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Salinity-induced alterations in protein expression pattern of wheat (*Triticum aestivum* L.) leaves

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Abstract

Salinity is a critical environmental factor that limits the growth and productivity of wheat. It induces physiological and biochemical changes which affects plant metabolism. This study's objective was to evaluate the effects of various sodium chloride concentrations (0, 50, 100, and 150 mM NaCl) on wheat leaf proteins using SDS-PAGE analysis. The total protein was not significantly affected by a modest dose of 50 mM sodium chloride salt. At the high doses (100 and 150 mM), a noticeable decrease is seen. Furthermore, salt stress also caused specific alterations in the protein expression patterns. The expression of some proteins (28 and 72 kDa) was up-regulated by 50 mM.

However, the relative expression of these proteins was reduced with the increase of salinity. And the proteins 53, 55 and 63 kDa expression were also decreased with increasing salt stress. These findings reveal that the response of wheat plant to salt stress is dependent on the degree of salinity and proteins affected. Elucidating these molecular mechanisms is instrumental in producing the wheat genotypes, which 17 can tolerate salt better.

Keywords: Salt stress, wheat, SDS-PAGE, protein profile

Introduction

Wheat is one of the most important crops and plays a vital role in feeding the world's population. Due to the limitations of arable land and the escalating demand for wheat, it is imperative to enhance productivity per unit area. To ensure the sustainability of this crop, it is imperative to develop cultivars with enhanced tolerance to environmental challenges (Koyro *et al.*, 2012) [20].

Salinity is expected to affect about 20% of worldwide land and more than 50% of irrigated regions (Kang *et al.*, 2010) [17]. The escalating salinization of agricultural regions presents significant consequences, with forecasts suggesting that over 50% of cultivable land would be impacted by salinity by 2050 (Wang *et al.*, 2003) [35]. Salt stress is a major abiotic environmental stress caused by saline soil in semi-arid and arid areas, which affects crop productivity (Sharbatkhari *et al.*, 2013) [30].

When plants are exposed to abiotic stressors, they undergo different changes, including physiological responses and alterations in gene expression (Flowers *et al.*, 2015) [11].

Plant stress tolerance is a polygenic trait linked to molecular, physiological, and biochemical processes. Accordingly, proteomic methods are thought to be crucial for elucidating the molecular basis of salt stress resistance at the protein level (Thiellement *et al.*, 2002) [33].

Proteins, lipids, nucleic acids (Apel & Hirt 2004) [6], metabolic and physiological processes are negatively influenced and lead to reduction in crop yield due to salinity stress. (Ahmad *et al*, 2019; Sadak et al., 2019) [4, 27]. Plant response to salinity is a complicated mechanism based on activation or modification of physiological, biochemical and molecular processes (Greenway & Munns, 1980) [13].

Several altered gene expression and many protein modifications mediated by salt stress, precipitate different physical injuries. Accurate and comprehensive description of protein changes in the crops under salt stress conditions would logically contribute to reveal the molecular mechanism underlying the salt responses. Adaptation to salt stress consists of complicated mechanisms involving developmental, morphological, physiological and biochemical processes (Taji *et al.*, 2004) [32].

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Furthermore, the expression of salt-stress-responsive genes is increased, resulting in modifications to the overall protein profile that facilitates plant adaptation to accumulating salt (Parker *et al*., 2006) [25]. Many types of stress result in the excessive synthesis of proteins known as stress-inducible proteins (Al-Whaibi, 2011) [5].

Although, analysis of changes in gene expression at the transcriptional level has helped to identify the genes that are involved in salinity stress tolerance, yet such assays are insufficient to have insight into mechanisms regarding what happens under salinity stress as some of the genes change during their transcriptome process and some at translation or post-translation level. The study of proteomic data in conjunction with physiological traits provides valuable information on which breeders can develop molecular breeding strategies to increase the resistance of crops to abiotic stresses. Therefore, proteomics is a powerful tool for analyzing the plant response to various types of stress (Abdalla & Rafudeen 2012) [1]. Understanding the genetic and molecular bases of tolerance is important for deciphering mechanisms underlying tolerance and providing a direction to create and develop tolerant genotypes through breeding. A strategy to identify the mechanisms of tolerance is to profile proteins in plants under stress. Biochemical methods such as sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), although inexpensive and simple techniques, have been widely used to visualize protein profiles of the plant under different conditions (Raza*viz*adeh, 2015; Abdelhaliem & Al-Huqail, 2016) [26, 2].

