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Leaf proteome alterations in tolerant pearl millet (*Pennisetum glaucum* L.) genotype under water stress

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Abstract

Drought tolerant pearl millet genotype was used for differential physiological and proteomic analysis. The water stress was imposed on 20 days seedling up to five days. The physiological parameters *viz.* soil moisture content, relative water content (RWC), shoot length (cm) were studied from drought and control seedling after 25 days. The results showed significant changes on RWC and soil moisture content was decreased under water stress. Proteome analysis of 2D gel electrophoresis indicates around 1262 well resolved spots within the 4-7 pH and 10-110 kDa ranges. Image analysis revealed the presence of both, qualitative and quantitative changes between two treatments. The proteomic changes were observed in tolerance genotype J-2340 resulted total 84 spots protein (22.5-97.4 kDa, pH- 4.00 to 6.73) matches with control and water stress treatments. However, 32 proteins up regulated (29.0-97.4 kDa, pH 4.20-7.00) and eight down regulated (57.9-97.4kDa, pH 4.00 to 6.68) were observed after imposing water stress.

Highlights

- The leaf proteome analysis in tolerant pearl millet J-2340 evident 1262 protein spots of which 32 up regulated and 8 down regulated proteins under water stress.

Keywords: Pearl millet, Relative water content, Water stress, 2D gel electrophoresis

Pearl millet is known to be susceptible to drought particularly at the seedling stage; however, unfavourable soil water conditions at the beginning of plant growth may also dramatically limit the biomass production and the photosynthetic ability of leaves and thus indirectly negatively affect the formation of reproductive organs and yield parameters. The most rapidly developing symptom of water stress in plants is a cessation of cell expansion caused by a decrease of turgor. Decrease of transpiration caused by partial or complete stomatal closure is associated with changes in both leaf water status and soil moisture content (Benesova *et al.* 2012). Abiotic stresses usually cause protein dysfunction (Kamal *et al.* 2010).

It is convenient to use a combination of biochemical and physiological measurements of stress response-relevant parameters and to monitor the qualitative and quantitative changes in the composition of proteins which represent the executive component of the protective response for study the mechanism of the plant stress response. Proline increased proportionately faster than any other amino acid in plants under water deficit stress conditions and

suggested that it is an evaluating parameter for selecting drought tolerance (Bates *et al.* 1973).

The aim of present study was to know response of pearl millet under mild or severe water deficiency at the early developmental stages. Pearl millet genotype J-2340 was chosen based on its tolerant capacity to water stress. Aconsolidated study on changes in physiological, biochemical and protein profile by 2D GE was carried out in present investigation to understand drought tolerance mechanism.

Materials and Methods

Experimental Materials

Drought tolerant pearl millet J-2340 genotype was procured by pearl millet research station, Jamnagar, Junagadh Agricultural University, Junagadh, Gujarat, India.

Experimental details

Experiment was conducted in summer season. Pearl millet seeds had sown in 2 kg polytheen plastic bag under small greenhouse and polytheen bag was filled with equal weight of soil mixture of sand, warmi compost and FYM in ratio of 40: 40: 20 respectively and 25 to 30 seeds sown per polytheen bag with three replication of one genotype to comparative study with control and drought stress (or water withhold).

Table 1: Experimental materials and weather information.

Soil pH, EC and maximum water holding capacity (MWHC %)				
	Average ± S D			
Soil - pH	7.39 ± 0.02			
Soil – EC	1.20 ± 0.01 (ms)			
MWHC (%)	30.15 ± 0.29			
Water pH and EC				
	Average ± SD			
Water – pH	7.16 ± 0.15			
Water – EC	0.42 ± 0.01 (ms)			
Whether or environmental condition				
	Maximum Temp. (°C)	**Minimum Temp. (°C)**	**Relative Humidity (%)**	
	(Day)	**(Night)**	**(Day)**	**(Night)**
Average	35-39	26 - 28	85 - 88	45 - 50

Soil mixture had pH - 7.39 ± 0.02 and EC - 1.20 ms ± 0.01. Maximum water holding capacity of soil mixture was 30.15 ± 0.29 %. Water had an average pH - 7.16 ± 0.15 and EC - 0.42 ± 0.01 ms. Water used for irrigation. Experiment was conducted in green house with maximum temperature (35°C - 39°C) in day and minimum temperature (26°C - 28°C) in night. The relative humidity was lies between 85% - 88% in day and 45% - 50% in night (Table 1).

About 10 to 15 seedlings were maintained by thinning af er 10 days of sowing. Regular irrigation was applied on alternate days up to 20 days. Af er 20 days water withhold for 5 days or drought stress and thus, leaf of 25days old seedling was used for analysis. Bulk leaf samples from 10 seedlings were collected in each treatment for analysis in duplicate. Physiological parameters were analyzed in three independent replications.

Physiological analysis

Soil moisture content (%)

Soil samples were collected from the depth of 5 and 10 cm in the soil moisture boxes. These boxes were weighed using digital weighing machine and their initial weights were noted down. The samples were brought to the laboratory and put in the oven for 24 hours at 105°C. Once the oven drying was complete the samples were weighed again and their weights were noted down. These are the weights af er oven drying. Af er oven drying, the empty weights of soil moisture boxes were measured (Shukla *et al.* 2014; Black, 1965; Kakumanu *et al.* 2012).

