BAHÇE 54 (1): 25-33 (2025)
https://doi.org/10.53471/bahce.1603329

Cyclic 3-Hidroksimelatonin'in Tuz ve Su Stresi Altında Biber Tohumu Çimlenmesi Üzerine Etkileri

Aygül KARACA*

Bingöl Üniversitesi, Gıda, Tarım ve Hayvancılık Meslek Yüksekokulu, Bingöl; ORCID: 0000-0001-9142-9678
Gönderilme Tarihi: 17 Aralık 2024 *Kabul Tarihi: 4 Mart 2025*

ÖZ

Abiyotik stres koşulları tohum kalitesini bozarak, tohumun canlılığını ve çimlenme potansiyelini azaltmaktadır. Bu nedenle, abiyotik stres koşulları altında tohum çimlenmesini iyileştirmek önemlidir. Melatonin'in bitkilerde çeşitli abiyotik stres faktörlerine karşı tolerans kazanılmasında etkin rol aldığı ortaya konmuştur. Ancak, melatonin metabolitlerin bitkilerdeki olası rolleri üzerinde sınırlı sayıda çalışma yürütülmüş ve sonuçlar bu metabolitlerinde melatonin benzeri görevlere sahip olduğu yönündedir. O nedenle bu çalışmada bir melatonin metaboliti olan cyclic 3-hidroksimelatoninin (3-ohm) stres koşulları altında (kuraklık ve tuz stresi) tohum çimlenmesi üzerine etkileri araştırılmıştır. Bu amaca yönelik değişik konsantrasyonlarda (0 µM, 10 µM, 50 µM ve 100 µM) 3-ohm uygulanmış biber tohumları, tuz (0, 75, 100 ve 125 mM NaCl) ve su (0, 75 ve 100 gL^{-1} PEG 6000) stresleri altında çimlenme testine tabi tutulmuştur. Çalışma sonucunda 3-ohm uygulamalarının tüm konsantrasyonlarının, tuz ve su stresi altındaki çimlenme testine tabi tutulan biber tohumlarının çimlenme yüzdelerini, çimlenme üniformitesini, çimlenme hızını, kökçük uzunluğu ve vigor indeksini kontrol uygulamasına kıyasla arttırdığı gözlenmiştir. Ayrıca 3-ohm'in biberde çimlenme aşamasında tuz ve su streslerine karşı toleransı arttırmada melatonin benzeri fonksiyona sahip olduğu ve antioksidan olarak görev yaparak stres toleransının artmasında aktif rol aldığı sonucunu ortaya çıkarmıştır.

Anahtar Kelimeler: Cyclic 3-hidroksimelatonin, tuzluluk stresi, su stresi, RNS, ROS, vigor indeksi

Effects of Cyclic 3-hydroxymelatonin on Pepper Seed Germination Under Salt and Water Stress

ABSTRACT
Abiotic stresses reduce seed germination by increasing deterioration of seed quality, reducing germination potential and seed viability. Therefore, it is important to improve seed germination under abiotic stress conditions. Melatonin has been shown to play an active role in the acquisition of tolerance to various abiotic stress factors in plants. However, limited studies have been conducted on the possible roles of melatonin metabolites in plants and the results suggests that these metabolites may also function similarly to melatonin. Therefore, in this study, the effects of cyclic 3-hydroxymelatonin (3-ohm), a melatonin metabolite, on seed germination under stress conditions (drought and salt stress) were investigated. For this purpose, pepper seeds treated with 3-ohm at different concentrations (0 µM, 10 µM, 50 µM and 100 µM) were subjected to germination test under salt (0, 75, 100 and 125 mM NaCl) and water (0, 75 and 100 gL^{-1} PEG 6000) stresses. As a result of the study, all concentrations of 3-ohm treatments increased the germination percentage, germination uniformity, germination rate, root length and vigor index of pepper seeds subjected to germination test under salt and water stress compared to control treatment. It was also concluded that 3-ohm has a melatonin-like function in increasing tolerance to salt and water stresses at the germination stage in pepper and plays an active role in increasing stress tolerance by acting as an antioxidant.

Keywords: Cyclic 3-hydroxymelatonin, salinity stress, water stress, RNS, ROS, vigour index

INTRODUCTION

Pepper plant belongs to the genus Capsicum of the *Solanaceae* family and is an annual crop in temperate climates and a perennial crop in tropical climates [1]. Drought stress causes severe reductions in crop productivity by reducing plant water uptake, leaf water content and gas exchange rates [2]. Salt stress disrupts the osmotic and ionic balance of plants, negatively affecting their growth and development and causing many damages [3]. In order to reduce the negative effects of these stress factors in cultivation, many plant growth regulators are the subject of research. One of them is melatonin and the metabolites released as a result of the breakdown of melatonin.

Seed germination is the first stage in the life of a plant and is of great importance in sustaining crop production. Abiotic stresses reduce seed germination by increasing seed quality deterioration, reducing

*Sorumlu yazar / *Corresponding author:* akaraca@bingol.edu.tr

germination potential and seed viability. Therefore, to achieve a sustainable level of crop yield, it is important to improve seed germination under abiotic stress conditions [4]. Melatonin, a potent source of antioxidants, has been reported to increase plant resistance to stress by directly or indirectly scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced under biotic and abiotic stressors such as low temperature [5], high temperature [6], drought [7], salinity [8] and oxidative stresses [9, 10, 11].

