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# Physiological and Molecular Effects of Exogenous Gibberellin (GA3) Treatment on Germination of Barley Seeds under Salt Stress

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## Abstract

Salinity is considered as one of the most important factors restricting germination parameters including rate and percentage of seed germination in crops. This study focused on the alleviating role of exogenously applied 10 mg/L gibberellic acid (GA3) on barley (*Hordeum vulgare* L.) seeds during germination under salt stress (120 mM NaCl). Physiological and morphological changes, and differential gene expression at 3 days after imbibition (DAI) were determined and compared with or without gibberellic acid (GA3) under salt stress. Exogenous GA3 was found to increase the shoot and root length of germinated barley seeds under salt stress by 67 and 15%, respectively, compared to those treated with salinity alone. On the other hand, exogenous GA3 treatment significantly reduced ion leakage, osmolyte accumulation, and proline content under salinity. NaCl was found to decrease the expression of the *HvABI5, HvABA7* and *HvKO1* by 3, 10, and 33 fold, respectively, at 3 DAI, whereas addition of GA3 in root medium rescued the expression of these genes to control levels. Besides, exogenous GA3 significantly decreased mRNA level of *HvGA2ox4* under salinity during germination. This study may give insight into the relationship between salinity stress and the genes involved in GA3 metabolism and germination.

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***Keywords:*** Barley; Germination; GA3; Salinity; Proline; *HvKO1; HvGA2ox4*.

**Eksojen Gibberellin (GA3) Uygulamasının Tuz Stresi Altındaki Arpa Tohumlarının Çimlenmesine Fizyolojik ve Moleküler Etkileri**

**Öz**

Tuzluluk, bitkilerde tohum çimlenme oranı ve yüzdesi gibi çimlenme parametrelerini kısıtlayan en önemli faktörlerden biri olarak kabul edilmektedir. Bu çalışma, tuz stresi (120 mM NaCl) altında çimlendirilen arpa (*Hordeum vulgare* L.) tohumlarına eksojen olarak uygulanan 10 mg/L giberellik asidin (GA3) stresi hafifletici rolüne odaklanmıştır. İmbibisyondan 3 gün sonra tuz stresi altında giberellik asit (GA3) ile veya onsuz gerçekleşen fizyolojik ve morfolojik değişiklikler ve farklılaşmış gen anlatımı belirlendi ve karşılaştırıldı. Eksojen GA3'ün, tuz stresi altında filizlenmiş arpa tohumlarının sürgün ve kök uzunluğunu, yalnızca tuz ile muamele edilenlere kıyasla sırasıyla %67 ve %15 arttırdığı bulundu. Öte yandan, eksojen GA3 uygulaması tuzluluk altında iyon sızıntısını, ozmolit birikimini ve prolin içeriğini anlamlı şekilde azaltmıştır. NaCl'nin imbibisyondan 3 gün sonra *HvABI5, HvABA7* ve *HvKO1* genlerinin anlatımını sırasıyla 3, 10 ve 33 kat azalttığı bulunurken, kök besiyerine GA3 eklenmesinin bu genlerin anlatım seviyelerini kontrol grubunun seviyesine çıkarmıştır. Ayrıca, eksojen GA3 tuz stresi altında çimlenen örneklerde *HvGA2ox4*'ün mRNA seviyesini anlamlı derecede azaltmıştır. Bu çalışma, tuzluluk stresi ile GA3 metabolizması ve çimlenme ile ilgili genler arasındaki ilişki hakkında fikir verebilir.

***Anahtar Kelimeler:*** Arpa; Çimlenme; GA3; Tuzluluk; Prolin; *HvKO1; HvGA2ox4*.

## 1. Introduction

Salinity is regarded as one of the most important stress factors lowering crop productivity by preventing or delay seed germination as well as seedling establishment in crops [1]. Salt stress exhibits adverse effects on plants by accumulation of $Na^+$ and $Cl^-$ causing an ionic imbalance and toxicity, and osmotically reducing water uptake in arid and semi-arid regions [2]. Salt stress causes detrimental physiological and biochemical changes including certain enzymatic or hormonal activities, protein mechanism, and gene expression profile in germinating seeds; therefore, led to delay or preventing seed germination [3, 4]. Seed germination and early seedling stages are the most sensitive to salinity during plant life-cycle [5]. In plants, salt-stress responsive genes can be classified into three groups, which are the genes involved in sensing and signalling of the stress, transcriptional regulators such as *DRF1*, and salt-stress related genes including *LOX, MT2,* and *BAS1* [6, 7].

Seed germination is a critical stage in establishing of a plant, and controlled by a number of factors comprising plant hormones such as gibberellins (GAs) and abscisic acid (ABA). GAs and ABA are the main endogenous factors, which act antagonistically in the control of seed germination and dormancy. While GAs promote seed germination, ABA induces and maintains seed dormancy [8]. Seed germination is regulated by differential expression of plant genes coding specific proteins necessary for germination, and also with activity of plant hormones which is regulated by the expression of plant genes [9]. Differential expression of ABA metabolic genes (e.g. *NCED1*), and ABA signalling genes including *ABI3, ABI5*, and *ABA7* regulate the ABA level in plant seeds, and therefore play roles in the control of dormancy and germination [8].

Gibberellins (GAs) are diterpenoids acting as a plant hormone and have important roles in seed germination, plant growth, development processes, as well as the mitigation of abiotic stresses including salt stress [10, 11]. Among more than 130 GAs, only four of them, namely GA1, GA3, GA4, and GA7 are thought to function as bioactive hormones [12, 13]. GA3 has been reported to alleviate the adverse effects of salinity on seed germination and seedling growth in crops [14, 15]. The biosynthesis of gibberellin is well clarified in model plants and mainly involves seven major enzymes including ent-kaurene oxidase (KO), ent-copalyl diphosphate synthase (CPS), ent-kaurenoic acid oxidase (KAO), ent-kaurene synthase (KS), gibberellin 20-oxidase (GA20ox), gibberellin 3-oxidase (GA3ox), and gibberellin 2-oxidase (GA2ox) which controls the inactivation of bioactive GAs [16, 17]. DELLA proteins, known as SLENDER1 (SLN1) in barley, are repressors of GA signalling in plants; thus, they repress the growth and germination in plants [18, 19].

