RESEARCH ARTICLE

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The role of agar concentrations on multiple shoot regeneration of *Alternanthera reineckii* Briq.

Muhammet Dogan[*1]

[*1]*Department of Nutrition and Dietetics, Faculty of Health Sciences, Karamanoglu Mehmetbey University, 70200, Karaman, Turkey.*

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ABSTRACT: *Alternanthera reineckii* Briq. is an aquatic plant with economic value. In this study, the effects of different agar levels on the multiple shoot formation of *A. reineckii* were investigated. Shoot tip explants were used in the experiments. Agar was added to Murashige and Skoog (MS) nutrient medium in varying proportions (5-7.5 g/L). It was observed that different agar concentrations significantly affected the shoot regeneration ability of explants (p<0.05). In terms of shoot regeneration frequency and shoot length, the best agar concentration was determined as 5 g/L. In terms of average shoot number, the most effective agar level was determined as 6 g/L agar. The lowest shoot number was recorded in MS nutrient media with 7.5 g/L agar. As the agar doses in the culture medium increased, the shoot length decreased. The plants were rooted in MS nutrient medium fortified with 0.25 mg/L 1-naphthaleneacetic acid (NAA) and then successfully acclimatized to aquarium conditions. As a result, the optimum agar level was determined for shoot regeneration of *A. reineckii*.

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

INTRODUCTION

Plant tissue culture is a technique for culturing cells, tissues or organs in an aseptic environment with a defined chemical composition. In other words, it is the growth of isolated plant cells or tissue fragments under controlled environmental conditions in a sterile growth medium. Tissue culture is based on the ability of a plant cell to form a whole plant (totipotency) (El-Sherif, 2018; Loyola-Vargas et al., 2018: Phillips and Garda, 2019).

Tissue culture techniques allow the development and rapid reproduction of plants with desired characteristics in a short time, and also help the development of new varieties with genetic modifications (Al-Mizory, 2013). In recent years, important plants such as *Limnophila aromatica* (Lamk.) Merr. (Dogan, 2018), *Rheum emodi* Wall. ex Meisn (Singh and Chaturvedi, 2022) and *Humulus lupulus* (Hirakawa and Tanno, 2022) have been successfully produced using the tissue culture technique.

The success of plant tissue culture technology is significantly affected by the chemical composition of the culture media. (Zhang et al., 2015; Dogan, 2017; Dogan, 2019; Demétrio et al., 2021; Khan et al., 2021; Uysal, 2021). Generally, agar is added to increase the density of the nutrient medium so that explants (plant parts/tissues) remain on the surface of the nutrient medium (Debergh, 1983). However, it is known that the level of agar to be used in the nutrient medium may affect the regeneration ability of the plant (Suthar et al., 2011; Saklani et al., 2015; Lebedev et al., 2018). Therefore, in the present study, the effects of different agar concentrations on *in vitro* multiplication of economically valuable *Alternanthera reineckii* Briq. were investigated.

MATERIALS AND METHOD

A. reineckii was used as plant material in the experiments. The plant surface sterilization was previously performed by Uğur et al. (2019). Sterile plants are available sterile in the Department of Biology Laboratory of Karamanoglu Mehmetbey University. These sterile plants were used in propagation works.

Shoot tip explants transferred to Murashige and Skoog (1962) (MS) nutrient medium without plant growth regulators. Cabin conditions were set as the temperature at 24°C and lighting for 16 hours. Shoot tip explants of four-week-old plants grown in this MS media were transferred to MS medium supplemented with 5-7.5 g/L agar (Duchefa). 0.50 mg/L Zeatin + 0.25 mg/L 1-naphthaleneacetic acid (NAA) was preferred as a growth regulator. Trials were completed in the sixth week.

The pH of the medium was adjusted to 5.7±1, followed by sterilization at 1.2 atmospheres pressure (120°C and 20 min). Cultures were incubated under white fluorescent light at a temperature of 24°C and a 16-hour light photodiode.

