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INFLUENCE OF DIFFERENT CONCENTRATIONS OF MURASHIGE AND SKOOG MEDIUM ON MULTIPLE SHOOT REGENERATION OF *Staurogyne repens* (Nees) Kuntze

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Abstract

Tissue culture applications help mass production for many plant species, especially horticultural plants, ornamental plants, medicinal and aromatic plants. Therefore, optimization of production techniques is very important. In this study, the effects of different Murashige and Skoog (MS) salt levels (25%-200%) on the *in vitro* production of *Staurogyne repens* (Nees) Kuntze were investigated. The shoot tip was used as explant. Different levels of MS salts significantly affected the regeneration abilities of shoot tip explants. The highest shoot regeneration rate was determined at 100% and 150% MS salt levels. The maximum regenerated shoots (9.13 shoots/explant) was determined in nutrient media supplemented with 150% MS salts. The minimum count of shoots (5.22 shoots/explant) was recorded in food media including 200% MS salts. Mean shoot lengths were between 1.65-2.27 cm. The highest length value (2.27 cm) was determined in the culture medium with 100% MS salts added, while the lowest length value (1.65 cm) was determined in the nutrient medium containing 25% MS salts. The shoots in the propagation medium were rooted in nutrient medium supplemented with 0.25 mg/L indole-3-acetic acid (IAA). Then they were successfully acclimatized to external conditions.

Keywords: Culture medium, *in vitro* propagation, shoot regeneration, tissue culture

1. Introduction

Plant tissue culture techniques enable the production of plants or herbal products in laboratories and under sterile conditions. The nutrient medium provides the necessary ingredients for the growth-development or regeneration of plants or plant parts. The propagation of plants can be increased with plant hormones added to the nutrient medium. In addition, the regeneration of plants is supported by making appropriate light and temperature settings [1]. Plant cells or

tissues have the potential to form a complete plant. So plant cells are totipotent. This totipotency feature is the basis of tissue culture studies [2].

The main nutrient media components in tissue culture are distilled/deionized water, sugar (primary carbon source), nutrient salts, vitamins and plant growth regulators. Murashige and Skoog (MS) basal salts [3] are generally used as a nutrient medium in plant tissue culture studies. MS salts contain macroelements and mycoelements [3]. Similarly, in vitro production of Cymbidium aloifolium (L.) Sw. [4] *Campomanesia phaea* [5] and *Broussonetia papyrifera* (L.) Hér. ex Vent [6] were done using MS salts. In addition, many ornamental plants such as *Passiflora edulis* [7], *Nelumbo nucifera* Gaertn [8] ve *Buxus sempervirens* L. [9] have been successfully produced using MS nutrient salts.

The mechanisms that trigger the development of a plant from a cell or tissue part depend on factors that vary according to the plant species, explant types, age, nutrient contents and environmental conditions [10]. Therefore, in the current work, the influences of various MS nutrient salts on in vitro multiple production of *S. repens* were investigated. Although tissue culture studies on *S. repens* are limited, they have generally been carried out on the functions of plant growth regulators on *in vitro* production [11-13]. In this context, the present study can contribute to the scientific literature. It can assist in the production optimization of commercially valuable *S. repens*. In addition, valuable bioactive compounds can be obtained at high levels by mass production of this plant.

2. Material and methods

All experimental studies were practised in the Department of Biology of Karamanoğlu Mehmetbey University. The plants were bought from the aquarium store. Surface sterilization of the plants was done according to Köse et al. [11]. Before starting the production studies, stocks were created from sterile and live shoots and the trials were carried out from the stock plants.

The shoot tip explants of the plant were used in the experiments. Nutrient media containing different Murashige and Skoog salts (MS - Duchefa) [3] were created. The addition of 4.4 g/L MS was determined as 100%. The ratios of MS salts used in nutrient media: 25% (1.1 g/L), 50% (2.2 g/L), 75% (3.3 g/L), 100% (4.4 g/L), 150% (6.6 g/L)) and 200% (8.8 g/L). In addition, 30 g/L sucrose (Duchefa) and 6.5 g/L agar (Duchefa) were supplemented to the MS nutrient medium. 0.50 mg/L Zeatin was added as a plant growth regulator.

