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# Differential Accumulation Patterns of Seed Proteins in Salt-Tolerant and Salt-Sensitive Rice Lines under Varying Salinity Levels

A. Singh[1*], B. Arora[1], and N. K. Matta[2]

## ABSTRACT

**Salt-induced changes in the accumulation pattern of seed proteins were studied in salt-sensitive (MI-48) and tolerant (CSR-10) rice lines. An increase in seed protein content was observed with progress in seed development from 4 to 12 Days After Flowering (DAF) and up to maturity at each salinity level (4, 7, and 10 dS $m^{-1}$). However, a 10-21, and 14-30% reduction in seed protein was noted when compared at a given developmental stage at all the salinity levels in 'CSR-10' and 'MI-48,' respectively. Among the four seed protein fractions, the proportion of glutelins revealed an increase (5-9%) in mature seeds of 'CSR-10' with a decrease (11-13%) in 'MI-48' under increasing salinity levels. Prolamins exhibited a reverse trend in both lines. Albumins and globulins revealed a decreased proportion in 'CSR-10' but an increase in 'MI-48' only at 10 dS $m^{-1}$ at a given developmental stage. In 'CSR-10', the accumulation pattern of the glutelin [Molecular weight (*Mr*.) 36-40.5 and 19-21.5 kDa] and prolamin (13 kDa) polypeptides was seen similar at the control, 4, and 7 dS $m^{-1}$ except for the higher concentration of these at later two. At 10 $dSm^{-1}$, a contradictory pattern of accumulation of these polypeptides was observed. In 'MI-48', a completely different trend (earlier and faster accumulation) of the above-mentioned polypeptides was seen at 4 and 7 dS $m^{-1}$ in comparison to the control from early stages. Prolamin polypeptide (13 kDa) showed a continuous decrease in its concentration at all the salinity levels; more pronounced at 10 $dSm^{-1}$. Therefore, both lines revealed a different mechanism in response to a given salinity condition.**

**Keywords**: Glutelins, *Oryza sativa*, Prolamins, Seed storage proteins.

## INTRODUCTION

Seeds from the two plant groups – cereals and legumes - have been the major source of dietary protein in human nutrition; however, the protein content varies in these two groups approximately 10-15% in cereals to 30-40% in legumes (Vliet *et al.,* 2015). These proteins are stored in the seeds during its development and are termed Seed Storage Proteins (SSPs). Among cereals, rice is one of the important crops next to wheat and maize and serves as a staple food for about 60% of the world's population (Wing *et al.,* 2018). SSPs, stored in specialized organelles known as Protein Bodies (PBs) in rice, have been classified into albumins, globulins, glutelins, and prolamins (Shewry and Halford, 2002). Glutelins, present in PB-II, constitute the major fraction with 80% of the total rice proteins while prolamins contribute only 5% and are deposited in PB-I (Tanaka *et al.,* 1980). Glutelins are known to be represented by the polypeptide pairs of Molecular weight (*Mr*.) 60, 58, 52, 49, and 25 kDa which on reduction, in the presence of 2-mercaptoethanol, break into a large acidic (18-40.5 kDa) and a small basic subunit

[1] Department of Botany, Akal University Talwandi Sabo (Bathinda), Punjab, India.
[2] Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India.
*Corresponding author; e-mail: arvinder_bot@auts.ac.in





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(16.5-25 kDa). Prolamins, the alcohol-soluble proteins, are constituted by the polypeptides of *Mr*. 21, 17, 15, 14, and 13 kDa (Singh and Matta, 2011). Accumulation of different polypeptides in rice grains has been reported to begin early during the seed development i.e. 4 Days After Flowering (DAF) reaching a maximum between 8 and 10 DAF. Whereas the glutelins and globulins are synthesized early and appear by 5 DAF, the prolamins could be seen at 10 DAF stage (Yamagata *et al.,* 1982). However, at the later stages of seed development i.e. 10 DAF, the synthesis and accumulation rate of prolamins exceed that of glutelins *vis-à-vis* early and mid-stages, but the overall content of glutelins remain higher in the mature rice grain (Huang *et al.*, 2019).

The accumulation of different seed protein fractions in rice endosperm is primarily dependent on genotype, although it is also affected by the prevailing environmental conditions, mainly the abiotic stresses viz. drought, temperature, salinity etc. Soil salinity is one of the major limiting factors affecting the physiological and biochemical processes resulting in poor productivity and growth (Maggio *et al.,* 2010; Tavakkoli *et al.,* 2011). Over the past and recent years, a good number of studies have been carried out to show the effect of salinity on agronomical, physiological and biochemical responses in rice, mainly during seed germination and seedling growth (Islam *et al.,* 2008; Momayezi *et al.,* 2009; Datir *et al.,* 2018). The ion toxicity with excess $Na^+$ ions causes cellular damage and metabolic dysfunction (Rains and Epstein, 1965), as well as detrimental effect on essential enzyme functions (Flowers and Lauchli, 1983) and protein synthesis (Hall and Flowers, 1973). They, in turn, adversely affect water uptake, transpiration and photosynthetic apparatus, $CO_2$ assimilation, physiologically important cell components, and ultimately growth and yield (Arzani and Ashraf, 2016). The reproductive stage has been shown to be the most susceptible stage for rice plant during the salt stress (Hussain *et al.,* 2017), and the salinity tolerance at this stage is necessary for the high yield. A large number of QTL for salt tolerance related traits has been identified at this stage affecting plant height, tiller numbers, panicle length, pollen fertility and $K^+/Na^+$ ratio in the flag leaf of rice (Hossain *et al.,* 2015).

