Effect of explant types and different basal nutrient media on in vitro growth of bitter almond cuttings during establishment and proliferation stages

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Abstract: This study was carried out in plant tissue culture Laboratory, Agriculture development Systems project (ADS) at Giza, Egypt during the period from 1999-2003. This study aimed to investigate the effect of different sources of explant and basal nutrient media on in vitro growth of bitter almond cuttings during establishment and proliferation stages. From the results of the study the following conclusions were derived: One node cuttings surpassed shoot-tip in in vitro growth (Survival percentage (96.67 and 63.33), No. of leaves / explant (11.0 and 6.33) and shoot length (cm.) / explant) (2.83 and 1.33) of bitter almond respectively. Murashige and Skoog medium (MS) (Liquid or solid) was superior in in vitro growth of explant establishment in (No. of shoots / explant, No. of leaves / explant and shoot length / explant cm.) of bitter almond nodal cuttings. DKW medium was the poorest medium for almond, while WP medium came in between of all measured parameters for explant establishment. Higher effect of solid MS basal nutrient medium on enhancing the activity of bitter almond established nodal cuttings for the production of shoots and leaves and also for stimulating the elongation of bitter almond proliferated shoots. [Journal of American Science 2010;6(10):408-411]. (ISSN: 1545-1003).

Keywords: basal nutrient; establishment; proliferation stages

1. Introduction

Selection of the promising explant is crucial step in the starting of any in vitro study 0.4-0.7mm shoot tips of almond are preferable as explants as it was stated by Antonelli and Chiariotti (1988) .

On the other hand, shoot – tip explants recorded lower percentage of survival as compared with one–node cuttings of bitter almond (Saeed 1995) Shoot-tips surpassed one-node cuttings in explant establishment of Neplus ultra and bitter almond (Zaied 1997).

In addition, shoot tips of Nonpareil and Neplus ultra almond cv. Surpassed nodal explant (Saeed 1998). Ingredients of the culture medium vary with kind of plant and the propagation stage of which one is working. These ingredients include (a) inorganic salts, (b) organic compound, (c) natural ingredients and (d) inert support (Hartmann and Kaster, 1992). Ms Medium proved to be the most suitable medium for successful explant development of stone fruit (almond, apricot and peach), (Zaied 1997). Shoot-tips nodal explants of Neplus ultra and Nonpareil almond cultivars were imitated on modified 8.MS free from hormones and supplemented with 0.5 mg / L BAP and 0.1 mg / L IBA.(Saeed 1998) .

On the other hand, single nodal explant of paradox walnut (Juglans hindsii x J. regia) performed better on woody plant medium (WPM) (Lloyd and McCown, 1980). (Driver and Kuniyuki 1984). WPM was more effective than MS basal nutrient medium on enhancing the activity of Canino and Amar established nodal cutting for the production of shoots and leaves (Kandil 2001).

2. Material and Methods

This study was carried out in plant tissue culture Laboratory, Agriculture development Systems project (ADS) at Giza, Egypt during the period from 1999-2003.

This study aimed to investigate the effect of different sources of explant and basal nutrient media on in vitro growth of bitter almond cuttings during establishment and proliferation stages.

Source of explant

Active growing new shoots of about 5cm in length were excised from one year old seedlings of bitter almond rootstock, at the orchard of faculty of Agriculture, Cairo University, Giza, Egypt. Shoot were collected in April and they were brought to the laboratory in plastic pags. In the laboratory were washed with detergent under running tap water for 60 min. The shoots were stripped of their leaves.

Tow types of explants were prepared:

a- Shoot tips were excised from terminal portion with 0.5-1.0cm. long containing apical meristem and 2-3 leaf primordial.

b- Nodal cuttings with one node were prepared by dividing the rest of the shoot into 1.0-1.5 cm segments.

Nutrient media

a- Effect of nutrient medium:

Different nutrient media i.e. MS, WPM and DKW were tested to find out the suitable starting nutrient medium supplemented with 3.0 % sucrose, 0.5 mg / L BA (6-benzyleadenine, 0.1 mg / L IBA (Indole butyric acid and 7 g / L difico bacto agar for 6 weeks for in vitro shoot-tips and nodal cuttings establishment.

b- Effect of liquid and solid medium:

Both liquid and solid MS media were tested to find out the suitable nutrient medium supplemented with 3.0 % sucrose, 0.5 mg/L BA , 0.1 mg/L IBA and 7.0 g/L difico bacto agar (for solid media) for 6 weeks for in in vitro shoot-tips and nodal cutting establishment of bitter almond rootstock .

Each treatment contained of three replicates (3 explant / each replicate). Data were recorded after 6 weeks. The following growth parameters were determined:

a- survival percentage

b- Number of leaves / explant c- Shoot length / explant

3. Results and Discussion

Establishment Stage

Effect of explant type

Results in Table (1) showed the effect of two different explant types shoot-tips and one-node cuttings of bitter almond rootstock on in vitro growth of explants.It was observed that, one- node cutting scored higher significant value ov survival percentage (96.67 %) as it was compared with shoot-tip (63.33 %). The same trend was obtained with both numbers of leaves / explant and shoot length / explant as one-node cutting resulted in greater number of leaves / explant (11.0) and longer explant (2.83 cm).

