



FINAL PROGRESS REPORT OF UGC_MRP
MAJOR-BIOC-2013-6699



Sanctioned to:-

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OBJECTIVES OF THE PROJECT

Aim of the present investigation was to study the effect of salt stress on key enzymes of chlorophyll biosynthesis, enzymatic components and non-enzymatic components of anti-oxidative system as well as proteomic study with a view to work out the possible correlation. In maize leaves interactive effects of salt and cadmium stress was also analysed on biochemical parameters.

DETAILED PROGRESS REPORT OF THE RESEARCH WORK

Effect of different concentrations of NaCl on chlorophyll a, chlorophyll b, total chlorophylls, Chlorophyll a/b ratio, protochlorophyllide and carotenoids in etiolated maize leaf segments during greening was performed. Results are shown in Table 1A and B.

Table 1A. Effect of salt stress imposed by using NaCl on total chlorophylls, carotenoids and protochlorophyllide content in etiolated maize leaf segments during greening.

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at $25 \pm 3^\circ\text{C}$.

NaCl conc. (mM)	Total Chlorophylls ($\mu\text{g g}^{-1}$ FW)	Carotenoids ($\mu\text{g g}^{-1}$ FW)	Protochlorophyllide (Fluorescence 674 nm g^{-1} FW)
0	432 \pm 28 (100)	71 \pm 3 (100)	1167.5 \pm 43 (100)
50	346 \pm 9** (80)	65 \pm 2* (92)	----
100	282 \pm 12** (65)	54 \pm 2** (76)	----
150	203 \pm 13*** (47)	50 \pm 2** (70)	----
200	136 \pm 14*** (32)	38 \pm 3** (54)	----
300	36 \pm 02*** (08)	38 \pm 2** (54)	667.8 \pm 19 (57)

Table 1B. Effect of salt stress imposed by using NaCl on chlorophyll a, chlorophyll b and chlorophyll a / b ratio in etiolated maize leaf segments during greening.

NaCl conc. (mM)	Chlorophyll 'a' ($\mu\text{g g}^{-1}$ FW)	Chlorophyll 'b' ($\mu\text{g g}^{-1}$ FW)	Chl a/b ratio
0	349 \pm 20 (100)	83 \pm 9 (100)	4.5 (100)
50	286 \pm 11** (82)	60 \pm 3** (72)	4.8* (107)
100	232 \pm 10** (66)	50 \pm 4** (60)	4.8* (107)
150	167 \pm 10*** (48)	36 \pm 4*** (43)	5.0* (111)
200	112 \pm 12*** (32)	22 \pm 2*** (27)	5.2** (116)
300	31 \pm 03*** (09)	05 \pm 0*** (06)	6.9*** (153)
R² value	0.99	0.97	0.81

Values relative to control are given in parentheses.

Level of significance: 'p' values <0.05*, <0.01**, <0.001*** compared with control.

Effect of different concentrations of NaCl on protein, RNA and DNA content in etiolated maize leaf segments during greening were performed. Results are shown in Figure 1A and B.

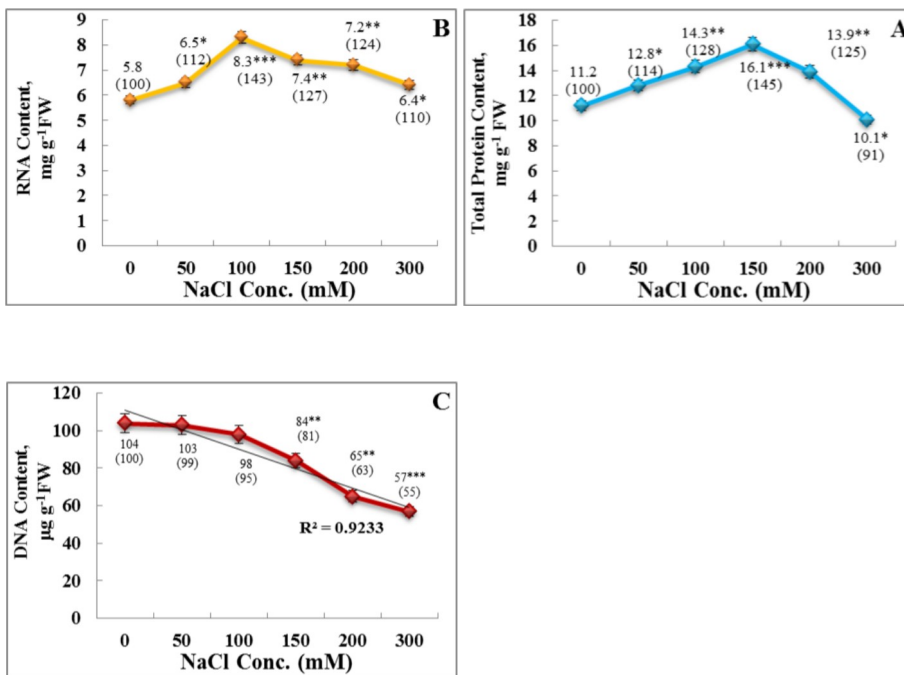


Figure 1. Effect of salt stress imposed by using NaCl on total protein, total RNA and DNA content in etiolated maize leaf segments during greening.

