Society for Advancement of Wheat Research
Directorate of Wheat Research
Karnal - 132 001, India

Journal of Wheat Research
6(1):41-44
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

*Research Article*

# Salt stress induced changes in protein profile of tolerant (Kh 65) and sensitive (HD 2009) wheat genotypes

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***Article history***

Received: 06 May, 2014
Revised: 09 May, 2014
Accepted: 15 May, 2014

***Citation***

Ram S, V Rana and I Sharma. 2014. Salt stress induced changes in protein profile of tolerant (Kh 65) and sensitive (HD 2009) wheat genotypes. *Journal of Wheat Research* 6(1):41-44.

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## Abstract

**In addition to drought and extreme temperatures, soil salinity represents a growing threat to wheat crop productivity. Therefore, understanding molecular mechanism involved in salinity stress can help in developing salt tolerant cultivars. In this investigation a comparative proteome analysis using 2-D electrophoresis was carried out between salt sensitive variety HD 2009 and salt tolerant variety Kh 65 to identify differentially expressed protein subunits. Salt treatment (12 ECe) was given to 2 week old seedlings under controlled conditions in Growth Chamber. Chemical and physical parameters were recorded after 72 hrs of salt treatment. 2D- electrophoresis of proteins extracts from root tissues was conducted under control and treated conditions. There was reduction in root biomass by 32 and 56 per cent in Kh 65 and HD 2009, respectively. A total of more than 200 spots were identified on the gel among them 60 spots showed differential expression in both the cultivars in combination. Among them, seventeen spots (3, 6, 8, 9, 11, 12, 14, 15, 21, 23, 25, 27, 30, 33, 30, 35 and 36) were up-regulated and while fifteen (2, 4, 5, 7, 13, 16, 17, 18, 20, 22, 26, 31, 37, 38 and 39) down-regulated in Kh65. Whereas only five spots (4, 35, 36, 58, 59) were up-regulated in HD 2009 and twenty one (1, 2, 3, 5, 9, 26, 37, 38, 40, 42, 43, 44, 45, 46, 47, 49, 52, 53, 54, 56, and 60)down-regulated. These differentially expressed spots can be isolated and sequenced for identifying protein subunits affected by salt stress.**

**Keywords:** Wheat, 2D electrophoresis, salt stress

## 1. Introduction

In addition to drought and extreme temperatures, soil salinity represents a growing threat to wheat crop productivity. In India, an area of about 5.5 mha is already under salinity and 3.6 mha under sodicity problem and still larger area is coming under potential salinity problem due to injudicious use of water under canal irrigation system. To meet the demand for food of growing population at global level, there is increased activity towards improving crops for salt tolerance. Among the cereal crops, wheat is considered as moderately salt tolerant and there are considerable variations among wheat cultivars for tolerance towards salinity stress (Munns *et al.,* 2006). Salt stress leads to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity. Recently, large-scale transcriptomic analyses have reported the expressional patterns of numerous salt-responsive genes in wheat (Kawaura *et al.,* 2006). Since many transcripts may undergo a number of post-transcriptional and post-translational modifications, the changes at the protein level, *i.e.,* proteotype can provide direct understanding of salt adaptive mechanisms (Jiang *et al.,*2007).

At molecular level, plants react to stress conditions by changing gene expression that produces several alterations in protein synthesis (with up- as well as down-regulation), and consequently in their biological functions. Alterations in the levels of proteins may be related to salt tolerance. Therefore, comparative proteomics approach may help in understanding the effect of proteins on cellular functions

