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# In Vitro Micropropagation of *Pogostemon erectus* (Dalzell) Kuntze in Liquid Culture Medium

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## Abstract

*Pogostemon erectus* (Dalzell) Kuntze is a valuable aquatic-ornamental plant. This study was designed for the multiplication of *P. erectus* in a liquid culture medium. In this context, shoot tip explants of P. erectus were placed in Murashige and Skoog (MS) food solutions including Zeatin (ZEA: 0.1-2.4 mg/L) and indole acetic acid (IAA: 1.2 mg/L). In addition, experiments were set up in hormone-free environments as controls. In general, high-frequency results were recorded. 100 % regeneration was determined in the treatment of 0.6 mg/L ZEA + 1.2 mg/L IAA. The best results for shoot count and shoot length were obtained with 0.6 mg/L ZEA + 1.2 mg/L IAA and 0.3 mg/L ZEA + 1.2 mg/L IAA, respectively. Generally, low regeneration values were seen in nutrient solutions using high or low doses of ZEA. Shoots were rooted in MS nutrient media with 0.25 mg/L IAA. Then they were transferred to an aquarium and successfully adapted to external conditions.

**Keywords:**
*MS medium, Shoot regeneration, Shoot tip explant, Tissue culture*

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## Introduction

Tissue culture applications have provided significant advantages over the multiple and rapid production of plants (Hussain et al., 2012; Espinosa-Leal et al., 2018). When tissue culture applications are combined with molecular biology techniques, it can become an important tool such as studying metabolic pathways, elucidating cellular processes, obtaining improved plants for the production of biotic and abiotic stress-resistant cell lines through genetic engineering (Hussain et al., 2012; Loyola-Vargas & Avilez-Montalvo, 2018).

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The achievement of tissue culture method is impressed by the content of the culture media. Under *in vitro* conditions, macronutrients, micronutrients, plant growth regulators, vitamins, amino acids and other nitrogen supplements and sugars (carbon sources) are required for healthy plant growth (Loyola-Vargas & Avilez-Montalvo, 2018; Yaseen et al., 2013).

In recent years, important papers have been published on the production of ornamental plant such as *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne (Dogan, 2018), *Hibiscus coddii* subsp. barnardii (Plessis et al., 2021), *Echinacea angustifolia* (Tyub et al., 2021), *Anubias heterophylla* (Rittirat et al., 2021), *Staurogyne repens* (Nees) Kuntze (Kose et al., 2021), *Campomanesia phaea* (Demétrio et al., 2021) and *Lindernia antipoda* (L.) Alston (Viella, 2021) by tissue culture methods.

*Pogostemon erectus* is a valuable ornamental plant (Dogan, 2019a). Therefore, it has an important economic value. In this study, *in vitro* micropropagation of *P. erectus* in liquid culture media containing different concentrations of Zeatin (ZEA) and indole acetic acid (IAA) was investigated.

**Materials and Method**

Sterile *P. erectus* plants were obtained according to the process previously performed by Doğan (2017). Shoot tip explants were preferred for propagation experiments. These explants were transferred to liquid Murashige and Skoog (1962) (MS) food medium with ZEA+IAA. Growth regulators and their concentrations are given in Table 1. 30 g/L sucrose was added as a carbon source. Agar was not added to the culture medium.

Nutrient solutions were prepared from ultrapure water. The solutions were pH set to 5.7±1 and sterilized using an autoclave (120°C, 20 min). Liquid cultures were incubated under white LED light (1500 lux) (24°C and 16 h illumination).

The elongated and growing shoots were cut about 3 cm long and rooting studies were carried out. 0.25 mg/L IAA was inserted to the MS media for root formation. Rooting studies lasted four weeks. Rooted shoots were then transposed to an aquarium (24ºC and 16 h illumination) to adapt to *ex vitro* conditions.

Experiments were carried out in three repetitions. Shoot regeneration data were evaluated with SPSS 21 for Windows. Duncan test was performed for Post Hoc.

