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Germination and Protein Patterns of Some Genotypes of two Species of Jute as Affected by NaCl Stress

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Abstract: Different genotypes of Jute (*Corchorus capsularis* UPC 94, UPC 7716 and IRC 212) and (*Corchorus olitorius* S₁ and S₂₅) seeds were selected to study the effects of salinity stress on germination and subsequent seedling growth and concomitant changes of protein banding pattern. Different concentrations, of NaCl (1000, 3000 and 5000 ppm) were used. Generally, seed germination and growth parameters of seedlings dropped below the control levels at high concentration of NaCl (5000 ppm). However the different genotypes of Jute show higher growth at (1000) ppm as compared with the control. Protein banding patterns of Jute genotypes which previously treated with the different concentrations (1000, 3000 and 5000 ppm) of NaCl were studied. It has been found that the contents of proteins in the seedling of some different Jute genotypes were considerably increased with increasing salt stress compared with the control. Qualitative and quantitative changes of protein pattern suggested a modulation of gene expression. The results were interpreted in the light of recent arguments on salinity stress and proteins.

Key words: Jute, *Corchorus capsularis*, *Corchorus olitorius*, germination, seed protein electrophoresis, salinity

INTRODUCTION

Jute (*Corchorus* spp.) belongs to family *Tiliaceae*, which contains about 35 genera and 370 species among which the more important jute are *C. capsularis* and *C. olitorius*, which are cultivated on a large scale, in many countries especially in Far East for the commercial production of Jute fibers.

The mechanisms that confer salt tolerance to plant cells need to be completely understood. Variation in salt tolerance could more clearly identify key molecular strategies involved in this trait King^[1], Howell^[2] mentioned that no stage in plant life is more vulnerable than seed germination and seedling growth. At present almost every physiological process in the life cycle of a plant is being scrutinized with molecular tools^[2,3]

The salinity problems in connection with seed germination and growth of plants have been studied extensively. However, a general conclusion is obtained that germinability and growth are commonly adversely affected in a negatively correlated manner with salinity levels. The literature in this respect is extremely voluminous, therefore, attention has been devoted to most recent work^[4-7].

Growth and distribution of plants are controlled chiefly by water stress induced either by salinization, drought or water logging affects partially every aspect of

plant growth, physiology and biochemistry of plants. Some of the effects are related to the decrease in turgor^[8].

Gadgil *et al.*^[9] showed that the seed of 7 cultivars of *C. olitorius* and 4 of *C. capsularis* were analysed for protein, oil and ash contents there were significant differences between cultivars in concentration of protein, oil and ash. Three zones were observed following SDS-PAGE of seed protein extracted in SDS buffer zone 1, 2 and 3 consist of polypeptides having M.wt. around 60000, 40000 and 20000, respectively. In zone 2, *C. olitorius* showed 2-3 major polypeptides of 38000 - 41000 M.wt., whereas *C. capsularis* had 5-6 polypeptides of 3900-44000 M.wt.

Pathak and Chattopadhyay^[10] examined five *C. olitorius* and 6 *C. capsularis* cultivars using PAGE of soluble seed proteins based on RP values 30 protein bands were observed. The cultivars were identified from the electrophoregrams of their soluble protein.

Continuous treatment with 80 mM NaCl induced several deteriorative changes in general metabolism of seedling of the Jute species *Corchorus capsularis* as decreased in biomass production, protein and potassium level and increase in free amino acids, proline and Na⁺ levels it characterized lower decreased in protein pigments^[11].

SarKer *et al.*^[12] stated that PAGE revealed the presence of heterogeneity in protein banding profiles

among 12 wild and cultivated species of Jute (*Corchorus* sp.). Electrophoretic patterns differed in respect of number, mobility and intensity of bands, thus electrophoretic technique can successfully be used to characterize wild and cultivated *Corchorus* species. Wang *et al.*^[13] found that the salt tolerance of an organism depends upon the range of external salinity over which it is able to sustain these conditions in the cytoplasm.

