

## **EFFECT OF GIBBERELIC ACID ON GERMINATION AND SEEDLING GROWTH OF BARLEY UNDER NaCl-INDUCED SALINITY**

 Onur Okumuş<sup>1\*</sup>,  Neslihan Doruk Kahraman<sup>2</sup>

<sup>1</sup> *University of Erciyes, Faculty of Agriculture, Department of Field Crops, Kayseri, Türkiye*

<sup>2</sup> *University of Selcuk, Faculty of Agriculture, Department of Field Crops, Konya, Türkiye*

*\*Corresponding Author:*

[okumus@erciyes.edu.tr](mailto:okumus@erciyes.edu.tr)

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**ABSTRACT.** Barley is a widely cultivated grain in the world. Barley is widely used in both human and animal nutrition. Uniform germination is one of the critical factors affecting yield. Salinity is one of the most common abiotic stress factors in the world. In recent years, various seed coating processes have provided more uniform germination of seeds. This study investigated the response of different doses of gibberellic acid applications to salt stress. Germination percentage, shoot, root length, and wet and dry weight data were analyzed. The study used two widely used barley varieties (Anka 06 and Cirit). Control, 5 and 10 EC were used as salt dose, and control, 100 and 200 ppm were used as gibberellic acid. As a result of the study, gibberellic acid positively affected germination data in both varieties at increasing salt doses.

**Keywords:** *Abiotic stress, germination, Hordeum vulgare*

### **INTRODUCTION**

Barley (*Hordeum vulgare*), one of the most widely cultivated cereals, plays a crucial role in global agriculture, particularly in regions where abiotic stress conditions such as salinity are common. Salt stress, mainly due to sodium chloride (NaCl), impairs seed germination and seedling growth in barley, reducing crop productivity [1, 2]. As a glycophytic plant, barley is sensitive to high salinity, which affects osmotic balance, ion toxicity, and nutrient uptake, ultimately disrupting critical physiological and biochemical processes during germination [3,4]. Therefore, improving the germination and growth of barley under saline conditions is a crucial research area aimed at sustaining crop yields in salt-affected soils [5,6].

Germination is a critical stage in the plant life cycle, and its success determines seedling establishment and subsequent crop yield. Under salt stress, high concentrations of Na<sup>+</sup> and Cl<sup>-</sup> ions inhibit water uptake by seeds, leading to osmotic stress that delays or prevents germination. Moreover, ion toxicity can damage cellular structures, affecting vital enzymes involved in metabolism and energy production [7]. Barley, a relatively salt-tolerant crop compared to other cereals, has shown variability in its response to salinity during the germination stage [8]. This highlights the importance of exploring strategies to mitigate the adverse effects of salt stress on germination.

In this scenario, the exogenous application of phytohormones and homeostasis modulators can be used to mitigate the effects of various abiotic stresses on plants [9]. For example, the external application of gibberellic acid (GA3) has shown the ability to alleviate some adverse impacts of environmental stresses, resulting in enhanced growth, improved photosynthetic efficiency, increased enzyme activity, altered gene expression, higher nutrient uptake, and greater yield in various crops facing challenging conditions. Additionally, GA3 interacts with

other plant growth regulators, influencing various metabolic processes in plants [10,11]. The protective effects of GA3 can be triggered through different application methods, including foliar spraying [12,13], substrate supplementation [14], and seed priming [15]. Gibberellic acid (GA3) is a key phytohormone essential in enhancing cereal crops' resistance mechanisms to various environmental stresses. Particularly under common abiotic stress conditions such as drought, salinity, and cold, the application of GA3 triggers numerous physiological changes that support growth and development in cereals. GA3 is effective in critical processes such as maintaining water balance, promoting root development, enhancing photosynthetic capacity, and optimizing nutrient uptake [13]. It modulates enzyme activity and gene expression under stress conditions, strengthening the plant's defense responses. Therefore, the exogenous application of GA3 stands out as an effective strategy for reducing yield losses and developing more resilient cereal crops [12].

Gibberellic acid (GA3), a plant growth regulator, has been extensively studied for its role in promoting seed germination by stimulating the synthesis of hydrolytic enzymes such as  $\alpha$ -amylase, which break down stored starch reserves to provide energy for the growing seedling [16]. In saline environments, applying gibberellic acid has been reported to counteract the inhibitory effects of salt stress by enhancing seedling vigor and improving germination rates [17]. GA3 promotes the synthesis of enzymes involved in cell wall loosening and expansion, which aids in radicle emergence, even under suboptimal conditions such as high salinity [18].