(A) Total protein (B) Total RNA (C) Total DNA

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at $25 \pm 3^\circ\text{C}$.

Values relative to control are given in parentheses

Level of significance 'p' values $<0.05^*$, $<0.01^{**}$, $<0.001^{***}$ compared with control.

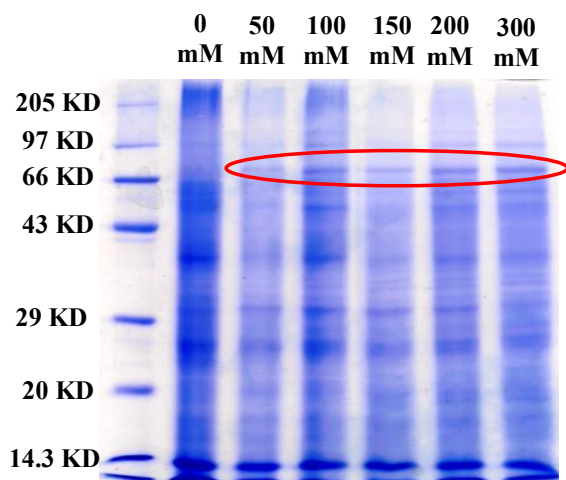


Figure 2. Effect of salt stress imposed by using NaCl on protein profile in excised etiolated maize leaf segments during greening.

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at $25 \pm 3^\circ\text{C}$.

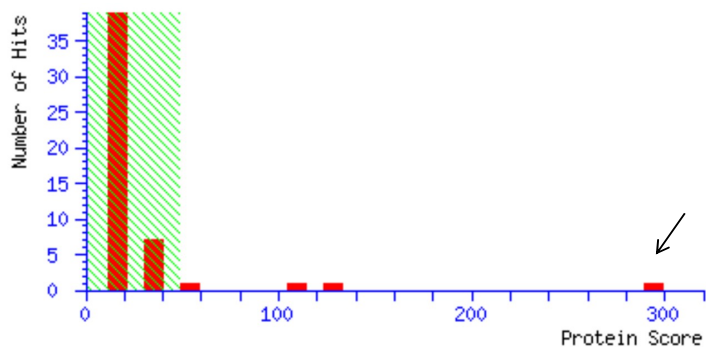


Figure 3. MALDI-TOF mass spectrum histogram.

It shows results from a protein database search with the MS spectrum peak list data using Mascot. The top score (295) matches the correct *Cenchrus americanus* accession.

Table 2A. The MALDI TOF mass spectrum sequence of peptides matched with [gi145388994](#) chloroplast heat shock protein 70 of *Cenchrus americanus* (Protein score: 295).

S. No.	Peptides Identified by MS / MS	Mr (expt)	Mr (calc)
1	K.DIDEVILVGGSTR.I	1372.9911	1372.7198
2	K.QFAAEEISAQVLR.K	1461.0489	1460.7623
3	K.AVITVPAYFNDSQR.T	1580.1024	1579.7995
4	R.QAVVNPENTFFSVKR.F	1735.2343	1734.9053
5	K.LQFKDIDEVILVGGSTR.I	1889.3760	1889.0258
6	K.SEVFSTAADGQTSVEINVLQGER.E	2436.6431	2436.1769

Table 2B. The MALDI TOF mass spectrum sequence of peptides matched with [gi302786294](#) hypothetical protein SELMODRAFT_267815 of *Selaginella moellendorffii* (Protein score: 125).

S. No.	Peptides Identified by MS / MS	Mr (expt)	Mr (calc)
1	K.QFAAEEISAQVLR.K	1461.0489	1460.7623
2	K.AVITVPAYFNDSQR.T	1580.1024	1579.7995
3	R.QAVVNPENTFFSVKR.F	1735.2343	1734.9053
4	K.KQDITITGASTLPQDEVER.M	2100.5066	2100.0699

Table 2C. The MALDI TOF mass spectrum sequence of peptides matched with [gi302786294](#) hypothetical protein VITISV_000728 of *Vitis vinifera* (Protein score: 118).

S. No.	Peptides Identified by MS / MS	Mr (expt)	Mr (calc)
1	K.DLDEVILVGGSTR.I	1372.9911	1372.7198
2	K.QFAAEEISAQVLR.K	1461.0489	1460.7623
3	R.QAVVNPENTFFSVKR.F	1735.2343	1734.9053
4	K.SEVFSTAADGQTSVEINVLQGER.E	2436.6431	2436.1769

Mr: Nominal Mass

Table 2D. The MALDI TOF mass spectrum sequence of peptides matched with [gi302764140](#) hypothetical protein SELMODRAFT_230659 *Selaginella moellendorffii* (Protein score: 51).