The effect of salt stress was determined by Glenn and Brown^[14]. They found that effect of salt on the growth and water use efficiency of the xerohalophytes *Atriplex canescens*, enhanced the growth performance of the plants in drying soil by increasing their days to wilting, ability to extract water from the soil, organic matter production and water use efficiency.

Delfine *et al.*^[15] reported that, environmental stresses such as drought, salt stress and leaf aging cause considerable losses in crop quality and productivity. Salinity generally inhibits the growth of plant affecting both water absorption and biochemical processes such as N and CO₂ assimilation and protein synthesis.

Zhao *et al.*^[16] found that six-day barley (*Hordeum vulgare*) seedlings when were treated with Hogland's solution applied to leaves increasing membrane protein and protease activity increased with increasing NaCl concentration.

The salt stress to *Cicer arietinum* induced changes in amino acids, glutamate and proline. The protein profile showed major alternations at salinity levels which inhibited growth. Also protein and protein contents increased rice calli with increasing salt concentration in the medium, but in seedlings, increasing the salt concentration decreased the protein content. Furthermore, drought and salinity increased protein content in fully grown tomato leaves, compared with well-watered plants^[17-19].

Moreover *Vicia faba* seeds when were subjected to a salinity level of 180 mM NaCl or treated with vitamin B₆ (100 ppm) showed increasing in the numbers of protein bands and seed treatments with vitamins induced the synthesis and increased the intensity of protein bands of control and caused the appearance of additional new bands in the two tested broad bean lines, moreover, the polypeptides in two tested line disappeared under salinity stress^[20].

Duca and Barsan^[21] found that salt stress delayed plant emergence and the initial stages of stress, increased the content of soluble protein fractions and reduced the amount of salt soluble protein fraction in sunflower lines. Also, the increase in salinity resistance correlated with a reduction in sodium uptake and pre-treatment caused new protein to be synthesized^[22].

Germination and subsequent growth under salt stress triggered an accumulation of six major stress proteins and

resulted in growth arrest of young seedlings of rice (*Oryza sativa* L.) Cv. Bura Rata, but expressed proteins were at low level. Seedlings stressed with salt for four days recovered immediately. Longer exposure to 100 Mm/L NaCl were progressively delayed recovery and reduced the number of seedling which could recover from salt stress^[23].

The main target of this study was to evaluate three *C. capsularis* and two *C. olitorius* genotypes. This study included germination and subsequent growth of seedlings of different genotypes of jute and the qualitative and quantitative changes of protein which suggested a modulation of gene expression under salinity stress conditions.

MATERIALS AND METHODS

Three Jutes (*C. capsularis*) genotypes UPC 94, UPC 7716 and IRC 212 introduced from Bangladesh in addition to two strains of *C. olitorius* namely S₁ and S₂₅ from Pakistan were used in this study.

The apparent uniformity of size and shape was considered in selecting, which were divided into four groups, each of 100 seeds. The seeds of the first group were soaked in aerated distilled water to serve as control whereas the other three groups were soaked in 1000, 3000 and 5000 mg L⁻¹ NaCl. Treatment continued 24 h at 25±°C. Each group was germinated in 20 cm diameter Petri-dish supplemented with 30 mL of either distilled water and 1000, 3000 and 5000 mg L⁻¹ NaCl. The Petri-dishes were incubated for 10 days at 25±°C under continuous illumination.

For germination character homogenous seeds from each variety were freshly collected for determination of the seedling length, fresh and dry weights of seedlings.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Protein extracts from seedlings of Jute grown under NaCl stress were subjected to SDS-PAGE according to the method of Laemmli^[24].

Using 10% acrylamide in the separating gel and 3% in the stacking gel. Samples each of µL containing 30 mg protein in equivolume of the extraction and denaturation buffers were denaturated for 3 min in boiling water bath; cooled; centrifuged for few seconds at 1140x9 and 15 µL were applied per well, the separation was carried out on EC mini gel unit at 60 volt for 4 h. Gels were stained with coomassie brilliant blue (R-250), destained with high methanol solution (40% methanol in 10% acetic acid), photographed and the molecular weights of polypeptide bands were calculated from a calibration curve of low molecular weight marker standard of Sigma.

