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RESEARCH ARTICLE

Alleviation of the Germination Inhibitory Effect of Salt Stress in Pepper (*Capsicum annuum* L.) Seeds by Serotonin

Oguzhan Araz • Ertan Yildirim* • Melek Ekinci

Atatürk University, Faculty of Agriculture, Department of Horticulture, Erzurum/Turkey

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ABSTRACT

Salinity stress is one of the important factors affecting all growth processes, from seed germination to seedling development, plant growth, yield and quality. In this study, the effects of serotonin treatments on the germination of pepper seeds (*Capsicum annum* L.) under salt stress were investigated. Different doses of salt (0, 75 and 150 mM NaCl) and serotonin (S0:0 µM, S1:5 µM, S2:10 µM, S3:15 µM, S4: 20 µM) were used. The applied seeds were placed between the papers in petri dishes, watered with the prepared salt solutions and left to germinate at 25 °C. In the study, parameters related to germination percentage, germination speed, mean germination time, daily mean germination time, peak value and germination value were investigated. As a result of the research, it was determined that the germination of pepper seeds decreased in parallel with increasing salt concentrations, and this negative effect of salt stress decreased with serotonin applications. Although it changes depending on the serotonin doses, it has been observed that significant effects occur on the measured germination parameters, and the best germination was observed at S1 and S2 doses. It is thought that the application of serotonin will have positive effects on the germination of pepper seeds under salt stress, and these effects may also occur during the plant growth period.

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Introduction

Plants are exposed to numerous stresses from seed germination to their entire life cycle. Salinity, which is one of these factors, affects all processes including seed germination, root, leaf, fruit and seed formation etc.

Salinity causes various morphological, physiological and biochemical reactions in plants by showing osmotic and ionic effects on plants. High salt concentration causes a decrease in the water potential in the soil and a decrease in the absorption of water by the plant roots. With the osmotic effect of salinity, water deficiency occurs and causes a decrease in water potential and turgor, fading, stomatal closure and decreased cell growth (Parihar et al., 2015; Ibrahimova et al., 2021). The ionic effect of salinity, on the other hand, changes the K^+/Na^+ ratio in plant cells, causing an increase in Na^+ concentrations and disruption of cellular homeostasis (James et al., 2011). With these effects, salinity can cause deterioration of plant metabolism and death of the plant.

Many researchers have done research on the effects of salinity stress on different plants, and it has been tried to determine the tolerance levels of plants to salt stress and ways to increase their tolerance. However, in recent studies, it has been focused on that some substances applied to plants can alleviate the damage caused by salt stress on the plant. The use of exogenous hormones to increase plant stress tolerance is one of these applications.

Serotonin (5-hydroxytryptamine; 5-HT) is an indoleamine (derived from tryptophan) with a strong antioxidant function, and it was first identified as a neurotransmitter signaling molecule in mammals and later determined to be present in all living things. Different amounts of serotonin accumulate in different parts of plants such as roots, stems, leaves, fruits and seeds (Abbasi et al., 2020; Roychoudhury, 2021). Serotonin is

* Corresponding author
E-mail address: ertanyil@atauni.edu.tr

involved in all vital activities in plants, affects plant growth and development, and also responds to biotic and abiotic stress in the plant (Erland et al., 2016; Roychoudhury, 2021). Serotonin, as a plant growth regulator and a stress defense molecule, affects plant morphogenesis, vegetative growth, reproduction, seed germination and ensures survival in abiotic and biotic stress conditions (Erland & Saxena, 2017).

The biosynthesis of serotonin in the plant takes place in the chloroplasts and mitochondria of various tissues, where highly reactive oxygen species (ROS) are produced in the tissue. With the antioxidative effect of serotonin, it detoxifies the ROS in the organelles and ensures better development (Roychoudhury, 2021).

