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Differential organ specific protein profiling in chickpea cultivars under water deficit stress

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ABSTRACT

Chickpea is an important legume crop but its yield is highly affected by water deficit stress. It has been reported that many proteins related to stress/defence/detoxification, carbohydrate metabolism and photosynthesis are crucial for imparting water deficit stress tolerance to crops but the correlation between the expression of induced proteins and the level of stress tolerance has not been explored. In this study, we have reported proteomic changes in different tissues of two chickpea cultivars differing in drought tolerance capacity ICC4958 (drought tolerant) and ILC3279 (drought susceptible) at different developmental stages under field and at 7 days after germination (DAG) under laboratory conditions. An insight into the proteomic changes of these two chickpea cultivars revealed that average polypeptide expression under stress was up regulated and down regulated in underground system of ICC4958 and ILC3279, respectively, as compared to control which affected the protein expression in aboveground tissues. More pronounced increase in polypeptide expression in leaves of ICC4958 under stress was observed at 80 DAS, the stage corresponding to initiation of reproductive development while stress in leaves of ILC3279 resulted in decreased overall protein expression. This was ultimately reflected in enhanced protein expression under water deficit stress in mature seeds of ICC4958 as compared to ILC3279. The study under laboratory condition also revealed that protein expression under water deficit stress is increased on an average in ICC4958 and reduced in ILC3279.

Keywords: Chickpea, Protein profile, Water deficit stress

Water stress is a major constraint limiting grain legume production particularly in arid and semi-arid regions. Different climate models have predicted changes in rainfall distribution and frequent drought spells for the future (Farooq *et al.* 2017). Chickpea (*Cicer arietinum* L.), an annual grain legume or "winter pulse crop", is the second most produced pulse worldwide after dry beans (Garg *et al.* 2016) which restores and maintains the soil fertility by its nitrogen fixing capability and fits very well in various cropping patterns (Hameed *et al.* 2012). The crop is grown in arid and semi arid regions characterized with varying intensities and distribution of crop season rainfall from almost nil to >400 mm. As a consequence, terminal drought of varied intensities is a major limitation to chickpea productivity (Ramamoorthy *et al.* 2016). Therefore, the need of the hour is to develop drought tolerant cultivars for an increased productivity and for this understanding the contribution of various traits to drought tolerance is necessary. A lot of research on the role of various enzymatic and non enzymatic antioxidants along with compatible osmolytes and various physiological adaptations in imparting water deficit stress tolerance in chickpea has been reported till date. However, one major aspect of drought tolerance ability in chickpea that needs more attention is differential protein expression in various vegetative and reproductive tissues under control and water deficit stress.

Proteome of the plants is sensitive to environmental conditions as a wide array of abiotic stresses have been shown to cause both the up regulation and down regulation of protein expression; and induction and suppression of protein synthesis in plants (Sengupta *et al.* 2011). A study by Kreps *et al.* (2002) in arabidopsis showed that up to 30% of the transcriptome is responsive to stress and 1008 mRNAs were specifically up regulated by water deficit stress. Zhou *et al.* (2013) revealed that differentially affected proteins in drought tolerant and susceptible plants are directly linked to the molecular mechanisms that plants would use to develop tolerance to dehydration stress. Induction of new proteins synthesis can enhance the survival under adverse environmental situations by causing differential expression of genetic information, resulting in changes in gene products, including mRNAs and proteins (Saijo *et al* . 2000, Hayano-Kanashiro *et al.* 2009). These stress induced proteins might be associated with a variety of cellular functions, *i.e.* signal transduction, protein processing, protein folding, protein degradation, redox homeostasis, oxidative stress detoxification, cell wall modification, metabolisms of carbon, energy, lipid, lignin and flavonoid (Zheng *et al.* 2014; Hajheidari *et al.* 2005). Stress induced signal pathways can either deactivate an active protein isoform or can activate a silent protein/ enzyme isoform, either directly or by changing gene expression (Hu *et al.* 2012).