Over the past few years, the use of proteomic tools have also been used to characterize several salt stress-responsive proteins in rice leaf sheath (Kong-ngern *et al.,* 2005), root (Malakshah *et al.,* 2007), seedlings (Liu *et al.,* 2017) and young panicle (Dooki *et al.,* 2006). The proteomic analysis of the rice seeds during germination also revealed several proteins in maintaining the levels of ROS, abscisic acid, and seed reserves (Zhang *et al.,* 2016). Similarly, the upregulation of metallothione-like proteins in the rice leaves has been reported to be associated with salt tolerance through efficient scavenging of ROS (Fukuda, 2011). Further, the proteome analysis of roots of salt sensitive (L7) and salt resistance (T07339) rice lines by Liu *et al.* (2017) have reported eight differently expressed proteins imparting salt tolerance to the rice plant. Baxter *et al.* (2011) have reported increase in glutelins in the mature rice seed from the plants grown at the salt concentration of 4 $dSm^{-1}$, which is under the limit of tolerance by the rice plants. In this way, most of the proteomic studies have focused on investigation of salinity responses in rice using roots, leaf, seedlings or mature seeds as the target material; very limited studies are there showing the effect of salinity on the seed protein fractions in the mature rice seeds. Moreover, no report is available on the accumulation pattern of seed storage proteins in the developing seeds of rice under varying salinity levels.

In the present study, a detailed analysis of seed protein characteristics viz. changes in seed protein content, four protein fractions, and accumulation pattern of polypeptides on SDS-gels was carried out in the seeds of salt-sensitive and salt-resistant rice variety collected at different developmental stages at varying salinity levels.





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## MATERIALS AND METHODS

### Plant Materials

Seeds of one sensitive (MI-48) and one tolerant (CSR-10) rice line procured from CSSRI, Karnal, were grown in non-saline soil in cemented pots (75 cm length, 45 cm width and 30 cm height) in three replicates. Then, $3/4^{th}$ of the pots were filled with fertilized soil on the day of transplanting, and 25-day old seedlings (three of each line) were transplanted in every pot. Plants were irrigated with distilled water daily two times (8am and 5pm) to maintain the 2 cm water level during the first 10 days after transplanting for the better recovery of the seedlings. Afterward, depending upon the soil condition, regular watering was made to maintain the humidity in soil throughout the experimental period. Keeping in view the range of salinity tolerance of rice plant, the three salinity levels were created using the following methods as described in U.S. Salinity Laboratory Hand Book No. 60 (Richard, 1954).

One of the sets was kept as control (without salt treatment) and in the other three sets, salt treatments were started just before the onset of flowering by irrigating the pots with the salt solution (NaCl) having ECs 4, 7, and 10 $dSm^{-1}$, and the desired salinity levels were maintained till maturity. Seeds of different developmental stages at each salinity level, including the control, were harvested at an interval of 2 days starting from 4 Days After Flowering (DAF) to 12 DAF and at maturity. Different seed harvesting stages were identified on the given basis. The seeds harvested at 4 DAF stage had watery content; however, at 6 DAF stage, these turned milky in consistency. At 8 DAF, the milky caryopsis turned into soft dough and on reaching 10 DAF stage converted into hard dough. At 12 DAF, the grain became slightly hard with some translucency and greenish tint; later on, the harvested mature seeds were harder, clearer and free from any greenish tint. The collected seeds were dried and stored in the deep freeze for various analyses.

### Preparation of Total Seed Protein Extracts

Total seed protein extracts were prepared as described by Matta (1981) with minor modifications. Forty mg of the seed meal was suspended in 400 µL of 0.2M Tris-HCl buffer (pH 6.8) containing 2% Sodium Dodecyl Sulphate (SDS) in Eppendorf tubes. The suspension was heated in a water bath at 80°C for about 40-45 minutes with frequent vortex mixing. The suspension was centrifuged at 2,000×g and the extract was used.

### Seed Protein Fractionation

The separation of four protein fractions was based on the method employed by Schaeffer and Sharpe (1990) with slight modifications. All aqueous extraction solvents were buffered with 10 mmol $L^{-1}$ Tris-HCl (pH 7.5). After extraction of albumins in water, the residue was used for separation of globulins, prolamins and glutelins sequentially with 0.5 mol $L^{-1}$ NaCl, 55% n-propanol and 0.5% SDS.

### Protein Estimation

For estimation of seed protein content, the semi-micro Kjeldahl method (Peach and Tracey, 1956) was followed. After digestion of seed meal with concentrated sulfuric acid in the presence of a catalyst, the digest was heated with 40% NaOH in Markham's distillation assembly. The ammonia evolved was titrated with N/40 HCl to determine the nitrogen present in the sample. The so determined nitrogen was multiplied by 6.25 to get the seed protein content value. Protein concentration in different protein fractions was determined using the method given by Bradford (1976).





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### SDS-PAGE and Gel Staining

SDS-polyacrylamide gel electrophoresis was carried out on 14% gels following the method of Laemmli (1970). For gel electrophoresis under reducing conditions, 2% 2-mercaptoethanol was added to the seed protein extract. The gels were stained with CBB R-250 (0.05%) dissolved in a solvent containing methanol, acetic acid, and distilled water in the ratio 50:7:43 (v/v) and destained in the same solvent mixture but lacking the dye.

### Densitometric Scanning of Gels

The relative concentration of the major polypeptides, separated by SDS-PAGE, was determined by densitometric scanning of the gel using 'TotalLab' software from Nonlinear Dynamics Ltd. (downloaded from www.nonlinear.com).

### Molecular Weight Determination and Statistical Methods

The standard proteins obtained from Sigma-Aldrich were run on SDS-gels for calibration of the 14% gels used for the purpose. To calculate the Molecular weight (Mr) of the bands that appeared on SDS-gel, standard curve was drawn according to molecular weight protein markers and their pixel position on the gel using Total lab TL software. Pearson's coefficient of correlation was carried out using SPSS 24.0 software.