The elongated shoots were cut to a length of approximately 2.5 cm and rooted in MS nutrient medium containing 0.25 mg/L NAA. After four weeks, rooted shoots were transferred to the aquarium to acclimate to external conditions (24ºC temperature and 16 hours lighting).

*Corresponding author : mtdogan1@gmail.com

Applications were carried out in three repetitions. SPSS 21 for Windows program was used for statistical calculations of shoot regeneration data. Values were considered significant at the 95% confidence interval ($p<0.05$). Data were analyzed by one-way ANOVA followed by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

In this study, the effects of different agar levels on the micropropagation of *A. reineckii* were investigated. Shoot tip explants were preferred in production studies. These explants are widely used in tissue culture studies and many researchers have successfully achieved shoot regeneration using shoot tip explants (Ghanbar et al., 2016; Dogan et al., 2016; Wada and Feyissa, 2021).

The first shoots in culture media were determined at the lowest agar level (5 mg/L). At the end of six weeks, the experiment was terminated and the data were recorded. It was determined that agar concentrations significantly affected the regeneration ability of the plant. The shoot regeneration frequencies of plants treated with different levels of agar were given in Figure 1. The data were statistically significant at the $p<0.05$ level. It was observed that the addition of a high level of agar reduced the shoot regeneration ability of the plant. While the highest regeneration value (100%) was obtained in MS medium with 5 g/L agar, the lowest regeneration value (50%) was determined in MS medium with 7.5 g/L agar. According to the results, the use of agar levels in the culture medium at 5-65 g/L levels did not affect the shoot regeneration percentages statistically significantly.

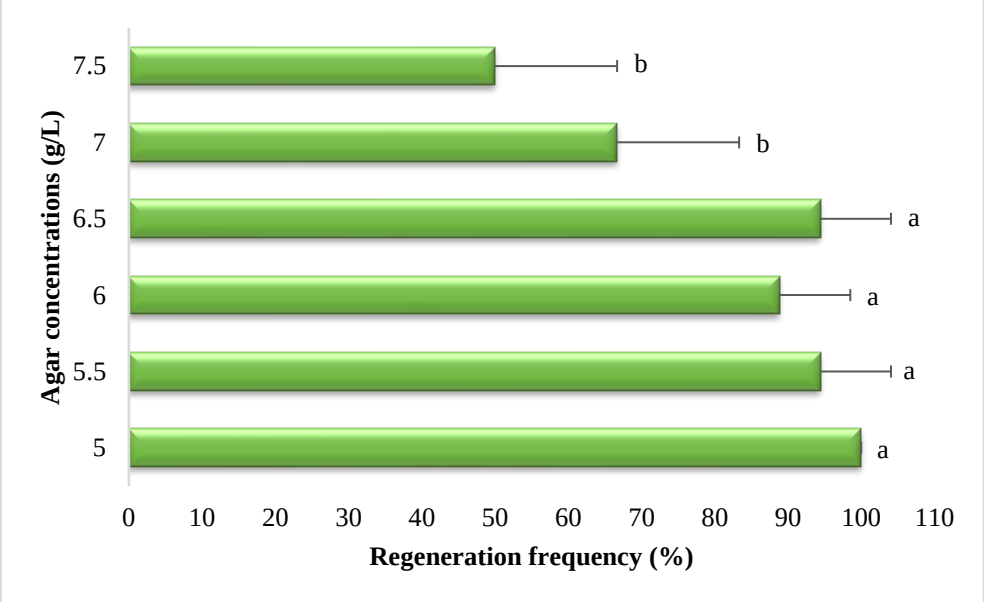

Figure 1. *The impacts of different agar concentrations on shoot formations in A. reineckii. Data show the average rates of three replicates (n = 3). Standard errors are given by bars. Different letters demonstrate statistical differences (p<0.05; DMRT).*

