

Full Length Research Paper

# ***In vitro* propagation of *Amsonia orientalis* Decne (Apocynaceae)**

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***In vitro* plant regeneration was achieved from shoot explants of *Amsonia orientalis* Decne. The seeds were germinated aseptically in Petri dishes containing growth-regulator-free MS medium. The shoot explants of 30-day-old grown seedlings were cultured on MS medium supplemented with 1 mg/l indole-3-butyric acid (IBA) plus 0.5 mg/l kinetin (Kin) for direct regeneration and subcultured at three week intervals. While induction of adventitious buds from shoot explants was achieved approximately 33% within four weeks, a few roots (around 15%) were induced on the same medium within eight weeks. In addition, shoot explants were transferred to MS medium containing 2 mg/l benzyl aminopurine (BAP) plus 0.2 mg/l naphthalene acetic acid (NAA) for indirect regeneration and after three weeks, proliferated actively growing calli was occurred.**

**Key words:** *Amsonia orientalis*, propagation, callus formation, IBA, Kin, NAA, BAP.

## INTRODUCTION

*Amsonia orientalis* Decne, a dicotyledon, is a medicinal plant which has very restricted distribution only in Turkey and Greece in the world (Tutin et al., 1968; Davis, 1978). Tutin et al. (1968) reported the presence of *A. orientalis* in three localities in Turkey, these being Apolyont (Lake Uluabat), Menekşe Deresi (Istanbul) and Hıdırlık Tepe, Balıkesir. However the plant was not encountered in these localities in the study by Özen (2006), only small populations were reported in Gazi Osman Paşa, Adnan Menderes, and Paşaalanı Districts in Balıkesir. *A. orientalis*, categorized as one of the rare plants of Turkey, were gathered from Hıdırlık Tepe, Balıkesir in 1988 by Turhan Baytop, and placed under preservation *ex situ* at the Botanic Garden, Faculty of Sciences, İstanbul University. İstanbul University Botanical Garden is the oldest botanical garden in Turkey. Several endangered plants from Turkey have been protected in the botanical garden and propagated *in vitro* certain rare plants.

*Amsonia* is a genus of clump-forming herbaceous perennials with multiple leafy stems growing from a semi-woody rootstock. Plant height varies from 12.5 cm to nearly 1.2 m. The alternate leaves are entire and range in shape from broadly ovate, up to 2.5 cm wide, to linear

and needle-like to only 2 mm wide. In spring and early summer, the flowers are borne in terminal paniced or corymbose cymes. The individual flowers, up to 1.5 cm in diameter, have funnel-shaped corollas with five sharply pointed, spreading petal lobes, and are star-like in overall appearance. Ranging from very pale to rich sky blue, flower colour is most concentrated in bud stage and sometimes fades to near-white when fully opened in strong sunlight. Stamens included, inserted on upper part of corolla, filaments very short, and anthers obtuse at base, without apical appendages. Disc glands are absent. Seeds are produced in cylindrical capsules (follicles) up to 10 cm in length, glabrous, oblong not comose.

Like most other members of the Apocynaceae (dogbane family), *Amsonia* species have milky sap (Darke, 2005). Although the sap of some relatives such as *Nerium* contains highly toxic alkaloids, the sap of *Amsonia* is relatively innocuous. It is not known to be harmful to humans but does seem to discourage predation by deer and other mammals - a good thing for many gardeners.

*A. orientalis* Decne. Syn. *Rhazya orientalis* (Decne.) A. D.C. is branched perennial, 30 - 60 cm. Leaves are almost sessile, narrowly ovate or lanceolate, 3 - 7 X 0.5 - 3.5 cm, base cuneate or rounded, apex generally acute, glabrous except for margin and midrib, pubescent when young, with numerous lateral veins. Inflorescence is com-

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pact or loose, many flowered. The calyx is 2-3 mm, while the corolla is pale blue, glabrous outside, with tube of 10-12 mm. The lobes are 4 - 5.5 mm, the seeds 8 - 10 mm and tuberculate, while the flowering time is 5-6.

A sweep of star-like sky-blue flowers in spring is certainly an appealing sight. Pretty as their flowers are, blue stars have many other characteristics that make them among the best garden perennials. Most would be worth growing for foliage alone. All blue stars produce masses of rich green leaves that remain neat and attractive throughout the growing season, generally free from any insect pests.

