Original Article

Effect of cadaverine on *Brassica juncea* (RH-30) seedlings under multiple stress - A quantitative analysis

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Abstract

Background: Plants, in general, are put to various kinds of stress, biotic and abiotic, both natural and manmade. Cadaverine (Cad) is a lysine catabolite and is involved in the growth and development of plants. The present study aims to determine the effect of cadaverine response on the induction of polypeptide profile under multiple stresses in leaf and root tissues of *Brassica juncea* and to diagnose the change in genetic expression modification (if any). The protein content has been analysed using SDS-PAGE and the results were further validated using Densitometer.

Results: The basal medium used for tissue culture was MS media formulated with different stress conditions and treatments without and with Cad (100mM NaCl, 1mM Cd or Pb, 5mM NH_4NO_3, 1mM cadaverine). Cad treatment showed increased protein content in the presence of NH_4NO_3 and even in seedling provided/ supplemented with multiple stresses. The protein content has been analysed using SDS-PAGE and the

results were further validated using densitometric analysis. Supplementation of Cad induced the expression of 5 more peptides in a leaf tissue which were not observed in a leaf of the seedling without Cad. Cad enrichment in a root tissue did not change the expression of any peptide in NaCl environment which got affected in the absence of Cad under saline conditions.

Conclusions: Due to the positivistic performance of Cad in increasing the expression of novel peptide and mitigating the different effects of multiple stresses suggesting its role in growth and development of leaf and root tissues of *Brassica juncea* (RH-30).

Keywords: *Brassica juncea*, Cadaverine, Densitometric, Polyamines, Protein, Quantification, Stress

1. Introduction

Urbanization and anthropogenic activities resulted in accumulation of waste material causing the significant and unfavorable modification to the surroundings (Chen, 2007). Accumulation of heavy metals is one of such modifications whose escalating toxicity raising the threat to both the biological system and environment (Nagajyoti, Lee, & Sreekanth, 2010). Due to the non-biodegradable nature of heavy metals, their endowment in soil results in their accumulation in exposed plants that ultimately affects the growth and development of plants. Further, they have shown to penetrate inside the body of human via food chain that results in their chronic accumulation in human liver and kidney, disrupting biological pathways (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). Heavy metals accumulation induces oxidative stress in plants due to generation of Reactive oxygen species leading to stunted growth, necrosis, chlorosis of leaf, poor quality and quantity of fruit, weakens

the development of roots-shoots and leaves (Emamverdian, Ding, Mokhberdoran, & Xie, 2015).

Brassica juncea is a member of Brassicaceae family (Szollosi, 2011). It has an intense flavour and consists of various healing properties (Fomina, 1962). Seed oil is obtained from B. juncea, whose crushed seeds is utilized in mustard production. In addition to the vegetable uses, its medicinal role has also been reported (Tomar, Lakra, & Mishra, 2013 a). It has diuretic, rubefacient, emetic and anti-septic properties. Also, it is known to be one of the valuable repositories of vitamins (vitamin A and C), iron. It also contains calcium, beta carotene, potassium, thiamine, riboflavin and antioxidants such as carotenes, indoles, flavonoids, zeaxanthin and lutein (Arena, Cacciola, Dugo, Dugo, & Mondello, 2020).

Cadaverine, a lysine catabolite, is a diamine involved in the growth and development of plants. Lysine decarboxylase (LDC) catabolize lysine, that results in an imino compound i.e., cadaverine. The presence of enzyme LDC in higher plants specifically in plants belonging to family Leguminosae, Gramineae and Solanaceae indicates the wide presence of Cadaverine (Tomar, Lakra, & Mishra, 2013 a,b). Cadaverine is also present in the environment; it can be produced by rhizosphere and phyllosphere microbes (Jancewicz, Gibbs, & Masson, 2016). Cadaverine is a product of bacterial decomposition, which is malodorous in nature and due to cadaverine, cadaver has foul rotting smell (Kusano, Berberich, Tateda, & Takahashi, 2008). It is involved in numerous activities such as it is a precursor of polyamides, its role in water absorption, it ensures the survival of cell in acidic conditions, protects cells that are starved of inorganic phosphate, Pi, under anaerobic conditions, regulates animal growth (Ma et al., 2017; Moreau, 2007; Samartzidou, Mehrazin, Xu, Benedik, & Delcour, 2003). Further

roles include response to stress, cell signaling, insect defense, tolerance to crop salt, improvisation of heavy metal stress (Jancewicz et al., 2016; Rajpal and Tomar, 2020).