While previous studies have shown promising results with the exogenous application of gibberellic acid in improving seed germination and seedling growth in crops exposed to salinity, including wheat and maize, further research is urgently needed. These studies suggest that gibberellic acid could be a potential solution to alleviate salt stress in barley, particularly during the germination phase [19]. However, more research is needed to fully understand the mechanisms by which GA3 interacts with NaCl-induced stress in barley and to determine optimal concentrations of GA3 for improving germination under varying salinity levels. This research is crucial for the future of barley production in salt-affected soils.

In this study, we evaluated the effect of NaCl-induced salinity on barley seed germination and the potential of gibberellic acid application to reduce the negative effects of salt stress. The results of this research are not just informative, but they also contribute significantly to our understanding of how GA3 enhances seed germination under saline conditions. This practical information will be invaluable in improving barley production in salt-affected soils, making this research particularly important and engaging for the scientific community.

## MATERIALS AND METHODS

The study used Anka 06 and Cirit barley cultivars as plant material. NaCl (Merck, Germany) was used for salt stress. Salt levels were adjusted as control, 5 EC, and 10 EC. The study was carried out under controlled conditions at 25°C.

The seeds to be used in the study were sterilised with 1% sodium hypochlorite for 5 minutes and then rinsed 3 times with pure water. The seeds were sown in 25 replicates between 3 filter papers and sealed with a ziplock bag to prevent moisture loss. For each filter paper, 7 mL of solution was added. Seeds were considered germinated when the root ( $\geq 2$  mm) emerged and germinated seeds were counted for 14 days. At the end of the 14th day, germination percentage (number of germinated seeds/25 x 100) was calculated, and shoot and root length, wet weight, and dry weight data were analyzed in 10 randomly selected seedlings.

### ***Seed pretreatment procedures:***

The study used 100 and 200 ppm GA solutions for seed pretreatment.

#### *Preparation of GA (100 ppm):*

To prepare 100 ppm GA3, 1g GA tablet was dissolved in 1 lt water, and 100 ml of this solution was taken with a measuring cylinder and added to 1 lt water.

#### *Preparation of GA (200 ppm):*

To prepare 200 ppm GA3, 1g GA tablet was dissolved in 1 lt water, and 200 ml of this solution was taken with a measuring cylinder and added to 1 lt water.

The experiment treated pretreated seeds with different doses of GA (100, 200 ppm) [20] for 4 hours, and untreated seeds were used as control.

### ***Statistical Analysis***

The research was established as a factorial trial design in random plots with 3 replications. The data obtained from the research were analyzed on the computer with the 'JMP 13.2.0 program according to the factorial trial design in random plots. Treatment means were compared using the Tukey Multiple Comparison Test [21].

## **RESULTS AND DISCUSSION**

Giberelic acid is widely preferred to reduce the negative effect of salt stress on the initial growth and development of plants. [22]. As a result of the study, different doses of GA3 and NaCl doses in barley varieties were subjected to variance analysis. The results of the variance analysis are given in Table 1.

The increase in salt dose negatively affected the seedling quality in all traits considered in the study. Earth's most important abiotic stress factor is salinity [6,23,24]. Excessive fertilization, unconscious irrigation, and faulty agricultural practices increase soil salinity [25]. Salinity negatively affects plant early development and prevents a uniform emergence [26]. With the increase of salt in the soil, plant roots are damaged, preventing them from utilizing the water in the soil. In recent years, seed coating with various chemical and biological methods has become widespread [27]. The priming process is widely preferred to get away from various stresses in the first development period of the seed or to break dormancy [28, 29]. GA has been widely evaluated in priming processes across multiple field crops [30].

When the germination percentage value was examined as a result of the study, the highest germination percentage was obtained in 100 ppm GA application in the control salt application of the Cirit variety, while the lowest germination rate was obtained in the control GA application in 10 EC salt application in Anka 06 variety. It was revealed that 100 and 200 ppm GA application decreased the salt stress at increasing salt doses in the Anka 06 and Cirit varieties. At the highest salt dose, the 70% germination rate in the Anka 06 variety increased to 77% with 100 ppm GA application. In Cirit variety, 71.5% germination rate increased to 77% with 100 ppm GA application (Table 1).