Table 1: The effect of salinity on some morphological criteria of different genotypes of Jute plants

Genotypes	Control			1000 mg L ⁻¹			3000 mg L ⁻¹			5000 mg L ⁻¹		
	Seedling length	Fresh wt.	Dry wt.	Seedling length	Fresh wt.	Dry wt.	Seedling length	Fresh wt.	Dry wt.	Seedling length	Fresh wt.	Dry wt.
<i>C. capsularis</i> (UPC 94)	6.00	5.32	0.36	7.24	6.60	0.60	3.74	7.40	0.76	2.40	4.41	0.23
<i>C. capsularis</i> (UPC 7716)	6.65***	6.30***	0.52***	6.74***	6.47***	0.55***	3.80***	7.28*	0.70***	2.90***	5.50***	0.45***
<i>C. capsularis</i> (IRC 212)	6.60***	6.25***	0.50***	7.05***	7.41***	0.75***	3.32***	8.65***	0.88***	2.20***	5.40***	4.40***
<i>C. olitorius</i> (S ₁)	5.90***	7.12***	0.66***	8.00***	7.30***	0.73***	2.80***	9.74***	0.97***	2.50***	6.90***	0.63***
<i>C. olitorius</i> (S ₂)	7.00***	5.61***	0.48***	7.50***	6.10***	0.47***	3.60**	7.63**	0.79**	3.40**	4.80***	0.30**
LSD	1%	0.07	0.10	0.03	0.01	0.10	0.03	0.16	0.23	0.04	0.07	0.02
	5%	0.05	0.07	0.02	0.07	0.08	0.02	0.11	0.16	0.03	0.04	0.14

***Highly significant, **Significant, *Non-significant

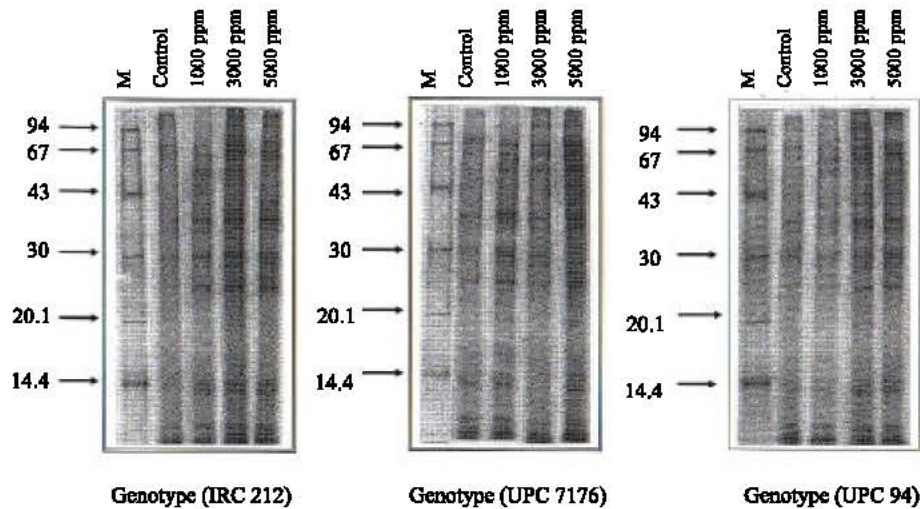


Fig. 1: SDS-PAGE diagrams of seed protein extracted from three salinized genotypes (*C. capsularis*) at different concentrations of NaCl

RESULTS AND DISCUSSION

The progressively retarded seed germination and seedling growth at higher levels of salinity (5000 ppm NaCl) compared with slight effect if any at the low concentration (1000 ppm) might be mainly attributed to ion disturbance in the radicle under osmotic stress^[7].