As a result of the researches on melatonin, which is a circadian rhythm sensor and antioxidant source, the fact that melatonin has many positive features about its physiological roles has led researchers to study on melatonin metabolites. Several metabolites of melatonin have been detected in plants, including 2-hydroxymelatonin (2-ohm), cyclic-3-hydroxymelatonin (3-ohm), and N^1-acetyl-N^2-formyl-5-methoxyquinuramine (AFMK) [12, 13]. It has been shown that melatonin is converted into AFMK by indoleamine 2,3-dioxygenase (IDO), into 2-hydroxymelatonin (2-ohm) by 2-hydroxylase (M2H) and into 3-ohm by 3-hydroxylase (M3H) [14]. In a study, they reported that exogenous melatonin application led to the production of several melatonin metabolites in paddy seedlings, including 2-ohm, 3-ohm and AFMK, and N^2-acetyl-5-methoxyquinuramine (AMK), suggesting the presence of a melatonin metabolism pathway. Furthermore, it has been demonstrated that, similar to melatonin, the amounts of 2-ohm and 3-ohm metabolites in tissues exhibit a diurnal rhythm that peaks at night [15]. In a study on the protection of melatonin and its precursors and metabolites against oxidative stress, it was reported that N-acetylseratonin (N-Ser), one of the precursors of melatonin, and 6-ohm, a melatonin metabolite, were more effective than melatonin in eliminating peroxyl free radicals [16].

It has been reported that the two dominant melatonin metabolites found in terrestrial plants are 3-ohm and 2-ohm [17] and that the enzymes involved in the synthesis of these metabolites (M2H and M3H) belong to the 2-oxoglutarate-dependent dioxygenase (2-ODD) superfamily [18, 19]. It was revealed that 2-ohm increased the tolerance of cucumber and tomato seedlings to both cold and water stresses when applied separately and together [20]. Similarly, it was reported that paddy seedlings treated with 2-ohm provided tolerance to stress factors in plants when cold and water stresses were applied together [21; 20]. In a study investigating the effects of 2-ohm on seed germination, it was reported that the germination of dormant Arabidopsis seeds treated with 2-ohm was significantly increased compared to control seeds [22]. Korkmaz et al. [23] reported that 2-ohm had a melatonin-like function in increasing tolerance to salt, water and chilling stresses during germination and seedling emergence in pepper and played an active role in increasing stress tolerance by acting as an antioxidant. Shah et al. [24] reported that 4-ohm reduced the effect of nickel stress on the roots and shoots of eggplant seedlings and increased their resistance to stress. In a study with rice plants, M3H genes that catalyze melatonin to 3-ohm via 2-ODD were cloned and 3-ohm was found in high amounts in melatonin-treated leaves. These results showed that melatonin is metabolized to 3-ohm by M3H genes in plants [25]. In addition, in a limited number of studies, it has been reported that 3-ohm responds to oxidative stress in adverse environmental conditions and exhibits high antioxidant activity against the harmful effects caused by this stress [15, 26, 27].

3-ohm, an important metabolite produced through melatonin interaction with oxygenated compounds, is thought to have an active role in increasing tolerance to various abiotic stress factors. However, there is a very limited number of studies on the physiological functions of 3-ohm in plants and no studies on its effects on seed germination, seedling emergence and yield were found in the literature. Therefore, in this study, its effects on germination of pepper seeds under various stress factors (drought and salinity stresses) were investigated. Pepper seeds exposed to drought and salt stresses were subjected to 3-ohm treatments and the possible effects of the treatments on seed germination under stress conditions were revealed. In addition, it will be an advantage for future studies to complete the deficiencies in the literature by revealing the effects of 3-ohm, a melatonin metabolite, on application doses and abiotic stress factors.

MATERIALS AND METHODS

Plant Material and Treatments

'Sera Demre' pepper seeds were superficially disinfected in water containing 1% (active ingredient) sodium hypochlorite for 10 minutes to eliminate microorganisms that could be carried on the seeds, washed in plenty of water and dried superficially on blotting paper for 2 hours under room conditions. For 3-ohm treatment, the superficially dried pepper seeds were kept at 20°C for 24 hours on double-layer blotting paper containing 30 mL of 0, 10, 50 and 100 µM 3-ohm in plastic containers. Control seeds (0 µM) were kept in pure water only. The seeds were then washed under tap water and dried on blotting paper for one day and subjected to germination tests under optimum conditions and salinity and water stress conditions.

Germination Test

Germination tests were carried out in 10 cm diameter petri dishes with 4 replicates and 50 seeds in each replicate in the dark. Seeds treated with different concentrations (10, 50 and 100 µM) of 3-ohm and control (0 µM 3-ohm) seeds treated with pure water were included in the experiment. In the water stress treatment, 5 ml of 0 gL^{-1} PEG 6000, 75 gL^{-1} PEG 6000, 100 gL^{-1} PEG 6000 solution (water stress) and 3-ohm concentrations were used in each petri dish, totaling 48 petri dishes. In the salt stress treatment, a total of 64 petri dishes were used with 0 mM NaCl, 75 mM NaCl, 100 mM NaCl and 125 mM NaCl solution (salt stress) and 3-ohm concentrations. For germination tests in water stress and salt stress treatments, the petri dishes were placed in a climate cabinet the at 25℃ in darkness (relative humidity: 65±5%). Petri dishes were counted daily during germination tests. Germination tests continued until germination became stable and lasted for 16 days under salt and water stress conditions. During this period, petri dishes were irrigated equally with appropriate solutions as needed. The appearance of the root (1 mm) was considered sufficient for germination, and germination continued until the number of germinated seeds became constant. Using Seed Germination Software v 1.0, the final germination percentage (FGP), mean germination time (MGT), and germination uniformity (G_{10-90}) an inverse indicator of germination rate, calculated based on the total number of germinated seeds. Root length and vigor index were also calculated after germination test. Root length (RL) was determined on 5 randomly selected seeds in each petri dish using a digital caliper. Vigor index was calculated by the formula (root length + shoot length) × germination percentage" [28].

