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RESEARCH ARTICLE

A Study on the Germination of *Origanum acutidens* L. Seeds Subjected to Pre-Treatment of Gibberellic Acid and Colchicine

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ABSTRACT

In Türkiye, the general name for aromatic plant species belonging to the Lamiaceae family is "thyme". However, species containing thymol/carvacrol type essential oil are considered "thyme". *Origanum acutidens* is one of the thyme species that grows endemic in the Northeastern Anatolia Region of Türkiye. The low germination rate of its seeds is one of the factors limiting the studies conducted on this plant. This study was conducted to investigate the effects of different doses of colchicine and gibberellic acid on germination in *O. acutident* seeds. Seeds collected from the plant's natural habitat at the end of the flowering period were used as plant material. The experiment was planned as control (only distilled water) and treatments consisting of three different gibberellic acid (GA_3) [100 ppm (GA1), 200 ppm (GA2) and 300 ppm (GA3)] and four different colchicine doses [0.01 mM (C1), 0.02 mM (C2), 0.04 mM (C3) and 0.08 mM (C4)]. The applications were kept at 25±1 ºC for 12 hours. After the waiting period, all seeds were filtered and placed, 50 seeds each, in 9 cm diameter petri dishes between two layers of sterile filter paper sheets. The experiment was carried out in 4 replications. Some parameters of the germination (Germination rate (GR), Germination time (GT), Average germination time (AGT)) and early seedling period (Embryonal root length (ERL), Number of embryonal roots (NER), Root fresh weight (RFW), Root dry weight (RDW), Grass sheath length (GSH)) were measured and the results were statistically evaluated. In general, the highest values obtained from all evaluated germination (92.0% GR and 1.7 day AGT) and early seedling parameters (10.4 cm ERL, 4.6 NER, 0.095 g RFW, 0.028 g RDW and 3.6 cm GSL) were found to belong to the GA3 application. The lowest values obtained from the relevant parameters were obtained with the C4 application. In our study, it was observed that gibberellic acid applications significantly increased germination in this plant and positively increased the parameters related to germination. Based on the study results, we think that colchicine stimulates germination at certain rates, but causes death by having a toxic effect in increasing doses.

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1. Introduction

Origanum known as oregano and marjoram (Davis, 1982; Baser, 2002) belongs to the Lamiaceae family. There are nearly 900 species of origanum in the world. Türkiye is also the gene center of this genus, and 22 species and 4 subspecies of Origanum. These species are of great economic importance (Kizil et al., 2009). Türkiye makes a significant contribution to the oregano trade, which is approximately 60 million dollars in the world, with its annual production of 15 thousand tons (Sari & Altunkaya, 2015). The use of *Origanum* species has been known for tens of thousands of years (Tepe et al., 2016). *Origanum* species are widely used in alcoholic beverages, as culinary herbs and to flavor food products (Aligiannis et al., 2001; Cetin et al., 2011).

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Origanum acutidens, an important member of the *Origanum* species, grows endemically in Türkiye, in the North East Anatolian region (Karagoz, 2018). *O. acutidens* is used as herbal tea by the people in the region where it grows (Tumen et al., 1995). It is known that this plant has antimicrobial and insecticidal effects, antitumor effects and antioxidant effects due to its high content of carvacrol (Karagoz & Parlakova Karagoz, 2019). In addition, it has the potential to become an ornamental plant with its magnificent appearance (Karagoz et al., 2022). It has very beautiful flowers (with white to pale yellow or pink corollas) that bloom between June and August (Ietswaart, 1980; Davis, 1982).