The seedlings growth parameters, length, fresh and dry weights of seedlings decline with increasing the external osmotic potential (Table 1). The leaf emergence was observed on 5th day in the case of control seedlings and it was delayed by about a two day in the seedlings in salt solutions. The different varieties of Jute show higher growth at (1000 ppm NaCl) as compared with the control, however there was gradual decreases in the growth with the highest concentration of NaCl in all Jute varieties.

The decreases of seedling lengths in response to the increase in the concentration of NaCl was reported by Parashar and Verma^[25] who observed an adverse effect on plumule and radicle growth in wheat during the early stages of seedling growth under saline conditions.

Moreover, Dubey^[26] attributed the decline in growth of roots and shoots of rice seedlings to the inhibition of hydrolysis of the endosperm reserves.

Salt damage to plants has been attributed to a combination of several factors including osmotic stress and the accumulation of toxic ions^[27]. Abo-Hamed^[28] also agree with this observation and discussed the effect of salt stress on plant growth might be ionic toxicity and ionic imbalance or combination of these factors

Dubey and Sharama^[29] reported that the presence of NaCl caused limited hydrolysis of endosperm reserves because of reduced uptake of water by seedlings and inhibited the mobilization of cotyledon reserves to the growing embryonic axis. Inal *et al.*^[30] noticed that fresh and dry weights of tomato plants decreased with increasing salinity.

Moreover, El-Bastawisy^[31] mentioned that the correlation between the retarded growth and salinization as well as water stress were attributed to the effect of salt and water shortage on plant metabolism such as osmotic adjustment, protein and nucleic acid synthesis, ion

Table 2: Comparative analysis of relative concentrations and molecular weight of three genotypes of salinized *C. capsularis* seed protein bands. These bands were separated using SDS-PAGE technique.

M.wt.	Genotype (UPC 94)				Genotype (UPC 7716)				Genotype (UPC IRC 212)			
	Control	1000 (ppm)	3000 (ppm)	5000 (ppm)	Control	1000 (ppm)	3000 (ppm)	5000 (ppm)	Control	1000 (ppm)	3000 (ppm)	5000 (ppm)
101		2.1										
100.7			3.3	6.6								
99.7						2.5	2.9	7.4			3.5	
98.9	9.2				9.1			3.9	10.5			7.9
97.5											4.1	
96.9			2.5	3.2			2.4	4.0				6.0
95.4	6.0	3.1			4.9	2.2	2.4		0.6			
78.8												
72.8				4.4								6.5
71.9								5.7			2.8	
71.0		2.6										
70.2	4.1											
69.4					5.2				8.9	2.4		
68.6		3.1				4.2						
62.8							2.6	4.2	8.4		2.7	6.2
61.5	5.3		3.1		6.3							
60.9		4.8							2.6			
54.1								4.9				
53.2		2.6										
51.5	4.7			2.7			1.4		6.1		0.6	2.9
50.4			1.7		5.6	3.9		3.5				
49.9		2.1							2.8			
46.1					3.7							
43.5						2.2						
42.9	2.1								1.6			
40.9								2.4				
36.3	3.2	3.1	4.6	1.8	3.6	3.6	2.4	3.9	3.4	3.4	2.3	2.7
34.5	2.1	2.7	1.5	0.8	1.6	1.1	0.7		1.3	1.7		0.4
32.0	1.8			0.9								
30.8		2.6	2.4	1.4	2.8	2.1	1.5	6.6	1.1	5.5	1.5	1.6
30.0				2.3			2.4	1.8			2.6	
29.5	2.6	3.4	4		2.1	2.9		1.9	2.4	3.4		2.6
28.5	1.5	2.2	2.2	1.2	1.2	1.4	2.6	1.9	1.3	1.1	2.4	1.7
27.1			1.3									
26.6								1.8				
25.0	1.7											
24.7		2.7	4.1	2.9	1.6	2.9	4.0	2.3	2.1	3.5	3.4	2.7
23.7								0.9		1.2		1.7
21.6	1.2	1	1.7	0.5	0.8		0.8	1.5	0.9	1.4	2.3	0.8
19.4	6.7			0.6	0.4			1.3	2.4		1.5	1.7
18.5							0.6	0.5		0.7		0.4
17.1							0.9					
16.5	1.9	1.5	1.7	2.2	2.7		2.7	2.4				
15.8	2.8		2.2	2.5				2.1	2.5	3.3	4.4	2.7
14.5		4.5	8.0	5.7	4.5		7.5	5.9	7.1	6.8	8.5	8.0
12.3		6.3			8.5		7.9				9.8	
11.8	3.2		12.7	5.5		12.8		6.7				8.4
9.1				2.8								4.3
7.1												
6.1				3.9			4.1					
6.1						5.2						
1.2	5.9			5.9	4.9				6.5			
0.8						8.0	8.7	4.6		6.2	8.5	7.1
0.5		6.3		11.3								
No. of bands	18	17	17	21	18	16	19	23	14	17	18	20

