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NITROGEN FERTILIZER ALLEVIATED NEGATIVE IMPACTS OF NACL ON SOME PHYSIOLOGICAL PARAMETERS OF WHEAT

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Abstract

Understanding salinity-fertilizer relationship is of considerable economic importance. Salinity is one of the abiotic stresses limiting crop productivity, especially in arid and semi-arid regions. This study was done to determine whether the nitrogen (N) could alleviate the negative effects of NaCl on several physiological attributes and antioxidative defense system. The results showed that high salinity significantly reduced chlorophyll content (SPAD reading), photosynthesis, stomatal conductance, protein content, and SOD. Moreover, salt treatment increased the activity of CAT and POD. Nitrogen application had significant effects on all the physiological parameters by enhancing chlorophyll content, photosynthesis, protein content, SOD, POD, and CAT. All parameters were significantly affected by the interaction between variety and nitrogen except CAT. The interactive effect between variety and salinity was significant for protein content, SOD, and CAT. The content of protein and the activities of SOD, POD, and CAT were significantly affected by the interaction between nitrogen and salinity. In this study, the application of nitrogen fertilizer was successful in alleviating the adverse effects of salinity. Of all the nitrogen levels used in this study, 210 kg $^{-1}$ha was most effective on most of the measured parameters. The results of this study will be helpful in getting increasing yield by choosing suitable varieties and nitrogen management on saline soils.

Key words: Abiotic stresses, Antioxidative enzymes, Physiological attributes, Salinity.

Introduction

Salinity stress can cause osmotic and ionic stresses in plant cells that lead to a deterioration in plant's growth and yield by influencing physiological processes. Salt stress also causes oxidative stress by generating high levels of oxygen radicals (Chawla *et al*., 2013). Plant species are different in salt tolerance and the mechanism of tolerance. It has been confirmed that under the saline condition some exogenous chemicals can mitigate the adverse effects of salinity on plants (Gupta & Huang, 2014).

Bread wheat (*Triticum aestivum* L.) is an essential source of food and livelihood for over one billion people in developing countries. It is reckoned among the "big three cereal crops" viz. rice, wheat, and maize (Metwali *et al*., 2011). China is one of the world's largest wheat-producing countries. However, Chinese wheat yield is among the highest in the world and its estimated production put it in second place after the European Union (Anon., 2014). Wheat has become an important staple food in Sudan, it is the second most important cereal crop after sorghum in Sudan. Wheat cultivated area in Sudan in irrigation schemes such as Gezira, Rahad, and New Halfa schemes (Anon., 2015). Salinity is the most important factor limiting wheat production in Sudan, particularly in the Northern States of the country (Elahmadi & Hamza, 2015).

Nitrogen (N) is a necessary nutrient in agricultural production, and its effective use to enhance crop production is more than any other chemical fertilizer (Malhi *et al*., 2001). Consequently, proper application of nitrogen fertilizer is essential to enhance crop yield. It has been determined that the most of nitrogen applied (40%-60%) is taken up by wheat (Guarda *et al*., 2004). It is understood that the adverse influences caused by salt stress could be alleviated by application of fertilizer (Chen *et al*., 2010).

Plants growing in saline soils are often N deficient. The lack of nitrogen is very common in crops and induces inhibition of plant development whether plants are growing under salinity stress or normal conditions. Addition of nitrogen to nitrogen deficient soils at moderate salt stress increased growth and yield of crops. In most cases, total N uptake declines, but N concentration increases under optimal N conditions (Machado & Serralheiro, 2017).

In this study, we hypothesized that adverse effects of salinity could be improved by nitrogen fertilizer and will help to promote the physiological performance. Therefore, the aim of this study was to investigate whether the nitrogen could alleviate the impact of salinity in the leaves of wheat.

Materials and Methods

Plant material: This study two wheat varieties, Elnilein and Xumai 30 that are routinely grown on saline soils in Sudan and China (Ibrahim, *et al*., 2016) were selected as model varieties. All seeds were less than 18-month old. The seeds were sterilized with 1% NaOCl solution for 2-3 min, then washed with distilled water three times and then air-dried and within each variety the seeds of uniform color, size, and shape were selected for the experiment.

Soil preparation: The soil used in this study was the 0-20 cm layer of a Typic fluvaquents Entisols. It was collected from the surface of sandy loam soil (0-20 cm) of the Experimental Farm of Yangzhou University (32°30'N, 119°25'E), Jiangsu Province, China. The soil was dried and sieved in a 5 mm sieve. Then the soil was spread at a thickness of about 50 mm over a piece of polyethylene sheet. Soil suspension was prepared in deionized water at a ratio of 1:2 (soil: water) (Sonneveld & Van Den Ende, 1971). The suspension was moved and allowed to stand overnight. After that, the electrical conductivity (EC) of the supernatant solution was 0.26 dSm^{-1} measured with an EC meter (TZS-EC-I, Zhejiang Top Instrument Co., Ltd., Hangzhou, China). The soil samples were analyzed for pH (1:1 in water) using a pH meter (Bench Top pH-meter by 3B Scientific - U33100 - 1011690 - pH meter), The determination of soil organic carbon is based on the Walkley-Black method (1934) described by (Nelson & Sommers, 1996), nitrogen (N) following the Kjeldahl method described by Labconco (1998), available phosphorus (P) following Micro-Vanadate-Molybdate method Olson (1954), available potassium (K) following neutral ammonium acetate extract method determined by flame photometer (Chapman & Pratt, 1962). The soil was tested containing 1.22% organic matter, 1.0 g kg^{-1} total N, 14.1 mg kg^{-1} P, and 77.3 mg kg^{-1} soil test K with pH 7.1. Water added to each treatment was 3.44 L/94.3 cm^2 (pot surface area) to promote germination. Tap water (EC of 0.4 dS m^{-1}) was the water source used in this experiment. The pots were weighted every 2 or 3 days to maintain the soil water content at 80% of field capacity. Each pot was without holes at the bottom to avoid drainage or leaching through the pots.