Relative water content (%)

The pre-weighed leaf samples of pearl millet were transferred in Petri dishes filled with at least 15-20 ml distilled water so that leaves remain submerged for minimum one hour. Then the leaves were taken out, dried by blot ing paper and weighed i.e. turgid weight. Af er that, turgid leaf samples were kept in oven at 80°C for 5 hours and weighted until constant weight was obtained. The RWC was estimated as per formula and expressed as per cent relative water

content, using method described by Smart and Bingham (1974).

$$\text{Relative Water Content}(\text{RWC}\%) = \frac{\text{Fresh weight}(\text{g}) - \text{Dry weight}(\text{g.})}{\text{turgid weight}(\text{g.}) - \text{Dry weight}(\text{g.})} \times 100$$

Shoot length (cm)

Shoot length of 29 days old seedling of control and water stress plants was measured according to the methods described by Jajarmi, (2009) and Kocheva *et al.* (2010). All the parameters were taken in three replications.

2D gel-electrophoresis of leaf protein

Fresh leaves of pearl millete (500 mg) were powdered in liquid nitrogen with a pre-cooled mortar and pestle. The powder was suspended in 500 µl rehydration buffer containing 8M Urea, 2% CHAPS, 40 mM DTT. Once it is completely homogenized, the volume was made up to 1.5 ml with buffer. The mixture was centrifuged at 12000 rpm for 30 min at 4°C. The supernatant was further treated with 10% v/v TCA in acetone at 4°C for overnight in order to precipitate protein. The precipitate was collected by centrifugation at 12000 rpm for 15 min at 4°C. The precipitated protein was washed with acetone to remove traces of TCA and finally acetone was removed by speed vacuum treatment. Precipitated protein was resuspended in sample solubilization buffer (SSB) (8 M urea, 4% w/v CHAPS, 40 mM w/v DTT and carrier 2% v/v ampholytes 4–7 NL, 24 cm) and stored at –80°C until further used (Damerval *et al.* 1986).

Rehydration of immobiline dry strips (IPG strip; GE Healthcare) was carried out employing an immobiline dry strip re-swelling Tray (GE Healthcare) according to manufactures instructions. IPG strips (pH 4–7 NL), 24 cm long, was used for the present study. Sample was centrifuged at 12000 rpm for 5 min and insoluble fraction was discarded. The Immobiline dry strips were allowed to rehydrate with the samples in 8 M urea, 2%w/v CHAPS, 2%IPG buffer v/v (GE Healthcare), traces of bromophenol blue and 40 mM DTT/2.5 mL of rehydration solution at 28°C for 16 h. Final sample load per strip was approximately 400 µg for 24 cm strip. The protein concentrations were measured by Lawry's reagent. The rehydrated strips were then subjected to IEF. IEF was performed using a Et an IPG Phore 3 electrophoresis unit at 20°C in gradient mode. IEF was performed using a Et an IPG Phore 3 electrophoresis unit at 20°C in gradient mode as follows:

Step	Mode	Voltage (V)	Time (Hour)
1	Step	200	1:00
2	Step	500	7:00
3	Step	1000	1:00
4	Gradient	8000	8:00
5	Step	8000	5:00
6	Step	5000	4:00

Briefly, 24 cm strips were focused at 0–200 V for 1.00 h, 200–500 V for 7.00 h and 8000 V for 8.00 h, with a total of 9 kVh accumulated. Af er focusing, the strips were stored at –80°C for later use. Prior to the second-dimensional SDS-PAGE, IPG strips were equilibrated for 15 min in equilibration solution containing 50mM Tris-HCl, pH8.8, 6M urea, 30%w/v glycerol, 2%w/v SDS and traces of bromophenol blue with 100 mg/10 mL w/v of DTT. A second equilibration was carried out for 15 min by adding iodoacetamide (250 mg/10 mL) instead of DTT in equilibration solution; 10 mL of equilibration solution was used for 24 cm strip. Second- dimensional vertical SDS-PAGE was performed using precast minigels (12% Tris-HCl), large gels (12% Tris-HCl), and gradient gels (4–20% Tris-HCl), all 1 mm in thickness (Bio-Rad). Electrophoresis was performed at constant current of 5 mA/gel for 20 min followed by 12 mA/gel for 1.5 h until the bromophenol band had exited the gel. Large gel 2-DEwas carried out in a Protean II xi system (Bio-Rad). Electrophoresis was performed at 16 mA/gel for 30 min followed by 24 mA/gel for 4 h and 40 min according to the manufacturer's instructions. Briefly, gels were stained with 0.2% Coomassie brilliant blue G 250 in methanol and acetic acid in ratio 8:2 respectively. The gels were distained in methanol, acetic acid and distilled water in ration 40:10:50 respectively (Nandkumar and

marten, 2002) and Spots were analyzed by Platinum Master sof ware (Kausar *et al.* 2012).

The relative mobility (Rm) of each band was measured in each zymogram for every sample tested (Eeswara and Peiris, 2001).

