https://doi.org/10.46341/PI2022018
UDC 581.19

RESEARCH ARTICLE

Investigating the using potential of Schiff base molecule as an exogenous antioxidant on barley seeds under salt stress conditions

Sertan Çevik

Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Harran University, 63300 Şanlıurfa, Turkey; srtncvk@gmail.com

Received: 03.09.2022 | **Accepted**: 27.09.2022 | **Published online**: 01.10.2022

Abstract

Salt stress is one of the most important environmental factors that affect agricultural lands and causes product loss. Today, the application of various natural or synthetic molecules exogenously to plants and efforts to increase plant tolerance against environmental stresses as a result of these applications have been widely investigated by scientists. In this study, a Schiff base molecule (0, 3, 6, and 9 µM), which has shown *in vitro* antioxidant properties, was applied to barley seeds under salt stress (0, 50, 150, and 250 mM NaCl). In order to evaluate the effects of this molecule on barley under salt stress, seed germination, growth parameters, lipid peroxidation, proline content, histochemical detection of superoxide and hydrogen peroxide radicals, and mitotic index analysis were conducted. According to the results, salt stress decreased germination parameters, plumule and radicle lengths, and mitotic index while it increased proline content, lipid peroxidation, and radical contents. Schiff base treatment clearly reduced lipid peroxidation and radical content in all groups. However, it also decreased germination and growth parameters and mitotic index. The obtained results showed that the antioxidant property of this molecule was also preserved in plants under stress, but it was also determined that the molecule had negative effects, primarily on cell division. If necessary modifications can be made to the molecule, the negative effects on cell division can be eliminated, and this molecule, which is very easy and cheap to obtain, may be widely used to increase the tolerance of plants against environmental stress.

Keywords: barley, Schiff base, reactive oxygen species, salt stress

Funding: The author declares no funding.

Competing Interests: The author declares no conflict of interest.

Introduction

Plants are exposed to various biotic and abiotic environmental stressors throughout their lives. These stresses negatively affect all metabolic events in plants and cause serious crop losses (Raza et al., 2019). Salinity, one of the most important abiotic factors, is one of the most restrictive stress factors for agricultural production today (Güzel Değer & Çevik, 2021). In addition to ion toxicity, low osmotic potential occurs in the soil, which makes it difficult for the plant to take water from the soil (Ahmad & Akhtar, 2019). Studies show that almost 20% of arable land is constantly exposed to salt stress. However, many studies have been conducted worldwide to understand the mechanism of salt stress tolerance and the responses given by plants,

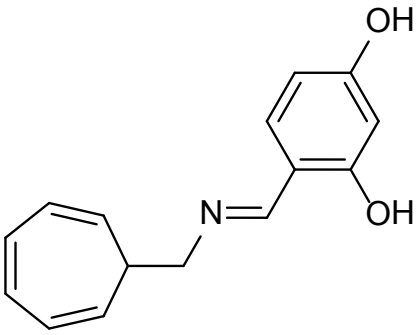

Figure 1. The Schiff base molecule (8e) applied in this study.

but little progress has been made in this regard. The most important reason for this is the complex physiological and genetic mechanism of salt stress tolerance and the lack of reliable screening methods (Zhu et al., 2020).

Environmental stresses (abiotic and biotic), which are common today due to severe and harsh climate change (Hasanuzzaman et al., 2020), can trigger oxidative stress in plants, causing the formation of reactive oxygen species (ROS). ROS are partially reduced or activated oxygen derivatives containing both free radical and non-radical forms that cause cellular damage and metabolic disorders (Jaleel et al., 2009). Plants cope with oxidative stress through an endogenous defense mechanism consisting of enzymatic and non-enzymatic antioxidants (Kaur et al., 2019).

Recent studies have shown that ROS are not entirely harmful and are necessary for the stabilization of the intracellular redox state at low concentrations (Circu & Aw, 2010; Schieber & Chandel, 2014). The ROS level is maintained by the balance between ROS generation and ROS scavenging. However, during stress conditions, excessive ROS production upsets the balance and causes cellular damage, reducing plant productivity (Hasanuzzaman et al., 2020). In order to minimize product loss due to environmental stress, researchers generally adopted two different approaches. One of them is to develop varieties resistant to environmental stresses through traditional or modern methods, and the other is to strengthen the antioxidant systems of plants by exogenous applications (Özkoku et al., 2019). Developing cultivars resistant to environmental stresses through traditional or modern techniques is time-consuming and quite difficult (Breseghello & Coelho, 2013). These situations have led researchers to make external applications that strengthen the antioxidant system, which is a more practical way. When the studies conducted in this context are examined, it is seen that synthetic or natural molecules are applied to plants in an extensive scope, especially under stress conditions (Çevik et al., 2014, 2019; Bekfelavi et al., 2021).

Schiff bases are important functional groups due to their wide biological and chemical functions. In addition to their broad chemical properties, the biological functions of Schiff bases, such as antibacterial, antifungal, antioxidant, antitumor, and antiviral action, have been reported by various researchers (Yılmaz, 2021). In this study, a Schiff base molecule, whose antioxidant properties were demonstrated by *in vitro* methods by Yılmaz (2021), was applied to barley seeds under salt stress, and the effects of this molecule were investigated with extensive morphological, physiological, and biochemical analyses. According to the literature, this is the first study to examine the effects of a Schiff base molecule, which has been shown to have antioxidant properties *in vitro* by applying to a plant under salt stress. The obtained data provided important information for the future use of such molecules.

