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Original article

The Effects of Chitosan Applications on Seed Germing and Early Seedling Period of Red Beet (*Beta vulgaris* L.) Under Salty Conditions

Ayşe Nur Şavkan [a,*] & Ayşe Çandar [b]

[a] Pilot University Coordinatorship of Agriculture and Geothermal Energy, Rectorship, Kırşehir Ahi Evran University, Kırşehir, Türkiye
[b] Department of Crop and Animal Production, Cicekdagi Vocational School, Kırşehir Ahi Evran University, Kırşehir, Türkiye

Abstract

Salinity is a global problem and can significantly reduce agricultural productivity and cause negative effects on plant growth. Chitosan is a natural biopolymer and is known to have plant growth promoting and stress reducing properties. This study aimed to determine the effect of chitosan applications on seed germination and early seedling growth in red beet under saline conditions. In the experiment, seeds of red beetroot were soaked in chitosan and pure water at concentrations of 100-200-300 ppm for one hour. Then the seeds were allowed to dry for 24 hours and germinated at 22/24°C at 100 mM salt concentration for 10 days. In the study, various growth parameters such as seed germination rate, velocity and vigour as well as root and shoot length were determined. At the end of the experiment, it was found that hydropriming and chitosan treatments significantly improved the parameters observed in red beet seeds compared to the control. In particular, 300 ppm chitosan dose was effective on germination parameters, while 200 ppm chitosan dose was the most effective in reducing the negative effects of salt and increasing the growth parameters compared to control and hydropriming. These findings suggest that chitosan can be used as a potential growth promoter and protective agent for plants exposed to salt stress.

Keywords: Chitosan, Germination, Priming, Red Beetroot, Salt Stress, Seed.

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* **Corresponding author:**

Şavkan A.N. is a Lecturer Dr. in the Pilot University Coordinatorship of Agriculture and Geothermal Energy at Kırşehir Ahi Evran University in Kırşehir, Türkiye. Her research interests include the Horticulture, Agricultural Plant Science and Plant Breeding. She has lived, worked, and studied in Kırşehir, Türkiye.
Email: ayse.cetin@ahievran.edu.tr

INTRODUCTION

Plants are negatively affected by different abiotic and biotic stress conditions at many stages of their life cycle and are particularly susceptible to various abiotic stresses during critical periods such as germination and seedling growth. Salinity is one of the major abiotic stress factors limiting plant growth and production (Demir and Mavi, 2008; Liang et al., 2018). The problem of soil salinity affects more than 800 million hectares worldwide, corresponding to about 6% of the total agricultural area (Yang and Guo, 2018). Salt stress experienced during germination and early seedling development, which is one of the most important stages for a healthy plant, causes significant decreases in germination rate, germination rate, root and shoot length (Ibrahim, 2016). In studies carried out in many plant species, it has been found that germination of plant seeds grown under salt stress is delayed or inhibited, thereby significantly reducing germination (Bybordi and Tabatabaei, 2009; Wu et al., 2015; Ahmed et al., 2017). In this regard, new methodologies to be applied and/or developed on stress management in agricultural production are crucial. Many methods are used for stress management, such as developing resistant varieties, water and nutrient management, use of mycorrhizal fungi and plant growth-promoting bacteria, and pre-sowing seed treatments (Jha et al., 2019; Nadeem et al., 2019). Each of these methods is an important part of stress management. However, these methods usually require long processes and high costs, and therefore do not provide a practical and sustainable solution. But priming applications stand out among these methods as a low-cost, effective and easy-to-apply method for stress management, as well as being sustainable and eco-friendly (Sheteiwy et al., 2020; Johnson and Puthur, 2021).

Priming technique is based on keeping the seed in water or a solution with low osmotic potential for a certain period and temperature before sowing and re-drying. Priming treated seeds can be sown directly after drying or stored for long periods at low temperatures without losing the pragmatic effects of priming. In general, the most used priming techniques are listed as hydro-priming, osmopriming, halo-priming, solid matrix priming, hormonal priming, nano-priming and bio-priming. Priming technique contributes to the improvement of germination characters, promotion of plant growth and crop yield, and development of tolerance to various biotic and abiotic stress factors. Studies carried out to combine seed coating technology with priming technique are extremely important for a sustainable approach in agriculture (Ceritoğlu et al., 2021).