under salt stress. High-throughput quantitative proteomic technologies have facilitated the study of global root proteomes expression. 2DE and DIGE approaches have been widely used for separating salt-responsive proteins in plant roots followed by MALDI-TOF/TOF MS and LC-MS/MS analysis. 2-DE provides an excellent means of comparing the expression of hundreds of proteins between samples, which show quantitatively substantial differences in expression (Witzel *et al.*, 2009, Irar *et al.*, 2010). Recently, few studies indicated up and down regulation of some of the protein units under salt stress in wheat (Guo *et al.,* 2012, Wang *et al.,* 2008, Peng *et al.,* 2009).In this investigation, proteomes of two contrasting wheat cultivars as salt tolerant (Kh 65) and susceptible (HD 2009) were compared by 2-D gel electrophoresis at seedling stage under control conditions and salinity level of 12 ECe. It is intriguing that so little is known about the genetics and physiology of the Indian landrace Kh 65, universally regarded as highly salt tolerant. Thus main objective of the present investigation was to identify genotype- and treatment-specific alterations in the protein complement, and to exploit these as potential candidate proteins involved in conferring salinity tolerance in wheat.

## 2. Material and methods

The seeds of two varieties namely Kh 65 (salt tolerant)and HD 2009 (salt sensitive) were grown in pots containing 400 g of sand treated with half strength of Hoagland solution.The pots were kept under controlled conditions in growth chamber (800 µmol/$M^2$S: 14h light 20$^0$C and 10h dark at 16$^0$C) at 70% relative humidity. Three days old seedlings at 1 leaf stage were treated with saline water containing 0.5 gram of NaCl and 80 mg of CaCl2. Similar saline water treatment was given after two days again to make ECe value as 12.0. Root samples were taken after 72 hours of final treatment.

*Protein extraction:* Proteins of root samples were extracted using phosphate saline buffer (Aghaei*et al.,* 2009). 200 mg of root and shoot samples were taken and homogenized in phosphate saline buffer (pH 7.6) containing 65 mM $K_2HPO_4$, 2.6 mM $KH_2PO4$, 400 mM NaCl and 3 mM $NaN_3$ at 4°C using a mortar and pestle on ice. The homogenate was centrifuged at 15,000x g for 10 min and trichloro- acetic acid was added to supernatant to final concentration of 10%. The solution was kept on ice for 30 min and then centrifuged for 10 min at 15,000x g. The resultant precipitate was washed with acetone and was dissolved in lysis buffer containing 7M urea, 2M Thiourea, 4%v/v Triton X-100 and 40 mM Tris (Gao*et al.,* 2011).

*Two- dimensional gel electrophoresis :* Electrophoresis of extracted proteins was conducted using two steps as IEF (Isoelectric focusing) and SDS-PAGE. IEF was performed using 7cm IPG strips with pH range 3-10. 500µg of protein sample was loaded on IPG strips and rehydrated passively with 250 ul of protein solution for 12 hr at 20°C. IPGphor III unit (GE Healthcare) was used for performing IEF with the following parameters: Grad 150V for 200Vhr, Grad 1000V for 300Vhr, Grad 5000V for 4000Vhr and 5000V for 1250Vhr with a total of approximately 5750Vhrs. The strips were then equilibrated for 15min in 50mM Tris-HCl, pH 8.8, 6M urea, 30%(v/v) glycerin, 2%(w/v) SDS, 20mM DDT, and 0.01% bromophenol blue followed by second equilibration step of 15 min with same equilibration buffer containing 2.5% w/v iodo-acetamide. The equilibrated strips were loaded on the top of 12% SDS-polyacrylamide gels and sealed with 0.5% w/v agarose (Gao *et al.,* 2011). The gels were stained with Commassie brilliant blue overnight followed by destaining with distilled water and then visualized under white light using Gel-Documentation System (Bio-Rad).

## 3. Result and discussion

*Plant response to salt stress:* Both HD 2009 and Kh 65 showed reduction in growth on imposition of salt stress (Fig. 1). To determine the appropriate NaCl concentration for subsequent proteome analysis plants were exposed to gradual salt stress. At the time of harvest both varieties suffered a decreased in biomass due to imposition of salinity stress, the decrease in biomass was more in susceptible variety (HD 2009). (Table 1). The observed reduction in root biomass was 32% and 56% in Kh 65 and HD 2009, respectively. Root shows greater decrease in biomass as compared to shoot. Salt stress was imposed gradually to the seedlings so as to determine an appropriate salt concentration for proteome analysis. The response of plants to salt stress occurs in two phases. In Initial phase plant shows drought like symptoms as uptake of water by roots is prevented by accumulation of excess salt near root zone (Seelig, 2000). After 2-3 days of salt treatment plant second growth phase response occurs from buildup of Na+ concentration inside the cell resulting in sodium toxicity (Munn, 2005).