Material and methods

Plant material and treatments

Hordeum vulgare L. seeds were obtained from ALATA Horticultural Research Station. Seeds were kept in 5% sodium hypochlorite for 5 min and washed five times with pure water for surface sterilization prior to use. Schiff base molecule (8e) (Fig. 1), which has high *in vitro* DPPH free radical scavenging activity, has been used in this study. Details on the synthesis steps of this molecule are given by Yılmaz (2021).

A preliminary experiment was performed to determine the optimum concentration of 8e molecules for barley seeds according to germination data. Solutions containing Schiff base molecule (0, 3, 6, and 9 µM of 8e) and salt (0, 50, 150, and 250 mM of NaCl) were added to Petri dishes in equal amounts, and water

Table 1. Experimental application groups.

Groups	NaCl (mM)	8e (µM)	Groups	NaCl (mM)	8e (µM)	Groups	NaCl (mM)	8e (µM)	Groups	NaCl (mM)	8e (µM)
1	0	0	5	0	3	9	0	6	13	0	9
2	50	0	6	50	3	10	50	6	14	50	9
3	150	0	7	150	3	11	150	6	15	150	9
4	250	0	8	250	3	12	250	6	16	250	9

was used as a control group. Ten seeds were placed in each Petri dish, and ten Petri dishes were used for each application group. Applied combinations are shown in Table 1.

Seed germination and seedling growth parameters

Seeds were imbibed in aerated water for one day at 22 °C and then transferred to Petri dishes. Seeds in Petri dishes were germinated at 24:18 °C day: night temperature, 16:8 day: night light period, 150 µmol $m^{-2}s^{-1}$ light intensity, and 60 ± 5 % humidity conditions for three days under controlled conditions in the climate room. The radicle and plumule lengths were measured with a digital caliper at the end of the third day.

Seeds were considered to have germinated when the radicles were ≥2 mm long. The number of germinated seeds was recorded daily, and the final germination percentage was determined after three days. Germination rate (M) was calculated according to the formula $M = n_1/d_1 + n_2/d_2 + n_3/d_3$, where n is the number of germinated seeds, and d is a day (Ranal & Santana, 2006). Mean germination time (MGT) was calculated by using the equation $MGT = \sum(n \times d)/N$, where n is the number of seeds germinated on each day, d is the number of days, and N is the total number of germinated seeds (Ellis & Roberts, 1981). Seed germination percentage (SG) was calculated using the following formula SG *[%] = Number of germinated seeds / Total number of seeds* × 100 (Czabator, 1962). Germination index (GI) was calculated according to the formula $GI = \sum(n_i \times T_i)/N$, where n is the number of newly germinating seeds, N is the total number of seeds; and n_i is the number of seeds germinated at day T_i (Aravind et al., 2019).

Lipid peroxidation

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content according to Ohkawa et al. (1979). Radicle and plumule tissue (0.2 g) was homogenized 1 mL (5 %) trichloroacetic acid (TCA) solution. The homogenate was centrifuged for 10 min at 8,000 rpm. After that, supernatant, thiobarbituric acid, and TCA solutions were mixed in equal volumes in tubes, and tubes were incubated at 96 °C for 25 min. The tubes were placed in an ice bath to terminate the reaction and centrifuged at 6,000 rpm for 5 min. The mixture was measured at 532 and 600 nm (Shimadzu 1800 240V). MDA content was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Free proline content

Free proline content was determined according to Bates et al. (1973). The radicle and plumule samples (0.25 g) were homogenized in 3 % sulfosalicylic acid. The homogenate was centrifuged for 3 min at 3,000 rpm, and then the supernatant was mixed well with acid ninhydrin and glacial acetic acid in equal volumes and incubated at 100 °C for 60 min. The reaction was terminated by adding cold toluene (4 mL) to the tubes. The toluene phase was evaporated and analyzed by spectrophotometry (Shimadzu 1800 240V) at 520 nm. Proline concentration was calculated by using a calibration curve and expressed as µmol proline g^{-1}FW.

Detection of superoxide and hydrogen peroxide radicals in barley roots

Superoxide radicals were detected following Piacentini et al. (2021). In this method, NBT is used to monitor the intracellular production of the superoxide anion. Roots were exposed

to NBT solution (0.5 mg/mL NBT in 10 mM Tris-HCl, pH 7.4) for 30 min. NBT is reduced by superoxide radicals, and blue color forms on the root surface as superoxide radicals reduce NBT.

H_2O_2 was detected by 3,3′-diaminobenzidine (DAB) staining method according to Thordal-Christensen et al. (1997) with minor modifications. Briefly, barley roots were treated with DAB staining buffer for 60 min (in the dark), and the reaction was stopped by adding ethanol:glycerol:acetic acid (3:1:1, v/v). The oxidized DAB formed a brown precipitate on the surface of the roots and was visualized by light microscopy (Olympus BX53).