## RESULTS

### Seed Protein Content and Statistical Studies

Under control conditions, protein content in the developing seeds of salt-sensitive line 'MI-48' followed an increasing trend (4.6 to 11.3%) from 4 DAF onwards till maturity (Figure 1). However, in salt-tolerant line 'CSR-10', protein content increased continuously during seed development, and after 12 DAF, it was reduced slightly in the mature seeds. It could also be observed that with an increase in the salinity level from 4 to 10 $dSm^{-1}$, the seed protein content of the lines 'MI-48' and 'CSR-10' exhibited a decrease at all the developmental stages. At maturity, seed protein content of 'MI-48' decreased from 11.3% under control condition to 10.5, 9.3 and 8.0% at 4, 7, and 10 dS $m^{-1}$, respectively. In line 'CSR-10', it decreased from 7.9 to 7.1%, 6.4, and 6.2% at three salinity levels in their increasing order. A highly negative significant correlation was observed between seed protein content and salinity level in these lines at $P< 0.05$ (Table 1).

### Relative Proportion of Four Seed Protein Fractions

Each protein fraction was studied for its relative proportion at each developmental stage and at all the salinity levels *vis-a-vis* control in both rice lines (Table 2):

Albumins: An increase in the relative proportion of albumins was exhibited in the line 'MI-48' at 4 and 7 $dSm^{-1}$ when compared with the control i.e. 19.1% (control) to 47.1 and 77.3%, respectively, and a decrease in the same was noticed at 10 dS $m^{-1}$ (19.1 to 10.4%) in the mature seeds. On the other hand, the line 'CSR-10' revealed a decreased proportion of albumins at 4 and 7 $dSm^{-1}$ salinity levels from the control in contrast to an increase at 10 dS $m^{-1}$ in its mature seeds. However, at each developmental stage at a given salinity level on comparing with the control, the proportion of albumins was found to be suppressed in 'CSR-10' at all the salinity levels in contrast to an enhanced level of albumins in 'MI-48' at 7 and 10 dS $m^{-1}$.

Globulins: The globulins showed an increase in their proportion at all the salinity levels in 'MI-48' and 'CSR-10' from 4 DAF up to maturity during the seed development. However, their ultimate accumulation in





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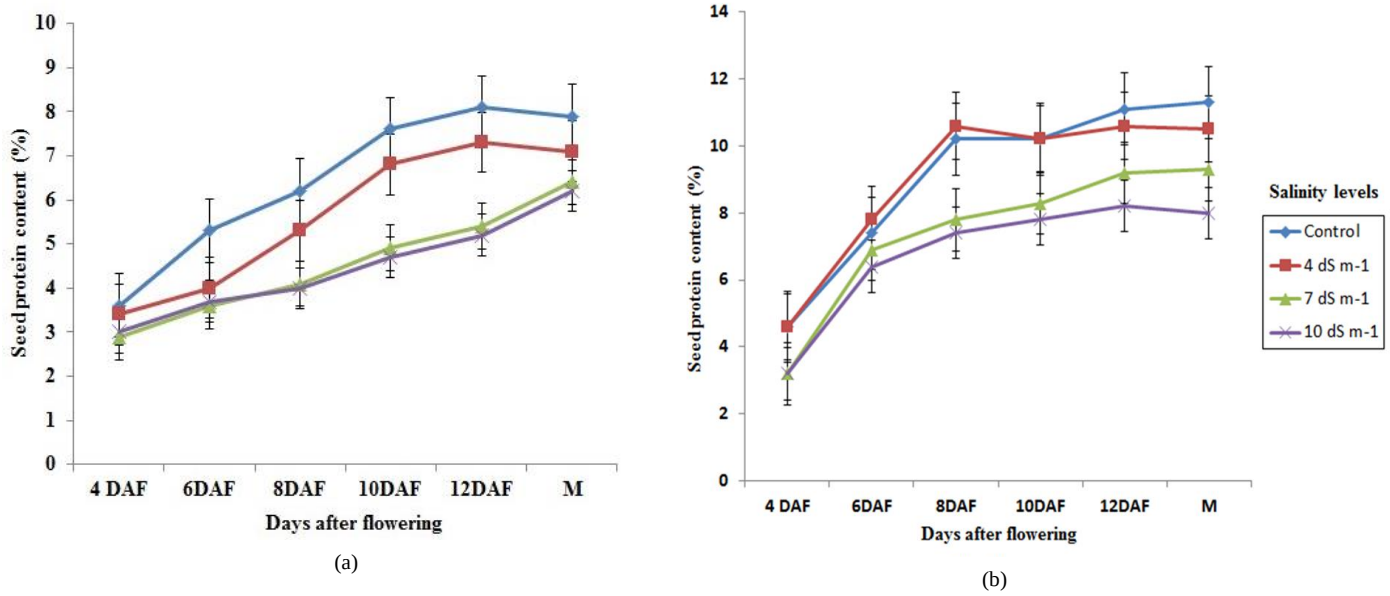


**Figure 1.** Seed protein content at different seed developmental stages at different salinity levels in rice lines 'CSR-10' & MI-48 respectively; 4 DAF, 6 DAF, 8 DAF, 10 DAF,12 DAF and M represent developmental stages. Error bars indicate SE.

**Table1.** Bivariate correlation between seed protein content, seed developmental stages and salinity in rice lines **'**CSR-10' and **'**MI-48'.

| | | | 'CSR-10' | 'MI-48' |
|---|---|---|---|---|
| | Salinity | Seed developmental stages | Protein content | Protein content |
| Salinity | 1.000 | ---------- | -0.416* | -0.492* |
| | | | 0.043 | 0.015 |
| Seed developmental | ------- | 1.000 | 0.336 | 0.497 |
| stages | | | 0.090 | 0.074 |

* Correlation is significant at 0.05 level.

mature seeds followed a decreasing trend from the control to 10 dS $m^{-1}$ i.e. Control> 4 dS $m^{-1}$> 7 dS $m^{-1}$>10 dS $m^{-1}$. The effect of salinity on globulin proportion in 'MI-48' was found reverse of the line 'CSR-10' at each developmental stage and at each salinity level when compared to the control; however, the effect was more pronounced at 7 and 10 dS $m^{-1}$ levels.