Many proteomic studies have been conducted to explore salt-induced changes in proteins. Most of the proteins affected by stress are involved in various cellular processes such as photosynthesis, photorespiration, metabolism, managing oxidative stress, ion channel regulation and protein folding (Joseph & Jini 2010) [16]. Changes of plant protein concentration respond to salinity tolerance stress in some treatment conditions, resulting in a decrease and an increase of protein expression or the inability to find the corresponding components (Yildiz 2007) [36]. In many plants, saline stress causes proteins to undergo special changes, potentially making it possible for the identified protein(s) to play a molecular role in the response of *Tricozia bracteata* to NaCl stress. Another study conducted by (Khalili *et al*., 2018) [18] investigated the proteomic profiles of two wheat cultivars (salinity-tolerant/salIint-sensitive one) after a 2-week application of 250 mM NaCl. After 2-DE with IPG strips and SDS-PAGE, a total of 198 (tolerant) and 203 spots (sensitive) were detected.

Some proteins which are shared commonly between the two have been observed that include oxidative dehydrogenases regulated proteins, Calvin cycle related, light reaction in photosynthesis and proton transport related as well as a heat shock protein. Saqib *et al*. (2006) [28] analyzed modifications of wheat proteome induced by 30 days of 125 mM NaCl application.

A negative correlation of salt tolerance with Na(+) in wheat stems was strongly found. The variation of protein expression level was larger than 5%, and the difference between groups with patterns for changed proteins (over-expressed, down-expressed, when disappeared and appeared) was ranging from 1 to 8% in salt-stressed samples.

Another study has shown that two doses of sodium chloride (0 and 150 mM) for a period of three weeks induced salt stress which resulted in changes in the protein profile in roots of sensitive as well as tolerant wheat cultivars, after analysis on two-dimensional electrophoresis (2-DE) polyacrylamide gels.

Another study reported 120 protein spots based on proteomic analysis. The difference in expression of 15 protein spots between the susceptible variety and resistant ones was significant. Downregulation of four protein spots was detected in the sensitive genotype. In the tolerant variety, eleven protein spots displayed highly significant changes in differential expression (five upregulated and six down-regulated proteins). (Shahbazi *et al*., 2023) [29].

Protein changes were induced under salt stress in several plants; Brassica 56.1-70.8 kDa (Jain *et al*.,1992) [15]; the rice leaf sheaths 22 and 31 kDa(Kong-Ngern *et al*.,2005) [19]; wheat leaf (Eid, 2019) [10].

However, Studies looking into the impact of salt stress on the protein profile in wheat leaves is notably rare; so, the research intended to examine the alterations in the protein composition of the local wheat cultivar, Ebba 99. by SDS-PAGE.

Materials and Methods

Plant material

Seeds of wheat (*Triticum aestivum* L.) Ebaa 99 cultivar were obtained from Agricultural Research Department / Al-Mishikhab, Ministry of Agriculture, Najaf, Iraq.

Plant Growth and NaCl Treatment

The experiment was conducted on the local cultivar of wheat, cv. Ebaa 99. The seeds were sterilized with 70% ethanol and 5% sodium hypochlorite. Then, the seed viability and germination rate were tested, and the seeds were planted in plastic pots containing a mixture of sand and peat moss at a ratio of 2:1, v/v, and irrigated with distilled water. After the seeds germinated and 7-day-old seedlings were obtained, the pots were classified into four categories: the first was irrigated with distilled water (control), the second was irrigated with 50 mM NaCl, the third with 100 mM NaCl, and the fourth with 150 mM NaCl.

The plants were grown in a growth chamber adjusted at 25 °C ± 2 °C, with a photoperiod of 16-8 hours of light and darkness, respectively for a duration of four weeks.

Leaf Protein Extraction

To extract leaf proteins, 1 g of leaves were ground in a mortar and pestle with liquid nitrogen and mixed with 2 ml of an extraction buffer containing 1 M Tris hydrochloride (pH 8.0), 250 mM NaCl, 0.25 mM EDTA, 0.5% (w/v) SDS, and 10 mM β-mercaptoethanol. The sample was then vortexed and centrifuged at 14,000 rpm for 15 minutes under cooling conditions. After that, the supernatants were collected and considered to be leaf protein extracts. Using a spectrophotometer, Protein content in the samples was determined using the Bradford method. (Bradford, 1976) [8].

Protein profiling using SDS-PAGE

SDS-polyacrylamide gels were used for protein profiling, following Laemmli's (1970) [21] procedure. A vertical gel electrophoresis device was used to conduct the electrophoresis on a 15% SDS-PAGE gel. After scanning the gels, densitometry was used to calculate the quantitative amounts of the bands by BIO-RAD (Image Lab 6.0.1).

Data analysis

Data were analyzed using analysis of variance (ANOVA), and mean differences were compared by the Least Significant Difference (LSD0.05) test using the GenStat program.

