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# Induction of callogenesis and rhizogenesis in *Perovskia abrotanoides* Karel., a little known medicinal plant

**Arehzoo Zaker[1], Parvaneh Abrishamchi[1], Javad Asili[2], Seyed Hadi Mousavi[3] and Azar Rezaee[1]**

[1]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.
[2]Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
[3]Department of Pharmacology, School of Medicine, Mashhad University of Medical Science, Mashhad, Iran.

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**The present study was carried out to investigate *in vitro* callogenesis and direct rhizogenesis of *Perovskia abrotanoides* Karel. for the first time. This medicinal plant grows in various regions of Iran. Leaf explants were incubated on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of auxins like IAA, IBA and NAA (0.5 to 4 mg/L) with cytokinins like Kin and BA (0.1 to 0.75 mg/L). Callus induction from leaf explants was achieved at all MS media containing auxins in combination with cytokinins. Most of these treatments revealed 100% callogenesis response. Callus initiated in MS medium supplemented with 0.5 mg/L IAA in combination with 0.75 mg/L Kin, showed the highest dry weight to fresh weight ratio (0.234±0.2054 mg). Calli grown at MS medium supplemented with 2 mg/L NAA and 0.75 mg/L BA, produced more dry (43.63±12.01) and fresh weight (1150.9± 86.4) than other treatments. Results showed that the MS medium supplemented with 2 mg/L NAA was the best medium to induce adventitious roots. In this treatment, percentage of rhizogenesis and the number of roots per explant were recorded as 84.61% and 46.400±14.258, respectively. Callus and adventitious root cultures of *P. abrotanoides* were established successfully.**

**Key words:** *Perovskia abrotanoides,* callogenesis, rhizogenesis, plant growth regulators.

## INTRODUCTION

*Perovskia abrotanoides*, is a medicinal plant species that belongs to Lamiaceae family, which is growing wild in Iran, Afghanistan, Pakistan and Turkmenistan (Safaee-Ghomi and Batooli, 2010). This perennial herb is distributed in North, East and Central regions of Iran. Its roots are used in Iranian traditional medicine for treatment of leishmaniasis (Jaafari et al., 2007). There are a few scientific reports about the *in vitro* cultures and biological activities of *P. abrotanoides.* Some of them are implicated to the pharmacological effects of this plant such as lishmanicidal, antiplasmodial, antinociceotive, anti-inflammatory, antibacterial and cytotoxic effects (Jaafari et al., 2007; Hosseinzadeh and Amel, 2001; Nassiri et al., 2002). It has been reported that the roots of this folkmedicinal herb, contain a large amount of diterpenoid tanshinones (Sairafianpour et al., 2001; Aoyagi et al., 2006) and its biological properties may be related to the high tanshinones content.

Recent studies showed that these compounds exhibited various pharmacological activities like antibacterial (Lee et al., 1999), antioxidant (Cao et al., 1996; Park et al., 2009), antidiabetes (Kim et al., 2007), anticancer (Wang et al., 2005; Liu et al., 2009; Chiu et al., 2010; Pan et al., 2010) and anti-inflammatory activities (Jang et al., 2003).

Plants and their products have long been used as drugs.

*Corresponding author. E-mail: abrisham@um.ac.ir or abrisham.parwaneh@gmail.com. Tel: +989151246726. Fax: +985118796416.

Medicinal plants are source of important therapeutics and medicinal compounds that play dominant roles in human health (Shoja et al., 2010; Zia et al., 2007). In recent years, they have provided precise stimulus for the development of natural products (Aslam et al., 2006). It has been estimated that approximately the roots of 66% of the medicinal plants, are the main source to prepare drug (Wadegaonkar et al., 2006). So, there is growing concern about reducing populations, loss of genetic diversity, local extinction and habitat degradation of medicinal plant because of their over exploitation (Canter et al., 2005). Therefore, the management of traditional medicinal plants resources is very important.

Plant tissue cultures, including root culture systems, would present unique opportunities to provide root drugs in the laboratory without resorting to field cultivation (Wadegaonkar et al., 2006). On the other hand, these techniques may be able to facilitate the large-scale produc-tion of valuable clones to avoid further depletion of plant species from its natural habitat. Besides, the culture of callus tissues provide an important tool for regeneration of whole plant and establishment of cell suspension cultures (Ling et al., 2013).

Taking into therapeutic importance attributed to *P. abrotanoides*, the present investigation was carried out to establish direct rhizogenesis and callogenesis of this plant for the first time.

## MATERIALS AND METHODS

Mature seeds of *P. abrotanoides* Karel. were collected in September from natural plants growing in Kalat, khorassane, Razavi province, Iran. Seed germination was achieved in distilled water. One week old seedlings were transferred to hydroponic culture using Hoagland medium. The plants were grown for 3 months in culture room under 16 h photoperiod, 45 $\mu molm^{-2}s^{-2}$ irradiance level and 25 ± 2°C.

