Effect of blue and white lights on physiological characteristics of two wheat cultivars under salinity stress

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Abstract

Light is an energy source for photosynthetic organs and the type of optical wavelength plays an important role in growth. The effect of blue light investigated on delta-1-pyrroline-5-carboxylate (proline precursor) in 2-wheat cultivar, BAM (resistant to salinity) and Tajan (sensitive to salinity) in a culture medium. There were 5 salinity treatments including 0 (control), 50, 100, 150, and 200 mM NaCl. In addition, there were two light treatments, namely exposure to blue and white light. After five days of growth of wheat seedlings, the growth indices (fresh weight, dry weight, and longitudinal growth), proline, chlorophyll a and b, sodium, and potassium contents, peroxidase enzyme activity, malondialdehyde, and pyrolin-5- Carboxylate (proline precursor) contents were measured. Data were analyzed using Duncan statistical test. Blue light increased the amount of proline and PSC in the salinity-resistant cultivar. In the sensitive cultivar, the amount of potassium under the white light was higher than that under the blue light in different levels of salinity. The amount of sodium accumulated under the influence of blue light in different concentrations of salt in the resistant cultivar was higher than that in the sensitive cultivar. Peroxidation activity of the resistant wheat cultivar was higher and blue light did not increase this attribute, but the amount of malondialdehyde in the sensitive cultivar increased under the blue light regime and with increasing level of salinity. Fresh and dry weight of plants in the resistant cultivar was the highest under the white light. In addition, plant length in the resistant cultivar under increasing concentration of salt was more than that in the sensitive cultivar under both light regimes.

Keywords: blue light, proline, proline precursor, salinity stress, wheat

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Introduction

Growth and yield of plants in many parts of the world is limited by several living and non-living environmental stresses. In non-polluting stresses, salinity stresses in the world have caused extensive damage to plants (Manzoor et al., 2021). The effects of salt stress on only one plant growth stage cannot be different depending on the severity of the stress, the type of tension, the degree of plant resistance, different stages of growth, the type of tissue, and plant organs (evolution)(Husain et al., 2003). The amount of soluble ions in the saline soil creates two major

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problems for plant growth. First, the absorption of water is reduced due to the high concentration of osmotic active substances inside the cell, and second, some ions such as sodium, accumulate in cells (Zörb et al., 2019). Since sodium and chlorine ions are abundant in saline soils, sodium chloride salts are often used in studies designed to investigate the physiological effects of salinity on plants (Liang et al., 2018).

A vast majority of plant species accumulate proline in response to salinity or other abiotic stresses as a factor in regulating intracellular osmosis (KAVI KISHOR and Sreenivasulu, 2014). Large amounts of proline can stabilize proteins and biological membranes. It can also stimulate antioxidant defense systems (Zouari et al., 2019). In plants, proline biosynthesis is a two-step process in which the intermediate pyrroline-5-carboxylate is first made by the enzyme P5C-synthetase and combining both glutamate kinase and γ-glutamylphosphate reductase activities in a single polypeptide(Turchetto-Zolet et al., 2009).

Blue light (390-500 nm) leads to a variety of physiologic and photomorphogenic responses in plants, and these responses are regulated by cryptochrome (Ahmad et al., 2002). It is essential for chlorophyll biosynthesis, stomata opening, enzymatic activity, chloroplast maturation, and photosynthesis (Tibbitts et al., 1983). Research indicates the presence of an adsorption peak in chlorophyll in the blue wavelength range (450 nm). In fact, plants require blue light for optimal growth (Okamoto et al., 1996).

Wheat (*Triticum* spp) is considered as one of the most important crops in Iran, and it is relatively tolerant to salinity, with a tolerance threshold of 6 Ds / m (Anoshee and Farzami Sepehr, 2016). A frequently assayed osmolyte in salinization studies is proline. Proline is available in a wide range of organisms, from bacteria to excellent plants (Sepehr and Ghorbanli, 2006). There is a very strong relationship between the increase in proline level of plants and their ability to survive in aqueous conditions and salt stress (Ashraf, 2009). Extensive research has been conducted around the world to increase crops' tolerance to salinity (Anoshee and Farzami Sepehr, 2016). However, this is an ongoing research achieve more and more tolerant crops. One of the factors affecting light plants. Plants are solar-dependent in their attempt to transform sunlight into a variety of sugars, and through photosynthesis they provide energy for their growth (Ingrao et al., 2019). In this project, the effect of blue and white light on physiological responses of two wheat cultivars, namely Bam (salt-tolerant) and Tajan (salt-sensitive) were studied under salinity stress.