Applications such as the stratification of seeds, classifying them according to their size, soaking them before sowing, etching them with acids, treating them with growth regulators, sowing them in gel form after germination, keeping them in nutrients or osmotic solutions, and coating are among the pre-treatments before sowing (Yamaguchi & Kamiya, 2002; Karakurt et al., 2010). Gibberellic acid is widely used as a priming method and/or the pre-treatment. Because gibberellic acid is effective in eliminating dormancy in seed production (Yamaguchi & Kamiya, 2002; Karakurt et al., 2010; Yıldız et al., 2017). Colchicine ($C_{22}H_{25}NO_6$) is an alkaloid found in the seed or bulb of *Colchicum autumnale*, belonging to the Liliaceae family (Kwon et al., 2016). Colchicine is used in biological research to promote polyploidy and in cultivation and as a positive control in tubulin binding assays (Trease & Evans, 1983). It interferes with the poleward movement of chromosomes and microtubule formation during the middle stage of cell division and induces polyploidization (Hadlaczky et al., 1983). Additionally, researchers have produced studies demonstrating the germination stimulating effects of colchicine (Bond, 1942; Pande & Khetmalas, 2012; Abd El-Latif et al., 2018; Lv et al., 2021).

As a result of our preliminary studies, we determined that the germination rate of *O. acutidens* seeds was very low (2.5-8%). An important factor limiting studies on this plant is its low seed germination rate. It was carried out to evaluate the effects of gibberalic acid and colchicine applications at different doses on the germination characteristics of wild *O. acutidens* seeds.

2. Materials and Methods

Seeds forming the plant material of the study were collected from Askale district of Erzurum province of Türkiye, which is located approximately 2170 m altitude (40°1'19" N, 40°38'28" E) in October (2022), at the end of the flowering period of *O. acutidens*. For the identification of plant *O. acutidens* was used as a main source "Flora of Turkey and the East Aegean Islands" (Davis, 1982). It was identified by Prof. Dr. Ramazan Çakmakçı. Before setting up the germination experiment, the seeds were surface sterilized with 20% sodium hypochlorite for 10 minutes. After, the seeds were passed through distilled water seven times and left to dry in sterile conditions (Yıldız et al., 2017).

In the research, three different gibberellic acid (GA_3) (100 (*GA1*), 200 (*GA2*) and 300 (*GA3*) ppm) and four different colchicine doses (0.01 mM (*C1*), 0.02 mM (*C2*), 0.04 mM (*C3*) and 0.08 mM (*C4*) Colchicine) and distilled water (Control) were applied to *O. acutidens* seeds. Experiments were carried out in 4 replications using a total of 32 petri dishes.

The sterilized and dried seeds were kept in GA (100, 200 and 300 ppm), colchicine (0.01, 0.02, 0.04 and 0.08 mM) and distilled water (H_2O) in dark conditions at 25±1 °C for 12 hours until the moisture content reached 12-13% (Mangena, 2021). After the waiting period, all seeds were filtered and placed, 50 seeds each, in 9 cm diameter petri dishes between two layers of sterile filter paper sheets (ISTA, 1996). A germination experiment was conducted based on the germination protocol of the *Origanum vulgare* species (ISTA, 1996) and the germination experiment was completed on the 21st day.

At the end of the experiment, the germination rate (GR) was determined by counting the seeds that germinated at the same time every day. When the radicle reached 1-5 mm, the seed was considered germinated and removed from the petri dish. At the end of 21 days, the GR, GT and AGT parameters were calculated through the equations below (Gosh et al., 2014; Nasırcılar et al., 2020). In addition, embryonal root length (ERL) was determined by measuring embryonal roots with a millimetric ruler. Number of embryonal roots (NER) was determined as the total number of embryonal roots formed in a plant. Root fresh weight (RFW) was the fresh weight of embryonal roots separated from the seed. Root dry weight (RDW) was determined by drying the roots, whose fresh root weight was determined, at 72 °C for 72 hours. Grass sheath length (GSL) was obtained by measuring the part of the plant between the seed and the tip of the grass sheath where the leaf emerges, with a millimetric ruler.

