Advances in Bioresearch
Adv. Biores., Vol 9 (1) January 2018: 181-186

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.1.181186

Advances in Bioresearch

ORIGINAL ARTICLE

Effect of Salinity Stress on Soluble Protein of wheat (*Triticum aestivum* L.) varieties

Shivraj Khalipe, Ganesh Kalibag, *Nileema Gore, Yogesh Bhavar and Sanjay Harke
MGM's Institute of Bioscience and Technology, N-6, Cidco, Aurangabad, MS, India.
***Correspondence authors Email:** goreneel@gmail.com

ABSTRACT
In the present study, two varieties of wheat (Lok 1 and HD 2189) were subjected to salt stress of 00 mM, 50 mM, 100 mM, 150 mM and 200 mM of NaCl concentrations, respectively. In the variety Lok 1, the highest protein content was observed in 100 mM (21.25 mg/ml ±4.02) as compared to control (15.91 mg/ml ±0.72) at 48 hr. In the variety HD 2189, the highest protein content was observed in 200 mM (22.83 mg/ml ±1.18) as compared to control (15 mg/ml ± 1.73) at 48 hr. In variety HD 2189, it was observed that the amount of protein was increased and further decreased at higher concentration as compared to control at 96 hr. After the protein profiling by using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis, the protein marker band was detected in the variety Lok 1 and HD 2189 in all the treatments of NaCl. In the variety HD 2189, similar banding pattern was observed in treatment 100 mM, 150 mM and 200 mM concentration of NaCl at 96 hr. The results concluded that the increase in the amount of the proteins content in the wheat varieties may lead to an increase in the tolerance mechanisms towards NaCl salinity.
Keywords: protein, SDS PAGE, protein marker, salt stress, wheat

Received 30.07.2017 Revised 23.09.2017 Accepted 20.12.2017

How to cite this article:
S Khalipe, G Kalibag, N Gore, Y Bhavar and S Harke.Effect of Salinity Stress on Soluble Protein of wheat (*Triticum aestivum* L.) varieties. Adv. Biores., Vol 9 [1] January 2018.181-186.

INTRODUCTION

Salt stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity. The tolerance to salt stress is accompanied by alterations in the levels of proteins. Salinity causes either decrease or increase in the level of soluble proteins or completely disappears in some proteins when compared to the control treatment. In addition, salt stress promotes a complete loss of present proteins and the synthesis of newly formed proteins [1].

Soluble protein is generally decreased in response to salinity [2]. It has also been reported that high salt concentration either causes an increase in the N-contents and high protein content in some glycophytic plants [3] or increase in soluble proteins [4]. The Number of N-containing compounds accumulating in plants subjected to environmental stress [5]. The most frequently accumulating compounds include amides (glutamine and asparagines), amino acids (arginine, proline, citrulline and ornithine) and the amine putrescine. Amino acids such as proline, asparagine and amino butyric can play important roles in osmotic adjustment of plant under saline conditions [6].

Biochemical techniques like Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isozyme markers has been particularly helpful in deducing systematic relationships between groups where morphological and cytological data were not corollary. SDS-PAGE is an economical, simple and extensively used technique for describing the protein diversity of crop germplasm. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique for separating proteins based on their ability to move within an 3electrical current, which is a function of the length of their polypeptide chains or of their molecular weight. This is achieved by adding SDS detergent to remove secondary and tertiary protein structures and to maintain the proteins as polypeptide chains. The SDS coats the proteins, mostly proportional to their molecular weight, and confers the same negative electrical charge across all proteins in the sample [7].

To find out soluble proteins of wheat under salt stress condition, the present investigation was carried out. Wheat is one of the major staple food in India and south Asian countries. Specially in India wheat yield is highly affected by increased salinity in farm soil. So the current research is directed towards producing the wheat varieties with better or improved tolerance towards salt stress. This study gives researchers a key base for identifying the stress induced proteins which help crop for the sustainable growth. Specific proteins can be identified and that particular variety can be utilised in breeding programme targeted towards salt tolerant wheat and also can be exploited for transgenic study. Hence, the effect on protein quantity and quality of Wheat varieties under salt stress condition was studied the present investigation.

MATERIALS AND METHODS

Experimental details

In the present study two wheat varieties (Lok 1 and HD 2189) were grown under pot culture. The two varieties were planted in 50 pots filled with soil. Five treatments were applied to the two varieties where each treatment were applied to five pots per replications, three plants per pots. Salt stress treatments *Viz.,* 00 mM, 50 mM, 100 mM, 150 mM and 200 mM of NaCl concentration. The experiment was conducted for one month. For qulitative and quantitative study of protein seedling stage of wheat varieties were used.