Materials and Methods

Seed germination experiments

Seeds of two cultivars of wheat, Bam (salt-tolerant) and Tajan (salt-sensitive) were used in the study. Seed germination and growth status were measured under five salinity treatments (0, 50, 100, 150, and 200 mM NaCl). There were three replicates for each treatment and each replicate contained 30 seeds placed in 15 cm diameter petri dishes. The seeds were kept in two incubators at 25± 2 ℃. Each incubator was randomly assigned to either blue LED light or white light, with an irradiance of 70 µE $m^{-2}s^{-1}$ in 12h photoperiod for both.

First, seeds of the two wheat cultivars were disinfected with 3% saline solution. Inside the cultivating plants, 10 seeds were placed in each of the petri dishes. Ten salt-tolerant seeds (BAM) were placed in each of the culture vessels with 0 (control), 50, 100, 150, and 200 mM salinity. Then the culture plates were placed under LED bulbs, 10 cm away from the light source. Three replications were considered for each of the white and blue light conditions. The same procedure followed for the sensitive to salinity cultivar (Tajan). All groups kept at an identical temperature of 25 ℃. The wheats harvested after 15 days and fresh and dry weights measured with precision scales.

Fresh and dry weight measurement

Following 15 days of treatments with light and salinity, the fully germinated wheat seedlings of the same length were harvested for fresh weight and dry weight measurements. Following the method suggested by Hunt Jr and Stabeno (2002), seedlings were kept at 70 ℃ in a hot air circulation

drying oven (LIYI, China) for 48 h, to determine the dry weights using a 0.0001 g electronic precision digital laboratory analytical balance (BA2104B, China).

Chlorophyll assay

Chlorophyll contents were determined following (Arnon, 1949). Accordingly, 0.2 g fresh plant material weighed and ground in a china mortar along with 80% acetone. Then, 5 ml acetone added to increase the volume to 15 ml. Three ml of the obtained solution was poured into a cuvette and its absorption intensity was read at 663, 647 nm using a spectrophotometer (Spectronic-20 Genesis TM). Acetone 80% used as witness to regulate the spectrophotometer. Chlorophyll contents were determined in terms of mg/g seedling fresh weight.

Enzyme activity assay

To obtain the extract, 2 g of wheat seedlings, frozen in liquid nitrogen, were homogenized in a cold phosphate buffer (0.05 M, pH:7) containing 0.5 g polyvinylpolypyrrolidone (PVP) in a 1:5 tissue to buffer ratio, using an ice cold mortar and pestle. The homogenates were mixed using a vortex mixer and insoluble materials were separated by centrifugation at 14,000 g for 20 min at 4 ℃. All extracts were stored at -70 ℃ for PO assay.

Peroxidase assay

The procedure for assaying peroxidase (PO) was adopted from (Mozzetti et al., 1995). Twenty micro-liters of the extract from each sample added to 3 ml of assay mixture consisting of a solution of 0.1 M sodium phosphate buffer (pH 6.0), 1 mM hydrogen peroxide, and 0.1 mM O-methoxyphenol (guaiacol). The mixture was blended thoroughly and the increase in the absorbance was recorded at 470 nm spectrophotometrically for 1 min. Peroxidase activity was determined as unit mg^{-1} protein (Ngo and Lenhoff, 1980).

Proline assay

The method suggested by (Bates et al., 1973) was used to measure the proline content. In brief, 100 mg of the frozen seedlings homogenized in 1.5 ml of sulphosalicylic acid 3%, and the residue removed by centrifugation. To make acid ninhydrin, 1.25 g ninhydrin added to 30 ml glacial acetic acid and warmed until dissolved. Then, 20 ml phosphoric acid (6 M) added to the solution. Next, 2 ml glacial acetic acid and 2 ml ninhydrin acid added to 100 µl of the extract for 1 h before the reaction mixture warmed at 100 ℃. The reaction then completed in an ice bath. One ml toluene added to the mixture, and it warmed to room temperature before its optical density measured at 520 nm. The proline content was determined from a standard curve within 20-100 µg.