Results and Discussion

Pearl millet (*Pennisetum glaucum*) J-2340 drought tolerant genotype was used as experimental material which procured by pearl millet research station, Jamnagar, J. A. U., Junagadh. 100 seeds weight and seed germination percentage (%) were recorded in J-2340 genotype. The results showed that an average 100 seeds weight was 0.57 gm ± 0.02 and seed germination percentage was an average 70.67 ± 3.06 % in genotype J-2340 had (Table 2). Germination percentage was varied with seed storage condition, crop maturity, different varieties and genotypes.

Table 2: Changes in physiological parameters of control leaves (CL) and treated leaves (TL) of pearl millet genotype J-2340 under water stress

Shoot length (cm)		
Treatment	Control (CL)	50.84 ± 5.73
	Water stress (TL)	40.51 ± 1.96
Relative water content (%)		
Treatment	Control (CL)	85.95 ± 1.23
	Water stress (TL)	55.54 ± 0.73 ↓
Soil moisture content (%)		
Treatment	Control (CL)	45.12 ± 0.63
	Water stress (TL)	12.20 ± 0.65

Values after ± indicates standard deviation between replications

Physiological parameters

The shoot length was decreased under water stress condition compared to control plants (Table 2). Shoot length of control seedlings of well watered genotype J-2340 was 50.84 cm ± 5.73 which was higher than water stressed seedling (40.51 cm ± 1.96) (Figure 1). Leaves of control had an average RWC 85.95 % ± 1.23 while of leaves of water stress treatment had significantly decreased RWC (55.54 % ± 0.73). Soil moisture content of soil of control group had an average 45.12 ± 0.63 while soil moisture content of soil of water stress treatment was significantly decreased (12.20 % ± 0.65). The similar results were obtained in case of shoot length, RWC and soil moisture content, when compared with results of other researchers (Kakumanu *et al.* 2012; Mujtaba *et al*. 2007; Talame *et al.* 2007, Gupta and Soni 2015)

Leaf proteome analysis

Drought tolerant pearl millet genotype J-2340 was selected for the study of protein profiling. There were two treatments, viz control and water stress. The 25 days old leaves of both the groups were selected for the proteomic study.

As per principle of 2D gel electrophoresis, proteins were separated on the basis of their isoelectric point (pI) on the IPG strips (pH 4-7, 24 cm Non Linear) and in second dimension, these IPG strips were subjected to SDS PAGE separation where protein was separated based on their mass. 12% SDS PAGE was stained with CBB R-250.

Total 1262 spots were detected in sof ware analysis (Table 3). Out of 1262 spots, 575 spots were present in control leaves and 687 spots were found in water stressed leaves. Out of 1262 spots, 152 spots in control treatment and 212 spots in water stress treatment were found between pH 4 to 5 and molecular mass ranged from 24.6 KDa to 97.5 KDa and 25.2 KDa to 97.5 KDa, respectively. Out of 1262 spots, total 364 spots found between pH 4 to 5. In water stress treatment 54 spots were recorded unique and were not found in control treatment indicating up regulated in water stress treatment or down regulate in control treatment. Out of 1262 spots, total 482 spots found between pH 5 to pH 6. Out of which 234 spots observed in control treatment and 248 spots in water stress treatment with molecular mass ranged from 22.0 KDa to 107.9 KDa and 26.5 KDa to 97.4 KDa respectively. In water stress treatment, 17 spots found unique in control treatment indicating up regulated in water stress treatment. Out of 1262 spots, total 424 spots found between pH 6 to 7 in which 189 spots in control treatment with molecular masses between 21.8 KDa

Fig. 1. Effect of drought stress on 24 days old seedling of pearl millet J-2340 genotype **(A)** Control and drought stress seedling in 2 kg polytheen bag and **(B)** Shoot length deference in Control and drought stress seedling with reference of scale (1 ft).

to 105.2KDa and 227 spots in water stress treatment with molecular masses range from 25.3 KDa to 97.4 KDa. In water stress treatment, 38 spots obtained under pH range 6 to 7 are up regulated or down regulated due to water stress treatment (Table 3).

Table 3: Summary of protein spots obtained by 2D gel electrophoresis with PI group, MW range up/down regulated spots of control and treated leaves of tolerant genotype J-2340 of pearl millet.

	Control Leaves	Treated Leaves (Water stress)	Total No. of Spots	Up regulated Spots (TL)	Down regulated Spots (CL)
PI (4-5)	152	212	364	54	54
Mole.Wt (KDa)	24.6-97.5	25.2-97.5	-	-	-
PI (5-6)	234	248	482	17	17
Mole.Wt (KDa)	22.0-107.9	26.5-97.4	-	-	-
PI (6-7)	189	227	424	38	38
Mole.Wt (KDa)	21.8-105.2	25.3-97.4	-	-	-
Total Spots	575	687	1262	112	112

In protein profiling, maximum (482) spots were found between pH 5 to 6 and minimum (364) spots were found between pH 4 to 5. But highest number of up regulated spots was found between pH 4 to 5. The maximum drought responsive protein spots were lies near acidic pH range.

Table 4. Match ID spots found in 2DE-Gel analysis of pearl millet genotype J-2340.