The pH of the nutrient media was arranged to 5.7 ± 1 via NaOH (1 N) and HCl (1 N). Sterilization of the nutrient medium was achieved in an autoclave (1.2 atmospheric pressure for 20 min). The petri dishes with the shoot tip explants were placed under White LED light (1500 lux) (16 h light - 8 h dark). The temperature of the culture chamber was set to 24°C. Different MS applications were completed after six weeks.

For *in vitro* rooting, shoots were cut to 2.5-3 cm long. They were then planted on nutrient medium supplemented with 4.4 g/L MS + 3% sucrose + 0.65% agar containing 0.25 mg/L indole-3-acetic (IAA). At the end of the fourth week, the rooted plants were replanted to the aquatic environment (aquarium) to adjust to *ex vitro* conditions. About 3 cm of sand was added to the aquarium. The water environment temperature was arranged to 24ºC and 16 hours of illumination was used.

Experiments were performed in petri dishes in triplicate. SPSS for Windows versions 21 was used for statistical data. Parameter were estimated via One Way ANOVA. Duncan multiple range test (DMRT) was preferred for Post Hoc.

3. Results and discussion

The shoot tip explants of *S. repens* were transferred to medium containing different concentrations of MS salts for multiple shoot regeneration. Different levels of MS nutrient salts affected the shoot regeneration values of the plant. Similarly, it has been reported that different MS nutrient salts affect the growth-development or regeneration values of plants such as *Mentha spicata* L. [14], *Bacopa monnieri* (L.) [15] and *Lophophora williamsii* (Lem.) Coult. [16].

While the first shoot formations from the shoot tip explants were monitored in the food media with 75% MS salts, the latest shoot emergence was determined in the food media with 25% MS salts. After the sixth week, the experiments were completed. In general, the best results were observed in culture media supplemented with 75% MS salts (Figure 1). Data were obtained for regeneration values (%), shoots count, and shoot lengths. Data were statistically analyzed.

Shoot regeneration values are shown in Figure 2. The data were statistically analyzed and found significant at the $p<0.05$ level. The MS salt levels in the culture medium were found to affect shoot emergence from explants. Regeneration percentages ranged from 60% to 100%.The highest shoot regeneration rate was recorded in nutrient media containing 100% and 150% MS salts. However, the percentages of regeneration in nutrient media containing 50-150% MS salts were not statistically significant ($p>0.05$). These results showed that the use of MS salt in the culture medium at a rate of 50-150% did not statistically differ on the percentage of shoot emergence from the explants. On the other hand, the lowest regeneration rate was determined in the food media using 200% MS salts (60% regeneration), followed by the culture medium using 25% MS salts (73,33% regeneration). These results showed that the addition of high or low levels of MS nutrient salts weakened the shoot ability of the explants.

Figure 1. Regenerated *S. repens* shoots in culture medium containing 75% MS nutrient salts.

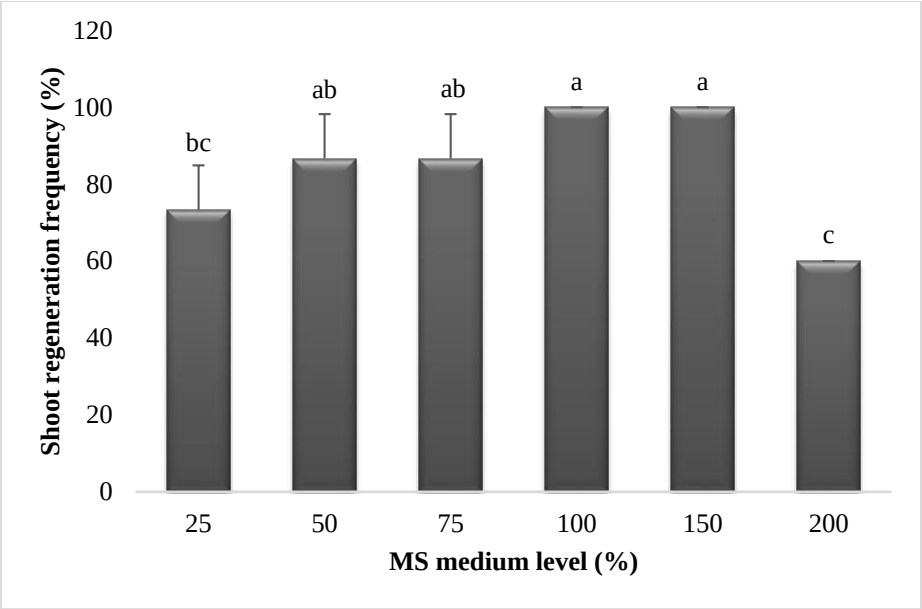

Figure 2. Influence of various MS salts on regeneration frequency of *S. repens*. All values are the mean of three replicates (n = 3). Vertical bars show standard errors. Different letters are statistically diverse (p<0.05; DMRT).

