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Impact of Gibberellic Acid and Naphthalene Acetic Acid on Axillary Shoot Multiplication in *Hygrophila polysperma* (Roxb.) T. Anderson

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Abstract

In this work, axillary shoot regenerations were investigated from nodal explants of *Hygrophila polysperma* (Roxb.) T. Anderson in MS medium (Murashige and Skoog) including different combinations of Gibberellic Acid (GA_3) and Naphthalene Acetic Acid (NAA). Nodal explants of H. polysperma were used in propagation studies. The 100% regeneration frequency was determined in MS plus 0.25 and 0.50 mg/L GA_3 + 0.15 mg/L NAA. When the shoot numbers were examined, the best nutrient medium was determined as 0.25 mg/L GA_3 + 0.15 mg/L NAA (14.72 shoots/explant). The best result in shoot length was reached in MS plus 0.50 mg/L GA_3 + 0.15 mg/L NAA (1.91 cm). Then the longest shoot was determined as 1.79 cm in nutrient media plus 0.25 mg/L GA_3 + 0.15 mg/L NAA. Longer shoots were obtained in GA_3+NAA nutrient media according to control. When the results were examined, it was determined that the number and length of shoots decreased as the amount of GA_3+NAA used in the culture medium increased. Regenerated shoots were rooted in MS media including 0.25 mg/L Indole-3-Acetic Acid and successfully acclimatized to aquarium conditions. As a result, multiple and rapid productions of *H. polysperma* under tissue culture conditions were achieved.

Keywords:

In vitro propagation, shoot regeneration, nodal explant, tissue culture

Article history:

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Introduction

Tissue culture is a culture process a whole plant or plant parts like cells, tissues and organs under sterile conditions and artificial nutrient media in order to obtain new tissue, plant or plant products

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(El-Sherif, 2018; Boopathi et al., 2021). The tissue culture method is applied for many purposes such as the production of new plant varieties, haploid plant production, germplasm preservation, protection of rare and endangered plants, production of secondary metabolites, obtaining transgenic plants and propagation of plants that are difficult to reproduce (Kaya et al., 2018; Tiku et al., 2021; Boopathi et al., 2021).

The plant tissue culture method has many advantages over the traditional method. Some of these are (i) the propagation process can be applied independently of the season, (ii) large-scale plants can be obtained in a short time (iii) the plants produced in vitro are free from diseases caused by microorganisms, and (iv) valuable genotypes can be protected from plant viruses (Kaya et al., 2018; Naik & Buckseth, 2018).

For this reason, several herbs like *Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne (Dogan, 2017), *Limnophila aromatica* (Lam.) Merr. (Dogan, 2018), *Bacopa monnieri* L. Pennell (Dogan & Emsen, 2018), *Glycine max* L. Merr. (Mangena, 2021), *Ruellia tuberosa* L. (Lakshmi et al., 2021), *Paulownia tomentosa* (Thunb.) Steud (Amirova et al., 2022), *Vanilla borneensis* Rolfe (Hasnu & Tanti, 2022) and *Vaccinium oldhamii* Miq. (Yun et al. 2022) have been produced by using tissue culture, which is a biotechnological production method.

In this work, axillary shoot regenerations were investigated from nodal explants of H. polysperma in MS medium (Murashige and Skoog) including different combinations of Gibberellic Acid (GA_3) and Naphthalene Acetic Acid (NAA).

Materials and Methods

H. polysperma was obtained from Konya (Türkiye). Surface sterilization was achieved with a 15% commercial bleach (NaOCI) application for 10 min. Nodal explants from sterile plants were used. Murashige and Skoog (1962) (MS) salts (Duchefa), 3% saccharose, 0.65% agar were used in the preparation of nutrient media. In the experiments, 0.25-1.25 mg/L GA_3 and 0.15 mg/L NAA were put in the culture media in different combinations (Table 1). In addition, trials were set up in a hormone-free environment and a control group was formed.

Table 1. Plant hormones placed in MS medium for axillary shoot regeneration

GA_3 (mg/L)	**NAA (mg/L)**
-	-
0.25	0.15
0.50	0.15
0.75	0.15
1.0	0.15
1.25	0.15

All trials were set up according to the randomized plot design. Tissue culture studies were carried out in 3 replications. SPSS 21 for the Windows program was used for statistical calculations. Data were analyzed by Duncan's multiple range test (DMRT), one of the Post Hoc tests ($p<0.05$).

Results and Discussion

In the work, nodal explants were isolated for axillary shoot regeneration of H. polysperma and then placed in MS nutrient medium containing GA_3+NAA in different combinations. With the effect of growth regulators, new shoots started to appear in the culture medium. After six weeks, the experiments were terminated and axillary shoots emerging from the nodal explant were recorded in terms of percentage, number and length values.