P5CS activity assay

The activity of P5CS (delta 1-pyrroline-5-carboxylate synthetase) was assayed following the method described in (Hayzer and Leisinger, 1980). In order to measure P5CS activity, the required extracts were obtained by homogenizing cladodes in an extraction buffer (pH 7.5, 100 mM Trise HCl, 10 mM MgCl2, 1 mM EDTA, 10 mM b-mercaptoethanol, 4 mM DTT, 2 mM PMSF, and 2% PVPP) in pre-chilled Eppendorf tubes in a cold room. The extracts were centrifuged at 4 ℃ for 20 min at 10,000 × g. The supernatants were further clarified by centrifugation at 10,000-× g for 20 min at 4 ℃. The activity of P5CS was determined as g-glutamyl kinase in the enzyme extract by recording the formation of g-glutamyl hydroxamate. The enzyme assay carried out in a mixture with the final volume of 0.5 ml containing Trise HCl (50 mM, pH 7.0), 50 mM L-glutamate, 20 mM MgCl2, 100 mM hydroxylamine HCl, 10 mM ATP, and the enzyme extract. The reaction mixture was incubated at 37 ℃ for 15 min, and the incubation was then stopped by adding 1 ml of the stop buffer (2.5 g of FeCl3 and 6 g of trichloroacetic acid in a final volume of 100 mL of 2.5 N HCl). The precipitated proteins removed by centrifugation and the absorbance read at 535 nm against a blank identical to the above but lacking ATP. The amount of g-glutamyl hydroxamate complex produced estimated from the molar extinction coefficient 250 mol^{-1} cm^{-1} reported for Fe^{3+} hydroxamate complex of the compound. The activity was expressed in U mg^{-1} protein, which represents the amount of enzyme required to produce one mmol

of g-glutamyl hydroxamate min^{-1}. Total protein content was determined according to Bradford method (Kruger, 2009).

MDA assay

The harvested seedlings were homogenized in 1% trichloroacetic acid and then centrifuged at 10,000 rpm for 15 min. The supernatant was heated with 0.05 thiobarbituric acid for 30 min at 95 ℃, re-centrifuged at 5000 rpm for 5 min, and the absorbance was measured at 532 and 600 nm on a UV–VIS spectrometer Specord 200 PC (Analytica Jena AG) (Heath and Packer, 1968).

K and Na Contents

Seedlings dried at 70 ℃ for 48 h and extracted by dry-ashing, using HCL (2 M) at 80 ℃ for measuring K and Na contents. The amounts of K and Na in extracts were measured by an atomic absorption spectrophotometer (Phoenix 896, England) using relevant standard solutions (Olawale and Oyawale, 2012).

Statistical Analysis

Experiments arranged in a complete randomized design. The obtained data analyzed with a one-way ANOVA using the Duncan test in SPSS (ver. 14).

Results

In this study, increasing salinity led to more decrease in chlorophyll a content of the salinity sensitive wheat cultivar as compared to the resistant wheat. Besides, an increase observed in chlorophyll a content of the salinity resistant seedlings under blue light irradiation and 100 mM concentration of NaCl (Fig. I). The amount of chlorophyll b in the salinity resistant cultivar was higher than that in the sensitive wheat. In addition, in salinity resistant cultivar under blue light irradiation, an increase in chlorophyll b observed in some concentrations (Fig. II).

Accumulation of proline in the resistant wheat was higher than that in the sensitive cultivar (Fig. III). In addition, the highest proline accumulation under blue light and the highest salinity level observed in the salinity resistant wheat cultivar.

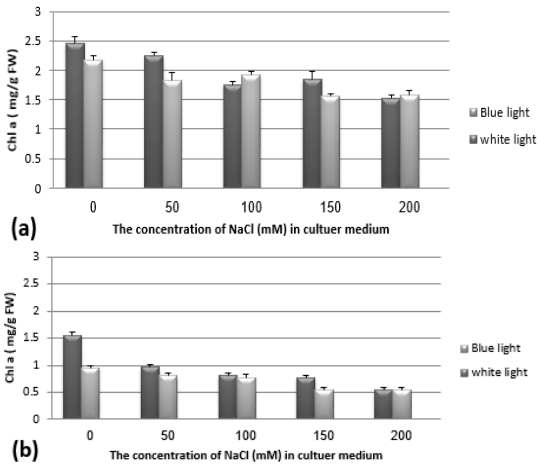

Fig. I. The effect of different salt concentrations on Chl a content in seedlings of Bam (a) and Tajan(b) cultivars under white and Blue lights.

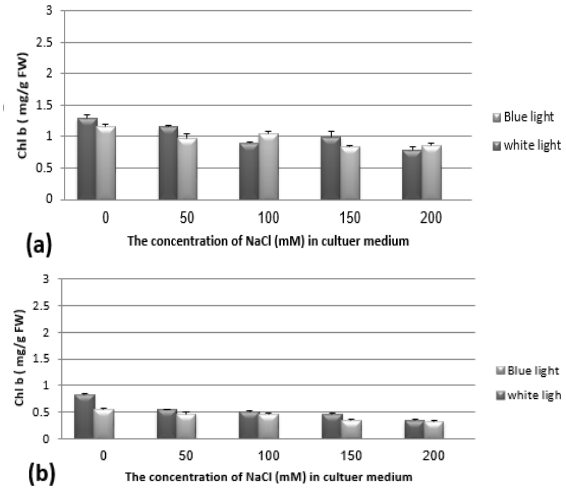

Fig. II. The effect of different salt concentrations on Chl b content in seedlings of Bam (a) and Tajan (b) cultivars under white and blue lights.