Statistical Analysis

The experiment was established according to the randomized plot experimental design with 4 replications. SPSS 20.0 package program two-way Anova and Duncan Multiple Range Test (5%) (to determine the differences between group averages) were used in the evaluation of the data obtained as a result of the research.

RESULTS

Germination Test of Seeds Under Salt Stress

The effects of 3-ohm treatments on FGP, MGT, G_{10-90}, RL and vigor indices of pepper seeds after germination test under salt stress (75 mM NaCl) and optimum conditions are shown in Table 1. It was determined that salt stress had a significant effect on the germination percentage of the seeds and the germination percentage, which was 98.4 under optimum conditions, decreased to 97.0 under the effect of stress. It was also observed that 3-ohm applied to the seeds also had a significant effect on germination percentage and seeds treated with 3-ohm at a concentration of 50 µM (98.3%) had a higher germination percentage compared to the 0 µM treatment (96.8%).

The effects of both stress main factor and 3-ohm concentration main factor on MGT were statistically significant, but the interaction effect of both main factors was found to be insignificant. In Table 1, salt stress negatively affected the mean germination rates of the seeds and the mean germination rate of seeds germinated in 0 mM NaCl conditions increased from 6.60 days to 8.10 days due to the effect of stress. As a result of 3-ohm treatments, 10 µM, 50 µM and 100 µM treatments positively affected the germination rates of the seeds and decreased the MGT value from 7.69 days to 7.12, 7.31 and 7.30 days, respectively.

Salt stress negatively affected the germination uniformity of the seeds and the germination uniformity, which was 2.94 days in seeds germinated under 0 mM NaCl conditions, increased to 3.32 days with the effect of stress (Table 1). Although it was not statistically significant, among the 3-ohm treatments, 10 µM treatment positively affected the germination uniformity of the seeds and decreased the G_{10-90} value from 3.36 days to 2.82 days in 0 µM treated seeds.

When the effects of salt stress and 3-ohm treatments on the RL of pepper seeds were examined, it was observed that the main factors were significant but their interaction effect was insignificant (Table 1). RL, which was 7.47 cm under optimum conditions, decreased to 5.13 cm under the effect of salt stress. Among the 3-ohm treatments at different concentrations, the smallest root length was obtained from 0 µM treatment (5.79 cm), whereas the root lengths of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher (6.86 cm, 6.63 cm, and 5.90 cm, respectively).

When the effects of salt stress and 3-OHM treatments on vigor index of pepper seeds were examined, it was observed that the main factors were significant but their interaction effect was insignificant. The vigor index, which was 735.06 under optimum conditions, decreased to 497.38 under the effect of salt stress. Among the 3-ohm treatments at different concentrations, the lowest vigor index was obtained from 0 µM treatment. The vigor indices of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher (Table 1).

Table1. Effects of salt stress (75 mM NaCl) and 3-ohm treatments on germination parameters of seeds

Treatments		FGP (%)	MGT (day)	$G_{10\text{-}90}$ (day)	RL (cm)	Vigor index
NaCl (mM)						
0	-	98.4 a	6.60 b	2.94	7.47 a	735.06 a
75	-	97.0 b	8.10 a	3.32	5.13 b	497.38 b
3-ohm (µM)						
0	-	96.8	7.69 a	3.36	5.79 b	560.55 c
10	-	98.0	7.12 b	2.82	6.86 a	672.93 a
50	-	98.3	7.31 b	3.19	6.63 ab	653.01 ab
100	-	97.8	7.30 b	3.16	5.90 b	578.39 bc
NaCl×3-ohm						
0 mM	0 µM	97.5	6.80	3.06	6.83	665.10
	10 µM	98.5	6.37	2.74	8.40	826.75
	50 µM	99.5	6.59	2.97	7.64	757.28
	100 µM	98.5	6.67	2.98	7.01	691.13
75 mM	0 µM	96.0	8.57	3.65	4.75	456.00
	10 µM	97.5	7.87	2.89	5.33	519.10
	50 µM	97.5	8.04	3.40	5.63	548.75
	100 µM	97.0	7.94	3.34	4.80	465.65
Sign.						
NaCl (mM)	-	*	***	N.S.	***	***
3-ohm (µM)	-	N.S.	***	N.S.	*	*
NaCl×3-ohm	-	N.S.	N.S.	N.S.	N.S.	N.S.

Sign. (significant), NS, *, **, ***, not significant, significant at P < 0.05, 0.01 or 0.001, respectively

The effects of 3-ohm treatments on FGP, MGT, $G_{10\text{-}90}$, RL and vigor indices of pepper seeds after germination test under salt stress (100 mM NaCl) and optimum conditions are shown in Table 2. It was determined that salt stress had a significant effect on the FGP of the seeds and the germination percentage, which was 98.4 under optimum conditions, decreased to 96.5 under the effect of stress. It was also observed that 3-ohm applied to the seeds had a higher effect on the germination percentage of seeds treated with 3-ohm at a concentration of 50 µM (98.0%) compared to the 0 µM treatment (96.5%).