Compared to most other legumes, root system of chickpea is known to be well adapted for growing under receding soil moisture conditions and a large genetic diversity has been reported on the root biomass as well as rooting depth in chickpea. Therefore, we selected two chickpea cultivars differing in their rooting system - ICC4958 (deep rooted) and ILC3279 (shallow rooted) for studying the effect of water deficit stress on protein expression. In our earlier study, we have reported different biochemical and physiological adaptations contributing to better

efficiency of ICC4958 to combat water deficit stress as compared to ILC 3279 chickpea cultivar (Kaur *et al.* 2016). The aim of the present research was to investigate the effect of water deficit stress on the protein expression at different days after sowing (DAS) in underground (roots and nodules), above ground (leaves) vegetative and at different days after flowering (DAF) in reproductive (pod wall and seeds) tissues under field conditions and at 7 DAG (days after germination) in roots, shoots and cotyledons under laboratory conditions in ICC4958 and ILC3279 chickpea cultivars.

MATERIAL AND METHODS

Sowing and germination of chickpea cultivars: Water stress tolerant (ICC4958) and susceptible (ILC3279) chickpea cultivar were sown in Randomised Block Design in the experimental fields of Plant Breeding and Genetics in four equal sized plots. Crop was irrigated upto 65 days after sowing (DAS) and at 70 DAS water deficit stress was created on two plots by withholding irrigation and using rain-out shelter. The plots that received irrigation were termed as control while there was continuous depletion of moisture content in water deficit stress plots under rainout shelter as they were neither irrigated nor received any rainfall. The control and water deficit plots were separated by wide path and black polythene sheet was inserted deep in the middle of the path to prevent horizontal leaching of water from control to stressed plots. Electrophoretic analysis of total proteins was performed in roots, leaves and nodules at different days after sowing (DAS). Uniformly developed flowers were tagged and proteomic analysis was carried out in pod wall and developing seeds at different days after flowering (DAF) till maturity with 7 days interval.

For studying the proteomic analysis of these two chickpea cultivars under laboratory conditions, these cultivars were germinated under control and water deficit stress conditions (3% mannitol) on agar based media. The seeds of these cultivars were washed with water, surface sterilized with 0.1 % $HgCl_2$ and again washed thoroughly with distilled water under aseptic conditions. The seeds were then germinated aseptically in 250 ml conical flasks on 0.8 % agar. The flasks were then kept in an incubator at 25 ± 1 ^oC in darkness for 7 days.

Extraction of proteins: Proteins from different tissues (100 mg) were extracted with 2 ml of 20 mM sodium phosphate buffer (pH 7.5) containing 0.5% NaCl and a pinch of PVP. The extract was centrifuged at 10,000×g for 25 minutes and the pellet was discarded. The supernatant was used for protein estimation by the method of Lowry *et al.* (1951). A part of the supernatant having equal amount of total protein was used for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE Electrophoresis: Supernatant samples with equal amount of protein (100 ìg) were mixed with equal volumes of solubilizing buffer (0.5M Tris-HCl, pH 6.8, glycerol, 10% (w/v) SDS, 2-mercaptoethanol and 0.5% bromophenol blue) and heated for 4 min at 95°C and then it cooled on ice. Polypeptide pattern was analyzed on 12% SDS polyacrylamide gels according to the method of Laemmli (1970). After completion of the electrophoresis, the resolving proteins were prefixed by keeping the gel for 2 hour in 12.5% trichloroacetic acid followed by immersing the gel in staining solution (0.1 g Comassie blue, 100 ml of methanol, 20 ml of acetic acid and 80 ml of distilled water). Then, destaining was done by immersing in a mixture of methanol: acetic acid: distilled water (125:35:340). Protein molecular weight marker was used to analyse polypeptide bands by Gel-Doc (Bio Rad).