The effects of serotonin in increasing tolerance or reducing the damage level against various abiotic stresses in plants have been determined in some plant species. In the studies, it was determined that the effect of serotonin in plants is important in the case of salt stress in sunflower (Mukherjee et al., 2014) and rapeseed (Liu et al., 2021), cold stress in rapeseed (He et al., 2021), and heat stress in soybean (Kumar et al., 2021). Exogenous application of 200 μmol/L serotonin in *Brassica napus* L. under salt stress alleviated the growth inhibition caused by salinity, increased the fresh and dry weights of roots and shoots, and activated the enzyme system (Liu et al., 2021). It has been determined that salinity prevents oxidative damage in the integrity of chlorophyll and cell membrane in the plant, with the application of serotonin, clearing the reactive oxygen species and osmotic pressure regulation, thus promoting growth (Liu et al., 2021). There are findings that serotonin application could alleviate the effects of salt stress on plants (Mukherjee et al., 2014; Liu et al., 2021). However, it is not known exactly what the effect of serotonin application is on alleviating the damage of salt stress in pepper. For this reason, the effects of serotonin application on the germination of pepper seeds under salt stress were investigated in this study.

Materials and Methods

Pepper (*Capsicum annuum* L. cv. Yalova) seeds were used as plant material in the study. Firstly, the seeds were kept in 3% sodium hypochlorite for 5 minutes for surface disinfection and then washed several times with distilled water. Surface disinfected seeds were kept in solutions of different serotonin (Sigma, CAS: 153-98-0) doses (S0: 0 μM, S1: 5 μM, S2: 10 μM, S3: 15 μM and S4: 20 μM) for 14 hours. Afterwards, the seeds were washed with distilled water and left to dry, and then placed between the paper in the petri dish. The seeds sown in petri dishes were irrigated with 10 ml solutions with different salt levels (I0: 0, I1: 75 and I2: 150 mM NaCl). The sown seeds were left to germinate at 25 °C. The study was performed in 4 replications for each application and 50 seeds in each replication according to ISTA (1996), and germination counts were started on the 7th day and completed on the 14th day. Germination percentage, germination speed, mean germination time, average daily germination, peak value and germination value were calculated by using the formulas given below by counting the germinated seeds every day (Czabator, 1962; Ellis & Roberts, 1981; Gairola et al., 2011).

$$Germination\ percentage\ (\%)(GP) = \left(\frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds}\right)\ x\ 100 \quad (1)$$

$$Germination\ speed\ (GS) = \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{s3} + \cdots \quad (2)$$

$$Mean\ germination\ time\ (MGT) = \frac{n1xd1 + n2xd2 + n3xd3 + \cdots}{Total\ days} \quad (3)$$

$$Mean\ daily\ germination\ (MDG) = \frac{Total\ number\ of\ germinated\ seeds}{Total\ days} \quad (4)$$

$$Peak\ value\ (PV) = \frac{Highest\ germination}{Number\ of\ days} \quad (5)$$

$$Germination\ value\ (GV) = PVXMDG \quad (6)$$

Where, n: number of germinated seeds, d: days.

After the Arcsin transformation of germination percentages, statistical analysis was performed. Variance analysis was made by SPSS. The average of the obtained data was taken and the comparison was made according to the Duncan Multiple comparison test at a confidence interval of 95.0% (SPSS, 2010).

Results and Discussion

In the study, the effects of serotonin treatments on seed germination properties under salt stress were investigated, and the results are given in Table 1 and Figure 1-5. Significant reductions in germination percentage, speed, daily germination, peak value and germination value have occurred with salt stress, and the mean germination time has increased (Table 1). When the application averages of serotonin were evaluated, the application of serotonin caused an increase in germination characteristics compared to the control without application, and significant increases in germination occurred especially with S1 and S2 applications (Table 1).

Table 1. Mean values of serotonin and salt treatment on parameters of seed germination

		GP	GS	MGT	MDG	PV	GV
Serotonin Mean	S0	83.50 c	2.60 b	13.32 ns	2.89 b	0.79 b	2.50 b
	S1	92.17 a	3.05 a	12.14	3.19 a	1.21 a	3.77 a
	S2	91.33 a	2.99 a	12.37	3.16 a	1.25 a	3.82 a
	S3	88.36 b	2.86 a	12.28	3.15 a	1.03 ab	3.66 a
	S4	88.31 b	2.91 a	12.47	3.12 a	0.86 b	3.53 a
Salt Mean	I0	89.80 A	3.12 A	11.70 B	3.19 A	1.33 A	4.36 A
	I1	90.60 A	3.01 A	12.48 B	3.12 AB	1.12 B	3.97 B
	I2	85.80 B	2.52 A	13.38 A	2.99 B	0.63 C	2.03 C