The effects of both stress main factor and 3-ohm concentration main factor on MGT were statistically significant, but the interaction effect of both main factors was found to be insignificant (Table 2). Salt stress negatively affected the mean germination rate of the seeds and the mean germination rate of the seeds germinated under 0 mM NaCl conditions increased from 6.60 days to 8.33 days due to the effect of stress. As a result of 3-ohm treatments, 10 µM, 50 µM and 100 µM treatments positively affected the germination rates of the seeds and decreased the MGT value from 7.76 days to 7.16, 7.45 and 7.50 days, respectively.

When the effects of salt stress and 3-ohm treatments on RL and vigor index of pepper seeds were examined, it was observed that the main factors were significant but their interaction effect was insignificant. In Table 2, the RL, which was 7.47 cm under optimum conditions, decreased to 3.86 cm under the effect of salt stress. Among the 3-ohm treatments at different concentrations, the smallest RL was obtained from 0 µM treatment (5.04 cm), whereas the root lengths of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher (6.14 cm, 5.95 cm, and 5.54 cm, respectively). The vigor index, which was 735.06 under optimum conditions, decreased to 372.99 under the effect of salt stress. Among the 3-ohm treatments at different concentrations, the lowest vigor index was obtained from 0 µM treatment, whereas the vigor indices of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher.

Table 2. Effects of salt stress (100 mM NaCl) and 3-ohm treatments on germination parameters of seeds

Treatments		FGP (%)	MGT (day)	$G_{10\text{-}90}$ (day)	RL (cm)	Vigor index
NaCl (mM)						
0	-	98.4 a	6.60 b	2.94 b	7.47 a	735.06 a
100	-	96.5 b	8.33 a	3.52 a	3.86 b	372.99 b
3-ohm (µM)						
0	-	96.5	7.76 a	3.69	5.04 b	487.80 b
10	-	97.8	7.16 c	2.86	6.14 a	601.25 a
50	-	98.0	7.45 b	3.20	5.95 a	584.89 a
100	-	97.5	7.50 b	3.17	5.54 ab	542.16 ab
NaCl×3-ohm						
0 mM	0 µM	97.5	6.80	3.06	6.83	665.10
	10 µM	98.5	6.37	2.27	8.40	826.75
	50 µM	99.0	6.59	2.97	7.64	757.28
	100 µM	97.5	6.67	2.98	7.01	691.13
100 mM	0 µM	95.5	8.72	4.32	3.25	310.50
	10 µM	97.0	7.95	2.97	3.88	375.75
	50 µM	97.0	8.31	3.43	4.25	412.50
	100 µM	96.5	8.34	3.36	4.08	393.20
Sign.						
NaCl (mM)	-	**	***	*	***	***
3-ohm (µM)	-	N.S.	***	N.S.	*	*
NaCl×3-ohm	-	N.S.	N.S.	N.S.	N.S.	N.S.

Sign. (significant), NS, *, **, ***, not significant, significant at P < 0.05, 0.01 or 0.001, respectively

Table 3 shows the effects of salt stress (125 mM NaCl) on FGP, MGT, $G_{10\text{-}90}$, RL and vigor index of pepper seeds after 3-ohm treatments and after germination test under optimum conditions. It was determined that salt stress had a significant effect on the germination percentage of the seeds and the germination percentage, which was 98.4% under optimum conditions, decreased to 95.5% under the effect of stress. It was also observed that 3-ohm applied to the seeds also had an effect on germination percentage, with seeds treated with 3-ohm at a concentration of 50 µM (97.5%) having a higher germination percentage compared to the 0 µM treatment (96.0%).

The effects of both stress main factor and 3-ohm concentration main factor on MGT were statistically significant, but the interaction effect of both main factors was found to be insignificant. Table 3 shows that salt stress negatively affected the mean germination rates of the seeds and the mean germination rate of seeds germinated under 0 mM NaCl conditions increased from 6.60 days to 8.58 days due to the effect of stress. As a result of 3-ohm treatments, 10 µM, 50 µM and 100 µM treatments positively affected the germination rates of the seeds and decreased the MGT value from 7.81 days to 7.29, 7.60 and 7.66 days, respectively.

Table 3. Effects of salt stress (125 mM NaCl) and 3-ohm treatments on germination parameters of seeds

Treatments		FGP (%)	MGT (day)	G_{10-90} (day)	RL (cm)	Vigor index
NaCl (mM)						
0	-	98.4 a	6.60 b	2.94 b	7.47 a	735.06 a
125	-	95.5 b	8.58 a	3.71 a	1.80 b	172.30 b
3-ohm (µM)						
0	-	96.0	7.81 a	3.80	3.79 b	368.05 b
10	-	97.3	7.29 c	3.00	5.20 a	509.38 a
50	-	97.5	7.60 b	3.29	4.98 a	490.24 a
100	-	97.0	7.66 ab	3.21	4.57 ab	447.06 a
NaCl×3-ohm						
0 mM	0 µM	97.5	6.80	3.06	6.83	665.10
	10 µM	98.5	6.37	2.74	8.40	826.75
	50 µM	99.0	6.59	2.97	7.65	757.28
	100 µM	98.5	6.67	2.98	7.01	691.13
125 mM	0 µM	94.5	8.83	4.55	0.75	71.00
	10 µM	96.0	8.20	3.26	2.00	192.00
	50 µM	96.0	8.62	3.60	2.33	223.20
	100 µM	95.5	8.65	3.43	2.13	203.00
Significant						
NaCl	-	***	***	***	***	***
3-ohm (µM)	-	N.S.	***	N.S.	**	**
NaCl×3-ohm	-	N.S.	N.S.	N.S.	N.S.	N.S.