Chitosan is a biopolymer produced from waste materials such as seashells (Rinaudo, 2006). Recently, there has been an increase in research on chitosan (Shamov et al., 2002), which has many uses in many different areas of life such as medical sciences and plant sciences (Kaya et al., 2015a; Kaya et al., 2015b; Rinaudo, 2008). The most important reason for the increase in studies on the use of chitosan in plant cultivation is that chitosan is an eco-friendly approach due to its biocompatibility, effectiveness against abiotic stress, anti-microbial effect and non-toxicity (Dash et al., 2011; Shukla et al., 2013). In plant cultivation, chitosan is applied for a wide variety of purposes, usually as a foliar spraying, post-harvest and seed coating. When applied as a seed coating, chitosan increases seed germination ability, promotes germination and helps to improve the quality of seedlings produced (Shahrajabian and Petropoulos, 2023). In addition, chitosan applied to the seeds increases plant growth and helps to increase biomass by affecting parameters such as root length, shoot height, vegetative and seedling growth vigor (Lizárraga-Paulín et al., 2013).

Application of chitosan to the seed can also increase the activity of important resistance markers such as phenols and defense-related enzymes that provide resistance to biotic or abiotic stress in plants Chitosan, used in seed treatment to enhance seed germination and seedling emergence, has been found to increase water absorption and nutrient uptake by forming a protective coating around the seeds, thus promoting healthier and earlier growth of seedlings (Ziani et al., 2010). As can be understood from the previous studies, chitosan application to seeds positively affects the germination parameters of the seeds, and the tolerance of seedlings obtained from chitosan-treated seeds to abiotic and biotic stress conditions increases. In addition, chitosan, which is both economical and biocompatible, is thought to be a good alternative to produce vegetables such as red beet, which is produced by direct seed sowing in open land under abiotic stress conditions and has high salt sensitivity (Şavkan and Çandar, 2024), as it is an environmentally friendly approach and safer for our ecosystem. In this study, the effect of different concentrations of chitosan, one of the bio-pirming methods, on the emergence rate, emergence speed, emergence viability and morphological parameters of red beet seeds under effective salt stress conditions was evaluated.

MATERIALS and METHODS

Plant Material

Standard seeds of red beet (*Beta vulgaris* L.) with the code 1684 of Biogen Seed Company were used as plant material in the study. In this study, the effect of chitosan treatment on the emergence rate and early seedling growth of red beet seeds under salt stress (100 mM) conditions was investigated. In the study, NaCl was used to prepare the salt solution.

Seed Coating and Emergence Test

Plant seeds were surface sterilized first with 10% bleach and then with distilled water. In the germination experiment, 2% chitosan solution of Adaga Company was diluted to 100, 200 and 300 ppm to prepare different concentrations of chitosan solution. Seeds were soaked in the mentioned solutions and pure water for 1 hour and allowed to dry at room temperature for 24 hours (Zohara et al., 2019). Afterwards, 25 seeds were sown at equal intervals in 3 replicates in each of the plastic containers sized 12*9*6 cm (width*length*height) with lids together with the untreated (control) seeds. As salt treatment, 10 ml NaCl at a concentration of 100 mM was added to the plastic containers, while the same volume of pure water was used for the control groups to complete the humidification process. The plastic containers were then tightly closed to reduce evapotranspiration. Consistent environmental conditions were provided throughout the germination and early seedling stage to ensure reliability and reproducibility of the results.

Emergence experiments were carried out in plastic containers containing sterile porgrand H peat at 20/25°C (day/night) temperature (Khodarahmpour et al., 2012) for 10 days. The emergence rate of the seeds was determined daily and the emergence of 0.5 mm of radicle was considered as germination criterion (Abro et al., 2009; Datta et al., 2009). Emergence percentage values obtained on the 10th day were used to compare the emergence rates. At the end of the experiment, total emergence rate (Ologundudu et al., 2014), emergence velocity (Abazarian et al., 2011), seedling vigor index (emergence vigor) (Sivritepe, 2012) and salt tolerance index (Khayatnezhad and Gholamin, 2011) were determined. On the 10th day of emergence, shoot and root lengths (mm) of germinated seeds were determined with the help of a digital caliper, while fresh weights of roots and shoots were measured in mg on a precision balance (Shimadzu/AY220). Root and shoot fresh weights were determined by separating the rootlets and shoots of 10 randomly selected plants from each petri dish and measuring them on a precision balance.

Statistical Analysis

Statistical analysis was performed to compare the emergence and seedling growth parameters of soaking in water and chitosan treatments under salt stress conditions. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 21.0 statistical software (IBM. Chicago. IL. USA) at 5% and 1% level of significance (IBM. Chicago. IL. USA) to determine significant differences among treatments and the difference between means was determined by Duncan multiple comparison test.