Sr. No.	Match ID	Control Leaves			Water stress Leaves			Coeffi-cient Variation
		% Volume	M.W. (KDa)	PI	% Volume	M.W. (KDal)	PI	
1	0	0.167	29.1	5.58	0.153	29.1	6.18	0.044
2	1	0.536	29.0	5.14	0.103	29.0	5.68	0.677
3	2	0.581	29.0	5.38	0.780	29.0	6.04	0.146
4	3	0.303	29.0	5.22	0.290	29.0	5.84	0.022
5	4	0.163	29.2	5.39	0.063	29.2	6.00	0.442
6	5	0.103	29.3	5.50	0.037	29.3	6.06	0.466
7	6	0.086	29.0	5.27	0.170	29.0	5.81	0.329
8	7	0.138	29.0	4.71	0.189	29.0	5.22	0.158
9	8	0.019	29.0	4.32	0.081	29.0	4.88	0.617
10	9	0.189	29.0	4.96	0.209	29.0	5.46	0.050
11	10	0.035	29.0	4.73	0.186	29.0	5.33	0.677
12	11	0.468	29.0	4.19	0.019	29.0	4.78	0.918
13	12	0.035	29.0	5.13	0.037	29.0	5.64	0.029
14	13	0.089	29.0	4.68	0.293	29.0	5.21	0.531
15	14	0.153	29.0	5.25	0.063	29.0	5.75	0.417
16	15	0.261	29.0	5.41	0.129	29.0	5.96	0.337
17	16	0.072	29.0	4.18	0.027	29.0	4.73	0.447
18	17	0.155	29.0	4.56	0.131	29.0	5.08	0.082
19	18	0.100	29.2	4.81	0.202	29.0	5.31	0.335
20	19	0.747	29.0	5.15	0.287	29.0	5.64	0.444
21	20	0.054	29.0	5.55	0.190	29.0	5.98	0.557
22	21	0.034	29.0	5.50	0.049	29.0	5.94	0.175
23	22	0.112	29.0	4.64	0.198	29.0	5.18	0.275
24	23	0.031	29.0	5.26	1.097	29.0	5.67	0.944
25	24	0.409	29.0	5.33	0.146	29.0	5.78	0.473
26	25	0.042	29.0	5.39	0.080	29.0	5.89	0.306
27	26	0.048	29.0	5.06	0.044	29.0	5.51	0.047
28	27	0.157	29.0	5.97	0.006	25.3	6.46	0.921
29	28	0.140	29.0	4.74	0.497	29.0	5.23	0.559
30	29	0.063	29.0	6.17	0.026	30.0	6.66	0.407
31	30	0.064	29.0	4.89	0.425	29.0	5.34	0.737
32	31	0.170	29.0	4.56	0.196	29.0	5.05	0.072
33	32	0.054	29.0	4.19	0.075	29.0	4.68	0.165
34	33	0.104	29.0	4.83	0.161	29.0	5.31	0.212
35	34	0.150	29.0	4.96	0.513	29.0	5.42	0.545
36	35	0.174	29.0	4.20	0.151	29.0	4.66	0.069
37	36	0.060	29.0	4.30	0.279	29.0	4.74	0.643
38	37	0.189	29.0	4.76	0.191	29.0	5.23	0.004
39	38	0.080	29.0	5.90	0.190	29.0	6.34	0.404
40	39	0.277	29.0	5.99	0.437	31.7	6.40	0.223
41	40	0.527	29.0	5.77	0.081	26.9	6.22	0.731
42	41	1.060	29.0	5.70	0.027	29.0	6.16	0.950
43	42	0.020	29.0	4.33	0.201	29.0	4.73	0.812
44	43	0.201	29.0	5.78	0.062	30.9	6.21	0.524
45	44	0.096	29.0	5.90	0.046	35.5	6.30	0.350
46	45	0.147	29.0	4.92	0.200	29.0	5.31	0.150
47	46	0.097	29.0	5.95	0.120	42.2	6.34	0.105
48	47	0.108	29.0	6.73	0.067	97.4	7.00	0.233
49	48	0.391	29.0	5.39	0.125	29.5	5.73	0.515
50	49	0.216	29.0	5.46	0.049	32.8	5.81	0.626