RESULTS AND DISCUSSION

Water deficit stress leads to quantitative and qualitative changes in the synthesis of polypeptides in plants by causing tissue and organ specific differential genomic expression (Oishi and Bewley 1992). In the present study, band intensities in root protein profile indicate that expression of 30.42 kDa (R_m = 0.63) and 19.36 kDa (R_m = 0.84) polypeptide was up regulated in roots of both the cultivars at 85 DAS with more prominent increase was observed in 19.36 k Da band in ICC4958 (Table 1). At 100 DAS, the expression of these two polypeptides was inhibited in roots of ICC4958, while expression of 30.42 k Da polypeptide was inhibited and that of 19.36 k Da polypeptide was reduced from 2.7 to 1.8% in roots of ILC3279 (Table 1). During water stress, much of plant's metabolism is diverted to synthesis of stress specific protective proteins, known as induced proteins. These proteins are responsible for various functions, thus, affecting multiple cellular functional pathways (Kakaei *et al.* 2010). In nodules of ICC4958, water deficit stress resulted in induction of 19.06 k Da (R_m = 0.85) polypeptide in nodules of ICC4958; 69.99 (R_m=0.25) and 50 .11 (R_m= 0.40) kDa polypeptide in nodules of ILC3279, and inhibition of 35.37 k Da polypeptide in nodules of ICC4958 at 80 DAS as compared to control (Table 1). Moreover relative percentage of 93.05 (4.1%), 69.99 (18.4%), 50.11 (12.0%) and 30.15 (11.0%) k Da polypeptides was increased in nodules of ICC4958 as compared to control 3.1, 2.2, 1.8 and 0.8% respectively and that of 30.15 was decreased from 16.0 to 6.8% in nodules of ILC3279. Thus, stress increased the percentage distribution of proteins in nodules of ICC4958 as compared to that of ILC3279 that might have contributed significantly in nodules of ICC4958 to strengthen the defence system to withstand more intense stress condition.100 DAS in chickpea is a critical period for reproductive development when proper water and nitrogen supply is essential for pod wall and seed establishment. Water deficit stress at this period induced 107.77 (R_m=0.05), 93.05 kDa (R_m=0.11) and 30.15 kDa (R_m=0.64) polypeptide in nodules of both the cultivars; and inhibited 35.37 kDa

Table 1. Relative percent distribution of total proteins in roots and nodules of chickpea cultivars (ICC4958 and ILC3279) under control and water deficit stress conditions at different DAS

Band no	Relative mobility	Molecular wt (kDa)	Per cent distribution of proteins in roots							
			85 DAS				100 DAS			
			1	2	1'	2'	1	2	1'	2'
1	0.11	92.55	0.5	0.4	0.5	0.5	0.8	0.7	0.6	0.9
2	0.63	30.42	1.6	2.3	2.3	3.1	10.8	13.4	0.0	0.0
3	0.84	19.36	0.8	0.9	7.5	2.4	1.5	2.7	0.0	1.8

Band no	Relative mobility	Molecular wt (kDa)	Per cent distribution of proteins in nodules											
			80 DAS				100 DAS				120 DAS			
			1	2	1'	2'	1	2	1'	2'	1	2	1'	2'
1	0.05	107.77	0.0	0.0	0.0	0.0	0.0	0.0	2.8	3.0	0.0	6.3	1.3	0.0
2	0.11	93.05	3.1	3.5	4.1	0.0	0.0	0.0	10.5	6.1	0.0	0.0	0.0	0.0
3	0.25	69.99	2.2	0.0	18.4	21.0	26.8	2.7	12.7	4.2	11.1	5.1	2.4	3.2
4	0.40	50.11	1.8	0.0	12.0	2.8	1.5	14.6	3.3	4.9	5.5	19.5	22.2	1.6
5	0.56	35.37	2.1	0.0	0.0	0.0	1.4	1.6	0.0	0.0	1.4	0.0	2.2	1.2
6	0.64	30.15	0.8	16.0	11.0	6.8	0.0	0.0	12.7	1.0	0.0	1.8	0.0	1.4
7	0.75	23.62	1.9	1.0	1.6	0.7	0.8	1.6	2.1	1.9	0.0	0.9	0.0	5.1
8	0.85	19.06	0.0	2.1	1.7	1.4	1.2	0.0	0.0	0.0	1.2	2.5	0.0	0.0

1,2,1'and 2' represent ICC4958 (control), ILC3279 (control), ICC4958 (water deficit stress) and ILC3279 (water deficit stress) respectively. The values represent the mean of three electrophoretic gel documentation.