Experimental design: The experiment was done on the Experimental Farm of Yangzhou University during two wheat growing seasons of winter of 2014/2015 and 2015/2016. Temperature and relative humidity during kernel filling were 27°C and 70% in the 1st season and 24°C and 80% in the 2nd season. The study was designed as a factorial experiment arranged in a randomized complete block design in the split split-plot with three factors. Main plots included wheat varieties; subplots included three different levels of nitrogen fertilizer as urea (46% N) 0, 86, and 210 kg N ha-1, designated as 0N, 1N, and 2N. The sub–sub-plot included four levels of salinity included 1.4, 2.5, 4.2, and 7.6 dS m^{-1} designated as S0, S1, S2, and S3 respectively. The saline soils at different salinity levels were made by incorporating NaCl solutions into the non-saline soil and mixed. The control treatment of soil was made by adding tap water. There were 24 treatments in the study with three replications for each treatment. There were 72 pots in total in the study. Each pot (30 cm in diameter × 32 cm in depth,) was filled with 12 kg dry soil. Twenty seeds were sown at the seeding depth of 1.5 cm. All the pots were placed in the open field. If rain was expected, a plastic film was placed over the pots. The seeding dates were October 20th and 25th in 2014 and 2015 respectively. One month after seeding, all the pots were thinned to five plants for each pot.

Observations and measurements

Assessment of chlorophyll content (SPAD reading): The penultimate leaves of each plant in each pot were used for SPAD determination with a chlorophyll meter (SPAD-502, chlorophyll meter, Minolta Camera Co., Ltd., Japan). The SPAD reading was recorded at the tip, middle, and base of each leaf. The average of SPAD readings of the leaves of each pot was calculated.

Assessment of photosynthetic parameters: Photosynthetic rate, transpiration rate, and stomatal conductance were measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, USA) at 8:00–11:30 am local time at anthesis stages. The flag leaf of the wheat plant was selected for the measurements of leaf photosynthetic parameters.

Physiological parameters: The leaves of each treatment were sampled and immersed in liquid nitrogen for 15 minutes and then saved at -75℃ for the determination of physiological parameters, including soluble protein, and enzymatic activities of peroxides (POD), catalase (CAT), and superoxide dismutase (SOD).

Determination of soluble proteins: The soluble protein was determined according to method of Bradford (1976). About 0.5 g of leaf sample was used. The sample was homogenized at 4°C in 5 ml Na-phosphate buffer (pH 7.2) and then centrifuged at 4°C. The supernatants were placed on ice for analysis. The soluble protein was determined using the Coomassie blue dye-binding assay (Bradford, 1976) by using bovine serum albumin (BSA) as the standard curve. Then the absorbance reading was converted into the content of protein. The absorbance was determined with a spectrometer by pipetted supernatants and stain in spectrophotometer cuvettes at 595 nm (Model 721, Shanghai Mapada Instruments Co. Ltd, Shanghai).

Determination of antioxidative enzymes activities: For enzyme extraction, frozen (–75ºC) leaves samples of wheat plant (0.5 g) were collected and ground at 4ºC in the extraction buffer containing 50 mM PBS, 0.1 mM EDTA, pH 7.8, 4% poly vinyl polypyrroidone (PVP), and 0.3% Triton X-100, and then centrifuged at 4ºC, 10,500 pm for 20 min. The supernatant was collected and placed on ice and used for the determinations of antioxidant enzymes activities. The activity of POD was determined using the method of Xu & Ye (1989) while the activity of SOD was determined according to the method of Koca *et al*., (2007).

Statistical analysis: This experiment was a factorial one arranged in a split-split-plot randomized complete block design including three variables, with three replications for each experimental unit. The investigation was performed two different seasons, and there were no significant differences in all factors between the two seasons. So, the mean of each variable of the two seasons was applied for statistical analysis. The data of variables were subjected to analysis of variance (ANOVA) following the method of Gomez & Gomez (1984) with the statistical package of Mstat-C (Freed *et al*., 1991) according to this design. Means compared by the LSD test when F values were significant ($p \leq 0.05$).

Results

Effect of the treatments on chlorophyll content (SPAD reading): Chlorophyll content was significantly affected by nitrogen, salinity, and the interaction between variety and nitrogen (Table 1). Chlorophyll content was decreased with the increased salinity level. At the high salinity level, chlorophyll content was decreased by 15.9% as compared with the control (Fig. 1A). In the interaction between variety and nitrogen, Elnilein at the high nitrogen level had higher chlorophyll content than Xumai 30 (Fig. 1B).

Effect of the treatments on photosynthetic rate of wheat: Photosynthetic rate, transpiration rate, and stomatal conductance were significantly affected by wheat variety, nitrogen, salinity, and interaction between varieties and nitrogen (Table 1). Photosynthetic rate, stomatal conductance, and transpiration rate were decreased gradually with increasing salinity level. The S3 salinity level caused reductions in photosynthetic rate by 43.4% as compared with the non-salinity level (Fig. 2). In the interaction between variety and nitrogen, all nitrogen treatments significantly increased the photosynthesis, stomatal conductance, and transpiration rate (Fig. 3A-C).

Effect of the treatments on soluble protein of wheat: Protein content was significantly affected by all the experimental factors and their interactions except variety and interaction among three experimental factors (Table 1). In the interaction between variety and nitrogen, at all nitrogen level variety, Elnilein had the highest protein content by 7.9% (Fig. 4A). In the interaction between salinity and variety, at high salinity level, the variety Elnilein had higher protein content than Xumai 30 by 23.8% (Fig. 4B). In the interaction between salinity and nitrogen, all nitrogen treatments significantly increased protein content in all the salinity treatment (Table 2).