Glutelins: The decrease in the relative proportion of glutelins in mature seeds was noticed from 31.3% in control to 25, 25.3 and 24.4% at low, moderate and high salinity levels, respectively, in line 'CSR-10'. On the other hand, in the line 'MI-48', it was observed in the range of 26.8% in the control to 26.2% at 4 dS $m^{-1}$, 28.5% at 7 dS $m^{-1}$ and 23% at 10 dS $m^{-1}$. However, the proportion of glutelins remained higher at each developmental stage (4 DAF up to maturity) at increasing salinity level vis-a-vis under the control conditions in 'CSR-10' line i.e. Control< 4 dS $m^{-1}$< 7 dS $m^{-1}$< 10 dS $m^{-1}$. An opposite trend was shown by the glutelins in the line 'MI-48'.

Prolamins: The prolamins, which were the lowest up to 8 DAF, followed a relatively higher rate of accumulation from 10 DAF onwards as compared to albumins and globulins in both lines. In line 'MI-48', the relative proportion of prolamins was found to increase at each salinity level and at each developmental stage as compared to the





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**Table 2**. Relative proportion of four seed protein fractions in rice lines 'CSR-10' and 'MI-48' at different salinity levels at different seed developmental stages.

| Salinity Level | Seed developmental stage | Proportion of four seed protein fractions (g 100 $g^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 'CSR-10' | | | | 'MI-48' | | | |
| | | Albumins | Globulins | Glutelins | Prolamins | Albumins | Globulins | Glutelins | Prolamins |
| Control | 4 DAF | 7.8 | 4.0 | 81.1 | 7.1 | 8.9 | 5.1 | 80.4 | 6.0 |
| | 6 DAF | 9.7 | 6.4 | 74.0 | 9.9 | 10.8 | 7.5 | 74.2 | 7.5 |
| | 8 DAF | 13.0 | 10.7 | 66.0 | 10.3 | 14.1 | 11.8 | 63.6 | 10.5 |
| | 10 DAF | 11.9 | 12.0 | 60.1 | 15.0 | 12.0 | 11.9 | 60.4 | 15.7 |
| | 12 DAF | 11.0 | 14.3 | 55.8 | 18.9 | 10.9 | 11.5 | 58.7 | 18.9 |
| | Mature | 10.8 | 14.5 | 55.7 | 19.0 | 10.6 | 11.6 | 58.8 | 19.0 |
| 4 dS $m^{-1}$ | 4 DAF | 7.5 | 5.1 | 80.0 | 7.4 | 7.0 | 7.0 | 78.0 | 8.0 |
| | 6 DAF | 7.1 | 7.2 | 76.4 | 9.3 | 9.6 | 9.8 | 71.2 | 9.4 |
| | 8 DAF | 9.2 | 11.6 | 68.0 | 11.2 | 13.6 | 12.7 | 65.0 | 13.3 |
| | 10 DAF | 12.9 | 12.2 | 60.1 | 14.8 | 12.5 | 11.1 | 60.0 | 16.4 |
| | 12 DAF | 10.5 | 15.1 | 56.3 | 18.1 | 10.1 | 9.9 | 58.1 | 21.9 |
| | Mature | 10.3 | 15.0 | 57.0 | 17.7 | 10.3 | 9.7 | 57.5 | 21.5 |
| 7 dS $m^{-1}$ | 4 DAF | 6.9 | 4.9 | 79.8 | 8.4 | 7.5 | 6.3 | 78.1 | 8.1 |
| | 6 DAF | 8.7 | 7.0 | 74.3 | 10.0 | 9.2 | 8.6 | 70.0 | 7.4 |
| | 8 DAF | 8.1 | 11.0 | 69.1 | 11.8 | 14.0 | 9.9 | 62.6 | 11.3 |
| | 10 DAF | 9.2 | 12.2 | 63.7 | 14.9 | 16.5 | 10.1 | 55.8 | 17.6 |
| | 12 DAF | 9.5 | 14.7 | 59.2 | 16.6 | 13.8 | 9.4 | 56.3 | 20.5 |
| | Mature | 9.4 | 14.5 | 59.0 | 17.1 | 13.6 | 9.2 | 55.8 | 21.4 |
| 10 dS $m^{-1}$ | 4 DAF | 6.7 | 5.0 | 80.1 | 8.2 | 12.4 | 7.4 | 70.0 | 10.2 |
| | 6 DAF | 6.3 | 6.0 | 77.3 | 10.4 | 11.3 | 8.7 | 66.3 | 13.7 |
| | 8 DAF | 8.4 | 10.1 | 69.2 | 12.3 | 12.2 | 9.1 | 61.6 | 17.1 |
| | 10 DAF | 9.3 | 11.0 | 64.1 | 15.6 | 11.6 | 10.3 | 58.2 | 19.9 |
| | 12 DAF | 9.6 | 11.4 | 60.0 | 19.0 | 13.4 | 10.0 | 54.8 | 22.2 |
| | Mature | 9.6 | 11.3 | 60.4 | 18.7 | 13.7 | 9.8 | 53.9 | 22.6 |

control; faster accumulation was at 10 $dSm^{-1}$. The salt-tolerant line exhibited a significant decrease in the relative proportion of prolamins after 8 DAF at all the salinity levels at a given developmental stage.