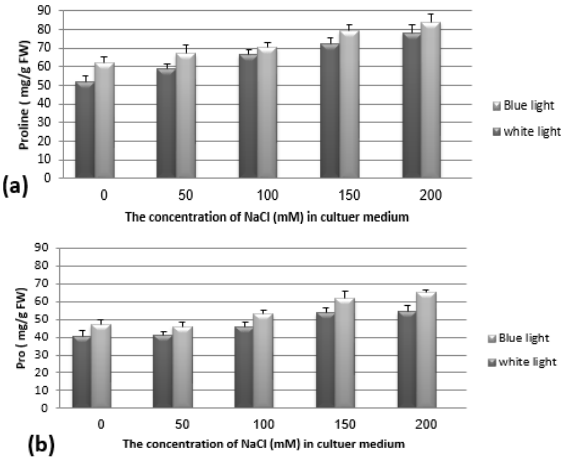

Fig. III. The effect of different salt concentrations on proline content in seedlings of Bam (a) and Tajan (b) cultivars under white and blue lights.

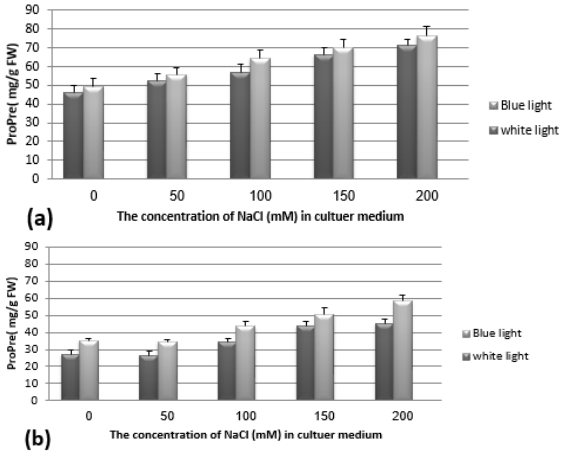

Fig. IV. The effect of different salt concentrations on P5CS activity in seedlings of Bam (a) and Tajan (b) cultivars under white and blue lights.

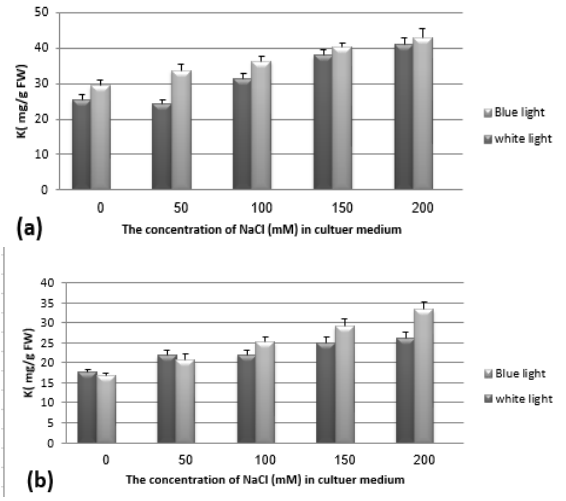

Fig. V. The effect of different salt concentrations on K content in seedlings of Bam (a) and Tajan (b) cultivars under white and blue lights.

As shown in Fig. (IV), increase in salinity level caused a greater increase in P5CS activity in the tolerant cultivar compared to the salinity sensitive wheat. In addition, blue light had a positive effect on the increasing trend of P5CS.

Salinity stress reduced the accumulation of potassium in the plants (Fig. V). The potassium content in the salinity resistant wheat was higher than that in the sensitive cultivar. In addition, the effect of blue and white lights on both cultivars was not significantly different. Results of sodium ion accumulation assays showed that salinity increased sodium contents in both cultivars under study (Fig. VI).

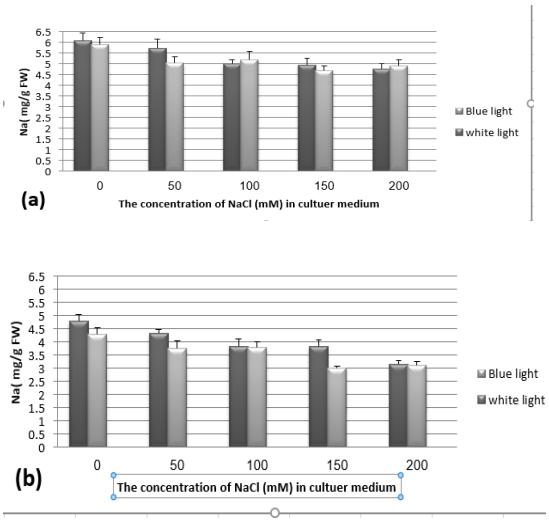

Fig. VI. The effect of different salt concentrations on Na content in seedlings of Bam (a) and Tajan (b) cultivars under white and blue lights.