(R_m = 0.56) polypeptide in both the cultivars; and 19.06 kDa polypeptide (R_m = 0.85) in nodules of ICC4958 (Table 1). At this stage, although relative percentage of 69.99 kDa polypeptide in nodules of ICC 4958, was reduced from 26.8% to 12.7% and was increased in nodules of ILC3279 from 2.7 to 4.2%, it was still much more than ILC3279 under stress conditions. Near maturity (i.e. at 120 DAS), water deficit stress resulted in induction of 107.77 kDa polypeptide in ICC4958, 35.37 kDa polypeptide in ILC3279 and inhibition of 19.06 kDa polypeptide in nodules of both the cultivars. Moreover, relative distribution of 69.99 kDa polypeptide was reduced from 11.1% to 2.4 % in nodules of ICC4958 and from 5.1 to 3.2 % in nodules of ILC3279. Relative percent distribution of 50.11 kDa polypeptide was increased from 5.5 to 22.2% in nodules of ICC4958 and reduced from 19.5 to 1.6 % in nodules of ILC3279. These results indicate that on an average polypeptide expression under stress was up regulated and down regulated in underground system of ICC4958 and ILC3279, respectively, as compared to control.

Variable polypeptide expression in roots and nodules affected the polypeptide expression in leaves of both the cultivars. Time of expression of certain proteins is also altered under stressed conditions because mobilization of certain polypeptides is slowed down (Samarah and Mullen 2006). In our study, initiation of stress induced 95.48 (R_m = 0.10), 45.47 (R_m = 0.45) and 39.82 (R_m = 0.51) kDa polypeptides and inhibited the expression of 32.21 polypeptide with R_m value 0.61 in leaves of ICC4958 while in leaves of ILC3279, initial stress induced the expression of 95.48 and 23.52 kDa (R_m = 0.75) polypeptides and inhibited the expression of 39.82 and 28.56 kDa polypeptides (Table 2). Thus, overall effect of initial application of stress was induction of new polypeptides in leaves of ICC4958 and decreased polypeptide expression in leaves of ILC3279. At 100 DAS, water deficit stress lead to induction of 32.21 (R_m = 0.61) kDa polypeptide in leaves of ICC4958 (Table 2). These newly expressed polypeptides in leaves might include proteins involved in photosynthesis, redox regulation, oxidative stress, signal transduction, and chaperone activities (Hajheidari *et al.* 2005). Severe stress (i.e. at 120 DAS) lead to induction of 39.82 and 28.56 kDa polypeptides and inhibition of 36.09 kDa polypeptide in leaves of ICC4958

Table 2. Relative percent distribution of total proteins in leaves of chickpea cultivars (ICC4958 and ILC3279) under control and water deficit stress conditions at different DAS

Band no	Relative mobility	Molecular wt (kDa)	Per cent distribution of proteins											
			80 DAS				100 DAS				120 DAS			
			1	2	1'	2'	1	2	1'	2'	1	2	1'	2'
1	0.10	95.48	0.0	0.0	1.6	1.6	2.4	2.3	1.3	2.7	0.7	1.7	2.9	1.7
2	0.45	45.47	0.0	1.2	2.1	2.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	4.4
3	0.51	39.82	0.0	2.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	0.0
4	0.54	36.09	0.0	1.9	0.0	1.0	0.5	0.3	0.5	0.5	0.6	0.9	0.0	0.8
5	0.61	32.21	1.3	1.6	0.0	0.0	0.0	1.2	0.5	0.7	0.8	1.1	1.8	0.0
6	0.66	28.56	2.7	1.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0
7	0.75	23.52	0.0	0.0	0.0	1.0	2.1	0.7	2.4	1.4	1.3	1.9	1.4	3.3
8	0.82	20.78	1.3	1.2	2.0	1.5	0.9	0.8	1.4	1.1	1.1	1.8	1.7	1.9

1, 2, 1' and 2' represent ICC4958 (control), ILC3279 (control), ICC4958 (water deficit stress) and ILC3279 (water deficit stress) respectively. The values represent the mean of three electropheretic gel documentation.