Mitotic index analysis

For mitotic index analysis, 1–1.5 cm barley root tips were cutted and fixed for 24 h in ethanol:glacial acetic acid (3:1, v/v), then stored in 70% ethanol at +4 °C until analysis. The root tips were hydrolyzed in 2M HCl for 20 min at 60 °C and stained by the Feulgen method (Tabur & Demir, 2010). The slides were examined under an optical microscope (Olympus BX53). A mean of 1000 cells was counted from each root to get a total of 5,000 cells per treatment. The mitotic index (MI) was determined using the formula: *MI [%]= the number of dividing cells/the number of totally examined cells* × 100.

Experimental design and statistical analysis

Salt stress and Schiff base treatment were carried out according to a completely randomized experimental design with two factors. Treatments had three replications for proline, MDA, and radical detection analysis; five replications for germination %, germination rate, germination index, mean germination time, and mitotic index analysis; ten replications for plumule and radicle length analysis. All quantitative data expressed as percentages were subjected to arcsine transformation. Data were subjected to analysis of variance (ANOVA), and the means were separated using the Least Significant Difference (LSD) multiple range test at $p \leq 0.05$. All the statistical analyses were performed using the JMP ver. 8 (SAS Institute Inc.) software package. The coefficients of variation were shown in the tables to demonstrate the reliability of the experiment.

Results and discussion

Salt stress affects plants and causes serious production problems in the field. The results of this study demonstrate that with an increasing degree of salt stress, germination percentage, germination rate, and germination index parameters of barley decreased, while mean germination time (MGT) increased (Table 2). Other researchers also reported similar results (Yildirim & Güvenc, 2006; Dehnavi et al., 2020). Salt stress can directly inhibit germination parameters by making difficult water uptake for seeds, or it can reduce germination rates due to ionic toxicity (Yildirim & Güvenc, 2006). Exogenous treatment by 8e increased MGT under control conditions and mild salt stress while decreased MGT under severe salt stress conditions. These interesting findings show that exogenous Schiff base application can reduce germination time, especially under severe stress conditions. A low MGT value indicates faster germination compared to a high MGT value (Yongkriat et al., 2020). The fact that MGT decreases under stress by Schiff base treatment may be an important advantage for plants to cope with salt stress.

As seen from Table 3, radicle and plumule lengths decreased due to increased salt concentration. Other researchers also reported similar results on different plants (Keshavarzi, 2011; El-Bastawisy et al., 2018). Generally, exogenous 8e applications decreased the radicle and plumule lengths. This result was also supported by mitotic index (MI) analysis. Especially under control conditions increasing 8e concentration decreased MI, but under severe salt stress conditions, 8e did not affect MI. These results are in good agreement with the radicle length results in this study. These findings present an important area to be investigated for the use of Schiff base molecules, which have antioxidant properties *in vitro*, before such exogenous applications. If the reasons for the negative effects of these molecules on cell division can be found, the obstacles to their use will be overcome with the necessary molecular modifications.

Table 2. Effect of salt stress and 8e treatment on germination of barley. Different letters indicate statistically significant differences between groups.

Treatment	Seed germination percentage (%)	Germination rate	Germination ındex	Mean germination time (days)
Control	92.60 ± 1.02 ^{a}	63.54 ± 1.12 ^{a}	2.06 ± 0.03 ^{a}	1.76 ± 0.02 ^{j}
0 NaCl : 3 µM 8e	89.00 ± 1.10 ^{b}	54.07 ± 0.57 ^{b}	1.81 ± 0.03 ^{b}	1.97 ± 0.02 ^{gh}
0 NaCl : 6 µM 8e	88.00 ± 2.00 ^{b}	51.04 ± 0.89 ^{c}	1.74 ± 0.03 ^{c}	2.02 ± 0.03 ^{f}
0 NaCl : 9 µM 8e	86.20 ± 0.75 ^{c}	49.64 ± 0.83 ^{d}	1.67 ± 0.02 ^{d}	2.06 ± 0.02 ^{e}
50 mM NaCl : 0 8e	80.80 ± 2.14 ^{d}	50.38 ± 0.53 ^{cd}	1.67 ± 0.01 ^{d}	1.94 ± 0.02 ^{h}
50 mM NaCl : 3 µM 8e	79.20 ± 1.60 ^{d}	53.16 ± 0.71 ^{b}	1.69 ± 0.02 ^{d}	1.87 ± 0.02 ^{i}
50 mM NaCl : 6 µM 8e	76.40 ± 1.02 ^{e}	39.92 ± 0.56 ^{f}	1.44 ± 0.03 ^{e}	2.11 ± 0.03 ^{d}
50 mM NaCl : 9 µM 8e	74.80 ± 1.33 ^{e}	44.14 ± 0.45 ^{e}	1.43 ± 0.01 ^{e}	2.09 ± 0.03 ^{de}
150 mM NaCl : 0 8e	57.20 ± 0.75 ^{g}	40.18 ± 0.35 ^{f}	1.28 ± 0.02 ^{f}	2.23 ± 0.02 ^{b}
150 mM NaCl : 3 µM 8e	64.20 ± 0.98 ^{f}	36.29 ± 0.36 ^{g}	1.18 ± 0.02 ^{g}	2.00 ± 0.02 ^{f}
150 mM NaCl : 6 µM 8e	48.00 ± 2.28 ^{h}	27.31 ± 0.43 ^{h}	0.87 ± 0.04 ^{h}	1.96 ± 0.03 ^{gh}
150 mM NaCl : 9 µM 8e	41.00 ± 1.10 ^{i}	21.78 ± 0.33 ^{i}	0.74 ± 0.03 ^{i}	1.99 ± 0.03 ^{fg}
250 mM NaCl : 0 8e	32.80 ± 1.60 ^{j}	18.18 ± 0.61 ^{j}	0.58 ± 0.02 ^{j}	2.24 ± 0.01 ^{ab}
250 mM NaCl : 3 µM 8e	26.20 ± 1.17 ^{k}	15.18 ± 0.48 ^{l}	0.49 ± 0.03 ^{k}	1.96 ± 0.02 ^{gh}
250 mM NaCl : 6 µM 8e	31.40 ± 1.02 ^{j}	16.17 ± 0.42 ^{k}	0.54 ± 0.03 ^{l}	2.26 ± 0.02 ^{a}
250 mM NaCl : 9 µM 8e	26.40 ± 1.50 ^{k}	13.34 ± 0.44 ^{m}	0.44 ± 0.01 ^{m}	2.19 ± 0.02 ^{c}
LSD, $p < 0.001$	1.428	0.534	0.034	0.032

