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Effect of salt stress on protein profile of different varieties of *Cajanus cajan* using SDS page

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Abstract
A laboratory experiment entitled Effect Of Salt Stress On Protein Profile of different varieties of *Cajanus Cajan* Using SDS PAGE was conducted using seeds of varieties BSMR-853, BDN-711, BSMR-736, BDN-708 collected from VNMKV, Parbhani (M.S.) Agriculture Research Station, Badnapur, District – Jalna (M.S.) and carried out during December 2014 to April 2015 in Department of Biochemistry and Molecular biology, MGM college of agricultural biotechnology, Gandheli, Aurangabad. The experiment was laid out in Factorial randomized block design with 20 treatments combination of varieties with sodium chloride concentration Viz (BSMR- 853, BDN-711, BSMR-736, BDN-708), (om M, 50mM, 100mM, 150mM, 200mM) with three replication. The seeds were germinated under different concentration of sodium chloride and it was found that seed germinated under different concentration of sodium chloride showed decrease in germination percentage. The seeds after germination were subjected to protein extraction. The extracted protein was quantified by using Folin`s Lowry method. The seed of variety BSMR-736 germinated at 150mM concentration NaCl concentration showed highest amount of protein i.e 3.38 mg/ml of protein, whereas the seeds of variety BDN-708 at 200mM concentration showed lowest protein i.e 0.46mg/ml. The amount of sugar increases with increase in concentration of sodium chloride and increase in saline condition was found to influence molecular weight of the protein on SDS PAGE. Hence from above experiment it is concluded that increase in concentration of sodium chloride increases the amount of protein and amount of sugar and significant amount of variation exist in banding pattern of protein this might be due to expression of stress protein. (Mintoo *et al*, 2014).

Keywords: Salt stress, Protein Profile, Cajanas Cajan, SDS-PAGE

Introduction
In Asia pigeon pea is grown over area of 4.33 million ha with production of 3.8 million tons. India has the largest area 3.8 million ha followed by Myanmar 580,000 ha, china 150,000 ha and Nepal 21,360 ha. Maharashtra state is leading producer of pigeon pea in India having 11.75 lakh ha area under cultivation with production of 10.83 lac tones and productivity is 921.70kg per ha (ICRISAT., 2013).

Pigeon pea is hardy deep rooted crop. The deep root system enhances its capacity to withstand drought condition. It is a kharif crop and grown in month of June to January. The optimum temperature required for growth of pigeon pea is about 28-30°C. It requires annual rainfall in a wide range of 600-1100 mm. It is sensitive to water logging condition and grown on wide range of soil but perform well in sandy and loamy type of soil. It can be grown in pH range of 4.5-8.4 and have maturity period of 120-180 DAS (Cook *et al.*, 2005) [5].

Some researcher reported that salt stress (NaCl) caused decrease in germination, shoot and root length in red gram and also emergence, fresh and dry mass of both shoot and yield decrease with increase in salinity and also reduce levels of N,P,K and Ca due to salinity is also found (Wahid *et al.*, 2006) [16].

Therefore, study and understanding the salt stress tolerance mechanisms of pigeon pea during the seedling stage are important in cultivating new varieties of salt stress-resistant pigeon pea. Understanding the salt stress tolerance mechanisms of pigeon pea involves isolation and characterization of stress-related proteins and genes (Salekdeh *et al.*, 2002) [14]. Many genes related to salt stress tolerance have been identified. However, findings from these genes are generally limited to the mRNA level and the mRNA characteristics of the genes cannot be completely accounted for the actual processes that occur in the abiotic-stressed pigeon pea. Complete elucidation of these processes requires a study of the proteins expressed by the genes because proteins are more physiologically and biochemically responsive to stress and better correlate with plant characteristics (Pandey and Mann, 2000; Bazargani *et al.*, 2011 [10]; Caruso *et al*, Therefore, a proteomic research concerning the structure and function of stress-induced proteins will provide a better understanding of the salt tolerance mechanism of pigeon pea.