Generally, it can be concluded that one-node cutting surpassed shoot-tip in terms of survival percentage, no. of leaves / explant and shoot length / explant. This result is in agreement with (Saeed, 1995; Zaied, 1997 and Saeed, 1998) on almond.

Effect of basal nutrient media

a- Liquid media

Data in Table (2) showed the effect of different liquid (L.) basal media i.e. (MS, WPM and DKW) on No. of shoots / explant,No. leaves / explant and shoot length of bitter almond nodal cutting rootstock. It was noticed that L.M.S. medium resulted in the significant highest number of shoot / explant (3.66) followed by WPM (2.50) and DKW (1.33) respectively.

Table (1): Effect of explant types on in vitro growth of bitter almond rootstock during Establishment stage

Growth Parameters / Explant type	Survival percentage	No. of leaves / explant	Shoot length (cm)
Shoot tip	63.33B	6.33B	1.33B
One-node cutting	96.67A	11.00A	2.83A

Means having the same letters are not significantly different at 5% level.

Table (2): Effect of different liquid basal nutrient media on in vitro growth of bitter almond nodal cutting during establishment stage

Growth measurement / Liquid basal media	No. of shoots / explant	No. of leaves / explant	Shoot length (cm)
MS	3.66A	12.00A	2.33A
WPM	2.50B	7.33B	1.40B
DKW	1.33C	3.33C	0.70C

Means having the same letters are not significantly different at 5% level.

As for number of leaves / explant, it is clear that L.M.S. medium gave the highest significant number of leaves / explant (12.00) compared with WPM or DKW (7.33 and 3.33) respectively. Concerning the effect of shoot length, it was obvious that, L.M.S. medium scored the highest significant shoot length / explant (2.33) followed by WPM orDKW (1.40 and 0.70) respectively.

Generally, it appeared that L.M.S. medium surpassed WPM and DKW as it gave the greatest

number of shoot / explant, highest number of leaves / explant and longest shoot compared with other media .This result is in harmony with Hammerschlag(1982a & b) and Sari El Deen et al (1998) who found that optimum growth of peach shoot tip and both shoot tip and nodal explant of Nemaguard and Okenawa peach was obtained on liquid MS medium .

b- Agar-gelled media (Solid)

Results in Table (3) showed the effect of three different basal solid nutrient media of number of shoot / explant, number of leaves / explant and shoot longest of bitter almond nodal cuttings. It appeared that, MS medium gave the highest significant average number of shoots / explant (3.50) followed by WPM and DKW (2.20 and 1.0) respectively.

As for both No. of leaves / explant and shoot length, it was notice that the same trend was obtained as MS agar-gelled medium resulted in the greatest number of leaves / explant and longest shoot / explant followed by WPM and DKW .

This result is in line with (Miller et al, 1982) ;(Hammerschlag 1982b); (Rugini and Verma 1983); (Zaied, 1997) ; (Saeed 1998) and (Sari El-deen et al 1998) . It was found that MS medium was suitable for the establishment of peach, almond and apricot as it gave best growth results.

Proliferation stage

Effect of different basal nutrient media

Results in Table (4) showed the effect of three different solid basal nutrient media (MS, WPM and DKW) on values of number of shoot / explant, number of leaves / explant and shoot longest (cm.) of bitter almond nodal cuttings.

Results showed that number of shoots developed from explants cultures on MS medium gave the highest significant average number of shoots / explant (47.0) followed by WP medium (30.0) while DKW medium scored the least number of shoots / explant (11.0) .

As for both average number of leaves and shoot length it was noticed that same trend was detected as MS medium gave the greatest significant number of leaves / explant and the longest explant compared with other media.

Generally, it could be concluded that, MS basal medium affected shoot proliferation as it recorded the highest number of shoots / explant, greatest number of leaves / explant and longest explant.

These result are in harmony with (Rugini and Verma 1983); (Caboni, 1994); and Saeed, (1998). They found that MS medium gave the highest shoot multiplication rate and growth of almond.

Table (3): Effect of different agar-gelled medium on in vitro growth of bitter almond Rootstock during establishment stage.

Growth measurement / Basal media	No. of shoots / explant	No. of leaves / explant	Shoot length (cm)
MS	3.50A	10.00A	1.83A
WPM	2.20B	4.50B	1.16B
DKW	1.00C	3.00C	0.43C

Means having the same letters in column are not significantly different at 5% level

Table (4) : Effect of different soiled basal media on in vitro growth of bitter almond Rootstock during proliferation stage .

Growth measurement / Basal media	No. of shoots / explant	No. of leaves / explant	Shoot length (cm)
MS	47.00A	8.01A	4.33A
WPM	30.00B	4.45B	3.10B
DKW	11.00C	0.84C	2.00C

Means having the same letters in column are not significantly different at 5% level

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