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Multiple shoot induction and plant regeneration of *Staurogyne repens* (Nees) Kuntze

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Abstract: *Staurogyne repens* (Nees) Kuntze is an aquatic plant belonging to Acanthaceae family. It is a valuable plant in the aquarium industry. Therefore, there has been a significant demand for this plant. This study was designed for multiple and efficient productions of *S. repens* under *in vitro* conditions. He used nodal meristems as the type of explant. Nodal meristems were preferred as explant type. The explants were transferred to Murashige and Skoog (MS) food media with TDZ single (0-1.50 mg/L) and TDZ (0.25-1.50 mg/L) + IAA (0.25 mg/L) combinations. In TDZ application, the most number of shoots per explant (15.36 shoots/explants) was recorded in cultures with 1.50 mg/L TDZ, while in TDZ + IAA application, the most number of shoots (9.44 shoots/explants) was determined in culture with 1.0 mg/L TDZ + 0.25 mg/L IAA. In general, shoot lengths in TDZ + IAA combination was measured higher than the single application of TDZ. The longest shoot (1.67 cm) was obtained in the culture medium with 1.0 mg/L TDZ + 0.25 mg/L IAA. The extended shoots were transposed to rooting media with 0.25 mg/L IAA, and multiple root formations were determined after four weeks. The rooted plants were transferred to the aquarium and successfully accustomed to *ex vitro* conditions.

Keywords: Acclimatization, propagation, shoot regeneration, tissue culture

1. Introduction

In vitro methods have been increasingly used since the 1960s for the development and production of plants. Plant tissue cultures studies have focused on cell biology, plant development, disease-free plant production, micropropagation and secondary metabolites (Gaspar et al. 1996; Efferth 2019). The propagation method applied to study cells, tissues and organs is a technique in which growth modifiers play an active role (Mahna et al. 2013; Pandey and Pandey 2018). Genetically engineered plants are obtained using tissue culture and techniques (Mineo 1990; El-Sherif 2018; Altman 2019).

Plant tissue culture is a system that allows the cultivation of a whole plant, organ, tissue or cells under aseptic conditions. This system provides all the nutrients, energy and water required for plant or explant growth in the nutrient medium. Besides, appropriate light and temperature settings are provided under controlled incubation conditions to promote growth. Plant growth can be manipulated by adding plant growth regulators (natural phytohormones or synthetic versions) (Gaikwad et al. 2017; Phillips and Garda 2019).

Plant hormones used in plant tissue culture have revolutionized production techniques. Because the balance between two or more hormones has been shown to reduce or increase the development of various organs in species (Hasancebi et al. 2011). Moreover, hormones may be applied to improve the production of phenolic compounds by not providing chemical stimulation in plants (Hasancebi et al. 2011). Effective breeding trials have been reported in many plant species, such as *Pistia stratiotes* L. (Aasim et al. 2013), *Ceratophyllum demersum* L. (Dogan 2019), *Campanula medium* (Gehl et al. 2020), *Nyctanthes arbor-tristis* L. (Kumar Mishra et al. 2020), *Vaccinium vitis-idaea* ssp. minus (Arigundam et al. 2020) and *Hemerocallis sp* (Matand et al. 2020) using growth regulators. In our current study, the efficacy of Thidiazuron (TDZ) and TDZ + Indole-3-acetic acid (IAA) on the *in vitro* micropropagation of *Staurogyne repens* (Nees) Kuntze, which is important in the aquarium industry. Thus, multiple productions of the plant in local sources can be achieved and the demand for plants in the domestic market can be met.

2. Materials and Method

These studies were carried out in the laboratories of the Department of Biology of Karamanoğlu Mehmetbey University. Surface sterilization of *S. repens* was achieved with an exposure of 5.5% Hydrogen peroxide (H_2O_2) for 20 minutes. Nodal meristems were used in reproduction studies.

The nodal explants were transposed to Murashige and Skoog (1962) (MS) food medium supplemented with TDZ single (0-1.50 mg/L) and TDZ (0.25-1.50 mg/L) + IAA (0.25 mg/L) combinations. In addition, 3% sucrose and 0.65% agar were added to the food medium.

The pH of the nutrient solution was made to 5.7 ± 1 through 1N NaOH and 1N HCl, and then it sterilized (1.2 atm and 120°C for 20 min). Plant parts were incubated under fluorescent lighting (16-hour and 24 °C). *In vitro* shoot regeneration trials were carried out for six weeks.