There is no statistical difference between means shown with the same lowercase and uppercase letters in the same column ($p<0.001$).
S0: 0 μM Serotonin, **S1:** 5 μM Serotonin, **S2:** 10 μM Serotonin, **S3:**15 μM Serotonin, **S4:** 20 μM Serotonin, **I0:** 0 mM NaCl, **I0:** 75 mM NaCl, **I0:** 150 mM NaCl

The germination percentage (GP) of pepper seeds decreased with salinity, and serotonin applications alleviated this negative effect on GP in salty conditions. In the highest salt (150 mM) condition, while the GP was 79% in control, it was 91% with S1, 91% with S2, 83.5% with S3 and 84.5% with S4 (Figure 1).

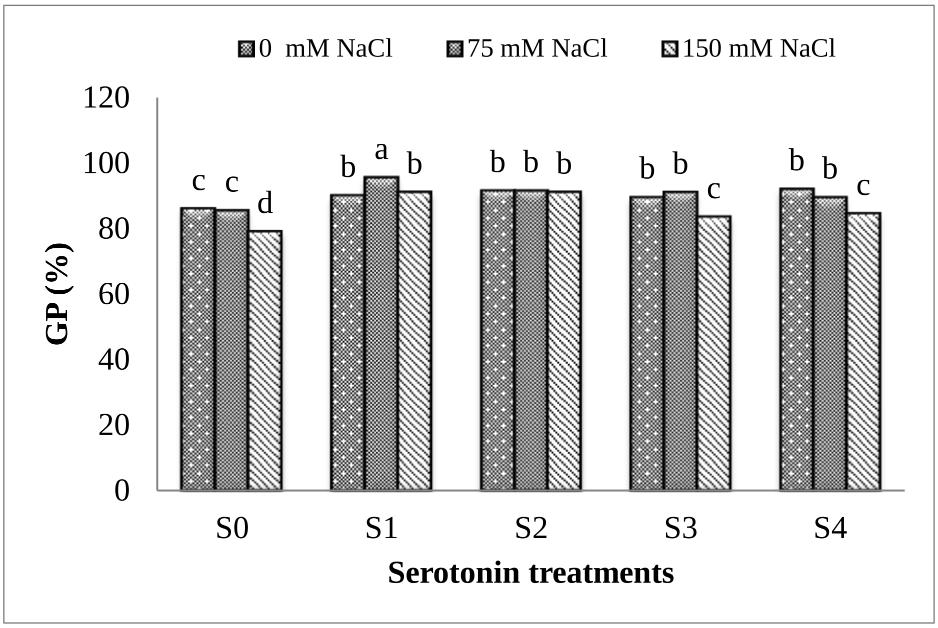

Figure 1. Effects of serotonin and salt treatment on seed germination percentage. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

In the study, it was determined that the germination speed (GS) decreased with salinity, and serotonin applications alleviated this negative effect on GS in salty conditions. While the GS was 2.11 in control, were 2.81, 2.82, 2.41 and 2.45 in the S1, S2, S3 and S4 treatments, respectively under 150 mM condition (Figure 2).