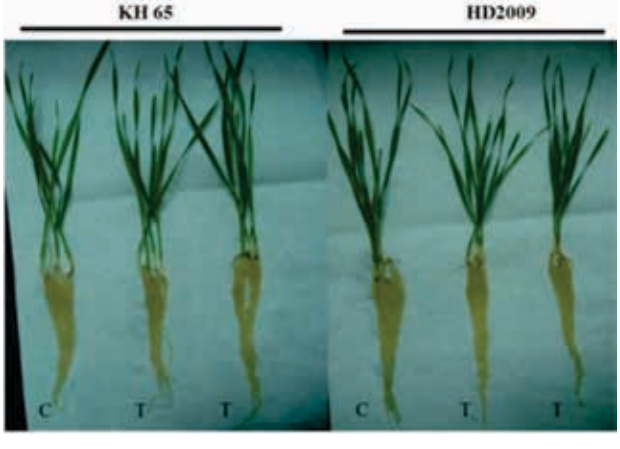


**Fig 1.** Effect of salinity on root biomass after 72 hours of treatment. C= control and T=treatment

**Table1.** Root biomass after 72hrs of salt treatment

| Variety | Control (g) | Treatment (g) | Reduction (%) |
|---|---|---|---|
| Kh 65 | 4.76 | 3.22 | 32 |
| HD 2009 | 3.67 | 1.61 | 56 |

*Differential proteome analysis:* 2-DE provides an excellent means of comparing the expression of hundreds of proteins between samples, which shows quantitatively substantial differences in expression. The comparison of wheat root proteome between the control and salt using 2D electrophoresis with pH range 3-10 revealed a broad distribution of protein subunits in the PI range from 4-7 that means most of the spots fall in the central area of the gel. Analysis of leaf proteome under controlled and treated conditions revealed minor changes (data not shown here), however, there was considerable change in root proteome under controlled and stressed conditions. Based on these observations a comparative proteome analysis was carried between root tissue of Kh 65 and HD 2009 under normal and salt stress. More than 200 spots were detected on 2D gels and many of them were cultivar dependent. The percentage of matching spots for the two varieties was 75%, demonstrating extensive homology between tolerant and sensitive genotypes. Only a low percentage of spots were ecotype-specific (less than 15%),meaning that it was not possible to find any match when compared to the remaining spots of the two varieties. There were salinity induced alterations in protein profile of both the cultivars. These induced differences were greater in HD 2009 than Kh 65. Many protein spots between 30 kDa-40 kDa showed down regulation in sensitive genotype. In contrast in resistant genotype new spots were observed in between 60 kDa-90 kDa.

For quantitative protein expression analyses, raw spot volumes were considered. Sixty spots showing quantitative or qualitative (presence/absence)variations among the two wheat varieties 2-DE gels were selected following the criteria described in materials and methods. Among them, seventeen spots (3, 6, 8, 9, 11, 12, 14, 15, 21, 23, 25, 27, 30, 33, 30, 35 and 36) were up-regulated and fifteen (2, 4, 5, 7, 13, 16, 17, 18, 20, 22, 26, 31, 37, 38 and 39) down-regulated in Kh 65. Whereas only five spots (4, 35, 36, 58, 59) were up-regulated in HD 2009 and twenty one (1, 2, 3, 5, 9, 26, 37, 38, 40, 42, 43, 44, 45, 46, 47, 49, 52, 53, 54, 56, and 60)down-regulated as shown in Fig. 2A &B. These selected spots need MALDI-TOF analysis for identification of protein. Understanding the role of protein subunits in tolerance to salinity will led to enhanced tolerance of wheat genotypes. Spots numbering 10, 15 and 19 were appeared as new spots under salinity stress in Kh 65 while 41, 51 and 57 in HD 2009.