S. No.	Peptides Identified by MS / MS	Mr (expt)	Mr (calc)
1	K.LEFKDINEVILVGGSTR.I	1890.3833	1889.3760

Mr: Nominal Mass

Table 3. Effect of salt stress imposed by using NaCl on ALA synthesizing activity in etiolated maize leaf segments during greening.

Leaf segments from dark grown maize seedlings were treated with ¼th strength Hoagland solution containing desired concentrations of NaCl in continuous light for 24 h at 25 ± 3°C. Afterwards leaf segments were incubated with phosphate buffer (50 mM, pH 6.0) containing levulinic acid (60 mM) in light and dark at 25 ± 3°C for 4 h.

NaCl conc. (mM)	ALA synthesizing activity (nmole ALA formed h ⁻¹ g ⁻¹ FW)		
	Light (L)	Dark (D)	L-D
0	139 ± 4.7 (100)	56 ± 2.2 (100)	83 ± 4.1 (100)
50	142 ± 4.8 (102)	43 ± 2.1** (77)	99 ± 4.7** (119)
100	148 ± 4.9 (106)	40 ± 2.0** (71)	108 ± 4.8** (130)
150	70 ± 2.4*** (50)	38 ± 1.7** (68)	32 ± 1.8*** (39)
200	48 ± 2.0*** (35)	31 ± 1.6*** (55)	17 ± 0.5*** (20)
300	33 ± 1.7*** (24)	27 ± 1.4*** (48)	06 ± 0.3*** (07)
R² value	0.81	0.89	0.71

Values relative to control are given in parentheses.

Level of significance: 'p' values <0.05*, <0.01**, <0.001*** compared with control.

Table 4. Effect of salt stress imposed by using NaCl on ALAD activity, PBGD activity and chlorophyllase activity in etiolated maize leaf segments during greening.

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at $25 \pm 3^\circ\text{C}$.

NaCl conc. (mM)	ALAD activity (nmole PBG formed $\text{h}^{-1} \text{g}^{-1}$ FW)	PBGD activity (nmole urogen formed g^{-1} FW)	Chlorophyllase activity (% chlorophyll degraded h^{-1})
0	154 \pm 5 (100)	19.3 \pm 0.1 (100)	92.8 \pm 2 (100)
50	159 \pm 5 (103)	--	93.1 \pm 2 (100)
100	176 \pm 4* (114)	--	91.9 \pm 2 (99)
150	120 \pm 4** (78)	--	89.6 \pm 2 (97)
200	89 \pm 6*** (58)	--	87.3 \pm 2* (95)
300	73 \pm 6*** (47)	8.6 \pm 0.2*** (45)	81.2 \pm 1* (88)
R² value	0.77	---	0.92

Values relative to control are given in parentheses.

Level of significance: 'p' values <0.05*, <0.01**, <0.001*** compared with control.

(ii) Second phase:

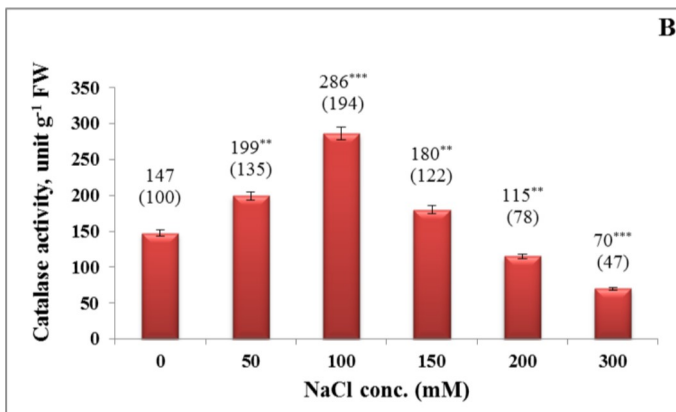
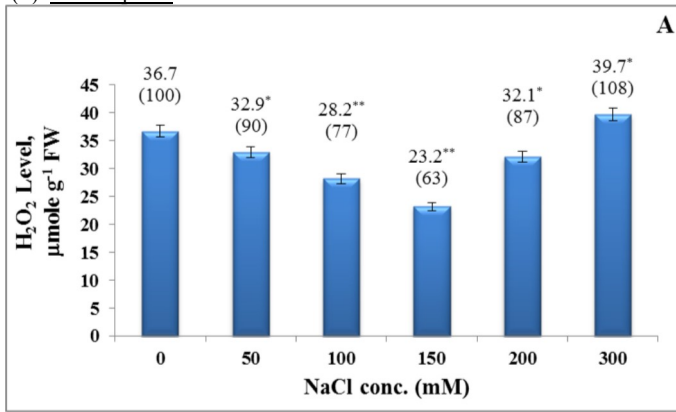


Figure 4. Effect of salt stress imposed by using NaCl on H₂O₂ level and catalase activity in etiolated maize leaf segments during greening.

(A) H₂O₂ level

(B) Catalase activity

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at 25 ± 3°C.