51	50	0.130	29.0	4.70	0.092	29.0	5.07	0.167
52	51	0.093	29.0	5.67	0.126	41.8	6.04	0.151
53	52	0.135	29.0	5.49	0.055	37.9	5.82	0.423
54	53	0.157	29.0	4.12	0.087	29.0	4.40	0.284
55	54	0.031	29.0	4.72	0.368	29.0	5.06	0.842
56	55	0.176	29.0	6.70	0.585	97.4	7.00	0.537
57	56	0.056	29.0	5.61	0.057	42.8	5.89	0.012
58	57	0.126	29.0	4.92	0.074	28.2	5.23	0.259
59	58	0.022	29.0	4.38	0.123	29.0	4.72	0.690
60	59	0.117	29.0	4.48	0.026	29.0	4.78	0.636
61	60	0.091	29.0	5.45	0.128	55.1	5.70	0.168
62	61	0.275	29.0	6.63	0.090	97.4	6.87	0.503
63	62	0.246	22.5	5.57	0.089	65.4	5.88	0.466
64	63	0.342	25.4	5.39	0.207	66.5	5.63	0.245
65	64	0.120	28.8	4.93	0.088	40.1	5.18	0.150
66	65	0.195	29.0	4.16	0.145	25.6	4.47	0.147
67	66	0.008	29.4	4.72	0.137	29.2	4.98	0.879
68	67	0.125	34.9	4.42	0.062	29.0	4.67	0.331
69	68	0.088	33.9	6.76	0.671	97.4	6.99	0.766
70	69	1.101	32.8	5.38	0.038	86.9	5.60	0.932
71	70	1.090	32.8	5.45	0.041	97.4	5.63	0.926
72	71	0.009	38.4	5.05	0.030	73.9	5.26	0.514
73	72	0.137	37.4	4.93	0.041	61.3	5.17	0.535
74	73	0.454	38.9	5.34	0.118	97.4	5.50	0.586
75	74	0.016	42.3	4.55	0.062	41.8	4.76	0.578
76	75	0.104	42.5	4.68	0.038	46.9	4.86	0.456
77	76	0.158	43.4	5.10	0.163	94.3	5.32	0.014
78	77	0.074	48.1	6.64	0.106	97.4	6.10	0.177
79	78	0.023	53.0	4.70	0.018	66.5	4.87	0.110
80	79	0.016	65.9	5.50	0.040	68.7	4.73	0.415
81	80	0.074	72.6	4.47	0.041	77.6	4.64	0.281
82	81	0.113	83.2	5.99	0.017	97.4	6.15	0.731
83	82	0.089	89.1	5.75	0.015	97.4	5.91	0.709
84	83	0.066	97.4	4.28	0.037	97.4	4.41	0.284

Eight four spots were matched between both the treatments. The match ID was given from 0 to 83 and Table 4 described % volume, molecular mass and PI of 84 spots. The different level of protein expression was expressed by the histogram of both the treatments (Figure 2). The molecular masses of 84 spots were identified with the range of 22.5 KDa to 97. 4 KDa with pH from 4.00 to 6.73. Among 84 matched spots, 14 protein spots (match ID number 20, 23, 24, 27, 34, 36, 38, 39, 40, 41, 54, 66, 69, 70 in Table 4) were significantly differentiated with expression level between two treatment groups and 2D gel photograph was shown in (Figure 3).

Table 5: Analysis of individual spot ID found unique in control leaves of pearl millet J-2340 genotype.

CONTROL LEAVES						
Spot ID	Spot Intensity	Area (µV. Sec^{-1})	Volume (µV.Sec^{-1})	% Volume	M. W. (KDa)	PI
A-6304	1521	37.76	22807.6	0.171	57.9	4.16
B-6282	1981	31.44	13558.6	0.101	73.6	4.75
C-6280	708	19.4	6327.36	0.047	75.1	4.87
D-6255	3074	53.6	53051.4	0.397	90.3	5.78
E-6246	5243	13.12	5946.72	0.044	97.4	6.68
F-6196	4280	9.68	6001.88	0.045	97.4	4.06
G-6176	4624	2.56	1002.16	0.007	97.4	5.24
H-6160	5773	4.56	3418.52	0.025	97.4	5.24

Many spots were found to be significant at different level of expression in both the treatment but among eleven spots (Spot ID number A(6304), B(6282), C(6280), D(6255), E(6246), F(6196), G(6176) and H(6160) were down regulated in water stress treatment which are indicated in Table 5. The molecular mass of these 11 protein spots were identified with the range of 57.9 KDa to 97.4 KDa with pH from 4.06 to 6.68. However 32 protein spots (Spot ID number A1(7547), A2(7038), B1(7545), B2(7032), C1(7509), C2(7011), D1(7445), D2(6975), E1(7432), E2(6970), G1(7366), G2(6967), H1(7357), H2(6938), I1(7349), I2(6932), J1(7339), K1(7313), K2(6896), M1(7293), M2(6893), N1(7213), O1(7196), P1(7193), Q1(7186), R1(7171), S1(7155), T1(7150), U1(7130), V1(7128), W1(7115) and Y1(7104) were up regulated in water stress treatment (Table 6) and the molecular mass of these 32 spot were identified with the range of 29 kDa to 97.4kDa and pH range from 4.20 to 7.00. Similarly, Rollins *et al.* (2013) studied leaf proteome alterations to drought and heat tolerance in barley in the content of physiological and morphological responses. Slibinskas *et al.* (2013) examined the