Table 1. Growth regulators in propagation experiments

| Zeatin (mg/L) | Indole Acetic Acid (mg/L) |
|---|---|
| 0 | 0 |
| 0.1 | 1.2 |
| 0.3 | 1.2 |
| 0.6 | 1.2 |
| 1.2 | 1.2 |
| 2.4 | 1.2 |

**Results and Discussion**

In this study, experiments on *in vitro* micropropagation of *P. erectus* in nutrient solutions with various ZEA + IAA were established. The first regenerated shoots were emerged on the 8th day. In the third week, shoots became more prominent (Figure 1a). Experiments were terminated with the elongated and developing shoots in the eighth week (Figure 1b and c). In the present study, shoot tips were preferred as explants. Similarly, successful shoot regeneration experiments with shoot tip explants have been reported in *Celastrus paniculatus* (Moola & Kumari, 2019), *Ceratophyllum demersum* (Dogan, 2019), *Pterocarpus marsupium* (Ahmad et al., 2020), *Xanthosoma sagittifolium* (Wada & Feyissa, 2021) and *Ipomoea batatas* (Abdalla et al., 2021).

The amount and variety of growth regulators used in the tissue culture medium are important for shoot regeneration (Oseni et al., 2018). Different effects of growth regulators on shoot regeneration values of plants have been previously reported in Triticum aestivum (Xhulaj & Doriana, 2019), Daphne mezereum (Nowakowska et al., 2019) and Lamprocapnos spectabilis (Kulus, 2020). The results in the present study were recorded and statistically evaluated.

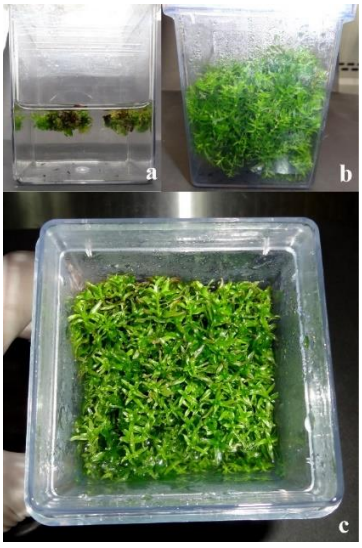


Figure 1. *In vitro* micropropagation of *P. erectus* in liquid culture media fortified with ZEA + IAA. (a) Regenerated shoots at the third week of culture (b and c) Shoot proliferating and elongating at eight weeks in nutrient solution including 0.6 mg/L ZEA + 1.2 mg/L IAA.

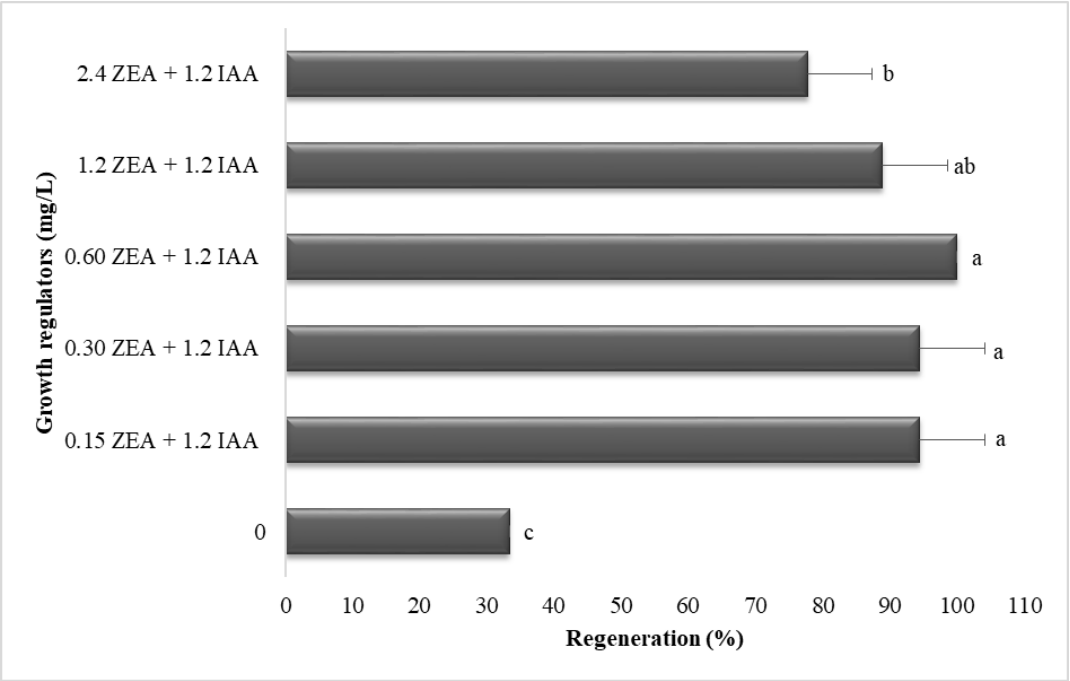


Figure 2. The influence of ZEA+IAA treatments on the regeneration value of *P. erectus*. Data are the mean of three replicates (n = 3). Vertical bars show standard errors. Different letters are statistically significant ($p$<0.05).