The shoots growing in the culture medium were cut (average 3 cm) and transposed to rooting medium with 0.25 mg/L IAA added. At the end of four weeks, the rooting trial was ended. Rooted shoots were then transposed to the aquarium environment to adapt to *ex vitro* conditions. Aquarium conditions were set at 24ºC and 16 hours of lighting.

The trials were carried out in six replicates in glass tubes. The analysis of the obtained data was carried out through the SPSS 21.0 statistical data package. Duncan (DMRT) was chosen from Post Hoc tests. Arcsin transformation was applied for per cent data (Snedecor and Cochran 1967).

3. Results and Discussion

3.1. The effects of different TDZ concentrations on in vitro propagation

In this trial, the nodal explants of *S. repens* were incubated *in vitro* food media with 0-1.50 mg/L TDZ for six weeks. Shoot development began to be observed three weeks later (Fig 1a). At the end of the six weeks, the experiment was terminated (Fig 1b) and shoot regeneration data were obtained and statistically analyzed (Table 1). Similarly, the successful cultivation of aquatic plants with tissue culture such as *Ipomoea aquatica* (Akaracharanya et al. 2001), *Spartina alterniflora* (Wang et al. 2003), *Bacopa monnieri* (Praveen et al. 2009) and *Limnophila aromatica* (Lamk.) Merr. (Dogan 2018) and have been reported in culture media with TDZ.

As can be seen in Table 1, the shoot regeneration rate was ranked between 77.77% and 100.00%. The use of high levels of TDZ reduced the degree of shoot regeneration. The number of shoots per explant was recorded between 2.42-15.36 shoots/explant. The maximum number of shoots per explant (15.36 shoots/explant) was determined in the MS nutrient medium containing 1.50 mg/L TDZ and then in the culture medium containing 1.25 mg/L TDZ (13.68 shoots/explant). The least number of shoots were obtained in the control group explants with 2.42 shoots/explant. The increase of TDZ in MS nutrient medium positively affected the number of shoots. Similarly, the lowering effect of high use of TDZ on the number of shoots has been previously reported by other researchers (Siwach and Gill 2011; Kapruwan et al. 2014; Dewir et al. 2018; Hesami and Daneshvar 2018). Kher et al. (2014) *Pluchea lanceolata* cultured in nodal explants in a nutrient medium containing 0.5-2.5 mg/dm^3 TDZ and obtained the number of shoots between 6.0±1.5 - 9.7±3.5 and found the highest number of shoots in 0.5 mg/dm^3 TDZ. *Paphiopedilum callosum*, *Paphiopedilum gratrixianum*, and *Paphiopedilum delenatii* were cultured in different TDZ-containing nutrients and a decrease in the number of shoots was reported with increasing TDZ concentration (Luan et al. 2019).

Shoot lengths were determined between 0.35-1.15 cm in the nodal explants (Table 1). The longest shoots were recorded in culture with 0.25 mg/L TDZ (1.15 cm) and then in control group explants with 1.02 cm. Short shoot lengths were seen in MS food media with 1.50 mg/L TDZ. The high use of TDZ had a negative effect on shoot length.

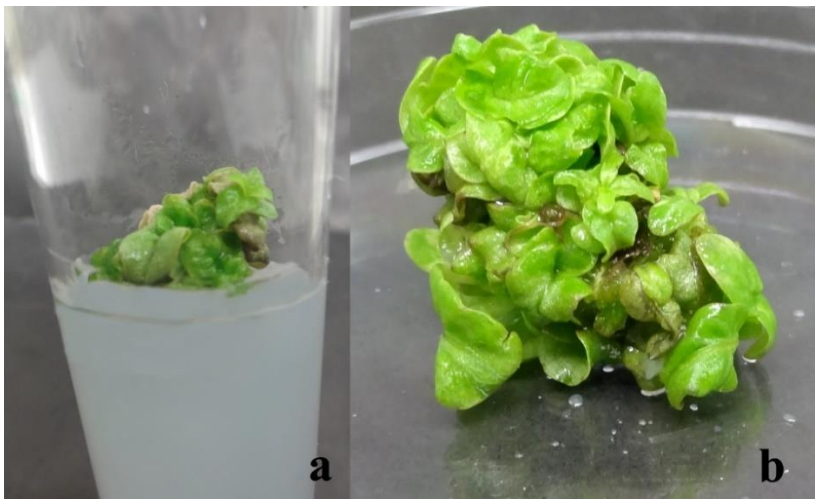

Fig. 1 Shoot regeneration of various TDZ concentrations from the nodal explants of *S. repens*. *In vitro* shoot regenerations from the nodal explants in culture with 1.50 mg/L TDZ after three weeks (a) and six weeks of culture (b).