Effect of the treatments on antioxidant enzyme activity of wheat: The activity of SOD was significantly affected by all experimental factors and their interactions (Table 1). In the interaction among the three experimental factors (variety, nitrogen, and salinity), 2N nitrogen treatment at the highest salinity level of S3 in Elnilein decreased SOD activity by 4.2% as compared with the control (Table 3).

POD was significantly affected by nitrogen treatment, salinity treatment, interaction between variety and nitrogen and between nitrogen and salinity (Table 1). Of the interaction between variety and nitrogen, at all nitrogen treatment variety, Xumai 30 had the greater value of POD than Elnilein (Fig. 5A). In the interaction between nitrogen and salinity, all nitrogen treatment significantly decreased POD at all salinity treatments, and at high salinity level 2N nitrogen treatment decreased POD by 33.7 % as compared with the control (0N) (Fig. 5B).

Table 1. Analysis of variance for effects of varieties (V), Nitrogen (N), Salt (S) and their interaction on seedling growth parameters of wheat.

Dependent variable	Independent variable						
	Variety (V)	Nitrogen(N)	Salt(S)	V×N	V×S	N×S	V×N×S
Chlorophyll content (SPAD reading)	ns	**	**	*	ns	ns	ns
Photosynthesis rate	ns	**	**	**	ns	ns	ns
Stomata conductance	**	**	**	**	ns	ns	ns
Transpiration rate	**	**	**	*	ns	ns	ns
Protein content	ns	**	**	**	**	**	ns
SOD	**	**	**	**	**	**	**
POD	ns	**	**	**	ns	**	ns
CAT	*	*	**	ns	**	**	ns

Ns = Insignificant difference; * = Significant the difference at p≤0.05; ** = Significant difference at p≤0.01

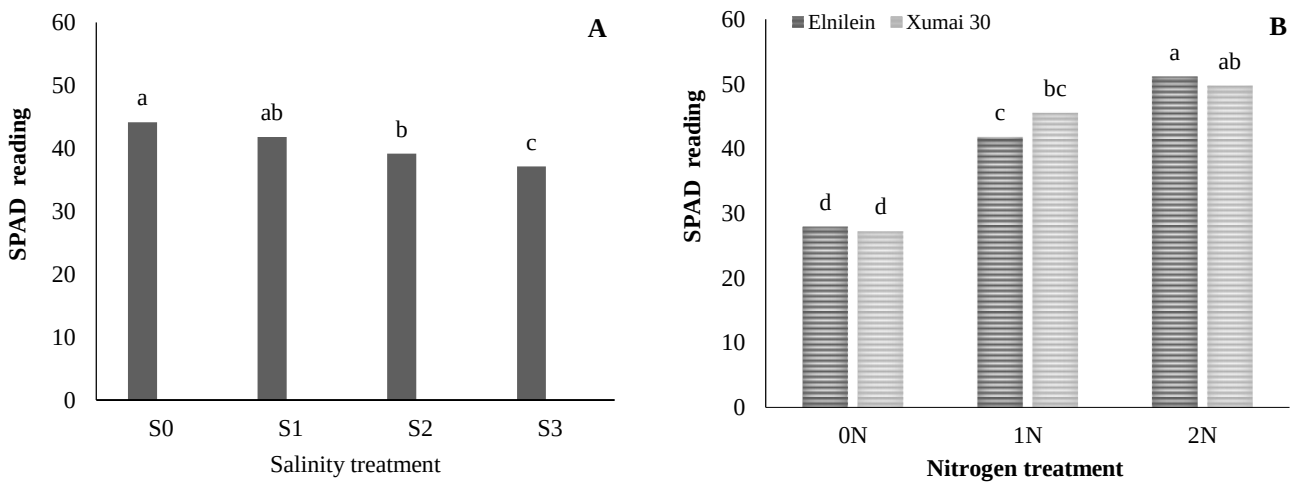
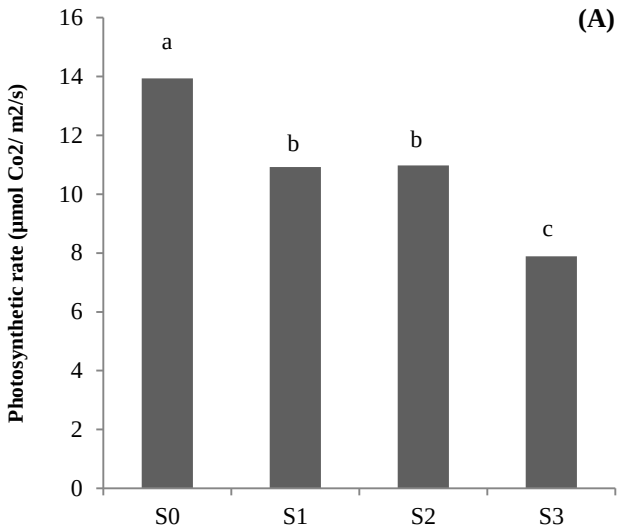
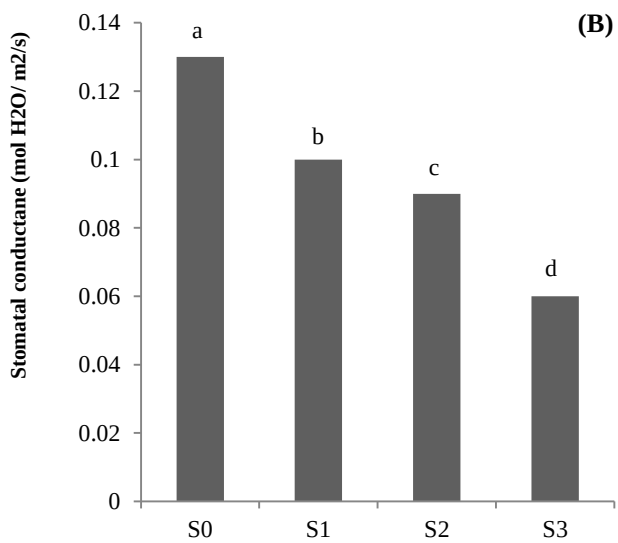


Fig. 1. Effects of salinity (A) and nitrogen (B) on leaf chlorophyll content (as leaf SPAD reading) of wheat plants of Elnilein and Xumai 30. Bars represent S.E. of the mean. Bars with the same letter are not different at the 0.05 probability level. Means were separated by the LSD test.