Table 3. Effect of salt stress and 8e treatment on growth of barley.. Different letters indicate statistically significant differences between groups.

Treatment	Plumule length (mm)	Radicle length (mm)	Mitotic ındex
Control	6.07 ± 0.70 ^{a}	6.78 ± 0.59 ^{a}	0.154 ± 0.005 ^{a}
0 NaCl : 3 µM 8e	4.73 ± 0.75 ^{b}	5.53 ± 0.25 ^{b}	0.123 ± 0.004 ^{b}
0 NaCl : 6 µM 8e	4.41 ± 0.63 ^{bc}	5.45 ± 0.58 ^{b}	0.121 ± 0.002 ^{b}
0 NaCl : 9 µM 8e	4.21 ± 0.72 ^{cd}	4.32 ± 0.34 ^{c}	0.094 ± 0.004 ^{c}
50 mM NaCl : 0 8e	3.94 ± 0.63 ^{d}	5.21 ± 0.61 ^{b}	0.116 ± 0.002 ^{b}
50 mM NaCl : 3 µM 8e	2.86 ± 0.41 ^{e}	4.48 ± 0.31 ^{c}	0.097 ± 0.002 ^{d}
50 mM NaCl : 6 µM 8e	2.21 ± 0.41 ^{f}	4.31 ± 0.70 ^{c}	0.093 ± 0.003 ^{d}
50 mM NaCl : 9 µM 8e	1.93 ± 0.21 ^{fg}	3.62 ± 0.51 ^{d}	0.073 ± 0.003 ^{e}
150 mM NaCl : 0 8e	2.03 ± 0.27 ^{f}	3.46 ± 0.82 ^{de}	0.072 ± 0.003 ^{fg}
150 mM NaCl : 3 µM 8e	1.52 ± 0.49 ^{gh}	3.33 ± 0.29 ^{de}	0.069 ± 0.003 ^{fg}
150 mM NaCl : 6 µM 8e	1.48 ± 0.34 ^{h}	3.13 ± 0.50 ^{e}	0.073 ± 0.004 ^{fg}
150 mM NaCl : 9 µM 8e	1.47 ± 0.34 ^{h}	3.40 ± 0.19 ^{de}	0.070 ± 0.001 ^{fg}
250 mM NaCl : 0 8e	0.71 ± 0.34 ^{i}	1.43 ± 0.31 ^{f}	0.032 ± 0.001 ^{h}
250 mM NaCl : 3 µM 8e	0.13 ± 0.05 ^{j}	1.31 ± 0.27 ^{fg}	0.030 ± 0.002 ^{hl}
250 mM NaCl : 6 µM 8e	0.13 ± 0.05 ^{j}	1.57 ± 0.24 ^{f}	0.036 ± 0.002 ^{h}
250 mM NaCl : 9 µM 8e	0.10 ± 0.01 ^{j}	1.07 ± 0.40 ^{g}	0.024 ± 0.002 ^{l}
LSD, $p < 0.001$	0.439	0.436	0.244

Figure 2. Distribution of hydrogen peroxide in barley roots visualized by 3,3'-diaminobenzidine staining. Dark-stained regions indicate hydrogen peroxide produced in cells.

