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Research Article

Effect of Salt (NaCl) Stress on Germination in Clary Sage (*Salvia sclarea* L.) Subjected to Gibberellic Acid Pre-treatment

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Abstract: The research aimed to ascertain how adding GA_3 to *Salvia sclarea* L. (clary sage) seeds before germination affected the process of seed germination in saline environments. Four different concentrations of NaCl (0, 50, 100, and 150 mM) and four different concentrations of GA_3 (0, 50, 100, and 150 ppm) were used in the study. This research was conducted with four replications according to the Randomized Blocks Split Plots Trial Design. The following criteria were examined in the study: root length (cm), stem length (cm), germination strength and germination rate (%). This investigation revealed that higher salt concentrations had a detrimental impact on germination. It was determined that GA_3 application applied before germination significantly eliminated the inhibitory effect of salt stress on the germination of Clary Sage seeds. In addition, the highest values in the criteria discussed were found in the averages of 0 mM NaCl and GA_{150} applications.

Keywords: *Salvia sclarea* L., Gibberellic Acid (GA_3), Germination, Salt Stress.

1. Introduction

Sage has been an important medicinal plant since ancient times. *Salvia* L. species, a member of the Lamiaceae family, was first recorded by the Romans in B.C. 753- A.D. They started to use it medicinally between 476 and transferred the data they obtained to the British (Arıhan, 2003). Plants of the Lamiaceae family are widely distributed in the Americas, Africa, and Asia continents, and there are also many species in the Mediterranean Region (Özhatay, 2000). The Lamiaceae family often consists of fragrant and aromatic plants. Due to this feature, it is widely used in pharmacology and perfumery industries (Ceylan, 1987). *Salvia sclarea* is a widely distributed cosmopolitan species in Türkiye.

The germination, emergence, and early seedling stages are when plants are most vulnerable to environmental influences. These environmental elements are collectively referred to as stressors. These elements frequently have separate or combined effects on the plant (Anonymous, 2015). In cases where the salinity problem in the soil cannot be solved in the short term, the most basic approach to be followed is the determination of salt-tolerant species (Kara et al., 2011). Wild irrigation practices, especially in regions with inadequate pressurized irrigation, have caused this problem to grow. A decrease in root and stem development is brought on by salinity in the soil, which also has an adverse effect on transpiration, respiration, and water uptake in plants. Consequently, inadequate water intake, nitrate intake, hormone imbalance, and transpiration disorders arise (Leopold and Willing, 1984; Dölarslan and Gül, 2012). Another issue is the poisonous consequences and ion balance disturbance brought on by the Na^+ and Cl^- ions found in saline soils (Siegel et al., 1980; Flowers and Yeo, 1981; İnal et al., 1995). For this reason, in determining salt tolerance, the effects of the seeds of the varieties on germination criteria by keeping them in salty environments are considered (Begum et al., 1992). In salinity studies, the germination and seedling development periods of plants were mostly examined. The main reason for this is that the early germination and seedling periods of plants are the most sensitive period in terms of salinity resistance (Shannon, 1984). In addition, the salt tolerance threshold of each plant varies periodically (Shannon, 1985).

In weeds and cultivars, germination is a critical stage that ultimately determines population survival (Keller and Kollmann, 1999). Seed germination varies depending on plant species and varieties, as well as environmental factors such as water, temperature, oxygen, and light. In some cases, due to reasons arising from the structure of the seeds (internal and external), there are delays in the germination and emergence of the seeds. To eliminate this problem, pre-treatments (priming) are applied to the seeds before planting. The most common among these are potassium nitrate, hydrogen peroxide, thiourea, auxins, gibberellins, and cytokinins (Heydecker and Coolbear, 1977).

Gibberellic acid (GA_3) concentrations have been shown to have a major impact on germination (Duman, 2006). Studies have reported that the GA_3 hormone used in seed germination initiates germination by increasing α-amylase activity (Wurzburger and Leshem, 1974). In addition, it has been suggested that GA_3 (gibberellic acid), applied externally to seeds in germination studies, promotes germination by providing embryo growth by replacing environmental stimuli (light and temperature) in dormant seeds. As a result of the research, although it is known that GA_3 increases seed germination, it has also been reported that it may inhibit germination depending on the method and concentration used. For this reason, it was concluded that the appropriate concentration and method differences for each species were examined separately (Baskin and Baskin, 2014). The purpose of this study was to ascertain the impact of adding GA_3 to *Salvia sclarea* (clary sage) seeds before germination on saline seed germination.