Considering above point in view to know stress proteins expressed under salt stress condition experiment entitled Effect Of Salt Stress On Protein Profile of different varieties of *Cajanus Cajan* Using SDS PAGE was conducted at Department of Biochemistry and Molecular biology, MGM College of agricultural biotechnology, Gandheli, Aurangabad during year 2014 to 2015 with following objectives. 1. To extract protein. 2. To study effect of salt stress on protein profile using SDS PAGE.

Materials and Methods

Experimental Details

The details of materials used and methods adopted for conducting the present investigation is described under appropriate heads.

Experimental site

The experiment was conducted in Biochemistry and Molecular Biology Laboratory of MGM College of Agriculture Biotechnology, Gandheli, Aurangabad**.**

Experimental Design

Factorial Randomized Block Design (FRBD)
1) No. of treatments: 20
2) No. of replication: 3

Treatment Details

Combination of different varieties of *Cajanus cajan* with different concentration of sodium chloride

Table 1: Factor A Different varieties of *Cajanus cajan*

Symbol	Treatments Varieties
V_1	BSMR-853
V_2	BDN-711
V_3	BSMR-736
V_4	BDN-708

Table 2: Factor B- Different concentrations of sodium chloride

Symbol	Treatments Sodium chloride concentrations (mM)
N_0	D/W (control)
N_1	50
N_2	100
N_3	150
N_4	200

Collection of sample

Seeds of four different varieties of *Cajanus cajan* (BSMR-853, BDN-708, BSMR-736, BDN-711) obtained from VNMKV, Parbhani (M.S.) Agriculture Research Station, Badnapur, District – Jalna (M.S.) was used throughout the experiments.

Germination of seed under salt stress

Pigeon pea seeds collected from Agriculture Research Station, Badnapur were surface sterilized in 0.01M $Hgcl_2$ solution for three minutes. Then the seeds were washed thoroughly with distilled water and the seeds were transferred on sterile petri dishes containing respective solution of treatment applied on filter papers and the seed were allowed for germination for five days and data for germination percentage was recorded and germination percentage was calculated (Mintoo *et al*, 2014) [9].

Extraction and quantification of protein from germinated seeds

Extraction

Before seeds subjected to protein estimation was defated using hexane. Estimation of protein from germinated seed was carried out by using 0.1M phosphate buffer. 500mg of the sample was grinded in 5 ml of buffer in mortal and pastle. Then sample was subjected to centrifugation at 10,000 rpm for 20 min and the supernatant was used as a protein sample for quantification and gel electrophoresis.
Estimation and quantification of protein concentration by Folins Lowry's method

Procedure

The working standard solution was pipette out in (0.2, 0.4, 0.6, 0.8, 1 ml) into a series of test tubes. Then 0.1 and 0.2ml of the sample extract was pipette out in two other test tubes. The volume in all test tubes was made to 1 ml with distilled water and a tube with 1ml was used as blank. Then 5 ml of alkaline copper reagent was added to including blank and allowed for 10 min. then fc reagent of 0.5ml was added to each test tube and mixed well. Test tubes were incubated at room temperature, in dark for 30 min and the optical density was measured at 660nm by using spectrophotometer. A standard graph was prepared from which amount of protein was calculated (Lowry *et al*, 1952).

Polyacrylamide gel electrophoresis of their extracted proteins

The separation of protein was carried out by using SDS PAGE. The extracted protein was used as a sample (Geok *et al*, 2012).
1. The glass plate and spacers was cleaned and dried thoroughly, then they was assembled properly and the assembly was held together with bulldog clip. The assembly was clamped in an upright position. White petroleum jelly of 2% agar (melted in boiling water bath) is then applied around the edges of the spacers to hold them in place and the chamber between the glass plates was sealed.