### Polypeptides Accumulation Pattern

Under non-saline conditions, seed protein extracts of mature seeds of the lines 'MI-48' and 'CSR-10' revealed a number of bands of *Mr*. 95, 88, 78, 65, 60, 57, 45, 40.5, 38, 36, 33, 31, 29, 27, 21.5, 20, 19, 16, 14 and 13 kDa on the gels (Figure 2-a and b). Out of these, polypeptides of 95, 88, 78, 65, 60, 38, 20 and 13 kDa with light intensity were seen at an early stage (4 DAF) in both lines and the rest of the bands could be observed clearly but with low intensity at 8 DAF. In the salt-tolerant line 'CSR-10', the intensity of all the polypeptides kept on increasing after 8 DAF and reached maximum at maturity. In salt-sensitive line 'MI-48', the maximum intensity of bands could be seen at 10 DAF, which decreased later on in mature seeds. Except for changes in the intensity of various polypeptides in mature seeds collected from the plants grown under non-saline conditions, no other qualitative change was observed on SDS-gels. The same was found true for the total seed protein extracts prepared from the plants grown under various saline conditions.





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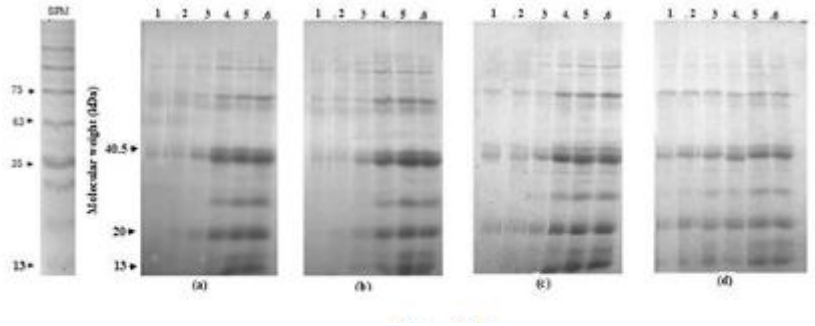
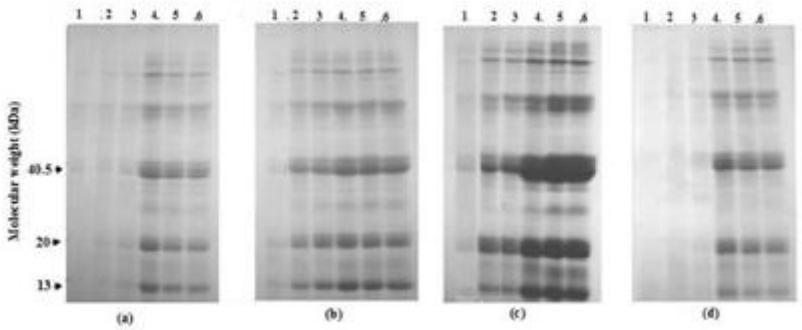


(a)



(b)

**Figure 2**. SDS-PAGE of total seed protein extracts of rice lines 'CSR-10' & 'MI-48' under reducing conditions (Tracks 1, 2, 3, 4, 5, 6 represent seed protein extracts at 4,6,8,10,12DAF and maturity respectively; a, b, c, d represent the gels at control and salinity levels (4, 7 and 10 Ds $m^{-1}$). '+2ME' stands for the presence of '2- Mercaptoethanol'. 'SPM' stands for Standard Protein Markers

Further, electrophoretic analysis of the control and salinity treated seed protein extracts of both lines revealed a number of major polypeptide bands undergoing significant changes (decreased or increased ) in their intensity. These polypeptides were classified as glutelins with *Mr.* 88, 65, 57, 40.5, 38, 36, 21.5 and 20 kDa, globulins with 27 kDa and prolamins with *Mr.*16, 14, and 13 kDa in accordance with our previous report (Singh and Matta, 2011).

On further observing the accumulation patterns of the various polypeptides at different salinity levels in the line 'MI-48' (Figure 2-b), the bands of *Mr.* 95, 88, 78, 65, 60, 38, 20, and 13 kDa could be seen at 4 DAF stage in the control as well as at 4 (low) and 7 dS $m^{-1}$ (moderate) levels; however, at 10 dS $m^{-1}$ these could be noticed at 8 DAF. Under 4 and 7 $dSm^{-1}$, the accumulation of these polypeptides (with other appeared late in the development process) kept on increasing and reached the maximum in the mature seeds. In the seeds harvested from the plants grown at 10 dS $m^{-1}$, the same polypeptides achieved their maximum accumulations at 10 DAF;





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afterward, a decrease in the intensity was noticed in the mature seeds.

'CSR-10' exhibited different trend for seed proteins accumulation at different growing conditions, varying from normal to 10 dS $m^{-1}$ (Figure 2-a). At the control, 4 and 7 dS $m^{-1}$, the accumulation pattern of the polypeptides was found to be more or less the same, except the bands with *Mr*. 38, 36, 33, 27, 20, 19, 16, and 13 kDa, which displayed slightly higher intensity at 7 dS $m^{-1}$ at all the developmental stages. With the seed development under non-saline and saline conditions (4 and 7 dS $m^{-1}$), the maximum accumulation of these polypeptides could be seen in mature seeds. However, a different accumulation pattern was observed at 10 dS $m^{-1}$ when compared with the control, 4 and 7 $dSm^{-1}$ levels. At the early stages of seed development (4 and 6 DAF), all the polypeptides with *Mr*. 95, 88, 78, 65, 60, 45, 40.5, 38, 36, 33, 31, 29, 20, 19, 16, 14 and 13 kDa could be seen with a higher intensity as compared to the polypeptide patterns in the seed extracts from the control, 4 and 7 $dSm^{-1}$ conditions at the same developmental stage. The intensity of these bands remained more or less the same up to 8 DAF and thereafter increased at the later seed developmental stages up to maturity.