Barley (*Hordeum vulgare* L.) is an important field crop after maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.) in terms of cultivated area (47 million hectares) and production (147.4 million tonnes) [7]. Global production of barley is mainly used for animal feed (more than 70%), malting in alcoholic beverages, seed, and human food [20]. While barley is considered the most salt-tolerant species among cereals, tolerance level of barley against salt stress varies depending on genotype and stage of plant growth [21]. Barley has been reported to be more sensitive to salinity during germination and young seedling stages but exhibits an increased tolerance with age [22].

The objectives of this study were to evaluate the alleviating effects of exogenous GA3 on seed germination of barley under salt stress, and determine the morphological and physiological changes as well as to determine the transcription pattern of 3 groups of genes. Group1 genes including *HvABA7, HvNCED1, HvABI3, HvABI5*, and *HvSLN1* are functional in suppression of

seed germination. Group2 genes involve *HvKO1, HvCPS1, HvKAO1, HvKS1, HvGA20ox1, HvGA3ox1* and *HvGA2ox4*, which are functional in GA3 metabolism and induction of seed germination. Group3 contains salt-stress responsive genes (e.g., *HvMT2, HvBAS1, HvDRF1,* and *HvLOX1*).

## 2. Materials and methods

### 2.1. Plant material and growth conditions

The seeds of barley (*Hordeum vulgare* L.), cv. Martı (a salt-tolerant barley cultivar) were kindly provided by Agricultural Research Institute (Edirne, Turkey). Trials in the range of 40-200 mM showed that an optimal salt concentration of 120 mM was the best for salinity stress during seed germination in barley. Besides, the preliminary studies have shown that 10 mg/L GA3 (Caisson labs-G001) dissolved in pure water was the best concentration among the tested GA3 concentrations (1, 10 and 100 mg/L) under salt stress during germination of barley seeds. Ten barley seeds were germinated in petri dishes containing filter papers soaked with 3 ml $dH_2O$ (control), 10 mg/L (~28.9 µM) GA3, 120 mM NaCl and 120 mM NaCl +10 mg/L GA3 for 3 days in dark at 25 °C in a climate chamber (Angelantoni, Ekochl 700).

### 2.2. Determination of osmolality, ion leakage, and proline content

In root and shoot tissues, the osmolyte measurement and ion leakage analysis were conducted according to Uçarlı and Gürel [7]. To determine the osmolyte content, 10 root or leaf samples (25-30 mg) were kept at −20 °C overnight. The cell sap was obtained by centrifugation at 4 °C for 30 min at 15000×g. An amount of 15 µl sap was taken from each sample, and after 10-fold dilution, osmolality content was measured using the osmometer (Semi-Micro Osmometer K-7400, Germany)

For ion leakage analysis, five 2 cm-length leaves (~30 mg) and 40-45 mg roots were harvested and washed three times with distilled water ($dH_2O$), then kept in glass tubes containing 5 ml $dH_2O$ in a controlled chamber at 25 °C in the dark. After 16 h, 50 µl of the liquid sample was taken from glass tubes including root or leaf samples and measured the conductivity (E1) using a conductivity meter (HORIBA Scientific, NJ, USA). The glass tubes were autoclaved at 121 °C for 15 min and then, conductivity measurement was repeated to record E2 values. Ion leakage was calculated using following formula. Ion leakage (%) = (E1/E2) × 100 [7].

Proline content was determined according to Carillo et al. [23]. 20-40 mg of shoot or root tissue were pulverized with liquid nitrogen in centrifuge tubes and then 200 µl $dH_2O$ was added into the tubes. The samples were incubated for 20 minutes in boiling water and then centrifuged at 6000 rpm for 1 minute. Supernatant was transferred into a tube and diluted 20-fold with 70%

ethanol. 150 µl aliquot of extract was added to reaction mixture (300 µl) containing 1% (w/v) ninhydrin in acetic acid 60% (v/v), and ethanol 20% (v/v). The resulting mixture was boiled in water bath at 95° C for 20 minute. After cooling at room temperature, mixture was spun down quickly at 2500 rpm for 1 minute. 200 µl of the mixture was transferred to a microplate well and read at 520 nm. The proline content was calculated using the following formula.

Proline in µmol.g-1FW = (Absextract – blank) / slope * Volextract / Volaliquot *1/FW**.** Absextract is the absorbance determined with the extract, blank (expressed as absorbance) and slope (expressed as absorbance·nmol-1) are determined by linear regression, Volextract is the total volume of the extract, Volaliquot is the volume used in the assay, FW (expressed in mg) is the amount of plant material extracted. It is assumed that Absextract is within the linear range.

### 2.3. Sampling, total RNA isolation, and qPCR

Shoot and root samples from were harvested 3 days after imbibition. Total RNA was extracted with the TRIzol® (Invitrogen, 15596-026) according to manufacturer's instructions using frozen 100 mg roots and shoots. cDNA synthesis, using SuperScript™ First-Strand Synthesis System (Invitrogen, 11904-018), and qPCR analyses were performed using CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) according to Uçarlı and Gürel [7] .

The transcription pattern of 3 groups of genes, functional in suppression of seed germination (group1), functional in GA3 metabolism and induction of seed germination (group2), and salt-responsive genes (group3), was investigated at 3 DAI in germinating barley seeds by qPCR (Table 1). The relative mRNA levels of the genes were normalized with respect to the internal control *HvGAPDH* and the relative expression level of each gene was analyzed by delta CT method [24]. All qPCRs were performed in three biological replicates and two technical replicates.

### 2.4. Statistical analysis

Statistical analyses were performed with SPSS21 (IBM). Tests for significant differences were conducted using one-way analysis of variance (ANOVA) with least significance difference (LSD) tests at the 0.05 level of confidence.