To investigate the effects of different plant growth regulators on callus and root induction, 2×1 cm leaf segments were excised from young leaves of hydroponically cultured plants. Explants were surface sterilized by ethanol 70% for 30 s and sodium hypochlorite 20% solution (containing 1% active chloride) for 5 min, followed by four times rinsing with sterilized distilled water. Then, the explants were placed on Murashige and Skoog (MS, 1962) basal medium containing 3% sucrose, 0.7% agar and supplemented with different concentrations (0, 0.5, 1, 1.5, 2, 3 and 4 mg/L) of IAA, IBA and NAA alone or in combination with various concentrations (0, 0.1, 0.5 or 0.75 mg/L) of BA or Kinetin. The pH of medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 min. All cultures were incubated in the dark at 25±2°C. After 40 days, the percentage of callogenesis and rhizogenesis were recorded for each explant. Besides, fresh and dry weights of calli and mean number of roots formed per explant, were measured.

For establishment of adventitious roots suspension culture, roots induced from leaf, were aseptically separated from the explants and sub-cultured into 75 ml liquid MS medium supplemented with 2 mg/L NAA in 250 ml Erlenmeyer flasks. The cultures were shaken at 80 rpm in the dark at 25±2°C. Adventitious roots were maintained by sub-culturing to fresh medium every 3 weeks.

Experiments were conducted in a completely randomized design. The data was analyzed by one-way analysis of variance (ANOVA) and the mean values were compared at 5% level of significance by Tukey's test.

**Figure 1.** Callogenesis by leaf explant of *P. abrotanoides* Karel. after 40 days in MS medium supplemented with 0.5 mg/L IAA and 0.75 mg/L Kinetin.

## RESULTS

### Callogenesis

In the present study, MS medium supplemented with different plant growth regulators including IAA, IBA and NAA in combination with Kinetin or BA were used for callus induction from leaf explants of *P. abrotanoides.*

Callogenesis from leaves explants was observed at all treatments tested independent of the auxin to cytokinin ratio. Callus initiation occurred within 12 to 14 days after transferring explants to MS basal medium containing plant growth regulators. The callogenic response at different concentrations of plant growth regulators varied from 62.5 to 100%. Maximum response (100%), was observed at most treatments especially media containing IBA or IAA as auxin (Table 1). The lowest amount of callus induction (62.5%), was found in medium containing 1.5 mg/L NAA in combination with 0.5 mg/L Kin. Explants in MS medium plant growth regulator-free, did not show any collogenesis. At all concentrations of BA and Kin, the callogenesis response was good. Data analysis of variance for dry weight to fresh weight ratio, showed significant differences between various concentrations of phytohormones (Table 1). The highest dry weight to fresh weight ratio (0.234±0.2054), that significantly differ from all other treatments ($P<0.05$), was obtained in MS medium containing 0.5 mg/L IAA and 0.75 mg/L Kin. There was no difference in texture and color of calli, except in the aforementioned medium, that calli were friable and less watery than other media (Figure 1).

Results showed that calli grown at MS medium supplemented with 2 mg/L NAA and 0.75 mg/L BA, produced more dry and fresh weight than other treatments. The difference of callus dry weight between this treatment and medium containing 0.5 mg/L IAA and 0.75 mg/L Kin which produced more dry weight to fresh weight ratio, was not significant.

The lowest means for callus dry weight and fresh weight

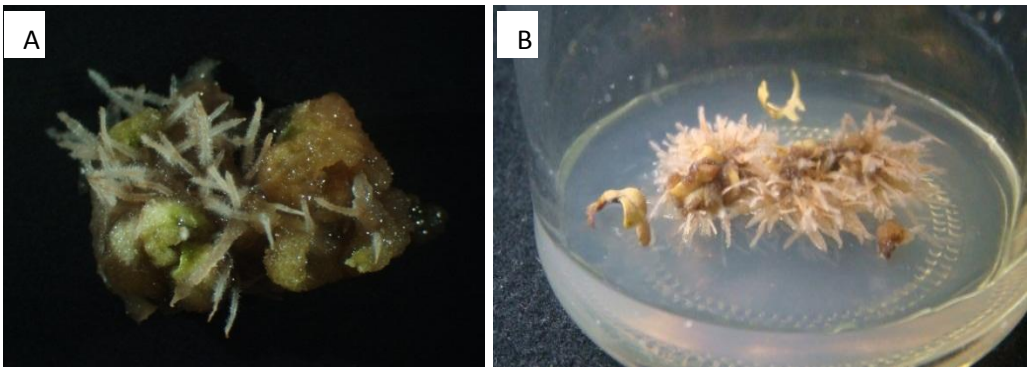


**Figure 2.** Rhizogenesis from leaf explant of P*. abrotanoides* in MS medium supplemented with 2 mg/L NAA, A) after 15 days and B) after 40 days.