Table 3: A sufficient volume of separating gel mixture was prepared by mixing following

S. No.	Chemicals	For 15% gel	For 10% gel
1	Stock acryl amide	20 ml	13.3 ml
2	Tris-HCl (pH 8)	8 ml	8 ml
3	Water	11.4 ml	18.8 ml
4	Ammonium persulphate solution	0.2 ml	0.2 ml
5	SDS 10%	0.4 ml	0.4 ml
6	TEMED	20 ul	20 ul

3. above chemicals was mixed gently and carefully, the gel solution was poured in the chamber between the glass plates. Distilled water was layered on the top of the gel and left to set for 30-60 min.

Table 4: The stacking gel (4%) was prepared by mixing the following solutions (total volume 10 mL).

S. No.	Chemicals	Volume
1	Stock acrylamide solution	1.35 ml
2	Tris-HCL(pH 6.8)	1 ml
3	Water	7.5 ml
4	Ammonium persulphate solution	50 ul
5	SDS 10%	0.1ul
6	TEMED	10 ul

Result and discussion

This study was designated to evaluate the changes in protein content of different varieties of *Cajanus cajan* seed germinated under various concentration of NaCl.

Effect of varieties on protein content of germinated seeds:

The treatment V_3 (BSMR-736) was recorded with highest protein content i.e 1.96 mg/ml and was significantly superior over rest of all. The treatment V_1 (BSMR-853) was at par with V_2 (BDN-711) and was significantly superior over V_4 (BDN-708). Treatment V_2 was at par with V_4.

Effect of Sodium Chloride on protein content of germinated seeds

The treatment N_2 (100mM NaCl) was recorded with highest protein content i.e 2.04 and was significantly superior over rest of all. The treatment N_1 (50mM NaCl) and N_3 (150mM NaCl) and N_0 was at par and significantly superior N_4 (200mM).

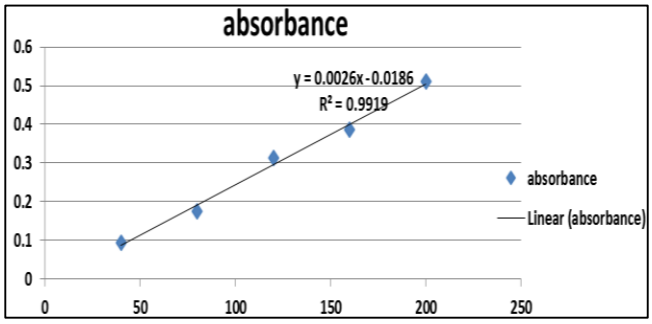


Fig 1: Standard graph of BSA for protein estimation

Table 5: Effect of Varieties combine with NaCl concentrations on protein content (mg/ml) of *Cajanus cajan*

Factor B\ Factor A	No (D/W)	N_1 (50mM)	N_2 (100mM)	N_3 (150mM)	N_4 (200mM)	Means
V_1 (BSMR-853)	1.29	1.62	2.19	1.02	0.84	1.39
V_2 (BDN-711)	1.11	1.75	2.01	1.05	0.86	1.35
V_3 (BSMR-736)	1.16	1.67	2.49	3.38	1.14	1.96
V_4 (BDN-708)	1.02	1.26	1.47	0.75	0.70	1.04
	1.14	1.57	2.04	1.55	0.88	GM=5.7
Factor A	SE= 0.129			CD= 0.389		
Factor B	SE= 0.145			CD= 0.435		
Factor A x Factor B	SE= 0.0.290			CD= 0.871		