2. Materials and Methods

This research was conducted in 2023 at Balıkesir University Altınoluk Vocational School Medicinal and Aromatic Plants Laboratory according to the Randomized Blocks Split Plots Trial Design with 4 replications. Plant seeds were obtained from BACEM (Balıkesir Farmer Training Center). The plant material used was the seeds of clary sage. Four distinct GA_3 concentrations (0, 50, 100, and 150 ppm) and four distinct salt concentrations (0, 50, 100, and 150 mM) were used in the research. Salt stress was produced using analytical grade NaCl (sodium chloride, Merck). Prior to germination, the seeds were surface sterilized for ten minutes in a 5% sodium hypochlorite solution (NaOCl, Sigma Aldrich) (Uyanık et al., 2014). For a whole day, seeds that had been surface sterilized were immersed in solutions varying in concentration of GA_3 (Nazarian, 2016). In petri plates with double-layer filter paper, 25 seeds were steeped in 5 ml of solution from each of the several salt concentrations independently. After these applications, the seeds in petri dishes were planted under 20±1 °C temperature conditions. Initial viability of seeds was determined according to ISTA (1996) rules. Germination tests were carried out for 14 days on 4 x 25 seeds in petri dishes between double-layered blotting papers (ISTA, 1996).

The values of germination power (%), germination rate (%), germination index (%), average germination time (cm), root length (cm), and stem length (cm) were investigated in this research. Germination power and germination rate were obtained by dividing the number of germinating seeds obtained on the 7th and 14th days to the total number of sown seeds (Akıncı and Çalışkan, 2010). The TARIST statistical program was used to do a statistical analysis on the research data. The averages were compared using the LSD test.

3. Results

3.1. Effect of Gibberellic Acid on Germination Power

According to studying’s findings this relationship is examined at the 5 % significiant level. These findings include the effects of NaCl doses on the capacity of Clary Sage seeds to germinate in solutions to containing varying concentrations of GA3, the variation in GA3 doses, and the relationship between NaCl and GA_3 (Table 1).

The germination power was found to be best at 94.00 % at 0 mM NaCl and lowest at 73.50 % at 150 mM NaCl when the average concentrations of NaCl were analyzed. Upon analyzing the mean GA_3 dosages, it was discovered that GA150 had the maximum germination power, measuring 86.38 %, while GA0 had the lowest potency, measuring 80.38 %.

The analysis of the NaCl x GA_3 interaction revealed that the 0 mM NaCl x GA_{150} interaction had the maximum germination power, 96.00 % conversely, the 150 mM x GA_{50} and 150 mM x GA_0 interaction had the lowest germination power, 71.00 %.

Table 1. Effect of gibberellic acid on germination power (%).

NaCl / GA_3	**GA_0**	**GA_{50}**	**GA_{100}**	**GA_{150}**	**Means**
0 mM	92.00	94.00	94.00	96.00	94.00 a
50 mM	82.50	86.00	88.00	90.50	86.75 b
100 mM	76.00	77.50	78.00	81.00	78.13 c
150 mM	71.00	71.00	74.00	78.00	73.50 d
Means	80.38 c	82.13 b	83.50 b	86.38 a	

LSD_{NaCl}: 0,128; LSD_{AsA}:0,235; $LSD_{Int.}$: 0,159

* The difference between means marked with the same letter is insignificant (5%).

3.2. Effect of Gibberellic Acid on Germination Rate

It is investigated at the 5% significant level, as indicated by the research findings about the effects of NaCl doses on the germination rate of Clary Sage seeds in NaCl solution at different concentrations, the variation in GA3 doses, and the NaCl x GA_3 interaction (Table 2).

Table 2. Effect of gibberellic acid on germination rate (%).

NaCl / GA_3	**GA_0**	**GA_{50}**	**GA_{100}**	**GA_{150}**	**Means**
0 mM	73.50	76.00	85.25	86.50	80.32 a
50 mM	60.00	65.00	68.00	76.50	67.38 b
100 mM	58.00	58.00	59.00	65.50	60.13 c
150 mM	51.00	54.50	55.50	58.50	54.88 d
Means	60.63 d	63.38 c	66.94 b	71.75 a	

LSD_{NaCl}: 0,128; LSD_{AsA}:0,235; $LSD_{Int.}$: 0,159

*The difference between means marked with the same letter is insignificant (5%).

After examining the average NaCl concentrations, it was shown that the germination rate was highest at 0 mM NaCl (80.32 %) and lowest at 150 mM NaCl (54.88 %). Based on an analysis of the average GA_3 doses, GA_{150} had the highest germination percentage (71.75 %), while GA_0 had the lowest germination rate (60.63 %). Upon analyzing the NaCl x GA3 interaction, it was found that the germination rate in the 0 mM NaCl x GA GA_{150} 150 interaction was 86.50 %, while in the 0 mM NaCl x GA_{100} interaction it was 85.25 %. The germination rate in the 150 mM x GA_0 interaction was 51.00 %.