Our results demonstrated that, after treatment for 2 days under a specific salt concentrations, HD 2009 was affected more than Kh 65. Salt stress inhibits plant growth for two main reasons; it reduces the ability of the plant to take up water (osmotic stress) and it accumulates to excessive levels in the tissues resulting in cellular injury (ionic stress) (Guo*et al.,* 2012). Under high salt concentrations, the regulatory functions of the plant appear to be lost resulting in cell death. In the present study, the sodium content accumulated in both cultivars. Sodium content in HD 2009 increased more drastically as compared to Kh 65 (Data not shown). Thus salt stress had a larger effect on HD 2009 than Kh 65 as expected since Kh 65 was known to have a higher salt tolerance than HD 2009. Because plant roots are exposed directly to salt conditions, the root is considered to be the first organ directly affected by salinity and the most sensitive organ to salt stress. Due to the ability of plants to activate a large number of stress related genes and to synthesize a variety of functional proteinsto counteract salt stress (Yan *et al.,* 2005), it is important to study differences in salt-tolerance mechanisms between tolerant and sensitive varieties by comparative proteome analysis of wheat seedling roots.

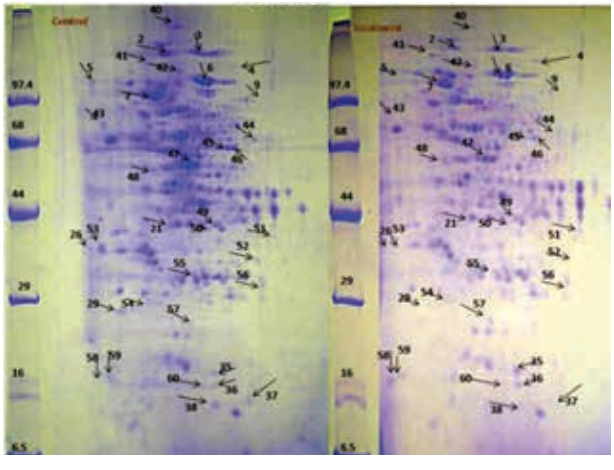

**Fig 2A.** Root proteome of HD 2009 under control and saline condition

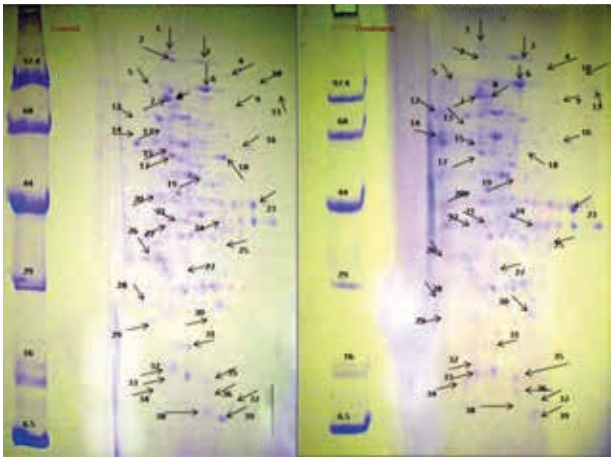

**Fig 2B.** Root proteome of Kh 65 under control and saline conditions

It may be concluded that for discovering of gene product specifically related to salt tolerance an appropriate salt level was chosen as high salt stress lead to unspecific stress response. Comparative proteome analysis led to the detection of proteins involved salt tolerance so the present study was conducted for comparing the protein profile of resistant and sensitive variety. The new spots that were identified in the resistant variety can be sequenced and the protein subunits involved in salt stress can be identified for better understanding the mechanism of salt tolerance.

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