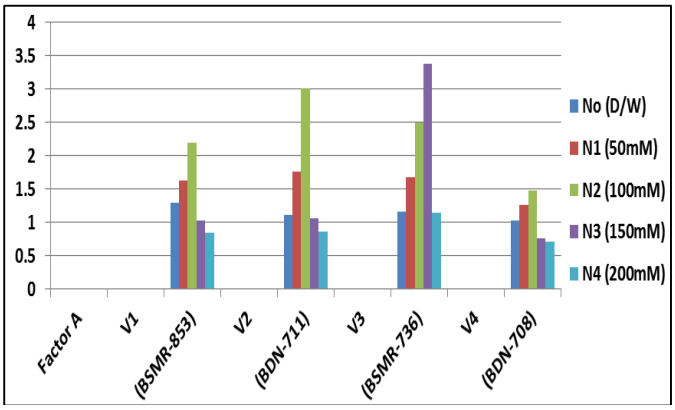


Fig 2: Influence of salt stress on amount of protein of different varieties of *Cajanus cajan*

Effect of interaction of Varieties with sodium chloride on protein content of germinated seeds

Data represented in above table reveal the amount of protein in different varieties *Cajanus cajan* was influenced significantly in response to salt stress. The treatment N_3V_3 (150mM NaCl + BSMR-736) was recorded with higher protein concentration i.e 3.338 mg/ml and significantly superior over rest of all. The treatments N_2V_2, N_2V_3, N_4V_3, N_2V_1, N_1V_3 were at par and significantly superior over rest of all except N_3V_3. The treatments N_4V_3, N_1V_2, N_1V_3, N_1V_1 and N_2V_4 were at par. The treatments N_3V_2, N_0V_2, N_3V_1, N_0V_4, N_4V_2, N_4V_1, N_3V_4 were at par and lowest protein content was recorded at N_4V_4 (D/W With 200mM NaCl) There was increase in protein content with increase sodium chloride this is due to expression of stress protein under salt stress. (Rangnakaylu *et al*, 2013) [11]

The Treatments with highest protein content along with their control was used for SDS PAGE analysis

Table 6: Effect of different concentration of Sodium chloride on protein profile of *Cajanus cajan*

Molecular weight of protein (Kda)	N_0V_1	N_2V_1	N_0V_2	N_2V_2	N_0V_3	N_3V_3	N_0V_4	N_2V_4
54		-	-	-	-	-	+	+
52	-	-	-	-	+	+		-
49	-	-	+	+	-	-	-	-
43	+	+	-	-	-	-	-	-
41	+	+	-	-	-	-	-	-
40	-	+	-	-	-	-	-	-
39	-	-	-	-	-	+	+	+
37	-	-	+	-	+	-	-	-
34	-	-	-	+	-	-	-	-
33	+	-	-	-	-	-	-	-
32.5	-	-	-	-	-	+	-	-
29	-	+	-	-	-	-	-	-
28.5	-	-	-	+	-	-	-	-
28	-	-	+	-	+	-	-	-
27	-	-	-	-	-	-	+	-
26	-	-	-	-	-	+	-	-
24	+	+	-	+	-	-	-	-

21.5	-	-	-	-	+	-	-	+
20	-	-	-	+	-	+	-	-
18	-	-	-	-	-	-	+	-
15.5	-	+	-	-	+	-	-	+
14	-	-	-	-	-	-	+	-

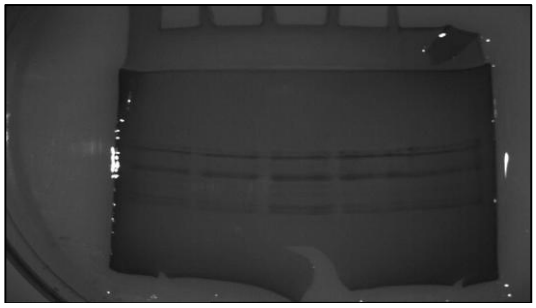
The protein profile of different varieties of *Cajanus cajan* under saline condition is contributed by various protein obtained in SDS PAGE analysis. In cultivar BSMR-853, total number of bands founds were four of of molecular weight 43 Kda, 41Kda, 33 Kda, 24 Kda and additional band of 40 Kda was found in N2V1 (BSMR-853 with 100mM NaCl).