while in leaves of ILC3279, stress induced the expression of 45.47 kDa polypeptide; and inhibited the expression of 32.21 kDa polypeptide (Table 2). Moreover, at this stage, stress resulted in increased relative percent distribution of 95.48 kDa polypeptide from 0.7 to 2.9%, 32.21 kDa polypeptide from 0.8 to 1.8% and 19.56 kDa polypeptide from 1.1 to 1.7% in leaves of ICC4958 and 23.52 kDa polypeptide from 1.9 to 3.3% in leaves of ILC3279. These results indicated that more pronounced increase in polypeptide expression in ICC4958 under stress was observed at 80 DAS as compared to 100 or 120 DAS, which might have helped the tissue to increase photosynthesis and fortify its defence system during initial stress stage only, and thus, with the progression of tissue development and stress continuation, stress adapted leaves did not require much alteration in polypeptide profile. On the other hand, stress in leaves of ILC3279 resulted in decreased overall protein expression which might have affected photosynthesis and defence system of tissue.

Based on the antioxidant response of chickpea, Raheleh *et al* . (2012) reported that the flowering and podding are more suitable stages for investigating tolerance to drought stress in chickpea. Stress in pod wall of ICC4958 at early developmental stages (i.e. at 7 and 14 DAF) resulted in induction of 77.98 (R_m = 0.19), 55.05 (R_m = 0.35), 34.70 (R_m = 0.56) and 30.86 (R_m = 0.57) kDa polypeptides and repressed the expression of 91.20 kDa (R_m = 0.12) polypeptide as compared to control (Table 3). On the other hand, in pod wall of ILC3279 stress at early developmental stages repressed the expression of 34.70 and 30.86 kDa polypeptides which were later expressed at 21 DAF. At early developmental stages (i.e. at 7 and 14 DAF), stress reduced the relative percent distribution of 19.84 kDa polypeptide from 9.0 to 2.5% and 18.48 kDa polypeptide from 7.2 to 2.4 % in pod wall of ICC4958 and increased the relative percent distribution of 19.84 kDa polypeptide from 2.4 to 10.2% and 18.48 kDa polypeptide from 2.5 to 4.7%. At 21 DAF, stress increased the relative percentage of 60.60 and 18.48 kDa polypeptide in pod wall of both the cultivars, 19.84 kDa in ILC3279, and reduced the percentage of 19.84 kDa polypeptide in ILC3279 (Table 3). Thus, on an average protein expression in pod wall of ICC4958 was enhanced and that in ILC3279 was down regulated during early developmental stages. Pod wall in chickpea in addition to providing protection from biotic and abiotic stresses provides assimilates and nutrients that are subsequently imported into the developing seeds (Bennett *et al.* 2011). Thus enhanced expression of polypeptides in young pod wall of ICC4958 under stress as compared to control might have aided seed establishment and development. Water deficit stress in pod wall of ICC4958 near maturity (i.e. at 28 and 35 DAF) lead to induction of 77.98 and 70.11(R_m = 0.24) kDa polypeptides and repression of 60.60 (R_m = 0.30), 34.70 and 30.86 kDa polypeptides. On the other hand, in pod wall of ILC3279, stress near maturity induced 70.11 and 55.05 kDa polypeptides and repressed the expression of 77.98, 60.60 and 34.70 kDa polypeptides (Table 3). Moreover at this stage, relative percentage of 19.84 kDa was increased; and that of 60.60 and 26.67 kDa was reduced in pod wall of ILC3279. When the pod wall reaches near maturity (i.e. at 28 and 35 DAF), pod wall had by and large fuelled seed development and in our study stress in pod wall of both the cultivars at this stage resulted on an average decreased protein expression.

Water deficit stress during early seed developmental stages (*i.e.* at 7 and 14 DAF) induced 59.23 (R_m = 0.32) kDa polypeptide and inhibited 68.96 (R_m = 0.25), 53.73 (R_m = 0.37) and 22.82 kDa (R_m = 0.77) polypeptides in seeds of ICC4958. On the other hand, in seeds of ILC3279 stress at early developmental stages induced 15.81 (R_m = 0.94), 22.82 and 64.48 (R_m = 0.28) kDa polypeptides and inhibited 68.96 kDa polypeptide. Stress increased the relative percentage of 64.48 and 17.93 (R_m = 0.87) kDa polypeptide in seeds of ICC4958, 30.65 (R_m = 0.63) kDa polypeptide in seeds of

