doi:10.30495/MEDHERB.2021.687755

***Journal of Medicinal Herbs***

journal homepage:**www.jhd.iaushk.ac.ir**

# The effect of different concentrations of BAP on micropropagation of two species of Paneer-booti (*Withania coagulans* L.) and (*Withania somnifera* L.)

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**ARTICLE INFO**

***Type:*** *Original Research*
***Topic:*** *Medicinal Plants*
***Received*** *September 05th 2021*
***Accepted*** *December 15th 2021*

***Key words:***
- ✓ *Branch number*
- ✓ *Branching*
- ✓ *Growth regulator*
- ✓ *Propagation rate*

**ABSTRACT**

**Background & Aim:** Withania genus has more than 200 species, of which two important species (*W. somnifera*) and (*W. coagulans*) have high medicinal value and their rapid and extensive reproduction with new methods is very important to expand the area of cultivation. Propagation of crops in vitro is an accessible method for mass propagation. Therefore, the selection of a suitable culture medium is necessary to motivate shoot regeneration from explants and proliferation. The main purpose of this study was to present a rapid and efficient method for mass propagation of virus-free and disease-free plants under in vitro culture conditions.

**Experimental:** The experiment was designed in a completely randomized design (CRD) with three replicatesin. The seeds were sterilized and placed on MS medium after being collected from their natural habitat in Sistan and Baluchestan province. It was then cultured on MS medium containing benzyl amino purine growth regulator at four levels of 0, 0.5, 1 and 1.5 mg/l for branching.

**Results:** The results showed that in coagulans specie in MS medium containing 0.5 mg/l of benzyl amino purine (BAP) with an average proliferation of 3.66 of shoots and a length of 2.85 cm, the highest branching was obtained. Also, the highest propagation coefficient and number of leaves were obtained in benzyl amino purine (BAP) treatment with a concentration of 0.5 mg/l. The lowest propagation rate was reported in control (without hormones). In this experiment, application of benzyl amino purine at concentrations of 0.5 and 1 mg/l improved seedling quality which was more effective in coagulans and contained higher quality and fresher seedlings.

**Recommended applications/industries:** In general, for the production of seedlings with high branching percentages, the use of benzyl amino purine at a concentration of 0.5 mg/l is recommended for optimal branching.

## 1. Introduction

Among all the Withania species, only two species: *Withania somnifera* and *Withania coagulans* are of economical and medicinal importance and their cultivation has been reported in the different regions. Paneer-booti is native of the Mediterranean region in North Africa and is widely distributed in Pakistan, India, Sri Lanka, South Africa, Iraq, Iran, Syria, and Turkey (Valizade *et al*., 2015). From the shoots, roots, and fruits of two species of *W. somnifera* and *W.coagulans*, 12 types of alkaloids, more than 35 types of withanolides, and a number of glycovitanolides called cytoindosides have been extracted and investigated (Ghorbani Ghozhdi, 2014). Withaferin A, Withanolide A, and Withanon are the most important

withanolides in these two species. among the available withanolides, the composition of vitafrin is highly appreciated in the pharmaceutical sector due to its inhibitory capabilities of cancer cells and tumors (Zhang *et al*., 2012;Yang *et al*., 2007). This plant is also known as plant renin, because its fruit extract is used as a milk coagulant. In some parts of Sistan and Baluchestan Province, as well as in the Punjab Province of Pakistan, the fruits of this plant are widely used as a source of milk coagulation enzyme. In this regard, biotechnology can be used for mass production of this plant (Valizade *et al*., 2015). Seeds are the most common way of propagation for the Paneer-booti. The seeds have a diameter of 2,3,5 mm, Dark to brown spots, and an angular capsule with mucilage (Gupta and Keshari, 2013). Harvesting the roots of the plant for medicinal purposes and using the leaves as forage has prevented the plant from reaching maturity and seed production and put its generation at risk (Hosseini *et al*., 2016). Plant tissue culture is a new approach for producing significant and high-quality medicinal plants, as well as an alternative to standard growing methods, to solve these problems. Plant tissue culture techniques are rapidly being employed for micropropagation, germplasm management, medicinal plant genetic improvement, and secondary metabolite production (Hussain *et al*., 2012). Several studies in the field of medicinal plant micropropagation, Germplasm preservation, medicinal plant genetic enhancement, and secondary metabolite production with the prospect of rapid and mass reproduction of some of their species have been conducted such as: *Ceropegia candelabrum*, (Beena *et al*., 2000), *Aloe chinensi* (Liao *et al*., 2004), *Curcuma longa* L. (Prathanturarug *et al*., 2003). Since some Solanum species have a high medicinal value, studies on in vitro cultivation in various species such as S.villosum L., Kaykha *et al*. (2017) and Others have been reported as well. According to the findings, factors such as growth regulators can have an impact on in vitro culture and propagation. The Murashig and Skoog (MS) medium, as well as varying quantities of the hormones Thidiazuron and benzyl amino purine, were employed in a study by Nahok (2013), the highest number of shoots were developed in culture media containing 1 mg/l BAP and 0.25 mg/l 2-4-D. According to the findings of this study, Super *et al.* (2006) analyzed the impact of several hormones on the branching and roots of Paneer-booti that 0.6 mg/l BAP and 0.4 mg/l BAP were particularly effective for branching.The influence of different hormones on the branching and roots of Paneer-booti seeds was also examined by Super *et al.* (2006). Benzyl amino purine 0.6 mg/l and auxin 0.4 mg/l were particularly successful in branching in this experiment. Because of the importance of Paneer-booti and the need for mass propagation in Iran, this study was carried out to assess the ability of in vitro propagation and the effect of different concentrations of the growth regulator BAP (benzyl amino purine) on the proliferationof two Paneer-booti species.