**Table 1:** Primer sequences used in qPCR analyses

| Gene | Accession No | Group No | Primer Sequences | Amplicon Size (bp) |
|---|---|---|---|---|
| *HvABA7* | X69817.1 | 1 | 5’ GAACAAGCTTGGCAGTGACA 3’<br>5’ CTTAGACGCCGGATCTCTGT 3’ | 228 |
| *HvNCED1* | AB239297.1 | 1 | 5′ CTCCATCCCTCCCATCTTCTC 3’<br>5' GCCGCTAACTGTTTCCTCTTC 3' | 126 |
| *HvABI3* | AK369681.1 | 1 | 5’ TGGTGATACCGAAGCAGAAC 3’<br>5’ CGCATCGTCCTCGAAGTT3' | 101 |
| *HvABI5* | AY150676 | 1 | 5’ GGTGAAGAAACAGACCGAGATAC 3’<br>5’ CTTCGTAAGCACTGCCTCTT 3’ | 96 |
| *HvSLN1* | AF460219.1 | 1 | 5’ CTACGAGTCCTGCCCCTACC 3’<br>5’ CCCTGCTTGATGCCGAAGTC 3’ | 114 |
| *HvGA2ox4* | AY551432 | 2 | 5’ TCCTAGCCAGCCAGCAACT 3’<br>5’ GGCATGGACAGGACACAGA 3’ | 205 |
| *HvGA3ox1* | AY551430 | 2 | 5' CCTCATAGATCAGCGCCAATA 3'<br>5' GGATGGAGAGACAGCAATGT 3' | 110 |
| *HvGA20ox1* | AY551428 | 2 | 5' CCAACTCGAACGTTGTTGTTATT 3'<br>5’ CATGCATCGCTACCACTCTAC 3’ | 84 |
| *HvCPS1* | AY551435 | 2 | 5' CCACCATTTCCTCCTTGGATAC 3'<br>5' GGACCAAACAACCAATCCAACTTG 3' | 110 |
| *HvKS1* | AY551436 | 2 | 5’ CATGCAAGGAGCTGTTCTGGAAGA 3’<br>5’ GGATCAAAGGTTCACTGCCGCTTC 3’ | 115 |
| *HvKO1* | AY551434 | 2 | 5' GACCCACATTCTCCTCTGAAC 3'<br>5' GAATCCACATCCTCGCCTAAA 3 | 92 |
| *HvKAO1* | AY326277 | 2 | 5’ CCACCATTTCCTCCTTGGATAC 3’<br>5’GGAGGAGAGTCTGGTGATCTT 3’ | 118 |
| *HvDRF1* | AY223807 | 3 | 5’ TCCTCTCGGTCAGATTTGCT 3’<br>5’ ACAGTCACCGGGTCAACTTC 3’ | 227 |
| *HvMT2* | BM816564 | 3 | 5’ TCAGTCGAATCAACACATGGA 3’<br>5’ CACGAGGACGGAACTAAAGC 3’ | 266 |
| *HvLOX1* | U83904.1 | 3 | 5’ AGCAGTGAAAGCGAGGAGAG 3’<br>5’ AAGTCGTTGAGGTCCAGCAC 3’ | 142 |
| *HvBAS1* | Z34917.1 | 3 | 5’ CGTCACCAAATCGATCTCAAA 3’<br>5’ TCCACACTACGGCCAATACC 3’ | 141 |
| *HvGAPDH* | AK251456 | - | 5’ TGTCCATGCCATGACTGCAA 3’<br>5’ CCAGTGCTGCTTGGAATGATG 3’ | 105 |

## 3. Results and Discussion

### 3.1. Physiological and morphological effects of NaCl and GA3 treatments

Seed germination is initiated by water imbibition and comprised with many different metabolic, cellular, and physiological processes [25]. Besides, the germination process can be

affected positively or negatively due to the environmental conditions including water, nutrition, temperature, and soil properties. Soil salinity negatively affects the germination process by retarding germination, lowering germination rate, and even preventing germination depending on salt concentration in soil. Germination and early seedling growth are two critical stages for plant establishment. Germination is the most sensitive plant growth stage to salinity stress and plants get tolerance against salt by age [3].

The effects of exogenous GA3 (10 mg/L), salt (120 mM NaCl), and their interactions during seed germination and post-germination of barley seeds were examined in terms of morphological changes (length of root and shoot) (Fig. 1), physiological changes including ion leakage, accumulation of osmolytes, and proline content (Table 2). In the present study, it has been found that salt (120 mM NaCl) has negative impact on barley germination and early seedling growth including retarding in root and shoot growth by 35% and 68%, respectively, compared to control condition ($dH_2O$) at 3 DAI. On the other hand, exogenous application GA3 (10 mg/L ≈ 28.9 µM) has alleviated the detrimental effects of salt stress on root and plant development, which resulted in a significant increase in length of shoot and root by 67% (from 0.84 to 1.40 cm) and 15% (from 3.60 to 4.25 cm), respectively, compared to salt-stressed seeds at $P<0.05$ level (Fig. 1). In another study, 10 µM GA3 application enhanced the final germination percentage up to 70%, and 31% at 100 mM and 200 mM NaCl, respectively, and the germination velocity compared to salt stress in the oilseed halophyte *Crithmum maritimum* [26]. Application of low concentration of GA3 (5 µM) were able to reverse the detrimental effects of salinity stress (100 mM NaCl) on 10-day-old mung bean seedlings with significant increase in shoot-root elongation as well as in biomass [27]. These results are in accordance with Abdel-Hamid and Mohamed [28] who reported that the exogenous applications of GA3 (100 µM ≈ 35mg/L) enhanced the germination of barley seeds under salt stress. Furthermore, 100 µM GA3 significantly increased the length of root and shoot under moderate salinity (100 mM NaCl) in barley cv. Giza 126, but had no effect on growth of root and shoot under higher salt stress (300 mM NaCl). Similarly, Maggio et al. [29] have reported that exogenous GA3 (100 mg/L) treatment decreased stomatal resistance and increased plant water use at low salinity (6.8 dS $m^{-1}$) after 141 days of treatment in tomato, while did not mitigate the salinity effects in moderate (11.7 dS $m^{-1}$) and high (16.7 dS $m^{-1}$) salt concentrations. Salinity stress causes the delay in germination and growth by reducing water potential, which resulting slower rate of imbibition. The increased growth of root and shoot with exogenous GA3 under salt stress may be attributed to the positive influence of gibberellic acid under salinity, which encourages cell division and cell elongation, and enhances efficiency of plant water use depending on salt concentration.