RESULTS and DISCUSSION

Salinity stress which is an abiotic stress negatively affects crop production and significantly limits the germination and growth of plants. Salt ions in the soil cause negative effects on germination, growth and development of plants (Li et al., 2016, Zhou et al., 2016, Kang et al., 2017). Therefore, different organic substances have recently been preferred to increase the stress tolerance of plants and to support plant defense mechanisms (Bulut, 2020). One of these organic substances is chitosan (Bulut and Öztürk, 2023). Chitosan is also used as a plant growth regulator, to protect plants against abiotic stresses and for priming seeds. In this study, the effects of hydropriming and different chitosan doses applied to seeds under salt stress conditions, which are reported to be effective in red beet, on both seed germination parameters and early seedling stage were investigated. Statistically significant differences were found between all morphological parameters examined after hydropriming and chitosan treatments under effective salt stress conditions at 5% and 1% significance levels.

In our study, the emergence rate of red beet was observed as 73.34% and 27.13% as a result of salt application to control (0 min) and hydropriming groups, while the emergence velocity was 73.26% and 26.69%, respectively. The best result among the chitosan treatments was obtained at 300 ppm dose, which showed the least decrease in emergence rate by 4.93% and emergence velocity by 5.31% (Table 1). As a result, hydropriming application significantly increased the emergence rate and velocity (63%) compared to the control group (0 min), while 300 ppm chitosan application was found to be approximately 80% more effective compared to hydropriming application. These findings indicated that salt stress conditions had a negative effect on all morphological characteristics of red beet seeds compared to the control group and hydropriming and chitosan treatments contributed significantly to reduce the negative effects of salt stress. It was observed that the application of chitosan to the seeds before sowing regulated the germination velocity and increased the germination percentage and seed resistance to stress conditions in tomato (Saharan et al., 2015). Similarly, the effects of different chitosan doses on germination and growth parameters were investigated in wheat (Lian-Ju et al., 2014) and maize (Öner, 2023) and it was observed that chitosan significantly improved these parameters. These studies show that chitosan positively affects germination parameters in seeds and this biopolymer has a wide potential in agricultural applications.

Table 1. Effect of priming treatments on emergence rate and velocity under salt stress conditions.

Treatments	Emergence Rate (%)			Emergence Velocity (Day)		
	Control (0 ppm)	**Salt Stress (100 ppm)**	**Change (%)**	**Control (0 ppm)**	**Salt Stress (100 ppm)**	**Change (%)**
Control (0 min)	80,00d	21,33g	-73,34	4,60a	1,23g	-73,26
Hydropriming	86,00c	62,67f	-27,13	3,56c	2,61f	-26,69
100 ppm-Chitosan	94,67a	74,67e	-21,13	3,94b	3,11e	-21,07
200 ppm-Chitosan	86,60b	81,36cd	-6,05	3,59c	3,39cd	-5,57
300 ppm-Chitosan	81,36cd	77,35de	-4,93	3,39cd	3,21de	-5,31
Stress		**			**	
Treatment		**			**	
Stress*Treatment		**			**	

F values: $p < 0.05$ (*), $p < 0.01$ (**) and N.S. not significant.

In our study, because of salt stress applied to the control group (0 min), 76.81% and 95.22% decrease was observed in salt tolerance index and emergence vigor parameters, respectively. This result shows that salt stress has serious negative effects on plants. In hydropriming treatments, lower reductions of 26.57% and 32.24% were observed in salt tolerance index and emergence vigor parameters, respectively (Table 2). These results suggest that hydropriming improves the performance of plant seeds under salt stress. Because hydropriming accelerates the germination process by increasing the water intake of the seeds and makes the seeds more resistant to stress conditions (Ceritoğlu et al., 2021).

However, the best results were obtained in 300 ppm chitosan application. The 300 ppm chitosan treatment was found to be the least affected by salt stress with a decrease of 4.92% in salt tolerance index and 13.32% in emergence vigor (Table 2). In another study on the subject germination index and germination vigor of chili pepper seeds treated with chitosan significantly increased, average germination time decreased, and quality parameters of chitosan-treated seeds also improved (Chookhongkha et al., 2012). Chitosan is one of the biopolymers that stimulates growth and enhance the resistance mechanisms of plants against stress conditions (Lizárraga-Paulín et al., 2013; Shahrajabian and Petropoulos, 2023). Therefore, chitosan treatment made the plants more resistant to salt stress and hydropriming treatment showed a significant effect on salt tolerance index (82%) and emergence vigor (66%) compared to the control group (0 min). However, 300 ppm chitosan dose was determined to be more effective than the hydropriming treatment, resulting in an 80% improvement in salt tolerance index and 58% increase in emergence vigor.