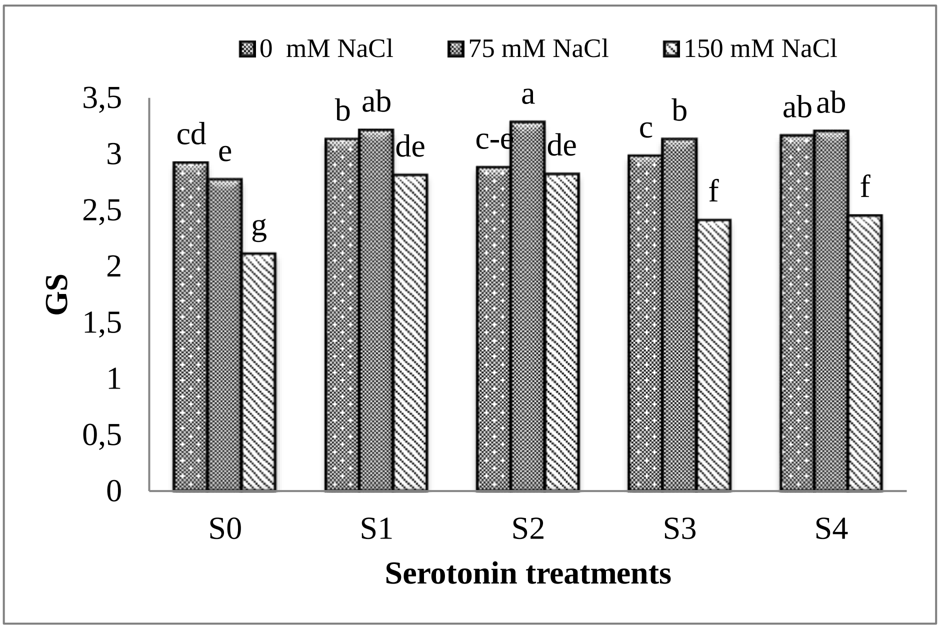

Figure 2. Effects of serotonin and salt treatment on seed germination speed. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

Mean germination time (MGT) increased with salinity, MGT was 11.37 at 0 mM NaCl, 12.08 at 75 mM NaCl and 13.76 at 150 mM NaCl. However, this increase in MGT was lower than the control with serotonin treatments. Especially in S3 application, MGT was lower in 150 mM salt environment compared to other treatments (Figure 3).

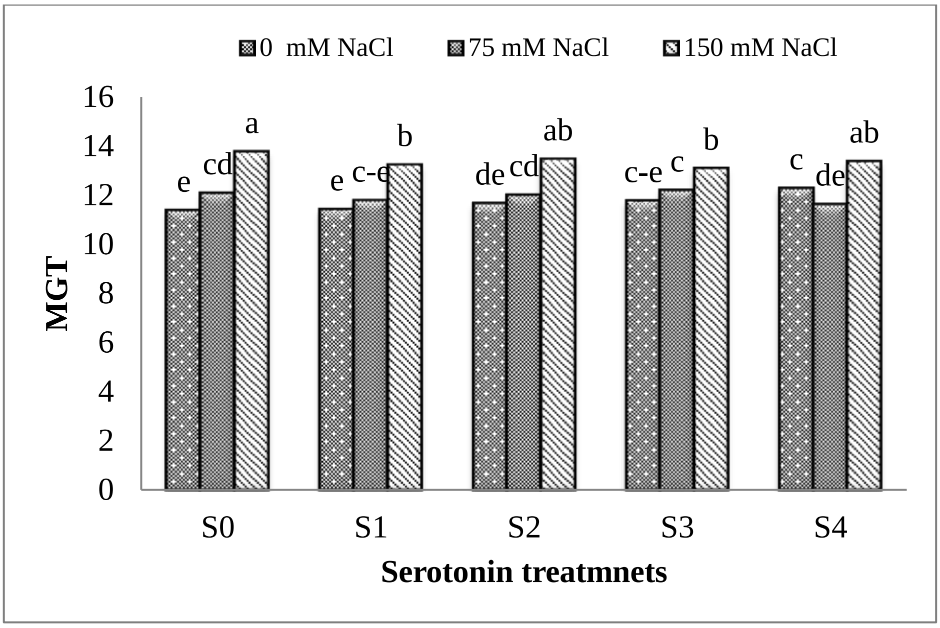

Figure 3. Effects of serotonin and salt treatment on mean germination time. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

In the study, it was determined that mean daily germination (MDG) decreased with salinity, and serotonin applications alleviated this negative effect on MDG in salty conditions. While MDG was 2.74 in control, were 3.07, 3.25, 2.96 and 2.93 in the S1, S2, S3 and S4 treatments, respectively under 150 mM condition (Figure 4).