In cultiar BDN-711, total number of bands found were four of molecular weight 49 Kda, 37 Kda, 28Kda, 20 Kda and additional band of 15.5 Kda also appeared in N2V2(BDN-711 with 100mM NaCl). In cultivar BSMR-736, total number of bands were five of molecular weight 52 Kda, 37 Kda, 28 Kda, 21.5 Kda, 15.5 Kda were found and additional band of 21.5 Kda also appeared in N3V3 (BSMR-736 with 150mM NaCl). In cultivar BDN-708, total number of band found were five of molecular weight 54 Kda, 39 Kda, 27Kda, 18 Kda, 14Kda and additional band of 15.5 Kda also appeared in N2V4 (BDN-708 with 100 mM NaCl) so the stress protein expressed under salt stress are15.5 Kda, 21.5Kda, 40 Kda.

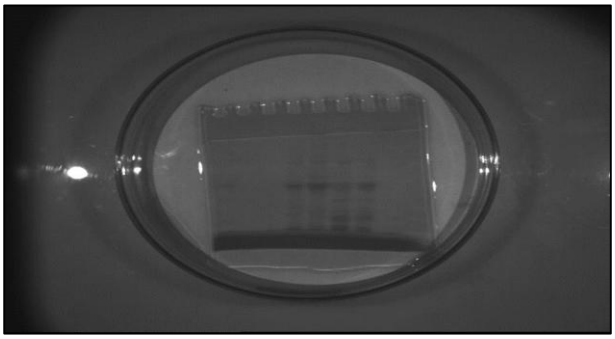
In pigeonpea biochemical parameters such as protein shown a decrease in salinity treated *Cajanas cajan* when compared with controlled plants. The decrease was more pronounced in 100mM NaCL salt stress treated plants than the 50mM NacL treated plants reported by Ramaiyapillai Mallika (2015). Similar findings in two pigeonpea genotypes under salt stress condition dusring seedling stage, radicle and plumule proteins were analysed by SDS PAGE reported by S.K. bishnoi *et al*. (2006) [13]

Above result shows that under different concentration of sodium chloride protein content was influenced due to expression of stress protein. Seeds under salt stress were utilizing their storage and all other form of protein to tolerate salt stress. The change in banding pattern and molecular weight of protein is due to expression of stress protein.

Plate 1: Quantification of extracted protein



2. A.) SDS PAGE analysis of varieties BSMR-853 and BDN-711



2. B.) SDS PAGE analysis of BSMR-736 and BDN-708

Outcome of the Research

Based on the finding from present investigation following outcome/Conclusions are drawn

1. Among the four cultivar of *Cajanus cajan* (BSMR-853, BDN-711, BSMR-736, BDN-708) BDN-711 can tolerate salt stress upto 150mM NaCl and hence it is more salt tolerant compared to rest of all
2. The highest protein content was recorded in BSMR-736 at 150mM sodium chloride hence can be used for cDNA libraries.
3. The increasing concentration of sodium chloride increases the amount of sugar in all four cultivar of *Cajanus cajan*.

Conclusion

The extracted protein was quantified by using Folin`s Lowry method. The seed of variety BSMR-736 germinated at 150mM concentration NaCl concentration showed highest amount of protein i.e 3.38 mg/ml of protein, whereas the seeds of variety BDN-708 at 200mM concentration showed lowest protein i.e 0.46mg/ml. The amount of sugar increases with increase in concentration of sodium chloride and increase in saline condition was found to influence molecular weight of the protein on SDS PAGE. Hence from above experiment it is concluded that increase in concentration of sodium chloride increases the amount of protein and amount of sugar and significant amount of variation exist in banding pattern of protein this might be due to expression of stress protein. (Mintoo *et al*, 2014) [9]. Future line of work 1. The extracted protein can be further purified by using different chromatography techniques.2. BDN-711 variety can be used by breeder to develope salt resistant variety. 3. The stress protein expressed under salt stress can be isolated, purified and sequenced

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