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Research Article

Phytohormone Pretreatment Mitigates the Negative Impacts of Salt Stress on the Germination of Common Buckwheat Seeds (*Fagopyrum esculentum*)

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Abstract

Salinity is a significant environmental factor that impacts agricultural output globally. Salinity has the greatest impact on seed germination, which is the most vulnerable stage. Common buckwheat, scientifically known as *Fagopyrum esculentum* Moench, is a minor crop that is extensively grown and holds significant economic value in the regions of southwest and northwest China. Nevertheless, there is limited knowledge regarding the impact of plant hormones on seed germination in the presence of salt stress. In this study, our results have shown that the germination rate decreased with increasing NaCl concentration. The impact of four distinct artificial plant hormones (ethephon; gibberellin, GA3; 6-benzylaminopurine, 6-BA; and naphthalene acetic acid, NAA) at varying levels on seed germination under low (50 mmol L^{-1} NaCl) and high (100 mmol L^{-1} NaCl) salt stress levels was examined. Pretreatments with ethephon, GA3 and 6-BA have alleviated the adverse effects of salt stress during seed germination, whereas pretreatment with NAA has aggravated the adverse effects due to salt stress. Ethephon at a concentration of 100 mg L^{-1}, GA3 at a concentration of 100 mg L^{-1}, and 6-BA at a concentration of 200 ug L^{-1} were found to be the most successful remedies for mitigating the negative impact of salt stress on the germination of common buckwheat seeds. This study provides a new insight for cultivation of common buckwheat especially the seed germination of common buckwheat in the saline environments.

Keywords: Common Buckwheat; Plant Hormones; Salt Stress; Seed Germination

Abbreviations

GA3: Gibberellin; 6-BA: 6-benzylaminopurine; NAA: Naphthalene Acetic Acid

Introduction

With the aggravation of global greenhouse effect and the use of a large number of pesticides and fertilizers, soil salinization has become a global problem. Approximately 7% of the Earth's surface is at risk of salinization, with the threat continuing to increase. China faces a significant salinization issue, with a widespread distribution of salinization affecting a large area, posing a major threat to the country's agricultural development and production [1]. High levels of salt in the soil greatly limit the ability to sustainably produce crops by impacting the germination of plant seeds, the growth of crops, and the overall yield. Saline soils cause osmotic stress and ion toxicity, leading to reduced seed germination rates and decreased crop productivity. Nevertheless, certain plants that can withstand high levels of salt and halophyte plants have evolved different physiological adaptations to thrive in harsh saline environments, such as seed germination, in order to survive and grow [2].

Common buckwheat and tartary buckwheat belong to the Fagopyrum genus within the Polygonaceae family. Common buckwheat, also known as *Fagopyrum esculentum*, is a minor crop that grows annually and can thrive in various geographical environments. It has a high tolerance for extreme climates and is primarily grown in temperate regions across Asia, Europe, and North America. Typically lasting around 3 months, its period of growth is brief. Common buckwheat is gaining popularity worldwide due to its high

protein, fat, starch, vitamins, rutin, minerals, and vegetable cellulose content, as well as its unique ability to prevent and treat cardiovascular diseases, diabetes, and constipation [3].

Synthetic analogs of plant hormones, known as exogenous synthetic phytohormones, have shown to reduce the negative impacts of salt stress on seed germination and plant metabolic growth. Various plant hormones have different impacts on the growth and development of plants. 6-benzylaminopurine (6-BA) and gibberellin (GA) are natural plant hormones that stimulate cell division, seed sprouting, and bud dormancy release in response to environmental stress [4]. Ethylene plays a crucial role in the gas atmosphere, controlling various plant growth processes such as seed germination and seedling establishment, and is also involved in breaking seed dormancy in many species [5]. The synthetic hormone NAA can boost the peroxidase and catalase levels in seeds, leading to improved cell membrane integrity and enhanced germination [6].

Common buckwheat exhibits excellent ecological flexibility, thriving in marginal areas with severe weather and soil conditions [7]. Nevertheless, limited research has been conducted on the impact of various plant hormones on the germination traits of Fagopyrum esculentum seeds in the presence of NaCl stress. Local common buckwheat seeds were pre-treated with varying concentrations of four plant hormones (NAA, ethephon, GA3, and 6-BA) to investigate their germination rate when exposed to NaCl stress. The primary goal of these trials was to determine levels of plant hormones that alleviate salt-induced stress in regular buckwheat seeds, aiming to gather data necessary for the growth of *Fagopyrum esculentum* in salty soil environments.