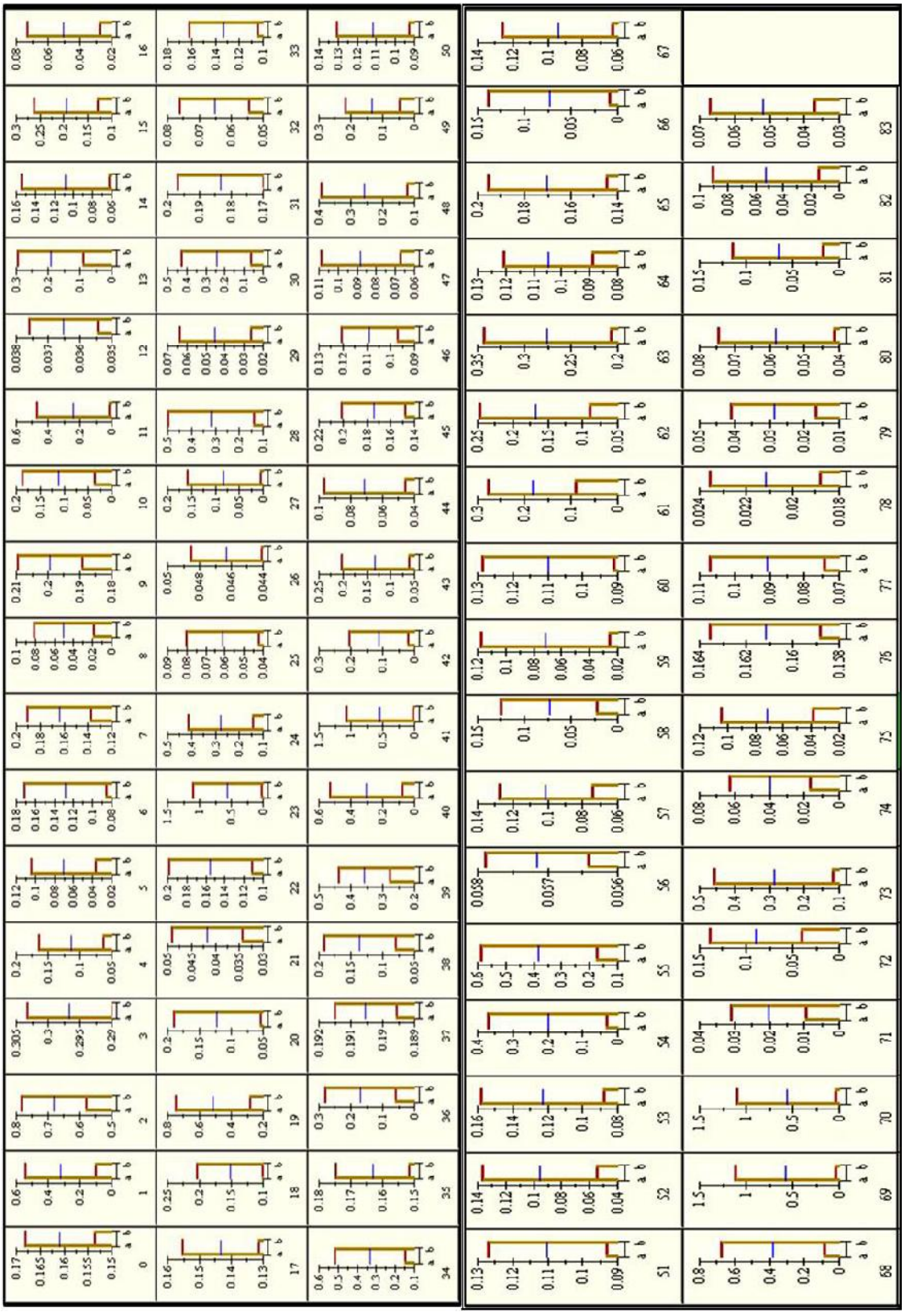

Fig. 2. Comparative Histogram of Match ID spots, **(a)** Control Leaves and **(b)** Drought stress Leaves of pearl millet **J-**2340 genotype.

