The Effect of Plant Growth Regulators on Callus Induction and Regeneration of *Amygdalus communis*

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Abstract

The Almond (*Amygdalus communis*) is one of the most important and oldest commercial nut crops, belonging to the *Rosaceae* family. Almond has been used as base material in pharmaceutical, cosmetic, hygienically and food industry. Propagation by tissue culture technique is the most important one in woody plants. In the current research, *in vitro* optimization of tissue culture and mass production of almond was investigated. In this idea, explants of actively growing shoots were collected and sterilized, then transferred to MS medium with different concentrations and combinations of plant growth regulators. The experiment was done in completely randomized blocks design, with 7 treatment and 30 replications. After 4 weeks, calli induction, proliferation, shoot length and number of shoot per explants were measured. Results showed that the best medium for shoot initiation and proliferation was MS + 0.5 mg/l IAA (Indol-3-Acetic Acid) + 1 mg/l BA (Benzy1 Adenine). Autumn was the best season for collecting explants. The shoots were transferred to root induction medium with different concentrations of plant growth regulators. The best root induction medium was MS + 0.5 mg/l IBA (Indol Butyric Acid).

Keywords: *in vitro*, *Amygdalus communis*, micropropagation, tissue culture, shoot proliferation

Introduction

The almond (*Amygdalus communis*) is one of the major tree crops of the world (Kester *et al.*, 1996). From Middle and West Asia, it has diffused to other regions and continents which include Middle East, China, Mediterranean region and America (Ladizinsky, 1999). Conventional breeding of woody fruit species is a slow and difficult process due to high levels of heterozygosity and long generation cycles (Sriskandarajah *et al.*, 1994).

Because almond is highly heterozygous, and most common commercial cultivars are self-incompatible, almond trees are virtually as variable as wild populations. Vegetation propagation via layerge or cutting is inefficient in order to minimize the problem of the enormous genetic variation and to obtain genetically identical populations, due to the great problems of this fruit species regarding rooting *in vivo* (Henry *et al.*, 1992).

Also, to maintain clonal purity, seed-derived material is not generally used for propagation. Thus, plant tissue culture techniques are more valuable for the clonal propagation of almond trees. Therefore, the aim of this study was to determine the conditions needed to optimize micropropagation methods for almond, from buds explants by *in vitro* culture. Yet, there have been no useful protocol on the optimization of propagation of almonds through tissue culture.

Material and methods

Plant materials

Actively growing shoots of almonds were collected in four seasons from suitable trees growing at the Agricultural and Natural Resource Research Center of Razavi Khorasan in Iran. After removing the leaves, the shoots were cut into segments of 1-2 cm in length, and then washed with running water for 1 hour. After that, the scions were surfaced sterilized in 0.02% (w/v) mercuric chloride for 3 min, and then rinsed with 70% ethanol for 2 min. Later, the segments were sterilized by immersion in 30% (w/v) calcium hypochlorite solution, containing 0.05% (v/v) Tween 20, for 15 min; finally they were rinsed four times with sterile distilled water.

Micropropagation

Shoot tips of 0.5 to 1 cm, containing a single bud, were established in tubes containing 20 mL of Murashig and Skoog (1962) medium (MS), supplemented with 30 gl⁻¹ sucrose, 7 gl⁻¹ agar (Agar-Agar, Sigma) and different concentrations and types of plant growth regulators (PGRs) (Tab. 3). The pH was adjusted to 5.8 prior to autoclaving at 120°C for 20 min. The cultures were maintained at 25±2°C with 16 h photoperiod (35 µmol m⁻²s⁻¹), provided by white fluorescent lamps. Proliferating axillary shoots were subcultured once every 3 weeks. In the second stage, proliferating shoots were separated and transferred to a
weeks, before transferring into the growth room. Relative humidity was slowly decreased by gradually removing beakers. Plantlets were acclimatized after 3 weeks in a green house at 25 ± 2°C under natural daylight conditions.

Experimental design and statistical analysis
A completely randomized blocks design was used for the study, with data from each experiment being analyzed separately. In micropropagation the treatments were replicated 30 times, and in rooting induction, treatments were replicated 10 times, with each replicate comprising one explants.