**Figure 3.** Establishment of adventitious root suspension culture in liquid MS medium containing 2 mg/L NAA.

weight, were observed at MS medium supplemented with 3 mg/L NAA and 0.1 mg/L Kin and medium containing 1.5 mg/L IAA and 0.1 mg/L Kin, respectively. In general, IBA and IAA were more effective than NAA for callus induction. Rhizogenesis after callogenesis (indirect rhizogenesis) occurred in a few treatments, although this was not significant, statistically.

## Rhizogenesis

Direct root induction was achieved by placing the leaf segments on root induction medium that was MS basal medium containing different concentrations (0.5, 1, 1.5, 2, 3 and 4 mg/L) of IAA, IBA or NAA. Initiation of roots were observed in all MS medium after 14 to 16 days. Among the three auxins tested, NAA was more effective than IBA and IAA in *P. abrotanoides* root induction (Table 2). Maximum percentage of rhizogenesis (84.6%), and the highest mean root number per explant (31.33 roots), were observed in MS medium containing 2 mg/L NAA (Figure 2). The lowest average number of roots per explant was achieved on the basal medium containing 0.5 mg/L IBA. In all treatments, callogenesis occurred 4 to 6 days after rhizogenesis, except in MS medium containing 0.5 mg/L IAA that only direct rhizogenesis was observed. The roots formed in medium containing NAA, were thicker and shorter than roots formed in other auxins treatments. In general, the effect of different concentrations of auxins favored rhizogenesis.

Adventitious roots suspension culture was established by placing adventitious roots initiated on the explants in liquid MS medium (Figure 3).

## DISCUSSION

Callus initiation is a primary and necessary step in many tissue culture processes like the establishment of cell suspension cultures, regeneration of plants and indirect somatic embryogenesis (Sundram et al., 2012). Synergetic effects of plant growth regulators play a critical role in callus induction and cell differentiation (Shoja et al., 2010).

The *in vitro* callogenesis and rhizogenesis of *P. abrotanoides* Karel., a little known medicinal plant, are reported here for the first time. Results of the present study showed that response of explant to induce callus or root, depends on different concentrations and types of plant growth regulators.

For callus induction, leaf explants were cultured on MS medium supplemented with 97 different combinations of auxins and cytokinins. All auxins in combination with cytokinins were successful for inducing callogenic responses. In all cases, it took 12 to 14 days to induce callus. Callus induction was established successfully on the MS medium. Results showed that media supplemented with IBA either with Kin or BA, produced 100% callogenic response from leaf explants. This is applicable to the most media containing IAA in combination with both cytokinins, too. Nin et al. (1996), had also reported 100% callogenesis in *Artemisia absinthium* L. explants, independent of the cytokinin to auxin ratio (Nin et al., 1996).