Values relative to control are given in parentheses

Level of significance 'p' values <0.05*, <0.01**, <0.001***

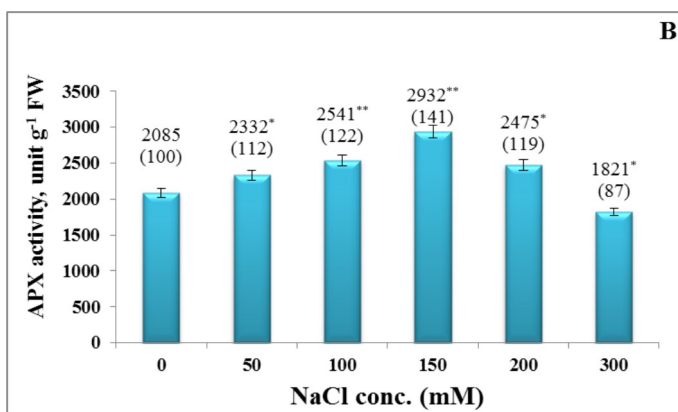
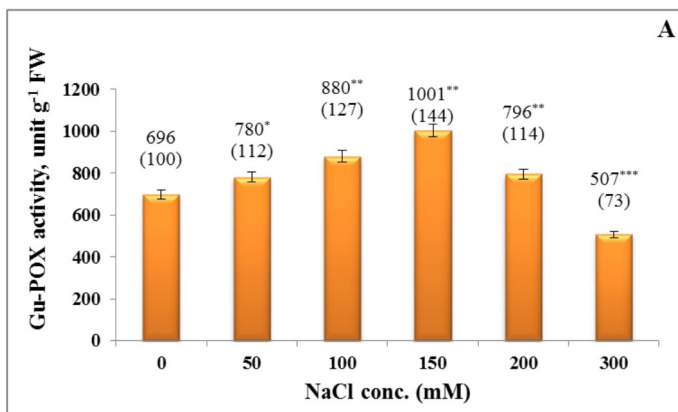


Figure 5. Effect of salt stress imposed by using NaCl on Gu-POX and APX activity in etiolated maize leaf segments during greening.

(A) Gu-POX activity (B) APX activity

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at 25 ± 3°C.

Values relative to control are given in parentheses

Level of significance 'p' values <0.05*, <0.01**, <0.001***

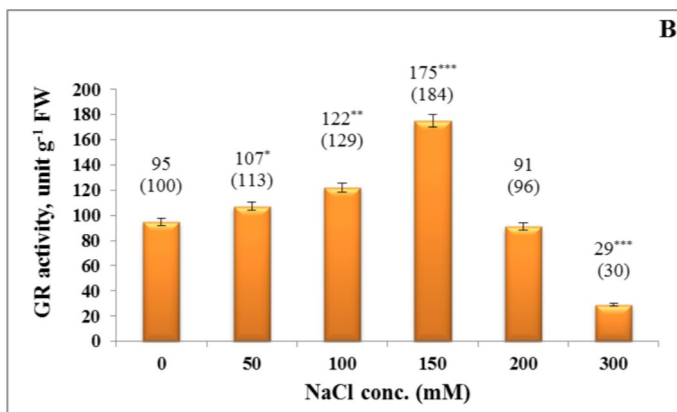
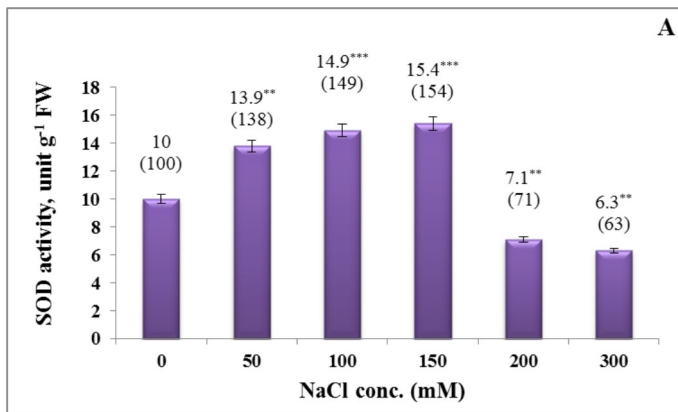


Figure 6. Effect of salt stress imposed by using NaCl on SOD and GR activity in etiolated maize leaf segments during greening.

(A) SOD activity (B) GR activity

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at $25 \pm 3^\circ\text{C}$.

Values relative to control are given in parentheses

Level of significance 'p' values <0.05*, <0.01**, <0.001.