Table 2. Effect of priming treatments on salt tolerance index (%) and emergence vigor under salt stress conditions.

Treatments	Salt Tolerance Index (%)			Emergence Vigor		
	Control (0 ppm)	Salt Stress (100 ppm)	Change (%)	Control (0 ppm)	Salt Stress (100 ppm)	Change (%)
Control (0 min)	100,00d	23,19g	-76,81	5773,85d	275,85e	-95,2224
Hydropriming	106,67bc	78,33f	-26,57	8367,13b	5669,62d	-32,24
100 ppm-Chitosan	118,33a	93,33e	-21,13	8531,49b	7393,38c	-13,34
200 ppm-Chitosan	108,63b	101,67cd	-6,41	9492,61a	7261,15c	-23,51
300 ppm-Chitosan	101,67cd	96,67de	-4,92	8207,98b	7113,57c	-13,31
Stress		**			**	
Treatment		**			**	
Stress*Treatment		**			**	

F values: $p < 0.05$ (*), $p < 0.01$ (**) and N.S. not significant.

Regarding shoot lengths, a decrease of 42.93% and 20.10% was observed as a result of salt stress applied to control (0 min) and hydropriming groups, respectively. When the treatments were evaluated among themselves, 200 ppm chitosan (4.12%) application showed the best result under stress conditions. As a result of salt application to control (0 min) and hydropriming groups, root lengths decreased by 35.49% and 13.76%, respectively. Among the treatments, the best result under stress conditions was observed in 200 ppm chitosan (5.38%) treatment and it was determined that it was less affected by salt stress (Table 3).

As a result, hydropriming treatment significantly increased shoot and root lengths compared to the control (0 min) and when compared with chitosan treatments, 200 ppm chitosan dose provided approximately 80% increase in shoot length and 61% in root length. In a similar study in which the effective chitosan dose was determined in tomato plants under different salt stress conditions, it was reported that plant length was suppressed as the dose of salt stress increased, the applied chitosan showed efficiency in length measurements against salt stress and the most effective chitosan dose in reducing salt stress was 150 ppm treatment (Bulut and Öztürk, 2023). In the studies investigating the effect of different doses of chitosan solution applied to wheat (Lian-Ju et al., 2014) and maize (Öner, 2023) seeds on root activities; it was determined that the highest dose of chitosan solutions applied provided the highest increase in root length. In a study conducted by Ziani et al. (2010) with artichoke seeds, it was observed that seedlings emerging after chitosan application to the seed had a longer and better developed radicle and greener hypocotyls, thus seedlings developing from chitosan-treated seeds grew better. In our study, different doses of chitosan applied to reduce the negative effects of salt stress on red beetroot plants contributed positively to the seedlings. As a matter of fact, the results of the studies given in the literature are compatible with our study and support the literature. These findings suggest that treatments such as chitosan and hydropriming have significant potential to improve plant growth and development parameters under salt stress.

Table 3. Effect of priming treatments on shoot and root lengths (mm) under salt stress conditions.

Treatments	**Shoot Length (mm)**			**Root Length (mm)**		
	Control (0 ppm)	**Salt Stress (100 ppm)**	**Change (%)**	**Control (0 ppm)**	**Salt Stress (100 ppm)**	**Change (%)**
Control (0 min)	31,30f	17,86g	-42,93	24,71c	15,94d	-35,49
Hydropriming	58,61a	46,83e	-20,1	40,99ab	35,35b	-13,76
100 ppm-Chitosan	57,59ab	55,04abc	-4,42	42,96a	40,51ab	-5,64
200 ppm-Chitosan	54,82a-d	52,56cd	-4,12	43,07a	40,75ab	-5,38
300 ppm-Chitosan	53,10bcd	50,84d	-4,25	42,04ab	38,44b	-8,56
Stress		**			**	
Treatment		N.S			N.S	
Stress*Treatment		**			**	

F values: $p < 0.05$ (*), $p < 0.01$ (**) and N.S. not significant.

In our study, 38.82% and 32.44% decrease was observed in shoot fresh weights because of salt stress applied to control (0 min) and hydropriming groups, respectively. Among the treatments, 200 ppm chitosan treatment had the best result under stress conditions with a 12.18% reduction. Root fresh weight decreased by 86.71% and 40.55% in the control (0 min) and hydropriming groups, respectively, as a result of salt treatment. Under stress conditions, the best result was obtained in 200 ppm chitosan application with 15.42% reduction (Table 4).