Results and Discussion

Plants respond to salinity stress by either degrading certain proteins or increasing the production of specific stress-related proteins (Wang *et al*., 2015) [34]. The results (Figure 1.) showed that plants treated with different salinity levels exhibited significant changes in the pattern of protein accumulation. There is a shift in pattern of protein synthesis and accumulation in salt grown plants. Salt Stress Soluble protein level decreases or increases either not so some proteins may disappear when it is compared with the controlled treatment (Yıldız, 2007).

The results of the current study (Fig 2.) showed that there is no significant effect on total protein at the low concentration (50 mM) of sodium chloride compared to the control. However, both treatments with 100 and 150 mM resulted in a significant decrease in total protein content when compared to the control. The salt stress may lead to the inhibition of various cellular activities, such as protein metabolism. Consequently, protein content decrease which might be due to plant inability to keep the level of protein balanced as affected under prolonged stress (Ahmad and Sharma 2008; Doganlar *et al*., 2010) [3, 9].

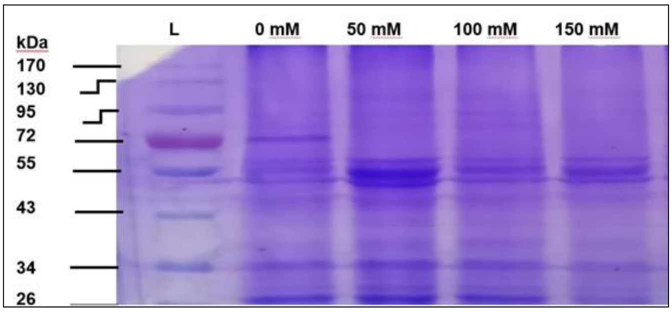

Fig 1: SDS-PAGE of treated and untreated samples

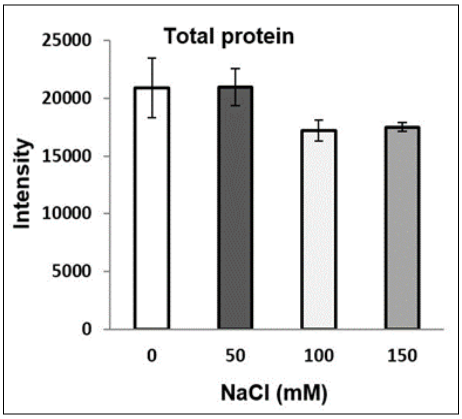

Fig 2: The effects of treatment with different concentrations of NaCl on total protein

Whatever the reason, it was elegantly shown by SDS-PAGE of treated and control samples (Figure 2). Influence of NaCl treatment on total protein Disease-free EMs were grown in different concentration of NaCl. This study also found (Figure 3.) that sodium chloride treatment had a significant effect on protein expression in wheat leaves, and the proteins of (28, 53, 55, 63 and 72) kDa were differently affected by salt treatment.

This suggests that the behavior of proteins in response to salt stress varies and depends on the specific type and function of each protein. Several proteins may be either up-regulated or down-regulated following exposure to salt stress. The identified proteins are recognized for their involvement in various biological processes: energy metabolism, reactive oxygen species (ROS) scavenging and detoxification, protein translation, processing and degradation; signal transduction; hormone and amino acid metabolism; the cell wall adaptations etc. These proteins all potentially cooperate to maintain cellular homeostasis of the osmotic pressure, salt stress and ionic toxicity.

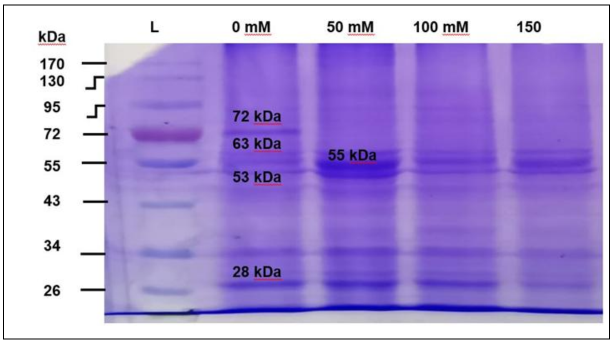

Fig 3: SDS- PAGE shows the molecular weights of the affected proteins.

Proteins are involved in regulation of these physiological processes either under normal or salt stress condition. Iqbal *et al*., 2019) [14]. Manaa *et al*., (2011) [23] Li *et al*. (2017) [22] Sonirajua and Aliakbarib.