The effects of agar concentrations on the average shoot numbers were shown in Figure 2. Maximum shoots per explant (12.62 shoots/explants) were determined in the culture medium supplemented with 6 g/L agar (Figure 3), followed by the culture medium containing 5.5 g/L agar. In contrast, the minimum number of shoots was determined in MS nutrient media supplemented with 7.5 g/L agar (4.13 shoots/explants). The least number of shoots were detected in the culture medium containing the highest agar (7.5 g/L). It was reported that the use of agar amount more than normal levels inhibited shoot regeneration and growth and limited the ability of explants to use water in the medium (Selby and Harvey, 1989). Suthar et al (2011) conducted experiments for *in vitro* multiplication of *Boswellia serrata* Roxb. in a culture medium supplemented with different concentrations of agar (1-0.2%). They found that there was a positive correlation between the decrease in agar concentration and the average shoot number. The highest shoot number was obtained in the culture medium with 0.2% agar. Ghashghaie et al. (1991) investigated the *in vitro* propagation of rose (*Rose hybrida* cv. Madame G. Delbard) in culture medium with different concentrations of agar (0-15 g/L), and they obtained a large number of shoots in a culture medium with 7.5 g/L agar. Al-Mizory (2013) investigated the combined impacts of various carbon sources (sucrose, glucose, fructose and galactose) and agar concentrations (4-12 g/L) on shoot regeneration from nodal explants of *Dahlia sp*. In general, the most shoots in all carbon sources were obtained in MS nutrient medium with 8 g/L agar. When carbon sources and agar applications were compared together, more shoots were recorded in glucose + 8 mg/L agar application. Araujo et al. (2016) experimented with different nutrient media, agar concentrations (0-10.5 g/L) and pH levels for *in vitro* multiplication of *Myrciaria dubia* and determined the most effective medium for shoot regeneration in the culture medium supplemented with 7 g/L agar.

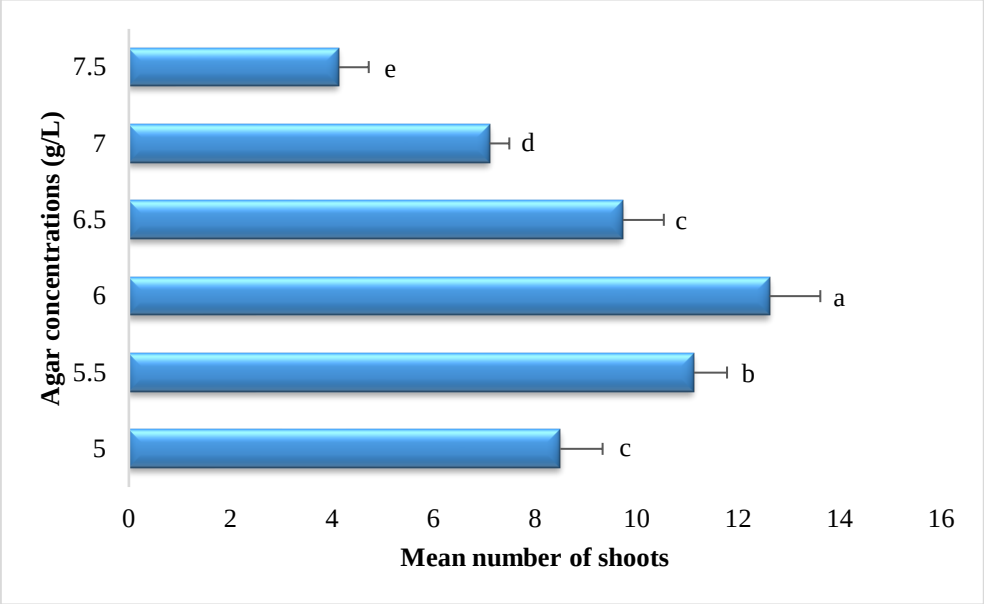

Figure 2. *The impacts of different agar concentrations on average number of shoots in A. reineckii. Data show the average rates of three replicates (n = 3). Standard errors are given by bars. Different letters demonstrate statistical differences ($p<0.05$; DMRT).*

Figure 3. *In vitro shoot regenerations from A. reineckii in culture medium containing 6 g/L agar*