## 2. Materials and methods

This experiment was conducted to determine the suitable concentration of growth regulators for branching in a completely randomized design, in the biotechnology laboratory of the Agricultural and Natural Resources Research and Education Center of Khorasan Razavi province. Plant samples were taken from the plant's natural habitat in the province of Sistan and Baluchestan. For sterilization of seeds, 70% alcohol and 10% sodium hypochlorite were used for 5 and 10 min, respectively. Then, it was washed in sterile water in three steps and the seeds were cultivated in MS medium (Fig. 1A).

Plant samples (single seedling) were activated in Fig. 1B and then transferred to MS culture medium containing benzyl amino purine at four levels (0, 0.5, 1, 1.5 mg/l) for branching (Fig. 1D). Each treatment had four replications, with five plant samples in each replication. Solid MS medium with 7 g/l agar and 30 g/l sugar was employed for explant culture. Before autoclaving, the culture medium was adjusted to pH of 5.7 with NAOH and HCL. Every three weeks, seedlings were transplanted into new culture medium.The culture dishes were placed in the growing room with a light level of 2000 to 2500 lux and 16 hours of light at 22°C and 8 hours of darkness at 18°C (Fig. 1E).

Number of branches, branch length, number of leaves, propagation coefficient (after transferring seedlings produced to culture medium containing hormone for branching and performing successive subculture, many branches are produced, which is calculated by increasing the number of branches per

harvest) and seedling quality were examined in this study after three subcultures at three-week intervals.

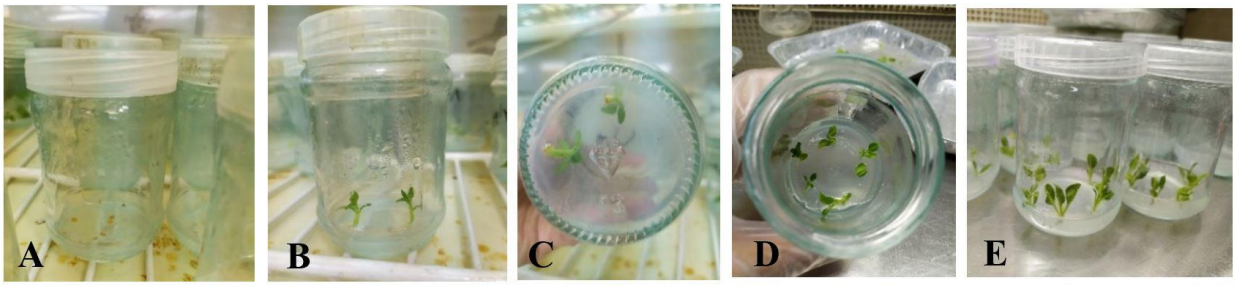


**Fig 1. (**A) Transfer of seeds of two spieces of Paneer-booti to hormone-free MS medium; (B,C) activation of seeds and growth of single seedlings; (D) transfer of activated seedlings to MS + BAP media; (E) transfer of seedlings to the growing room.