3.3. Effect of Gibberellic Acid on Root Length

It is investigated at the 5 % significant level, as indicated by the research findings about the effects of NaCl doses on the root length of Clary Sage seeds in NaCl solution at different concentrations, the variation in GA3 doses, and the NaCl x GA_3 interaction (Table 3).

Table 3. Effect of gibberellic acid on root length (cm).

NaCl / GA_3	GA_0	GA_{50}	GA_{100}	GA_{150}	Means
0 mM	0.10	1.60	1.80	4.90	2.10 a
50 mM	0.30	0.80	1.50	4.70	1.83 b
100 mM	0.10	1.00	1.30	3.10	1.38 d
150 mM	0.50	1.30	1.50	2.60	1.48 c
Means	0.25 d	1.18 c	1.53 b	3.83 a	

LSD_{NaCl} : 0,128; LSD $_{AsA}$:0,235; LSD $_{Int.}$: 0,159

* The difference between means marked with the same letter is insignificant (5%).

The longest root measured 2.10 cm in 0 mM NaCl and the shortest 1.38 cm in 100 mM NaCl when the average NaCl concentrations were examined. Upon analyzing the mean GA3 dosages, it was shown that GA_{150} had the longest roots (3.83 cm), while GA_0 had the shortest ones (0.25 cm). Furthermore, the longest root length measured in the NaCl x GA_3 interaction was 4.90 cm in the 0 mM NaCl x GA_{150} interaction, whereas the shortest root length measured in the NaCl x GA_0 and 100 mM x GA_{100} interaction was 0.10 cm.

3.4. Effect of Gibberellic Acid on Stem Length

The results of the study show that the NaCl doses of Clary Sage seeds in various concentrations of NaCl solution in relation to stem length, the variation in GA_3 doses, and the NaCl x GA_3 interaction are investigated at the 5 % significance level (Table 4). The longest stem measured 1.45 cm in 0 mM NaCl and the shortest 0.93 cm in 150 mM NaCl when the average NaCl values are examined.

Table 4. Effect of gibberellic acid on stem length (cm).

NaCl / GA_3	GA_0	GA_{50}	GA_{100}	GA_{150}	Means
0 mM	0.60	1.50	1.70	2.10	1.45 a
50 mM	0.30	1.30	1.50	1.80	1.23 b
100 mM	0.40	1.10	1.20	1.70	1.10 c
150 mM	0.10	1.20	1.10	1.30	0.93 d
Means	0.35 d	1.28 c	1.38 b	1.78 a	

LSD_{NaCl} : 0,128; LSD $_{AsA}$:0,235; LSD $_{Int.}$: 0,159

* The difference between means marked with the same letter is insignificant (5%).

The average GA3 dose was analyzed, and the results showed that GA_{150} had the longest stem (1.78 cm), while GA_0 had the shortest stem (0.35 cm). The largest stem length in the 0 mM NaCl x GA_{150} interaction was 2.10 cm, and the lowest stem length in the 150 mM x GA_0 interaction was 0.10 cm, according to the analysis of the NaCl x GA3 interaction.

4. Discussion

Previous study showed that that gibberellic acid (GA3) concentrations have a significant effect on germination (Duman, 2006). Another study reported that the GA3 hormone used in seed germination initiates germination by increasing α-amylase activity (Wurzburger and Leshem, 1974). In this study, it is found that as gibberellic acid dosage increased, germination power and germination rate values increased in contrast to decreasing with increasing salt concentrations.

Studies in the literature (Okçu et al., 2005; Çavuşoğlu and Kabar, 2007; Yaver and Paşa, 2009; Azizi et al., 2011; Tepe et al., 2011; Haiping et al., 2012; Yıldız et al., 2017; Paşa, 2022) are also in parallel with the findings. It has been found that root length reduces at low salt concentrations, and that seeds' capacity to absorb water is reduced in plants subjected to high salt concentrations because of strong osmotic pressure (Kızılgeçi et al., 2010). In sunflower plants, GA3 increases seedling length (Erdemli and Kaya, 2015). It has been reported that 150 mg-l GA3 concentration in corn plants increases plant height (Akter et al. 2014). GA3 is more effective in increasing cell length than other hormones; In their research on triticale plants, they found that increased GA3 application increased stem length (Altuner et al., 2019). The findings of this study are consistent with previous research.

5. Conclusions

As a result of this research, it was determined that GA3 application in Clary Sage seeds had a positive effect on increasing salt concentrations. It was determined that high salt concentrations during the germination period of the plant limited growth in all criteria considered, and a regression in growth was observed due to high salt slowing down water uptake and enzymatic activations in plants. According to the research results, the highest values in the criteria were determined in the averages of 0 mM NaCl and GA_{150} applications.

Conflicts of Interests
Authors declare that there is no conflict of interests

Financial Disclosure
Author declare no financial support.

Statement contribution of the authors
This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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