*In vitro* plant tissue culture may be involved in

**Table 1.** Callogenesis response of leaf explant at different combinations of plant growth regulators.

| Auxins (mg/L) | BA (mg/L) | Kinetin (mg/L) | Response (%) | DW (mg) | FW (mg | DW/FW (mg) |
|---|---|---|---|---|---|---|
| **NAA** | | | | | | |
| 0 | 0.0 | 0.0 | 00.00 | 00.00± 00$^{q}$ | 00.00±00.00$^{n}$ | 00.000± 00.00$^{c}$ |
| 0.5 | 0.1 | - | 75 | 12.10±6.70$^{g-q}$ | 212.3±135.4$^{f-n}$ | 0.0596±0.0075$^{bc}$ |
| 0.5 | 0.5 | - | 88.9 | 7.80±2.17 k-$^{q}$ | 84.4±30.3$^{k-n}$ | 0.0941±0.0074$^{b}$ |
| 0.5 | 0.75 | - | 100 | 22.20±4.35 $^{b-p}$ | 440.8±63.6$^{c-n}$ | 0.0501±0.0042$^{bc}$ |
| 0.5 | - | 0.1 | 70 | 14.63±7.75$^{e-q}$ | 342.1±191.6$^{c-n}$ | 0.0438±0.0031$^{bc}$ |
| 0.5 | - | 0.5 | 100 | 13.23±5.71$^{e-q}$ | 295.3±274.4$^{d-n}$ | 0.0593±0.0242$^{bc}$ |
| 0.5 | - | 0.75 | 100 | 33.27±1.99$^{a-f}$ | 675.9±7.0$^{b-e}$ | 0.0492±0.0025$^{bc}$ |
| 1 | 0.1 | - | 83.3 | 11.37±1.35$^{h-q}$ | 178.9±39.4$^{h-n}$ | 0.06450±0.0076$^{bc}$ |
| 1 | 0.5 | - | 100 | 26.30±14.94$^{a-m}$ | 564.1±346.4$^{c-i}$ | 0.0474±0.0020$^{bc}$ |
| 1 | 0.75 | - | 100 | 28.47±4.74$^{a-k}$ | 598.0±162.8$^{b-h}$ | 0.0488±0.0064$^{bc}$ |
| 1 | - | 0.1 | 100 | 7.00±5.89$^{l-q}$ | 192.0±182.2$^{g-n}$ | 0.0428±0.0181$^{bc}$ |
| 1 | - | 0.5 | 80 | 5.80±1.30$^{m-q}$ | 119.9±41.2$^{i-n}$ | 0.0496±0.0055$^{bc}$ |
| 1 | - | 0.75 | 100 | 39.03±11.63$^{a-c}$ | 744.1±297.9$^{a-c}$ | 0.0537±0.0053$^{bc}$ |
| 1.5 | 0.1 | - | 100 | 23.83±12.97$^{a-o}$ | 509.8±346.2 $^{c-l}$ | 0.0497±0.0065$^{bc}$ |
| 1.5 | 0.5 | - | 88.9 | 19.63±3.55$^{c-q}$ | 378.9±86.8$^{c-n}$ | 0.0523±0.0036$^{bc}$ |
| 1.5 | 0.75 | - | 88.9 | 17.70 ±7.50$^{d-q}$ | 300.9±127.4$^{c-n}$ | 0.0587±0.0053$^{bc}$ |
| 1.5 | - | 0.1 | 100 | 42.47±2.5$^{ab}$ | 1038.2±38.00$^{ab}$ | 0.0410±0.0040$^{bc}$ |
| 1.5 | - | 0.5 | 62.5 | 14.83±17.90$^{e-q}$ | 239.0±289.0$^{e-n}$ | 0.0630±0.0031$^{bc}$ |
| 1.5 | - | 0.75 | 100 | 19.23±7.48$^{c-q}$ | 401.2±179.0$^{c-n}$ | 0.0491±0.0042$^{bc}$ |
| 2 | 0.1 | - | 100 | 8.83±2.81$^{j-q}$ | 194.4±62.3$^{g-n}$ | 0.0456±0.0023$^{bc}$ |
| 2 | 0.5 | - | 85.7 | 13.63±9.02$^{e-q}$ | 180.1±162.3$^{h-n}$ | 0.0871±0.0190$^{bc}$ |
| 1 | 0.75 | - | 100 | 43.63±12.01$^{a}$ | 1150.9±86.4$^{a}$ | 0.0375±0.0076$^{bc}$ |
| 2 | - | 0.1 | 100 | 13.07±0.91$^{e-q}$ | 348.6±23.7$^{c-n}$ | 0.0375±0.0019$^{bc}$ |