Sign. (significant), NS, *, **, ***, not significant, significant at $P<0.05$, 0.01 or 0.001, respectively

When the effects of 3-ohm treatments on G_{10-90} of pepper seeds under salt stress and optimum conditions were examined, it was observed that only the effect of salt stress was significant, but the interaction effects of 3-ohm treatments and the two main factors were found to be insignificant (Table 3). Salt stress negatively affected the germination uniformity of the seeds and the average value of 2.94 days in seeds germinated under 0 mM NaCl conditions increased to 3.71 days with the effect of stress.

When the effects of salt stress and 3-ohm treatments on RL and vigor index of pepper seeds were examined, it was observed that the main factors were significant but their interaction effect was insignificant. When the effects of salt stress and 3-ohm treatments on the RL of pepper seeds were examined, the RL, which was 7.47 cm under optimum conditions, decreased to 1.80 cm under the effect of salt stress. Among the 3-ohm treatments at different concentrations, the smallest RL was obtained from 0 µM treatment (3.79 cm), whereas it was significantly higher in seeds treated with 10 µM, 50 µM and 100 µM 3-ohm (5.20 cm, 4.98 cm, and 4.57 cm, respectively).

When the effects of salt stress and 3-ohm treatments on the vigor index of pepper seeds were examined, the vigor index, which was 735.06 under optimum conditions, decreased to 172.30 under the effect of salt stress. Among the 3-ohm treatments at different concentrations, the lowest vigor index was obtained from 0 µM treatment, whereas it was significantly higher in seeds treated with 10 µM, 50 µM and 100 µM 3-ohm.

Germination Test of Seeds Under Water Stress

The effects of 3-ohm treatments on FGP, MGT, G_{10-90}, RL and vigor index of pepper seeds after germination test under water stress (75 gL^{-1} PEG) and optimum conditions are presented in Table 4. When the results on the effects of water stress on FGP of seeds were analyzed, the main factors, stress and 3-ohm treatments and the interaction relationship between them were found statistically insignificant. However, water stress negatively affected the FGP of the seeds and the germination percentage decreased from 98.1% under optimum conditions to 97.4% under the effect of stress. Moreover, seeds treated with all 3-ohm concentrations had higher germination percentages compared to 0 µM treatment.

The effects of stress and 3-ohm treatments on MGT were statistically significant, but the interaction effect of both main factors was found to be insignificant. When the data presented in Table 4 are analyzed, water stress negatively affected the germination rates of the seeds and the germination rate, which was 6.59 days under optimum conditions, increased to 7.46 days with the effect of stress. As a result of 3-ohm treatments, 10 µM and 50 µM treatments positively affected the germination rates of the seeds and decreased the MGT value from 7.19 days to 6.79 and 7.03 days, respectively.

When the effects of 3-ohm treatments on G_{10-90} of pepper seeds under water stress and optimum conditions were examined, stress negatively affected the germination uniformity of the seeds and the germination uniformity of the seeds germinated under 0 gL^{-1} PEG conditions increased from 2.95 days to

3.26 days with the effect of stress. Although it was not statistically significant, it was determined that among the 3-ohm applications, 10 µM application positively affected the germination uniformity of the seeds and decreased the G_{10-90} value from 3.45 days to 2.80 days in 0 µM treated seeds.

Table 4. Effects of water stress (75 gL⁻¹ PEG) and 3-ohm treatments on germination parameters of seeds

Treatments		FGP (%)	MGT (day)	G_{10-90} (day)	RL (cm)	Vigor index
PEG						
0 gL⁻¹	-	98.1	6.59 b	2.95	7.50 a	735.61 a
75 gL⁻¹	-	97.4	7.46 a	3.26	5.76 b	561.85 b
3-ohm (µM)						
0	-	96.5	7.19 a	3.45	5.65 b	546.33 b
10	-	97.8	6.79 b	2.80	7.55 a	738.23 a
50	-	98.5	7.03 a	3.12	7.10 a	699.43 a
100	-	98.3	7.10 a	3.06	6.22 b	610.94 b
PEG×3-ohm						
0 gL⁻¹	0 µM	97.0	6.84	3.22	6.80	660.15
	10 µM	98.0	6.25	2.59	8.35	817.95
	50 µM	99.0	6.60	3.00	7.83	774.10
	100 µM	98.5	6.68	2.98	7.01	690.23
75 gL⁻¹	0 µM	96.0	7.54	3.67	4.50	432.50
	10 µM	97.5	7.33	3.01	6.70	658.50
	50 µM	98.0	7.47	3.24	6.38	624.75
	100 µM	98.0	7.52	3.13	5.43	531.65
Sign.						
PEG	-	N.S.	***	N.S.	***	***
3-ohm (µM)	-	N.S.	***	N.S.	***	***
PEG×3-ohm	-	N.S.	N.S.	N.S.	N.S.	N.S.

Sign. (significant), NS, *, **, ***, not significant, significant at $P < 0.05$, 0.01 or 0.001, respectively

The effects of stress and 3-ohm treatments on RL and vigor index were statistically significant, but the interaction effect of both main factors was found to be insignificant. RL, which was 7.50 cm under optimum conditions, decreased to 5.76 cm under the effect of water stress. Among the 3-ohm treatments at different concentrations, the smallest RL was obtained from 0 µM treatment (5.65 cm), whereas the root lengths of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher (7.55 cm, 7.10 cm, and 6.22 cm, respectively).

