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EFFECT OF CADAVERINE ON PROTEIN PROFILING OF BRASSICA JUNCEA (RH-30) SEEDLINGS UNDER MULTIPLE STRESS

Pushpa C.Tomar *, S.N.Mishra[1] and Charu Rajpal[2]
***** *Associate Professor,* Department of Biotechnology Engineering, Faculty of Engineering and Technology,
Manav Rachna International University, Faridabad, Haryana, (INDIA) -121004
[1]*Dean,* Faculty of Lifesciences, Maharishi Dayanand University, Rohtak, Haryana (INDIA)-124001
[2]*Research Scholar,* Department of Biotechnology Engineering, Faculty of Engineering and Technology,
Manav Rachna International University, Faridabad, Haryana, (INDIA) -121004

[*]Email: pushpa.fet@mriu.edu.in

ABSTRACT

Cadaverine (Cad), diamine a species of polyamines group synthesized independently through different pathway effects growth of the plant, but antistress role is obscure. In view of this, Cad effect on protein profile of leaf and root tissues were examined cultured on modified half strength of Hoagland's solution contained with NaCl (100 mM), NH_4NO_3 (5mM), Cd, Pb (1mM) and added with Cad (1mM). In view to determine the quantity of protein present in SDS gel. The Cad treatment of the seedling under either salinity or Pb and Cd stress increased the protein content significantly in the leaf as well as in root tissue. In leaf tissues of seedling showed presence of 66, 56, 51.8, 50, 40.6, 35, 24.6, 22, 14, 12 and 11 kDa peptides among these 66 and 56 kDa were highly expressed peptides. In root tissues 56, 36, 34, 22, 20, 13.4 and 12.5 kDa peptides were highly expressed. Salt stress either caused complete suppression of 44, 40 or little suppression of 56, 36, 34 whereas, slightly elevated the expression of 12.5 kDa peptide. Cad ameliorates the growth of the leaf and root tissues under stress conditions and these stress induced proteins in the presence of Cad may help plant to grow under stress condition.

Keywords: B. juncea, Cadaverine, Metal, Polyamines, Protein, Salinity, Stress

1. INTRODUCTION

The polyamines including cadaverine (Cad) wide acceptance as anti senescence activity [1-6] in one hand and no effect on senescence in few cases [7] on the other warrant extensive experimentation to elucidate its role in plant growth in general and under stress in specific. Liu et al. [3] are also of opinion that the physiological relevance of polyamines increment under stress is not fully explained except that sensitive species deficient in PA over tolerant. The same kind of reports in case of Cad is also noted. As per literature survey, however, it appears that Cad has been not evaluated extensively like to that of putrescine or spermine or spermidine in plant growth management, especially under multiple stress condition in Indian mustard. The stress related proteins are believed to be involved in the proper folded structure of essentially required proteins. For instance, LEA and dehydrins are considered to be acting as substitute for water satisfying hydrogen bonding for polar amino acids at protein surface and thus maintains folded structure [8,9], which is implied in desiccation tolerance. It is also suggested that some proteins might be involved in pH regulation, ionic balance and hormone homeostasis [10]. The stress specific proteins could be protease inhibitors as well [11].

The Cad no effect on chloroplast outer envelop porins (OEP) involved in transport of triosephosphate, dicarboxylic acids, charged amino acid, sugars, ATP and Pi further indicates specific role in metabolic processes during plant growth. Whereas, Cad effect on bacterial porins are mentioned [12]. In fact, no effect on uricase enzyme in Cicer arientinum, V.faba and T.aestivum leaves [13] further demonstrates Cad specific role in metabolism of plants. Like Cad checks protein degradation either in dark or in light in Chinese cabbage leaf, while as spermidine and spermine increased protein degradation [14]. In general, PAs carrying cationic charges tend to attach with negatively charged proteins or nucleic acids thereby interfering in possible breakdown and provide stability under stress conditions, and simultaneously suppressing the DNases, RNases and proteases enzymes [15]. PAs are capable to bind A- or B-DNA form, the Cad binding to the sugar-phosphate backbone is proposed thus tendering stability to DNA [16]. In stress tolerant plant species, the decreased level of Put are compensated by Spm and Cad. The Cad also tends to accumulate under osmotic/salt stress like other polyamines [2]. For instance, accumulation of Cad in rice under water stress [17] and in maize under salt stress, a little increased compared with other PA [18]. Bouchereau et al. [2] have suggested osmotic and salt stress induced production of Cad in higher plants. The rap and tomato leaf disc subjected to 100-300 mM NaCl accumulated Cad, which doubled on the increase in concentration of KCl [1]. Root Cad increased several times with heat shock and diverts the Cad translocation to shoot [19]. This was unlike to Put which is translocated to leaf tissues [20-22]. Cad increased many fold in ice plants under heat shock [23]. The studies indicate that Cad synthesis and translocation appears to be heat labile.

2. MATERIAL AND METHODS

Seeds of mustard (Brassica juncea CV RH-30) were surface sterilized with bleaching powder ($CaOCl_2$) for 5-

min and sown in Whatman's filter paper lined Petri - plates. The seedlings were grown in controlled condition (light 75 Wm^2, Temp. 25± 2ºC, RH 65%) and watered with half strength Hoagland nutrient solution contained with NaCl (100 mM), Cd or Pb (1mM) and blended with cadaverine (Cad 1mM) and NH_4NO_3 (5mM) as per experimental plan. The pH 6.4 of the nutrient solution (containing salts and cadaverine) was kept constant for all treatments (T_0-Control, T1-NaCl, T2 - NH_4NO_3, T3 - NaCl+ NH_4NO_3, T4 - Cd , T5 – Pb, T6 - Cd+NaCl, T7 - Pb+NaCl, T8 - Cd+NaCl+ NH_4NO_3, T9 - Pb+NaCl+NH_4NO_3) with and without Cad. Random sampling of morphologically and physiologically similar seedlings was done on 7th day for various measurements.