Table 3. Relative percent distribution of total proteins in pod wall of chickpea cultivars (ICC4958 and ILC3279) under control and water deficit stress conditions at different DAF

Band no	Relative mobility	Molecular wt (kDa)	Per cent distribution of proteins																			
			7 DAF				14 DAF				21 DAF				28 DAF				35 DAF			
			1	2	1'	2'	1	2	1'	2'	1	2	1'	2'	1	2	1'	2'	1	2	1'	2'
1	0.12	91.20	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.19	77.98	0.0	1.3	2.4	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.6	0.0
3	0.24	70.11	2.4	0.0	0.0	0.0	2.5	2.3	2.2	2.4	0.0	0.0	0.0	0.0	0.0	0.0	7.3	2.4	2.4	0.0	0.0	2.4
4	0.30	60.60	0.0	2.5	2.6	2.6	8.8	0.0	0.0	0.0	2.4	2.4	4.9	6.8	2.4	3.2	0.0	2.4	2.4	2.5	2.7	0.0
5	0.35	55.05	0.0	0.0	0.0	0.0	0.0	2.1	2.1	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4
6	0.41	47.86	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.56	34.70	0.0	0.0	0.0	0.0	0.0	2.2	2.2	0.0	0.0	0.0	0.0	1.9	2.2	2.2	0.0	0.0	0.0	0.0	0.0	0.0
8	0.57	30.86	0.0	0.0	0.0	0.0	0.0	2.3	2.2	0.0	0.0	0.0	0.0	2.4	2.3	2.2	0.0	2.2	3.1	0.0	2.7	0.0
9	0.65	28.72	2.3	2.2	2.0	2.0	2.1	2.3	2.2	2.2	2.2	6.8	2.6	2.4	2.2	2.2	2.1	2.3	3.4	2.5	2.0	2.2
10	0.69	26.67	2.4	2.2	2.0	2.1	2.1	2.2	2.2	2.1	2.1	4.7	2.1	4.1	2.5	3.9	2.1	2.4	5.2	2.1	0.0	2.3
11	0.83	19.84	9.0	2.4	2.5	10.2	2.1	2.3	2.2	2.0	2.2	2.2	2.6	7.9	2.5	0.2	7.8	2.6	10.2	2.7	2.4	8.3
12	0.86	18.48	7.2	2.5	2.4	4.7	2.3	2.3	2.2	2.1	2.3	2.9	5.0	11.3	2.5	4.1	6.2	2.6	6.6	2.8	2.4	7.2

1, 2, 1' and 2' represent ICC4958 (control), ILC3279 (control), ICC4958 (water deficit stress) and ILC3279 (water deficit stress) respectively. The values represent the mean of three electropheretic gel documentation.

ILC3279, and reduced the percentage of 20.43 (R_m = 0.82) and 30.65 kDa polypeptide in seeds of ILC3279 (Table 4). At 21 DAF, stress induced 64.48 and 22.82 kDa polypeptide in ICC4958, 59.23 and 20.43 (R_m = 0.82) kDa polypeptide in ILC3279, inhibited 68.96, 43.41 (R_m = 0.47) and 15.81 kDa polypeptide in ICC4958, and 64.48 and 15.81 kDa polypeptide in ILC3279 (Table 4). Stress at this stage increased the percent distribution of 30.65 kDa polypeptide in both the cultivars, 15.06 (R_m = 0.95) kDa polypeptide in ICC4958, 17.93 kDa polypeptide in ILC3279, and reduced the distribution of 20.43 kDa polypeptide in ICC4958. Thus, seeds of ICC4958 and ILC3279 responded to stress at early developmental stage by down regulating and up regulating protein expression, respectively. The reason for this can be the opposite trend of protein expression observed in pod wall of both the cultivars at this stage as certain transporters in pod wall like *AAP2* have been reported to translocate proteins to seeds (Bennett *et al* . 2011). Stress near maturity (i.e. at 28 and 35 DAF) induced 68.96, 53.73 and 30.65 kDa polypeptide in seeds of ICC4958, and 73.57, 53.73, 22.82 and 17.93 kDa polypeptide in ILC3279; inhibited the expression of 20.43 kDa polypeptide in seeds of both the cultivars, 68.96 kDa polypeptide in seeds of ILC3279 (Table 4). Moreover, relative per cent of 20.43 kDa polypeptide was increased in ICC4958; and 30.65, 64.48 and 15.06 kDa polypeptide was reduced in seeds of ILC3279 at 28 and 35 DAF. Thus, polypeptide expression was increased in mature seeds of ICC4958 and reduced in seeds of ILC3279 near maturity.