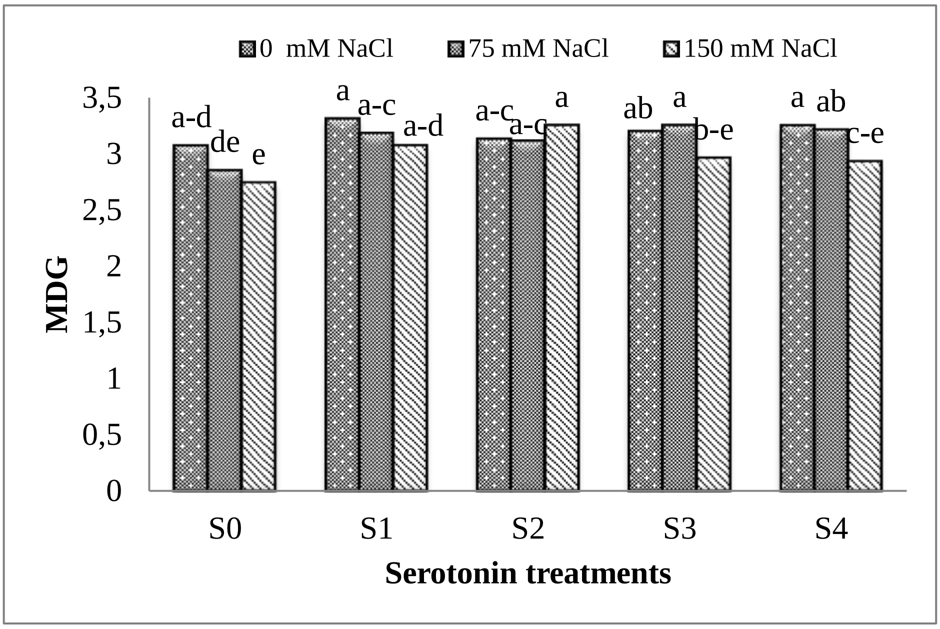

Figure 4. Effects of serotonin and salt treatment on mean daily germination. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

The peak value (PV) of pepper seeds decreased with salinity, and serotonin applications alleviated this negative effect on PV in salty conditions. PV was 1.15 at 0 mM NaCl, 0.95 at 75 mM NaCl and 0.28 at 150 mM NaCl. PV increased with increasing salinity in serotonin treatments, in the highest salt (150 mM) condition, PV was 0.73 with S1, 0.88 with S2, 0.58 with S3 and 0.67 with S4 (Figure 5).

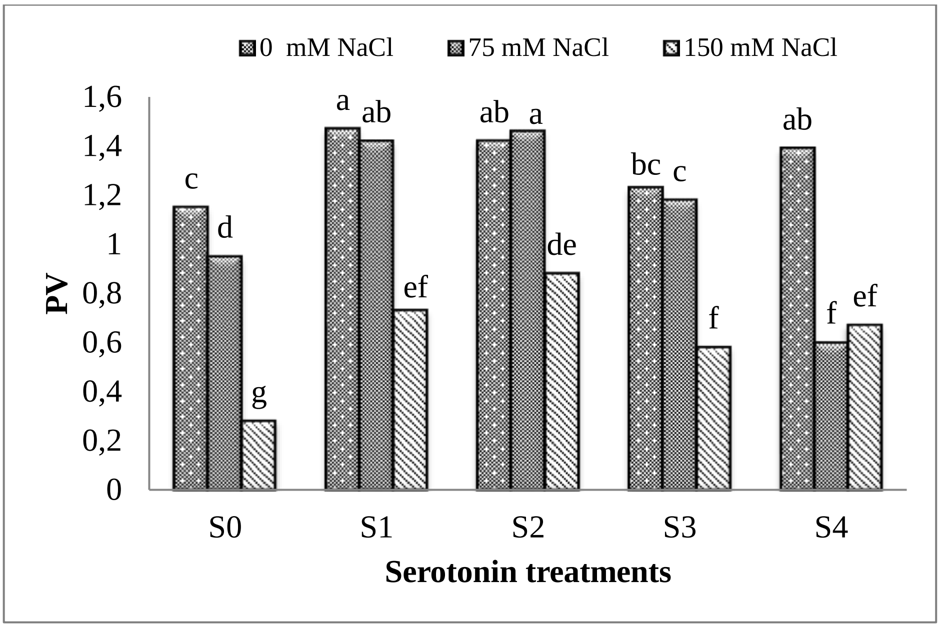

Figure 5. Effects of serotonin and salt treatment on peak value. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

Similarly, germination value (GV) of pepper seeds decreased with salinity, and serotonin applications alleviated this negative effect on GV in salty conditions. GV was 3.51 at 0 mM NaCl, 3.12 at 75 mM NaCl and 0.88 at 150 mM NaCl. GV increased with increasing salinity in serotonin treatments, in the highest salt (150 mM NaCl) condition, GV was 2.48 with S1, 2.85 with S2, 1.84 with S3 and 2.12 with S4 (Figure 6).