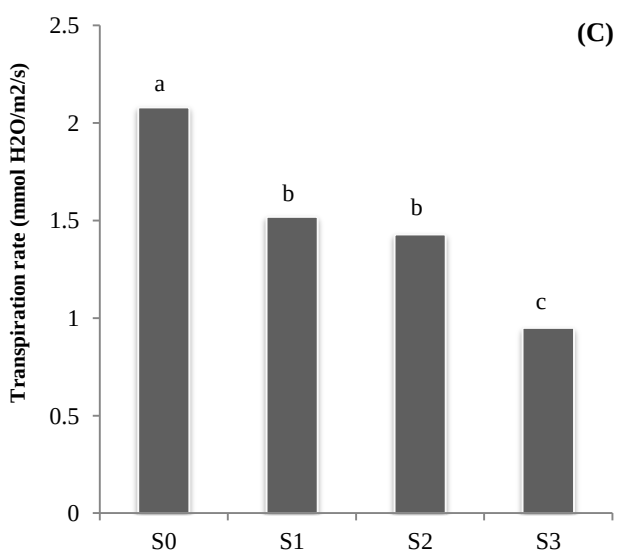
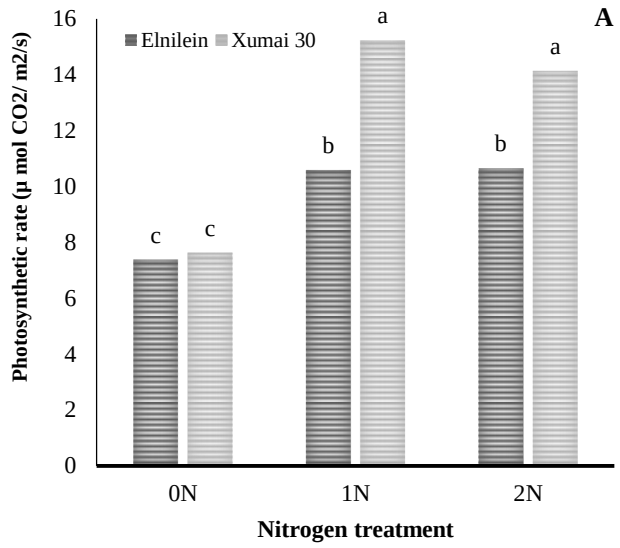
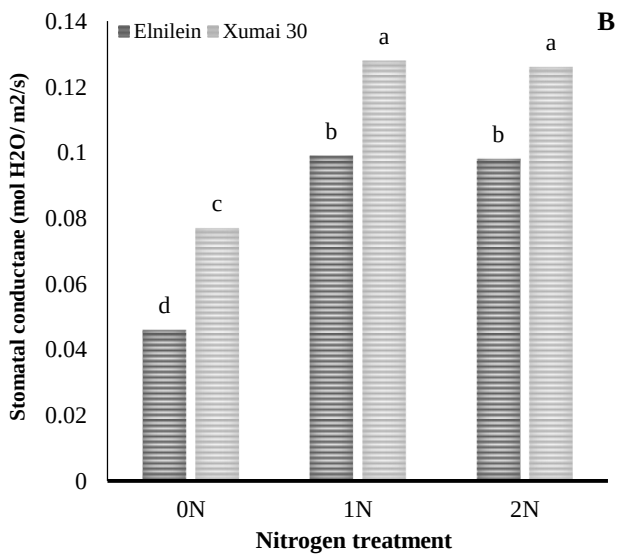


Fig. 2. Effects of salinity on photosynthetic rate (µmol CO_2/ m^2/s) (A), stomatal conductance (mol H_2O/ m^2/s) (B) and transpiration rate (mmol H_2O/m^2/s) (C) of wheat leaves. Bars with the same letter are not different at the 0.05 probability level. Means were separated by the LSD test.





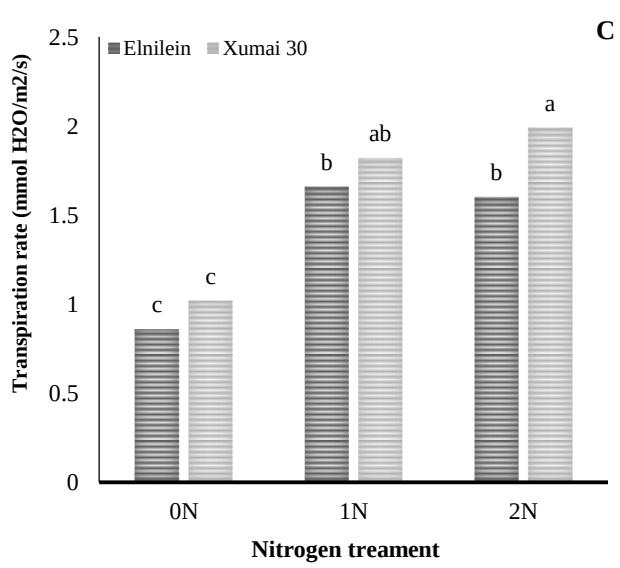


Fig. 3. Effects of the interaction variety and nitrogen on photosynthetic rate (µmol CO_2/ m^2/s) (A), stomatal conductance (mol H_2O/ m^2/s) (B) and transpiration rate (mmol H_2O/m^2/s) (C) of wheat leaves. Bars represent S.E. of the mean. Points marked with the same letter are not different at the 0.05 probability level. Means were separated by the LSD test.