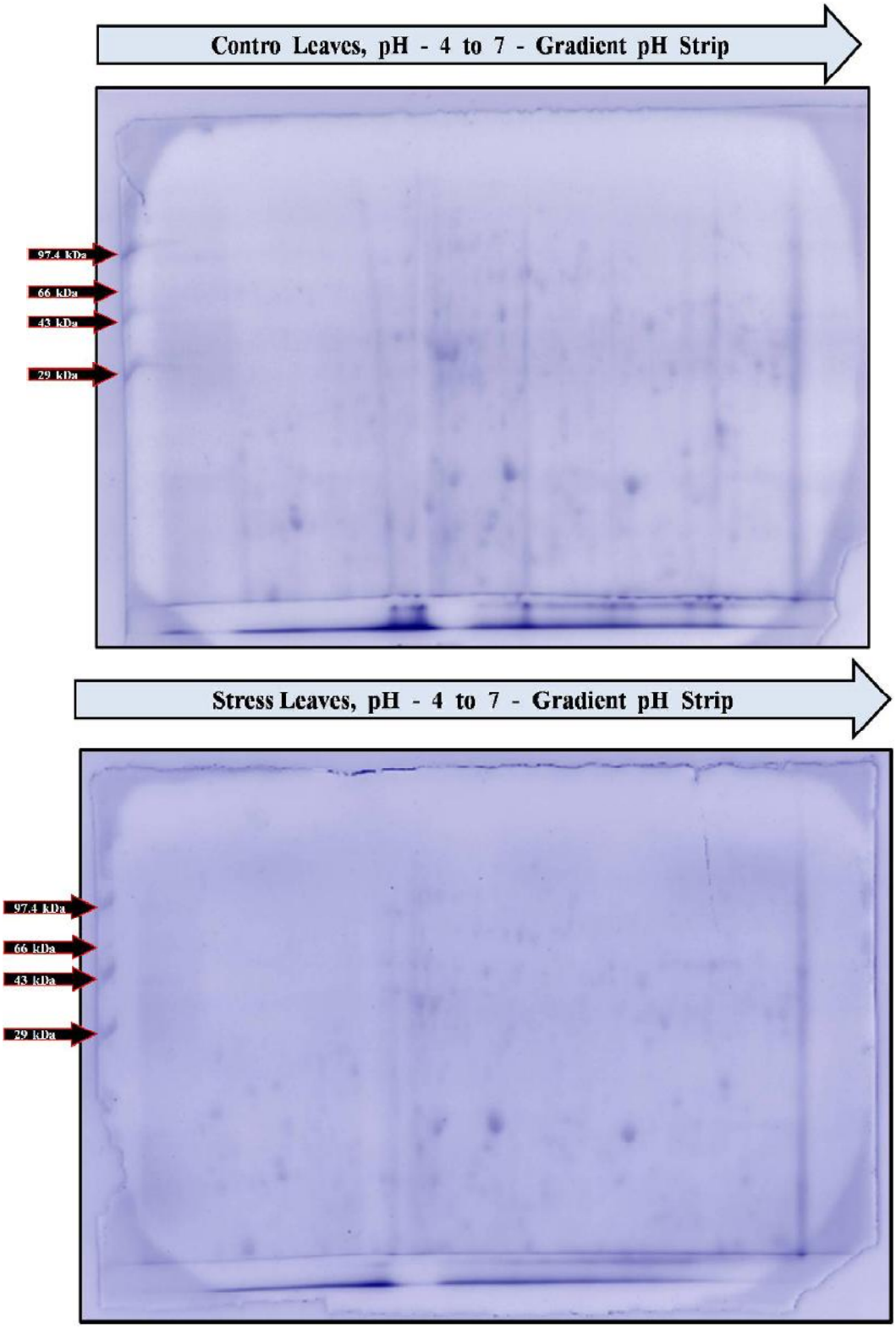

Fig. 3. 2D second dimension 12% Polyacrylamide gel electrophoresis by stained using CBB-R-250 of control leaves and Drought stress leaves of pearl millet J-2340 genotype.

comparison of first dimension IPG and NEPHGE techniques in two-dimensional gel electrophoresis experiment with cytosolic unfolded protein response in Saccharomyces cerevisiae. The down regulated and up regulated protein spots between same pH ranges were studied by Slibinskas *et al.* (2013) who examined the comparison of first dimension IPG and NEPHGE techniques in two-dimensional gel electrophoresis experiment with cytosolic unfolded protein response in Saccharomyces cerevisiae. Sumathi and Balamurugan (2013) examined seed protein profiling to discriminate oat cultivars based on number, intensity and specific presence or absence of bands.

Table 6. Analysis of individual spot ID found unique in water stress leaves of pearl millet J-2340 genotype.

WATER STRESS LEAVES						
Spot ID	Spot Intensity	Area (µV. Sec^{-1})	Volume (µV.Sec^{-1})	% Volume	M. W. (KDa)	PI
A1-7547	10921	25.56	63306.6	0.455	29.0	5.22
A2-7038	1171	9.12	3698.8	0.026	97.4	4.49
B1-7545	16227	40.52	140268	1.009	29.0	7.00
B2-7032	4943	4	1876	0.013	97.4	6.80
C1-7509	16350	1.44	4644.88	0.033	29.0	5.03
C2-7011	5434	4.24	3102.92	0.022	97.4	6.61
D1-7445	2862	58.04	35712.2	0.256	29.0	4.49
D2-6975	20277	1.28	4028.32	0.028	97.4	5.22
E1-7432	4620	9.44	5940.32	0.042	29.0	4.20
E2-6970	1785	8.52	3791.72	0.027	97.4	5.67
G2-6967	2493	3.84	1920.84	0.013	97.4	4.65
G1-7366	1493	13.44	4438.19	0.031	29.0	4.96
H1-7357	2396	4.72	2788.52	0.020	29.0	4.40
H2-6938	2219	12.16	5975.24	0.042	97.4	4.65
I1-7349	5622	44.6	99509.6	0.715	29.0	6.25
I2-6932	2041	88.92	44569.2	0.320	97.4	5.83
J1-7339	4106	4.92	1994.56	0.014	29.0	4.31
K1-7313	1923	26.72	19291.7	0.138	29.0	6.46
K2-6896	1758	5.12	1510.52	0.010	97.4	5.86
M1-7293	2902	34.48	39673.3	0.285	29.0	6.66
M2-6893	1517	3.6	1173.12	0.008	97.4	6.72
N1-7213	761	20	7668.2	0.055	32.5	5.97
O1-7196	2148	52.88	42263.5	0.304	37.0	6.85
P1-7193	5199	3.88	2541.32	0.018	40.5	5.14
Q1-7186	1669	12.56	4890.21	0.035	42.2	4.66
R1-7171	2805	50.68	53583.1	0.385	40.5	5.41
S1-7155	4519	4.88	2285.44	0.016	52.9	5.85
T1-7150	5243	1.8	2110.64	0.015	54.7	6.69
U1-7130	2410	40.8	35775.5	0.257	57.4	6.31
V1-7128	1554	8.6	2879.72	0.020	67.0	4.95
W 1 - 7115	1580	22.08	12663	0.091	72.1	6.39
Y1-7104	2698	27	28195.7	0.202	73.9	6.61

Conclusion

Physiological parameters were differed significantly RWC and soil moisture content. The proteomic changes were observed in tolerant genotype J-2340. Coomassie staining of the gels allowed visualization of around 1262 well resolved spots within the 4-7 pH and 10–110 kDa ranges. Image analysis revealed the presence of both, qualitative and quantitative changes between two treatments. In plant, changes in a number of proteins during stress application have been observed, with different level of numbers in up-regulated protein spots compared with down-regulated ones throughout stress progression.