This study is a basis of determination of the effect of cadaverine response on the induction of polypeptide profile under multiple stresses in leaf and root tissues of *Brassica juncea*, to diagnose the change in genetic expression modification, if any

2. Materials and Methods

2.1 Estimation of Total Soluble Protein and SDS-PAGE Analysis

Lowry method was used to estimate the total soluble protein present in leaf and root tissues (Lowry, 1951). Determination of protein Quality of protein content was done by plotting the standard curve against bovine serum albumin. Protein's molecular weight was determined by performing SDS-PAGE (Laemmli, 1970). Standard protein markers were used for the characterization of molecular weight. Using Migration distances molecular weight of sample proteins was determined.

2.2 Densitometric Analysis

For the determination of the quantity of polypeptide obtained in SDS gel and to interpret accurate data, densitometric analysis was done using a densitometer. The retention time was recorded for each protein band. UV gel documentation and UV gel star MV were used for their density measurement. Depending upon the density of the particular peptide, different graphical peaks were obtained. Densitogram (protein bands' intensity) was quantified in accordance with area percentage and variation in protein samples upon treatment was estimated by calculating the protein area.

3. Results and Discussion

3.1 PAGE analysis of proteins

3.1.1 Leaf Tissue of 7th day Old Seedling under Stress Conditions without Cad

A total of 11 bands of polypeptides i.e., 66, 56, 51.8, 50, 40.6, 35, 24.6, 22, 14, 12 and 11 kDa was observed in leaf tissues of seedling, out of which the peptides of 66 kDa and 56 kDa expressed highly (Figure 1). The peptides of molecular weight 35, 14, 12 and 11 kDa disappeared upon saline treatment whereas the expression of 56 kDa peptide increased a little.

Upon NH_4NO_3 supplementation, more peptides of 90, 88, 85, 80, 75, 51.8, 48 and 44 kDa were expressed. Also, expression of peptides 66 and 56 kDa increased and expression of salt induced peptide of 50 kDa suppressed upon NH_4NO_3 enrichment. Supplementation of NH_4NO_3 to NaCl stressed plants results in the disappearance of high molecular peptides such as 90, 88, 85, 80, 75 and 29.6 kDa and reduced expression of peptides 66 and 56 kDa. The expression of peptides of molecular weight 90, 88, 80, 75 and 50 kDa induced by Cd treatment over control whereas the expression of two more peptides i.e., 85 and 44 kDa induced upon Pb treatment over Cad and rest all proteins were same induced by Cd treatment.

The expression of peptides 85, 51.8 and 44 kDa was observed when the combinatorial treatment (Cd+NaCl) was induced as compared to the plants exposed to Cd only whereas suppressed expression of peptides 90, 44, 40.6, 37, 35 and 29.6 kDa observed upon Pb+NaCl treatment over plants exposed to Pb only.

Further, stress combinations (Metal+ NaCl) were supplemented/amalgated with NH_4NO_3 so that profile of peptide in leaf tissue can be examined. This resulted in the suppressed expression of peptides 90, 85, 44, 40, 40.6, 37 and 29.6 kDa in plants

exposed to Cd+ NaCl and suppressed expression of peptides 85, 44, 37 and 29.6 kDa in plants exposed to Pb+ NaCl.

3.1.2 Leaf Tissue of 7th Day Old Seedling under Stress Conditions with Cad

A total of 16 peptides was observed upon Cad supplementation over control (without Cad) where only 11 peptides observed (Figure 2). The hierarchy in terms of highest expression is as follows: 56>60>48.7, 46=44, =39, =34.5, =28, =26, =22>73.8, 68, 31, 14.9, 13, 11.8. The peptides either disappeared i.e., 73.8 and 60 kDa or little suppressed i.e. 63.8, 48.7, 44, 39, 34.5, 28, 26 kDa or showed no change in remaining peptides under Cad+ NaCl environment.

No change in the peptide expression was observed upon application of NH_4NO_3 to the plants but peptides of 73.8 and 68 kDa got suppressed in Cad+NaCl environment (T_3). However, peptide expression did not change upon Cad supplementation to plants stressed with Cd as compared to Cad treatment while no expression was suppressed by metal expressed due to Cad. Plants exposed to Pb also showed a similar response.