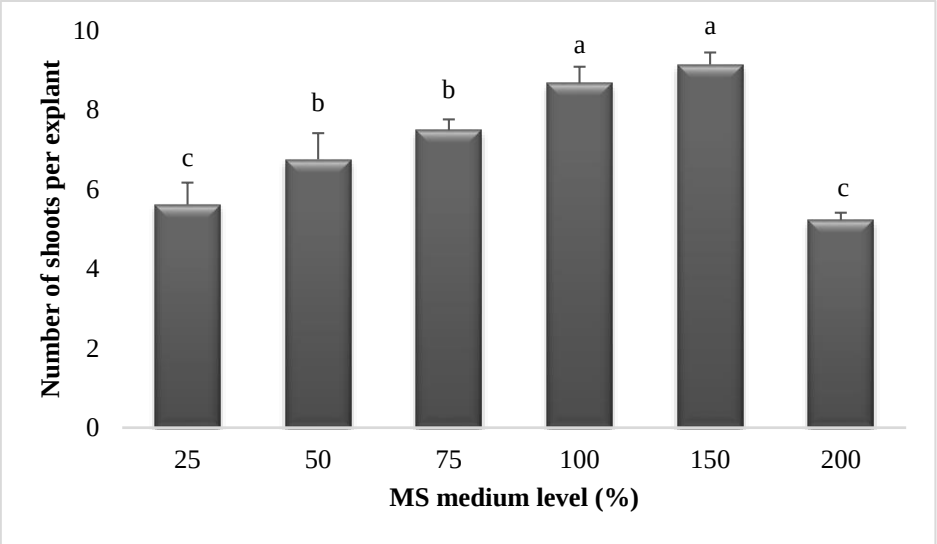

Figure 3. Influence of various MS salts on average number of shoots per explant of *S. repens*. All values are the mean of three replicates (n = 3). Vertical bars show standard errors. Different letters are statistically diverse (p<0.05; DMRT).

The effects of MS salts on shoot numbers per explant are shown in Figure 3. As can be seen, the values obtained were statistically significant at the 95% confidence interval. Different levels of MS salts significantly affected the number of shoots emerging from the explants. The number of shoots emerging from the shoot tip explants varied between 5.22 and 9.13 shoots/explant. Maximum regenerated shoots were recorded in nutrient media fortified with 150% MS salts (9.13 shoots/explant), followed by nutrient medium supplemented with 100% MS salts (8.67 shoots/explant). The shoot numbers in the nutrient media containing 100% and 150% MS salts

were found to be statistically insignificant. Also, there wasn't statistically significant variation in cultures containing 50% and 75% MS salts (p>0.05). The minimum shoot count (5.22 shoots/explant) was recorded in food media including 200% MS salts. These results revealed the negative effects of the use of high levels of MS salts on the number of shoots.

Fadel et al. [14] investigated the effect of different levels of MS nutrient salts (1/4, 1/2 and full MS) on the multiple production of *M. spicata*. In the culture medium, the highest value in terms of the number of leaves was recorded in the nutrient medium containing full MS. The highest shoot number was obtained in the culture medium containing 1/2 MS salts. Naik et al. [15] examined shoot regeneration values from leaf explants of *Bacopa monnieri* in culture medium containing different levels of MS salts (0.25, 0.50, 0.75, 1.0, 1.50 and 2.0) and compared the fresh and dry weights. The highest shoot number was obtained at 1.0 MS level. On the other hand, it determined the lowest shoot number at 1.5 MS level. They determined the maximum fresh and dry weight values in the medium containing 1.0 MS. Cortés-Olmos et al. [16] investigated the germination of *L. williamsii* in eight different media. Different MS (full and half-strength), sucrose (20 and 30 g/L) and agar (8 and 10 g/L) were used as nutrient media contents. The most suitable medium for germination was determined as half-strength MS + 20 g/L sucrose and 8 g/L agar. Ebrahimi [17] studied the effects of different growth regulators and two different levels of MS (1 and 1.2) on shoot regeneration of *Rosa foetida*. He reported that no statistically significant difference was detected at both MS levels in terms of shoot regeneration. Tetsumura et al. [18] reported that the decrease in MS level in the culture medium increased shoot formation in *Vaccinium corymbosum* and *Vaccinium virgatum*. All these results showed that the effects of different MS levels may vary according to the plant species.