### *2.1. Statistical analysis*

Data analysis was performed using JMP software after the data was recorded in excel program, and mean comparison was done using the Tukey HSD test.

## 3. Results and discussion

### *3.1. Number of branches*

According to the results of the mean comparison, different concentrations of benzyl amino pourine generated significant difference in the number of branches, branch length, propagation coefficient, number of leaves, and seedling quality (Table 2). In addition, the results revealed that MS culture media containing 0.5 mg/l BAP produced the most branches (3.66) in coagulans species (Table 1) (Fig. 2 B).

The number of branches (2.54) was reported in *somnifera* (Table 1). The smallest number of branches was recorded in hormone-free MS medium (1.02). When the BAP concentration exceeded 0.5 mg/l, the number of shoots decreased (Table 1). Benzyl amino purine is a cytokine that promotes cell growth and division, and when added to the culture media, it causes regeneration.

### *3.2. Branch length*

The average shoot length varied with BAP different concentrations (Table 1). As shown in Fig. 2C the highest branch length was achieved in *Withania Coagulans* containing 0.5 mg/l and the lowest branch length was recorded in the control (non-application of hormone), which was 1.43 cm. Shoot length reduced by increasing BAP concentrations.

### *3.3. Proliferation rate*

In this experiment, the different Concentrations of benzyl amino purine was significant at the probability level of 1% (Table 2). By increasing the benzyl amino purine content, the rate of proliferation was reduced. So that, 0.5 mg/l benzyl amino purine increased the rate of coagulans proliferation by 79.02 percent, compared to the control (Table 1).

### *3.4. Number of leaves*

The average number of leaves was variable In different concentrations of benzyl amino purine (Table 1). According to the findings, the MS medium with 0.5 mg/l benzyl amino purine produced the most leaves. The least number of leaves were found in the growth medium without growth regulator (control) or with higher concentrations of BAP (Table 1).

### *3.5. Seedling quality*

Based on the findings of this study, the highest seedling quality belonged to the benzyl amino purine treatment at concentrations of 0.5 and 1 mg/l (Table 1). In the present study, low concentrations of BAP stimulated branching. In Solanum nigrum L. The highest percentage of branching was observed at a concentration of 0.5 mg/l of BAP. However, increasing hormone level up to 2 mg/l had a somewhat negative effect on regeneration percentage and number of shoots in this plant (Pashm Foroush *et.al*., 2016), which is consistent with the results of the present experiment. Researchers have also studied the effect of growth regulators on micro-propagation of Paneer-booti and reported that application of 0.5 mg/l of benzyl amino

purine and 0.5 mg/l of tidiazone increased branching (Nathiya *et al*., 2013).

Another study on *Lippia alba* showed a connection between an increase in the number of shoots and an increase in the average concentration of BAP (Gupta *et al*., 2001). Which confirms the current findings.The researchers claim that benzyl amino purine is a component of cytokines that stimulates cell division and proliferation, and that adding it to culture media causes regeneration (Bagheri *et al*., 2005). According to the researchers, the treatments lacking naphthalene acetic acid and moderate concentrations of benzyl amino purine had the highest percentage of branching in leaf microsamples (Sanjida *et al*., 2011).

According to research, Paneer-booti has a high level of endocrine cytokinin hormone, which is probably why the plant's intermediate tissue achieves cytokinin saturation in an environment with lower levels of benzyl amino purine and Being able to regenerate new shoots. Internal hormones not only influence shoot regeneration, but they also influence hormone concentration during in vitro culture (Hussain *et al*., 2012). Accordingly, in the present study, application of 0.5 mg/l of this growth stimulant showed better results compared to other levels tested.