Moreover, metal exposed plants when subjected to saline conditions supplemented with Cad, only the expression of peptide of 13 kDa suppressed while the expression of all other peptides remained same in Cd environment. Also, no change in the expression of peptides observed upon NH_4NO_3 and Cad supplementation to multiple stressed plant (T_8 and T_9).

3.1.3 Root Tissue of 7th Day Old Seedling under Stress Conditions without Cad

A total of 12 bands appeared in controlled tissues of seedlings root, out of which peptides of molecular weight 56, 36, 34, 22, 20, 13.4 and 12.5 kDa expressed remarkably (Figure 3). The peptides either suppressed completely i.e., 44, 40 kDa or little suppressed i.e., 56, 36, 34 kDa or showed elevated expression i.e., peptide of 12 kDa under saline conditions. Also, expression of peptides 56, 44, 40, 36, 34, 22, 20, 13.4 kDa got affected in the presence of NH_4NO_3.

The non-expression of 44, 40 and 13.4 kDa in the root tissue with NH_4NO_3 over control seedlings was intriguing phenomenon. The peptides 36, 34, 13.4 and 12.5 kDa got suppressed in NaCl+ NH_4NO_3 environment. Similar response was observed on the expression of peptides in Cd and Pb stress conditions. However, expression of peptides 44, 30 kDa got suppressed by Cd but it induced a novel peptide of molecular weight 18kDa. The expression of peptide 40 kDa was found to be completely suppressed.

The peptide 56 kDa got over expressed along with the induction of a novel protein of 50 kDa under Cd+ NaCl stress conditions while in case of Pb+ NaCl environment the peptides 56, 44, 36, 34, 30 kDa suppressed completely and the peptides 22, 20, 13.4, 12.5 and 12 kDa expressed a little. Inclusion of NH_4NO_3 to metal+ NaCl environment in the seedlings showed almost similar response over only metal in saline (metal+ NaCl).

3.1.4 Root Tissue of 7th Day Old Seedling under Stress Conditions with Cad

Expression of protein changed upon cad enrichment to the plants that were in stress environment (Figure 4). The expression of peptides 70, 68, 49, 44, 36, 32, 30, 26, 22,18,16.8 and 14 kDa induced by Cad whose expression did not change when NaCl

was added to the seedlings. However, a little suppression was observed in the peptides of 32 and 30 kDa under Cad+NaCl condition (T_1).

Also, protein expression induced by Cad did not alter upon NH_4NO_3 supplementation to those seedlings but the expression of peptide 84 kDa was observed. Interestingly, few variations were observed due to Cad in the presence of metals Cd and Pb. The change in protein bands was observed under metal+NaCl+NH_4NO_3 environment that too depend upon type of metal (Pb or Cd) used along with Cad.

3.2 Densitometric analysis of protein band of leaf and root tissues

In order to support the data of proteins (which changed with treatments identified on SDS-PAGE), to identify differential proteins and to remove falsifying results, the densitometric analysis was done. This revealed some specific bands which have high density in Cad treated tissues. Different peaks in the densitogram confirm the increased/decreased intensity of the band (Table 1, 2, 3, 4 and Figure 5, 6, 7, 8).

The leaf of the seedlings without Cad exhibited 66, 56, 40.6, 35 and 22 kDa protein peaks in the densitograph. According to the protein band area, 66 kDa peptide' s expression found maximum with NH_4NO_3 and even in multiple stress conditions. While 56 kDa peptide expressed maximum with NH_4NO_3 and minimum with NaCl stressed leaf. The most strikingly 22 kDa protein as discussed earlier considered to be a member of protease family (Downing et al., 1992) showed increased level with non-Cad treated leaves and its level decreased significantly with Cad. In Cad treated leaf the 60, 56, 46, 39, 26 and 22 kDa protein showed clear peaks in the densitograph indicates the modulation specifically.

According to band area calculated in non-Cad treated root, the 56, 36, 30, 22 and 13.4 kDa peptides showed clear peaks and in Cad treated root i.e., 84, 49, 36, 22 and 14 kDa pepides had high of peaks (showed increased intensity). It is again worth to mention that 22 kDa peptide appeared to be common both in leaf and root with or without Cad. The 22 kDa peptide assigned as protease in number of studies (Downing et al., 1992) was very prominent in leaf and root tissues, which was otherwise increased by stresses and modulated by Cad. Several changes in proteins in B. napus leaf under water stress have been also noted (Reviron et al. 1992). The further experimentation to define the role of Cad induced proteins is required.