Proteomic analysis of these two chickpea cultivars under laboratory conditions also revealed similar results. Stress in roots of ICC4958 induced 47.14 (R_m = 0.42) kDa polypeptide and inhibited the expression of 51.90 kDa (R_m = 0.38) polypeptide. On the other hand, in roots of ILC3279, stress induced 47.14 kDa polypeptide and repressed 51.90, 16.06 (R_m = 0.93) and 14.42 (R_m = 0.98) kDa polypeptide (Table 5). Moreover, stress increased the relative percentage of 32 kDa polypeptide from 2.0 to 4.2% and decreased the percentage of 26.36 and 16.06 kDa polypeptide from 3.0 and 6.9% to 1.8 and 1.7 %, respectively, in roots of ICC4958. On the other hand, in roots of ILC3279, stress increased the relative percentage of 32.00, 26.36, 21.77 kDa polypeptide from 3.1, 3.3 and 2.8% to 9.5, 8.7 and 8.3%, respectively, and decreased the percentage of 17.90 kDa polypeptide from 1.5% to 0.8 % as compared to control. Thus, water deficit stress increased the protein expression in roots of ICC4958 while in roots of ILC 3279, stress down regulated the polypeptide expression by either reducing the percent distribution of some polypeptides or by inhibiting expression of some polypeptides. Further research on the differentially expressed polypeptides in chickpea roots under control and water deficit stress conditions can provide useful information on individual enzymes or transporters, measuring their stress-dependent changes in quantity, activity as well as modifications of structural polypeptide, polypeptide-polypeptide interactions, stress dependent polypeptide movements, *de novo* synthesis and controlled degradation (Kakaei *et al.* 2010). In shoots of ICC4958, 47.14 kDa polypeptide was induced and 51.90 kDa polypeptide was repressed while on an average polypeptide expression in shoots of ILC3279 was unchanged. In cotyledons of both the cultivars, 16.06 and 14.42 kDa polypeptides were repressed, 51.90 and 47.14 kDa polypeptides were repressed in cotyledons of ICC4958 and ILC3279, respectively, while 36.30 kDa (R_m = 0.55) polypeptide was induced in cotyledons of ILC3279 (Table 5). Moreover stress increased the relative distribution of 26.36 kda polypeptide from 1.0 to 2.1 % in cotyledon of ICC4958 and 86.04 kDa polypeptide from 0.9 to 1.2 % in cotyledons of ILC3279. These differentially expressed

Table 4. Relative percent distribution of total proteins in seeds of chickpea cultivars (ICC4958 and ILC3279) under control and water deficit stress conditions at different DAF