The Duncan’s test was adjusted at p = 0.01 probability level to separate mean differences when significant treatment effects were detected.

Results and discussion
The best multiple shoot initiation was obtained on the MS medium supplemented with BA at 1 mg L⁻¹ and IAA at 0.5 mg L⁻¹ (Table 1 and 3) with a shoot number of 3.15 per explants, on the 4th week of culture (Table 3). The higher length of shoots, with a shoot length of 1.21 cm and the most callus initiation, with 9.5, were observed on MS medium supplemented with the same PGPs as the above (Table 3). Conclusions show that BA treatments caused better shoot proliferation and better shoot multiplication (Fig. 1, 2, 3).

The results indicated that between the different seasons, autumn was the best time for collecting explants from trees on the field (Table 4). There were significant differences between the rate of contaminations and the dates of collecting the explants (Table 2).

| Treatment Codes | Medium Composition | Mean rate contamination | Mean callusing | Mean shoot length (cm) | Mean shoot number | Mean Shoot induction |
|-----------------|--------------------|------------------------|----------------|------------------------|------------------|----------------------|
| N               | IAA(0/5mg/l)+BA(1mg/l) | 42/91 a              | 9/5 a           | 1/21 a               | 3/15 a               | 9 a               |
| H               | BA(2mg/l)+IBA(0/1mg/l) | 42/91 a              | 7 ab            | 0/77 cb              | 2/57 b               | 6/25 b             |
| J               | TDZ(4mg/l)         | 39/58 a              | 5/75 b           | 0/84 b               | 1/93 c               | 6 bc               |
| K               | IBA(0/01mg/l)+BAP(1/27mg/l) | 37/50 a               | 5/5 b           | 0/6 cd               | 1/52 cd              | 4/5 bc             |
| F               | IBA(0/01mg/l)+BAP(0/68mg/l) | 37/91 a               | 5/75 b          | 0/73 cb               | 1/57 cd              | 4/5 bc             |
| C               | IAA(0/1mg/l)+BA(1/5mg/l) | 40/41 a               | 4/75 b           | 0/75 cb              | 1/67 cd              | 3/8 c              |
| Control         | ---                | 40/41 a                | 0.0 d           | 0.0 d                | 0.0 d                | 0.0d               |

Values with the same letters in the same column are not significantly different (p≤0.01) according to Duncan's test.
The best root formations were observed in MS medium supplemented with 0.5 mgL\(^{-1}\) IBA (Tab. 6). The maximum number of root induction was 7, the mean value for roots per seedling was 1.92 and for root length was 2.8 cm, data obtained on MS medium supplemented with 0.5 mgL\(^{-1}\) and 1.0 mgL\(^{-1}\) IBA, for the first two (treatments D and E, respectively) and with MS medium + 0.5 mgL\(^{-1}\) IAA+1.0 mgL\(^{-1}\) IBA for the last experiment (Tab. 3). No root development was observed in control groups.

The present results are in agreement with Tabachnic and Kester (1977) observations. They reported that the use of BA in shoot multiplication was absolutely necessary.

Hisajima (1982) reported that the best result for proliferation of the almond was obtained from MS medium supplemented with 0.2 mgL\(^{-1}\) BA + 0.005 mgL\(^{-1}\) IBA.

Isikalan et al. (2008) determined that the best multiple shoot initiation for almond was obtained on the MS medium supplemented with BA 2 mgL\(^{-1}\), with a shoot number of 5.7 ± 1.04 mgL\(^{-1}\) per explant.

Increasing BA concentration from 1 mgL\(^{-1}\) to 3 mgL\(^{-1}\) may significantly reduce the length of shoots, as observed by Shekafandeh and Khush-khui (2008) in guava (Psidi-
Prunus dulcis micropropagation of almond (Prunus dulcis Mill). In vitro Cell Develop Biol-

Cultural and Natural Resources Research Center.

The present experiments demonstrated that shoot multiplication could be achieved on MS medium supplemented with 1 mgL⁻¹ BA and 0.5 mgL⁻¹ IAA. BA treatments resulted in a better elongation of almond scion. However, MS medium with 0.5 mgL⁻¹ IBA was also effective for root induction and root elongation.