2.1 Estimation of Total Soluble Protein

The fresh mass of leaf tissues 250mg and root tissues 500mg obtained at the specified period contained homogenized in chilled mortar in protein extraction buffer pH 7.0 (Tris- HCl 30mM, DTT 1mM, Ascorbic acid 1mM, PMSF 1mM with PVP 6(mg/ml). The homogenate was centrifuged at 10,000g for 30min in refrigerated centrifuge (Plasto Crafts Superspin R-V/ F_M). Total soluble protein was estimated by the Lowry method [24].

For the measurement of total protein, the reaction mixture was 0.1ml of protein extract, 0.9ml of distilled water and 5ml of reagent C (Reagent A+ Reagent B) and kept for 10-15min. followed by adding 0.5ml of Folin's Phenol reagent (half strength). This reaction mixture was allowed to stand for 30 min for the development of color. The bluish green color developed was measured using spectrophotometer (double beam UV Vis–Cecil at 750nm. The protein content was determined by calculating the standard curve drawn for the pure commercial bovine serum albumin (Fig. 3.1).

2.2 SDS-PAGE

For evaluation of quality of protein the separation of protein SDS PAGE was done. The leaf and root tissues were homogenized and centrifuged to collect the supernatant as described above. The supernatant was added with equal volume of 2 × gel loading buffer. The mixture was heated at 100°C for 3-5 min. The protein sample was stored at -20°C until used for SDS-PAGE.
SDS-PAGE with discontinuous buffer system was used as described by Laemmili [25].
The reagents used in SDS-PAGE are indicated in Annexure-1.

2.2.1 Vertical Slab Preparation

Glass slab of 18 × 18 × 0.1cm were cleaned properly and space bars adjusted on the 2 sides before sealing with brown tape. A sufficient volume of resolving gel mixture of 12.5% (12.5ml acrylamide, 0.3ml SDS, 4.5ml distilled water, 1.5ml of 15% APS, 11.25ml Tris HCl (0.1mM), 20 µl TEMED) degassed the mixture and poured in between the glass plate's assembly, leaving about 4cm space at the top for a stacking to be polymerized later on. Then the 2% stacking gel mixture (2.0ml acrylamide, 7.4ml distilled water, 1.825 Tris HCl, 0.3ml of 10% SDS, 1.5ml of 1.5% APS and 15µl TEMED) was poured and the comb was inserted immediately into the stacking gel mixture. The assembly was left undisturbed overnight for the maturation of the gel at 4°C. After polymerization, the comb was removed carefully without distorting the shape of the wells. The glass plates were installed in the apparatus. The lower and upper chamber of the apparatus was filled with electrophoresis buffer in such a manner that no air bubbles are formed between the gel and buffer system.

2.2.2 Sample Loading and Electrophoresis

Prior to loading, the stored (at -20^{0}C) samples were heated in a boiling water bath for 3 min. The samples were loaded in the wells in a predetermined pattern. Electrophoresis was performed using electrophoresis electrode buffer solution (pH-8.3) at a voltage 8V/Cm for stacking gel and 15V/cm for resolving gel. At the end of the electrophoresis, the polypeptide bands were visualized by staining with Coomassie brilliant blue R-250 (CBB) or silver salts as described below.

2.2.3 Staining with Coomassie Brilliant Blue (CBB)

The gels were stained in a staining solution 3% w/v CBB R-250 dye in methanol: acetic acid: water (4:1:5) for 5-6 hours at room temperatures. The gels were destained in a destaining solution with continuous shaking on a platform rocker. During the process, the destaining solution was changed about 3-4 times. After complete destaining, the gels were stored in 7% ac ic acid solution for further observation and study.

2.2.4 Silver Staining of Gels

The method followed for silver staining was originally devised by Sammons et al., 1981 and later on modified by Schonle et al., [26]. The gels were incubated after electrophoresis with gentle shaking for 1 h to 1:30 h (may be overnight) in the fixative solution (methanol: glacial acetic acid: water) (25:12:63). Discarded the fixative solution and washed the gel in ethanol: water solution 1:1 for three times 20 minutes each. After washing, pretreat the gel in sodium thiosulphate (.02%). Discarded the above solution and then washed the gel with distilled water for 15 seconds about 3-4 times. Gel was incubated in silver nitrate (0.1 % solution of $AgNO_3$) for 20 minutes and washed again for 20 seconds under a stream of deionized water. In the last step of staining, the gel was placed in freshly prepared developer solution consists of sodium carbonate: sodium thiosulphate:

formaldehyde (6g: 1.6mg: 25 µl). The gel was incubated with gentle agitation. Stained bands of polypeptides appeared within minutes. The reaction was stopped after the desired contrast and it was obtained by washing the gel in stopper solution methanol: glacial acetic acid: distilled water (50:12:38). Finally, the gel was washed several times with distilled water and stored.

3. RESULTS

3.1 Protein Content

Protein Contents under Stresses in the Presence and Absence of Cad The Cad treatment of the seedling under either salinity or Pb and Cd stress increased the protein content significantly in the leaf. While this increase under multiple stresses was little in either of the multiple stresses condition like Cd plus NaCl, Pb plus NaCl except Cad plus NaCl. The increasing trend of proteins under Cad treatment of plants was observed in the presence of NH_4NO_3 also in the multiple stressed seedling leaves (Fig. 1).This was unlike to other response on total organic nitrogen, root/shoot length etc. A similar trend in protein content of the root of the seedlings was observed except that the magnitude of the protein increase was considerably higher in the seedlings exposed to either of the stresses alone. But the Cad response on proline content under multiple stresses (T_8, T_9) in leaf and root tissue was almost found same.