Materials and Methods

- **Plant material**: In this research, the study utilized plant material from the Local J-2 strain of common buckwheat (*Fagopyrum esculentum*).
- **Seeds germination treatments**: Various methods were used to promote the germination of disease-free common buckwheat seeds that were chosen for their consistent size and full grains. The seeds were cleansed using distilled water, sanitized by immersing in a 1% NaClO solution for 10 minutes, washed once more with distilled water, and finally patted dry to eliminate any remaining moisture on the surface.

There were 43 treatments in the study, comprising one control, two NaCl treatments, eight phytohormones treatments, and 32 hormones + NaCl treatments, with 50 common buckwheat seeds used in each treatment. All synthetic phytohormones and NaCl were ordered from Merck, China. The treatments were repeated three times and the seeds germination rate was assessed. The control (H_2O) consisted of distilled water. Solutions containing 50 mmol L-1 and 100 mmol L-1 of NaCl were utilized as treatments with NaCl. Seeds were soaked in solutions containing different concentrations of NAA, ethephon, GA3, and 6-BA for 24 hours as part of phytohormone treatments, with the process taking place at room temperature in the absence of light [8]. Seeds from different treatments were evenly distributed between two layers of quantitative filter paper (90 × 90 mm) in sterilized Petri dishes (90 × 90 mm). Each Petri dish received 3 mL of either 50 mmol L-1 or 100 mmol L-1 NaCl solution (NaCl treatment). For hormones + NaCl treatments, the seeds were presoaked with different hormones with different concentrations for 24h and transferred to Petri dishes with two different NaCl concentrations. Petri dishes were kept in a dark incubator at a temperature of 22 ± 3 °C for a week, with the NaCl solution being regularly refreshed. After seven days, germination rate was calculated [9].

Calculation of seed germination

Germination rate (Gr) = n/N × 100% (n: germination number; N: total number of seeds). There were three replicates for each treatment.

Statistical analysis

Data input, processing, and basic analysis were performed using Microsoft Excel 2010 for statistical purposes. Three replications were used for each treatment, and statistical significance was determined through ANOVA analysis using Data Processing System (V7.05). Mean values along with standard errors are reported in the results. Variance analysis charts were created with Microsoft Excel 2010.

Results and Discussion

The effect of different NaCl concentration to seed germination

The germination rate was evaluated and calculated manually after 7 days. Our research revealed that when exposed to 100 mmol L-1 NaCl, the average germination rate decreased significantly to 55.3%, which was approximately 25% lower than the control rate of 69.3%. Nevertheless, a higher number of sprouted seeds were observed under the 50 mmol L-1 NaCl treatment, achieving an average germination rate of 86%. Furthermore, there was a 24.6% increase in germination rate compared to the control, and a 56.4% increase compared to the treatment with 100 mmol L-1 NaCl (see Figure 1 and Supplementary Table 1). Our results showed that low concentration of NaCl treatment could facilitate buckwheat seed germination speed, and similar results had also been reported in Xanthoceras sorbifolia Bunge [10].

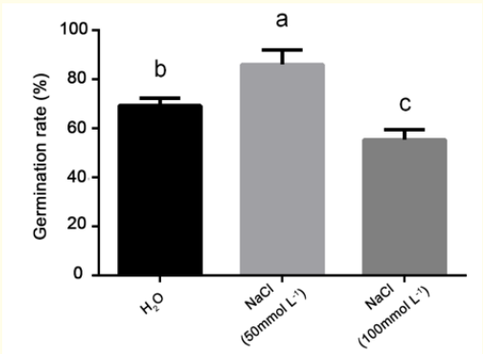

Figure 1: Effects of different NaCl concentrations on the seed germination. Column shows means values of three replicates and bar shows the standard error of means. Lowercase letters on each column indicate significant differences between treatments at $p < 0.05$.