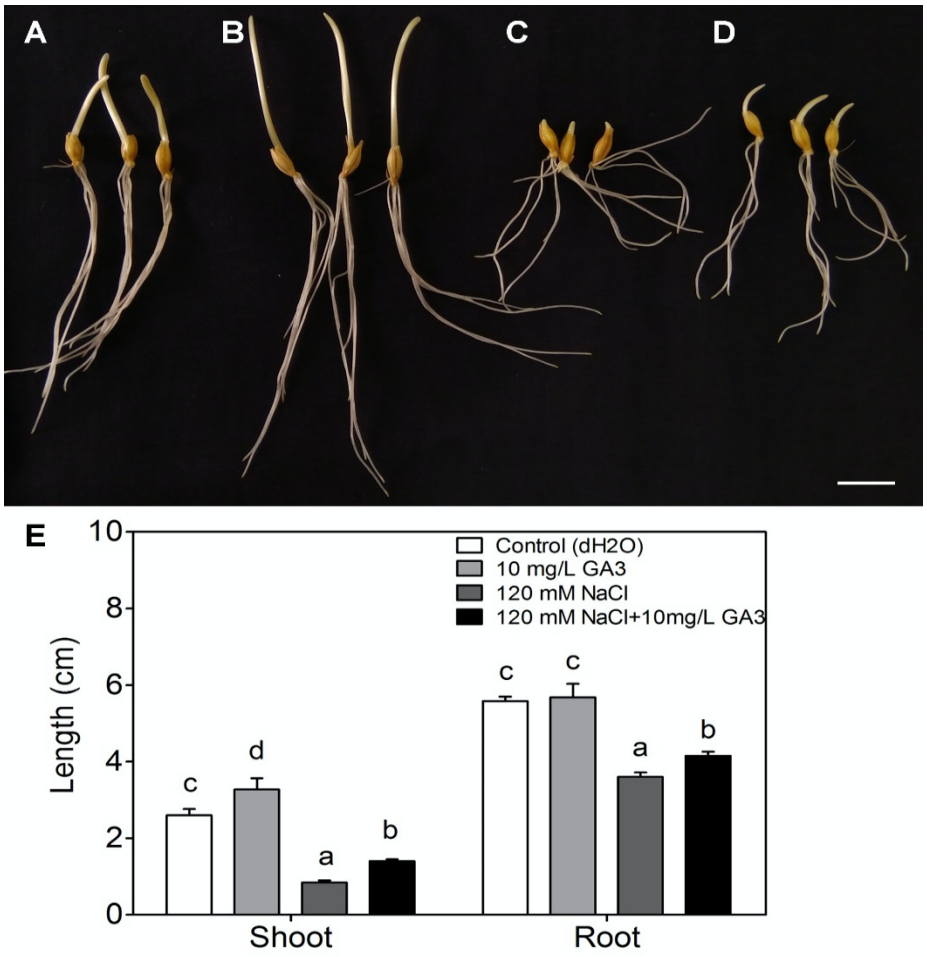


**Figure 1:** Phenotypes of 3-day-old barley sprouts germinated under different conditions: (A) $dH_2O$ (Control), (B) 10 mg/L GA3, (C) 120 mM NaCl, and (D) 120 mM NaCl+10 mg/L GA3. The bar length is 1cm. (E) Average shoot and root lengths of three-day-old barley sprouts from six replicates consisting of three biological and two technical replicates (n=10) with standard error of mean. Values denoted with the different letters differ significantly ($P < 0.05$, LSD post hoc test)

To cope with osmotic stress due to salinity, plants synthesize and accumulate osmolytes or compatible solutes, which are low-molecular-weight, highly soluble compounds, including proline, glycine betaine, and soluble sugars to increase osmotic potential of the cells, so to participate in osmotic adjustment [30]. In the present study, osmolyte content was significantly increased from 352 to 670 and 495 mosmol.kg$^{-1}$ by 120 mM NaCl treatment and 10 mg/L GA3, respectively, at 3 DAI. When 10 mg/L GA3 was applied with 120 mM NaCl, osmolyte content was found higher than GA3-treated seeds and lower than salt-treated seeds. No synergetic effect was observed.

Proline plays a protective role acting as an important osmoprotectant, enzyme protectant, and free radical scavenger against salinity stress in plants [31]. 120 mM NaCl significantly

increased proline accumulation in both shoot and root tissues by 6.5 fold and 7.5 fold, respectively, compared to controls ($P<0.05$). Exogenous 10 mg/L GA3 application did not alter the proline content in both shoot and root tissues compared with non-stressed sprouts at 3 DAI. Furthermore, combined application of 10 mg/L and 120 mM NaCl was observed to reduce proline accumulation in shoot and root by 57% and 66%, respectively, compared to seeds treated with 120 mM NaCl alone (Table 2). These results suggested that exogenous GA3 may induce the accumulation of other osmolytes instead of proline. Tuna et al. [32] have reported that foliar application of GA3 (50 mg/L) in maize seedlings under salt stress (100 mM NaCl) for 5 weeks slightly decreased in proline content compared to salt-stressed seedlings whilst higher GA3 concentration (100 mg/L) stimulated proline content leading to a big increase in similar conditions. In the present study, after soaking of seeds with 10 mg/L GA3 under salt stress for 3 days, proline content was found to have decreased by 58 and 65% in shoots and roots, respectively, compared to seeds soaking with 120 mM NaCl alone. Similarly, seedlings grown from seeds pre-soaked in GA3 (150 mg/L) was reported to exhibit less accumulation of proline than rice cultivars exposed to saline conditions (150 mg/L NaCl) for 10 days [33]. Manjili et al. [14] also have reported negative effects of GA3 (50 mg/L) application on proline content in three wheat cultivars in comparison with control and salinity conditions (3.5 and 7 $dSm^{-1}$). Consequently, both positive and negative interactions between GA and proline have been reported in the literature.