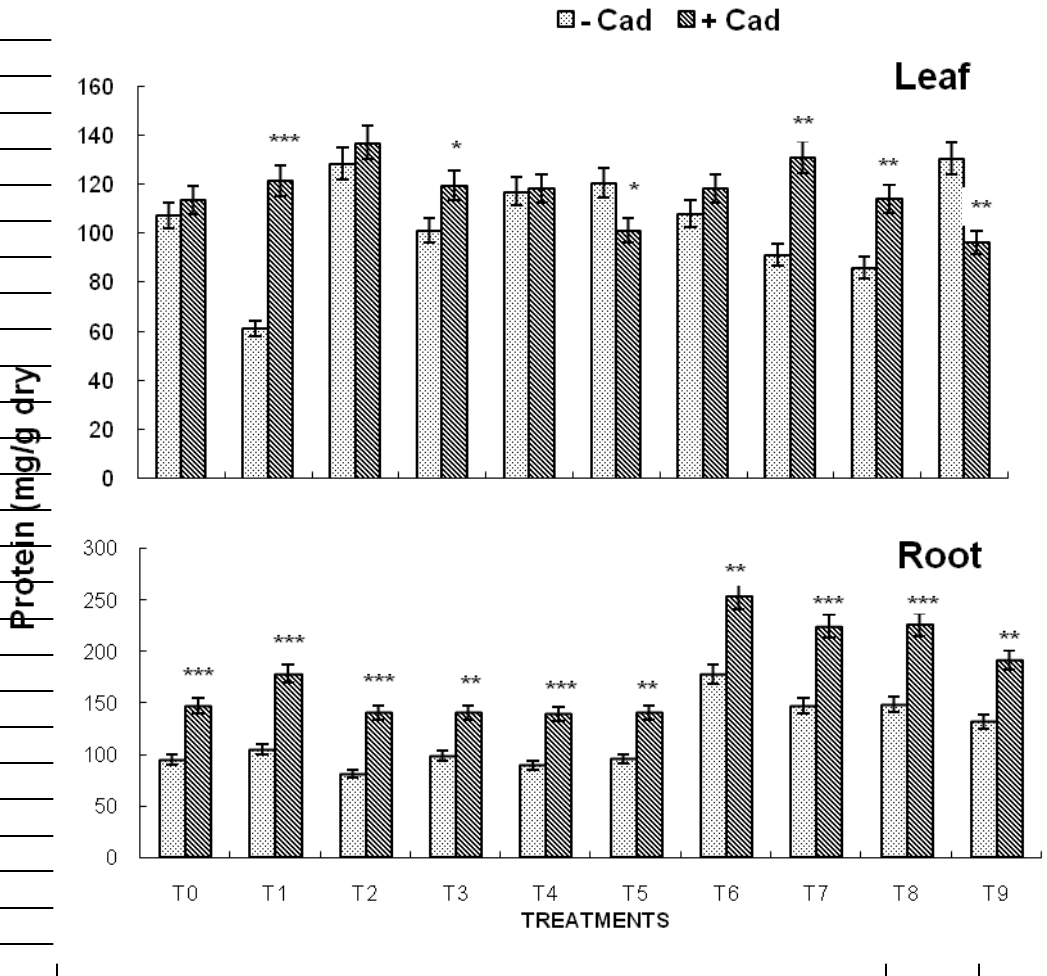


Fig 1:Protein content in the leaf and root tissues of 7 day old seedlings grown under salinity, metal and multiple stress with or without Cad. Data are mean value of replicas with (n=3) ± SD

Data are mean value of replicas with (n=3) ± SD. Asterisks indicate the significance of difference at $p<0.05$% (*) probably significant, $p<0.01$ (**) definitely significant, $p<0.001$ (***) highly significant and No asterisks indicate insignificant $p >= 0.05$.

	T0 - Control T1 - NaCl (100mM) T2 - NH_4NO_3 (5mM) T3 - NaCl+NH_4NO_3 T4 - Cd (1mM)	T5 - Pb (1mM) T6 - Cd+NaCl T7 - Pb+NaCl T8 - Cd+NaCl+NH_4NO_3 T9 - Pb+NaCl+NH_4NO_3			