**Table 1.** Analysis of variance and tukey grouping

Cultivar	NaCl	GA3	Germination Percent	Shoot Length	Root Length	Fresh Weight	Dry Weight
Anka 06	Control	Control	94.67 ab	7.24 fgh	6.36 bcd	3.25 b	0.47 ab
		100 ppm	95.33 ab	8.23 de	7.47 a	4.43 a	0.63 a
		200 ppm	91.33 bc	8.00 ef	6.80 abc	4.90 a	0.70 a
	5 EC	Control	78.67 fgh	6.54 h	5.73 abc	2.68 cd	0.39 cde
		100 ppm	86.00 de	7.10 gh	6.77 abc	3.08 bc	0.44 bc
		200 ppm	81.33 f	7.80 efg	6.23 cd	3.00 bc	0.43 bcd
	10 EC	Control	70.00 k	4.03 j	2.80 i	1.65 f	0.24 f
		100 ppm	76.67 ghi	4.80 ij	3.10 hi	1.83 ef	0.26 ef
		200 ppm	74.66 hij	5.10 i	3.76 gh	2.00 ef	0.28 def
Cirit	Control	Control	92.00 ab	9.10 bc	7.40 a	4.40 a	0.63 a
		100 ppm	96.00 a	10.77 a	7.43 a	4.84 a	0.69 a
		200 ppm	91.33 bc	9.74 b	7.23 ab	4.82 a	0.69 a
	5 EC	Control	82.66 ef	8.87 cd	6.23 cd	2.96 bc	0.42 bcd
		100 ppm	87.33 cd	9.40 bc	6.50 bcd	3.09 b	0.44 ab
		200 ppm	80.00 fg	8.23 de	6.17 cd	3.22 bc	0.46 bcd
	10 EC	Control	71.50 jk	5.10 i	4.40 fg	2.09 ef	0.30 ef
		100 ppm	76.60 ghi	5.40 i	5.23 ef	2.10 ef	0.30 ef
		200 ppm	73.00 ijk	4.97 i	5.06 ef	2.21 de	0.32 d
	Cultivar	nsg	**	**	**	**	
	NaCl	*	**	**	**	**	
	GA3	*	**	**	**	**	
	Cultivar x NaCl	nsg	**	**	*	*	
	Cultivar x GA3	nsg	**	*	**	**	
	NaCl x GA3	**	**	*	**	**	
	Cultivar x NaCl x GA3	**	**	*	**	**	

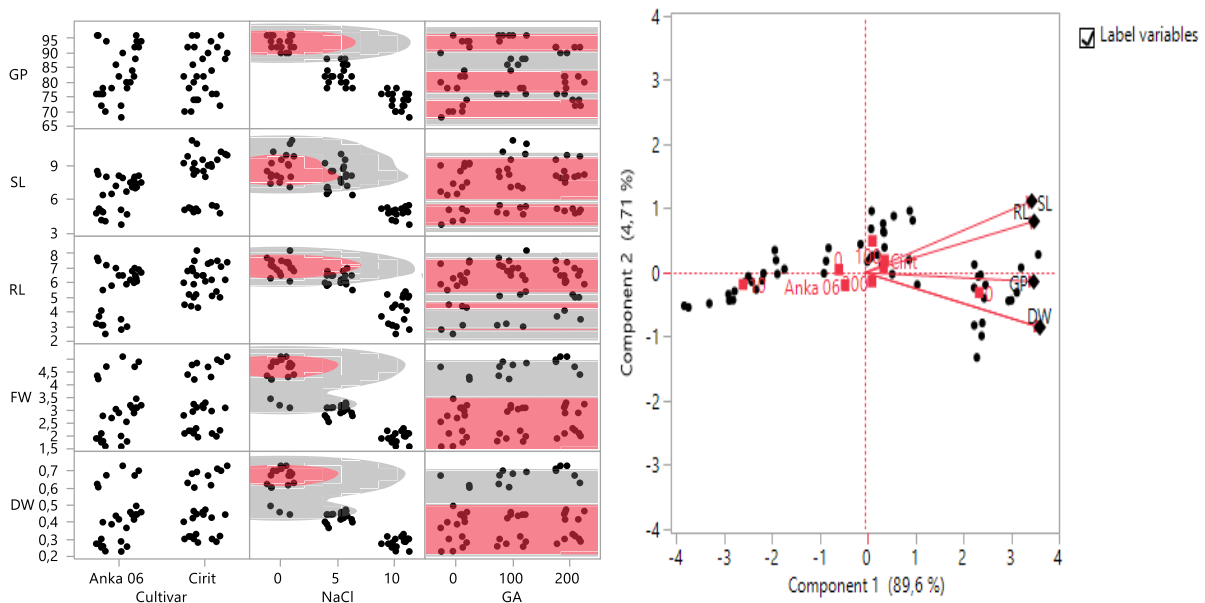
\*  $p < 0.5$ , \*\*  $p < 0.01$ , nsg: non significant

When other studies were examined, 12% germination rate was determined in control GA application at 225 mM salt dose in oat plant and 28% germination rate was determined in 480 mM GA3 application, and GA3 was effective in increasing salt doses [31]. When the results of the research in which similar findings were obtained with our study were examined; it was reported that increasing gibberellic acid doses caused an effect on germination properties in the opposite direction of salt [32]. [33], in their study to determine the germination conditions of seeds, the highest germination percentage (99%) was obtained from 100 ppm GA3 dose, while the lowest germination percentage (69%) was obtained from seeds treated with pure water.