4. Conclusions

In order to elaborate our understanding of the mechanism of stress tolerance in Brassica species in presence of Cad, the protein profile was done. The SDS analysis revealed the appearance of several specific polypeptides. However, due to salt and metal stress peptides 60, 56, 51.8 and 22 kDa observed. Cad stimulated the synthesis of 46 and 14.9 kDa peptides in root tissue. Whereas induction of 56, 36, 34, 23, 20, 13.4 and 12.5 kDa peptides with Cad were remarkable and concomitantly suppressed the 22 kDa peptide. During stress, it is quite possible that the change in peptide pattern due to salinity/metals may be involved in the number of growth-related biochemical functions. Densitometric analysis supports the results of protein expression with treatment. The identification and characterization of some of the relevant proteins and their genes may provide some insight regarding the regulation mechanism of the salt stress at molecular level.

Acknowledgments

We would like to acknowledge Dr. S.N. Mishra for his valuable guidance and support.

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SJST MANUSCRIPT TEMPLATE FOR A FIGURE FILE

A figure caption list:

Figure 1 Protein profile of leaf tissue of 7th day old seedling under various stress conditions without cad
M-Marker
T_0- Control
T_1- NaCl (100mM)
T_2- NH_4NO_3
T_3- NaCl+ NH_4NO_3
T_4- Cd (1mM)
T_5- Pb (1 mM)
T_6- Cd+ NaCl
T_7- Pb+ NaCl
T_8- Cd+ NaCl+ NH_4NO_3
T_9- Pb+ NaCl+ NH_4NO_3

Figure 2 Protein profile of leaf tissue of 7th day old seedling under various stress conditions with cad

Figure 3 Protein profile of root tissue of 7th day old seedling under various stress conditions without cad

Figure 4 Protein profile of root tissue of 7th day old seedling under various stress conditions with cad

Figure 5 Densitometric spectra of control and sample protein of leaf tissue on the SDS-PAGE gel without cad

Figure 6 Densitometric spectra of control and sample protein of leaf tissue on the SDS-PAGE gel with cad

Figure 7 Densitometric spectra of control and sample protein of root tissue on the SDS-PAGE gel with Cad

Figure 8 Densitometric spectra of control and sample protein of root tissue on the SDS-PAGE gel with Cad

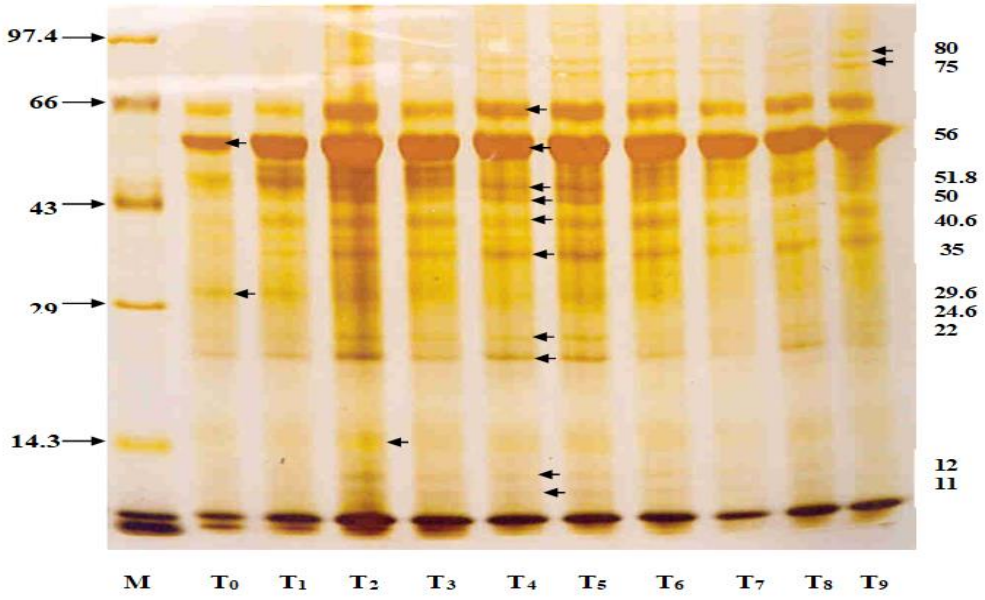

Figure 1 Protein profile of leaf tissue of 7th day old seedling under various stress conditions without cad.