- Cad

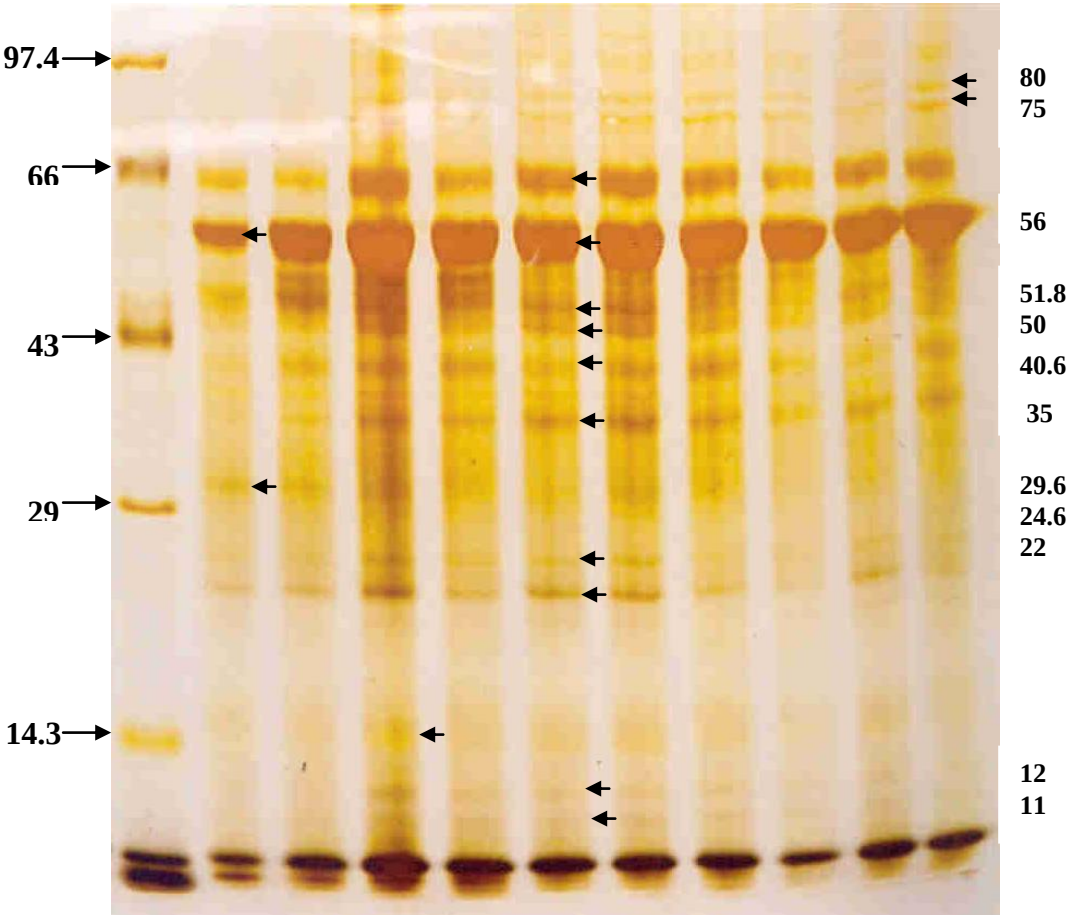


Fig. 2: Protein profile of leaf tissue of 7th day old seedling under various stress conditions without Cad.

M - Marker

T_0- Control
T_1 - NaCl (100mM)
T_2 - NH_4NO_3 (5mM)
T_3- NaCl+NH_4NO_3
T_4- Cd (1mM)
T_5 - Pb (1mM)
T_6- Cd+NaCl
T_7- Pb+NaCl
T_8 - Cd+NaCl+NH_4NO_3
T_9- Pb+NaCl+NH_4NO_3

3.2 PAGE Analysis of Protein

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

In leaf tissues of seedling showed presence of 66, 56, 51.8, 50, 40.6, 35, 24.6, 22, 14, 12 and 11 kDa peptides among these 66 and 56 kDa were highly expressed peptides (Fig. 2; Table 1). Under the saline condition, there was disappearance of 35, 14, 12 and 11 kDa peptide, whereas 56 kDa peptide expressions were little increased. The seedlings enriched with NH_4NO_3 in the nutrient induced the expression of few more peptides over control, such as 90, 88, 85, 80, 75, 51.8, 48 and 44 kDa peptides. Further, the NH_4NO_3 increased the expression of 66 and 56 kDa peptide as well. The salt induced 50 kDa peptide was suppressed by NH_4NO_3 supplementation. Simultaneously, when NH_4NO_3 treatment applied to salt stressed plants the high mol. wt. peptides like 90, 88, 85, 80, 75 and 29.6 kDa disappeared and also reduced the expression of 66 and 56 kDa peptides. The Cd treatment (lane T_4) only induced the 90, 88, 80, 75 and 50 kDa peptides over control. Where as, Pb caused expression of two more protein over Cd i.e. 85 and 44 kDa peptides; otherwise all other proteins similar to Cd induced one.

However, when Cd environment was blended with salinity, the 85, 51.8 and 44 kDa pepetides appeared over only Cd exposed plants. Though, Pb plus salinity (T_7) environment depressed 90, 44, 40.6, 37, 35 and 29.6 kDa peptides and slightly induced 48 kDa expression over only Pb exposed plants. In view of these cross talks of different metal plus salinity stress at molecular level, the stress combinations were blended with NH_4NO_3 to examine the peptide profile in leaf tissues. This showed the suppression of 90, 85, 44, 40, 40.6, 37 and 29.6 kDa peptides in Cd plus salinity exposed plants, while suppression of 85, 44, 37 and 29.6 was observed with Pb combination.

- Cad

M	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
90	-	-	+	-	+	+	+	-	-	+
88	-	-	+	-	+	+	+	+	+	+
85	-	-	+	-	-	+	+	+	-	-
80	-	-	+ +	-	+	+	+	+	+	+
75	-	-	+	-	+	+	+	+	+	+
66	+ +	+ +	+ + +	+	+ +	+ + +	+ +	+	+ +	+ +
56	+ +	+ + +	+ + + +	+ + +	+ + +	+ + + +	+ + + +	+ + +	+ + +	+ + +
53.2	+	++	+++	++	+	+	+	+	+	+
51.8	-	+	+ +	+ +	-	+ +	+	+	+	+
50	-	+	-	-	+	+	+	+	+	+
48	-	-	+	+	-	-	+	+	+	+
44	-	-	+ +	+	-	+ +	+	-	-	-
40.6	+	+	+ +	+	+	+	+	-	-	+
37	+	+	+ +	+	+	+	+	-	-	-
35	+	-	+ +	+	+	+	+	-	+	+
29.6	+	+	+ +	-	-	+	-	-	-	-
24.6	+	+	+ +	+	+	+	+	+	+	+
22	+	+	+ +	+	+	+	+	+	+	+
14	+	-	+	+	+	+	+	+	+	+
12	+	-	+	+	+	+	+	+	+	+
11	+	-	+	+	+	+	+	+	+	+

Table 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 (5mM)
T_3- NaCl+NH_4NO_3
T_4 - Cd (1mM)
T_5 - Pb (1mM)
T_6- Cd+NaCl
T_7- Pb+NaCl
T_8 - Cd+NaCl+NH_4NO_3
T_9- Pb+NaCl+NH_4NO_3