uptake, hormonal balance, enzyme activities and photosynthetic activity.

The reduction in growth may be due to reduction in cell division and/or in cell enlargement Hopkins^[32].

The contents of proteins in the seedlings of some different Jute genotypes were considerably increased with

increasing salt stress (Table 2 and 3). It is appear that the observed changes of each genotype of Jute seedlings were both qualitative and quantitative and may be illustrated by the disappearance of some bands, changes in bands intensity or changes in both and changes in some fractionation of some bands (Fig. 1 and 2).

Table 3 : Comparative analysis of relative concentrations and molecular weight of two genotypes of salinized *C. olitorius* seed protein bands. These bands were separated using SDS-PAGE technique

M.wt.	Genotype (S ₁)				Genotype (S ₂₅) control			
	Control	1000 (ppm)	3000 (ppm)	5000 (ppm)	Control	1000 (ppm)	3000 (ppm)	5000 (ppm)
100.7			6.6					
99.6				7.5	6.2		10.4	7.7
98.5			5.7					
96.7			3.6	7.9			4.4	
95.4	5.7				3.9	2.7		
72.8			3.8	3.8			5.3	
71.9								5.6
69.9		3.1						
69.3					3.5	2.5		
68.5	6.6							
63.5			3.6				4.9	6.2
62.8				4				
61.5					3.4			
60.9	5.8	1.8				2.8		
52.3			1.0	1.4				
51.5	4.5						2.0	3.5
50.7						1.8		
49.9		0.7						
46.5		0.9						
40.9							0.6	
36.4	1.7	3.2	2.3	0.8			1.8	1.5
35.9					2.9	3.2		
34.4					0.7	1.0	1.0	
31.4		0.9						
30.6	0.9	2	2.3		0.8	1.9	2.9	0.7
30.0			2.8	0.9			1.8	
29.5	1.6	3.0			1.9	3.1		1.7
28.5		1.1	1.8	1.3			0.8	1.1
27.9	0.9				1.4	2.8		
25.0			2.1	0.9				
24.7	1.4	2.1			1.9	2.6	1.9	1.0
21.7	0.5	1.2	0.7		0.4	1.5	0.8	0.3
19.3		0.5	1.8	1.6		2.0	0.9	0.9
17.1				0.7				
16.4	1.9	4.4	3.7		2.1	4.9	3.0	1.5
15.8							3.2	2.9
15.2		4.7						
14.5	4.4	8.6	4.1	9.9	6.7	8.9	7.2	4.7
12.4								5.9
11.8		10.3	8.2			12.5	8.2	
9.4								4.1
6.3	5.8		5.9	5.9				5.8
5.8					6.3			
1.3	4.9				7.6		7.9	5.4
0.8	9.3	8.2	7.6					
No. of bands	14	16	17	12	14	15	19	18

The total number of the recorded bands are (19) having M.wt. ranging between 101-0.5 KDa, these bands show variability in their expression with all genotypes of jute plants. i.e. not expressed in similar way in all genotypes. (UPC94, UPC 7716 and IRC 212) for *Corchorus capsularis* and S₁, S₂₅ for *Corchorus olitorius*).