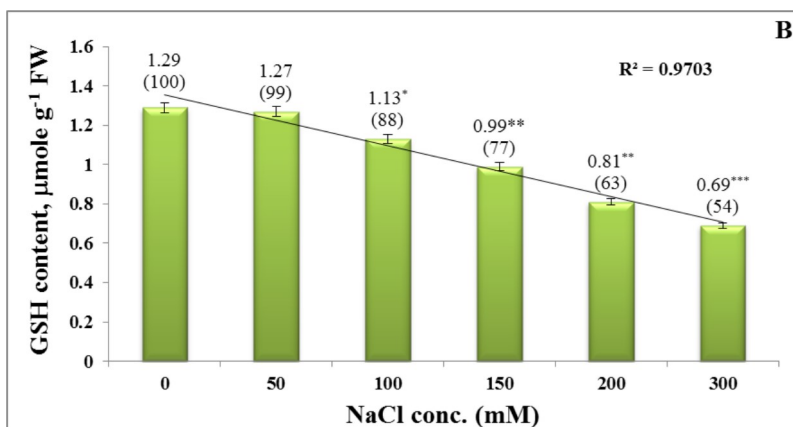
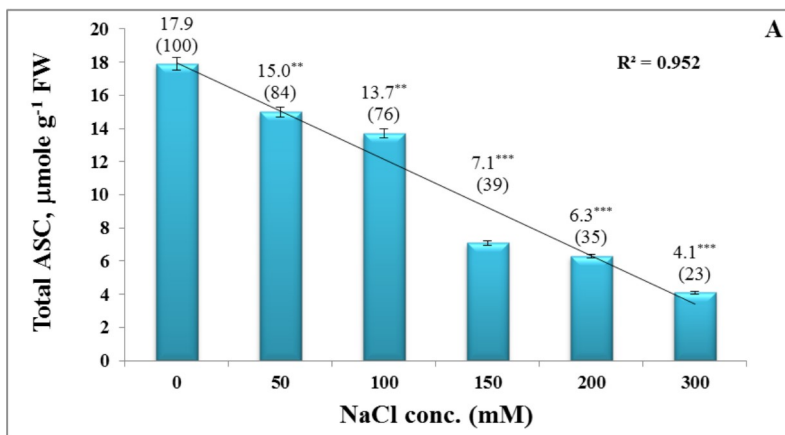


Figure 7. Effect of salt stress imposed by using NaCl on total ascorbate and GSH content in etiolated maize leaf segments during greening.

(A) Total ASC (B) GSH content

Leaf segments from dark grown maize seedlings were treated with varying concentrations of NaCl in continuous light for 24 h at 25 ± 3°C.

Values relative to control are given in parentheses

Level of significance 'p' values <0.05*, <0.01**, <0.001***

**Interactive effects of salt and cadmium stress on biochemical parameters in
maize seedlings (*Zea mays*)**

The details of the work are as follows:-

Salinity and heavy metals are two important abiotic stress factors for maize crop. The present study was aimed to evaluate the effects of salinity (NaCl) and heavy metal (Cd) stress individually and in combination on maize (*Zea mays*, Ganga safed-2) seedlings under controlled conditions.

1. Effect of CdCl₂ (μM)

Table 1A. Effect of Cd stress imposed by using CdCl₂ on germination %, length of root and shoots (cm), fresh weight of root and shoots. Maize seedlings were grown with varying concentration of CdCl₂ solution under continuous light.

Treatment	Control	100μM	200μM	300μM	500μM
Germination %	86±3.3 (100)	76±8.8 (88.5)	66±8.8 (76.92)	66±3.30 (76.92)	63±8.80 (73.08)
Length of root (cm)	10.5±0.42 (100)	8.6±0.28 (81.65)	6.8±0.20 (65.14)	6.4±0.18 (61.05)	5.4±0.19 (51.65)
Length of shoot (cm)	5.7±0.22 (100)	5.6±0.28 (97.72)	5.5±0.20 (96.32)	4.9±0.18 (85.81)	4.6±0.19 (80.33)
Fresh weight of root (mg)	183.79±10.02 (100)	153.49±10.82 (83.51)	141.63±10.06 (77.06)	136.36±7.49 (74.19)	96.93±5.79 (52.14)
Fresh weight of shoot (mg)	200.06±9.59 (100)	209.90±10.52 (104.95)	211.36±9.16 (105.68)	180.96±9.27 (90.48)	167.96±8.40 (83.98)

Table 1B. Effect of Cd stress imposed by using CdCl₂ on dry weight, % phytotoxicity of root and shoot. Maize seedling was grown with varying concentration of CdCl₂ solution under continuous light.

Treatment	Control	100µM	200µM	300µM	500µM
Dry weight of root (mg)	23.11±1.05 (100)	20.83±7.02 (90.16)	19.67±1.20 (85.16)	19.63±1.20 (84.92)	18.66±0.88 (80.91)
Dry weight of shoot (mg)	25.12±0.99 (100)	23.62±1.01 (94.03)	22.21±0.99 (88.43)	21.99±1.05 (87.57)	21.54±1.07 (85.75)
% Phytotoxicity of root	0	18.35	34.86	38.95	48.35
% Phytotoxicity of shoot	0	2.28	3.68	14.19	19.67

Table 1C. Effect of Cd stress imposed by using CdCl₂ on vigour index-I and II, tolerance index of root and shoot. Maize seedling was grown with varying concentration of CdCl₂ solution under continuous light.