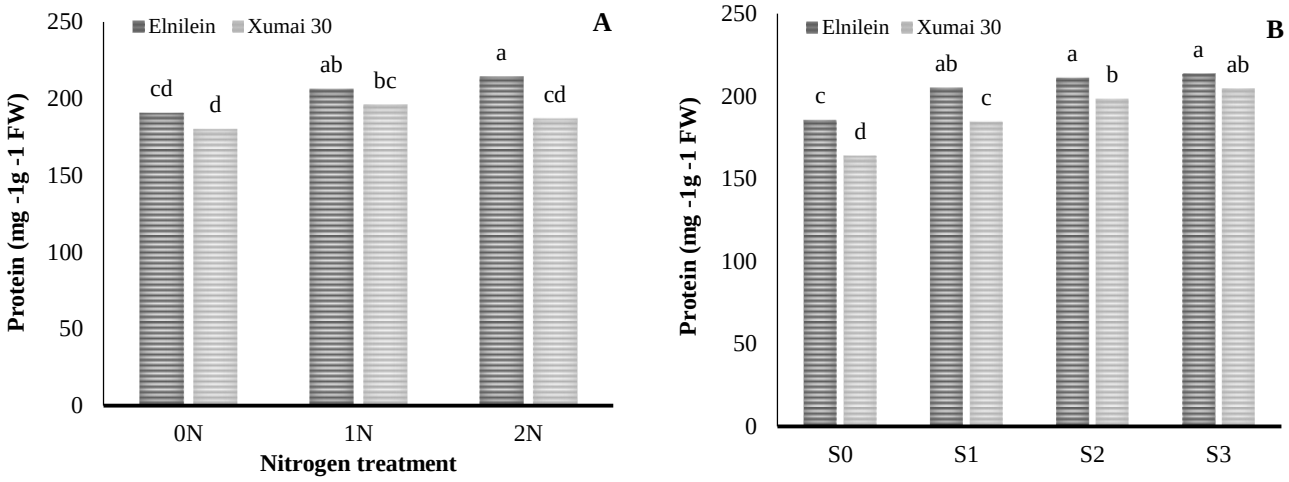


Fig. 4. Effects of the interaction between variety and nitrogen (A) and the interaction between variety and salinity (B) on the protein content of wheat plants of Elnilein and Xumai 30. Bars with the same letter are not different at the 0.05 probability level. Means were separated by the LSD test.

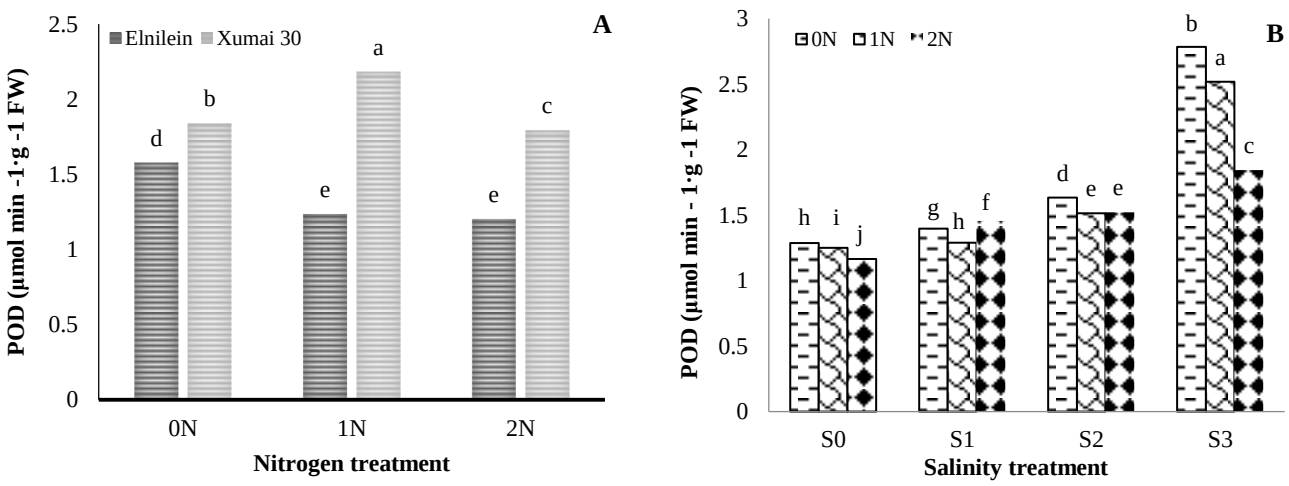


Fig. 5. Effects of the interaction between variety and nitrogen (A) and the interaction between nitrogen and salinity (B) on POD (µmol min $^{-1}$·g^{-1} FW) of wheat varieties of Elnilein and Xumai 30. Bars with the same letter are not different at the 0.05 probability level. Means were separated by the LSD test.

Table 2. Effect of interaction between nitrogen and salinity on Protein, and CAT of wheat leaves at anthesis stage.

	0N	Protein (mg/g FW)		0N	CAT (min $^{-1}$·g^{-1} FW)	
		1N	2N		1N	2N
S0	190.9c	216.9a	213.5a	24.12fg	22.62g	31.5ef
S1	199.9b	211.3a	211a	22.75g	35.95de	35.68de
S2	178.7de	191.1c	209.8a	29.52efg	39.18cd	59.9b
S3	171.4ef	184.3cd	167.8f	33.23de	46c	67.72a

Different letters in the table show significant differences at 0.05 probability level

Table 3. Effect of interaction among variety (Elnilein, and Xumai 30), nitrogen (0N, 1N, and 2N), and salinity (S0, S1, S2, and S3) on SOD (min $^{-1}$·g^{-1} FW) of wheat leaves at anthesis stage.