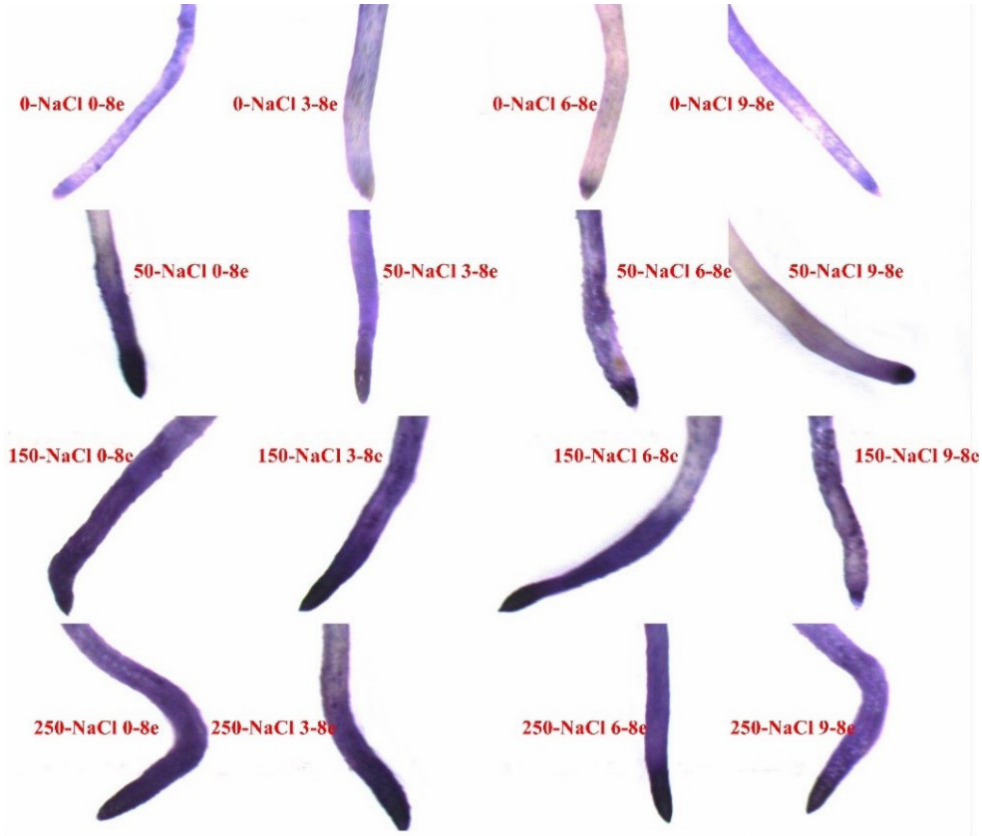

Figure 3. Distribution of superoxide radicals in barley roots visualized by nitroblue tetrazolium staining. Dark-stained regions indicate superoxide produced in cells.

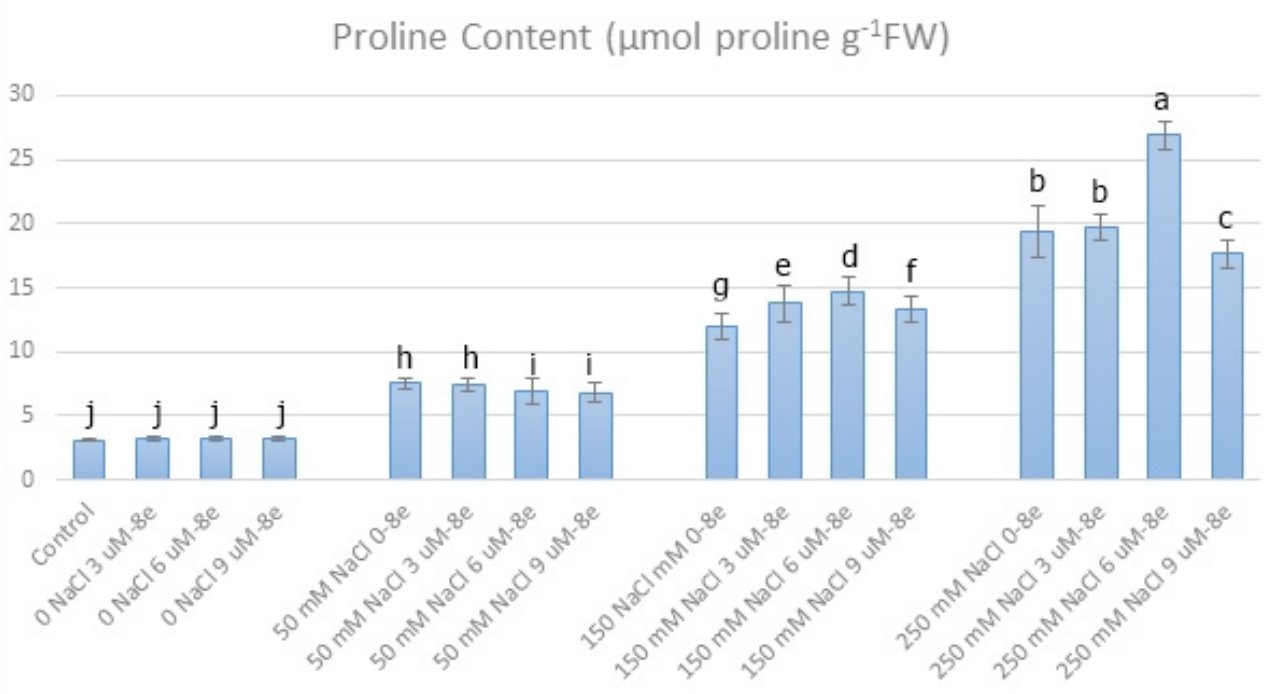

Figure 4. Effect of salt stress and 8e treatment on proline content of barley. LDS = 0.428, $p < 0.001$. Different letters indicate statistically significant differences between groups.