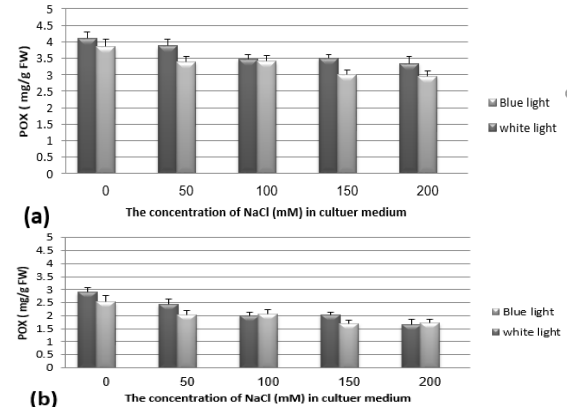

Fig. VII. The effect of different salt concentrations on POX content in seedlings of Bam (a) and Tajan (b) cultivars under white and blue lights.

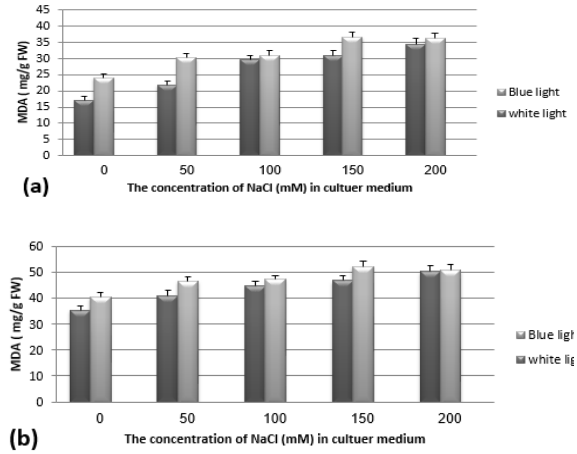

Fig. VIII. The effect of different salt concentrations on MDA content in seedlings of Bam (a) and Tajan (b) cultivars under white and blue lights.

Peroxidation activities of Bam (salinity resistant) and Tajan (salinity sensitive) cultivars decreased with increasing salinity levels (Fig. VII). However, the mean peroxidase activity was higher in salinity resistant cultivar under white light, and blue light decreased peroxidase activity in both cultivars under increasing concentrations of salt. The amount of malondialdehyde increased with increasing salinity level, and blue light caused a greater increase in malondialdehyde content than white light in both wheat cultivars. Furthermore, the sensitive cultivar accumulated more malondialdehyde under blue light irradiation (Fig. VIII).

Discussion

Blue light increased the amount of chlorophyll a and b in Tajan (salinity sensitive) more than in Bam (salinity resistant). Also, under the white light, the pigments increased in both cultivars with an even more noticeable increase in the salinity resistant cultivar. Negative effects of salinity on plant growth include destruction of chloroplast structure and lower chlorophyll content due to more chlorophyll activity (Stefanov et al., 2016). Also, the reduction in chlorophyll content can be due to the alteration of nitrogen metabolism in the production of compounds such as proline, which is used for osmotic regulation (Salehi et al., 2016). The reducing effect of salinity on the chlorophyll contents of wheat has been reported although reduction of chlorophyll a and b in tolerant cultivars is far less than salinity sensitive cultivars (Kafi, 2009).

Proline and PSC (proline precursor) contents increased under the blue light compared to the white light treatment in both cultivars, but the increase was greater in salinity-resistant cultivars. Accumulation of compatible salts may help maintain a relatively high amount of water needed for plant growth and cellular function (Verslues et al., 2006). Plants are opened up by the accumulation of certain specific metabolites, most prominent of which are amino acids and in particular proline (Zadehbagheri et al., 2014). There is direct evidence for proline accumulation under salt stress using a bacterial gene, engineered into plants, and it has been shown that excess proline production can increase the plants' ability to tolerate salinity (Rojas-Tapias et al., 2012). Maize cultivar Shandan 609 showed under the condition of moderate and severe salinity stress, the activities of superoxide, peroxidase, catalase, and proline content increased significantly. Studies show that various abiotic stresses, including salinity and drought, decrease material production by reducing the yield of photosystem II, and in the meantime, increasing proline plays a positive role in induction of stress resistance (Gao et al., 2015).