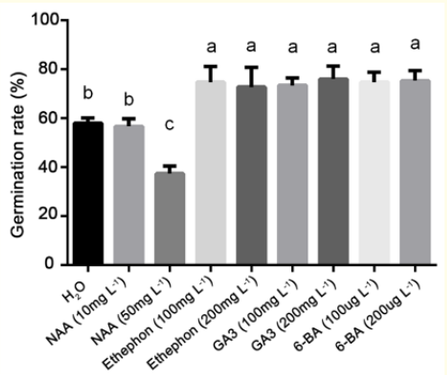

Figure 2: Effects of different hormones treatments on the germination rate of common buckwheat seeds. Column shows means values of three replicates and bar shows the standard error of means. Different lowercase letters on each column indicate significant differences between treatments at $p < 0.05$.

Supplementary Table 1

Treatment	Germination rate
H_2O	69.3% ± 2% b
NaCl (50mmol L^{-1})	86% ± 3% a
NaCl (100mmol L^{-1})	55.3% ± 2% c

The effects of exogenous phytohormones treatments on the seed germination

Different doses of external plant hormones were applied to common buckwheat seeds in this study to observe their impact on seed germination. The treatments included NAA at 10 mg L-1 and 50 mg L-1, ethephon at 100 mg L-1 and 200 mg L-1, GA3 at 100 mg L-1 and 200 mg L-1, and 6-BA at 100 ug L-1 and 200 ug L-1.H2O was used as control. The outcomes showed all seeds pretreated with H_2O and NAA germinated significant less than ethephon, GA3, and 6-BA pretreated seeds (Figure 2). Additionally, the germination rate of the high concentration of NAA (50 mg L-1) pretreatment showed the lowest (37.3%), significantly lower than the control and low concentration of NAA (10 mg L-1) conditions. There was no obvious difference between control (58%) and 10mg L-1 NAA pretreatment (56.7%). Following treatment with 100 mg L-1 and 200 mg L-1 of ethephon, 100 mg L-1 and 200 mg L-1 of GA3, and 100 ug L-1 and 200 ug L-1 of 6-BA, the mean germination percentages were 74.7, 72.7, 73.3 76, 74.7 and 75.3% correspondingly, showing a rise of 28.7, 25.3, 26.4, 31.0, 28.7 and 29.9% compared to the untreated control. Similar increased trends were also observed between 10 mg L-1 NAA and ethephon/ GA3/ 6-BA treatments (Figure 2, Supplementary Table 2). Our results indicated appropriate concentration of ethephon/ GA3/ 6-BA could promote seed germination, which are similar with earlier studies [11,12].

Supplementary Table 2

Treatment	Germination rate	$p < 0.05$
H_2O	58% ± 2%	b
NAA (10mg L^{-1})	56.7% ± 3%	b
NAA (50mg L^{-1})	37.3% ± 3%	c
Ethephon (100mg L^{-1})	74.7% ± 6.4%	a
Ethephon (200mg L^{-1})	72.7% ± 8%	a
GA3 (100mg L^{-1})	73.3% ± 3%	a
GA3 (200mg L^{-1})	76% ± 5%	a
6-BA (100ug L^{-1})	74.7% ± 4%	a
6-BA (200ug L^{-1})	75.3% ± 4%	a

The effects of different phytohormones pretreatments and low concentration NaCl stress (50mmol L-1) on the seed germination

Seed germination was influenced by various phytohormone pretreatments and exposure to low levels of NaCl stress (50mmol L-1). Pretreatment involved the use of ethephon, GA3, 6-BA, and NAA on all seeds before subjecting them to the stress of 50 mmol L-1 NaCl. H_2O was performed as empty control. The mean germination percentages for the untreated control, 50mmol L-1 NaCl alone, 10 mg L-1 and 50 mg L-1 of NAA, 100 mg L-1 and 200 mg L-1 of ethephon, 100 mg L-1 and 200 mg L-1 of GA3, 100 ug L-1 and 200 ug L-1 of 6-BA, with 50mmol L-1 NaCl treatments were 51.3%, 76%, 18%, 9.3%, 83.3%, 71.3%, 82%, 83.3%, 88%, and 88%, respectively. A noticeable decrease in germination compared to the control was observed under 50 mmol L-1 NaCl stress after being treated with 10 mg L-1 and 50 mg L-1 NAA. When exposed to NaCl stress levels