| 2 | - | 0.5 | 100 | 8.57±0.99$^{j-q}$ | 107.3±11.4$^{j-n}$ | 0.0801±0.0087$^{bc}$ |
| 2 | - | 0.75 | 88.9 | 33.13±6.89$^{a-f}$ | 702.2±217.4$^{b-d}$ | 0.0482±0.0067$^{bc}$ |
| 3 | 0.1 | - | 100 | 5.67±1.32$^{m-q}$ | 91.3±40.2$^{k-n}$ | 0.0655±0.0122$^{bc}$ |
| 1 | 0.5 | - | 100 | 22.40±6.85$^{b-p}$ | 368.4 ±119.2$^{c-n}$ | 0.0612±0.0026$^{bc}$ |
| 3 | - | 0.1 | 100 | 2.37±0.83$^{pq}$ | 83.4±21.3 $^{k-n}$ | 0.0279±0.0027$^{bc}$ |
| 3 | - | 0.5 | 100 | 27.10±3.74$^{a-l}$ | 551.4±41.5$^{c-j}$ | 0.0490±0.0040$^{bc}$ |
| 2 | 0.1 | - | 100 | 12.77±13.97$^{e-q}$ | 296.6±448.4$^{d-n}$ | 0.0987±0.0561 $^{b}$ |
| 4 | 0.5 | - | 100 | 8.37±1.63$^{j-q}$ | 136.1±51.8$^{i-n}$ | 0.0644±0.0137$^{bc}$ |
| 4 | - | 0.1 | 100 | 8.87±1.50$^{j-q}$ | 211.4±33.4$^{f-n}$ | 0.0419±0.0019$^{bc}$ |
| 4 | - | 0.5 | 88.9 | 24.83±8.89$^{a-n}$ | 523.6±122.7$^{c-k}$ | 0.0466±0.0101$^{bc}$ |
| **IAA** | | | | | | |
| 0 | 0.0 | 00.0 | 00.0 | 00.00± 00.00$^{q}$ | 00.00±00.00$^{n}$ | 00.000±00.000$^{c}$ |
| 0.5 | 0.1 | - | 100 | 20.80±3.05$^{c-q}$ | 317.4±29.7$^{c-n}$ | 0.0653±0.0035$^{bc}$ |
| 0.5 | 0.5 | - | 100 | 21.13±6.35$^{c-p}$ | 376.0±148.6$^{mn}$ | 0.0583±0.0079$^{bc}$ |
| 0.5 | 0.75 | - | 100 | 24.07±2.44$^{a-n}$ | 383.9±68.5$^{c-n}$ | 0.0638±0.0119$^{bc}$ |
| 0.5 | - | 0.1 | 100 | 6.13±0.97$^{m-q}$ | 92.4±23.5$^{k-n}$ | 0.0677±0.0084$^{bc}$ |
| 0.5 | - | 0.5 | 100 | 10.17±0.06$^{j-q}$ | 205.7±12.4$^{g-n}$ | 0.0495±0.0028$^{bc}$ |
| 0.5 | - | 0.75 | 100 | 24.00±8.41$^{a-n}$ | 160.0±134.3$^{h-n}$ | 0.234±0.2054$^{a}$ |
| 1 | 0.1 | - | 100 | 16.10±1.47$^{e-q}$ | 242.4±27.1$^{e-n}$ | 0.0665±0.0014$^{bc}$ |
| 1 | 0.5 | - | 100 | 28.93±2.71$^{a-j}$ | 357.2±23.7$^{c-n}$ | 0.0809±0.0025$^{bc}$ |
| 1 | 0.75 | - | 100 | 12.20±2.70$^{g-q}$ | 177.0±53.2$^{h-n}$ | 0.0700±0.0059$^{bc}$ |
| 1 | - | 0.1 | 100 | 8.40±3.06$^{j-q}$ | 208.1±68.1$^{g-n}$ | 0.0402±0.0034$^{bc}$ |
| 1 | - | 0.5 | 100 | 13.10±3.10$^{e-q}$ | 165.9±20.9$^{h-n}$ | 0.0782±0.0092$^{bc}$ |
| 1 | - | 0.75 | 100 | 12.90±3.22$^{e-q}$ | 278.6±57.7$^{d-n}$ | 0.0461±0.0026$^{bc}$ |
| 1.5 | 0.1 | - | 100 | 25.23±5.16$^{a-n}$ | 656.2±72.0$^{b-f}$ | 0.0385±0.0079$^{bc}$ |
| 1.5 | 0.5 | - | 100 | 37.17±2.48$^{a-d}$ | 693.0±52.1$^{b-d}$ | 0.0537±0.0013$^{bc}$ |
| 1.5 | 0.75 | - | 100 | 31.63±4.74$^{a-h}$ | 549.0±99.4$^{c-j}$ | 0.0579±0.0029$^{bc}$ |
| 1.5 | - | 0.1 | 100 | 3.03±0.42$^{o-q}$ | 33.1±4.0$^{mn}$ | 0.0916±0.0019$^{b}$ |