	0N	Elnilein		0N	Xumai 30	
		1N	2N		1N	2N
S0	186.3l	185.3l	131.3n	200.7jk	181.3l	123.3o
S1	214.3h	195.0k	169.3m	226.7g	247.7e	196.7k
S2	236.7f	205.3ij	250.0e	231.0fg	257.0d	204.0ij
S3	257.7cd	234.0f	269.0b	290.0a	263.3bc	207.3i

Different letters in the table show significant differences at 0.05 probability level

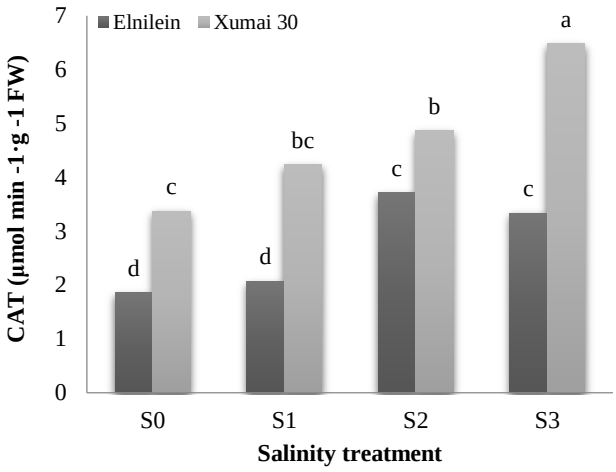


Fig. 6. Effect of salinity on CAT (µmol min $^{-1}$·g^{-1} FW) of wheat varieties of Elnilein and Xumai 30. Bars with the same letter are not different at the 0.05 probability level. Means were separated by the LSD test.

The CAT activity was significantly affected by all experimental factors and their combinations except the interaction between variety and salinity and interaction among the three experimental factors (Table 1). In the interaction between variety and salinity, at high salinity level, variety Xumai 30 had the higher CAT than Elnilein by 48.6% (Fig. 6). In the interaction between salinity and nitrogen, all nitrogen treatments significantly increased the CAT activity, at the high salinity level of S3, 2N nitrogen treatment increased CAT by 50.9 % as compared with the control (Table 2).

Discussion

Understanding salinity-fertilizer relationships is essential, and many studies have been conducted to examine the physiological responses by plants under saline conditions (Alam *et al*., 2015; Ali *et al*., 2004; Dhanapackiam and Ilyas, 2010; Nimir *et al*., 2014; Zhang *et al*., 2014). The damaging impacts of the salt stress on plants could be recognized at the all plant growth stages as the death of plants and reductions in production. High salinity affects plant growth by changing their physiological parameters (Alam *et al*., 2015, Muscolo *et al*., 2003). In this study, we investigated the adverse effects of different salinity levels, including 0, 1, 2 and 3 g NaCl/kg dry soil on physiological responses of the wheat plant. Our results indicated that salinity stress inhibited physiological parameters in term of chlorophyll content as SPAD reading, photosynthetic rate, and protein content as compared with the non-saline conditions. These findings were in agreement with (Jamil *et al*., 2014), who reported that chlorophyll content was significantly decreased with the increase in salinity in Cauliflower (*Brassica oleracea*), whereas a significant increase was observed in other Brassica species The decrease in chlorophyll content is partially because of the inhibitory effects of ions of several salts on the biosynthesis of different chlorophyll molecules (Kaya *et al*., 2015). Under saline conditions, the activity of chlorophyll-degrading enzymes could also be increased (Nimir *et al*., 2016). Besides in this study, the chlorophyll content of wheat in N treatments was higher than other without N application. These results were in conformity with those of Zhang *et al*., (2013) who found that increasing levels of nitrogen increased chlorophyll content in soya bean (*Glycine max* L.). Similar finding was described by (Vafadar *et al*., 2014) in wheat who reported that there was a positive relationship between nitrogen supply and the leaf chlorophyll content and biomass production.

One of the essential biochemical pathways is photosynthesis by which plants transform solar energy into chemical energy and plant growth. The decrease in photosynthetic rates in plants under salinity stress is mostly because of the decrease in water potential (Parihar *et al*., 2015). In the present study, as the salt concentrations increased, the photosynthetic rate, stomatal conductance, transpiration rate) were decreased. These results were in agreement with those of Liu *et al*., (2014). Also our results are in agreement with Khan *et al*., (2014) who report that the overall decreased growth of yield under salt stress is a result of the cumulative effect of disruption of ion homeostasis, water imbalance, and reduction in photosynthetic capacity of plants. The significant combination of nitrogen and salinity treatment showed a positive impact of nitrogen on the photosynthetic rate of plants due to the highest photosynthetic rate observed in plants treated with nitrogen fertilizer. Related results were reported by Živčák *et al*., (2014), Zhang *et al*., (2013), and Vafadar, *et al*., (2014).

In this investigation, soluble protein content was significantly affected by salt stresses. Moreover, protein content was increased when the plants were treated with nitrogen fertilizer. These findings agree with the results reported by Ayala-Astorga & Alcaraz-Meléndez (2010) and Mahboobeh & Akbar (2013). They are also in agreement with those who indicated that protein content reduced under salt condition in sunflower (*Helianthus annuus*) and corn (*Zea mays* L.) (Ebrahimian & Bybordi, 2012; Gautam & Singh, 2011), but in disagreement with those who reported that the soluble protein was considerably higher in the NaCl treatments than that in non-saline treatments (Nimir *et al*., 2015).