Literature on *A. orientalis*, which is critically endangered in nature according to IUCN categories, is very limited in number. Nevertheless, anatomy, morphology, palinology of *A. orientalis* have been investigated and it was found that extracts of this plant have strong antimicrobial activity against microorganisms (Akyalçın et al., 2006). Furthermore, it is known to have anti-tumor and anti-carcinogenic effects because of its various alkaloids (Dabine Lengyel et al., 1986; Rahman and Zaman, 1988; Rahman et al., 1989; Ekim et al., 2000; Akyalçın et al., 2006; Özen, 2006). In addition, it has been shown that *A. orientalis* Decne preferred soils with sandy-loamy, saltiness, slightly alkaline, mid-calcerous, poor organic material, and with very rich iron and magnesium (Özen, 2006). The author also established that the levels of other macro elements except phosphorus and potassium were generally sufficient for healthy feeding. Studies regarding plant regeneration by organogenesis from tissue cultures of *A. orientalis* plant have not been reported. That is why this study is of utmost significance in terms of the establishment of tissue culture systems of *A. orientalis*.

Studies on *A. tabernaemontana* Walter include germination tests, growth and alkaloid production in tissue culture (Furmanowa and Rapczewska, 1977; Scocco et al., 1998). In addition, Stano et al. (2005) reported intra- and extracellular distribution of aminopeptidase activity when a cell suspension culture of *A. tabernaemontana* Walt. as the plant material was used.

Here, we established a simple protocol for clonal propagation from shoot explants of medicinal *A. orientalis* Decne. These results could serve as guide for further regeneration study of this important plant.

## MATERIALS AND METHODS

Seeds were surface-sterilized in 70% alcohol for 2 min, then in 5% commercial bleach (Domestos) for 10 min, followed by three rinses for 15 min with sterile distilled water. Seeds were germinated aseptically in Petri dishes containing growth-regulator-free MS (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) sucrose and solidified with 0.8% agar (w/v). The pH of MS medium was adjusted to 5.8 before sterilization by autoclaving at 121°C at 105 kPa for 20 min. The pH of MS medium was confirmed after sterilization. Seedlings were grown in culture tubes containing MS medium without plant hormones. For the subsequent treatments,



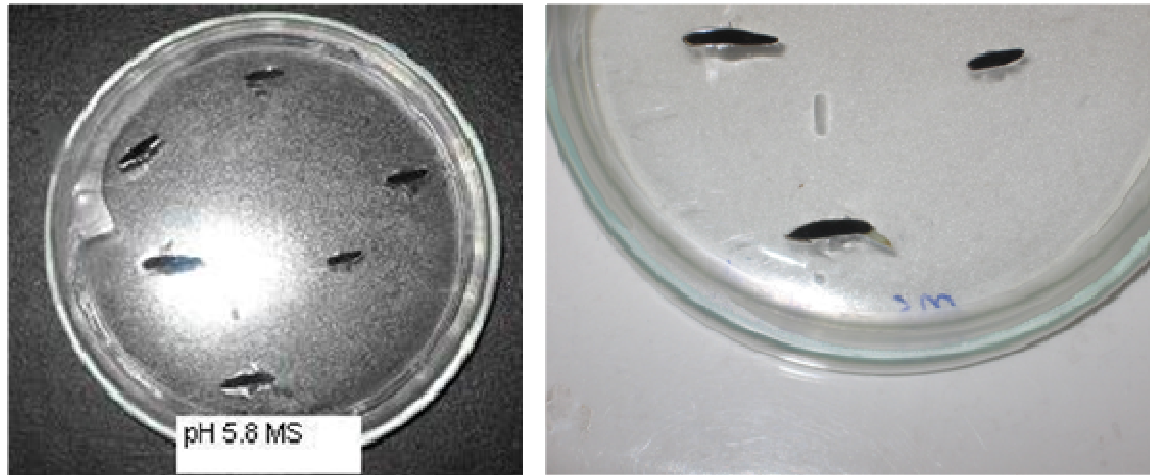
Figure 1. *A. orientalis* seed.

shoot explants from approximately 4 week-old-plants which are grown from seeds were cut into 0.5 or 1 cm and then cultured on MS medium containing 1 mg/l indole-3-butyric acid (IBA) plus 0.5 mg/l kinetin (Kin) for direct regeneration. For callus induction, shoot segments were placed in 2.2 mg/l benzyl aminopurine (BAP) plus 0.2 mg/l naphthalene acetic acid (NAA). Seed germination, callus induction and subculture were carried out in a growth chamber illuminated with cool-white fluorescent light with a 12-h photoperiod at 25 ± 2°C.