Germination rate (GR): (Total number of seeds germinated /50) x 100 (1)

Germination time (GT): n1/t1 + n2/t2 + ……. + nn/tn (2)

In the formula, n1, n2… n = number of seeds germinated on day counted from the beginning of the test.

t1, t2….. denote the number of days in which germination occurs (Ellis & Roberts, 1981; Nasırcılar et al., 2020).

Average germination time (AGT): Σ Dn / Σn (3)

In the formula, D = days counted from the beginning of the test, n = number of seeds germinated on day D (Nasırcılar et al., 2020).

2.1. Statistical Analysis

Results obtained from the end of the experiment were evaluated according to analysis of variance in the SPSS

statistical program (Statistical Package for Social Sciences, Version 22.0). Supplementary statistics and significance levels of the analysis results are given in tables. Duncan (p=0.05 or 0.01) test was performed to determine the significance of the difference between applications. Also online heatmapper software was used for heatmapper of all treatments.

3. Results

In our study, the highest value for the germination rate (GR), which was determined by dividing the number of germinated seeds by the total number of seeds allowed to germinate, was determined in the GA3 application (92.0%). The lowest value for the GR was obtained from the C4 (4.0%) application. The highest germination time *(*GT) was determined in the GA3 (1.7) application, while the lowest GT was obtained from the C4 (0.07) application. While the maximum embryonal root length (ERL) was determined in GA3 (10.4 cm) application, the minimum ERL value was detected in C4 (4.6 cm) application. In terms of the number of embryonal roots (NER) parameter, the highest value was found in the GA3 (4.6) application (Table 1).

Table 1. Results of germination and early seedling development parameters of *O. acutidens* seeds.

Level	GR (%)**	GT**	ERL (cm)**	NER (Number)**	RFW (g)**	RDW (g)**	GSL (cm)**	AGT (day)*
Control	8.0f	0.16f	5.5d	3.5e	0.05e	0.016e	2.5f	13.0bc
C1	10.0ef	0.19ef	5.6d	3.6d	0.04f	0.011g	2.8d	13.0bc
C2	16.0d	0.31d	5.9d	3.7c	0.06d	0.019c	2.9c	13.1bc
C3	12.0e	0.24e	5.4d	3.6d	0.038f	0.012f	2.4g	12.5c
C4	**4.0g**	**0.07g**	**4.6e**	**3.1f**	**0.02g**	**0.006h**	**1.6h**	**14.5 a**
GA1	38.0c	0.75c	7.2c	3.7c	0.065c	0.018d	2.7e	12.8bc
GA2	72.0b	1.31b	9.4b	4.2b	0.075b	0.023b	3.2b	13.8ab
GA3	**92.0a**	**1.7a**	**10.4a**	**4.6a**	**0.095a**	**0.028a**	**3.6a**	13.7ab
Mean	31.5	0.59	6.75	3.75	0.060	0.020	2.71	13.31

According to the Duncan test, the averages shown with the same letter are not important in their group (p<0.01) and (*p<0.05).

In terms of highest NER, GA2 (4.2) and GA1 (3.7) applications followed the GA3 (4.6) application, respectively. The highest values in the RFW and RDW parameters, which are parallel to each other, were determined in the GA3 application (0.095 g and 0.028 g, respectively). The highest GSL value was determined in the GA3 (3.6 cm) application. The lowest GSL value was determined in C4 (1.6 cm) application. The highest AGT was found in C4 application and G2 and G3 applications were in the same statistical group with the C4 application. The lowest AGT value was in the C3 application (Table 1).