The effects of water stress and 3-ohm treatments on the vigor index of pepper seeds showed that the vigor index, which was 735.61 under optimum conditions, decreased to 561.85 under the effect of water stress. Among the 3-ohm treatments at different concentrations, the lowest vigor index was obtained from 0 µM treatment, whereas the vigor indexes of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher. When the interaction effect of stress and 3-ohm concentrations was analyzed, it was observed that the lowest vigor index was obtained from 0 µM 3-ohm treatment under 75 gL⁻¹ PEG conditions, while the highest was obtained from 10 µM, 50 µM and 100 µM 3-ohm treatments under 0 gL⁻¹ PEG conditions.

The effects of 3-ohm treatments on FGP, MGT, G_{10-90}, RL and vigor index of pepper seeds after germination test under water stress (100 gL⁻¹ PEG) and optimum conditions are shown in Table 5. It was determined that water stress had a significant effect on the FGP of the seeds and the average value of 98.1% under optimum conditions decreased to 96.8% with the effect of stress. Furthermore, it was observed that 50 µM (98.3%) 3-ohm concentration applied to the seeds had a higher germination percentage compared to the 0 µM treatment (96.3%). However, the interaction relationship between the main factors, stress and 3-ohm treatments were found to be statistically insignificant.

The effects of stress and 3-ohm treatments on MGT were statistically significant, but the interaction effect of both main factors was found to be insignificant. According to the data presented in Table 5, water stress negatively affected the mean germination rate of the seeds and the mean germination rate of the seeds germinated under 0 gL⁻¹ PEG conditions increased from 6.59 days to 7.89 days due to the effect of stress. As a result of 3-ohm treatments, 10 µM, 50 µM and 100 µM 3-ohm treatments positively affected the germination rates of the seeds and decreased the MGT value from 7.52 days to 7.00, 7.17 and 7.26 days, respectively.

When the effects of 3-ohm treatments on G_{10-90} of pepper seeds under water stress and optimum conditions were examined, only the effect of water stress was found to be significant, while the interaction effects of 3-ohm treatments and two main factors were found to be insignificant. When the effects of 3-ohm treatments on G_{10-90} of pepper seeds under water stress and optimum conditions were examined, stress negatively affected the germination uniformity of the seeds and the germination uniformity, which was 2.95 days in seeds germinated under 0 gL⁻¹ PEG conditions, increased to 3.71 days with the effect of stress.

When the effects of water stress and 3-OHM treatments on RL and vigor index of pepper seeds were examined, it was observed that the main factors were significant but the interaction effects were insignificant. Water stress and 3-ohm treatments decreased the root length of pepper seeds from 7.50 cm under optimum conditions to 4.91 cm under the effect of stress. Among the 3-ohm treatments at different concentrations, the smallest RL was obtained from 0 µM treatment (5.53 cm), whereas the root lengths of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher (6.93 cm, 6.51 cm, and 5.85 cm, respectively).

When the effects of water stress and 3-ohm treatments on the vigor index of pepper seeds were examined, the vigor index, which was 735.61 under optimum conditions, decreased to 475.43 under the effect of water stress. Among the 3-ohm treatments at different concentrations, the lowest vigor index was obtained from 0 µM treatment, whereas the vigor indices of seeds treated with 10 µM, 50 µM and 100 µM 3-ohm were significantly higher.

Table 5. Effects of water stress (100 gL^{-1} PEG) and 3-ohm treatments on germination parameters of seeds

Treatments		FGP (%)	MGT (day)	$G_{10\text{-}90}$ (day)	RL (cm)	Vigor index
PEG						
0 gL^{-1}	-	98.1 a	6.59 b	2.95 b	7.50 a	735.61 a
100 gL^{-1}	-	96.8 b	7.89 a	3.71 a	4.91 b	475.43 b
3-ohm (µM)						
0	-	96.3	7.52 a	3.52	5.53 c	532.95 c
10	-	97.3	7.00 c	3.18	6.93 a	674.35 a
50	-	98.3	7.17 bc	3.26	6.51 ab	640.60 ab
100	-	98.0	7.26 b	3.35	5.85 bc	574.16 bc
PEG×3-ohm						
0 gL^{-1}	0 µM	97.0	6.84	3.22	6.80	660.15
	10 µM	98.0	6.25	2.59	8.35	817.95
	50 µM	99.0	6.60	3.00	7.83	774.10
	100 µM	98.5	6.68	2.98	7.01	690.23
100 gL^{-1}	0 µM	95.5	8.20	3.81	4.25	405.75
	10 µM	96.5	7.76	3.78	5.50	530.75
	50 µM	97.5	7.75	3.52	5.20	507.10
	100 µM	97.5	7.85	3.71	4.70	458.10
Sign.						
PEG	-	*	***	**	***	***
3-ohm (µM)	-	N.S.	***	N.S.	**	**
PEG×3-ohm	-	N.S.	N.S.	N.S.	N.S.	N.S.

Sign. (significant), NS, *, **, ***, not significant, significant at $P < 0.05$, 0.01 or 0.001, respectively

DISCUSSION

3-ohm is one of the metabolites released as a result of the degradation of melatonin and melatonin. Melatonin 3-hydroxylase (M3H), a member of the 2-oxoglutarate-dependent dioxygenase (2-ODD) enzyme family, is responsible for 3-ohm biosynthesis. In this study, the effects of 3-ohm, which is a universal antioxidant and has been suggested to be a plant hormone in recent years, on the germination of pepper seeds treated with different concentrations of salt and water stress conditions were investigated. As mentioned in the previous studies section, there is a very limited number of studies on the physiological functions of 3-ohm in plants and there is no study on its effects on seed germination under stress.