**Table 1.** Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| 1.5 | - | 0.5 | 100 | 9.93±2.59$^{i-q}$ | 144.1±44.4$^{i-n}$ | 0.0697±0.0075$^{bc}$ |
| 1.5 | - | 0.75 | 100 | 18.97±3.68$^{c-q}$ | 220.5±71.4$^{f-n}$ | 0.0882±0.0106$^{bc}$ |
| 2 | 0.1 | - | 100 | 27.80±6.07$^{a-l}$ | 513.1±83.1$^{c-l}$ | 0.0539±0.0028$^{bc}$ |
| 2 | 0.5 | - | 100 | 14.60±2.86$^{e-q}$ | 203.3±37.1$^{g-n}$ | 0.0717±0.0023$^{bc}$ |
| 2 | 0.75 | - | 100 | 24.40±2.89$^{a-n}$ | 384.0±30.0$^{c-n}$ | 0.0635±0.0039$^{bc}$ |
| 2 | - | 0.1 | 100 | 14.47±6.79$^{e-q}$ | 222.6±103.0$^{f-n}$ | 0.0649±0.0017$^{bc}$ |
| 2 | - | 0.5 | 100 | 4.93±1.99$^{n-q}$ | 83.7±33.1$^{k-n}$ | 0.0588±0.0008$^{bc}$ |
| 2 | - | 0.75 | 100 | 11.63±2.00$^{g-q}$ | 141.8±21.1$^{i-n}$ | 0.0819±0.0035$^{bc}$ |
| 3 | 0.1 | - | 100 | 14.53±2.90$^{e-q}$ | 183.9±20.3$^{h-n}$ | 0.0789±0.0129$^{bc}$ |
| 3 | 0.5 | - | 100 | 16.43±5.61$^{d-q}$ | 238.5±80.8$^{e-n}$ | 0.0688±0.0077$^{bc}$ |
| 3 | - | 0.1 | 100 | 6.13±1.40$^{m-q}$ | 84.0±19.2$^{k-n}$ | 0.0738±0.0119$^{bc}$ |
| 3 | - | 0.5 | 100 | 13.63±4.62$^{e-q}$ | 195.8±74.4$^{g-n}$ | 0.0705±0.0037$^{bc}$ |
| 4 | 0.1 | - | 88.9 | 12.37±2.18$^{f-q}$ | 196.9±4.0$^{g-n}$ | 0.0627±0.0100$^{bc}$ |
| 4 | 0.5 | - | 75 | 13.20±3.77$^{e-q}$ | 208.8±68.6$^{g-n}$ | 0.0645±0.0088$^{bc}$ |
| 4 | - | 0.1 | 100 | 6.17±2.00$^{m-q}$ | 125.9±30.2$^{i-n}$ | 0.0491±0.0128$^{bc}$ |
| 4 | - | 0.5 | 100 | 12.73±4.63$^{e-q}$ | 180.3±59.9$^{h-n}$ | 0.0700±0.0026$^{bc}$ |
| **IBA** | | | | | | |
| 0 | 0.0 | 0.0 | 00.0 | 00.00±00.00$^{q}$ | 00.00±00.00$^{n}$ | 00.000±00.000$^{c}$ |
| 0.5 | 0.1 | - | 100 | 5.70±0.46$^{m-q}$ | 73.0±3.4$^{l-n}$ | 0.0781±0.0068$^{bc}$ |
| 0.5 | 0.5 | - | 100 | 14.63±4.57$^{e-q}$ | 181.5±50.2$^{h-n}$ | 0.0809±0.0116$^{bc}$ |
| 0.5 | 0.75 | - | 100 | 22.23±4.97$^{b-p}$ | 281.7±26.3$^{d-n}$ | 0.0786±0.0135$^{bc}$ |
| 0.5 | - | 0.1 | 100 | 10.33±1.33$^{i-q}$ | 220.8±32.8$^{f-n}$ | 0.0479±0.0121$^{bc}$ |
| 0.5 | - | 0.5 | 100 | 15.97±3.79$^{e-q}$ | 231.3±93.4$^{e-n}$ | 0.0732±0.0157$^{bc}$ |
| 0.5 | - | 0.75 | 100 | 17.60±1.92$^{d-q}$ | 195.0±17.0$^{g-n}$ | 0.0903±0.0069$^{b}$ |
| 1 | 0.1 | - | 100 | 7.10±1.04$^{l-q}$ | 89.4±6.5$^{k-n}$ | 0.0792±0.0072$^{bc}$ |
| 1 | 0.5 | - | 100 | 18.23±1.30$^{c-q}$ | 271.7±49.7$^{d-n}$ | 0.0688±0.0141$^{bc}$ |
| 1 | 0.75 | - | 100 | 23.43±5.00$^{a-o}$ | 447.3±141.9$^{c-m}$ | 0.0542±0.0098$^{bc}$ |
| 1 | - | 0.1 | 100 | 21.50±3.39$^{c-p}$ | 453.5±64.8$^{c-m}$ | 0.0476±0.0067$^{bc}$ |
| 1 | - | 0.5 | 100 | 15.03±2.17$^{e-q}$ | 241.3±17.4$^{e-n}$ | 0.0621±0.0046$^{bc}$ |
| 1 | - | 0.75 | 100 | 32.30±5.93$^{a-g}$ | 549.3±118.9$^{c-j}$ | 0.0591±0.0035$^{bc}$ |
| 1.5 | 0.1 | - | 100 | 13.93±0.86$^{e-q}$ | 159.7±40.4$^{h-n}$ | 0.0904±0.019$^{b}$ |
| 1.5 | 0.5 | - | 100 | 26.13±2.11$^{a-m}$ | 363.3±48.7$^{c-n}$ | 0.0729±0.0128$^{bc}$ |
| 1.5 | 0.75 | - | 100 | 19.60±4.36$^{c-q}$ | 267.1±61.2$^{d-n}$ | 0.0735±0.0048$^{bc}$ |
| 1.5 | - | 0.1 | 100 | 17.37±2.30$^{d-q}$ | 410.3±38.8$^{c-n}$ | 0.0422±0.0017$^{bc}$ |
| 1.5 | - | 0.5 | 100 | 22.17±0.32$^{b-p}$ | 358.6±59.2$^{c-n}$ | 0.0628±0.0094$^{bc}$ |
| 1.5 | - | 0.75 | 100 | 12.97±2.71$^{e-q}$ | 164.2±10.4$^{h-n}$ | 0.0785±0.0111$^{bc}$ |
| 2 | 0.1 | - | 100 | 24.07±2.06$^{a-n}$ | 441.9±76.0$^{c-n}$ | 0.0553±0.0084$^{bc}$ |
| 2 | 0.5 | - | 100 | 27.13±3.70$^{a-l}$ | 381.7±78.3$^{c-n}$ | 0.0717±0.0051$^{bc}$ |
| 2 | 0.75 | - | 100 | 19.80±2.05$^{c-q}$ | 201.5±38.4$^{g-n}$ | 0.0993±0.0087$^{b}$ |
| 2 | - | 0.1 | 100 | 16.80±7.05$^{d-q}$ | 235.1±107.2$^{e-n}$ | 0.0727±0.0041$^{bc}$ |
| 2 | - | 0.5 | 100 | 17.93±0.42$^{d-q}$ | 290.2±12.3$^{d-n}$ | 0.0619±0.0025$^{bc}$ |
| 2 | - | 0.75 | 100 | 23.10±1.91$^{a-p}$ | 372.3±21.7$^{c-n}$ | 0.0620±0.0038$^{bc}$ |
| 3 | 0.1 | - | 100 | 17.10±14.03$^{d-q}$ | 267.5±237.8$^{d-n}$ | 0.0665±0.0044$^{bc}$ |
| 3 | 0.5 | - | 100 | 15.17±1.76$^{e-q}$ | 220.3±17.7$^{f-n}$ | 0.0694±0.0118$^{bc}$ |
| 3 | - | 0.1 | 100 | 10.73±0.42$^{h-q}$ | 214.6±65.3$^{f-n}$ | 0.0530±0.0149$^{bc}$ |
| 3 | - | 0.5 | 100 | 33.53±4.28$^{a-e}$ | 635.0±62.4$^{b-g}$ | 0.0532±0.0089$^{bc}$ |
| 4 | 0.1 | - | 100 | 25.80±1.37$^{a-n}$ | 441.0±36.5$^{c-n}$ | 0.0586±0.0017$^{bc}$ |
| 4 | 0.5 | - | 100 | 30.20±7.71$^{a-i}$ | 552.8±216.5$^{c-j}$ | 0.0565±0.0083$^{bc}$ |
| 4 | - | 0.1 | 100 | 20.30±8.55$^{c-q}$ | 546.9±61.3$^{c-j}$ | 0.0363±0.0112$^{bc}$ |
| 4 | - | 0.5 | 100 | 27.33±2.39$^{a-l}$ | 635.9±9.3$^{b-g}$ | 0.0430±0.0042$^{bc}$ |