The total number of bands in *C. capsularis* genotypes ranges between (15-18) and ranges between (16-18) in *C. olitorius* genotypes.

There are existence of a number of protein bands having molecular weight 36.3, 28.2 and 21.6 KDa,

respectively were recorded in all genotypes of *C. capsularis* for all treatments and control. The rest of number bands differ from one genotype to another in *C. capsularis*.

On comparing with the protein bands number of the control for *C. capsularis* seedlings treatment induced the appearance of newly bands i.e. increased number of bands comparing with number bands of control in all genotypes (*C. capsularis*). Moreover, protein band with M.wt. 30 KDa was disappear in control and all genotypes as a function of the same treatment. In addition the intensity of protein bands varied according to the

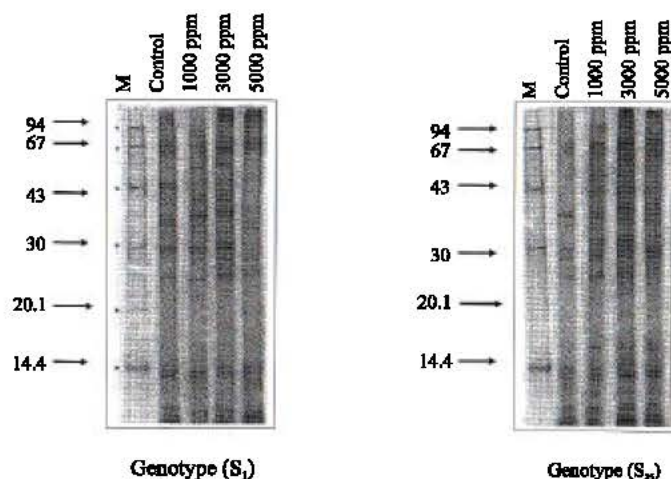


Fig. 2: SDS-PAGE diagrams of seed protein extracted for two salinized genotypes (*C. oltorius*) at different concentrations of NaCl.

genotype studied, this indicated that the presence of wide genetic variations among the studied genotypes. Therefore Paradies and Ohms^[33], were suggested that the differences in electrophoretic pattern could be used effectively in the identification of different cultivars.

As regards to genotypes of *Corchours oltorius*, there was induction in the disappearance of protein band with M.wt. 72. 8 KDa in two genotypes (S_1 , S_2) at low concentration of NaCl. Also the protein band with M.wt. 11.8 KDa exhibited the highest intensity of protein. Also, a number of characteristic bands were recorded in all genotypes and the number of these bands increased with low concentration of NaCl compared with control.

Generally, protein content of Jute genotypes showed gradual decreases with increasing NaCl concentration and the number of total bands in all genotypes of Jute plants increase at the higher concentrations of NaCl used, as comparing with those of the control. Moreover, this observation might indicate a changed pattern of gene expression on salinization treatment with lapse of time^[34].

Thus the qualitative and quantitative changes in the pattern of protein synthesis in the different genotypes of jute during germination and subsequent growth of seedlings under salinity stress, suggested that new genes might be transcribed and others might be repressed or, at least, the products of some genes were increased or decreased^[32]. In addition, treatment with salinity, induced shocked proteins were synthesized under stress condition and modulate the production of selected groups of proteins in germinating seedlings^[35,36].

Protein was considered physiological compatible solute or osmoprotectant that increase as needed to maintain a favorable osmotic potential between the cell and its surrounding^[37], this substance stabilize some

macromolecules or molecular assemblies, thus decreasing the loss of either enzyme activity or membrane integrity that occur when water is limiting^[38,39].

This agreement with the suggestion that, these stress specific proteins possibly gave plants the ability to tolerate salt stress through physiological and biochemical adjustment Lusardi *et al.*^[40]. From the above facts, it may be concluded that study of protein using SDS-PAGE is a more reliable tool and accurate technique to discriminate between cultivars.

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