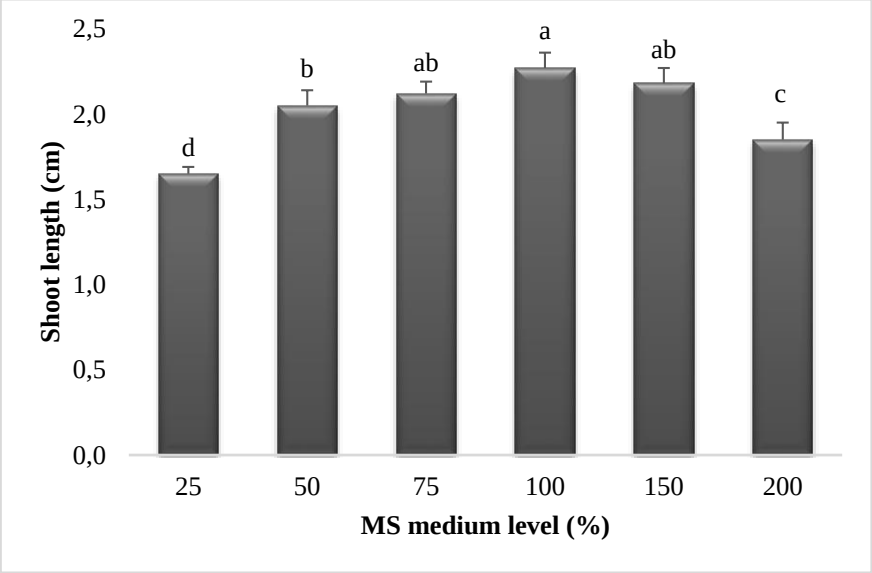

Figure 4. Influence of various MS salts on average shoots length of *S. repens*. All values are the mean of three replicates (n = 3). Vertical bars show standard errors. Different letters are statistically diverse (p<0.05; DMRT).

The average lengths of the regenerated shoots in the culture medium containing different MS salts are shown in Figure 4. The shoot length values changed with the MS levels and were obtained between 1.65-2.27 cm. Length data were statistically significant at the p<0.05 level. The highest length value (2.27 cm) was recorded in the culture medium with 100% MS salts added, while the lowest length value (1.65 cm) was determined in the nutrient medium containing 25% MS salts. When the average length of the shoots was examined, it was seen

that the use of MS salts between 75-150% did not affect the length results statistically ($p>0.05$). Addition of more or less MS salts of more than 100% to the nutrient medium affected the length of shoots negatively. *In vitro* multiplication of *M. spicata* was investigated in food media fortified with various levels of MS salts and the longest shoots were recorded at 1/2 MS level [14].

Regenerated shoots taken from culture medium were rooted in nutrient medium with 0.25 mg/L IAA. Then the nutrient medium on the plants was removed and they were transplanted to the water environment for acclimatization to *ex vitro* conditions. Within two weeks, improvements were observed in the leaves of *S. repens*. Acclimatization of *S. repens* to *ex vitro* conditions was achieved successfully in the third week.

4. Conclusion

The effects of different MS salts on multiproduction of *S. repens* were investigated. When examined in terms of shoot regeneration percentage and shoot number per explant, it was observed that the optimum MS level was 150%. The best results in terms of shoot length values were recorded in culture media containing 100% MS salts. It was determined that the addition of high or low levels of MS salts to the nutrient medium adversely affected the regeneration ability of the shoots. This study presents optimization of MS salts level for *in vitro* propagation of *S. repens*. These results can aid large-scale production of this plant.

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