To cope with oxidative harm under salinity stress, plants can develop an antioxidant defense system (Foyer & Noctor, 2005; Kala, 2015). In the present investigation, activity of antioxidant enzymes was increased accordingly under salt stress condition in the wheat plants and the highest SOD activity was observed in the variety Xumai 30 and the lowest was in the variety Elnilein under salt stress. Many researchers have also reported the increased activity of SOD under salt stress in canola (*Brassica napus* L.) (Ebrahimian & Bybordi, 2012) and wheat (Kahrizi *et al*., 2012). In this study significant increase in CAT and POD activity in leaves with increasing salt stress was observed. Approximate increase in CAT activity under salinity has been observed in several plants, including maize (Kholová *et al*., 2009; Kholova *et al*., 2010), wheat (Abdel Latef, 2010), and canola (Bybordi, 2010). They identified a deterioration in CAT activity under salt stress as compared to control in wheat cultivars. Increased POD activity under salt stress has been reported

in wheat (Abdel Latef, 2010, Kahrizi *et al*., 2012). An increase in the anti-oxidative enzymes under salt stress could be suggestive of an increased result of ROS and improvement of a protective mechanism to decrease oxidative harm triggered by stress in plants. Catalase in peroxisomes breaks down H_2O_2. Peroxidase in cytosol and chloroplast can correctly scavenge H_2O_2. An increase in peroxidase activity by salt treatment in plants has also been reported by Kahrizi, *et al*., (2012). We observed that the activity of antioxidant enzymes (POD, and SOD) was reduced slightly in all the nitrogen treatments (Table 3 and Fig. 6B). Related findings have been observed by Huang *et al*., (2004), Rios-Gonzalez *et al.,* (2002), and CAT was initially increased with increasing nitrogen rate (Table 3). Related increases in the CAT have been also observed by Huang *et al*., (2004).

Conclusion

In this study we found that salt stress had various effects on physiology and antioxidant enzymes of wheat plants. However, the effects of salinity differed significantly among different measurements. The use of nitrogen fertilizer under salinity stress has been determined to be useful to mitigate salinity induced damages. The 86 and 210 kg N ha^{-1} increased chlorophyll content, photosynthetic rate, transpiration rate, and stomatal conductance. More investigation is needed to optimize the effectiveness of nitrogen treatments on more varieties of wheat.

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References

Abdel Latef, A. 2010. Changes of antioxidative enzymes in salinity tolerance among different wheat cultivars. *Cereal Res. Commun*., 38: 43-55.

Alam, M.A., A.S. Juraimi, M. Rafii and A.A. Hamid. 2015. Effect of salinity on biomass yield and physiological and stem-root anatomical characteristics of purslane (*Portulaca oleracea* L.) accessions. *Bio. Med. Res. Int*., 2015: 1-15.

Ali, Y., Z. Aslam, M. Ashraf and G. Tahir. 2004. Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. *Int. J. Environ. Sci. Technol*., 1: 221-225.

Anonymous. 2015. Agristat, www.fao.org (accessed 27 January. 2016).

Anonymous. 2014. Favorable Conditions for China's 2015/16 Winter Wheat Crop http://pecad.fas.usda.gov/ highlights /2014/12/China/index.htm (accessed 10.11. 2016).

Ayala-Astorga, G.I. and L. Alcaraz-Meléndez. 2010. Salinity effects on protein content, lipid peroxidation, pigments, and proline in Paulownia imperialis (Siebold & Zuccarini) and Paulownia fortunei (Seemann & Hemsley) grown *In vitro*. *Electron. J. Biotechnol*., 13: 13-14.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72: 248-254.

Bybordi, A. 2010. Influence of NO3: NH4 ratios and silicon on growth, nitrate reductase activity and fatty acid composition of canola under saline conditions. *Afr. J. Agric. Res*., 5: 1984-1992.

Chapman, H.D. and P.F. Pratt. 1962. Methods of analysis for soils, plants and waters. *Soil Sci.*, 93: 68.

Chawla, S., S. Jain and V. Jain. 2013. Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). *J. Plant Biochem. Biotechnol*., 22: 27-34.

Chen, W., Z. Hou, L. Wu, Y. Liang and C. Wei. 2010. Effects of salinity and nitrogen on cotton growth in arid environment. *Plant Soil*, 326: 61-73.

Dhanapackiam, S. and M.M. Ilyas. 2010. Effect of salinity on chlorophyll and carbohydrate contents of Sesbania grandiflora seedlings. *Ind. J. Sci. Technol*., 3: 64-66.

Elahmadi, A.B. and F.E. Hamza. 2015. Evaluation of salt tolerant bread wheat genotypes in Sudan. *Mycopath*., 12: 103-111

Ebrahimian, E. and A. Bybordi. 2012. Effect of salinity, salicylic acid, silicium and ascorbic acid on lipid peroxidation, antioxidant enzyme activity and fatty acid content of sunflower. *Afr. J. Agric. Res*., 7: 3685-3694.

Foyer, C.H. and G. Noctor. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell Environ.*, 28: 1056-1071.

Freed, R., S. Eisensmith, E. Everson, M. Weber, E. Paul and E. Isleib. 1991. MSTAT-C: A Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments. East Lansing, MI, USA: Michigan State University.

Gupta, B. and B. Huang. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genomics*, 2014: 1-18.

Gautam, S. and P. Singh. 2011. Effects of salicylate on growth and biochemical changes in maize seedlings under salt stress. *IIOAB J*., 2: 16-20.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research John Wiley & Sons.

Guarda, G., S. Padovan and G. Delogu. 2004. Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *Eur. J. Agron*., 21: 181-192.

Huang, Z.A., D.A. Jiang, Y. Yang, J.-W. Sun and S.H. Jin. 2004. Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. *Photosynthetica*, 42: 357-364.

Ibrahim. M.E.H., X. Zhu, G. Zhou and N.E. Ahmed Nimir. 2016 Comparison of Germination and Seedling Characteristics of Wheat Varieties from China and Sudan under Salt Stress. *Agron. J*., 108: 85.

Jamil, M., S. Rehman and E. Rha. 2014. Response of growth, PSII photochemistry, and chlorophyll content to salt stress in four brassica species. *Life Sci. J*., 11: 139-145.

Kahrizi, S., M. Sedighi and O. Sofalian. 2012. Effect of salt stress on proline and activity of antioxidant enzymes in ten durum wheat cultivars. *Ann. Biol. Res*., 3: 3870-3874.