Table 1 The influence of various TDZ doses on shoot regeneration in *S. repens*

TDZ (mg/L)	Regeneration Rate (%)	Number of shoots (shoots / explant)	Shoot length (cm)
0	77.77[a]	2.42[c]	1.02[ab]
0.25	100.00[a]	4.66[c]	1.15[a]
0.50	100.00[a]	5.11[c]	0.80[bc]
0.75	77.77[a]	6.15[cd]	0.65[cd]
1.00	83.33[a]	9.59[bc]	0.56[cd]
1.25	77.77[a]	13.68[ab]	0.39[d]
1.50	77.77[a]	15.36[a]	0.35[d]

Important differences in the same column were shown in letters. (p <0.05)

3.2. The effects of different TDZ+IAA concentrations on in vitro propagation

The nodal explants of *S. repens* were incubated in media with 0.25 mg/L IAA and 0.25-1.50 mg/L TDZ for shoot regeneration. At the end of six weeks, the trial was terminated (Fig 2) and statistical analysis was applied for hoot regeneration data (Table 2).

Fig. 2 Shoot regeneration of various TDZ+IAA combinations from the nodal explants of *S. repens*. *In vitro* shoot regenerations from the nodal explants in culture medium containing 1.0 mg/L TDZ + 0.25 mg/L IAA.

Shoot regeneration percentages in culture media containing TDZ + IAA ranged from 44.44% to 100.00%.

Maximum shoot regeneration percentage (100%) was obtained in culture medium with 1.0 mg/L TDZ + 0.25 mg/L IAA. Minimum shoot regeneration percentage (44.44%) was determined in 1.50 mg/L TDZ + 0.25 mg/L IAA application. The shoot counts were ranked between 4.42-9.44. More number of shoots was obtained as 9.44 shoots/explant in culture medium with 1.0 mg/L TDZ + 0.25 mg/L IAA. The minimum number of shoots were determined in MS nutrient medium with 1.50 mg/L BAP + 0.25 mg/L IAA. Cheruvathur et al. (2010) transferred the internodal explants of *Malaxis acuminata* D. Don in a culture medium with 1-4 mg/L TDZ + 0.5 mg/L NAA and detected maximum shoots per explant in a culture medium with 3.0 mg/L TDZ + 0.5 mg/L NAA.

Shoot lengths were recorded between 0.72-1.67 cm. The longest shoot (1.67 cm) was obtained in the culture medium with 1.0 mg/L TDZ + 0.25 mg/L IAA. However, the shortest shoots (0.72 cm) appeared in media with 0.25 mg/L TDZ + 0.25 mg/L IAA. Siddique et al. (2010) added TDZ + IAA in different combinations to culture media for *in vitro* production of *Cassia angustifolia* Vahl. and obtained the longest shoots in culture with 5.0 mg/L TDZ + 1.5 mg/L IAA. These results reveal that the lengths of shoots vary according to the amount of growth regulator used.

The extended shoots were transposed to the rooting environment with 0.25 mg/L IAA and multiple root formations were determined after four weeks. Rooted plants were transferred to aquariums to acclimate to *ex vitro* conditions. Elongations in plant lengths and leaves were determined within two weeks. After four weeks, they were successfully accustomed to water conditions.

Table 2. The influence of different combinations of TDZ + IAA on shoot regeneration in *S. repens*

Growth regulator (mg/L)		Regeneration Rate (%)	Number of shoots (shoots / explant)	Shoot length (cm)
TDZ	IAA			
0.25	**0.25**	88.88[a]	5.24[bc]	0.72[d]
0.50	**0.25**	83.33[a]	7.13[abc]	1.11[c]
0.75	**0.25**	88.88[a]	7.51[ab]	1.54[a]
1.00	**0.25**	100.00[a]	9.44[a]	1.67[a]
1.25	**0.25**	55.55[b]	6.58[bc]	1.49[ab]
1.50	**0.25**	44.44[b]	4.42[c]	1.17[bc]

Important differences in the same column were shown in letters. (p <0.05)

5. Conclusion

Tissue culture is an important technique for mass production of plants. Many commercial and medicinal plants are produced with this technique. In this study, multiple reproduction of *S. repens* was successfully achieved in MS nutrient medium with TDZ and TDZ + IAA. Generally, high usage of TDZ was found more useful for shoot regeneration. The single-use of TDZ according to the number of shoots was more effective than TDZ-IAA combination. However, longer shoots were obtained in TDZ-IAA applications compared to single-use of TDZ. These study results provide an important protocol for the multiple productions of *S. repens*.

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