## RESULTS AND DISCUSSION

In previous experiments it was observed that the seeds of *A. orientalis* were characterized by slow and uneven germination. The seeds have a thick testa (Figure 1). Seed dormancy may be due to the presence of the seed coat or may require chilling treatments for germination to occur. Sodium nitroprusside (SNP) was used as a NO (Nitric oxide) donor to overcome seed dormancy. The seeds of *A. orientalis* were pre-treated with SNP concentration of 100 µM overnight (12 h) and germinated in Petri dishes containing MS medium without plant growth regulators. Germination frequency in *A. orientalis* seeds was around 15% (Figure 2). Fairly healthy plants were obtained from germinated seeds that were transferred into the test tubes without hormones (Figure 3). Shoot explants from 4-weeks old, young and healthy seedlings were cultured on MS medium supplemented with 1 mg/l IBA plus 0.5 mg/l Kin. After 10-15 days, shoot explants became larger. Direct shoot and root regeneration from explants are shown in Figure 4. While shoot regeneration (approximately 33%) obtained after 4 weeks from explants, root initials appeared (around 15%) after 8 weeks. The callus formation was originally induced from shoot explants on MS medium containing 2 mg l<sup>-1</sup> BAP plus 0.2 mg l<sup>-1</sup> NAA (Figure 5).

*In vitro* direct plant regeneration was successfully obtained by organogenesis from shoot explants of this ornamental plant. In addition, callus formation was also observed in shoot explants. The results obtained in this study could not be put under comparative evaluation, due to lack of any previous reports related with regeneration of this plant in tissue culture.

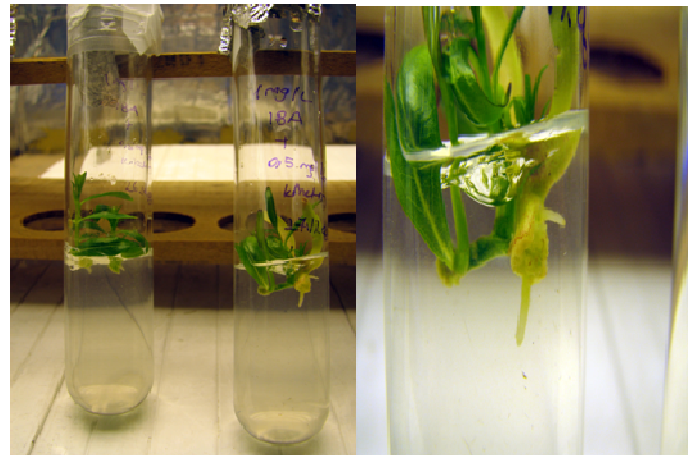


**Figure 2.** Germination of *A. orientalis* Decne. seeds.

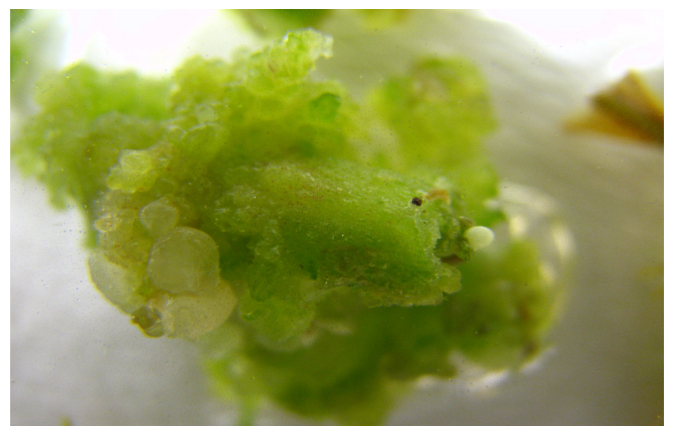


**Figure 3.** *A. orientalis* seedlings in the test tubes containing MS medium without plant hormones.

This study shows the preliminary results on the regeneration capability of *A. orientalis* in tissue culture. In addition, for the first time explants regeneration in *A. orientalis* plant was also carried out.



**Figure 4.** Shoot and root regeneration from shoot explants of *A. orientalis* on MS medium supplemented with 1 mg/l IBA and 0.5 mg/l Kin.



**Figure 5.** Callus formation on MS supplemented with 2 mg/l BAP plus 0.2 mg/l NAA.

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