P5CS is one of two specific enzymes in the proline biosynthesis of the glutamate precursor. This compound plays an important role in salinity stress and proline accumulation (Amini et al., 2015). Our observations showed an increase in P5CS activity as a result of salt stress synchronous with proline. Genes of P5CS have been shown to increase activity under high salt levels in Vigna plant leaves and induce root tolerance to increasing amounts of salt, so it has been suggested that it plays a key role in proline biosynthesis, ultimately regulating osmosis in plants (Hu et al., 1992). In this study, proline precursor increased in salinity resistant wheat than the sensitive cultivar. This was also the case with blue-irradiated wheat showing a significant difference is as compared with the plants treated with the white light. In their study on canola, Martel and Qaderi (2019) also found that increased levels of P5C in all cultivars and at all salinity concentrations were higher under blue light treatment.

Sodium accumulation has been observed in the resistant cultivar more than in the sensitive wheat. The amount of sodium accumulated under the influence of blue light in different salinity levels was higher in the resistant cultivar than in the sensitive cultivar; however, under the influence of blue light the amount of accumulated sodium was higher than that under the white light. The vast majority of salt-tolerant plants usually accumulate significant amounts of sodium in their various organs while sensitive plants do not (Levigneron, 1995).

The potassium ion content of the plant increased in the salinity resistant cultivar although no significant difference observed between the white

and blue light regimes. In the sensitive cultivar, the amount of potassium under white light was higher than that in the blue light under different salinity treatments.

Various studies in the field of salt-resistant halophytes and glycophytes have shown that the high ratio of potassium ion to sodium ion can be considered as an indicator of tolerance to salt (Yeo et al., 1990). The study of the K/Na ratio of the plants is considered as an appropriate trait for determining tolerant varieties (Abbasi et al., 2016).

Our results show a decrease in peroxidase activity along with an increase in salinity, regardless of the degree of cultivar tolerance, confirming the findings of other research, e.g. Meloni et al. (2003) in rice and Vaidyanathan et al. (2003) in cotton. On the other hand, peroxidase activity of the resistant wheat cultivar was higher than that in the sensitive wheat, and blue light did not increase peroxidation activity. Peroxides are a catalyst for the reduction of H_2O_2 by taking electrons from different donor molecules such as phenolic compounds, lignin precursors, auxins, or secondary substrates (Bavi et al., 2011). Polyphenol oxidases and peroxidase are involved in the use of reactive oxygen species for the production of lignin and other phenolic oxidized compounds as a defense barrier to strengthen the cellular structure. Two enzymes of peroxidase and catalase divert two different forms of H_2O_2 from the environment (Chandra and Dubey, 2010). The production of reactive oxygen species under a variety of abiotic stresses is one of the processes that disrupts a variety of cellular activities. The activity of antioxidant enzymes in response to salinity stress has shown significant differences in different studies (El-Shabrawi et al., 2010). In a study on different rice genotypes, catalase activity increased with increasing salt in salinity-resistant genotypes while susceptible genotypes showed decreased activity (Kibria et al., 2017). Another study suggests that peroxidase activity decreases in response to increasing levels of salt in all genotypes, regardless of their tolerance to salt (Hasanuzzaman et al., 2014).

Malondialdehyde is generated in plant cells when the unsaturated fatty acids in the cell membrane are oxidized. This phenomenon itself indicates an oxidative damage (Yaghoubian et al., 2021). Increase in salinity showed an increase in MDA in both wheat cultivars. A number of studies have shown that salt stress alters the structure and composition of cytoplasmic membranes. These changes include an increase in the degree of saturation of free fatty acids and an increase in the amount of free sterols, which lead to a decrease in membrane fluidity (Mansour et al., 2005). Salinity seems to cause a lower level of lipid peroxidation in Bam than in Tajan cultivar, which is related to higher tolerance of Bam cultivar. There are similar results by increasing lipid peroxidation with salinity in corn-sensitive genotypes (Carrasco-Ríos and Pinto, 2014).

The amount of malondialdehyde in the sensitive wheat increased under the blue light regime and with increasing level of salinity. Furthermore, malondialdehyde contents of the resistant cultivar was high and low under the blue and white lights, respectively. Blue light with a wavelength of 480 nm in the sensitive and resistant samples led to a significant increase in malondialdehyde contents. According to (Li et al., 2010), wavelengths of 450-550 nm have a destructive effect on membranes, especially photosynthetic membranes, which cause their destruction by increasing malondialdehyde.

The fresh and dry weights of the studied plants follow a constant trend under increasing levels of salinity. In fact, increased level of salinity resulted in no appreciable decrease in FW and DW of both tolerant and resistant wheat cultivars. However, FW and DW in the sensitive seedlings were lower compared to the tolerant wheats. In the resistant cultivar, the maximum FW and DW levels were observed under the white light. Plant lengths in both cultivars and under increasing salinity concentrations were higher than the sensitive cultivar under both blue and white light. Blue light (480 nm) falls within the range of 450-550 nm. This makes it possible to reduce the active photons that reduce the opening of the stomachs and thus reduce the gas exchange rate.