Table 4. Effect of priming treatments on shoot and root weights (g) under salt stress conditions.

Treatments	Shoot Fresh Weights (g)			Root Fresh Weights (g)		
	Control (0 ppm)	Salt Stress (100 ppm)	Change (%)	Control (0 ppm)	Salt Stress (100 ppm)	Change (%)
Control (0 min)	0,322e	0,197g	-38,82	0,0700c	0,0093e	-86,7143
Hydropriming	0,373c	0,252f	-32,44	0,0757c	0,0450d	-40,5548
100 ppm-Chitosan	0,404b	0,346d	-14,36	0,0950ab	0,0777c	-18,9474
200 ppm-Chitosan	0,435a	0,382bc	-12,18	0,1037a	0,0877b	-15,4291
300 ppm-Chitosan	0,392bc	0,343de	-12,5	0,0953ab	0,0713c	-25,1836
Stress		**			**	
Treatment		**			**	
Stress*Treatment		**			**	

F values: $p < 0.05$ (*), $p < 0.01$ (**) and N.S. not significant.

As a conclusion, hydropriming treatment caused a 16% increase in shoot fresh weight compared to the control (0 min) and was found to be 62% more effective compared to the effective chitosan dose. In root fresh weights, hydropriming treatment provided an increase of 53% compared to control (0 min), while the most effective result was obtained with an increase of 61% compared to 200 ppm chitosan dose. It has been reported in many literatures that shoot and root development of plants of different species are adversely affected under saline conditions (Şavkan and Çandar, 2024). In a study on rice, the effects of these interactions were investigated by adding chitosan to seed, leaf and growing medium and the highest growth parameters were obtained in the seed treatment and soil application interactions (Boonlertnirun et al., 2008). Similarly, five different doses of chitosan were applied to okra plants, and it was found that the highest chitosan dose was recommended for the most effective growth parameters (Mondal et al., 2012). In another study, 0, 125, 250 and 500 ppm concentrations of chitosan were applied to cucumber seeds to evaluate the effects of chitosan on seed germination, fresh root and shoot weight of seedlings emerging from treated seeds. As a result of the application, it was found that chitosan significantly ($p \leq 0.05$) increased seed germination, fresh root and shoot development of cucumber seeds depending on the dose (Zohara et al., 2019). It was determined that when different concentrations of chitosan were applied to tomato seeds, seed germination rate, germination vigor index increased; radicle and hypocotyls developed better, root and shoot development was better. In addition to the beneficial effects of chitosan on seed germination parameters, it was reported that seedlings obtained from chitosan-treated seeds showed more tolerance to salt stress than the control groups (Seth, 2023). Our study agrees with these literature data and shows that applied chitosan tends to improve the negative effects on both seedling and root development.

Conclusion

In this study, which represents an important step in expanding the use of chitosan as a stress-reducing agent in agriculture, investigated the potential benefits of chitosan treatment compared to hydropriming and control on germination and early seedling stage of red beet (*Beta vulgaris* L.) seeds under salt stress. It also provides invaluable information for the agricultural sector looking for alternative and environmentally friendly solutions for the management of salt-stressed crops. The study data show that hydropriming at 100 mM salt concentration significantly increased emergence and growth parameters compared to control (0 min). When compared among chitosan treatments, the most effective dose in reducing the negative effect of salt stress on emergence parameters was determined to be 300 ppm, while the most effective dose on seedling parameters was determined as 200 ppm chitosan. As a matter of fact, it is thought that the positive effect of 300 ppm chitosan dose on germination may be related to the soaking time of the seeds and that it promotes germination by corroding the seed coat. It is thought that the negative effects of 300 ppm dose on growth parameters can be reduced by adjusting this period in further studies. In addition, 100 and 200 ppm chitosan doses exhibited positive effects on plant growth parameters compared to both control and hydropriming, suggesting that this dose is in an optimal range for this plant species. In contrast, higher doses such as 300 ppm, demonstrated deviations from the expected benefits in certain cases. Therefore, it is important to carefully adjust the dosage of chitosan treatments and optimize it according to the plant species, developmental stage and environmental conditions.

In general, the results obtained in our study indicate that chitosan and hydropriming treatments improved the growth and development of red beet seedlings under salt stress and these treatments can be used in agricultural production to increase plant resistance to negative environmental conditions such as salt stress. Further research into the positive or negative effects of chitosan in agricultural applications and in the development of stressed plants will allow us to better understand the potential of this biopolymer in agricultural applications and develop more effective utilization strategies.

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