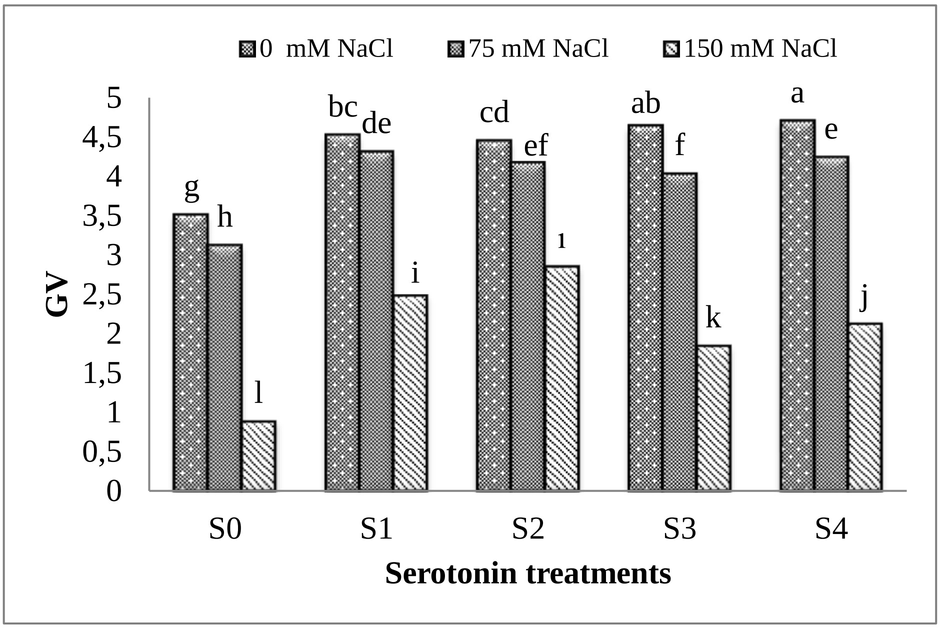

Figure 6. Effects of serotonin and salt treatment on germination value. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

There was a significant decrease in the germination properties of pepper seeds with salinity. This negative effect of salt stress on germination of pepper seeds was also determined by Yilmaz et al. (2004), Aloui et al. (2014) and Aminifard & Bayat (2020). Salt stress shows its effects on the germination and germination percentage, which is the first and most important stage of plant development, and causes changes in some important processes in plant development such as root dry weight, shoot dry weight, Na^+/K^+ ratio (Parida & Das, 2005). Salinity causes osmotic or ion toxicity and changes in enzyme activities in seed germination stage (Singh et al., 2012). However, it was determined that the negative effect of salt stress on germination was alleviated with the treatment of exogenous serotonin in this study. As a matter of fact, serotonin plays a role as a plant growth regulator at every stage of plant life such as germination, vegetative phase, reproduction and aging, and provides a defense or anti-stress effect with its antioxidative effect (Roychoudhury, 2021). As a plant growth regulator with cytokinin-like activity, serotonin is involved in various growth processes such as root and shoot formation, cell division and differentiation, germination, somatic embryogenesis and senescence (Roychoudhury, 2021). Serotonin can also provide salt stress tolerance by reducing ROS detoxification with the effect of antioxidant enzyme activity in the plant. It has been stated that serotonin provides its effect in reducing salinity in rapeseed seedlings by scavenging ROS, regulating osmotic pressure and promoting growth (Liu et al., 2021). Similarly, serotonin, with its ability to mediate the influx of ions into the chloroplasts, has been suggested to have the capacity to improve survival in response to salinity challenge (Erland & Sexana, 2017).

Conclusion

The effects of serotonin application on the germination of pepper seeds under salt stress were investigated. The germination of pepper seeds decreased in parallel with increasing salt concentrations, and this negative effect of salt stress decreased with serotonin applications. Although it is known that serotonin is involved in various metabolic, physiological and biochemical processes in plants and has an effect against various stresses in plants, its effect on abiotic stresses, especially salt stress, is not well known. In this study, it is concluded that serotonin could alleviate the effects of salinity in pepper seeds. No study has been found on the application of serotonin in salt stress in pepper. Therefore, the data obtained from this study can be a reference for other studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

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