Ion leakage demonstrates membrane damage as a result of salt-induced oxidative damage. It has been reported that ion leakage increases under salt stress in the vegetative stage of plant [34]. In the shoot tissues, the ion leakage increased significantly from 38 to 55% after 120 mM NaCl treatment for 3 days during germination compared to control ($dH_2O$) at $P<0.05$ level. On the other hand, when seeds were treated with 10 mg/L GA3 + 120 mM NaCl, ion leakage was significantly reduced from 55 to 21 % in shoot at 3 DAI ($P<0.05$). Ion leakage was measured as 93% in roots of sprouts in response to salt stress. On the other hand, application of GA3 (10mg/L) was seen to increase membrane stability by lowering the elevated ion leakage level from 93 to 79% under salt stress. Tuna et al. [32] have reported that 50 and 100 mg/L GA3 treatments ameliorated negative effects of salt stress (100 mM NaCl) on membrane permeability by decreasing ion leakage upon GA3 concentration from 18 to 13 and 11%, respectively, in maize leaves. The significant reduction in ion leakage suggested that GA3 alleviated the harmful effects of salt stress on cell membrane, resulting in enhanced salt tolerance during germination.

**Table 2:** Proline content, ion leakage, and osmolality in shoot and root tissues of germinated seeds at 3 DAI under different conditions: $dH_2O$ (C), 10 mg/L GA3 (GA3), 120 mM NaCl (NaCl) and 120 mM NaCl+10 mg/L GA3 (NaCl+GA3)

| Treatment | Proline Content (μmol.g$^{-1}$FW) | | Ion Leakage (%) | | Osmolality (mosmol.kg$^{-1}$) | |
|---|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Shoot | Root |
| C | 3.21±0.64 a | 4.10±0.77 a | 37.62±3.33 b | 82.86±2.86 a | 352±30 a | NA |
| GA3 | 3.85±0.26 a | 4.36±0.51 a | 26.84±2.22 a | 83.23±1.56 a | 495±50 b | NA |
| NaCl | 20.77±5.38 b | 30.90±1.15 b | 55.27±2.37 c | 93.18±2.06 b | 670±10 c | NA |
| NaCl+GA3 | 8.98±0.26 a | 10.64±5.25 a | 20.88±0.66 a | 79.02±2.23 a | 545±5 b | NA |

Within each column, values denoted by same letters are not significantly different according to the LSD test at $P<0.05$ level ± Standard error.

### 3.2. Differential gene expression profiles of barley seeds after NaCl and/or GA3 treatment

Effects of salt (120 mM NaCl), GA3 (10 mg/L) and interaction of GA3 and salt on the expression profiles of genes at 3 DAI in germinating barley seeds were analyzed by qPCR (Fig. 2). The genes to be analyzed were classified into 3 groups; group1 genes containing *HvABA7, HvNCED1, HvABI3, HvABI5, and HvSLN1* which are functional in suppression of seed germination, group2 genes including *HvGA2ox4, GA3ox1, GA20ox1, HvCPS1, HvKS1, HvKO1,* and *HvKAO1,* which are functional in GA3 biosynthesis and induction of germination and group3 genes are functional in response to salt stress including *HvMT2, HvBAS1, HvDRF1*, and *HvLOX1*.

Germination of seeds is a critical stage in a plant's life and involves multi-stage processes which are checked and regulated by differential expression of numerous genes in seed tissues [25]. GA20-oxidase (GA20ox) and GA3-oxidase (GA3ox) are major enzymes in the biosynthesis of Gas, whereas GA2-oxidase (GA2ox) has a role inactivation of GA [8].). In this study, application of exogenous GA3 (10 mg/L) was found to increase the expression of *HvGA2ox4* in shoot while decreasing mRNA levels of *HvGA20ox1* and *HvGA3ox1*. It is suggested that the expression of these genes are differentiated by exogenous GA3 to balance the endogenous GA level in cells. Furthermore, it has been showed that the active forms of gibberellins such as GA3 could feedback regulate the expression levels of the *GA20ox* and *GA3ox* genes, which are the downstream genes in the pathway of GA biosynthesis [35]. Salt stress slightly decreased the expression of these three GA-oxidase genes compared to control conditions in shoot at 3DAI. On the other hand, only *GA2ox4* was up-regulated among these genes after 10 mg/L GA3+120 mM NaCl treatment at 3 DAI (Fig. 2). Magome et al. [36] have reported six *GA2ox* genes, which are involved in the repression of growth under high-salinity conditions, were upregulated under high-salinity stress (150 mM NaCl) in Arabidopsis. In another study, after 150 mM NaCl treatment the

level of GA biosynthesis genes *GmGA3ox1* was also decreased in germinating soybean seeds at 9 h after sowing while expression of *GmGA2ox8,* which inactivates GA, increased compared with control [37]. In roots of germinated seeds under salt stress, exogenous GA3 was found to have no significant effect in expressions of *HvGA2ox4, GA20ox1*, and *HvGA3ox1* compared to salinity conditions (Fig. 2).