Treatment	Control	100µM	200µM	300µM	500µM
Tolerance index of root	100	81.65	65.14	61.05	51.65
Tolerance index of shoot	100	97.72	96.32	85.81	80.33
Vigor index-I	1405.41±49.42 (100)	1085.56±45.13 (77.24)	823.52±21.22 (58.60)	754.60±20.58 (53.69)	633.63±25.75 (45.09)
Vigor index-II	4.20±0.12 (100)	3.41±0.56 (81.26)	2.78±0.12 (66.25)	2.79±0.10 (66.51)	2.55±0.08 (60.70)

2. Effect of NaCl (mM)

Table 2A.Effect of salt stress imposed by using NaCl on germination %, length (cm), fresh weight of root and shoots taken at different concentration of sodium chloride solution. Maize seedling was grown with varying concentration of NaCl solution under continuous light.

Treatment	Control	50mM	100mM	150mM	200mM
Germination %	93±3.33 (100)	73±8.82 (78.57)	60±5.77 (64.28)	47±3.33 (50)	43±6.67 (46.43)
Root length (cm)	9.07±0.74 (100)	5.20±0.36 (57.34)	3.59±0.35 (39.63)	2.24±0.23 (24.74)	1.64±0.13 (18.13)
Shoot length (cm)	5.21±0.59 (100)	2.70±0.17 (51.80)	1.77±0.17 (33.93)	0.70±0.08 (13.43)	0.59±0.12 (11.27)
Fresh weight root(mg)	257.79±63.56 (100)	88.74±9.13 (34.42)	44.39±7.03 (17.22)	18.61±2.63 (7.22)	15.06±3.74 (5.84)
Fresh weight shoot (mg)	291.32±64.41 (100)	95.39±6.97 (32.75)	48±6.45 (16.48)	33.66±4.15 (11.56)	25.58±2.80 (8.78)

Table 2B. Effect of salt stress imposed by using NaCl on dry weight, percent phytotoxicity of root and shoots taken at different concentration of sodium chloride solution. Maize seedling was grown with varying concentration of NaCl solution under continuous light.

Treatment	Control	50mM	100mM	150mM	200mM
Dry weight root (mg)	16.41±2.15 (100)	10.83±0.96 (65.96)	5.85±0.79 (35.64)	3.07±0.38 (18.69)	2.74±0.50 (16.68)
Dry weight shoot (mg)	17.09±2.45 (100)	10.97±1.19 (64.19)	6.03±0.66 (35.28)	4.48±0.42 (26.62)	3.64±0.52 (21.32)
%phytotoxicity root	0.00	42.66	60.37	75.26	81.87
% phytotoxicity shoot	0.00	48.20	66.07	86.57	88.73

Table 2C. Effect of salt stress imposed by using NaCl on vigor index-I and II, tolerance index of root and shoot taken at different concentration of sodium chloride solution.

Treatment	Control	50mM	100mM	150mM	200mM
Tolerance index root	100	57.34	39.63	24.74	18.13
Tolerance index shoot	100	51.80	33.93	13.43	11.27
Vigor index-I	1332.44±121.20 (100)	660.16±36.28 (49.55)	321.75±29.41 (24.15)	137.47±13 (10.32)	96.62±6.95 (7.25)
Vigor index-II	3.12±0.41 (100)	1.59±0.14 (51.11)	0.71±0.08 (22.79)	0.35±0.03 (11.29)	0.27±0.04 (8.84)

3. Interactive effect of cadmium and salt

Table 3A. Interactive effect of cadmium and salt stress imposed by using NaCl and CdCl₂ on germination percentage, length (cm), fresh weight of root and shoot taken at different concentration of cadmium chloride and sodium chloride solution.

Treatment	Control	100µM+50mM	500µM+50mM	100µM+100mM	500µM+100mM
Germination %	86.67±2.7 (100)	66.67±2.7 (76.92)	86.67±2.7 (100)	53.33±2.7 (61.54)	50±4.7 (57.69)
Root length (cm)	10.5±0.55 (100)	5.9±0.38 (56.14)	2.7±0.14 (25.91)	3.5±0.20 (33.09)	3.5±0.23 (33.64)
Shoot length (cm)	5.5±0.30 (100)	3.5±0.33 (62.39)	2.3±0.15 (41.14)	2.3±0.11 (41.05)	4.1±0.17 (73.32)
Fresh weight root(mg)	186.28±13.70 (100)	55.33±7.21 (29.70)	46.21±0.92 (24.81)	66.52±1.48 (35.71)	83.75±1.44 (44.96)
Fresh weight shoot(mg)	198.69±12.97 (100)	113.89±6.72 (57.32)	82.18±3.54 (41.36)	75.08±2.10 (37.79)	130.24±1.97 (65.55)

Table 3B. Interactive effect of cadmium and salt stress imposed by using NaCl and CdCl₂ on dry weight, % phytotoxicity of root and shoot taken at different concentration of cadmium chloride and sodium chloride solution.