+ Cad

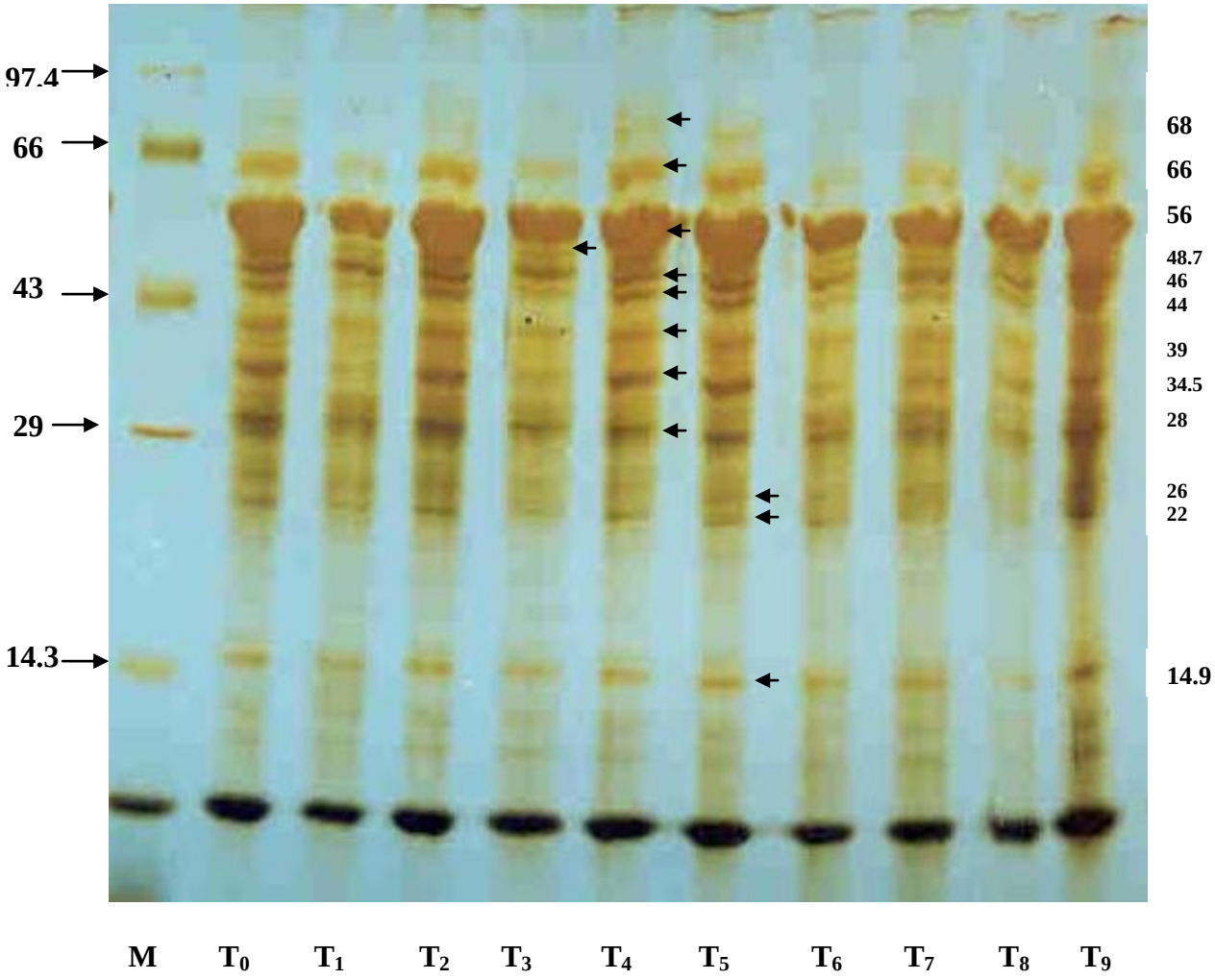


Fig. 3: Protein profile of leaf tissue of 7th day old seedling under various stress conditions with Cad.

Rest legend same as in Fig. 2

+ Cad

M	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
73.8	+	-	+	-	+	+	-	-	-	+
68	+	-	+	-	+	+	-	-	-	+
60	+ +	+	+ +	+	+ +	+ +	+	+	+	+ +
56	+ + + +	+++	+ + + +	+ ++	+ + + +	+ + + +	+ + +	+ + +	+ ++	+ + + +
48.7	+ +	+	+ +	+	+ +	+ +	+	+	+	+
46	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+ +
44	+ +	+	+ +	+	+ +	+ +	+	+	+	+
39	+ +	+	+ +	+	+ +	+ +	+	+	+	+
34.5	+ +	+	+ +	+	+	+	+	+	+	+
31	+	+	+	+	+	+	+	+	+	+
28	+ +	+	+ +	+	+ +	+ +	+	+	+	+ +
26	+	+	+	+	++	+	+	+	+	+ +
22	+ +	+	+ +	+	+	+	+	+	+	+ +
14.9	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	-	+	-	+
11.8	+	+	+	+	+	+	+	+	-	+

Table 2 : Protein profile of leaf tissue of 7th day old seedling under various stress conditions with Cad.

Rest legend same as in Table 1

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

The Cad supplementation to the seedlings induced sixteen peptides over control (without Cad), which was having 11 peptides only (Fig. 3; Table 2). The higher expression was in order of 56>60>48.7, 46=44, =39, =34.5, =28, =26, =22>73.8, 68, 31, 14.9, 13, 11.8. When these plants were subjected to salinity either caused disappearance of 73.8 and 60 kDa or little depressed 63.8, 48.7, 44, 39, 34.5, 28, 26 kDa or no change in rest of peptides.

Inclusion of NH_4NO_3 in those treatments to the plants did not alter the expression of all those peptides appearing under salinity except diminishing 73.8 and 68 kDa peptides (T_3). However, when Cad was supplemented to Cd stressed plants, no change in peptide expression over Cad treated one, while none of metal suppressed peptide expressed due to Cad. A similar response was noticed in Pb exposed plants.

Moreover, when these metal exposed plants under saline environment supplied with Cad, the total response in peptide expression remained same in both cases except that 13 kDa peptide suppression in Cd environment. The status of peptide expression again did not change even when multiple stressed plant was supplied with NH_4NO_3 as well as Cad (T_8 and T_9).

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

The study of protein profile in root tissues revealed that the numbers of low mol. wt. proteins were either suppressed or induced depending on the stress condition. The total 12 peptides (Fig. 4; Table 3) band were observed in controlled seedlings root tissues. Out of which, 56, 36, 34, 22, 20, 13.4 and 12.5 kDa peptides were highly expressed. Salt stress either caused complete

suppression of 44, 40 or little suppression of 56, 36, 34 whereas, slightly elevated the expression of 12.5 kDa peptide. NH_4NO_3 presence in the seedlings environment did affect the expression of 56, 44, 40, 36, 34, 22, 20, 13.4 kDa peptides. 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 salt plus NH_4NO_3 showed suppression of 36, 34, 13.4 and 12.5 kDa peptides. The Cd and Pb had almost similar kind of effect on peptides expression except that Cd suppressed the appearance of 44, 30 kDa, but simultaneously induced a novel protein of 18 kDa; where as Pb suppressed 40 kDa completely.