References

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil* **39**: 205-207

Benesova, M., Hola, D., Fischer, L., Jedelsky, P.L., Hnilicka, F., Wilhelmova, N., Rothova, O., Kocova, M., Prochazkova, D., Honnerova, J., Fridrichova, L. and Hnilickova, H. 2012. The physiology and proteomics of drought tolerance in maize: Early stomatal closure as a cause of lower tolerance to short-term dehydration?. *PLoS ONE* **7**(6): 1-17.

Black, C.A. 1965. Methods of Soil Analysis: Part I Physical and mineralogical properties. J Am Soc Agron.

Damerval, C., Devienne, D., Zivy, M. and Thiellement, H. 1986. Technical improvement in two dimensional electrophoresis increase the level of genetic variation detected in wheat seedling proteins. *Electrophoresis* **7**: 52-54.

Eeswara, J.P. and Peiris, B.C.N. 2001. Isoenzymes as a marker for identification of mungbean (*Vigna radiata*). *Seed Science and Technology* **29**: 249-254.

Jajarmi, V. 2009. Effect of Water Stress on Germination Indices in Seven Wheat Cultivar. World Acad Sci, *Eng Technol* **49**.

Kakumanu, A., Ambavaram, MMR, Klumas C, Krishnan A, Batlang U, Myers E, Grene R, Pereira A 2012. Effects of drought on gene expression in maize reproductive and leaf meristem tissue revealed by RNA-Seq. *Plant Physiology* **160**: 846-867.

Kamal, A.H.M., Kim, K.H., Shin, K.H., Choi, J.S., Baik, B.K., Tsujimoto, H., Heo, H.Y., Park, C.S. and Woo, S.H., 2010. Abiotic stress responsive proteins of wheat grain determined using proteomics technique. *Australian Journal of Crop Science* **4**(3): 196-208.

Kausar, R., Arshad, M., Shahzad, A. and Komatsu, S. 2012. Proteomics analysis of sensitive and tolerant barley genotypes under drought stress. *Amino Acids* **44**: 345-359.

Kocheva, K., Kartseva, T., Landjeva, S. and Georgiev, G. 2010. Parameters of cell membrane stability and levels of oxidative stress in leaves of wheat seedlings treated with PEG 6000. *General and Applied Plant Physiology* **35**(3-4): 127–133.

Mujtaba, M., Ali, M., Ashraf, M.Y., Khanzada, B., Farhan, S.M., Shirazi, M.U., Khan, M.A., Shereen, A. and Mumtaz, S. 2007. Physiological responses of wheat (Triticum aestivum L.) genotypes under water stress conditions at seedling stage. *Pakistan Journal of Botany* **39**(7): 2575-2579.

Raziuddin, Z., Swati, A., Bakht, J., Naqib, U., Shafi, M., Akmal, M. and Hassan, G. 2010. In situ assessment of morpho-physiological response of wheat (*Triticum aestivum* L.) genotypes to drought. *Pakistan Journal of Botany* **42**(5): 3183-3195.

Rollins, J.A., Habte, E., Templer, S.E., Colby, T., Schmidt, J. and Von Korff, M. 2013. Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (*Hordeum vulgare* L.). *Journal of Experimental Botany* **64**(11): 3201-3212.

Sarwar, M.K.S., Ullah, I., Urrahman, M., Ashraf, M.Y. and Zafar, Y. 2006. Glycinebetaine accumulation and its relation to yield and yield components in cot on genotypes grown under water deficit condition. *Pakistan Journal of Botany* **38**(5): 1449-1456.

Shukla, A., Panchal, H., Mishra, M., Patel, P.R., Srivastava, H.S., Patel, P. and Shukla, A.K. 2014. Soil Moisture Estimation using Gravimetric Technique and FDR Probe Technique: A Comparative Analysis. *American International Journal of Research in Formal, Applied and Natural Sciences* **8**(1): 89-92.

Slibinskas, R., Razanskas, R., Zinkeviciute, R. and Ciplys, E. 2013. Comparison of first dimension IPG and NEPHGE techniques in two-dimensional gel electrophoresis experiment with cytosolic unfolded protein response in Saccharomyces cerevisiae. *Proteome Science* **11**(36): 1-15.

Talame, V., Ozturk, N.Z., Bohnert, H.J. and Tuberosa, R. 2007. Barley transcript profiles under dehydration shock and drought stress treatments: a comparative analysis. *Journal of Experimental Botany* **58**(2): 229-240.

Sumathi and Balamurugan, 2013. Identification of oats (*Avena sativa* L.) cultivars by seed and seedling protein electrophoresis. *International Journal of Agriculture Environment and Biotechnology* **6**: 521-526.

Gupta, R. and Soni, S. 2015. Perchlorate uptake and its effect on physiological, biochemical and growth parameters of eucalyptusplant under ammonium perchlorate stress. *International Journal of Agriculture Environment and Biotechnology* **8**: 335-346.