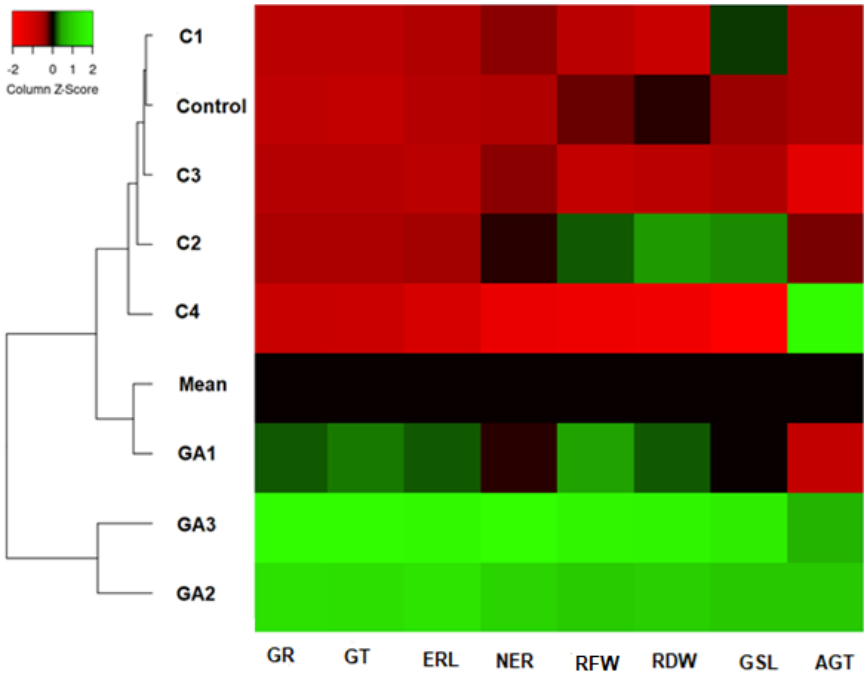

Figure 1. Heatmapper graph of applications, germination and early seedling development parameters.

Heat mapper analysis, which is an analysis method that classifies the averages of measured parameters by color, was created with our study data and the results are given in Figure 1. According to the heatmapper chart, red colors represent low values, green colors represent high values, and black color represents average values. According to this graph, while GA2 and GA3 applications have turned green and green tones in all parameters and it has been observed that these applications have the highest values. GA1 application received values close to the average and was in the same statistical group with the general mean. On the other hand, control and all colchicine applications received lower than average values, and it was observed that control and all colchicine applications were in the same statistical group (Figure 1).

When the correlation table showing the relationship between the germination and early seedling development parameters examined in the current study is evaluated, it can be stated that there is a positive relationship between all parameters except AGT (Table 2). There was a strong correlation (r=0.9995, p<0.01) between GT and GR (Table 2). There were highly significant (p<0.01) positive correlations with very strong values for the correlation of ERL with each of GR (r=0.9945) and GT (r=0.9946). There were highly significant (p<0.01) positive correlations with very strong values for the correlation of NER with each of GR (r=0.9411), GT (r=0.9416) and ERL (r=0.9575) (Table 2).

Table 2. Pearson's correlation coefficients among the germination and early seedling development parameters.

Traits	GR	GT	ERL	NER	RFW	RDW	GSL	AGT
GR	1							
GT	0.9995**	1						
ERL	0.9945**	0.9946**	1					
NER	0.9411**	0.9416**	0.9575**	1				
RFW	0.903**	0.9092**	0.9305**	0.943**	1			
RDW	0.8809**	0.8849**	0.91**	0.935**	0.9912**	1		
GSL	0.8011**	0.8047**	0.8485**	0.9387**	0.923**	0.9145**	1	
AGT	0.2744	0.2525	0.213	0.0328	-0.0131	-0.0101	-0.1727	1

*,** Significant at p<0.05 or 0.01, respectively.

Pearson's correlation analyses indicated that there were highly significant (p<0.01) positive correlations with very strong values for the correlation of RFW with each of GR (r=0.903), GT (r=0.9092), ERL (r=0.9305) and NER (r=0.943). RDW had highly significant positive correlations with GR (r=0.8809), GT (r=0.8849), ERL (r=0.91), NER (r=0.935) and RFW (r=0.9912). Also, there were highly significant (p<0.01) positive correlations with very strong values for the correlation of GSL with each of GR (r=0.8011), GT (r=0.8047), ERL (r=0.8485), NER (r=0.9387), RFW (r=0.923) and RDW (r=0.9145) (Table 2).