Band no	Relative mobility	Molecular wt (kDa)	Per cent distribution of proteins																			
			7 DAF				14 DAF				21 DAF				28 DAF				35 DAF			
			1	2	1’	2’	1	2	1’	2’	1	2	1’	2’	1	2	1’	2’	1	2	1’	2’
1	0.22	73.57	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	2.4	0.0	1.6	0.0
2	0.25	68.96	2.5	2.5	0.0	0.0	0.0	1.1	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	2.4	0.0	0.0
3	0.28	64.48	2.5	2.4	2.4	2.4	1.1	0.0	2.4	2.4	0.0	2.4	2.4	0.0	0.0	2.3	0.0	2.7	2.4	2.4	2.4	1.7
4	0.32	59.23	0.0	0.0	0.0	0.0	0.0	1.5	2.4	2.4	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.37	53.73	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	3.2	1.5	2.3	1.3	0.0	2.4	2.4	0.0	0.0	0.0	0.0	2.7
6	0.47	43.41	2.5	2.7	2.3	2.3	2.4	2.4	2.4	2.4	2.2	0.0	0.0	0.0	1.7	1.3	2.3	1.3	2.4	2.0	2.4	2.2
7	0.63	30.65	2.5	2.5	2.8	7.1	2.6	2.7	2.5	1.8	1.4	1.6	2.3	6.2	0.0	7.9	2.6	2.5	2.5	2.5	2.5	2.5
8	0.71	25.89	2.0	1.3	2.3	1.2	2.4	0.9	2.1	2.4	2.2	1.3	2.2	1.1	2.2	1.0	2.0	2.4	2.3	2.3	2.7	2.4
9	0.77	22.82	2.3	5.2	3.4	5.2	6.0	0.0	0.0	1.3	0.0	6.4	3.6	1.8	0.0	0.0	0.0	2.5	2.4	2.4	2.0	1.7
10	0.82	20.43	2.5	11.5	2.0	1.2	3.9	2.6	2.4	2.4	5.4	0.0	2.9	2.3	2.4	2.5	4.0	2.4	2.3	2.5	0.0	0.0
11	0.87	17.93	6.7	2.1	8.5	1.9	1.9	2.3	2.3	2.4	1.1	1.6	0.9	2.3	2.3	2.2	2.0	2.3	1.7	0.0	2.2	11.3
12	0.94	15.81	0.0	0.0	0.0	2.3	1.7	4.1	2.0	0.0	3.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	0.95	15.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.7	2.7	1.8	2.3	2.2	2.4	1.3	2.3	2.3	2.2	2.1	1.5

1, 2, 1’, 2’ represent ICC4958 (control), ILC3279 (control), ICC4958 (water deficit stress) and ILC3279 (water deficit stress) respectively. The values represent the mean of three electropheretic gel documentation.

Table 5. Relative percent distribution of total proteins in seedling of chickpea cultivars (ICC4958 and ILC3279) under control and water deficit stress conditions at 7 DAG

Band no	Relative mobility	Molecular wt (kDa)	Per cent distribution of proteins											
			Control						Water deficit stress					
			1R	1S	1C	2R	2S	2C	1R	1S	1C	2R	2S	2C
1	0.14	86.04	1.2	1.0	1.5	0.9	3.2	0.9	8.0	0.4	1.3	1.5	0.6	1.2
2	0.38	51.90	2.1	1.7	2.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.42	47.14	0.0	0.0	0.0	0.0	1.8	2.3	2.1	2.0	0.0	1.9	1.1	0.0
4	0.55	36.30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5
5	0.60	32.00	2.0	0.0	1.9	3.1	0.0	1.0	4.2	0.0	1.3	9.5	0.0	0.7
6	0.69	26.36	3.0	10.6	1.0	3.3	2.2	1.9	1.8	2.4	2.1	8.7	1.6	1.8
7	0.79	21.77	2.2	2.3	1.2	2.8	2.2	1.8	2.1	2.3	0.9	8.3	2.5	1.7
8	0.88	17.90	0.9	2.2	0.8	1.5	2.2	1.0	1.4	1.0	0.8	0.8	1.8	0.6
9	0.93	16.06	6.9	4.3	3.0	4.3	1.9	4.2	1.7	5.5	0.0	0.0	1.8	0.0
10	0.98	14.42	1.5	1.9	5.6	1.8	2.0	1.6	1.7	1.2	0.0	0.0	0.8	0.0

1R, 1S, 1C, 2R, 2S and 2C represent ICC4958 (roots), ICC4958 (shoots), ICC4958 (cotyledons), ILC3279 (roots), ILC3279 (shoots) and ILC3279 (cotyledons) respectively. The values represent the mean of three electropheretic gel documentation.

polypeptides can, be assessed for their function and role in drought tolerance and the high level of these polypeptides can be useful as a marker for drought tolerance.

This study on differential protein expression in different vegetative and reproductive tissues during crop growth and in cotyledons and seedlings in tolerant and susceptible chickpea cultivars under water stress could be the basis for further research in identifying potential protein markers that can be potential targets for MAS (marker assisted selection). Differentially expressed proteins in different tissues can be further identified and analysed by 2D-electrophoresis and mass spectroscopy to find out the role of individual proteins in imparting water deficit stress tolerance.

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