*CPS, KS, KO*, and *KAO* are the upstream genes in the GA biosynthesis pathway. CPS and KS, terpene cyclases, are located in the plastids and involved in conversion of geranylgeranyl diphosphate (GGDP) to ent-kaurene. KO and KAO are the cytochrome P450 monooxygenases, are located in the outer membrane of the plastid and the endoplasmic reticulum, respectively, catalyze ent-kaurene to GA12 aldehyde through a series of oxidation reactions [16, 35]. Salt stress slightly increased the mRNA level of *HvCPS1* and *KAO1* 2 and 1.9 fold, respectively, while lowering the expression of *HvKO1* by 33 fold compared to control in shoot of germinated barley seeds at 3 DAI. 10 mg/L GA3 application was found to recover the decreased mRNA level of *HvKO1* due to the salt stress as the level of control conditions in shoot of germinating sprouts at 3 DAI. The expression patterns of *HvCPS1* and *HvKAO1* were found to be similar in both shoot and root tissues (Fig. 2). It has been reported that Gas down-regulated *GA20ox* and *GA3ox,* did not change expression of *CPS, KS, KO*, and *KAO* genes [16]. Huang et al. [35] have reported that *TaCPS, TaKS, TaKO,* and *TaKAO* genes were constitutively expressed in leaves, young spikes and stem of wheat, but their relative expression levels changed in different tissues. Furthermore, the homologs of those genes also showed the different expression pattern in wheat seedlings after GA3 treatment. In the present study, GA3 (10mg/L) altered the expression level of *HvCPS1, HvKS1, HvKAO1*, and *HvKO1* in barley sprouts at 3 DAI and those genes showed different expression levels in different tissues. These results indicate that genes involved in GA biosynthesis may play important role in germination of seeds and may function differently in tissues and different development stages of plants.

GA and ABA have antagonistic role in germination of seeds. GA enhances seed germination while ABA prevents or delays seed germination [8]. It has been reported that the reciprocal modulation of the expression of genes involved in ABA and GA metabolism and signaling plays role in coordination of changes in the ABA/GA balance [8, 38]. Salt stress has been reported to induce ABA biosynthesis by promoting expression of the NCEDs (9-cis-epoxycarotenoid dioxygenase) that codes a rate-limiting enzyme for ABA biosynthesis [39]. Increased accumulation of endogenous ABA content by induction of *HvNCED1* with salt (120 mM NaCl) delayed seed germination and retarded shoot growth in barley. Addition of exogenous GA3 (10 mg/L) into root medium could help to reduce enhanced *NCED1* level due to salinity in

shoot so recovered shoot growth in salt-stressed barley seeds. Intriguingly, salinity stress was found to resulted in a significant reduction in expression of *HvABA7* (10 fold) and *HVABI5* (3 fold) in shoots of germinated barley seeds at 3 DAI. These genes are participated in suppression of seed germination and generally upregulated by salinity stress. At present, only a few studies have been published on *HvABA7* gene and it has been reported that *HvABA7* was upregulated in response to salinity and drought stress in barley in contrast to this [40, 41]. Besides, application of exogenous GA3 resulted in an 18-fold increase in expression of *HvABA7* while no alteration was determined in expression of *HvABI5*. Enhanced expression of *OsNCED1* and *OsABI5* resulted in increased ABA level and response, which caused a delay in germination in rice [39]. Increased ABA content disturbs the balance between ABA and GA levels which leads to retardation in germination and growth. In the study, application of exogenous GA3 to barley seeds under salt stress was found to decrease the mRNA level of *HvNCED1* compared to salinity conditions at 3 DAI (Fig. 2).

Salinity shows the physiological effects on seed germination by differentiating the expression of some plant genes including group3 genes. It has been found that application of exogenous GA3 alone induced the expression of the salt-stress responsive genes including *HvMT2, HvDRF1, HvBAS1*, and *HvLOX1* in shoots of barley sprouts at 3 DAI. Even the expression level of *HvBAS1* and *HvDRF1* was higher in GA3-treated sprouts than in salt-treated sprouts (Fig. 2). *HvBAS1* play important role in detoxification of $H_2O_2$ as an antioxidant enzyme [7]. Exogenous GA3 may induce the germination and growth by enhancing the expression of the genes coding ROS scavenging enzymes including *HvBAS1* and *HvMT2*, which resulting in the reduction of the oxidative damage through accumulation of ROS.