When plants were subjected to Cd plus salinity, the over expression of 56 kDa peptide and novel peptide of 50 kDa was observed. On the other, Pb in the saline environment caused complete suppression of 56, 44, 36, 34, 30 along with little expression of 22, 20, 13.4, 12.5 and 12 kDa peptides and almost similar kind of trend was observed when metal plus salinity plus NH_4NO_3 environment was provided to the seedlings over only metal plus salinity.

- Cad

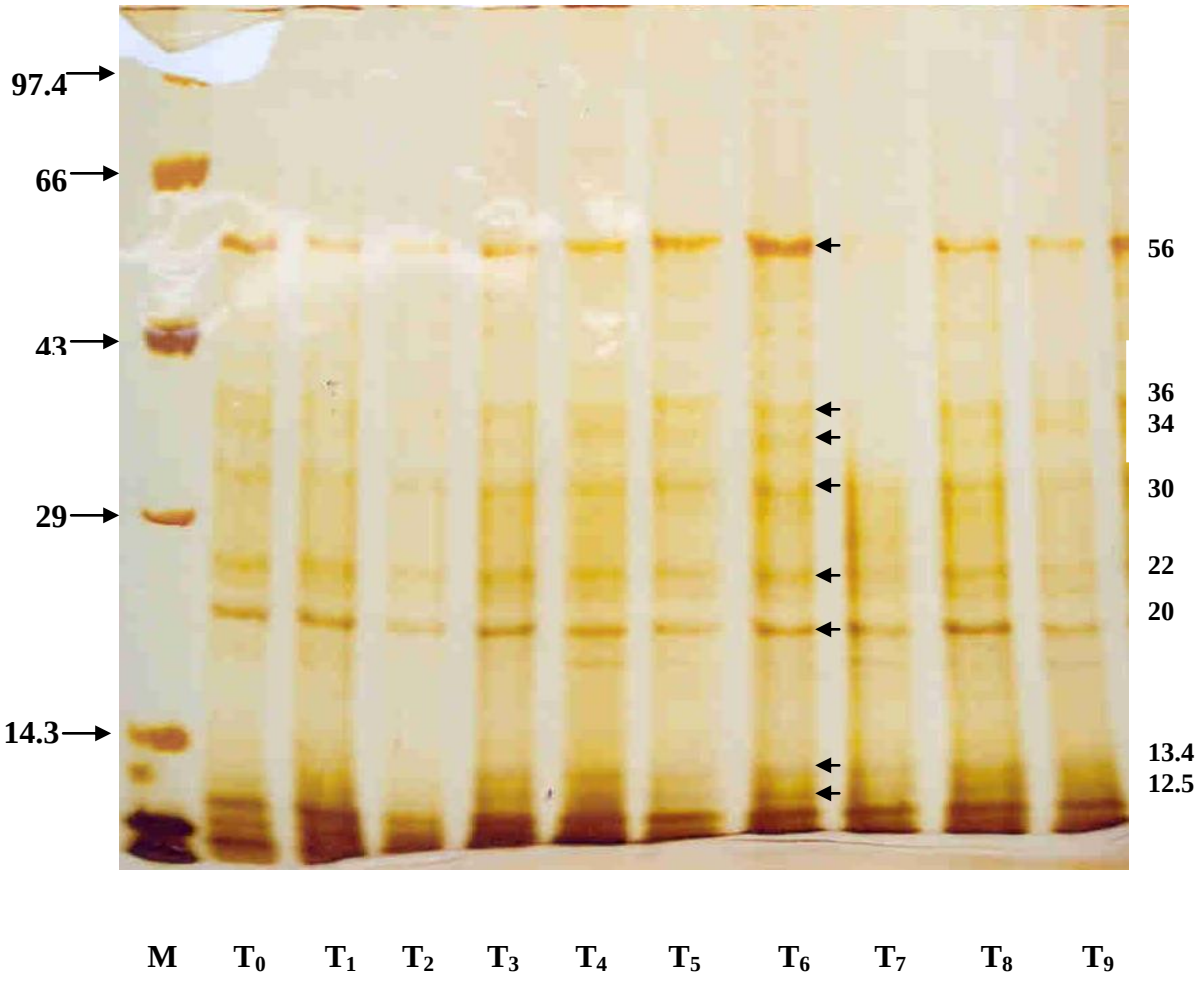


Fig. 4: Protein profile of root tissue of 7th day old seedling under various stress conditions without Cad.

