Research article
Curr. Pers. MAPs, (2021); 4(2): 103-107

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

The Effects of GA_3 Treatments and Nutrient Media on *In Vitro* Seed Germination of *Allium tuncelianum* (Kollman), Özhatay, Matthew, Şiraneci

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https://doi.org/10.38093/cupmap.1034719

Received: 09/12/2021
Accepted: 30/12/2021

Abstract

In this study, the effects of GA_3 and nutrient media (BDS and MS) on *in vitro* seed germination of *Allium tuncelianum* (Kollman), N. Özhatay, D. Matthew, Şiraneci, which is one of the endemic plants in Turkey, were investigated. In order to promote seed germination, GA_3 was applied to seeds under sterile conditions for 48 hours at different concentrations (2 mg L^{-1}, 3 mg L^{-1} and 4 mg L^{-1}). Then the seeds were cultured in BDS and MS medium. The results showed that the germination rate decreased with the increase of the applied concentrations of GA_3. The highest germination rate (17.73%) was obtained from seeds treated with 2 mg L^{-1} GA_3. It was determined that the effects of nutrient media on the germination of *Allium tuncelianum* seeds were important, and the highest germination values were obtained from the BDS medium.

Key Words: *Allium tuncelianum*, BDS, GA_3, Germination, MS

1. Introduction

Allium tuncelianum (Kollman), Özhatay, Matthew, Şiraneci, which is locally called as "Tunceli garlic" or "Ovacık garlic", is an endemic species unique to Turkey. It is widely found in especially in Ovacık and Pulümür districts of Tunceli province located at the foot of Munzur Mountain. *Allium tuncelianum* has single cloved, cream-white bulbs and also has the ability to flower and give fertile black seeds different from common garlic (Yanmaz and Ermiş, 2005; Yanmaz et al., 2010; Yarali and Yanmaz, 2016; Kizil et al., 2017; Yarali Karakan, 2019).

It has been reported that consumption of *Allium tuncelianum* stimulates the body's immune system, lowers blood sugar and cholesterol levels, improves blood circulation and thus reduces the risk of heart attack (Agbas et al., 2013; Atila et al., 2017; Yarali Karakan, 2019). In addition, Sehitoglu et al. (2018) stated that *Allium tuncelianum* has a stronger antioxidant, antiradical activity and effective essential omega acid levels than common garlic in terms of fatty acid compositions.

Allium tuncelianum is usually propagated by seeds and cloves. However, there is a problem of germination in the seeds due to dormancy that occurs after the seeds mature.

This situation makes it difficult to propagate the plant by seed (Alper, 2005; Kizil et al., 2017; Yarali Karakan, 2019). Therefore, extensive studies are needed to develop tissue culture techniques for *in vitro* propagation and seed germination of *Allium tuncelianum* (Kizil et al., 2014; Yarali Karakan, 2019). In this study, the effect of GA_3 as a germination stimulant and nutrient media were investigated *in vitro* conditions in order to ensure the propagation of *Allium tuncelianum* by seed.

2. Material and Methods

Allium tuncelianum seeds were kept in sodium hypochlorite solution (containing 5% NaOCl) for 20 minutes to ensure surface sterilization, and then washed three times for three minutes with distilled water (Kizil et al., 2017). In order to promote germination, different concentrations of GA_3 (2 mg L^{-1}, 3 mg L^{-1} and 4 mg L^{-1}) were applied to the seeds for 48 hours under sterile conditions. Then the seeds were cultured on BDS (Dunstan and Short, 1977) and MS (Murashige and Skoog, 1962) medium supplemented with 2.0% sucrose at 4 °C (Kizil et al., 2017), with 15 replications from each application and 15 seeds in each replication under *in vitro* conditions (Figure 1). 2 mm rootlets were accepted as germination criterion (Anonymous, 1985; Figure 2).

Figure 1. a) Sterilization of seeds, b) Filter sterilization of GA_3 solutions, c) Seeds treated at different GA_3 concentrations for 48 hours, d) Cultured seeds.