Treatment	Control	100µM+50mM	500µM+50mM	100µM+100mM	500µM+100mM
Dry weight of root (mg)	21.82±1.42 (100)	12.55±0.66 (57.53)	8.45±0.16 (38.72)	6.92±0.12 (31.73)	8.70±0.02 (39.87)
Dry weight of shoot (mg)	23.40±1.38 (100)	17.70±0.98 (75.66)	13.17±0.22 (56.29)	9.09±0.23 (38.83)	20.18±1.36 (86.23)
% Phytotoxicity root	0.00	43.86	74.09	66.91	66.36
% Phytotoxicity shoot	0.00	37.61	58.86	58.95	26.68

Table 3C. Interactive effect of cadmium and salt stress imposed by using NaCl and CdCl₂ on Vigor Index-I and II, Tolerance Index of Root and Shoot taken at different concentration of cadmium chloride and sodium chloride solution.

Treatment	Control	100µM+50mM	500µM+50mM	100µM+100mM	500µM+100mM
Tolerance index root	100	56.14	25.91	33.09	101.65
Tolerance index shoot	100	62.39	41.14	41.05	178.62
Vigor index-I	1388.03±66.84 (100)	622.53±45.18 (44.85)	432.67±17.36 (31.17)	305.84±13.51 (22.03)	379.05±14.47 (27.31)
Vigor Index-II	3.92±0.22 (100)	2.02±0.11 (51.48)	1.88±0.02 (47.98)	0.85±0.01 (21.77)	1.44±0.07 (36.83)

4. Proline content ($\mu\text{g/g}$ fresh weight)

Table 4. Individual and combined effect of salt and cadmium imposed by using NaCl and CdCl₂ on the total proline content with varying concentration of Cadmium chloride and sodium chloride solution.

Treatment	Proline content root	Proline content shoot
Control	161.77 \pm 4.5 (100)	183.37 \pm 3.98 (100)
50mM Nacl	162.82 \pm 10.29 (100.65)	149.65 \pm 5.58 (81.61)
100mM Nacl	491.10 \pm 3.80 (303.58)	357.26 \pm 0.91 (194.83)
100 μ M CdCl ₂	365.16 \pm 49.85 (225.71)	124.88 \pm 7.24 (60.34)
500 μ M CdCl ₂	658.14 \pm 30.58 (406.84)	140.69 \pm 2.41 (76.72)
100 μ M+50mM	245.55 \pm 16.86 (151.79)	217.62 \pm 3.21 (118.68)
500 μ M+50mM	313 \pm 5.08 (193.49)	113.82 \pm 1.83 (62.07)
100 μ M+100mM	372.01 \pm 4.12 (229.97)	295.08 \pm 8.63 (160.92)
500 μ M+100mM	512.71 \pm 41.27 (316.94)	250.82 \pm 3.80 (136.78)

5. H₂O₂ Content ($\mu\text{m H}_2\text{O}_2/\text{gram Fresh Weight}$)

Table5. Individual and combined effect of salt and cadmium imposed by using NaCl and CdCl₂ on the H₂O₂ content with varying concentration of cadmium chloride and sodium chloride solution.

Treatment	H ₂ O ₂ content in root	H ₂ O ₂ content in shoot
Control	0.25±0.07 (100)	0.28±0.06 (100)
50mM Nacl	0.32±0.01 (129.50)	0.47±0.12 (167.86)
100mM Nacl	0.35±0.06 (139.44)	0.56±0.07 (196.98)
100 μM CdCl ₂	0.36±0.02 (145.65)	0.55±0.12 (194.51)
500 μM CdCl ₂	0.48±0.5 (193.79)	0.56±0.01 (199.45)
100 μM +50mM	0.35±0.04 (140.06)	0.28±0.09 (97.80)
500 μM +50mM	0.50±0.13 (200)	0.43±0.09 (153.85)
100 μM +100mM	0.45±0.11 (179.19)	0.26±0.10 (91.48)
500 μM +100mM	0.34±0.12 (136.02)	0.31±0.13 (108.24)

ACHIEVEMENTS FROM THE PROJECT:-

- Two research papers have been published in reputed journals and One is under communication. Two manuscripts are in the process of preparation.
- A piece of work entitled “Salt Stress Induced Effects on Biochemical Parameters in Etiolated Maize Leaf Segments during Greening” was presented in the international conference 3rd international world research journals congress (WRJC-2018) held in the ‘Art of living’ Bangalore from January 10 to January 13, 2018 for which the PI received “Best paper presentation Award”.
- One research paper entitled “Salt stress induced inhibition of δ -Amino levulinic acid dehydratase activity in etiolated maize leaf segments during greening” was presented by PI in the international conference ‘Biosangam-2020 held in MNNIT, Prayagraj from February 21 to 23, 2020.