Pepper seeds treated with different concentrations (0, 10, 50 and 100 µM) of 3-ohm were subjected to germination tests under 4 different NaCl concentrations (0 mM, 75 mM, 100 mM and 125 mM) under salt stress and 2 different PEG-6000 concentrations (0 gL^{-1}, 75 gL^{-1} and 100 gL^{-1}) under water stress. All concentrations (10, 50 and 100 µM) of 3-ohm treatments before sowing increased the germination percentages of pepper seeds subjected to germination tests under salt and water stress compared to 0 µM concentration. The highest germination percentage of seeds under salt stress conditions was obtained from all 3-ohm treatments regardless of concentration. Germination rate and germination uniformity were positively affected by 3-ohm treatments under both stress conditions and all 3-ohm treatments significantly increased germination rate and germination uniformity. In addition, 3-ohm treatments positively affected the root length and vigor index of pepper seeds under both stress conditions and caused an increase in both parameters.

The 3-ohm molecule, a metabolite of melatonin, is thought to significantly improve seed germination performance, but there is very little information on this subject in the literature. For example, it was reported that 3-ohm significantly increased the germination of Arabidopsis seeds compared to control seeds and promoted plant growth and development [22]. Choi and Back [15], reported that 3-ohm levels in paddy plant tissues exhibited a circadian rhythm like melatonin, but significantly increased the tillering number by increasing the expression level of the MOC1 gene. Similarly, M3H genes catalyzing melatonin to 3-ohm via 2-ODD were cloned in paddy plants and 3-ohm was found in high amounts in melatonin-treated leaves [17]. It was also reported by Tan et al. [29] that 3-ohm is a powerful antioxidant in eliminating hydroxyl radicals (HO-). Galano et al. [30] suggested that the protective effects of melatonin against peroxyl radicals become important after metabolizing to 3-ohm. Accordingly, the results presented in this study support not only the sustained protection of melatonin against oxidative stress through its free radical scavenging but also its important role of 3-ohm in the peroxyl radical scavenging activity of melatonin. In a study of rice plants, M3H genes catalyzing melatonin to 3-ohm via 2-ODD were cloned, and 3-ohm was found at high levels in melatonin-treated leaves [25]. Also, 3-ohm promotes growth and flowering in Arabidopsis [31]. The researchers also observed that 3-ohm exhibited 15 times higher antioxidant activity than melatonin. However, further studies on 3-ohm, a melatonin metabolite, will shed light on its physiological functions in seed germination under different stress factors.

CONCLUSIONS

The results of the study showed that 3-ohm treatments of pepper seeds promoted germination under salt and water stress conditions. All 3-ohm concentrations used in the study gave the most successful results compared to control seeds. Moreover, 3-ohm treatment significantly increased seed germination under different concentrations of salt and drought stress conditions compared to non-3-ohm treated seeds. In conclusion, the effects of 3-ohm on seed germination performance of pepper seeds under various abiotic stress factors were undeniably significant. The results obtained suggest that 3-ohm has a melatonin-like effect and promotes the antioxidant system in increasing stress tolerance. However, in order to reach more conclusive results, further studies on 3-ohm, a metabolite of melatonin, are needed.

ACKNOWLEDGEMENTS

This study is a part of the project numbered 121C409 carried out with the support of TUBITAK/BİDEB-2218. I would like to thank TUBITAK for their support. I would also like to express my endless respect and gratitude to my esteemed teacher Assoc. Prof. Dr. Gökçen YAKUPOĞLU for his scientific support in my study.

REFERENCES

1. Kanal, A., Balkaya, A., Karaağaç, O., 2021. *Capsicum baccatum* türüne ait biber genotiplerinin fenotipik kök özellikleri yönünden seleksiyonu. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 26(1):19-33.
2. Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L., Nawaz, M., Rasheed, A., Afzal, A., Liu, Y., Guoqin, H., 2020. The critical role of zinc in plants facing the drought stress. Agriculture, 10(9):396.
3. Yang, Y., Guo, Y., 2018. Unraveling salt stress signaling in plants. Journal of Integrative Plant Biology, 60(9):796-804.
4. Wang, L., Tanveer, M., Wang, H., Arnao, M.B., 2024. Melatonin as a key regulator in seed germination under abiotic stress. Journal of Pineal Research, 76(1):e12937.
5. Li, R., Jiang, M., Song, Y., Zhang, H., 2021. Melatonin alleviates low-temperature stress via ABI5-mediated signals during seed germination in rice (*Oryza sativa* L.). Frontiers in Plant Science, 12, 727596.
6. Jahan, M.S., Guo, S., Sun, J., Shu, S., Wang, Y., Abou El-Yazied, A., Alabdallah N.M., Hikal M., Mohamed M.H.M., Ibrahim M.F.M., Hasan, M.M., 2021. Melatonin-mediated photosynthetic performance of tomato seedlings under high-temperature stress. Plant Physiology and Biochemistry, 167, 309-320.
7. Tiwari, R.K., Lal, M.K., Kumar, R., Chourasia, K.N., Naga, K.C., Kumar, D., Das, S.K., Zinta, G., 2021. Mechanistic insights on melatonin-mediated drought stress mitigation in plants. Physiologia Plantarum, 172(2):1212-1226.
8. Li, J., Liu, J., Zhu, T., Zhao, C., Li, L., & Chen, M., 2019. The role of melatonin in salt stress responses. International journal of molecular sciences, 20(7), 1735.
9. Khan, M.N., Zhang, J., Luo, T., Liu, J., Rizwan, M., Fahad, S., Xu, Z., Hu, L., 2019. Seed priming with melatonin coping drought stress in rapeseed by regulating reactive oxygen species detoxification: Antioxidant defense system, osmotic adjustment, stomatal traits and chloroplast ultrastructure perseveration, Industrial Crops and Products, 140, 111597.
10. Düver, E., 2019. Biberde tohum melatonin içeriği ile üşüme stresi koşulları altında çimlenme ve çıkış performansı arasında ilişkinin araştırılması. KSÜ Fen Bilimleri Enstitüsü Bahçe Bitkileri Bölümü, Yüksek Lisans Tezi, 82s.
11. Yakupoğlu, G., Köklü, Ş., Korkmaz, A., 2018. Bitkilerde melatonin ve üstlendiği görevler. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 21(2):264-276.
12. Erland, L., Saxena, P.K., 2017. Beyond a neurotransmitter: The role of serotonin in plants. Neurotransmitter, 4, e1538
13. Hardeland, R., 2021. Melatonin, its metabolites and their interference with reactive nitrogen compounds. Molecules, 26(13):4105.
14. Tan, D.X., Reiter, R.J., 2020. An evolutionary view of melatonin synthesis and metabolism related to its biological functions in plants. Journal of Experimental Botany, 71(16):4677-4689.
15. Choi, G.H., Back, K., 2019. Suppression of melatonin 2-hydroxylase increases melatonin production leading to the enhanced abiotic stress tolerance against cadmium, senescence, salt, and tunicamycin in rice plants. Biomolecules, 9(10):589.
16. Álvarez-Diduk, R., Galano, A., Tan, D.X., Reiter, R.J., 2015. N-Acetylserotonin and 6-hydroxymelatonin against oxidative stress: Implications for the overall protection exerted by