Protein Extraction

Protein extraction was performed from leaf tissues according to the method described by [8]**.** The leaf tissues were ground in Tris-HCl buffer (pH 7.2) using mortar and pestle. Centrifuged the homogenate at 10,000 rpm for 10-15 minutes at 4^0c and collected the supernatant. Precipitated the proteins using 10% TCA and incubated it at -20^0c for one hour. Supernatant was discarded, and the pellet was resuspended with 5 ml of 80% acetone to extract residual TCA and centrifugated at 10,000 rpm for 10 minutes at 4^0c. Pellets were air dried, and dissolved it in Tris-HCl buffer (pH 7.2).

Protein Estimation

The amount of protein in the samples was estimated by using Lowry's method [9]. Protein concentration of each sample was determined using Bovine Serum Albumin (BSA) standard curve. Optical density was recorded at 660 nm.

2.5 SDS-PAGE Electrophoresis

Separation of protein was done with SDS-PAGE electrophoresis according to [10]. The leaf protein extracts was mixed with sample buffer containing 1.25ml Tris-HCl (pH6.8), 150mg SDS, 0.5 ml β-mercaptoethanol, 1ml glycerol and bromophenol blue (0.05%) and then heated at 100°C for 2 min. About 100 µg of proteins were separated as polypeptides, by 8% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Gels were stained with the solution of 0.2% coomassie brilliant blue R-250, 50ml methanol, 7ml glacial acetic acid and 43ml distilled water for 8-12h, and then distained in the solution containing 30ml methanol, 7ml glacial acetic acid and 63ml distilled water. The stained gel was documented in gel documenting.

RESULTS AND DISCUSSION

Protein content of variety Lok 1

The significantly highest protein content was observed in 100mM (21.25mg/l ±4.02) followed by 200mM concentration (20.08mg/ml ±1.12) as compared to control (15.97mg/ml ±0.78) at 48 hr of NaCl treatment. But in 50mM concentration (11.47mg/ml ±2.86) and 150mM concentration (8.5mg/ml ±2.29) amount of protein was decreased compared with control at 48 hr of NaCl treatment. At 96 hr of NaCl treatment, the amount of protein content was decreased as compare with 48 hr of NaCl treatment. Highest protein content was observed in 100mM concentration (8.83mg/ml ±1.88) followed by 200mM concentration (6.83mg/ml ±1.89). Treatment 150mM concentration (5mg/ml ±1.14) showed lowest protein content.

This study supposed match with the authors [11], who found the protein degradation under saline environment has been attributed to the decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acid and denaturation of enzyme involved in protein synthesis.

Protein content of variety HD 2189

At 48 hr of NaCl treatment, the pattern of protein biosynthesis was increased with increasing NaCl concentration. The highest protein content was observed in 200mM concentration, followed by 100mM (19.57mg/ml ±3.17), 150mM (18.73mg/ml ±2.75) as compared with control (15mg/ml ±1.73). It was observed that in 50mM (15.82mg/ml ±3.12) treatment showed similar result with control. Similar results have been recorded by [12], who found that an increase of protein content in the vegetal tissues

especially under the action of high concentration of salts It is supposed that this phenomenon is determined by the concentration of proteins in the cells because the processes of growth are repressed much strongly than the processes of protein synthesis under salinity conditions.

At 96 hr of NaCl treatment, protein content was decreased compared with 48 hr of NaCl treatment. In the treatment 100mM concentration was observed that the protein content increased by 16.75mg/ml ± 2.82. followed by 50mM (15.08mg/ml ± 2.65). But treatment 150mM (12.25mg/ml ± 2.95) and 200mM (10.67mg/ml ± 2.33) NaCl concentration of protein content was decreased as compared to control (13.33mg/ml ± 2.33).