After 3 weeks, rooted shoots of almond were acclimated and then were successfully transferred to natural condition. Channuntapipat et al. (2003) showed that the maximum rooting of shoots for some almond hybrid rootstocks occurred on half strength MS medium with 2.4 mgL⁻¹ IBA. Ainsley et al. (2001) determined that IBA and NAA are the most suitable auxin for rooting seedlings of 'Nec plus ultra' and 'Nonpareil' almond cultivars, in vitro conditions.

The type and concentration of auxin during rooting period strongly influenced the quality of the root system during rooting period. The application of NAA resulted in poor rooting of the almond shoots. This might be explained by the NAA resistance to degradation by the auxin-oxidase enzyme (Smulders et al., 1990). Nissen and Sutter (1996) have shown that, in tissue culture, media IAA is rapidly photo-oxidized (50% in 24 h), while the IBA oxidized slowly (10%) and NAA is very stable.

Conclusions

The results obtained in the present research can be used as guidelines for improving propagation of almond as a commercial fruit tree. In addition, the results demonstrated which is the optimized stage for root induction. Since in micro propagation rooting of micro cutting is often a challenging step, losses at this stage have vast economic consequences. In conventional propagation via cuttings many woody plants are also recalcitrant to root. Thus, a research on root formation is highly important from the practical point of view. It can be conclude that proliferation and multiplication of almond by tissue culture is a fast, economic and valuable method.

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References

Ahmad T. Ur-Rahman H, Laghari MH (2004). Effect of different auxin on in vitro rooting of peach rootstock GF677. Sarhad J Agricult 20(3):373-375.

Ainsley PJ, Collins GG, Sedgley M (2001). In vitro rooting of almonds (Prunus dulcis Mill). In vitro Cell Develop Biol Plant 37(6):778-785.

Channunapipat C, Sedgley M, Collins G (2003). Micropropagation of almond cultivars “Nonpareil” and “Nec plus ultra” and the hybrid rootstock, “Titan×Nemgard”. Sci Hortic 98:473-484.

Henry PH, Blazich FA, Hinesley LE (1992). Vegetative Propagation of eastern red cedar by stem cuttings. Hort Sci 27(12):1272-1274.

Hisajima S (1982). Multiple shoot formation from almond seeds and an excised single shoot. Agricult Biol Chemi 46:1091-1093.

Isikalak C, Adiyaman Akbas F, Namli S, Tikat E, Basaran D (2008). In vitro micropropagation of almond (Amygdalus communis L. Cvs. “Nonpareil”). Afr Biotechnol 7(12):1875-1880.

Kester DE, Gradziel TM (1996). Almonds. In: Janick J, Moore JN (Eds.). Fruit breeding, Vol III: NUTS. New York: John Wiley and Sons, Inc.

Ladzinsky G (1999). On the origin of almond. Genetic Resour Crop Evolut 46:143-147.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473-497.

Nasiri M (2000). Micropropagation of olive (Olea europaea). Iranian Rangeland and Forest Plant Breeding and Genetic Res J 230:37-79.

Nissen SJ, Sutter EG (1990). Stability of IAA and IBA in nutrient medium of several tissue culture procedures. Hort Sci 800-802.

Sharzad SH, Emam M (2000). Micropropagation of populus euphratica using tissue culture. Iran Rangeland Forest Plant Breed Genetic Res J 230:11-36.

Shekafandeh A, Khush-Khui M (2008). Effect of bud position and culture medium on prolifiratin from nodal culture of two guava cultivars. Asian J Plant Sci 7:177-182.

Smulders MJM, Van Devan ETWM, Croes AF, Wullems GJ (1990). Metabolism of 1-naphthaleneacetic acid in explants of tobacco: evidence for release of free hormone from conjugates. J Plant Growth Regul 9:27-34.

Sriskandarajah S, Goodwin PB, Speirs P (1994). Genetic transformation of the apple scion cultivar delicious via Agrobacterium tumefaciens. Plant Cell Tissue Organ Cult 36:317-329.

Tabachnik L, Kester DE (1977). Shoot culture for almond and almond-peach hybride clones in vitro. Hort Sci 12:545-547.