In the experiments, GA_3 and NAA were used together and axillary shoots were obtained successfully. Similarly, positive impacts of cytokinin and auxin combinations on shoot formation have been shown in various plants like Blepharispermum subsessile DC. (Nayak & Kalidass, 2016), *Enicostema axillare* (Lam.) (Sasidharan & Jayachitra, 2017), *Saussurea costus* (Falc.) Lipsch (Khan et. al., 2021), *Solanum torvum* Sw (Malothu et al., 2022) and *Ammannia baccifera* L. (Basha et al., 2022).

In the current study, the nodal explants were preferred as explant type in in vitro propagation studies. Similarly, shoot regenerations have been reported in *L. aromatica* and *B. monnieri* (Dogan, 2020), *Ipomoea batatas* (L) Lam. (Abdalla et al., 2021) and *Corynandra chelidonii* var. pallae (Sirangi et al., 2022) plants using the nodal explant.

Regeneration percentages of explants exposed to GA_3+NAA revealed statistically significant differences. In general, the increase of growth regulators caused a decrease in the regeneration percentage of shoots. The 100% regeneration frequency was noted in MS media with 0.25 and 0.50 mg/L GA_3 + 0.15 mg/L NAA (Figure 1). The lowest percentage of regeneration was determined in culture medium without a growth regulator (control), followed by the lowest percentage of regeneration (61.11%) in media including 1.25 mg/L GA_3 + 0.15 mg/L NAA.

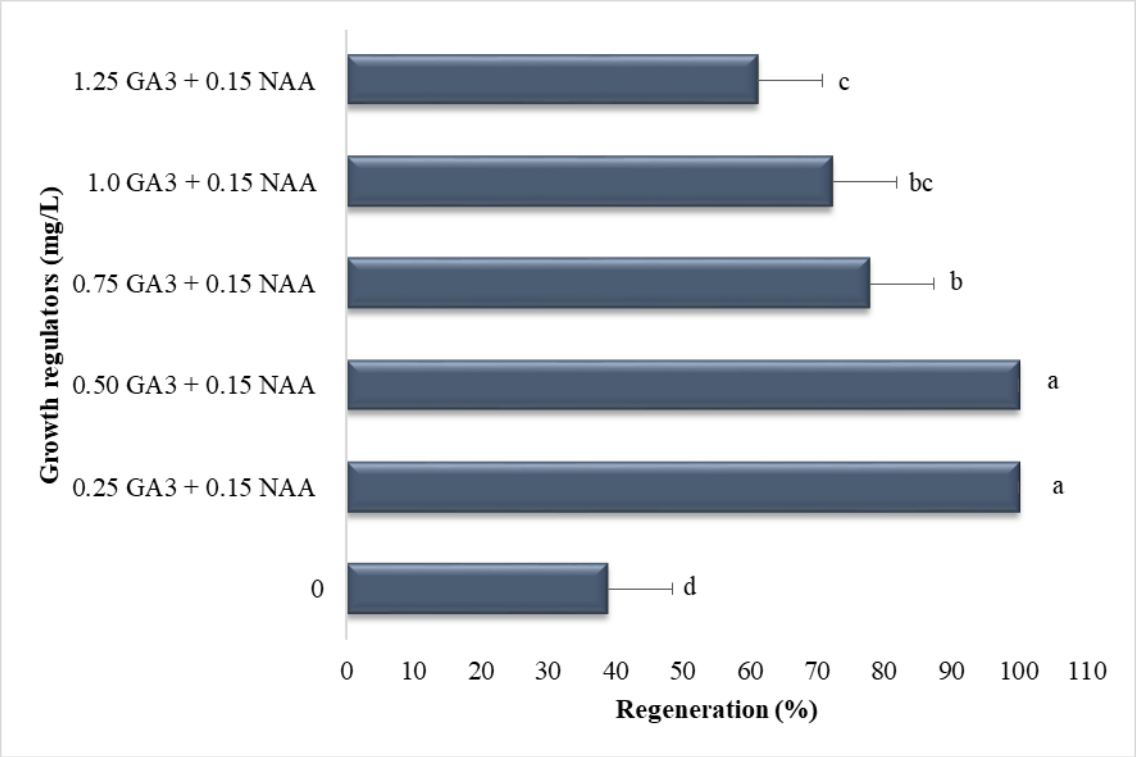

Figure 1. Impact of GA_3+NAA on shoot regeneration frequency. Vertical bars show standard deviation (n = 3). Various letters show significantly different values (DMRT, $p < 0.05$)

The effects of GA_3+NAA combinations in culture medium on shoot numbers of *H. polysperma* were shown in Figure 2. Statistically significant differences were founded between the concentrations of growth regulators and the number of shoots at the 95% confidence interval. In addition, GA_3+ NAA nutrient media produced significantly superior results compared to the culture medium without growth regulator (control group). In terms of shoot numbers, the best nutrient medium was founded as 0.25 mg/L GA_3 + 0.15 mg/L NAA (14.72 shoots/explant) (Figure 3).