### Densitometric Scanning Studies

In the line 'CSR-10', the relative concentration of the polypeptide of *Mr*. 88 kDa was seen to decrease after 10 DAF in plants under control condition and at different salinity levels viz. 4, 7, and 10 dS $m^{-1}$ from 1,564 to 1,088, 2,000 to 1,280, 1,292 to 1,120 and 1,104 to 930, respectively (Figure 3-a). The relative concentration of the polypeptides in the range of *Mr*. 36–40.5 kDa (large glutelin subunits) was observed to be increased in the control, which was more or less comparable for the same polypeptides at 4 and 7 dS $m^{-1}$. At 10 $dSm^{-1}$, the concentration of these polypeptides revealed an increase from 10 DAF to maturity. On the other hand, the polypeptides in the range of *Mr*. 19–21.5 kDa (small glutelin subunits) revealed a gradual increase in their relative concentrations at all the salinity levels from 10 DAF up to maturity; however, a great suppression in the accumulation of these polypeptides could be observed at 10 $dSm^{-1}$. The band of 13 kDa (prolamin) revealed a comparative increase in its concentration from 800 to 1,150 at 10 dS $m^{-1}$ *vis-a-vis* the control, 4, and 7 dS $m^{-1}$.

Whereas the concentration of polypeptides in 'CSR-10' increased gradually till the seed attained maturity, in the line 'MI-48' it was seen to decrease after 10 DAF stage in plants grown under controlled conditions. The decline in the relative concentration of polypeptides of *Mr*. 88, 65, 36, 21.5, 20 and 13 kDa could be seen from 1,300 to 750, 1,700 to1,550, 1,550 to 900, 950 to 400, 600 to 450 and 1,350 to 1,050, respectively, in the line 'MI-48' (Figure 3-b). At 7 and 10 dS $m^{-1}$ as compared to the control, the maximum alteration was observed for the polypeptides in the region of *Mr*. 36 – 40.5 kDa. However, the bands with *Mr*. 19-21.5 kDa showed enhanced cumulative concentration from approx. 5,218 to 6,800 at 7 dS $m^{-1}$, but under 10 dS $m^{-1}$, the concentration of these decreased from 1,780 to 1,376. The polypeptide of 13 kDa also exhibited a decrease in its concentration at all the salinity levels as compared to the control, low and moderate salinity levels. On comparing the whole trends, 4 and 7 dS $m^{-1}$ salinity levels showed little effects on the pattern of synthesis of various polypeptides in the line 'CSR-10' in contrast to that seen in the line 'MI-48'; however, at 10 dS $m^{-1}$ both lines were affected but in a different way.

## DISCUSSION

In the present study, the degree of suppression of protein content differed to the extent that the salt-sensitive line experienced more effect of salt stress as compared to the





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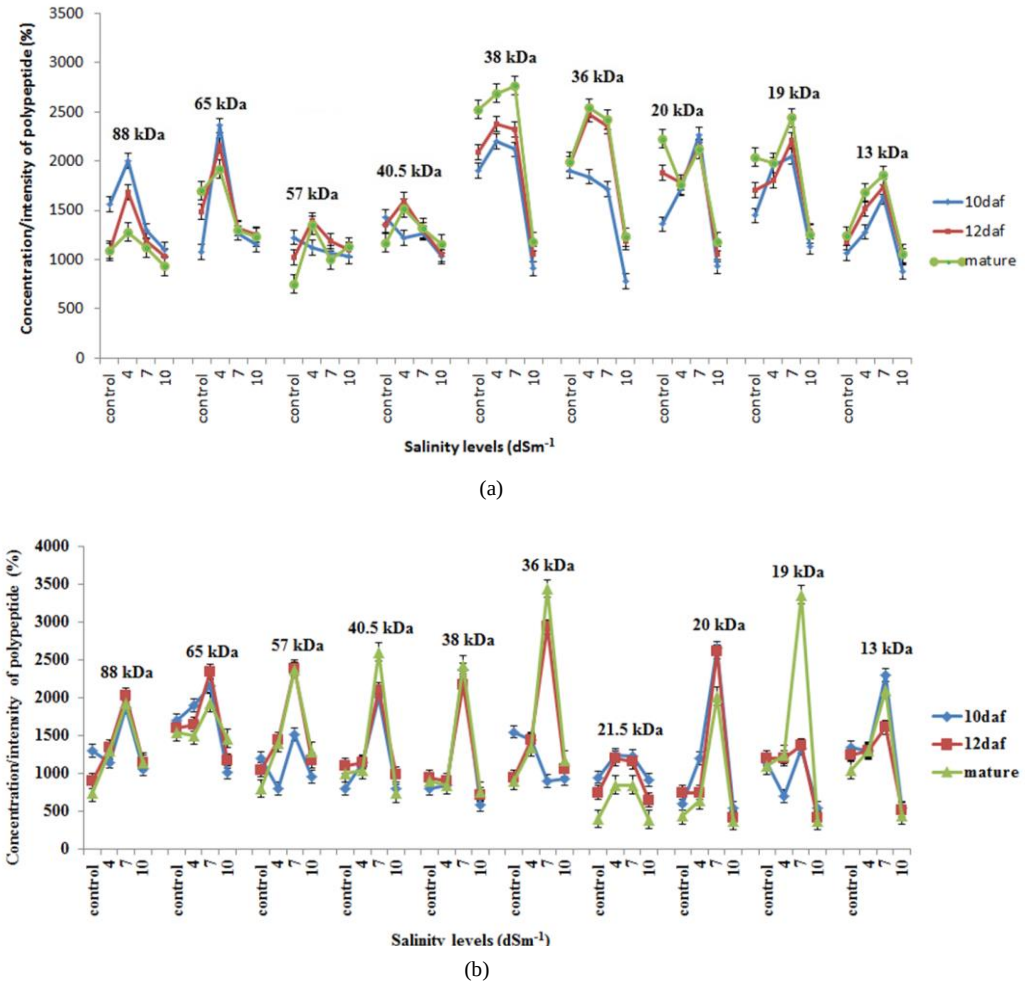


**Figure 3**. Densitometric scanning profiles of major polypeptides undergoing significant changes on SDS-gel at different salinity levels in rice lines 'CSR-10' and 'MI-48' respectively. Error bars indicate SE.