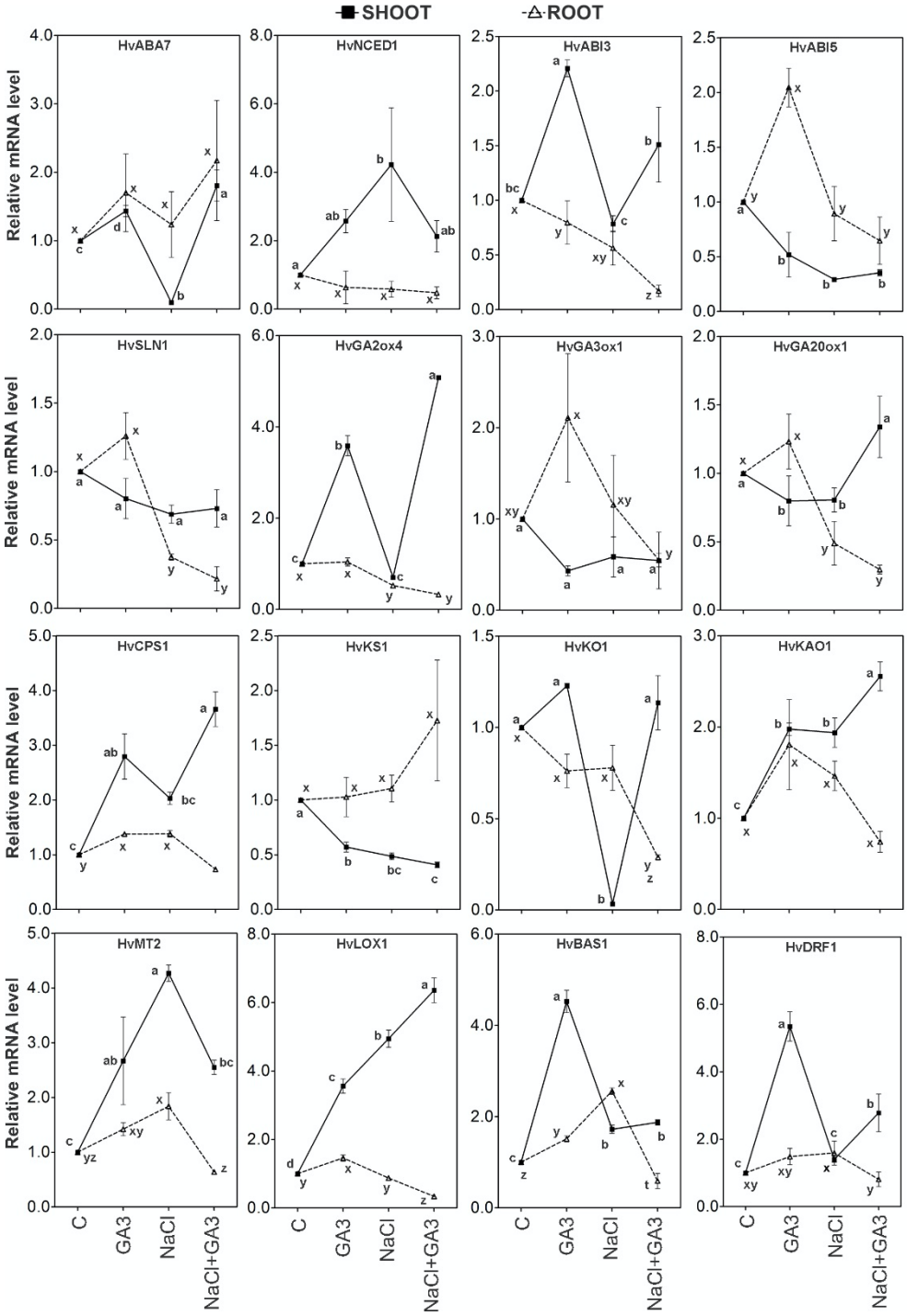


**Figure 2:** Gene expression profiles in shoot and root tissues of germinated seeds at 3 DAI. Barley seeds were treated with $dH_2O$ (C), 10 mg/L GA3 (GA3), 120 mM NaCl (NaCl) and 120 mM NaCl+10 mg/L GA3 (NaCl+GA3) during germination. *HvGAPDH* was used as a reference gene. The mean values from six replicates consisting of three biological and two technical replicates with standard error**.** Values represented by different letters (a, b, c, d, and t, x, y, and z in shoot and root, respectively, are significantly different according to the LSD post hoc test at $P<0.05$ level

## 4. Conclusion

Salinity is one of the most important abiotic stress limiting plant development and productivity in arid and semi-arid regions. Plants are most susceptible to salt stress in germination and young seedling formation stages. The exogenous application of GA3 significantly mitigated salt stress-induced delay of germination and growth inhibition in barley seeds. The results of the present study suggest that exogenous GA3 application depending on concentration could be helpful to improve germination and early seedling stages in crops under salt stress conditions. The expressions of the genes involved in GA3 metabolism and seed germination including the *HvABI5, HvABA7*, *HvKO1,* and *HvGA2ox4* were significantly differentiated after exogenous GA3 treatment. This study may give insight into relationship between salinity stress and genes involved in GA3 metabolism during germination and post-germination of barley seeds. However, high-throughput transcriptome analysis would reveal the mechanism underlying the role of GA3 in germination of seeds under salt stress.

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## References

[1] Zhu, J.K., *Abiotic stress signaling and responses in plants*, Cell 167, 313-324, 2016.

[2] Munns, R., Tester, M. *Mechanisms of salinity tolerance*, Annual Review of Plant Biology, 59, 651-681, 2008.

[3] Hasanuzzaman, M., Nahar, K., Fujita, M., *Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages,* In: Ahmad, P., Azooz, M.M., Prasad, M.N.V., (editors), Ecophysiology and responses of plants under salt stress, Springer, New York, pp. 25-87, 2013.

[4] Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., et al., *An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation*, International Journal of Molecular Sciences, 21(1), 148, 2020.

[5] Ibrahim, E.A., *Seed priming to alleviate salinity stress in germinating seeds,* Journal of Plant Physiology 192, 38-46, 2016.

[6] Amirbakhtiar, N., Ismaili, A., Ghaffari, M.R., Nazarian F., Shobbar, Z.S., *Transcriptome response of roots to salt stress in a salinity-tolerant bread wheat cultivar*, PLoSONE, 14(3), e0213305, 2019.

[7] Uçarlı, C., Gürel, F., *Differential physiological and molecular responses of three-leaf stage barley (Hordeum vulgare L.) under salt stress within hours,* Plant Biotechnology Reports, 14, 89-97, 2020.

[8] Tuan, P.A., Kumar, R., Rehal, P.K., Toora, P.K., Ayele, B.T., *Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals*, Frontiers in Plant Science, 9, 668, 2018.

[9] Miransari, M., Smith, D.L., *Plant hormones and seed germination*, Environmental and Experimental Botany, 99, 110–121, 2014.

[10] Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd-Allah, E.F., Hashem, A., *Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness*, Frontiers in Microbiology, 8, 1-14, 2017.

[11] Liu, X., Hou, X., *Antagonistic regulation of ABA and GA in metabolism and signaling pathways*, Frontiers in Plant Science, 9:251, 2018.

[12] Hedden, P., Sponsel, V., *A century of gibberellin research,* Journal of Plant Growth Regulation, 34, 740-760, 2015.

[13] Binenbaum, J., Weinstain, R., Shani, E., *Gibberellin localization and transport in plants*, Trends in Plant Science, 23, 410-421, 2018.

[14] Manjili, F.A., Sedghi, M., Pessarakli, M., *Effects of phytohormones on proline content and antioxidant enzymes of various wheat cultivars under salinity stress*, Journal of Plant Nutrition 35(7),1098-11, 2012.

[15] Zehra, A., Shaikh, F., Ansari, R., Gul, B., Khan, M.A., *Effect of ascorbic acid on seed germination of three halophytic grass species under saline conditions,* Grass and Forage Science, 68, 339-344, 2013.

[16] Yamaguchi, S., *Gibberellin metabolism and its regulation*, Annual Review of Plant Biology, 59, 225-251, 2008.