As the amount of GA_3 increased, the shoot number decreased. The lowest shoot numbers in culture media containing growth regulators were determined in culture media including 1.25 mg/L GA_3 + 0.15 mg/L NAA. On the other hand, shoot numbers in nutrient media including 1.25 mg/L GA_3 + 0.15 mg/L NAA and 1.0 mg/L GA_3+0.15 mg/L NAA were not statistically significant ($p>0.05$). In general, the results demonstrated that media with 0.25 mg/L GA_3 gave the best results and revealed that shoot numbers were reduced in nutrient mediums containing more than 0.25 mg/L GA_3. Similarly, Sasidharan & Jayachitra (2017) investigated the in vitro growth of *E. axillare* in a culture medium containing BAP + IBA in different combinations. In general, high shoot numbers were obtained in culture medium with low BAP + IBA, while few shoots were obtained in culture medium with a high level of BAP + IBA. Majumder & Rahman (2016) conducted an experiment for the micropropagation of Stevia rebaudiana Bertoni in media including different BAP + IAA,

they achieved maximum shoot formation in culture media including 1.5 mg/L BAP + 0.5 mg/L IAA (15.30 ± 0.15). In contrast, Eshghi Khas et al. (2020) cultured *Passiflora edulis* in nutrient media with different combinations of BA (0-8.9 µM), TDZ (2.3-9.1 µM), GA_3 (0 and 2.9 µM), and IBA (0-8.9 µM) and recorded the highest number of shoots in the nutrient medium where growth regulators were used most intensively. These results revealed that the effects of growth regulators may differ according to the plant species.

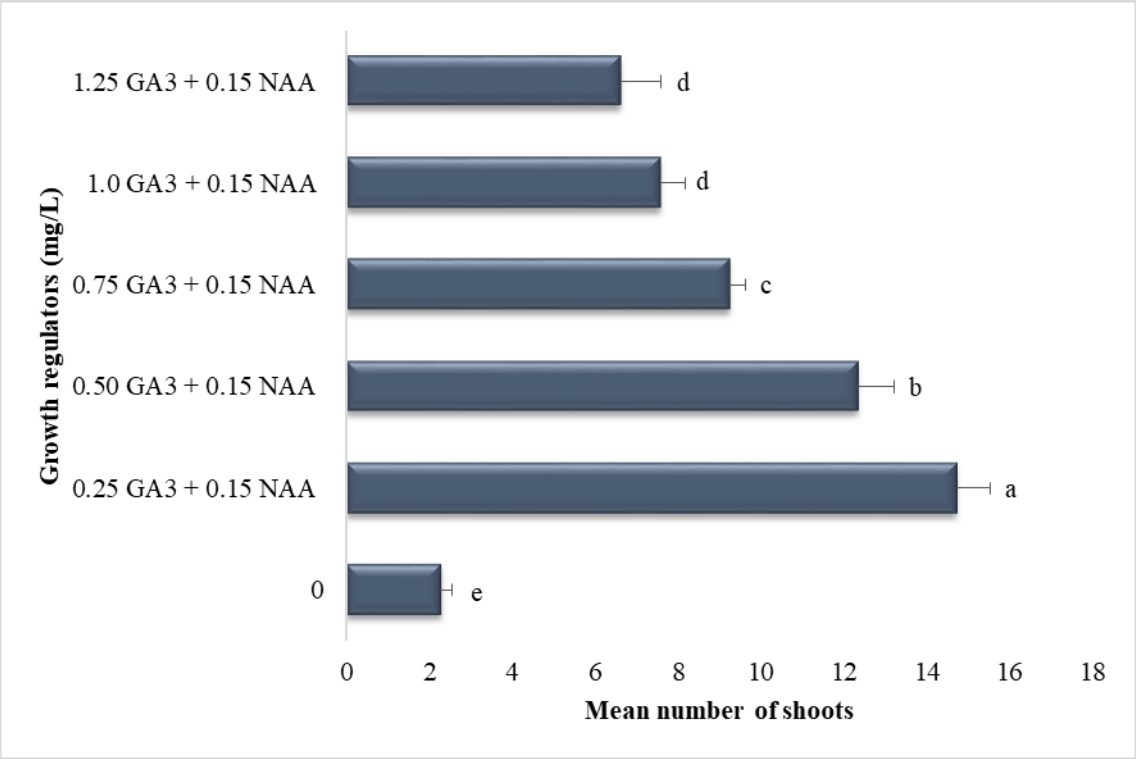

Figure 2. Impact of GA_3+NAA on mean number of shoots per explant. Vertical bars show standard deviation (n = 3). Various letters show significantly different values (DMRT, $p < 0.05$)