References

Abbasi, H., M. Jamil, A. Haq, S. Ali, R. Ahmad, Z. Malik and Z. Parveen. 2016. Salt stress manifestation on plants, mechanism of salt tolerance and potassium role in alleviating it: a review. *Zemdirbyste-Agriculture,* 103, (2) 229-238.

Ahmad, M., N. Grancher, M. Heil, R. C. Black, B. Giovani, P. Galland and D. Lardemer. 2002. Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in Arabidopsis. *Plant Physiology,* 129, (2) 774-785.

Amini, S., C. Ghobadi and A. Yamchi. 2015. Proline accumulation and osmotic stress: an overview of P5CS gene in plants. *Journal of Plant Molecular Breeding,* 3, (2) 44-55.

Anoshee, M. and M. Farzami Sepehr. 2016. The effect of salinity pretreatment of Glomus mosseae on induction of salinity tolerance in Lycopersicum esculentum L. *Iranian Journal of Plant Physiology,* 6, (4) 1815-1824.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology,* 24, (1) 1.

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology advances,* 27, (1) 84-93.

Bates, L. S., R. P. Waldren and I. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and soil,* 39, (1) 205-207.

Bavi, K., B. Kholdebarin and A. Moradshahi. 2011. Effect of cadmium on growth, protein content and peroxidase activity in pea plants. *Pak J Bot,* 43, (3) 1467-1470.

Carrasco-Ríos, L. and M. Pinto. 2014. Effect of salt stress on antioxidant enzymes and lipid peroxidation in leaves in two contrasting corn,'Lluteno'and'Jubilee'. *Chilean journal of agricultural research,* 74, (1) 89-95.

Chandra, A. and A. Dubey. 2010. Effect of ploidy levels on the activities of Δ1-pyrroline-5-carboxylate synthetase, superoxide dismutase and peroxidase in Cenchrus species grown under water stress. *Plant Physiology and Biochemistry,* 48, (1) 27-34.

El-Shabrawi, H., B. Kumar, T. Kaul, M. K. Reddy, S. L. Singla-Pareek and S. K. Sopory. 2010. Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. *Protoplasma,* 245, (1) 85-96.

Gao, J., R. Zhang, W. Wang, Z. Li and J. Xue. 2015. Effects of drought stress on performance of photosystem II in maize seedling stage. *Ying yong sheng tai xue bao= The journal of applied ecology,* 26, (5) 1391-1396.

Hasanuzzaman, M., M. Alam, A. Rahman, M. Hasanuzzaman, K. Nahar and M. Fujita. 2014. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (Oryza sativa L.) varieties. *BioMed Research International,* 2014,

Hayzer, D. J. and T. Leisinger. 1980. The gene-enzyme relationships of proline biosynthesis in Escherichia coli. *Microbiology,* 118, (2) 287-293.

Heath, R. L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics,* 125, (1) 189-198.

Hu, C., A. J. Delauney and D. Verma. 1992. A bifunctional enzyme (delta 1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. *Proceedings of the National Academy of Sciences,* 89, (19) 9354-9358.

Hunt Jr, G. L. and P. J. Stabeno. 2002. Climate change and the control of energy flow in the southeastern Bering Sea. *Progress in Oceanography,* 55, (1-2) 5-22.

Husain, S., R. Munns and A. T. Condon. 2003. Effect of sodium exclusion trait on chlorophyll retention and growth of

durum wheat in saline soil. *Australian Journal of Agricultural Research,* 54, (6) 589-597.

Ingrao, C., J. Bacenetti, J. Adamczyk, V. Ferrante, A. Messineo and D. Huisingh. 2019. Investigating energy and environmental issues of agro-biogas derived energy systems: A comprehensive review of Life Cycle Assessments. *Renewable Energy,* 136, 296-307.

Kafi, M. 2009. The effects of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt-tolerant and salt-sensitive wheat (Triticum aestivum L.) cultivars.

Kavi Kishor, P. B. and N. Sreenivasulu. 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant, cell & environment,* 37, (2) 300-311.

Kibria, M. G., M. Hossain, Y. Murata and M. A. Hoque. 2017. Antioxidant defense mechanisms of salinity tolerance in rice genotypes. *Rice Science,* 24, (3) 155-162.

Kruger, N. J. 2009. The Bradford method for protein quantitation. *The protein protocols handbook,* 17-24.

Levigneron, A. 1995. Plants toward salt stress. *Cahiers d'Agricultures,* 4, 263-273.

Li, G., S. Wan, J. Zhou, Z. Yang and P. Qin. 2010. Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (Ricinus communis L.) seedlings to salt stress levels. *Industrial crops and products,* 31, (1) 13-19.