[17] Kai, K., Kasa, S., Sakamoto, M., Aoki, N., Watabe, G., et al., *Role of reactive oxygen species produced by NADPH oxidase in gibberellin biosynthesis during barley seed germination*, Plant Signaling and Behavior, 11: e1180492.35, 753-759, 2016.

[18] Chandler, P.M., Marion-Poll, A., Ellis, M., Gubler, F., *Mutants at the Slender1 locus of barley cv. Himalaya. Molecular and physiological characterization,* Plant Physiology, 129:, 181-190, 2002.

[19] Davière, J.M., Achard, P., *A pivotal role of DELLAs in regulating multiple hormone signals*, Molecular Plant, 9, 10-20, 2016.

[20] Zhou, M., *Barley production and consumption,* In: Zhang, G., Li, C., (editors), Genetics and improvement of barley malt quality, Springer-Verlag, Berlin Heidelberg, pp. 1-17, 2010.

[21] Gao, R., Duan, K., Guo, G., Du, Z., Chen, Z., et al., *Comparative transcriptional profiling of two contrasting barley genotypes under salinity stress during the seedling stage*, International journal of Genomics, 972852, 2013.

[22] Al-Karaki, G.N., *Germination, sodium, and potassium concentrations of barley seeds as influenced by salinity*, Journal of Plant Nutrition, 24(3), 511-522, 2001.

[23] Carillo, P., Mastrolonardo, G., Nacca, F., Parisi, D., Verlotta, A., et al., *Nitrogen metabolism in durum wheat under salinity: accumulation of proline and glycine betaine*, Functional Plant Biology, 35(5), 412-426, 2008.

[24] Livak, K.J., Schmittgen, T.D., *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) method*, Methods, 25, 40-408, 2001.

[25] Potokina, E., Sreenivasulu, N., Altschmied, L., Michalek, W., Graner, A., *Differential gene expression during seed germination in barley (Hordeum vulgare L.)*, Functional and Integrative Genomics, 2, 28-39, 2002.

[26] Atia, A., Debez, A., Barhoumi, Z., Smaoui, A., Abdelly, C., *ABA, GA3, and nitrate may control seed germination of Crithmum maritimum (Apiaceae) under saline conditions*, Comptes Rendus Biologie 332, 704-710, 2009.

[27] Ghosh, S, Mitra, S., Paul, A., *Physiochemical studies of sodium chloride on mungbean (Vigna radiate L. Wilczek) and its possible recovery with spermine and gibberellic acid,* The Scientific World Journal, Article ID 858016, 2015.

[28] Abdel-Hamid, A.M.E., Mohamed, H.I., *The effect of the exogenous gibberellic acid on two salt stressed barley cultivars*, European Scientific Journal, 10, 228-245, 2014.

[29] Maggio, A., Barbieri, G., Raimondi, G., De Pascale, S., *Contrasting effects of GA3 treatments on tomato plants exposed to increasing salinity*, Journal of Plant Growth Regulation, 2, 63–72, 2010.

[30] Manivannan, P., Jaleel, C.A., Sankar, B., Kishorekumar, A., Somasundaram, R. et al., *Growth, biochemical modifications and proline metabolism in Helianthus annuus L. as induced by drought stress*, Colloids and Surfaces. B, Biointerfaces, 59, 141-149, 2007.

[31] Verbruggen, N., Hermans, C., *Proline accumulation in plants: a review*, Amino Acids, 35(4), 753-759, 2008.

[32] Tuna, A.L., Kaya, C., Dikilitas, M., Higgs, D., *The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants*. Environmental and Experimental Botany, 62(1), 1-9, 2008.

[33] Chunthaburee, S., Sanitchon, J., Pattanagul, W., Theerakulpisut, P. *Alleviation of salt stress in seedlings of black glutinous rice by seed priming with spermidine and gibberellic acid*, Notulae botanicae Horti Agrobotanic Cluj-Napoca, 42, 405-413, 2014.

[34] Li, Q.Y., Niu, H.B., Yin, J., Wang, M.B., Shao, H.B., et al., *Protective role of exogenous nitric oxide against oxidative-stress induced by salt stress in barley (Hordeum vulgare)*, Colloids and Surfaces. B, Biointerfaces, 65, 220-225, 2008.

[35] Huang, Y., Yang, W., Pei, Z., Guo, X., Liu, D., et al., *The genes for gibberellin biosynthesis in wheat. Functional and Integrative Genomics*, 12, 199-206, 2012.

[36] Magome, H., Yamaguchi, S., Hanada, A., Kamiya, Y., Oda, K., *The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA2ox7, under high-salinity stress in Arabidopsis*, Plant Journal, 56, 613-626, 2008.

[37] Shu, K., Qi, Y., Chen, F., Meng, Y., Luo, X., et al., *Salt stress represses soybean seed germination by negatively regulating GA biosynthesis while positively mediating aba biosynthesis*, Frontiers in Plant Science, 8, 1372, 2017.

[38] Izydorczyk, C., Nguyen, T.N., Jo, S., Son, S., Tuan, P.A., et al., *Spatiotemporal modulation of abscisic acid and gibberellin metabolism and signaling mediates the effects of suboptimal and supraoptimal temperatures on seed germination in wheat (Triticum aestivum L.),* Plant Cell Environment, 41, 1022-1037, 2017.

[39] Zeng, D.E., Hou, P., Xiao, F., Liu, Y., *Overexpression of Arabidopsis XERICO gene confers enhanced drought and salt stress tolerance in rice (Oryza Sativa L.),* Journal of Plant Biochemistry and Biotechnology, 24, 56-64, 2015.

[40] Walia, H., Wilson, C., Wahid, A., Condamine, P., Cui, X., et al., *Expression analysis of barley (Hordeum vulgare L.) during salinity stress,* Functional and Integrative Genomics, 6, 143-156, 2006.

[41] Gürel, F., Öztürk, N.Z., Yörük, E., Uçarlı, C., Poyraz, N., *Comparison of expression patterns of selected drought-responsive genes in barley (Hordeum vulgare L.) under shock-dehydration and slow drought treatments*. Plant Growth Regulation, 80, 183-193, 2016.