When the shoot length value was examined, the highest shoot length was obtained in 100 ppm GA application in the control salt application of Cirit variety, while the lowest shoot length was obtained in the control GA application in 10 EC salt application in Anka 06 variety. At the highest salt dose, the 4.03 cm shoot length of the Anka 06 variety increased to 5.10 cm with 200 ppm GA application. In Cirit variety, 5.10 cm shoot length increased to 5.40 cm with 100 ppm GA application (Table 1). When other studies were analyzed, [34] reported that GA increased the shoot length at increasing salt doses in the study they carried out in Bülbül 89 variety in barley plant in 2007. When the root length value was examined, the highest root length was obtained in 100 ppm GA application in the control salt application of Cirit variety, while the lowest shoot length was obtained in the control GA application in 10 EC salt application in Anka 06 variety. At the highest salt dose, shoot length of 2.80 cm in Anka 06 variety increased to 3.76 cm with 200 ppm GA application. In Cirit variety, 4.40 cm shoot length increased to 5.23 cm with 100 ppm GA application (Table 1). Their study, conducted in

2020 [32], reported that GA increased root length at increasing salt doses. In the study in which they examined the root lengths at different GA3 doses in *Verbascum linearilobum* species, they reported that increasing GA doses affected root length [35].

When the wet weight value was analyzed, the highest wet weight was obtained in control, 100 and 200 ppm GA application in control salt application of Cirit variety, while the lowest wet weight was obtained in control GA application in 10 EC salt application in Anka 06 variety. At the highest salt dose, the seedling wet weight of 1.65 mg in the Anka 06 variety increased to 2.00 mg with 200 ppm GA application. In the Cirit variety, the wet weight of 2.09 mg seedlings increased to 2.21 mg with 200 ppm GA application (Table 1). When the dry weight value was analyzed, the highest dry weight was obtained in control, 100 and 200 ppm GA application in the control salt application of Cirit variety. The lowest wet weight was obtained in control GA in 10 EC salt applications in the Anka 06 variety. At the highest salt dose, 0.24 mg seedling dry weight of Anka 06 variety increased to 0.28 mg with 200 ppm GA application. In Cirit variety, 0.30 mg seedling dry weight increased to 0.32 mg with 200 ppm GA application (Table 1). It was determined that the first developmental stages of plants were inhibited by increasing salt concentrations [36, 37]. Other studies found that increasing gibberellic acid doses positively affected seedling growth despite salt's suppressive effect on growth parameters [32].



*Fig 1. Biplot and scarp lot analysis*

The PCA graph, which evaluates the interaction of variety, salt, and GA3 treatments on the germination parameters of barley seeds, also supports the findings obtained. While germination rates vary according to variety, germination parameters are positively affected under conditions where increasing salt and GA3 dose treatments are applied together (Fig 1).

## CONCLUSION

In this study, we evaluated the effect of NaCl-induced salinity on barley seed germination and the potential of gibberellic acid application to reduce the adverse effects of salt stress. The results of this research are not just informative, but they also contribute significantly to our understanding of how GA3 enhances seed germination under saline conditions. This practical

information will be invaluable in improving barley production in salt-affected soils, making this research particularly important and engaging for the scientific community.

This study demonstrated that gibberellic acid (GA3) is a potent promoter of uniform and robust germination in barley seeds, even under saline conditions. The findings show that both Anka 06 and Cirit barley varieties exhibited improved germination percentages, shoot length, root length, and biomass when treated with 100 and 200 ppm GA3 despite increasing salt concentrations. Notably, in the Anka 06 variety, the germination rate increased from 70% to 77% with 100 ppm GA3 application, even at the highest salt dose. Similarly, in the Cirit variety, the germination rate rose from 71.5% to 77% under the same conditions. These results underscore the potential of GA3 in mitigating the negative effects of salt stress, particularly in barley, which is sensitive to salinity. Thus, GA3 is a promising solution for enhancing seed germination and early growth stages in saline soils. This enhanced conclusion better emphasizes the findings' significance and suggests future research directions, adding depth to the study's practical applications.

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