Rest legend same as in Fig. 1

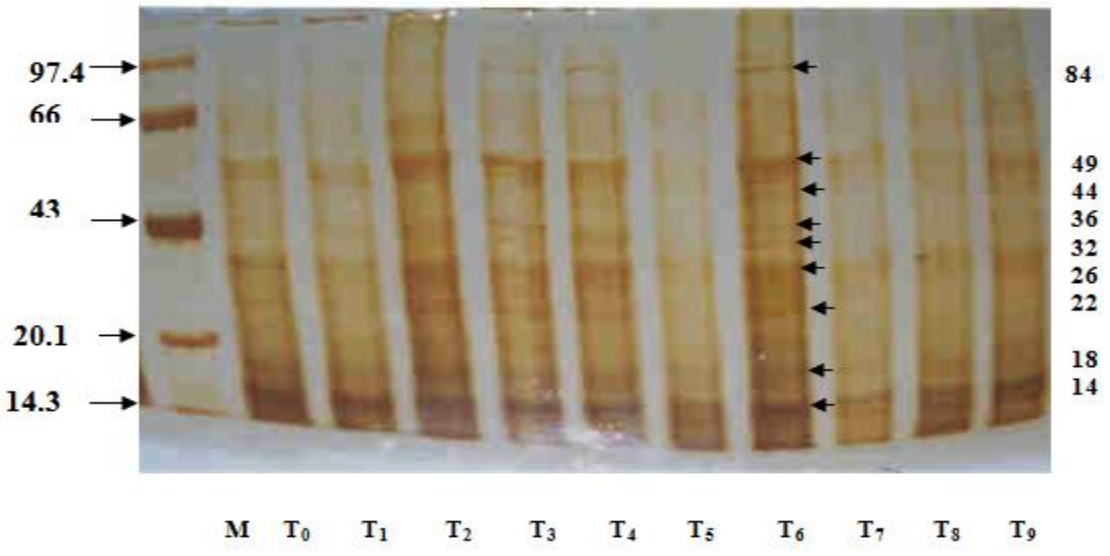
- Cad

M	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
56	+ +	+	+	+ +	+ +	+ +	+ + +	-	+ +	+ + +
50	-	-	-	-	-	-	+	-	-	+
44	+	-	-	+	-	+	+	-	+	+
40	+	-	-	-	+	-	+	-	+	+
36	+ +	+	+	+	+ +	+ +	+ +	-	+ +	+ +
34	+ +	+	+	+	+	+ +	+ +	-	+ +	+
30	+	+	+	+ +	-	+	+ +	-	+	+ +
28	-	-	-	-	-	-	-	-	+	+ +
23	+ +	+ +	+	+ +	+ +	+	+ +	+	+ +	+ +
22	+	+	+	+ +	+ +	+	+	+	+	+ +
20	+ +	+ +	+	+ +	+	+	+ +	+	+ +	+ +
18	-	-	-	-	+	-	+	+	+	+
13.4	+ +	+ +	-	-	+	+	+	+	+	+
12.5	+	+ +	+ +	-	+	+	+	+	+ +	+
12	+ +	+ +	+ +	+ +	+ +	+	+ +	+	+ +	+

Table 3 : Protein profile of root tissue of 7th day old seedling under various stress conditions without Cad.

Rest legend same as in Table 1

+ Cad



+ Cad

M	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
100.2	-	-	-	-	-	-	+	-	-	-
90	-	-	-	-	-	-	+	-	-	+
84	-	-	+	+	+	-	+ +	-	-	+
70	+	+	+	+	+	-	+	-	+	+
68	+	+	+	+	+	-	+	-	+	+
49	+	+	+	+	+	+	+	+	+	+
44	+	+	+	+	+	-	+	-	-	+
36	+	+	+	+	+	-	+	-	-	-
32	+ +	+	+ +	+	+	-	+	-	-	+
30	+ +	+	+ +	+	+	-	+	+	+	+
26	-	-	+	+	-	-	+	-	-	+
22	+	+	+	+	+	+	+ +	-	-	+
19	-	-	+	-	-	-	+	-	-	-
16.8	+	+	+	+	+	-	+	-	+	+
14	+	+	+ +	+	+	+	+ +	+	+	+
13.5	-	-	+	+	+	+	+	+	+	+

Table 4 : Protein profile of root tissue of 7th day old seedling under various stress conditions with Cad.

Rest legend same as in Table 1

4. DISCUSSION

4.1 Protein Content

Protein level in leaf and root tissues was enhanced with the supplementation of NH_4NO_3 and Cad under salinity. In root tissue, effect of Cad and NH_4NO_3 was high as compared to leaf tissue (Fig.6 a,b). The metal [27] or salinity [27-29] caused decline in protein has been observed (Table 2.1).

The reason for the higher level of protein content in RH-30 may be the synthesis of additional stress proteins, involved in alleviating NaCl stress. The several new proteins which are synthesized in response to an altered environment have been reported as "stress proteins" or "shock proteins" in *Bouvardia ternifolia* plants adapted to NaCl (Leon *et al.,* 1980). Polyamines having polycationic in nature at physiological pH and binding with several negatively charged molecules, such as DNA [30-31] and proteins [32] may modify the synthesis of proteins. Polyamines effect on protein could be through inhibiting protease activity which has been demonstrated *in vitro* [33]. The increased protein level under salinization has been reported in crops like *mungbean* [34] and *Brassica* [35] which may be due to increased synthesis of pre-existing as well as new set of proteins [36]. Sureena *et al.,* [37] reported increase in protein content in the cultivar of *B. juncea* in comparison to *B. carnita* and *B. napus* under salinity. The elevation in protein content (Fig.6 a,b) could considered to be due to either general enhancement of organic nitrogen content [38] or increase in some fractions of proteins [39]. Increase in protein content under stress conditions, especially the induction of new proteins which might have role in stress tolerance and thus it provides a scope to identify and characterize the respective proteins especially with Cad. As it could be linked with the alteration of gene expression due to any stress may change the over all response of the plant in general and protein level in particular either through *denovo* synthesis or degradation process.

A few studies have been conducted to identify polypeptides having possible role in salt stress tolerance, both in cell culture [40] and in whole plants [41]. Among these polypeptides, a few polypeptides were thought to be important for the tolerance/resistance of the plants. Under the multiple stresses, inorganic nitrogen or polyamine seems to be inducing elementary response.

The data of experiments here is pointing out that the photosynthetic pigments and protein can be an estimate of plant growth under stress (es) which can be ameliorated on supply of NH_4NO_3 but more efficiently with Cad.