M-Marker

T_0- Control

T_1- NaCl (100mM)

T_2- NH_4NO_3

T_3- NaCl+ NH_4NO_3

T_4- Cd (1mM)

T_5- Pb (1 mM)

T_6- Cd+ NaCl

T_7- Pb+ NaCl

T_8- Cd+ NaCl+ NH_4NO_3

T_9- Pb+ NaCl+ NH_4NO_3

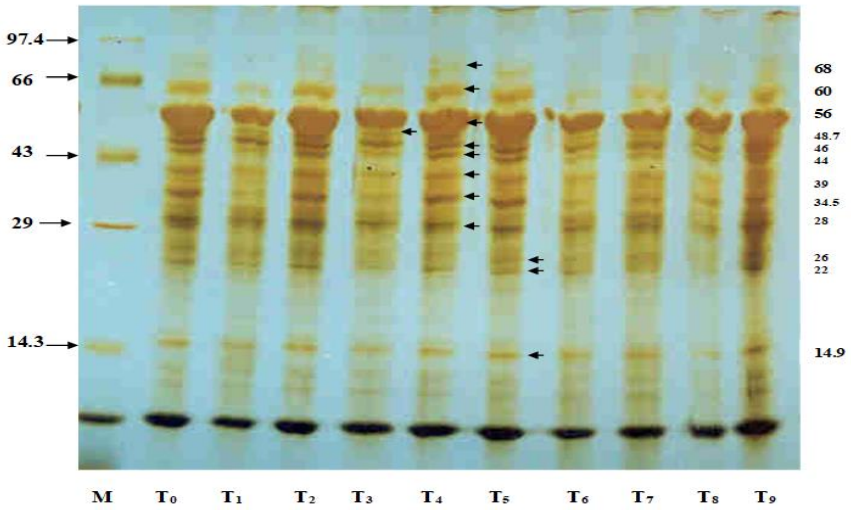

Figure 2 Protein profile of leaf tissue of 7th day old seedling under various stress conditions with cad.

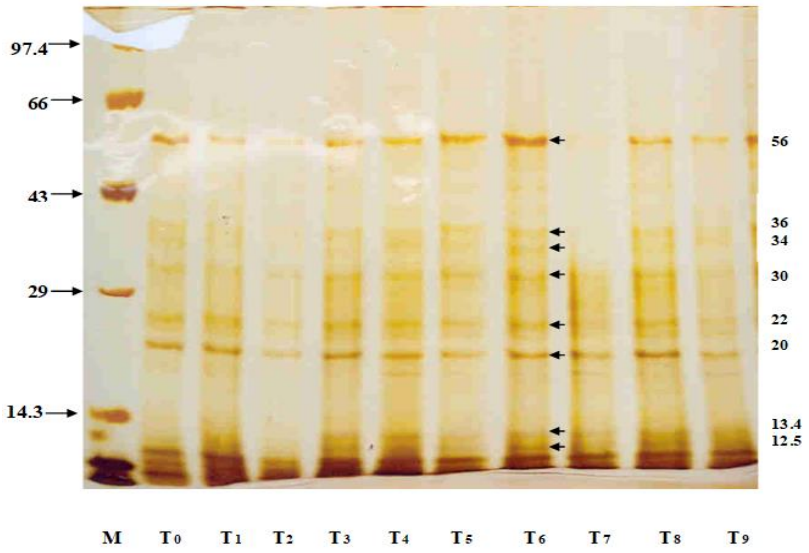

Figure 3 Protein profile of root tissue of 7th day old seedling under various stress conditions without cad.