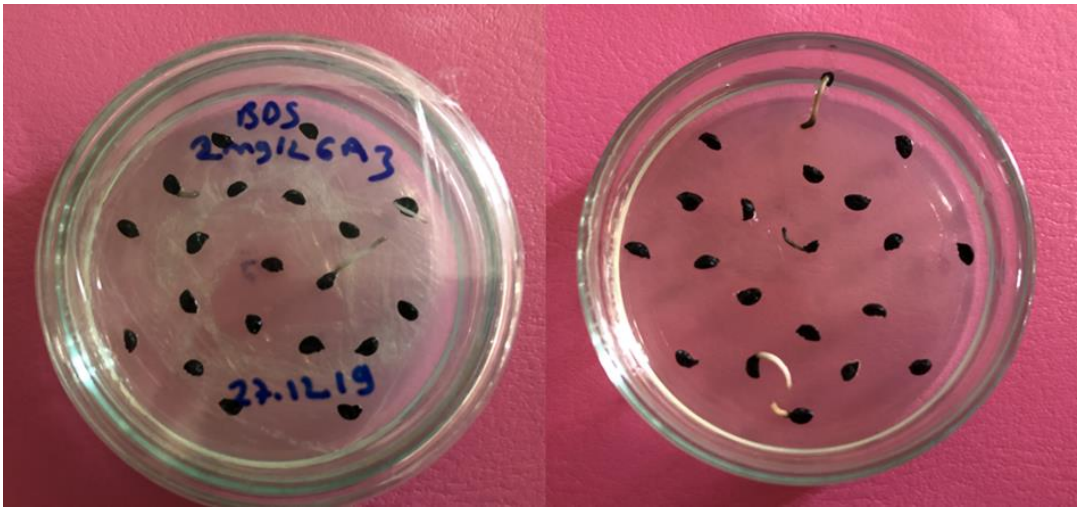

Figure 2. Germinated seeds in BDS medium treated with 2 mg L^{-1} GA_3

2.1. Statistical Analysis

The experiment was established according to completely randomized design (CRD) with 15 replications. Tukey test was used to identify different groups after analysis of variance.The level of statistical significance was taken as 5% and calculations were made with JMP software package version 5.0.1.

3. Results and Discussion

In this research, in order to reveal the effects of plant growth regulator application, the seeds were treated with gibberellic acid solution at different concentrations (2 mg L^{-1}, 3 mg L^{-1} and 4 mg L^{-1} GA_3) prepared under sterile conditions for 48 hours. Then, all seeds were cultured in BDS and MS medium at 4 °C. The effects of different concentrations of GA_3 applications and nutrient media on the germination of *Allium tuncelianum* seeds are shown in Table 1. When Table 1 was examined, it was determined that the seeds applied with 2 mg L^{-1} GA_3 had the highest germination rate of 17.73%. The germination rate decreased with the increase of the applied dose. Thus, the lowest germination rate was obtained from the application of 4 mg L^{-1} GA_3 in both nutrient media. It has been reported that storage at 4°C results in hydrolysis of seed starch resulting in mobility of carbohydrates in *Allium* species. Thus, macro-carbohydrate molecules are converted into sucrose, glucose and fructose, which are utilized during cell metabolism and energy required for the growth of plants. These have significant effects to break seed dormancy of garlic (Kizil et al., 2017). Similarly, it was reported that garlic seeds treated with low temperature at 4°C and gibberellic acid (GA_3) were helpful in seed dormancy break (Arguello et al. 2001). Yanmaz and Ermis (2005) used 0.5, 1.0, 2.0 mg L^{-1} doses of GA_3 for 24 and 48 hours in their study aiming to solve the germination problem in *Allium tuncelianum* seeds. They stated that GA_3 application for 48 hours was more effective on the germination and also it has been determined that 1.0 mg L^{-1} and 2.0 mg L^{-1} doses of GA_3 stimulate germination and increase the germination rate up to 18-20% depending on the low temperature period. In the study, it was determined that the effects of nutrient media on the germination of *Allium tuncelianum* seeds were also important. When BDS and MS media were compared, the highest germination values were obtained from BDS medium (Table 1). On the other hand, in their study to break dormancy in *Allium tuncelianum* seeds, Kizil et al., (2017) reported that it was higher in MS medium.