4. Discussion

In present study, it was determined that all applications except to C4 application were increased in values of all parameters except to AGT parameter. All doses of gibberellic acid applied in our study positively affected the germination and early seedling development characteristics of *O. acutidens* seeds. The highest values obtained for the parameters in the present study were generally determined at the highest gibberellic acid dose (GA3) application. Gibberellins are generally applied directly to the seeds and increase germination (Jacobsen et al., 2002). Also, gibberellins are used to eliminate dormancy. It promotes growth by increasing the plastids in the cell walls, converts carbohydrates into sugar and reduces the pressure on the cell wall. Thus, cell elongation occurs as water is taken into the cell (Arteca, 2013). İpek et al. (2008) reported that gibberellin application to seeds also promoted the production of some hydrolase enzymes, such as α-amylase. In another study, Yıldız et al. (2017) reported that the application of gibberellic acid increased the germination rate and germination-related parameters in *Dianthus barbatus* L. species under salt stress. In our study, it was observed that there was a significant increase in ERL, NER, RFW and RDW parameters with gibberellic acid application when compared to the control. The application of gibberellic acid positively affected peas (Okcu et al., 2005), pepper (Tepe et al., 2011), radish (Cavusoğlu & Kabar, 2007), carrots and spinach (Mufwanzala & Dikinya, 2010) seed germination. The aforementioned our results are consistent with the results of studies performed by Okcu et al. (2005), Cavusoğlu and Kabar (2007), Mufwanzala and Dikinya (2010), and Tepe et al. (2011).

In our study, colchicine applications have increased the parameters up to a certain point. According to some researchers tested different doses and durations of colchicine on the germinated seeds of different plants and increased the vegetative parameters by producing polyplioid plants (El-Nashar & Ammar, 2016; Sadat Noori et al., 2017; Khalili et al., 2020; Talei & Fotokian, 2020; Mo et al., 2020). The results we

obtained in our research, colchicine stimulates germination at certain rates, but colchicine causes death by having a toxic effect in increasing doses. Most seeds of bamboo (*Dendrocalamus brandisii)* could germinate normally after being inoculated in the basic Murashige and Skoog (1962) (MS) medium in culture tubes, supplemented with different concentrations of colchicine. It was observed that colchicine had no significant morphological effect on germination (Lv et al., 2021). Additionally, Lv et al. (2021) stated that some bamboo seeds showed abnormal germination phenomenon, particularly for high concentrations. Lv et al. (2021) determined that increasing colchicine dose increased the mortality rates, especially the highest mortality rate was 0.6%. At the same time as, they stated that seedling survival rates, germination rates and germination potential decreased constantly (Lv et al., 2021). It is known that the physiological level coupled with chromosomal damage causes a decrease in seed germination and an increase in mortality rate. Negativities caused by chemical mutagens may have caused deteriorations in the enzyme structure (Kulkarni, 2011). Previous studies (Bakry et al., 2007; Sourour et al., 2014) support the negative effect of high concentrations of colchicine on seed germination. The results of our study are parallel to Bakry et al. (2007), Grouh et al. (2011), Sourour et al. (2014), and Lv et al. (2021).

5. Conclusion

According to our findings, it was determined that GA2 and GA3 applications provided a significant increase in germination-related parameters. It was concluded that colchicine applications stimulated germination at a minimal level. It is thought that the application of gibberellic acid may be effective in solving the low germination problem in wild species such as *Origanum acutidents*. This study has identified some important new clues regarding the germination of *O. acutidents* seeds. In addition, it may be recommended to conduct detailed studies on both seed germination and colchicine doses and exposure times of *O. acutidens*.

Conflict of Interest

The author has no conflict of interest to declare.

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