Kala, S. 2015. Effect of Nacl salt stress on antioxidant enzymes of isabgol (*Plantago ovata* Forsk.) Genotypes. *Int. J. Food Sci. Tech. Res*., 4: 40-43.

Kaya, C., O. Sönmez, M. Ashraf, T. Polat, L. Tuna and S. Aydemir. 2015. Exogenous application of nitric oxide and thiourea regulates on growth and some key physiological processes in maize (*Zea mays* L.) plants under saline stress. *Soil-Water Journal*, 61-66.

Khan, M.I.R., M. Asgher and N.A. Khan. 2014. Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna radiata* L.). *Plant Physiol. Biochem*., 80: 67-74.

Kholová, J., R. Sairam, R. Meena and G. Srivastava. 2009. Response of maize genotypes to salinity stress in relation to osmolytes and metal-ions contents, oxidative stress and antioxidant enzymes activity. *Biol. Plant*, 53: 249-256.

Kholova, J., R.K. Sairam and R.C. Meena. 2010. Osmolytes and metal ions accumulation, oxidative stress and antioxidant enzymes activity as determinants of salinity stress tolerance in maize genotypes. *Acta Physiol. Plant*, 32: 477-486.

Koca, H., M. Bor, F. Özdemir and I. Türkan. 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot*., 60: 344-351.

Labconco, C. 1998. A guide to Kjeldahl nitrogen determination methods and apparatus. Labconco Corporation: Houston, TX, USA.

Liu, S., Y. Dong, L. Xu and J. Kong. 2014. Effects of foliar applications of nitric oxide and salicylic acid on salt-induced changes in photosynthesis and antioxidative metabolism of cotton seedlings. *Plant Growth Regul*., 73: 67-78.

Machado, R.M.A. and R.P. Serralheiro. 2017. Soil salinity: Effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae*, 3: 1-13

Mahboobeh, R. and E.A. Akbar. 2013. Effect of salinity on growth, chlorophyll, carbohydrate and protein contents of transgenic Nicotiana Plumbaginifolia over expressing P5CS gene. *J. Environ. Res. Manag*., 4: 0163-0170.

Malhi, S., C. Grant, A. Johnston and K. Gill. 2001. Nitrogen fertilization management for no-till cereal production in the Canadian Great Plains: a review. *Soil Tillage Res*., 60: 101-122.

Metwali, M., M.H. Eid and T.Y. Bayoumi. 2011. Agronomical traits and biochemical genetic markers associated with salt tolerance in wheat cultivars (*Triticum aestivum* L.). *Aust. J. Basic Appl. Sci*., 5: 174-183.

Muscolo, A., M.R. Panuccio and M. Sidari. 2003. Effects of salinity on growth, carbohydrate metabolism and nutritive properties of kikuyu grass (*Pennisetum clandestinum* Hochst). *Plant Science*, 164: 1103-1110.

Nelson, D.W. and L.E. Sommers 1996. Total Carbon, Organic Carbon, and Organic Matter. In: *Methods of Soil Analysis.* (Eds.): Sparks, D.L. Part 3. Chemical Methods. No. 5, SSSA and ASA, Madison, WI. 961-1010.

Nimir, N.E.A., S. Lu, G. Zhou, W. Guo, B.L. Ma and Y. Wang. 2015. Comparative effects of gibberellic acid, kinetin and salicylic acid on emergence, seedling growth and the antioxidant defence system of sweet sorghum (*Sorghum bicolor*) under salinity and temperature stresses. *Crop & Pasture Science*, 66: 145-157.

Nimir, N.E.A., S. Lu, G. Zhou, B.L. Ma, W. Guo and Y. Wang. 2014. Exogenous Hormones Alleviated Salinity and Temperature Stresses on Germination and Early Seedling Growth of Sweet Sorghum. *Agron. J*., 106: 2305-2315.

Nimir, N.E.A., G. Zhou, W. Guo, B. Ma, S. Lu and Y. Wang. 2016. Effect of foliar application of GA3, kinetin, and salicylic acid on ions content, membrane permeability, and photosynthesis under salt stress of sweet sorghum [*Sorghum bicolor* (L.) Moench]. *Can. J. Plant Sci*., 97: 525-535.

Olson, S.R. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Government Printing Office. Washington 25. D. C. 1-19

Parihar, P., S. Singh, R. Singh, V.P. Singh and S.M. Prasad. 2015. Effect of salinity stress on plants and its tolerance strategies: a review. *Environ. Sci. Pollut. Res*., 22: 4056-4075.

Rios-Gonzalez, K., L. Erdei and S.H. Lips. 2002. The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. *Plant Sci*., 162: 923-930.

Sonneveld, C. and J. Van Den Ende. 1971. Soil analysis by means of a 1: 2 volume extract. *Plant Soil*, 35: 505-516.

Vafadar, F., R. Amooaghaie and M. Otroshy. 2014. Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *J. Plant Interact*., 9: 128-136.

Walkley, A. and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci*., 37: 29-38.

Xu, L. and M. Ye. 1989. A measurement of peroxidase activity using continuous recording method. *Nanjing Nongye Daxue Xuebao*, 12: 82-83.

Zhang, H., G. Zhang, X. Lu, D. Zhou and X. Han. 2014. Salt tolerance during seed germination and early seedling stages of 12 halophytes. *Plant Soil*, 388: 229-241.

Zhang, X., G. Huang, X. Bian and Q. Zhao. 2013. Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. *Plant, Soil Environ*., 59: 80-88.

Živčák, M., K. Olšovská, P. Slamka, J. Galambošová, V. Rataj, H. Shao and M. Brestič. 2014. Application of chlorophyll fluorescence performance indices to assess the wheat photosynthetic functions influenced by nitrogen deficiency. *Plant, Soil Environ*., 60: 210-215.

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