melatonin. The Journal of Physical Chemistry B, 119(27):8535-8543.

17. Lee, K., Zawadzka, A., Czarnocki, Z., Reiter, R.J., Back, K., 2016. Molecular cloning of melatonin 3-hydroxylase and its production of cyclic 3-hydroxymelatonin in rice (*Oryza sativa*). Journal of Pineal Research, 61(4):470-478.
18. Bugg, T.D., 2003. Dioxygenase enzymes: catalytic mechanisms and chemical models. Tetrahedron, 59(36):7075-7102.
19. Back, K., 2021. Melatonin metabolism, signaling and possible roles in plants. The Plant Journal, 105(2):376-391.
20. Lee, H.J., Back, K., 2019. 2-Hydroxymelatonin confers tolerance against combined cold and drought stress in tobacco, tomato, and cucumber as a potent anti-stress compound in the evolution of land plants. Melatonin Research, 2(2):35-46.
21. Lee, H.J., Back, K., 2016. 2-Hydroxymelatonin promotes the resistance of rice plant to multiple simultaneous abiotic stresses (combined cold and drought). Journal of Pineal Research, 61(3):303-316.
22. Lee, H.Y., Back, K., 2022. 2-hydroxymelatonin promotes seed germination by increasing reactive oxygen species production and gibberellin synthesis in Arabidopsis thaliana. Antioxidants, 11(4):737.
23. Korkmaz, A., Sözeri, E., Ardıç, Ş.K., Havan, A., 2023. 2-hydroxymelatonin (2-OHM), a major melatonin metabolite, confers multiple stress tolerance in pepper at seed germination stage. South African Journal of Botany, 162:830-837.
24. Shah, A.A., Yasin, N.A., Ahmed, S., Abbas, M., Abbasi, G.H., 2021. 4-Hydroxymelatonin alleviates nickel stress, improves physiochemical traits of *Solanum melongena*: Regulation of polyamine metabolism and antioxidative enzyme. Scientia Horticulturae, 282, 110036.
25. Lee, K., Zawadzka, A., Czarnocki, Z., Reiter, R.J., Back, K., 2016. Molecular cloning of melatonin 3-hydroxylase and its production of cyclic 3-hydroxymelatonin in rice (*Oryza sativa*). Journal of Pineal Research, 61(4):470-478.
26. Mannino, G., Pernici, C., Serio, G., Gentile, C., Bertea, C.M. 2021. Melatonin and Phytomelatonin: Chemistry, Biosynthesis, Metabolism, Distribution and Bioactivity in Plants and Animals-An Overview. International Journal of Molecular Sciences, 22(18):9996.
27. Reiter, R. J., Mayo, J.C., Tan, D.X., Sainz, R.M., Alatorre-Jimenez, M.A., Qin, L., 2016. Melatonin as an antioxidant: under promises but over delivers. Journal of Pineal Research, 61(3):253-278.
28. Hu, J., Zhu, Z.Y., Song, W.J., Wang, J.C., Hu, W.M., 2005. Effects of sand priming on germination and field performance in direct-sown rice (*Oryza sativa* L.). Seed Science Technology, 33:243-248.
29. Tan, D.X., Hardeland, R., Manchester, L.C., Galano, A., Reiter, R.J., 2014. Cyclic-3-hydroxymelatonin (C3HOM), a potent antioxidant, scavenges free radicles and suppresses oxidative reactions. Current Medicinal Chemistry, 21:1557-1565.
30. Galano, A., Tan, D.X., Reiter, R.J., 2014. Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin. RSC Adv, 4:5220-5227.
31. Lee, H.Y., Back, K., 2022. The antioxidant cyclic 3-hydroxymelatonin promotes the growth and flowering of Arabidopsis thaliana. Antioxidants, 11(6):1157.