Regeneration values are shown in Figure 2 and they demonstrated important differences ($p$<0.05). The maximum percentage of shoot formation of explants was determined in the nutrient solution including 0.6 mg/L ZEA + 1.2 mg/L IAA. Subsequently, high regenerations (94.44 %) were obtained in cultures including 0.1 mg/L ZEA + 1.2 mg/L IAA and 0.3 mg/L ZEA + 1.2 mg/L IAA. Nutrient solutions supplemented with ZEA+IAA showed significantly higher regeneration values compared to control. When the growth regulator media were compared, the lowest shoot formation percentage (77.78 %) was seen in the nutrient solution with 0.3 mg/L ZEA + 1.2 mg/L IAA. As ZEA concentration increased, it had a negative effect on shoot emergence.

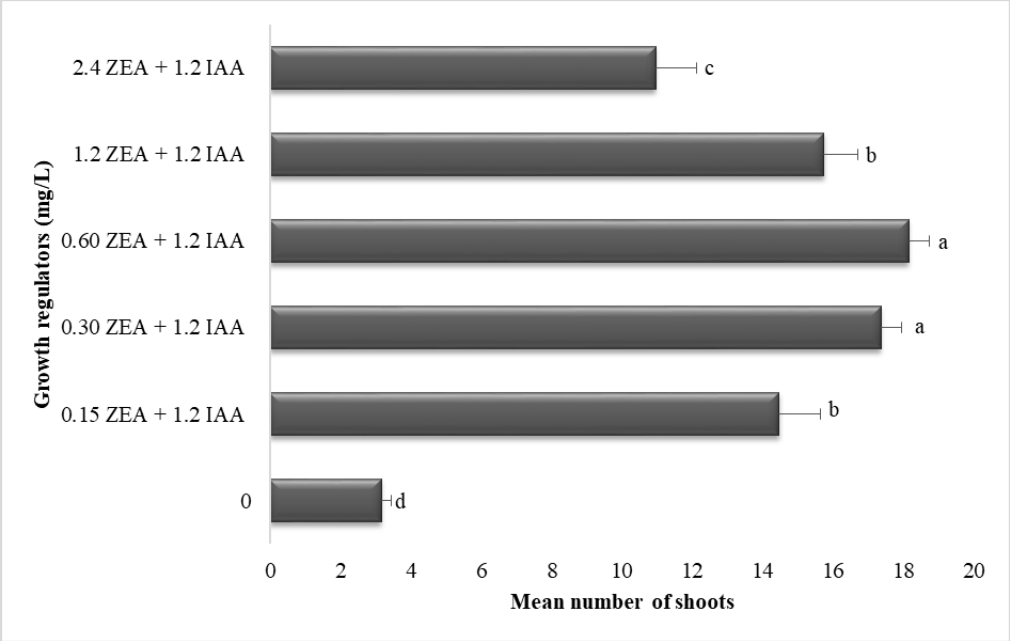


Figure 3. The influence of ZEA+IAA treatments on the shoot counts of *P. erectus*. Data are the mean of three replicates (n = 3). Vertical bars show standard errors. Different letters are statistically significant ($p$<0.05).

Shoot numbers recorded in liquid culture media ranged from 3.17-18.14 (Figure 3). Statistically significant differences were found in terms of shoot numbers ($p$<0.05). The best practice for shoot numbers was recorded as nutrient solution with 0.60 mg/L ZEA + 1.2 mg/L IAA (18.14), followed by nutrient solution including 0.3 mg/L ZEA + 1.2 mg/L IAA (17.34). The use of ZEA above or below 0.6 mg/L had a negative effect on the shoots emerging from the explants ($p$>0.05). Among the growth regulators applied, the worst result (10.95) was determined as 2.4 mg/L ZEA + 1.2 mg/L IAA. The successful effect of liquid culture medium with ZEA+IAA on *in vitro* micropropagation of shoots was observed. Similarly, successful production of *Jatropha curcas* (Singh, 2018), *Dendrocalamus longispathus* (Borpuzari & Bisht, 2018), *Vaccinium vitis-idaea* ssp. *minus* (Arigundam et al., 2020) and *Aerva lanata* (Varutharaju et al., 2021) have been reported under tissue culture conditions using liquid culture media.