below 50 mmol L-1, the germination rates of seeds pretreated with 10 mg L-1 and 50 mg L-1 NAA decreased by 183% and 467% in comparison to the control group. Compared to the control group under 50 mmol L-1 NaCl stress, the germination rates decreased by 322% and 744% with pretreatments of 10 mg L-1 and 50 mg L-1 NAA, respectively. In addition to NAA pretreatments, such as 50 mmol L-1 NaCl stress alone, all other pretreatments involving ethephon (100 mg L-1 and 200 mg L-1), GA3 (100 mg L-1 and 200 mg L-1), and 6-BA (100 ug L-1 and 200 ug L-1) combined with 50 mmol L-1 NaCl stress exhibited notably increased germination rates compared to the control, aligning with earlier findings. When compared to a control group with no substance, the germination rates under stress conditions of 50 mmol L-1 NaCl, ethephon (100 mg L-1 and 200 mg L-1) + 50 mmol L-1 NaCl, GA3 (100 mg L-1 and 200 mg L-1) + 50 mmol L-1 NaCl, and 6-BA (100 ug L-1 and 200 ug L-1) + 50 mmol L-1 NaCl increased by 49%, 62.7%, 39.2%, 60.8%, 62.7%, 72.5%, and 72.5%, respectively (See Figure 3 and Supplementary Table 3).

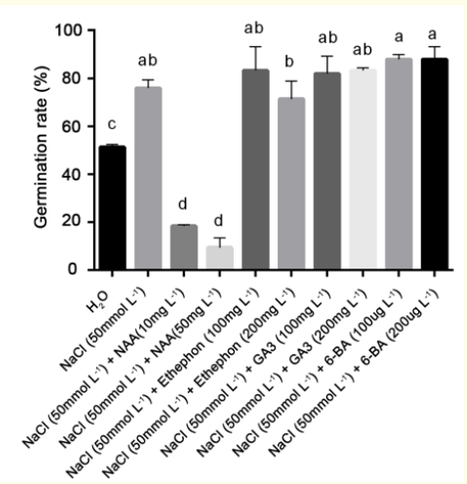

Figure 3: Effects of different phytohormones pretreatments and low concentration NaCl stress on the seed germination rates of common buckwheat seeds. Column shows means values of three replicates and bar shows the standard error of means. Different lowercase letters on each column indicate significant differences between treatments at $p < 0.05$.

The effects of different phytohormones pretreatments and high concentration NaCl stress (100mmol L-1) on the seed germination

The impact of various phytohormone treatments and high levels of NaCl stress (100mmol L-1) on seed germination was studied. Under the high NaCl stress, the germination rates varied for different treatments, including control, 100 mmol L-1 NaCl alone, as well as NAA at 10 mg L-1 and 50 mg L-1, ethephon at 100 mg L-1 and 200 mg L-1, GA3 at 100 mg L-1 and 200 mg L-1, and 6-BA at 100 ug L-1 and 200 ug L-1 combined with 100 mmol L-1 NaCl, with rates ranging from 51.3% to 82.7%. When exposed to 100 mmol L-1 NaCl stress, the germination rate decreased by 37.8%, significantly lower than the control group, consistent with earlier findings. When subjected to pretreatments with NAA at concentrations of 10 mg L-1 and 50 mg L-1 before being exposed to 100 mmol L-1 NaCl stress, the germination rates were notably reduced compared to both the control group and the group exposed solely to 100 mmol L-1 NaCl. Compared to the control group with no treatment and exposure to 100 mmol L-1 NaCl, the germination rates of seeds treated with NAA (at concentrations of 10 mg L-1 and 50 mg L-1) decreased by 155%, 665%, 76.2%, and 455% respectively. On the other hand, compared to the control group without any treat-