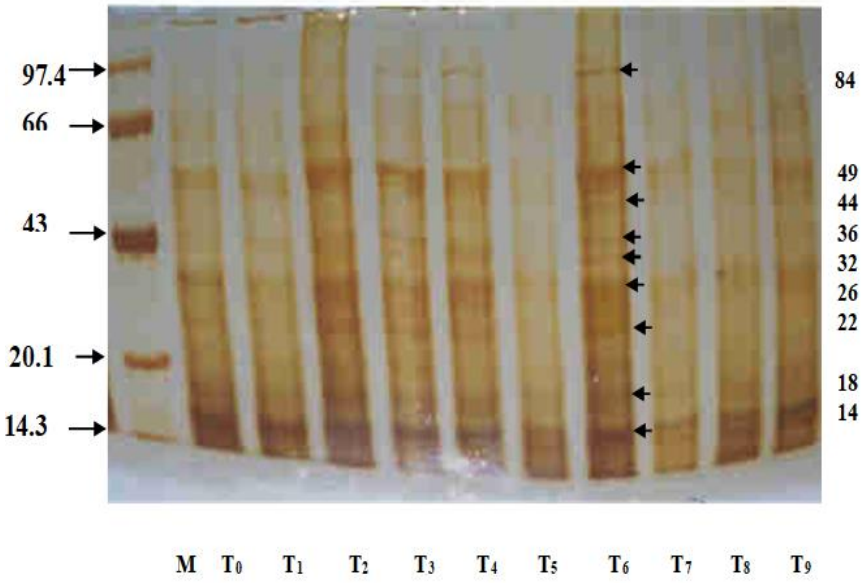

Figure 4 Protein profile of root tissue of 7th day old seedling under various stress conditions with cad.

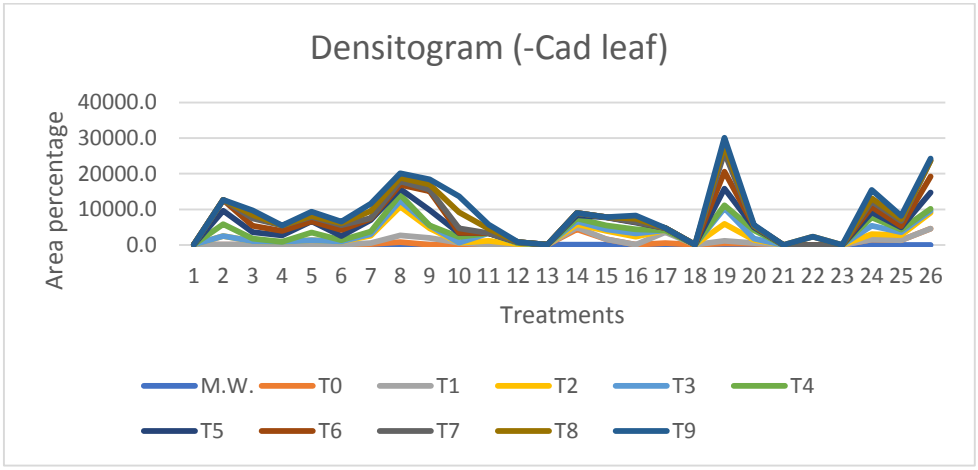

Figure 5 Densitometric spectra of control and sample protein of leaf tissue on the SDS-PAGE gel without cad.

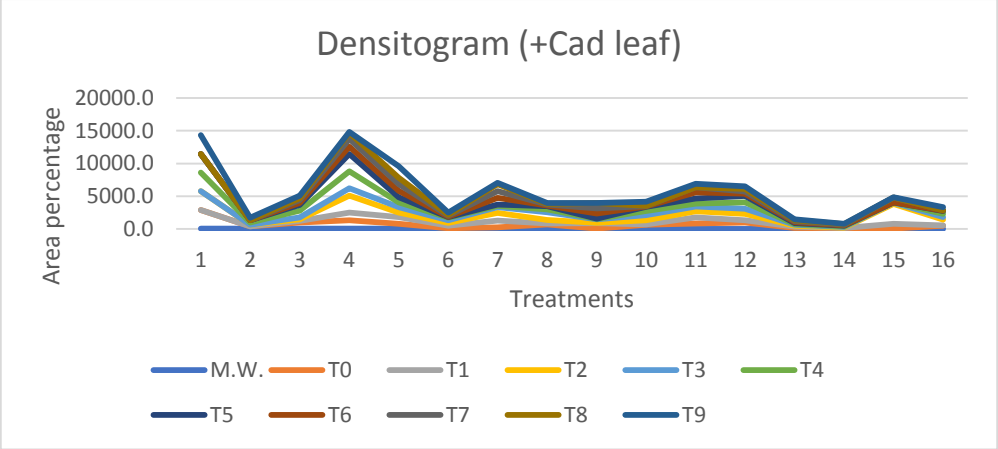

Figure 6 Densitometric spectra of control and sample protein of leaf tissue on the SDS-PAGE gel with cad.