Each value is mean of three replicates (±SD). Mean followed by different letters in the same column differ significantly at P≤ 0.05 (according to Turkey's test).

**Table 2.** Effect of plant growth regulators on rhizogenesis of leaf explant.

| NAA (mg/L) | IAA (mg/L) | IBA (mg/L) | Rhizogenesis (%) | Roots formed per explant |
|---|---|---|---|---|
| 0.0 | - | - | 00.00 | 00.00±00.00$^{d}$ |
| 0.5 | - | - | 69.20 | 5.400±1.517$^{d}$ |
| 1 | - | - | 75.00 | 19.600±2.510$^{bc}$ |
| 1.5 | - | - | 83.34 | 9.000±4.359$^{cd}$ |
| 2 | - | - | 84.61 | 46.400±14.258$^{a}$ |
| 3 | - | - | 72.70 | 7.000±3.742$^{d}$ |
| 4 | - | - | 53.34 | 28.400±14.328$^{b}$ |
| - | 0.5 | - | 50.00 | 4.000±2.550$^{d}$ |
| - | 1 | - | 30.00 | 4.600±1.140$^{d}$ |
| - | 1.5 | - | 66.67 | 5.400±2.510$^{d}$ |
| - | 2 | - | 63.64 | 10.600±3.782$^{cd}$ |
| - | 3 | - | 76.92 | 12.400±5.639$^{cd}$ |
| - | 4 | - | 76.92 | 5.800±2.049$^{d}$ |
| - | - | 0.5 | 41.18 | 3.200±1.924$^{d}$ |
| - | - | 1 | 66.67 | 5.200±2.490$^{d}$ |
| - | - | 1.5 | 61.54 | 11.400±2.191$^{cd}$ |
| - | - | 2 | 58.82 | 8.800±2.490$^{cd}$ |
| - | - | 3 | 66.67 | 10.200±1.643$^{cd}$ |
| - | - | 4 | 53.85 | 5.800±1.789$^{d}$ |

Each value is mean of five replicates (±SD). Mean followed by different letters in the same column differ significantly at P≤0.05 (according to Turkey's test).

organogenesis and development of roots or shoots depending on morphogenic potential of the cells (Bagadekar and Jayaraj, 2011). The ratio of auxins to cytokinins influences the balance between root and shoot formation (Sinha and Bandyopadhyay, 2011).