Liang, W., X. Ma, P. Wan and L. Liu. 2018. Plant salt-tolerance mechanism: A review. *Biochemical and biophysical research communications,* 495, (1) 286-291.

Mansour, M., K. Salama, F. Ali and A. Abou Hadid. 2005. Cell and plant responses to NaCl in Zea mays L. cultivars differing in salt tolerance. *Gen Appl Plant Physiol,* 31, (1-2) 29-41.

Manzoor, N., T. Ahmed, M. Noman, M. Shahid, M. M. Nazir, L. Ali, T. S. Alnusaire, B. Li, R. Schulin and G. Wang. 2021. Iron oxide nanoparticles ameliorated the cadmium and salinity stresses in wheat plants, facilitating photosynthetic pigments and restricting cadmium uptake. *Science of the Total Environment,* 769, 145221.

Mozzetti, C., L. Ferraris, G. Tamietti and A. Matta. 1995. Variation in enzyme activities in leaves and cell suspensions as markers of incompatibility in different Phytophthora-pepper interactions. *Physiological and Molecular Plant Pathology,* 46, (2) 95-107.

Ngo, T. and H. M. Lenhoff. 1980. A sensitive and versatile chromogenic assay for peroxidase and peroxidase-coupled reactions. *Analytical biochemistry,* 105, (1) 389-397.

Okamoto, K., T. Yanagi, S. Takita, M. Tanaka, T. Higuchi, Y. Ushida and H. Watanabe. 1996. Development of plant growth apparatus using blue and red LED as artificial light source. *Proc. International Symposium on Plant Production in Closed Ecosystems 440*, *1996*:111-116:

Olawale, O. and F. A. Oyawale. 2012. Characterization of rice husk via atomic absorption spectrophotometer for optimal silica production. *International Journal of science and Technology,* 2, (4) 210-213.

Rojas-Tapias, D., A. Moreno-Galván, S. Pardo-Díaz, M. Obando, D. Rivera and R. Bonilla. 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). *Applied Soil Ecology,* 61, 264-272.

Salehi, A., H. Tasdighi and M. Gholamhoseini. 2016. Evaluation of proline, chlorophyll, soluble sugar content and uptake of nutrients in the German chamomile (Matricaria chamomilla L.) under drought stress and organic fertilizer treatments. *Asian Pacific Journal of Tropical Biomedicine,* 6, (10) 886-891.

Sepehr, M. F. and M. Ghorbanli. 2006. Physiological responses of Zea mays seedlings to interactions between cadmium and salinity. *Journal of Integrative Plant Biology,* 48, (7) 807-813.

Stefanov, M., E. Yotsova, G. Rashkov, K. Ivanova, Y. Markovska and E. L.

Apostolova. 2016. Effects of salinity on the photosynthetic apparatus of two Paulownia lines. *Plant Physiology and Biochemistry,* 101, 54-59.

Tibbitts, T., D. Morgan and I. Warrington. 1983. Growth of lettuce, spinach, mustard, and wheat plants under four combinations of high-pressure sodium, metal halide, and tungsten halogen lamps at equal PPFD. *J Amer Soc Hort Sci,* 108, (4) 622-630.

Turchetto-Zolet, A. C., M. Margis-Pinheiro and R. Margis. 2009. The evolution of pyrroline-5-carboxylate synthase in plants: a key enzyme in proline synthesis. *Molecular genetics and genomics,* 281, (1) 87-97.

Verslues, P. E., M. Agarwal, S. Katiyar-Agarwal, J. Zhu and J. K. Zhu. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal,* 45, (4) 523-539.

Yaghoubian, I., S. Ghassemi, M. Nazari, Y. Raei and D. L. Smith. 2021. Response of physiological traits, antioxidant enzymes and nutrient uptake of soybean to Azotobacter Chroococcum and zinc sulfate under salinity. *South African Journal of Botany,* 143, 42-51.

Yeo, A., M. Yeo, S. Flowers and T. Flowers. 1990. Screening of rice (Oryza sativa L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theoretical and Applied Genetics,* 79, (3) 377-384.

Zadehbagheri, M., A. Azarpanah and S. Javanmardi. 2014. Proline metabolite transport an efficient approach in corn yield improvement as response to drought conditions. *Nature,* 566, 76-485.

Zörb, C., C. M. Geilfus and K. J. Dietz. 2019. Salinity and crop yield. *Plant biology,* 21, 31-38.

Zouari, M., A. B. Hassena, L. Trabelsi, B. B. Rouina, R. Decou and P. Labrousse. 2019. Exogenous proline-mediated abiotic stress tolerance in plants: Possible mechanisms. In *Osmoprotectant-mediated abiotic stress tolerance in plants*:99-121: Springer. Number of 99-121 pp.