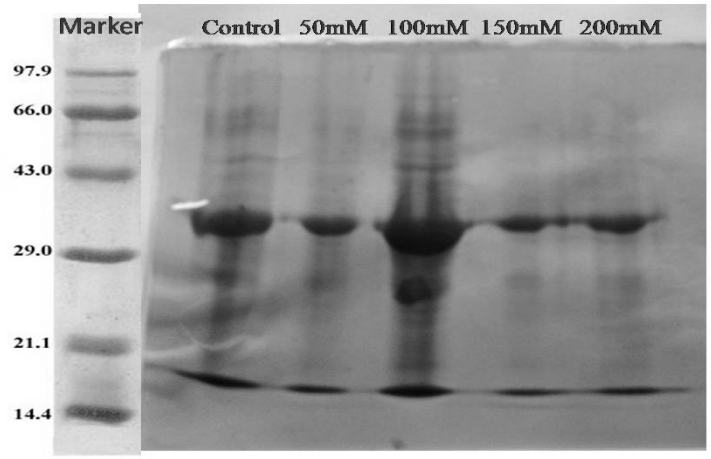

Plate 1; Protein profile of wheat variety Lok 1 at 48 hr of NaCl treatment

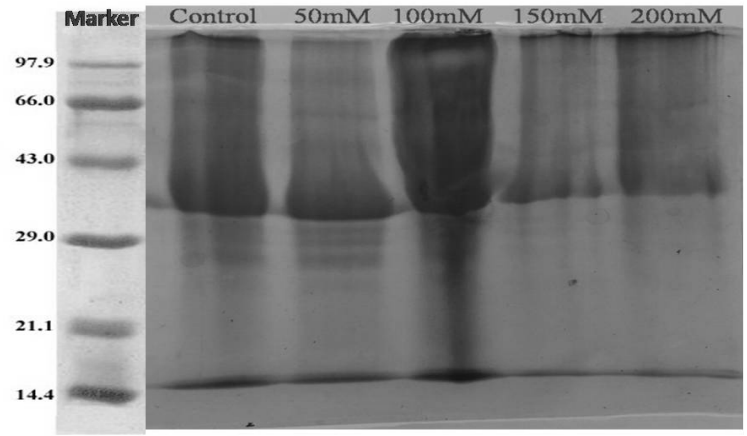

Plate 2; Protein profile of wheat variety Lok 1 at 96 hr of NaCl treatment

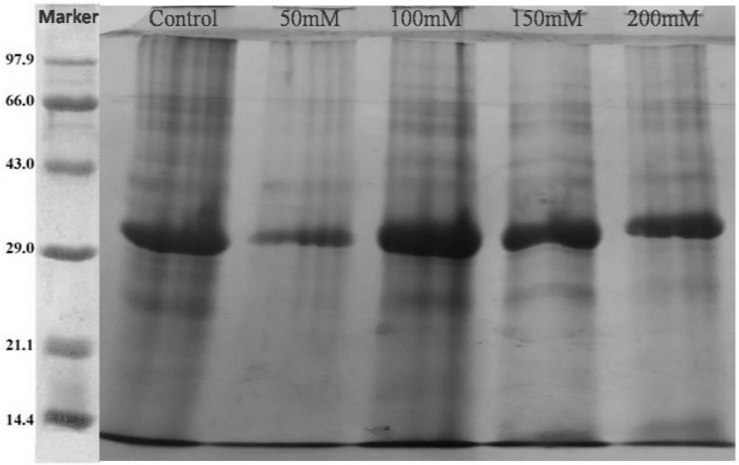

Plate 3; Protein profile of wheat variety HD 2189 at 48 hr of NaCl treatment

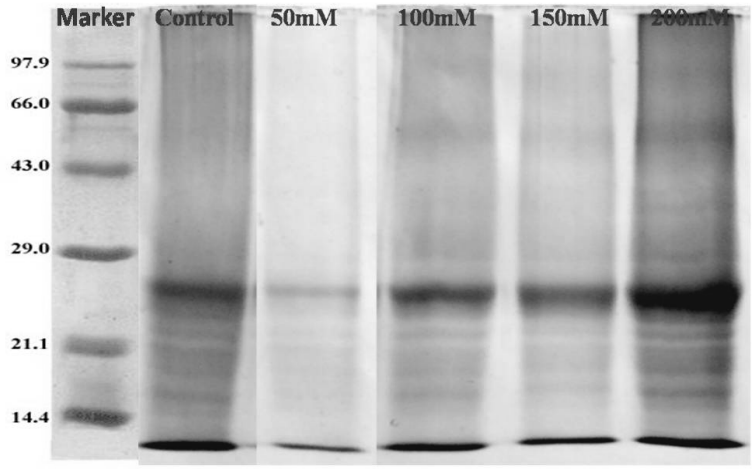

Plate 4; Protein profile of wheat variety HD 2189 at 96 hr of NaCl treatment

Table 1; Average and SD of soluble protein content in wheat variety Lok 1 at 48 and 96 hr of NaCl treatment