salt-tolerant line. As reported in earlier studies on changes in seed protein content under salinity treatment in different crops like wheat (Abdul Qados, 2009; Houshmand *et al.*, 2014), oats (Kumar *et al.*, 2010), soybean (Ghassemi-Golezani *et al.*, 2010), barley (Kumar *et al.*, 2017) and triticale (Salehi and Arzani, 2013)., the seed protein content in our studies also decreased in the mature seeds of both lines with an increase in the salinity level. This decrease in total soluble protein could be explained on the basis that higher salinity might affect the gene expression, which further resulted in the reduced RNA content, and eventually affected the protein synthesis. The alteration in the nitrogen assimilation under varying salinity levels might be another factor responsible for this reduction in the protein content at higher salinity in the mature seeds (Ghassemi-Golezani *et al.*, 2010). However, the pattern of protein accumulation in mature seeds was found different in both lines 'MI-48' and 'CSR-10' during seed developmental stages at different salinity levels. It could be divided into an initial period of rapid synthesis (up to 8 DAF) and a final period of slow synthesis (after 8 DAF) in 'MI-48' in contrast to a constant increase in the protein content during all the developmental stages in 'CSR-10'. Some workers have shown the synthesis of





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endopeptidases viz. aspartic, serine, threonine, or metalloproteases with their proteolytic activities at different seed developmental stages in some crop plants (Miernyk and Johnston, 2013). These enzymes were found to be active at one stage and inactive at other stages of grain development, thus, controlling the overall protein content in the mature seeds. Therefore, the differential accumulation trends of protein content in both lines could be attributed to the temporal and spatial expression of these proteases at varying developmental stages at a given salinity concentration. It could be further suggested to analyze the activities of these proteases at each developmental stage at a given salinity level. Moreover, to face the adverse conditions of salinity stress, the occurrence of high proline concentration has been observed in different plant parts, which might be the result of more protein degradation than synthesis during seed development, which eventually can lead to a decreased protein content in the mature seeds (Singh, 2016). Another way of proline accumulation has shown to be under the control of *P5CS* and *P5C* genes, the expression of which results in the proline synthesis via Glu pathway under salt stress conditions (Karthikeyan *et al.,* 2011). Therefore, this increase in the proline concentration along with comparatively lesser increase in other Amino Acids (AAs), has been correlated with the salinity tolerance in plants; however, the amount of elevation in the proline was reported to be dependent upon the genotypes employed for the study (Kibria *et al.,* 2017; Xie *et al.,* 2020). The proline generally improves the salinity tolerance by amelioration of antioxidative enzyme activities, photosynthetic activity and plant growth, and maintaining the suitable water status under salt stress in plants (Ahmed *et al.,* 2010). Further, the increased proline concentration was shown to regulate the *SOS1* and *HKT* gene expressions under salt stress, which are responsible for reduced $Na^+$ and $Cl^-$ content and increased K+/Na+ ratio in many plant species, thus, enhancing the salt tolerance (de Freitas *et al.,* 2019). Prior to the salt stress exposure, the presence of higher average AA contents along with some other metabolites with protective functions in salt-tolerant line impart it an intrinsic difference from the salt-sensitive line (Xie *et al.,* 2020).

Although the changes in the proline or other metabolites were not studied in the present study, these could be responsible for providing differential tolerance to these rice lines under the salinity stress. It is likely that the enhanced proline accumulation at the cost of more protein reduction in the mature seeds (due to more breakdown of the protein during seed development) as well as higher expression of Glu pathway genes in salt-sensitive line 'MI-48' might have helped it to withstand the high salinity stress. On the other hand, the presence of already higher proline content in the salt tolerant rice line resulted in lesser reduction in protein content in the mature seeds (due to less protein breakdown during seed development) and lesser expression of *Glu* pathway genes. Therefore, it can be concluded that the salt-tolerance by a plant species is the result of its intrinsic biochemical composition as well as the expression of its machinery at molecular level under various abiotic stresses.

The four seed protein fractions were seen to follow different synthesis rates and accumulation patterns during seed development under control and varying salinity levels conditions in the current study. The seed protein fractions extracted from the rice seeds showed albumins to be present at a higher concentration at all the salinity levels in the line 'MI-48', while it remained suppressed at the same salinity levels in 'CSR-10'. More increase in the albumins at 7 and 10 $dSm^{-1}$ in the line 'MI-48', because of their enzymatic functions, probably allow a mechanism for working hard under the salinity stress. Further, this increase in albumin content may be an indicator of tolerance against salt stress in sensitive genotype MI-48. On the other





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hand, the glutelins exhibited maximum concentration under controlled conditions but decreased constantly at increasing salinity levels in salt-sensitive line 'MI-48'. In contrast, an increased amount of glutelins at all the higher salinity levels in tolerant line 'CSR-10' point towards a mechanism providing enhanced tolerance capability by synthesizing higher glutelins. It is well known that the albumins are metabolic (enzymatic) in nature and the rest of the three (globulins, glutelins and prolamins) are storage in their function. In the present work, increase in metabolically active protein (albumins) at the cost of decreased energy-rich storage protein (glutelins) in the rice line 'MI-48' indicates the role of the former in defence. It is likely that being soluble in water, it contributes to stress avoidance by hydrating various cellular structure like many other stress proteins. Further, because of its enzymatic nature, the albumins might play an important role in energy metabolism required for $Na^+$ exclusion, and maintaining the membrane potential under the salinity stress. However, the direct evidence for the latter is generally lacking, so, this link between energy metabolism and avoidance mechanism could be the topic of investigation for future research. The salt-tolerant rice line is already having intrinsic mechanism of salinity tolerance as discussed earlier. It would spend most of its energy in redistributing the nitrogen, released by the protein breakdown during seed development under salinity stress, towards more synthesis of glutelins rather than fighting heavily to efflux the salt from its tissue. Moreover, increased glutelins due to its high proline and glutamine content might be involved in osmotic adjustment when plants are subjected to water shortage due to NaCl treatment. Still, there are no concrete evidences for the role of these proteins in providing tolerance against the salinity; it is further suggested to carry out the study on the structural and functional roles of these salt-stress responsive proteins to add-on our knowledge.