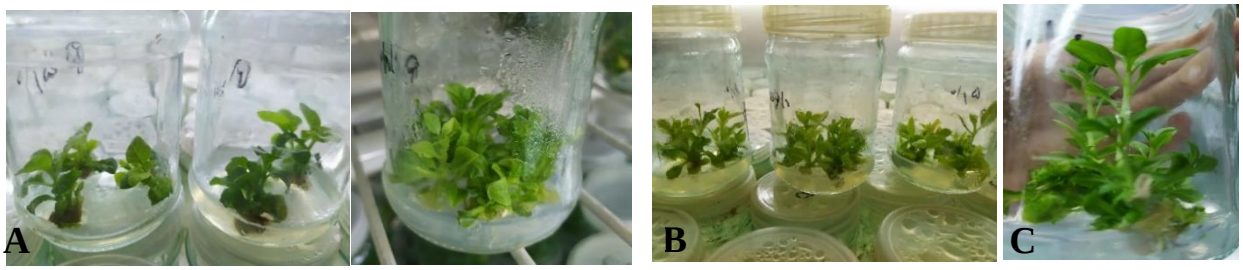


**Fig 2.** Comparison of proliferation of two species of Panee-booti. (A) Effect of MS medium containing 0.5 mg/l BAP on the number of regenerated shoots per *W.somnifera* explant; (B) Effect of MS medium containing 0.5 mg/l BAP on the number of regenerated shoots per *W. Coagulans* explant; (C) The effect of 0.5 mg/l BAP on the branch length of *W.coagulans.*

**Table 1.** Mean comparison effect of BAP on some traits of *Withania coagulans* and *Withania somnifera* in prolifertion stage.

| species | BAP concentrations (mg/L) | Proliferation rate | Number of branches | Shoot length | Number of leaves | Quality of seedlings |
|---|---|---|---|---|---|---|
| ***W.coagulans*** | 0 | $1.25\pm0.13^{d}$ | $1.02\pm0.08^{c}$ | $1.43\pm0.14^{d}$ | $6.94\pm0.64^{d}$ | $2.52\pm0.53^{a}$ |
| | 0.5 | $4.48\pm0.61^{a}$ | $3.66\pm0.54^{a}$ | $2.85\pm0.04^{a}$ | $21.00\pm2.0^{a}$ | $1.05\pm0.25^{d}$ |
| | 1 | $4\pm0.59^{a}$ | $3.49\pm0.11^{a}$ | $2.63\pm0.13^{a}$ | $19.75\pm1.1^{ab}$ | $1.25\pm0.29^{dc}$ |
| | 1.5 | $3.72\pm0.53^{b}$ | $1.28\pm0.25^{c}$ | $2.31\pm0.14^{b}$ | $11.75\pm0.85^{c}$ | $2.02\pm0.13^{b}$ |
| | LSD | 1. 25 | 0.84 | 0.28 | 2.24 | 1.2 |
| ***W.somnifera*** | 0 | $1.04\pm0.14^{d}$ | $1.02\pm0.13^{d}$ | $1.72\pm0.25^{dc}$ | $7.02\pm1.03^{d}$ | $2.82\pm0.53^{a}$ |
| | 0.5 | $3.12\pm0.35^{bc}$ | $2.54\pm0.53^{b}$ | $2.05\pm0.12^{c}$ | $18.35\pm0.03^{b}$ | $1.75\pm0.25b^{c}$ |
| | 1 | $3.08\pm0.04^{c}$ | $2.41\pm0.16^{b}$ | $2.33\pm0.29^{ab}$ | $18.02\pm1.1^{b}$ | $1.27\pm0.13^{c}$ |
| | 1.5 | $2.32\pm0.08^{c}$ | $1.15\pm0.04^{dc}$ | $2.31\pm0.08^{ab}$ | $11.32\pm0.81^{c}$ | $2.35\pm0.29^{b}$ |
| | LSD | 1.02 | 0.61 | 0.27 | 2.03 | 0.98 |

Values (means of three replicates ± SE) of each parameter followed by at least one same letter are significantly different at $p < 0.01$ based on least significant difference (LSD).

**Table 2.** Variance analysis of different concentrations of BAP on some traits of *Withania coagulans* and *Withania somnifera* in prolifertion stage.

| S.O.V | DF | Mean squares | | | | |
|---|---|---|---|---|---|---|
| | | Proliferation rate | Number of branches | Shoot length | Number of leaves | Quality/plant |
| Treatmentt | 4 | $0.71^{**}$ | 0.28** | 2.85** | 1.51** | 0.17** |
| Error | 15 | 0.02 | 0.02 | 0.06 | 7.05 | 0.02 |
| C.V (%) | | 7.19 | 10.25 | 6.28 | 11.23 | 10.20 |

** Significant at 0.01 probability levels.

## 4. Conclusion

Increasing the concentration of benzyl amino purine from 0.5 to 1.5 mg/l in the branching stage had a negative effect on the studied traits and the application of 0.5 mg/l of benzyl amino purine not only increased the number of branches, branch length, propagation rate and number of leaves but also improved the quality of seedlings. According to the results, the use of benzyl amino purine at a concentration of 0.5 mg/l is recommended for optimal branching.

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