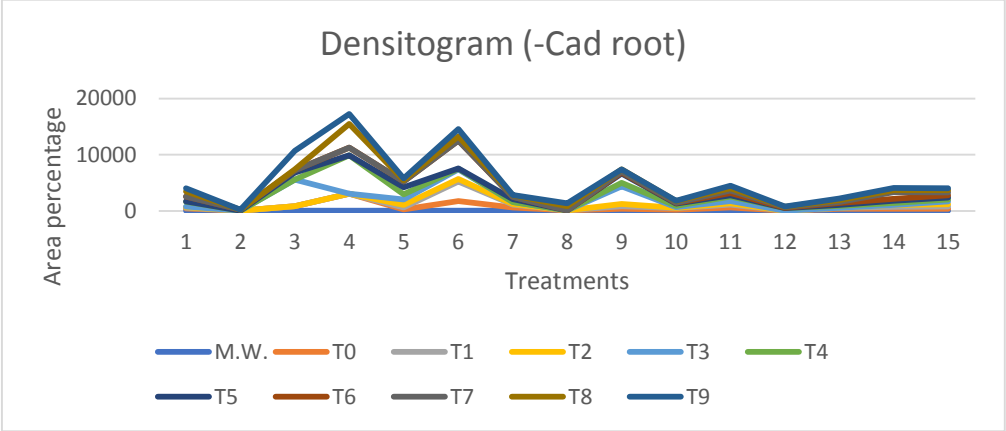

Figure 7 Densitometric spectra of control and sample protein of root tissue on the SDS-PAGE gel with cad.

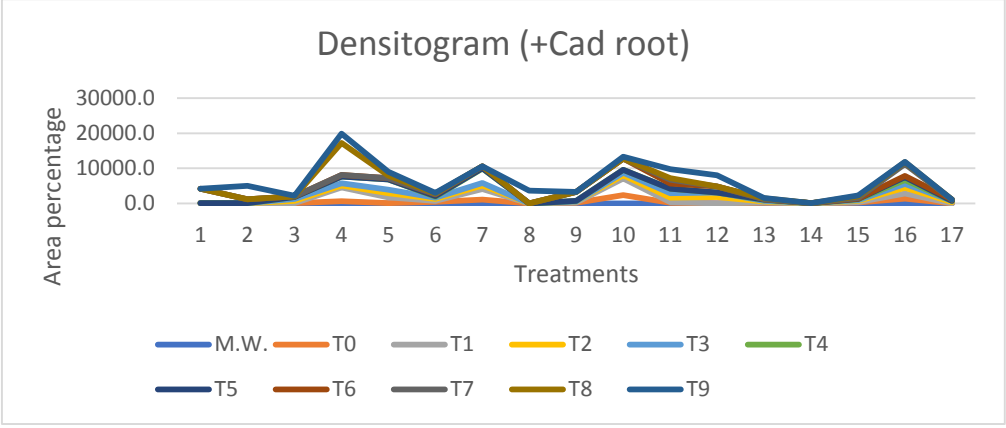

Figure 8 Densitometric spectra of control and sample protein of root tissue on the SDS-PAGE gel without cad.

Table

TABLE FILE

Table 1 Statistical data of densitogram in control and sample protein of leaf tissue on the SDS-PAGE gel without Cad (area percentage).

M.W.	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
97.4	-	-	-	-	-	-	-	-	-	-
90	-	-	2415	-	3298	3839	3060	-	-	-
88	-	-	945	-	748	1760	1836	2052	980	1320
85	-	-	805	-	-	1800	1224	1548	-	-
80	-	-	1260	-	2142	3160	36	1188	280	1200
75	-	-	910	-	306	1240	1692	1332	525	440
66	210	280	2000	476	756	3185	385	180	2000	2030
56	740	1920	8050	1632	1728	1632	1152	952	1000	1280
51.8	-	1880	2765	735	-	4352	5328	468	1440	1440
50	-	520	-	-	1632	1085	108	1224	4520	4520
48	-	-	1085	2170	-	-	-	36	1200	1200
44	-	-	245	210	-	245	36	-	-	-
43	-	-	-	-	-	-	-	-	-	-
40.6	4352	540	920	805	805	920	630	-	-	-
37	1560	-	2310	445	1085	2310	36	-	-	-