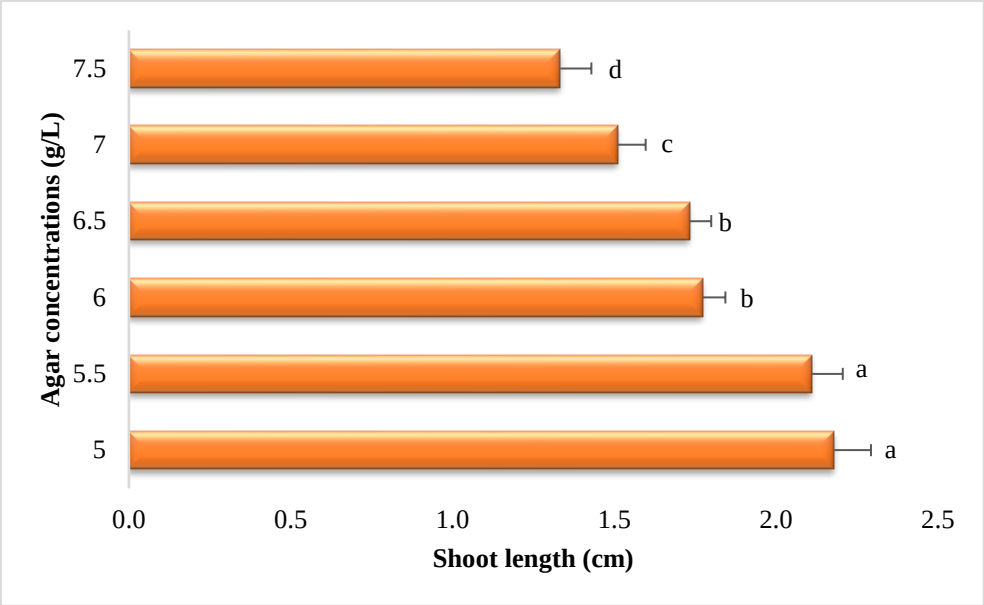

Figure 4. *The impacts of different agar concentrations on shoot lengths in A. reineckii. Data show the average rates of three replicates (n = 3). Standard errors are given by bars. Different letters demonstrate statistical differences (p<0.05; DMRT).*

The lengths of the shoots under the influence of different agar levels were given in Figure 4 and it was found to be statistically significant at the $p<0.05$ level. The results revealed that shoot lengths decreased with increasing agar levels. The longest shoots were obtained in culture medium supplemented with 5 g/L agar (2.18 cm), while the shortest shoots were determined in the culture medium supplemented with 7.5 g/L agar (1.33 cm). Similarly, Al-Mizory (2013) looked at the effect of agar and carbon sources on the *in vitro* growth of *Dahlia sp*. and determined the shortest shoots on MS medium with the highest agar added. In other words, the use of agar at a high level caused the shoots to be short. On *in vitro* micropropagation of *B. serrata*, solidified with agar and liquid nutrient mediums were compared and the longest shoots were determined in the liquid culture medium (Suthar et al., 2011). Among the reasons for this may be that the high agar hardens the nutrient medium and as a result, the explants do not benefit enough from the culture medium. The agar level in the culture medium should provide support to the explants, but it should be noted that very harsh nutrient media may prevent contact between the explant and the medium (Berrios et al., 1999).

As a result of the experiments, *A. reineckii* propagated in the culture media were rooted in MS nutrient medium containing 0.25 mg/L NAA and successfully acclimatized to the aquarium conditions.

CONCLUSION

In this study, *A. reineckii*, an important aquatic plant, was successfully grown *in vitro* on different agar levels. It was observed that different agar levels significantly affected the shoot regeneration ability of the plant. While the best agar concentration was determined as 6 g/L in terms of shoot number, the most effective medium for shoot length was determined as 5 g/L. This study is important in that it offers the optimum agar level for the *in vitro* propagation of *A. reineckii*.

CONFLICT OF INTEREST

No conflict of interest was declared by the author.

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