Supplementary Table 3

Treatment	Germination rate	$p < 0.05$
H_2O	51.3% ± 1%	c
NaCl (50mmol L-1)	76% ± 3%	ab
NaCl (50mmol L-1)+NAA (10mg L-1)	18% ± 0%	d
NaCl (50mmol L-1)+NAA (50mg L-1)	9.3% ± 4%	d
NaCl (50mmol L-1)+Ethephon (100mg L-1)	83.3% ± 9%	ab
NaCl (50mmol L-1)+Ethephon (200mg L-1)	71.3% ± 7%	b
NaCl (50mmol L-1)+GA3 (100mg L-1)	82% ± 7%	ab
NaCl (50mmol L-1)+GA3 (200mg L-1)	83.3% ± 1%	ab
NaCl (50mmol L-1)+6-BA (100ug L-1)	88% ± 2%	a
NaCl (50mmol L-1)+6-BA (200ug L-1)	88% ± 5%	a
Treatment	**Germination rate**	**$p < 0.05$**
H_2O	51.3% ± 1%	d
NaCl (100mmol L-1)	37% ± 2%	e
NaCl (100mmol L-1)+NAA (10mg L-1)	20.7% ± 8%	f
NaCl (100mmol L-1)+NAA (50mg L-1)	6.7% ± 1%	g
NaCl (100mmol L-1)+Ethephon (100mg L-1)	80.7% ± 3%	ab
NaCl (100mmol L-1)+Ethephon (200mg L-1)	66.7% ± 3%	c
NaCl (100mmol L-1)+GA3 (100mg L-1)	88.7% ± 3%	a
NaCl (100mmol L-1)+GA3 (200mg L-1)	69.3% ± 4%	bc
NaCl (100mmol L-1)+6-BA (100ug L-1)	74.7% ± 2%	bc
NaCl (100mmol L-1)+6-BA (200ug L-1)	82.7% ± 8%	ab

ment, the seeds treated with ethephon (100 mg L-1 and 200 mg L-1), GA3 (100 mg L-1 and 200 mg L-1), and 6-BA (100 ug L-1 and 200 ug L-1) exhibited significantly higher germination rates under high levels of NaCl stress. And the germination rates increased 58.8, 31.4, 74.5, 35.3, 47.1 and 62.7% respectively (Figure 4, Supplementary Table 3).

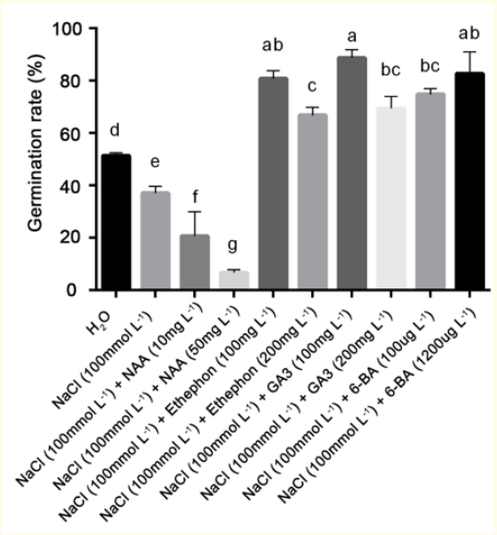

Figure 4: Effects of different phytohormones pretreatments and high concentration NaCl stress on the seed germination rates of common buckwheat seeds. Column shows means values of three replicates and bar shows the standard error of means. Different lowercase letters on each column indicate significant differences between treatments at $p < 0.05$.

In this assay, we investigated if various hormones (NAA, GA3, 6-BA, ethylene) could reduce the effects of salt stress by consulting relevant sources to determine suitable doses for treating common buckwheat seeds under varying NaCl stress conditions [11]. Exogenous ethephon, an ethylene releasing compound, has been reported to function as improving germination in many plant species. Under suboptimal environmental conditions like osmotic stress and salinity, it promotes the germination of seeds that are not in a dormant state when placed in an incubator [13,14]. The research found that exogenous ethephon, at a concentration of 100 mg L-1, exhibited increased resistance to salt stress and resulted in a higher number of germinated seeds compared to the control group in conditions with 50 mmol L-1 and 100 mmol L-1 NaCl. This result is consistent with Chang and Lin's reports [15,16]. That might be due to the effect of exogenous ethephon, which could reduce the accumulation of malondialdehyde (MDA) and H_2O_2 under NaCl stress, and increased germination percentage in common buckwheat.