Statistically different results were found between shoot length values and GA_3+NAA levels ($p<0.05$) and were shown in Figure 4. The best result in shoot length was reached in a nutrient medium with 0.50 mg/L GA_3+0.15 mg/L NAA. Then the longest shoot was determined as 1.79 cm in MS medium with 0.25 mg/L GA_3+0.15 mg/L NAA. Longer shoots were obtained in GA_3+NAA nutrient media compared to the control group. The shortest shoot (utrient medium except for the control group) was recorded at 1.40 cm in MS plus 1.25 mg/L GA_3+0.15 mg/L NAA. More GA_3+NAA combinations negatively affected shoot lengths.

Figure 3. Axillary shoot regenerations in MS media including 0.25 mg/L GA_3 + 0.15 mg/L NAA

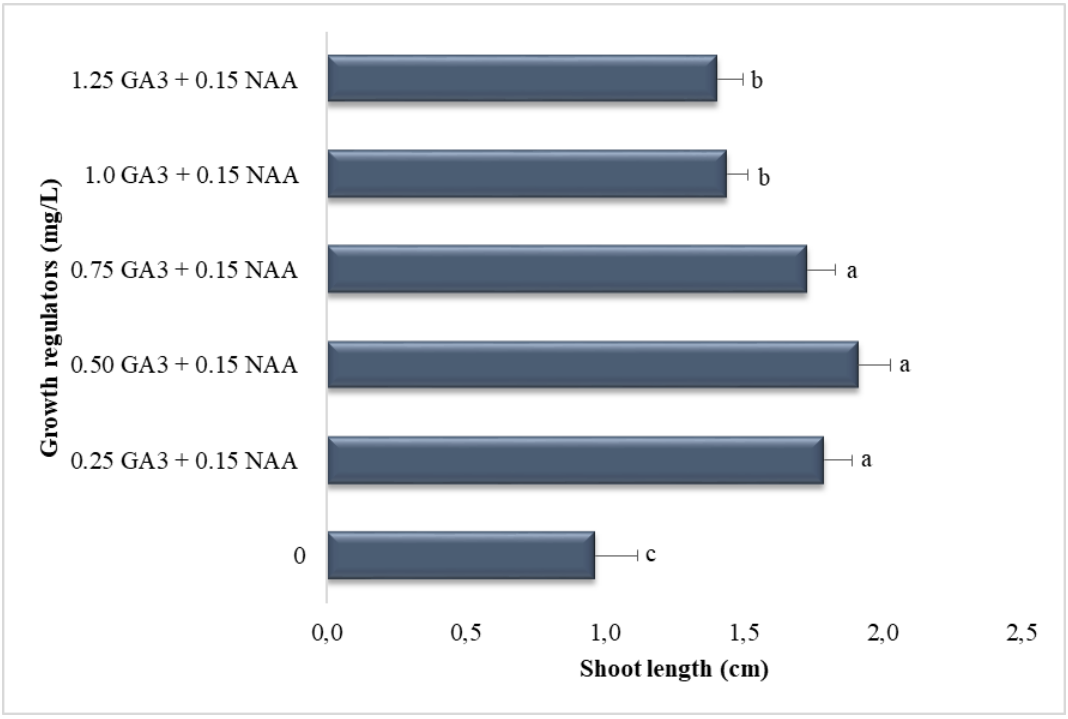

Figure 4. Impact of GA_3 + NAA on average shoot length. Vertical bars show standard deviation (n = 3). Various letters show significantly different values (DMRT, $p < 0.05$)

In order to root the regenerated shoots, nutrient medium with 0.25 mg/L IAA was prepared. The shoots cut to a length of about 2.5 cm were successfully rooted in this rooting medium. Then, *H. polysperma* plants were successfully acclimatized to aquarium conditions.

As a conclusion, the effects of various GA_3+NAA combinations on regeneration of *H. polysperma* were investigated and multiple shoots were successfully obtained. Significant advantages of culture media with GA_3+NAA compared to control have been demonstrated. The best results in shoot number were determined at 0.25 mg/L GA_3 + 0.15 mg/L NAA, while the best values for shoot length were obtained at 0.50 mg/L GA_3 + 0.15 mg/L NAA. The plants were rooted and acclimated to external conditions. The demand for this plant can be met thanks to multiple and rapid productions. Gene transfer studies can be done with this plant in the future.

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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