Salt stress increased the content of hydrogen peroxide (Fig. 2) and superoxide radical (Fig. 3) in the barley roots compared to control groups. It was observed that there is a strong correlation between the radical content and the increase in the salt concentration. Exogenous application of 8e decreased both superoxide and hydrogen peroxide radical contents. The *in vitro* antioxidant properties of this molecule were demonstrated by Yılmaz (2021). In this study, the fact that the exogenous application reduces the internal radical content shows that the antioxidant properties of the molecule are also preserved in-plant applications. However, as was emphasized before, the negative effect of the exogenous application of this molecule on cell division reduces its potential for use as an exogenous antioxidant. Therefore, if a specific cause affecting cell division of this molecule is found, it may be used as an exogenous antioxidant in such conditions.

Salt stress increased proline content in barley roots. Exogenous Schiff base treatment also increased proline content under severe salt stress conditions (Fig. 4). It has been demonstrated in different studies that the amount of proline increases under many environmental stresses (Vendruscolo et al., 2007; Lum et al., 2014; Chun & Chandrasekaran, 2018; Çevik et al., 2019). The best-known feature of proline is that it is a good osmotic preservative. In addition to being a good osmotic protector, it has been reported in recent years that proline reduces radical formation and shows antioxidant properties (Szabados & Savoure, 2010). Increasing proline content by Schiff base application may be important for plants under stress conditions. However, how exogenous Schiff base application increases the amount of proline under stress is another important question. The fact that this increase occurred only under severe salt stress conditions should also be investigated.

The MDA content, which increased with salt stress, decreased significantly by the exogenous Schiff base treatment (Fig. 5). MDA is the end product of lipid peroxidation and a good marker of oxidative damage. With the increase in the amount of intracellular radicals, lipid peroxidation occurs (Gawel et al., 2004). In this study, the fact that the Schiff base application decreased the content of H_2O_2 and superoxide radicals may have caused a decrease in MDA content. Protecting cell membranes under stressful conditions

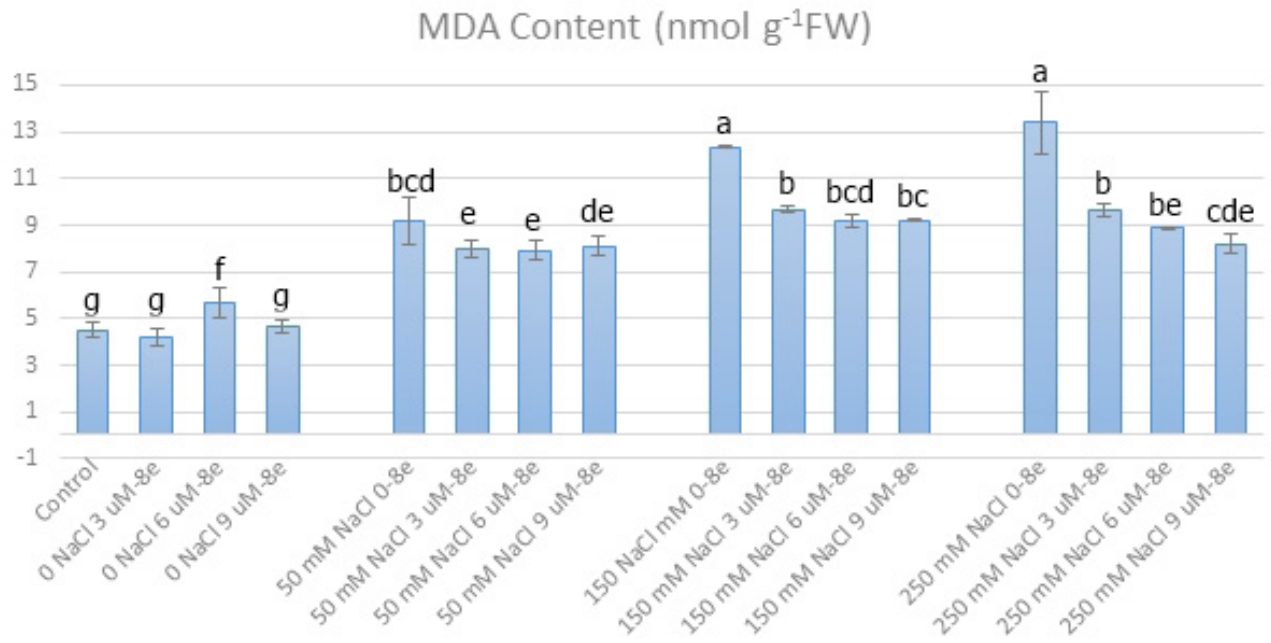

Figure 5. Effect of salt stress and 8e treatment on MDA content of barley. LDS = 1.066, $p < 0.001$. Different letters indicate statistically significant differences between groups.

gives the plant a significant advantage in coping with stress. Reducing MDA content by exogenous 8e treatment is important in plant stress tolerance.

Conclusions

Salt stress, which is one of the most important environmental factors, is a chronic stress. The difficulty of remediation of saline soils necessitates increasing plant tolerance to this stress. However, approaches increasing salt tolerance by exogenous applications have recently become very popular due to genetic constraints and insufficient time. In this study, a Schiff base molecule, whose *in vitro* antioxidant properties were shown in another study, was exogenously applied to barley plants under salt stress for the first time. According to the results, the application of this molecule decreased the amount of radicals and MDA that increased by salt stress. However, it was determined that the mitotic index and the growth parameters were also negatively affected by this application. The findings revealed that to avoid the negative effect, Schiff base molecules, which have antioxidant properties, need to be modified before application in a salt stress regulation. If the factors affecting cell division can be eliminated, these molecules, which are easy and cheap to obtain, may be widely applied to modify plant salt tolerance.