Treatments	48 hr Sample		96 hr Sample	
	Average	SD	Average	SD
T_0 (Control)	15.91667	± 0.721688	7.75	± 1.984313
T_1 (50mM)	11.41667	± 2.897556	4.333333	± 1.040833
T_2 (100mM)	21.25	± 4.023369	8.833333	± 1.876388
T_3 (150mM)	8.5	± 2.222049	5	± 1.145644
T_4 (200mM)	20.08333	± 1.127312	6.833333	± 1.842779

Table 2; Average and SD of soluble protein content in wheat variety HD 2189 at 48 and 96 hr of NaCl treatment

Treatments	48 hr Sample		96 hr Sample	
	Average	SD	Average	SD
T_0 (Control)	15	± 1.7320508	13.33333	± 2.3228933
T_1 (50mM)	15.81667	± 3.1226324	15.08333	± 2.6020825
T_2 (100mM)	19.56667	± 3.1722757	16.75	± 2.8394542
T_3 (150mM)	18.73333	± 2.7501515	12.25	± 2.9474565
T_4 (200mM)	22.83333	± 1.1814539	10.66667	± 2.3228933

Similar result found that the authors [13],who studied increases protein hydrolytic enzyme activity, decreases amino acid synthesis and interferes with tertiary and quaternary enzyme structures leading to decreases in soluble protein content. Increasing the salinity level caused decreases in the soluble protein content of shoots and roots, with the highest and the lowest levels found at salinity levels of 00mM (control) and 200 mM, respectively.

Protein profiling by SDS-PAGE

The proteins were separated based on molecular weight by SDS-PAGE in different samples collected at different concentration of NaCl treatment. In control and other NaCl treatments, qualitative and quantitative protein marker band was identified. At 48 hr of NaCl treatment of variety Lok 1, the protein profile of treatment 50mM, 150mM and 200mM concentration of NaCl there was disappearance of polypeptides and in treatment 100mM and control, there was appearance of polypeptides. After the application of 100mM NaCl concentration similar banding pattern of protein was observed as compared with control treatment.

The protein profile of Variety Lok 1 at 96 hr of NaCl treatment showed that the expression of protein marker bands was reduced in 200mM and 150mM concentration of NaCl treatment as compared with control. After the application of 50mM NaCl concentration similar banding pattern of protein was observed as compared with control treatment. In 150mM and 200mM concentration of NaCl treatment was observed that the polypeptide bands were disappeared. This result is agreement with [14], who studied changes in protein synthesis under salt stress may be due to changes in the efficiency of mRNA translation or the regulation of RNA transcription, transport and stability. The expression of salt-stress

proteins is related to the adaptation process of seedlings to salinity as well as to the genetic constitution of selected salt tolerant genotypes.

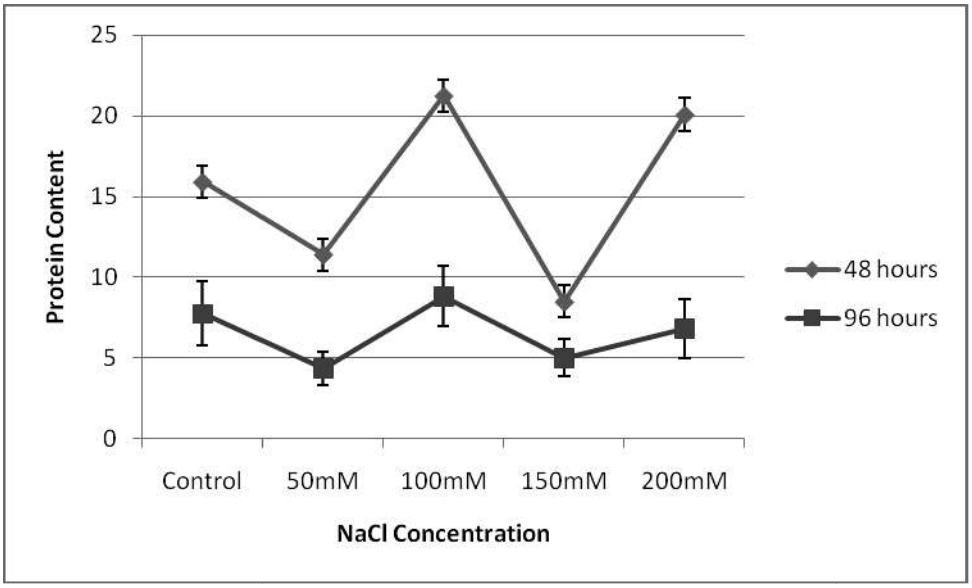

Figure 1; The variation of protein amount in wheat variety Lok 1 after NaCl treatment

