature of 25 ± 5°C under cool white fluorescent light (3000 lux) with 16 hr. light and 8 hr. dark regime.

RESULTS

Effect of various concentrations of growth hormones on shoot tips of almond and its varying morphogenetic responses are summarized in Table 1. Shoot tips (1 cm) without apices showed very encouraging results and on an

average 9 adventitious shoots developed per explant after 4- weeks of culture period as compared to shoot tips (1cm) with apices which developed only 6 adventitious shoots when both were cultured in presence of BAP (4.4 μM) [Fig. 1]. Similarly shoot tips (0.5 cm) without apices developed on an average 6 adventitious shoots compared to those with apices which developed only 5 adventitious shoots at BAP (4.4 μM) concentration. The overall response of explants (for shoot multiplication) without apical buds was superior to explants with apical buds under different phytohormonal treatments. Moreover, explant length of 1 cm has also been found more responsive for shoot multiplication as compared to 0.5 cm length on all BAP concentrations (Table 1a). Further, the proliferation and multiplication continued up to five subcultures approximately at the same rate.

Small adventitious shoots (0.25 cm) were isolated and then subcultured on different BAP concentrations for their elongation (Table 1b, Fig. 2). Maximum shoot length observed on cytokinin supplemented medium was 1.2 cm, whereas when a combination of BAP (0.44 μM) + GA_3 (0.37 μM) + NAA (0.53 μM) was used, shoot length increased upto average of 1.5 cm (Table 1b).

Table 1. Morphogenetic response of almond shoot tips and adventitious shoots to different concentrations of cytokinins and auxins.

a. Multiplication Phase

Medium	Shoot tip length	Response*		
		+ apex	- apex	% age
MS (x ½) basal (control)	1 cm	-	-	-
	0.5 cm	-	-	-
MS + BAP (4 μM)	1 cm	5	7	65
	0.5 cm	3	5	70
MS + BAP (4.4 μM)	1 cm	6	9	90
	0.5 cm	5	6	85
MS + BAP (5 μM)	1 cm	6	6	70
	0.5 cm	4	5	75

* Mean of ten replicates / treatment; data scored after 4 weeks of culture period.

b. Elongation Phase

Medium	Initial adventitious Shoot length	Average shoot length *
MS + BAP (1 μM)	0.25 cm	0.5 cm
MS + BAP (0.5 μM)	--	1. cm
MS + BAP (0.44 μM)	--	1.2 cm
MS + BAP (0.44 μM)+ GA_3 (0.37 μM) + NAA (0.53 μM)	--	1.5 cm

* Mean of ten replicates / treatment; data scored after 4 weeks of culture period.

c. Rooting Phase

Medium	Shoot length	Response*		% age
MS (x1/2) + IBA (2.5 μM)	1.5 cm	3-4 root initials were developed at the cut end.	followed by their elongation on hormone free medium	90
do	0.5 cm	1-2 root initials developed at the cut end.	-do-	55
MS (x ½) + IAA (10μM)	1.5 cm	4-5 thick root initiated at the cut end.	followed by their elongation on the same medium	95
do	0.5 cm	1-2 thin and short roots initiated at the cut end.	-do-	60

* Mean of ten replicates / treatment; Data scored after 4 weeks of culture period.

Adventitious shoots (1.5 and 0.5 cm) were used for rooting trials and the rooting was achieved either in one phase or in two phases. In one trial root initiation was achieved within 14 days on MS (x ½) medium supplemented with IBA (2.5 μM). Further elongation of these root initials was inhibited and hence were sub-cultured on hormone free medium for elongation (Table 1c, Fig.3). In

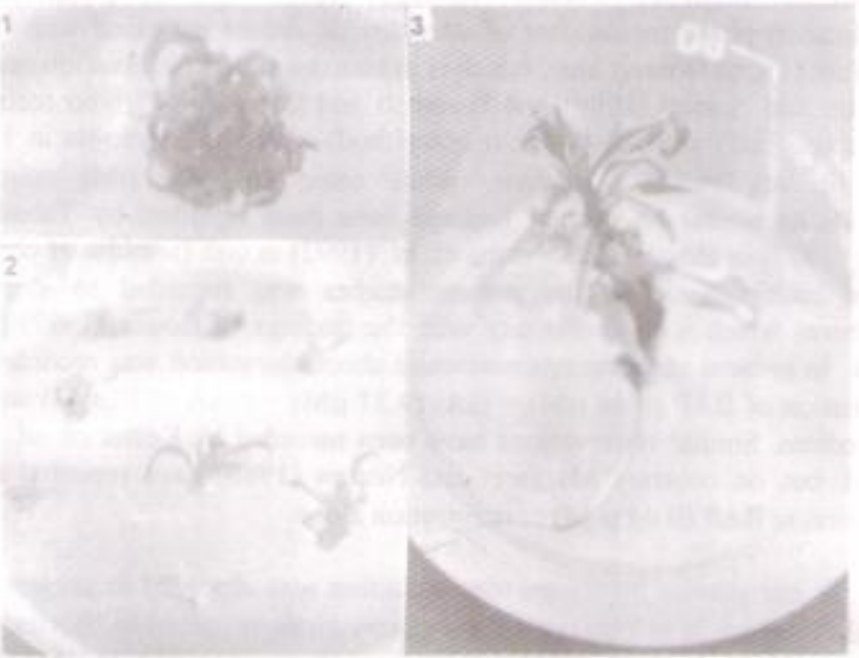

Fig. 1 - 3. In vitro response of almond shoot tip to various phytohormones.
1. Shoot multiplication on MS + BAP (4.4 µM). (After 4 weeks);
2. Sub-culturing of isolated shoots on MS + BAP (0.44 µM) + GA_3 (0.37 µM) + NAA (0.53 µM);
3. Rooted plantlet on MS x (1/2) + IBA (2.5 µM) (After 4 weeks).

the second trial root inititation as well as elongation was observed on MS (x ½) medium fortified with IAA (10 μM). Smaller shoots (0.5 cm) responded poorly to both methods of rooting (Table 1c).

DISCUSSION

The technique of micropropagation applied to Kagzzi variety of almond will be helpful to solve the important problems of vegetative propagation by non-conventional methods. Present findings reveal that in proliferation and multiplication phase the number of adventitious shoots increased with the length of explant (1 cm) without apex which is in contrast to the observation recorded by Margaret and Norton (1986) and Hammatt and Grant (1997) who recorded 4.5 (with apical bud) and 6.8 (without apical bud) adventitious shoots in 1 cm long shoot tip of *Prunus cerasifera*. While using BAP(4.4 μM) concentration observations similar to present findings have been reported by Tabachnik and Kester (1977) in almond and Sharma *et. al.* (1992) in colt (semidwarf cherry). The rate of multiplication in the present studies was recorded to stop after 5 subcultures which is in conformity with the findings of Bouza (1997) in *Prunus tenella*. In present observations maximum shoot elongation was recorded when a combination of BAP (0.44 μM) + GA_3 (0.37 μM) + NAA (0.53 μM) was used in MS medium. Similar observations have been recorded by Kester *et. al.* (1986) in almond, but on contrary Margaret and Norton (1986) have reported maximum elongation at BAP (0.44 μ M) concentration alone.

In our studies maximum root induction was observed in presence of IBA (2.5 μM) which is in contrast to the observations recorded by Bouza (1997) in *Prunus tenella* where maximum rooting was observed at IBA (5 μM). Thick and long roots observed in present studies with IAA (10μM) are confirmed by the observations of Caboni & Damiano (1994) in almonds.

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