Acknowledgements

I thank Dr. Sara Yasemin (Siirt University) for her contributions to statistical analyses and Dr. Özgür Yılmaz (Mersin University) for Schiff base molecule supply.

References

Ahmad, I., & Akhtar, M.S. (2019). Use of nanoparticles in alleviating salt stress. In M.S. Akhtar (Ed.), *Salt stress, microbes, and plant interactions: causes and solution* (pp. 199–215). Springer, Singapore. https://doi.org/10.1007/978-981-13-8801-9_9

Aravind, J., Vimala, D., Radharani, J., Jacob, S., & Srinivasa, K. (2019). *The germination metrics package: a brief introduction.* ICAR-National Bureau of Plant Genetic Resources, New Delhi, India.

Bates, L.S., Waldren, R.P., & Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil, 39*(1), 205–207. https://doi.org/10.1007/BF00018060

Bekfelavi, E.Y., Yildizli, A., Kuş, N.Ş., Çevik. S., & Ünyayar, S. (2021). Osmoprotectant and antioxidant effects of new synthesized 6-(2-hydroxyethyl)cyclohex-3-enol on barley under drought stress. *Biologia Futura, 72*(2), 241–249. https://doi.org/10.1007/s42977-020-00058-w

Breseghello, F., & Coelho, A.S.G. (2013). Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). *Journal of Agricultural and Food Chemistry, 61*(35), 8277–8286. https://doi.org/10.1021/jf305531j

Çevik, S., Güzel Değer, A., Yıldızlı, A., Doğanyiğit, N., Gök, A., & Unyayar, S. (2019). Proteomic and physiological analyses of dl-cyclopentane-1,2,3-triol-treated barley under drought stress. *Plant Molecular Biology Reporter, 37*, 237–251. https://doi.org/10.1007/s11105-019-01151-8

Çevik, S., Yıldızlı, A., Yandım, G., Göksu, H., Gultekin, M.S., Güzel Değer, A., Çelik, A., Şimşek Kuş, N., & Ünyayar, S. (2014). Some synthetic cyclitol derivatives alleviate the effect of water deficit in cultivated and wild-type chickpea species. *Journal of Plant Physiology, 171*(10), 807–816. https://doi.org/10.1016/j.jplph.2014.01.010

Chun, S.C., & Chandrasekaran, M. (2018). Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. *Frontiers in Microbiology, 9*, Article 2525. https://doi.org/10.3389/fmicb.2018.02525

Circu, M.L., & Aw, T.Y. (2010). Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radical Biology and Medicine, 48*(6), 749–762. https://doi.org/10.1016/j.freeradbiomed.2009.12.022

Czabator, F.J. (1962). Germination value: an index combining speed and completeness of pine seed germination. *Forest Science, 8*, 386–395. https://academic.oup.com/forestscience/article-abstract/8/4/386/4746567

Dehnavi, R.A., Zahedi, M., Ludwiczak, A., Cardenas Perez, S., & Piernik, A. (2020). Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy, 10*(6), Article 859. https://doi.org/10.3390/agronomy10060859

El-Bastawisy, Z.M., El-Katony, T.M., & Abd El-Fatah, S.N. (2018). Genotypic variability in salt tolerance of Vicia faba during germination and early seedling growth. *Journal of King Saud University-Science, 30*(2), 270–277. https://doi.org/10.1016/j.jksus.2017.04.004

Ellis, R.H., & Roberts, E.H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology, 9*, 373–409.

Gawel, S., Wardas, M., Niedworok, E., & Wardas, P. (2004). Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiadomości Lekarskie, 57*(9–10), 453–455.

Güzel Değer, A.G., & Çevik, S. (2021). Impacts of nano-TiO_2 on the initial development stages of barley seedlings under salinity. *Mediterranean Agricultural Sciences, 34*(1), 109-116. https://doi.org/10.29136/mediterranean.816107

Hasanuzzaman, M., Bhuyan, M.H.M., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., & Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. *Antioxidants, 9*(8), Article 681. https://doi.org/10.3390/antiox9080681

Jaleel, C.A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R., & Panneerselvam, R. (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. *International Journal of Agriculture and Biology, 11*(1), 100–105.

Kaur, N., Kaur, J., Grewal, S.K., & Singh, I. (2019). Efect of heat stress on antioxidative defense system and its amelioration by heat acclimation and salicylic acid pre-treatments in three pigeonpea genotypes. *Indian Journal of Agricultural Biochemistry, 32*(1), 106–110. https://doi.org/10.5958/0974-4479.2019.00014.5

Keshavarzi, M.H.B. (2011). Effect of salt stress on germination and early seedling growth of savory (*Satureja hortensis*). *Australian Journal of Basic and Applied Sciences, 5*(12), 3274–3279.