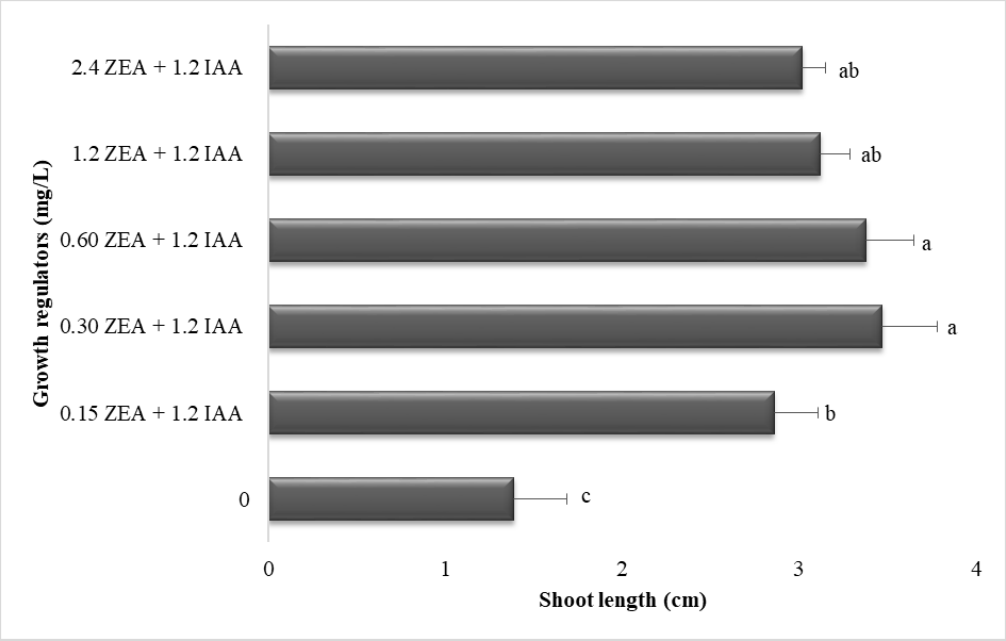


Figure 4. The influence of ZEA+IAA treatments on the shoot lengths of *P. erectus*. Data are the mean of three replicates (n = 3). Vertical bars show standard errors. Different letters are statistically significant ($p$<0.05).

Shoot lengths in nutrient solutions with ZEA + IAA are given in Figure 4. Length values were statistically significant ($p$<0.05). The nutrient solutions with ZEA+IAA had very high length values compared to the control. The best value on length data was obtained at 3.47 cm with 0.3 mg/L ZEA + 1.2 mg/L IAA. In contrast, the worst value for length data was determined in the control application (1.39 cm), followed by the application of 0.1 mg/L ZEA + 1.2 mg/L IAA (2.86 cm). When hormonal media were examined, the lowest or highest doses of ZEA showed adverse effects for length. In general, elongation of shoots was achieved successfully in the liquid culture medium.

The shoots were cut about 3 cm and successfully rooted in nutrient medium with 0.25 mg/L IAA at the end of the fourth week. After removing the nutrients on the new plants, they were placed in aquarium conditions with water, and at the end of three weeks, the plants were successfully acclimatized to the external conditions.

*P. erectus* is known as an important ornamental plant. In this study, micropropagation from shoot tip explants of *P. erectus* was achieved in nutrient solutions with ZEA+IAA. Higher regeneration values were obtained compared to the control. The best results on regeneration percentage and shoot number were obtained with 0.6 mg/L ZEA + 1.2 mg/L IAA. The best length was obtained in nutrient solution with 0.3 mg/L ZEA + 1.2 mg/L IAA. In general, the use of high and low ZEA decreased the regeneration capacity of explants.

**Author Contributions**

The article applications, design and writing were done by MD.

**Conflict of Interest**

The author declares that no conflict of interest.

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