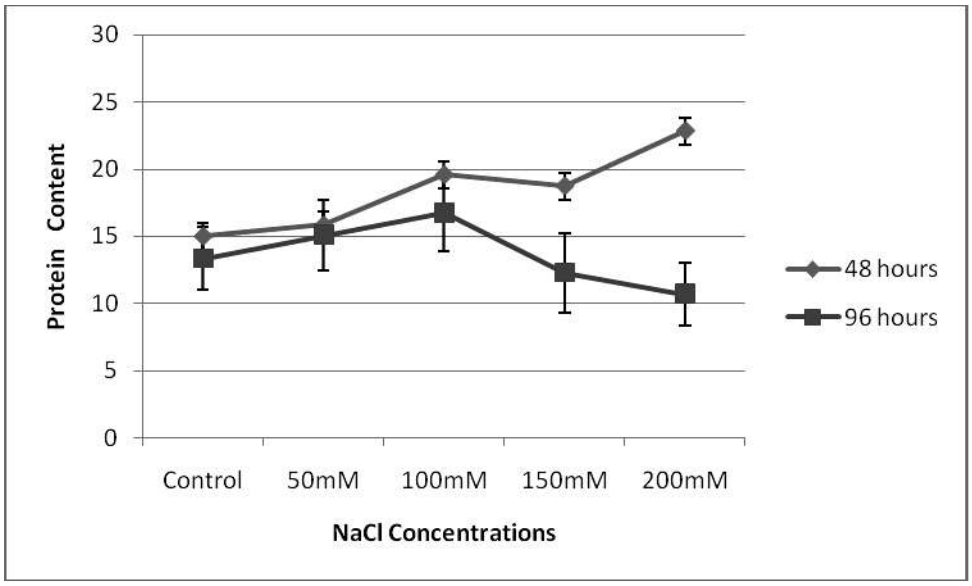

Figure 2; The variation of protein amount in wheat variety HD 2189 after NaCl treatment

The leaf soluble proteins SDS-PAGE profile of wheat variety HD 2189 at 48 hr NaCl treatment is shown in plate 5. In total 35-37 polypeptides band observed in NaCl treatments including control. In control and NaCl treatments, 2-4 fast migrating bands were indentified and 1-5 slow migrating bands were identified. The protein marker band was detected in control and NaCl treatments. The expression of protein was reduced at 50mM NaCl treatment but in 100mM, 150mM and 200mM NaCl treatment, protein expression was relatively constant compared to control. Protein turnover in stressed plants were observed at early time, followed by the induction of known stressed–responsive transcripts within hours, and the induction of transcripts for defense-related function later. So, it can be suggested that the proteins with molecular weights of 11, 18, 33, 35, 43, 46, 52 and 68 KD could play an important role in triggering a system to tolerate sever stress of NaCl [15].

The protein profile of wheat variety HD 2189 at 96 hr NaCl treatment is shown in plate 6. In control and NaCl treatments, fast migrating bands were disappearance but slow migrating bands were appearance. The protein profile showed that the expression of protein was increased in 200mM concentration of NaCl treatment followed by 150mM and 100mM concentration of NaCl treatment and the expression of protein

was decreased as compared with control. The protein marker was detected in NaCl treatments including control. The study of barley seedlings under salinity stress observed that increasing of salinity caused strongly reduction in the level of 55 KDa polypeptide band which is related to the rubisco enzyme [16].
In the conclusion, in wheat variety Lok 1 at 48 hr, the protein content was increased in 100mM and 200mM NaCl treatment. The pattern of protein content was increased with increasing NaCl concentration in wheat variety HD 2189 at 48 hr of salt stress and concluded that an increase in the amount of the proteins may lead to an increase in the tolerance mechanisms towards NaCl salinity of wheat varieties. The application of NaCl treatments at 96 hr showed that the similar banding pattern was observed in 50mM concentration of Lok 1 wheat variety and in 100mM, 150mM and 200mM of HD 2189 wheat varieties as compared to control treatment and it may be used for further study. The protein changes by electrophoretic analysis under salinity treatment may be useful for understanding the salinity tolerance of genotypes. Protein marker bands were identified, which may be useful for further sequencing.

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