35	-	-	2310	885	1120	1885	245	-	885	885
29.6	480	3080	558	-	-	558	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-
24.6	440	720	4720	4410	805	4720	4720	5400	2080	2080
22	280	245	885	380	2310	805	480	-	-	280
20.1	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	2310	-
14.3	-	-	-	-	-	-	-	-	-	-
14	1520	-	1520	2310	2310	1520	1520	1520	885	2310
12	1360	-	1360	885	685	660	360	1360	558	885
11	4520	-	4520	558	558	4520	4520	4520	-	558

M.W. -Molecular weight

T_0- Control

T_1- NaCl (100mM)

T_2- NH_4NO_3

T_3- NaCl+ NH_4NO_3

T_4- Cd (1mM)

T_5- Pb (1 mM)

T_6- Cd+ NaCl

T_7- Pb+ NaCl

T_8- Cd+ NaCl+ NH_4NO_3

T_9- Pb+ NaCl+ NH_4NO_3

Table 2 Statistical data of densitogram in control and sample protein of leaf tissue on the SDS-PAGE gel with Cad (area percentage).

M.W.	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
73.8	2840	-	2880	-	2840	2840	-	-	-	2840
68	360	-	200	-	360	360	-	-	-	360
60	920	280	240	280	1000	920	280	420	290	410
56	1280	1160	2600	1140	2600	2600	1140	1160	920	160
48.7	760	1000	680	840	760	760	1000	1000	1000	1760
46	80	400	400	400	400	80	80	120	180	320
44	200	1040	1160	880	200	200	1040	1040	1040	200
39	680	120	520	1200	420	500	120	120	120	120
34.5	40	800	80	520	40	40	800	800	800	40
31	640	40	640	760	640	640	40	40	40	640
28	840	920	920	740	420	780	920	780	40	500
26	960	360	960	800	960	960	360	360	380	385
22	160	160	160	148	80	160	148	148	150	148
14.9	92	40	80	80	88	96	80	80	80	80

13	40	720	3040	200	40	40	-	720	-	40
11.8	480	40	920	360	480	480	40	40	-	480

Table 3 Statistical data of densitogram in control and sample protein of root tissue on the SDS-PAGE gel without Cad (area percentage).

M.W.	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**	**T_9**
56	390	117	117	117	840	42	835	-	939	539
50	-	-	-	-	-	-	140	-	-	-
44	780	-	-	4719	-	1248	420	-	160	3315
40	3003	-	-	-	6825	-	1400	-	4200	1755
36	234	320	482	925	950	1224	1053	-	209	352
34	1677	3510	429	1716	156	39	4934	-	760	1326
30	546	546	109	312	-	560	351	-	288	104
28	78	-	-	-	-	-	-	-	720	507
23	351	429	429	3120	663	1601	178	178	400	35
22	200	259	39	118	265	390	76	78	288	78
20	560	560	187	390	819	209	429	429	429	420
18	-	-	-	-	468	-	39	156	39	39
13.4	351	167	-	-	351	167	351	167	351	265

12.5	351	351	351	-	351	351	351	1232	351	351
12	351	429	351	546	351	351	351	556	351	351

Table 4 Statistical data of densitogram in control and sample protein of root tissue on the SDS-PAGE gel with Cad (area percentage).

M.W.	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**	**T_9**
100.2	-	-	-	-	-	-	4120	-	-	-
90	-	-	-	-	-	-	1080	-	-	3816
84	-	-	655	596	492	-	240	-	-	188
70	616	3919	540	572	1936	-	440	-	9100	2700
68	44	1600	1215	1012	2948	-	320	-	735	1116
49	390	327	540	400	274	180	412	132	104	233
44	1056	3040	990	748	4048	154	520	-	-	-
43	-	-	-	-	-	-	-	-	-	3600
36	136	110	252	60	192	-	2520	-	-	-
32	2332	4800	855	748	855	-	3160	-	-	520
30	176	176	1260	1276	1305	-	1480	1080	440	2520
26	-	-	1800	1276	-	-	1760	-	-	3160
22	148	130	290	135	290	74	117	-	320	85

19	-	-	90	-	-	-	-	-	-	-
18	244	144	351	144	305	52	90	240	400	315
14	1408	1428	1677	1056	1120	810	351	3360	520	90
13.5	-	-	351	351	45	-	351	-	-	-