4.2 Protein Profile

4.2.1 Leaf Protein

In an attempt to understand the molecular basis of salt and metal tolerance by the supplementation of Cad, SDS-PAGE was done in order to identify proteins involved. The seedlings enriched with NH_4NO_3 in the nutrient induced the expression of few peptides of 90, 88, 85, 80, 75, 51.8, 48 and 44 kDa over control (Fig. 1; Table 1). Some specific polypeptides were also observed with exogenous supplementation of Cad (63.8, 56, 48.7, 46, 44, 39, 34.5, 28, 26, 22 and 14.9 kDa). Thus Cad supplementation to metal and salt stressed seedlings also showed variation in polypeptides (Fig. 2; Table 2). Hence, the differential expression of peptides in response to Cad supplementation indicates the differential regulation which may probably render stress tolerance. Cad specific polypeptide seems to be positively linked to salt tolerance and therefore, it is tempting to speculate the expression of gene(s) of this protein may be linked to salt tolerance. A 53.2 kDa peptide accumulates only with salt stress at vegetative stage in *B.juncea* has been observed by other also [42]. The specific polypeptides of different strains can be due to genetic make up as well different ecological adaptation and /or due to differential status of stress tolerance is envisioned. The analysis of total protein extracts from *A. philoxeroides* leaves revealed that three peptides of mol. wt. 80, 39 and 28 kDa had appeared in Cd-treated leaves [43] and the 80 kDa pepetide observed in *Brassica juncea* here in the presence of Cd (Fig.1; Table 1)

Polyamines stimulation of the synthesis of protein like OppA protein [44] and ribosomal proteins [45] are known and implicated in cell growth. Cad stimulated the synthesis of 46 kDa polypeptide and its expression was up regulated under salt and metal stress. A distinct protein of 60 kDa peptides has been found abundantly in the regenerated plantlets of *Dactylis glomerata* at higher salinity [46]. Similar peptide was observed with Cad under salinity stress (Fig. 8; Table 2). Role of this protein was presumed to be in signal transduction and involved in the number of stress responses. Induction of polypeptide of similar molecular weight 60 kDa under saline stress (Fig. 2; Table 2) may be correlated with the protein kinase (RPK1). A 44 and 39 kDa were also induced with Cad has to be looked for stress regulation.

Cad might be alleviating salt stress toxicity through pH regulation, osmoprotection by ionic balance, detoxification of enzymes and other proteins or even by playing role in hormone metabolism [10] where a number of peptides involved. A 22 kDa peptide appeared with Cad treat with NH_4NO_3 to Cd/Pb stressed plant leaf that presence has been observed in adapted tolerant cultures of *Shamuti* orange exposed to NaCl, ABA and PEG [47]. This peptide was not cross reactive with osmotin (26 kDa) was associated with the soluble fraction of the cell and has been found in tissue of growing trees as well [30]. Moreover, the Cad modulation of 22 kDa peptide assigned as protease in number of studies [48] was very prominent in leaf tissues, which was otherwise increased by stresses (Fig.1, 2; Table 1,2). Several changes in protein profile *B.napus* leaf under water stress have been also noted [49]. It is tempted to speculate that *B.juncea* seedlings also tend to accumulate 22 kDa peptide under salt, metal or combined stress. A 26 kDa protein in tissues (Fig. 2; Table 2) seems to be equivalent as reported in barley, rice, *Hordeum vulgare*, and *Mesembryanthemum crystallinum* by salt stress [50]. Thus, it could be conferred that some of specific peptides being induced by Cad may be able to confer tolerance to plants. It could be further suggested that diamines induced protein(s) besides stabilizing the molecular structure of nucleic acids or growth related protein, may promote the association of ribosomal subunits and may also affect the elongation of nascent polypeptide chains [30].

4.2.2 Root Protein

Root system plays a pivotal role in ion and water uptake and co-ordinate in ion regulation. Sodium chloride presumably enters into root cell by apoplastic and symplastic pathways [51]. Ion uptake, transport and exclusion all may be regulated by genetic makeup of plants [52]. The induction of 44 kDa appearently may be implicated in the maintenance of V-ATPase subunit, an equivalent protein is isolated from red beet which helps in non integral association with the tonoplast membrane [53]. The induction of 44 kDa protein which appeared prominently under salinity in presence of Cad indicated that the species is tending to adopt which is further facilitated by Cad (Fig. 3; Table 3).

The putative 44 kDa peptides in red beet appear to be structurally similar to yeast Vma6P which is homologous to V-ATPases. These are electrogenic proton pumps involved in the acidification of endomembrane compartments in all eukaryotic cells [54]. Thus, a 44 kDa peptide expressed (Fig. 4; Table 4 in gel) with the supplementation of Cad in metal stressed plants indicates that this protein might have some protective role, which needs to be further characterized. The Cad could also act as an N-source, as suggested in case of putrescine another diamine of PA family [55]. It is also reported that exogenous labeled Spd binds to a specific 18 kDa protein in thin layer tobacco tissue cultures [32] and to a larger protein in oat protoplasts [56]. Further, it could be deduced here that Cad could induce synthesis of 18 kDa protein, thereby inducing gene expression related for stressed plant growth. Rodriguez *et al.,* [57]

discovered ~12 kDa protein in *Pisum sativum*, similar peptide was found under Pb and Cd stress with Cad. Didierjean *et al.*, [58] had discovered a 15 kDa peptide in *Zea mays*, a near by peptide of 14 kDa was appeared in the presence of Cad under metal stress. These observations add to that Cad may have role in mitigating the plant under stress condition.

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AUTHORS PROFILE

Dr. Pushpa C.Tomar received her **B.Sc.** (Medical) degree from Maharishi Dayanand University (MDU), Rohtak, Haryana (India) in 1998, her **M.Sc.** (Biotechnology) degree from Guru Jambheshwar University, of Science & Technology, Hisar, Haryana (India) in 2000 and her **Ph.D** in Plant biotechnology and stress physiology from Maharishi Dayanand University (MDU), Rohtak, Haryana (India) in 2008. She has more than 10 years of experience in research and teaching. She has authored several research papers in National and International journals.

Dr. Shyam Narayan Mishra received his **B.Sc.** degree from Gorakhpur University, Gorakhpur in 1977, his **M.Sc.** (Botany) from Gorakhpur University in 1979, Gorakhpur and his **Ph.D.** (Life Sciences) from Indore University, Indore in 1985. He has more than 35 years of experience in research and teaching. He has authored several books and research papers in National and International journals.

Charu Rajpal received her **B.Tech.** (Biotechnology) degree from Maharishi Dayanand University (MDU), Rohtak, Haryana (India) in 2012, her **M.Tech.** (Biotechnology) degree from Amity University, Noida in 2014. She is now pursuing Ph.D from Manav Rachna International University Faridabad, Haryana. Her area of interest is plant stress physiology.