SUMMARY OF THE FINDINGS:-

When etiolated maize leaf segments were treated with 0-300 mM NaCl during greening, the osmotic potential of leaf sap decreased significantly. Na⁺ content of the leaf tissue increased substantially and significantly on supplying different concentrations of salt. Realative water content of the leaf tissue was marginally affected by NaCl. Total protein and RNA content of the maize leaf segments gradually increased when treated with low concentrations of NaCl and thereafter decreased at higher concentrations. Decrease in DNA content was noted with increasing salt treatment in a concentration dependent manner. SDS-PAGE analysis of salt treated maize leaf tissue revealed appearance of one protein band (approx.73kDA) in samples incubated with \geq 100 mM NaCl as compared to control. Protein identification performed with the Mascot search engine in NCBI nr database indicated extensive homology of this protein with chloroplast heat shock protein 70 of *Cenchrus americanus* (Protein score: 295), hypothetical proteins SELMODRAFT_267815 of *Selaginella moellendorffi* (Protein score: 125), VITISV_000728 *Vitis vinifera* (Proteinscore:118) and SELMODRAFT_230659 of *Selaginella moellendorffi*(Protein score: 51). The results revealed that the dark grown maize leaf segments exhibit high degree of stress due to NaCl treatment and it affects the biochemical parameters governing the metabolic activities of the leaf tissue. Further, it also seems that the stress induced proteins are being synthesized in leaf tissue in response to salt stress.

Treatment of leaf segments with NaCl during greening decreased the total chlorophylls, carotenoids and protochlorophyllide content. Decline in the chlorophyll ‘b’level was to a greater extent than chlorophyll ‘a’ at all the concentrations of salt. Marginal inhibition of chlorophyllase activity at higher concentration of NaCl indicates that degradation of chlorophyll is not the target site for NaCl. Earlier enzymes of the chlorophyll biosynthetic pathway, such as ALA synthesizing activity, ALAD activity and PBGD activity were inhibited by the treatment of leaf segments with higher concentration of NaCl. Difference in ALA accumulation in light and dark (L-D), which corresponds to the chloroplastic ALA synthesis, was more severely inhibited at 150,200and 300 Mm NaCl concentration. These results suggest that inhibition of earlier steps of chlorophyll biosynthesis contribute significantly in decreasing the total chlorophylls in NaCl stressed leaf segments.

Treatment with 50-300 mM NaCl substantially enhanced the MDA and proline content in maize leaf segments. Electrolyte leakage measurement of treated leaf tissue demonstrated

gradual decrease in cell membrane stability which confirms the injury of cell membrane by salt.

Further the work was done to investigate the individual and interactive effect of salt and cadmium (Cd) on biochemical parameters in maize seedling. The results revealed that with increasing Cd concentration there was gradual decrease in root length, vigor index-I, root fresh weight and a gradual increase in % phytotoxicity in roots. Substantial decrease in germination and growth parameters was noted with increasing NaCl and CdCl₂ concentration. Counteracting effect of NaCl for 100µM and 500µM Cd was found with the growth parameters. Supply of 100mM NaCl increased the proline content in roots 3 folds while 100µM and 500µM CdCl₂, 2 and 4 folds respectively. In roots counteractive effect of NaCl and CdCl₂ was observed for proline content however in shoots a prominent effect of NaCl was observed than CdCl₂. H₂O₂ content increased with the supply of NaCl and CdCl₂ singly in roots however, when supplied together prominent effect of 100mM NaCl was noticed. In shoots counteracting effect of two stresses was observed. and the result obtained, revealed that the maize seedlings exhibit some degree of stress due to NaCl as well as due to Cd treatment and it affects the biochemical parameters leading the metabolic activities of the plant growth.

CONTRIBUTION TO THE SOCIETY (GIVE DETAILS):-

- Presently, more than 800 million hectares of land throughout the world are salt affected equating to more than 7% of the world's total land area. Increase salinization of arable land is expected to have devastation global effects, resulting in 30% land loss within the next 25 years and up to 50% by the year 2050.
- Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soil has become an important environmental concern. Further, in developing countries, rapid increase in the population demands an expansion of crop areas to raise food production.
- The results of the present study will be helpful in understanding the mechanism of salt stress induced inhibition of chlorophyll biosynthesis and general biochemical status of the maize plant under salinity condition.
- As salt and heavy metal stress is a major obstacle for global food security insurance, the present study will be helpful in predicting the growth behavior of plants under saline soils.
- Further extension of the study at proteomic level will be helpful in giving clues for developing salt and heavy metal tolerant varieties of crop plants.

NO. OF PUBLICATIONS OUT OF THE PROJECT (COPIES ATTACHED)

1. Jayesh Vaishnav and Meeta Jain. "Influence of halopriming and hydropriming on seed germination and growth characteristics of Zea mays L. cv. GSF-2 under salt stress". Research Journal of Chemistry and Environment 19(10): 1-6 (October 2015). ISSN: 0972-0626.
2. Meeta Jain and Jayesh Vaishnav. "Salt Stress Induced Effects on Biochemical Parameters in Etiolated Maize Leaf Segments during Greening". African Journal of Biological Sciences, Vol.1, No. 3, (July 2019), pp. 22-31., Available at SSRN: <https://ssrn.com/abstract=3422312>