Lum, M.S., Hanafi, M.M., Rafii, Y.M., & Akmar, A.S.N. (2014). Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *Journal of Animal and Plant Sciences, 24*(4), 1487–1493.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Özkoku, G., Cevik, S., & Ünyayar, S. (2019). The effect of exogenous application of synthetic inositol derivative (allo-inositol) on salt tolerance of *Capsicum chinense. Anadolu Tarim Bilimleri Dergisi, 34*(3), 319–326. https://doi.org/10.7161/omuanajas.556100

Piacentini, D., Falasca, G., Canepari, S., & Massimi, L. (2019). Potential of PM-selected components to induce oxidative stress and root system alteration in a plant model organism. *Environment International, 132*, Article 105094. https://doi.org/10.1016/j.envint.2019.105094

Ranal, M.A., & Santana, D.G. (2006). How and why to measure the germination process? *Brazilian Journal of Botany, 29*(1), 1–11. https://doi.org/10.1590/S0100-84042006000100002

Raza, A., Razzaq, A., Mehmood, S.S., Zou, X., Zhang, X., Lv, Y., & Xu, J. (2019). Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. *Plants, 8*(2), Article 34. https://doi.org/10.3390/plants8020034

Schieber, M., & Chandel, N.S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology, 24*(10), R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

Szabados, L., & Savoure, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Science, 15*(2), 89–97. https://doi.org/10.1016/j.tplants.2009.11.009

Tabur, S., & Demir, K. (2010). Role of some growth regulators on cytogenetic activity of barley under salt stress. *Plant Growth Regulation, 60*(2), 99–104. https://doi.org/10.1007/s10725-009-9424-6

Thordal-Christensen, H., Zhang, Z., Wei, Y., & Collinge, D.B. (1997). Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *The Plant Journal, 11,* 1187–1194. https://doi.org/10.1046/j.1365-313X.1997.11061187.x

Vendruscolo, E.C.G., Schuster, I., Pileggi, M., Scapimd, C.A., Molinarie, H.B.C., Marure, C.J., & Vieira, L.G.E. (2007). Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *Journal of Plant Physiology, 164*(10), 1367–1376. https://doi.org/10.1016/j.jplph.2007.05.001

Yildirim, E., & Güvenc, İ. (2006). Salt tolerance of pepper cultivars during germination and seedling growth. *Turkish Journal of Agriculture and Forestry, 30*(5), 347–353.

Yılmaz, Ö. (2021). Synthesis of new Schiff bases; investigation of their *in situ* catalytic activity for Suzuki C-C coupling reactions and antioxidant activities. *Journal of the Chinese Chemical Society, 68*(5), 917–928. https://doi.org/10.1002/jccs.202000433

Yongkriat, K.O., Leksungnoen, N., Onwimon, D., & Doomnil, P. (2020). Germination and salinity tolerance of seeds of sixteen Fabaceae species in Thailand for reclamation of salt-affected lands. *Biodiversitas – Journal of Biological Diversity, 21*(5), 215–222. https://doi.org/10.13057/biodiv/d210547

Zhu, J., Fan, Y., Shabala, S., Li, C., Lv, C., Guo, B., & Zhou, M. (2020). Understanding mechanisms of salinity tolerance in barley by proteomic and biochemical analysis of near-isogenic lines. *International Journal of Molecular Sciences, 21*(4), Article 1516. https://doi.org/10.3390/ijms21041516

Дослідження потенціалу використання молекули основи Шиффа як екзогенного антиоксиданту для насінні ячменю в умовах сольового стресу

Сертан Чевік

Кафедра молекулярної біології та генетики, факультет мистецтв і наук, Харранський університет, 63300 Шанлиурфа, Туреччина; srtncvk@gmail.com

Сольовий стрес є одним із найважливіших факторів навколишнього середовища, який впливає на сільськогосподарські угіддя та спричиняє втрати продукції. Сьогодні вчені широко досліджують екзогенне застосування різних природних або синтетичних молекул щодо рослин і намагаються підвищити стійкість рослин до стресів навколишнього середовища. У цьому дослідженні молекула основи Шиффа (0, 3, 6 та 9 μM), яка показала антиоксидантні властивості *in vitro*, була застосована до насіння ячменю під сольовим стресом (0, 50, 150 та 250 mM NaCl). Для того, щоб оцінити вплив цієї молекули на ячмінь в умовах сольового стресу, було досліджено схожість насіння, параметри росту, перекисне окислення ліпідів, вміст проліну, а також гістохімічне виявлення радикалів супероксиду та пероксиду водню та аналіз мітотичного індексу. Згідно з результатами, сольовий стрес зменшував параметри проростання, довжину брунечок та корінців, а також мітотичний індекс, водночас збільшував вміст проліну, перекисне окислення ліпідів та вміст радикалів. Обробка основою Шиффа явно зменшила перекисне окислення ліпідів і вміст радикалів у всіх дослідних групах. Однак водночас це знизило параметри проростання і росту, а також мітотичний індекс. Отримані результати показали, що антиоксидантна властивість цієї молекули збереглася в рослинах під час стресу, але також було визначено, що молекула негативно впливає, насамперед на поділ клітин.

Якщо в молекулу було б можливо внести необхідні модифікації, можна було б усунути її негативний вплив на поділ клітин, і тоді цю молекулу, яку дуже легко і дешево отримати, можна було би широко використовувати для підвищення стійкості рослин до стресу навколишнього середовища.

Ключові слова: ячмінь, основа Шиффа, активні форми кисню, сольовий стрес