As an essential endogenous signaling molecule, GA3 is playing important role in seed dormancy-breaking for many plants [17]. The chemical signal that triggers the production of amylase, breaking down starch in the endosperm and aiding in the growth of the embryo and germination of the seed [18]. During this research, the application of GA3 at various concentrations was found to mitigate the adverse effects of salt stress on germination in common buckwheat. The most effective GA3 concentration in mitigating the inhibitory effects of NaCl on buckwheat seed germination was 100 mg L-1, particularly when exposed to 100 mmol L-1 NaCl treatment. However, diverse outcomes have been documented for various other types of organisms. During cucumber seed germination, it was discovered that GA3 acted as an inhibitor [19]. The results emphasize that various species exhibit varying reactions to plant hormones. Typically, a high concentration of NaCl reduces water flow, hindering water movement in the radicle. The current research shows that treating buckwheat seeds with GA3 at varying concentrations (100 mg L-1 and 200 mg L-1) has a beneficial impact on seed germination, resulting in more seeds germinating under NaCl stress. This finding aligns with earlier studies [20]. The increased rate of seed sprouting after being treated with GA3 could be attributed to GA3's role in activating cellular enzymes, enhancing cell wall flexibility, and promoting water absorption [21].

In the process of seed germination, 6-BA can promote the functions of a-amylase and protease, facilitating the breakdown and transformation of stored substances, boosting the level of soluble sugars in the endosperm, and elevating the levels of soluble protein and free amino acids in the seed, all of which support the growth of seedlings [22]. Thus, it was anticipated that 6-BA would serve as a crucial component in promoting germination in common buckwheat seeds. Under NaCl stress, we found more germinated seeds had been determined when pretreated with 6-BA compared with control. Furthermore, the 200 ug L-1 6-BA pretreatment behaved the better effect to relieve NaCl suppression with the higher germination rate, no matter under low or high salt stress conditions (Supplementary Table 3). These results were similar as found in Betula platyphylla seed [23] and in Arabidopsis seed [24] germinations. Both researches showed higher germination rates when treated with exogenous 6-BA. The external application of 6-BA before the experiment could have influenced various biochemical processes, transmission of signals, movement of internal seed components, and production of numerous substances to promote seed germination under salt-induced stress.

NAA, an auxin compound, acts as a plant regulator with a wide range of effects, including stimulating cell growth and division, as well as triggering the development of adventitious roots [25]. Nevertheless, there have been limited studies examining the effects of

NAA on seed germination under NaCl-induced stress in common buckwheat. Based on our results, no matter whether there were low or high salt conditions, NAA at both tested concentrations remarkably inhibited common buckwheat germination when compared with control. Our findings differ from results reported in chickpea [26], but are consistent with Arabidopsis [27] and Betula platyphylla seeds [23]. In these experiments, using of NAA exacerbated the NaCl suppression impact on buckwheat seed. Na^+ inhibits the activities of numerous hydrolytic enzymes under conditions of salt stress, leading to a reduction in hydrolysis and the prevention of enzyme activation. Nevertheless, NAA is recognized for its involvement in cellular proliferation and growth. Due to the inhibition of cell division by NaCl, the breakdown of starch was prevented, leading to the inability to synthesize proteins and nucleic acids. It is also reported that exogenous auxin could repress seed germination by mediating ABA and GA biosynthesis [28]. During seed germination under salt stress, common buckwheat was more strongly inhibited by the increased NAA concentration of 50 mg L-1 in this research.

Conclusion

The rising levels of saline soil are causing salt stress to become a critical abiotic factor impacting seed germination in crop production. It is crucial for crops grown in salty conditions to have ways to mitigate the harmful impacts of salt stress and ensure successful seed germination. Supplements containing phytohormones like ethephon, GA3, and 6-BA may help reduce the adverse effects of salt stress on the germination of common buckwheat seeds. The recommended concentrations are 100 mg L-1 of ethephon, 100 mg L-1 of GA3, and 200 ug L-1 of 6-BA. To be practical, it is important to modify the levels of ethephon, GA3, and 6-BA based on the salt concentration in the soil. Applying ethephon, gibberellic acid (GA3), and benzyladenine (6-BA) can help reduce the negative effects of salt, preserve seed quality, and promote successful germination. In our study, NAA exacerbated the negative effects under salt stress on common buckwheat seed germination.

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Conflict of Interest

No conflict of interest.

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