There are three distinct stages during organogenesis, namely dedifferentiation, induction of organogenesis pathway and development of organs (Bagadekar and Jayaraj, 2011). Specific phytohormones supplemented exogenously, can trigger the process of differentiation and induction pathway (Praveen and Murthy, 2010). It has been reviewed that hormonal metabolism operate in an integrated manner and that several potential and functional interacting points exist between various hormones (Afrasiab and Jafar, 2011). Bellamine et al. (1998), reported that auxin apply primary role in root formation by its involvement in expression phase (Bellamin et al., 1998). The exogenous application of auxins is one of the most important and key factors to increase the rooting ability and adventitious root formation in various plant species (Pop et al., 2011; Abu-Zahra et al., 2012). Although, different plant species vary in their responses to auxins ( Khalafalla et al., 2009). Earlier studies have shown that the auxins treatments either stimulated or inhibited root formation depending on their concentration (Vesperinas, 1998). Thus, in this experiment, only auxins (IAA, IBA and NAA) were selected to induce adventitious root from *P. abrotanoides*.

In our investigation, IBA, IAA and NAA stimulated adventitious rooting. An overall comparison of three different auxins tested at different concentrations (0, 0.5, 1, 1.5, 2, 3, and 4 mg/L), showed that NAA was the best auxin for *P. abrotanoides* in adventitious roots formation. The relation between NAA concentrations and percentage of root formation was nonlinear.

It has been reported that rooting ability of some plants species respond better to NAA treatments than to other auxins. For example, better root formation in NAA treatment was shown for Mung bean (Kollarova et al., 2005). It has been demonstrated that NAA was more effective than IBA to induce root formation in some *Gerarillea* species (Watad et al.,1992). Leonardi et al. (2001), have shown the positive effect of NAA on *in vitro* rooting of *Gerarillea rosmarinifolia*, too (Leonardi et al., 2001). Similar results have been reported by Abdulmalik et al. (2012) in microshoots culture of *Arachis hypogaea* (Abdulmalik et al., 2012). Abu-Zahra et al. (2012) reported that application of NAA, effectively resulted in rooting of *Virginia creeper* cuttings.

It was shown that besides the types of auxins used, the concentration was also important to induce adventitious rooting. As shown in this investigation, MS medium supplemented with 2 mg/L NAA, showed the best percentage of rhizogenesis response and rooting ability in terms of number of roots induced per explant, whereas concentrations higher or lower than 2 mg/L NAA, reduced rooting potential in explants. This observation is in agreement with results of Ling et al. (2013), who found that the percentage of root induction from *Labisia pumila* leaf explants, declined as the concentration of NAA increased

(Ling et al., 2013). Lower rooting efficiency of high concentrations of NAA (3 and 4 mg/L) could be explained by herbicidal property of high auxin concentrations which may inhibit the adventitious root induction from leaf explants (Ling et al., 2013). Besides, high concentrations of auxins in medium, will actually inhibit cell elongation, because of the production of ethylene. Abdulmalik et al. (2012), reported that the induction of ethylene biosynthesis at high concentration of NAA could be the reason for root growth inhibition in *Arachis hypgaea* tissue culture (Abdulmalik et al., 2012). High concentration of auxins will also inhibit the root growth and development and resulting in root apical dormancy (Chao et al., 2006).

The results showed that auxin is important to induce rhizogenesis in the leaf explant of *P. abrotanoides*, because no adventitious root formation was observed in the lack of auxins. Combination of auxins with BA or Kin stimulated callus formation and relatively moderate concentrations of auxins were essential for rooting response. Lower rooting ability at lower concentrations of auxins (0.5 and 1 mg/L) may be due to inadequate supply of auxins that was necessary for cell divisions and roots induction in leaf explants.

Adventitious roots are attended as commercial interest, because it is an alternative technique for the plant propagation industry (Ling et al., 2013). Besides, adventitious roots are highly differentiated and could cause stable and extensive production of secondary metabolites. Moreover, *in vitro* production of secondary metabolites, can be improved by different approaches like treatment with various biotic and abiotic elicitors.

Thus, further studies could be carried out by focusing on the identification and quantification of some pharmacologically important secondary metabolites (like tanshinones), in the adventitious roots and callus derived cell suspension cultures of *P. abrotanoides* and the effects of elicitors on their production (Zaker et al., unpublished data)

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### Abbreviations

**NAA,** Naphthalene acetic acid; **IAA,** indole acetic acid; **IBA,** indole butyric acid; **BA,** benzyladenine; **Kin,** kinetin (6-Furfurylaminopurine); **MS,** Murashige and Skoog medium.

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