Table 1. Effects of GA_3 treatments and different nutrient media on germination of *Allium tuncelianum* seeds

GA_3 treatments	Nutrient media			
	BDS		MS	
	Germinated seeds (number/petri)	Germination rate (%)	Germinated seeds (number/petri)	Germination rate (%)
Control	0,80b	5,33	1,13 ab	7,53
2 mg L^{-1}	2,66a	17,73	1,33ab	0,86
3 mg L^{-1}	1,26ab	8,40	1,53ab	10,20
4 mg L^{-1}	0,40b	2,66	0,46b	3,06

Significant at P = 0.05 level

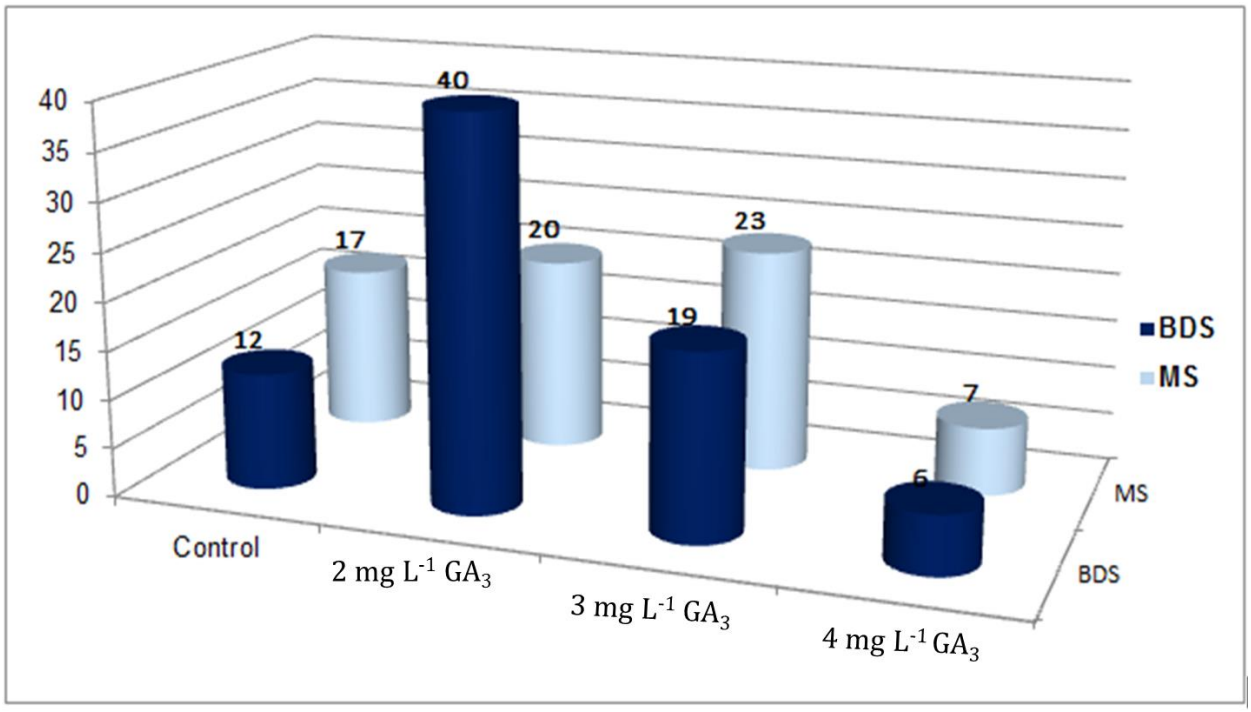

Figure 3. Number of germinated seeds in different GA_3 treatments and nutrient media.

4. Conclusion

The effects of GA_3 treatments and nutrient media on seed germination were found to be significant. The seed germination rate was higher at 2 mg L^{-1} GA_3 treatment than the other treatments. And also it was determined that the effects of nutrient media on the germination of *Allium tuncelianum* seeds were also important and the highest germination values were obtained from BDS medium. The results obtained from this research will contribute to future research for protection and propagation of *Allium tuncelianum.*

Author Contribution

Faika YARALI KARAKAN designed the experiments and wrote the paper.

Acknowledgements

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Conflicts of Interest

The author declares no conflict of interest.

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