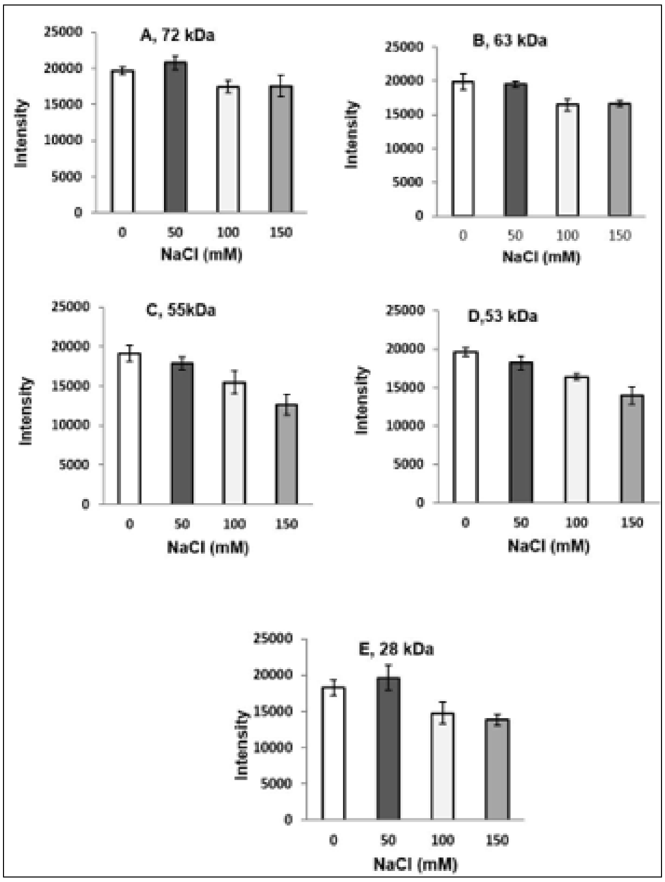

Fig 4: The effects of different concentrations of NaCl on the 72, 63, 55, 53, and 28 kDa proteins.

The results (Figure 4.) reported a marked up-regulation in the 28 and 72 kDa proteins at 50 mM of TA. And at higher salinities this stimulation did not continue. Their expression was distinctly reduced at 100 and 150 mM. These results accurately represent the way in which plants respond to salinity stress.

Proteins that play in the initially salinity response can become overburdened and ineffective when salt concentrations overwhelm a plant's capacity to acclimate, interfering with the cellular function of the plant. This disorder stresses the inability of the plant's native defense system during exposure to a high extent of salt (Athar *et al*.,2022) [7]. The 53, 55, and 63 kDa proteins were obviously a showcased protein in the DP.pattern their expression consistently declined with rising salt stress, highlighting their pronounced sensitivity to salinity driven by salt accumulation.

This reduction may result from many expected reasons like disrupted protein translation, diminished protein stability, metabolic pathway inhibition, or cellular damage caused by salt exposed.

Also, present results were agree with what Yildiz & Terzi (2008) [37] results through their study of wheat varieties growing under the impact of NaCl stress, since the study showed quantitative changes in some proteins, as the medium molecular weight proteins decreased with a specific increase in some proteins.

This come in line with Singh *et al*. (2024) [31], findings who studied two varieties of wheat: sensitive and tolerant. He observed an increase in of the 23 kDa protein abundantly in the sensitive variety at a concentration of 100 mM, whereas the prevalently of proteins in 20-30 kDa range were observed in the tolerant variety.

At a concentration of 100 mM, while proteins within the range of 20-30 kDa were more abundant in the tolerant variety, while proteins with a molecular weight of (38, 44, 66, 68) kDa decreased in both varieties. These studies suggested that the production of new proteins and increased protein content may contribute to enhancing wheat plant tolerance to salt stress. Figure 4.

The impacts of various NaCl concentrations on the 72, 63, 55, 53, and 28 kDa proteins. Results from this research show that wheat leaves' protein expression and content are both strongly impacted by salt stress. notanly, Total protein levels were not statistically affected by exposure to low sodium chloride concentrations (50 mM). whereas higher concentrations (100 and 150 mM) resulted in a significant reduction.

Protein expression patterns varied according to salinity concentration. The levels of 28 and 72 kDa proteins increased at low salinity, while 53, 55, and 63 kDa proteins decreased progressively as salt stress intensified. These data demonstrate the variety of functional roles of proteins whose expression levels fluctuate in plants due to salinity, underscoring the significance of discovering proteins linked to processes of salt stress tolerance.

Conclusion

This study's findings indicated that HSP 23.6 gene regulation in wheat leaves under normal growth circumstances exhibited elevated gene expression in response to salt stress induced by sodium chloride (NaCl). This underscores the importance of this protein's expression in consistently growing plants and its function in assisting plants to manage stress caused by salinity. It may be essential for plants to exhibit stress resistance, which could facilitate the development of transgenic plants.

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