The alterations in respect to polypeptide patterns did not show any qualitative changes in these two lines, except clear quantitative changes when observed on SDS-gels. In the line 'CSR-10', intensity of almost all the large glutelin subunits (*Mr*. 36-40.5 kDa) was found to be increased at all the salinity levels; that of salt-sensitive line followed a decrease at salinity levels above 7 dS $m^{-1}$. On the other hand, the prolamin band of 13 kDa exhibited an increasing trend in its concentration from control to 10 dS $m^{-1}$ level in 'CSR-10', it exhibited reversible trend in the line 'MI-48'. The different response of these polypeptides, belonging to glutelins and prolamins fractions in term of their accumulation patterns in both rice lines, at varying salinity levels and at different developmental stages might be one of the indicators of the genes responsible for imparting sensitivity or tolerance to the plants. The time as well as strength of exposure of the salt conditions has been shown to affect the salt-sensitive and tolerant rice varieties differently for the expression of genes for different protein fractions (Abbasi and Komatsu, 2004). In the present study, greater accumulation of glutelins and prolamins at all the salinity levels as compared to the control conditions in salt-tolerant line 'CSR-10' indicated the more expression of the genes for these protein fractions under a given saline conditions. However, the expression of these genes could remain suppressed in salt sensitive line 'MI-48' at higher salinity levels as compared to the control. Further, the higher accumulation of glutelins/prolamins might be the result of higher degree of translatability and stability of a specific mRNA for these proteins. So, from the current data on the proportion of four fractions as well as the polypeptide patterns analysis, and from various studies on mRNA synthesis and stability in different crops (Zhu et al; 2003; Xu et al., 2012), it may be stated that factors for molecular regulation of storage protein genes are functionally different for the four fractions under various





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salinity levels at different developmental stages. Alterations in the intensity of polypeptides should be a function of various factors regulating the rate of protein synthesis and protein degradation during seed development.

Based on a large number of studies, salt tolerance has been explained to be governed by complex mechanisms involving different morphological, physiological, and biochemical changes. As stated earlier, the proteins coded by different genes are classified into two groups. One group of proteins represent the functional proteins that work for salinity tolerance and the other group represents regulatory proteins that participate in gene expression and signal transduction pathways (Hasegawa *et al.*, 2000). Therefore, it will be of interest to further characterize various glutelin (Glu α and Glu β), albumins as well as prolamin polypeptides involving diverse sets of rice genotypes for their specific roles as functional or regulatory proteins, and for their relationship with alterations seen in various characteristics under salt stress. In the present study, major polypeptides constituting a given seed storage protein fractions followed a different pattern of alteration in their accumulation/intensity. Keeping in view such variation in their response, it may be stated that genes for these storage protein fractions in rice are coordinately regulated with independent regulatory mechanisms for each of the fractions under salt stress.

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## الگوی انباشت افتراقی پروتئین دانه در لاین های برنج متحمل و حساس به شوری در سطوح مختلف شوری

**ا. سینگ، ب. آرورا، و ن. ک. ماتا**

## چکیده

در این پژوهش، تغییرات ناشی از شوری درالگوی انباشت پروتئین دردانه لاین‌های برنج حساس به شوری (MI-48)و متحمل به شوری (CSR-10)بررسی شد. با پیشرفت مراحل تکامل رشد دانه از ۴ روز تا ۱۲ روز بعد از گلدهی (DAF) و تا مرحله رسیدن کامل، در هر سطح شوری (که شامل ۴، ۷، و ۱۰ دسی زیمنس برمتر بود)، درپروتئین دانه افزایش مشاهده شد. با این وجود، درهرمرحله تکامل رشد و





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در همه سطوح شوری، پروتئین دانه کاهشی برابر ۲۱-۱۰٪ و۳۰-۱۴٪ به ترتیب برای (CSR-10) و (MI-48)نشان داد. همراه با افزایش سطح شوری، در میان ۴ دسته پروتئین دانه، مقدارglutelins در دانه های کاملا رسیده برنج، افزایشی برابر ۹-۵ ٪ در (CSR-10) و کاهشی برابر ۱۳-۱۱٪ در (MI-48)داشت. مقدار Prolamins در هر دو لاین روندی برعکس نشان داد. در هر مرحله رشد تکاملی، آلبومین ها و گلوبولین‌ها در(CSR-10) کاهش و در (MI-48) افزایش فقط در سطح شوری ۱۰دسی زیمنس برمتر داشتند. در لاین (CSR-10) الگوی انباشت glutelins ( وزن ملکولی با نماد *Mr* برابر 36-40.5 kDa و 19-21.5 kDa ) و prolamin (13 kDa) پلی پپتید در تیمار شاهد، ۴، و ۷ دسی زیمنس بر متر مشابه بود، به استثنای غلظت بالاتر دو مورد اخیر. در شوری ۱۰دسی زیمنس برمتر ، الگوی انباشت این پلی پپتیدها متناقض بود. در (MI-48) روندی کاملا متفاوت( انباشتی زودتر و سریعتر) از پلی‌پپتیدهای پیشگفته در سطوح شوری ۴ و ۷ دسی زیمنس برمتر در مقایسه با تیمار شاهد در مراحل اولیه مشاهده شد. غلظت پلی پپتید Prolamin (13 kDa) کاهشی پیوسته در همه سطوح شوری نشان داد و کاهش آن در ۱۰دسی زیمنس برمتر شدیدتر بود. بنا براین نتایج، در واکنش به یک شرایط شوری معین، این دو لاین برنج سازوکار متفاوتی آشکار ساختند.



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