International Journal of Current Research and Academic Review

ISSN: 2347-3215 Special Issue-3 (August-2016) pp. 51-66

Journal home page: http://www.ijcrar.com

Effect of Salt and Ozone Stress on Cowpea (*Vigna unguiculata* (L) WalP.) Secondary Metabolite Production

Manickam Akila, Ravanappan Srinivasan Ramya*, Selvakumar Dharmaraj, Balaji Poojashree and Shibi Nivetha

Department of Biotechnology, Kumararani Meena Muthiah College of Arts and Science, Adyar, Chennai - 600020, Tamil Nadu, India

Corresponding author

KEYWORDS

Cowpea,
ozone,
salinity,
secondary
metabolite.

A B S T R A C T

To study the effect of salinity and ozone exposure on the growth and biomass production in cowpea plant. The present study focuses on the effect of salinity and ozone exposure on growth, photosynthetic metabolism, physiological parameters and biomass production. The photosynthetic metabolism of chlorophyll a, b, c and total chlorophylls were estimated and leaf area was measured using a scanner and the T-Scan program. Protein content was estimated by dye binding method. The biochemical content of proline was measured using L-pro as a strandard, the ascorbic acid was estimated by the visual titration method and phenol was measured. Cowpea seedlings on exposure to salinity stress at different concentrations showed gradual decrease in shoot length, root length and leaf area. With increasing concentration of Nacl (10-50mM) the chlorophyll content was also found to be altered. The chlorophyll a/b ratio increases and decreases with increase in saline concentration. The protein content of the vigna leaves increased at varied saline concentration. Ozone stress showed a decrease in protein level at low concentration with gradual increase on increasing concentrations. The level of ascorbic acid showed a fivefold increase under ozone stress and two fold increase on salinity stress. The phenolic compounds also increased both under ozone and salinity stress with noticeable proline accumulation under salt stress alone. Exposure of the cowpea plant to ozone and salinity altered the morphology and physiology of the plant system enabling the plant to promote several biosynthetic pathways resulting in high secondary metabolite yield.

Introduction

Cow pea (*Vigna unguiculata* (L) Walp) belongs to Fabaceae, its vernacular name is ThattaiPyaru, it is an annual plant growth in arid and semi-arid soil with a life-cycle of 6-7 months.Cow peas are primarily a substituence crop grown by people for their

own use, some species are well adapted to semiarid, sub-humid and humid tropic climate, cowpea are more susceptible to the attack of storage pest. The normal plant breeding method or genetically engineered plant shows great improvement in pest control and plant production potential. In 1972 Jacob Levit proposed a definition of biological stress derived from physical science and then suggested that biological stress is any change in environmental condition which may reduce/ alter a plant growth or its development. The economic aspects of this crop are mainly used as a food material and fodder for cattle. This group of plants can fix the atmospheric nitrogen with rhizobium but in stress condition this activity varies from one plant to other. The high salt concentration leads to reduction in plant growth and photosynthetic activities.

Salinity is a major barrier for growth and crop yield. Salinity and drought stresses constitute a permanent and increasing agronomical problem plant cells must be able to adjust their osmolarity to survive under saline condition (Flower *et al*., 1997). Plants have evolved many types of adaptation to resist the stress conditions depending on their developmental changes. The morphological changes that occur in plant part includes leaf, stem, root etc, physiological changes were observed in photosynthetic pathway, and metabolic changes such as variation in gene expression, membrane desaturation and signalling pathways. During such stress condition plants maintain the water content and metabolic activities in normal and steady state by accumulating various solutes that are non-toxic.

The term saline soil is normally used to indicate soil with an electrolyte concentration which inhibits plants growth the irrigated land has been found turning saline leading to loss of agricultural land. Wyn Jones (1981) has summarized three major factors that limit the growth of plants in saline habitat that were 1. Water stress was due to osmotic effect, 2. Specific ion toxicity. 3. Ion imbalance or induced nutrient deficiency.

Ozone is the most wide-spread air pollutant in many industrialized area and its concentration increase during the recent years by anthropogenic activity (Krupa, 1988). Ozone is considered to be the most phytotoxic of common pollutant, which reduces photosynthesis, growth and enhances premature senescence in plant (Darral, 1989; Reich, 1987, Pell *et al.*, 1991). Once generated they induce structural and functional alteration of plasma membrane of plant,which respond to O_3^- induced oxidative stress by activating a number of antioxidative stress- related to the defense mechanisms that induces changes in biochemical plant machinery (Sharma *et al*., 1997) . Hence, the present investigation was carried out to see the effect of salinity and ozone exposure on the (i) growth characteristic and biomass production of cow pea, (ii) photosynthetic metabolism and other physiological parameter such as ascorbic acid, phenol and protein metabolism, (iii) Comparison of abiotic stress salinity and ozone stress on proline metabolism.

Materials and Methods

Seeds

Cowpea seeds (*Vignaunguiculata.L.Walp*) Co6 was purchased from Tamilnadu Pulses Research Institute in Vamban, Puthukottai district. Seeds were washed thrice in deionized water. After soaking the seeds for overnight in Deionized water, they were

sown in pots containing vermiculite substratum.

Nutrient Solutions

The cow pea(*Vignaunguiculata .L.Walp*.) Co6 grown in vermiculite soil were irrigated with Hoagland's medium (Arnon and Hoagland, 1949).

Solution 1

100x	g/100ml
KH_2PO_4	13.6
KNO_3	10.2
$Ca(NO_3)_2$	4.92
Mg $SO_4.7H_2O$	4.9

Solution 2

100x	g/100ml
$NH_4H_2PO_4$	2.3
KNO_3	10.2
$Ca(NO_3)_2$	4.92
$MgSO_4$	4.9

Solution A

100x	mg/100ml
H_3BO_3	28.6
$MnCl_2$ $4H_2O$	18.1
$ZnSO_4.4H_2O$	0.8
H_2Mo $O_4.H_2O$	0.9

Solution B

Dissolve 26.18g EDTA in 268 ml of 1N KOH, ADD 24.98 g of $FeSO_4.7H_2O$ and dilute to 1 liter.Aerate overnight to produce stable ferric complex.The pH of the solution should be about 5.5.

Hoagland's Medium

Solution 1 or 2	10 ml
Solution A	1ml
Solution B	1ml

Above mixture was made upto 1000 ml distilled water.The pH of the medium should be around 6.0.

Ozone Treatment

Ozone used for the experiment was generated using ozone generator model M221 manufactured by V-can networks.Corona discharge generators pass dry O_2 enriched air across a high electric voltage (>5000) or Corona similar to a spark plug.Single O rapidly combines with available O_2 to form the very reactive O_3.Ozone fumigation given in the concentration of 10 ppb,20 ppb,30 ppb,40 ppb and 50 ppb using Electric discharge vacuums generator.Plants were placed in the chamber for 10 min before the addition of ozone to allow equilibrium with chamber.After treatment plants returned to open growth chamber.The controlled plant

placed were exposed non- filtered air in chamber through Aquarium air pump.

Sodium chloride (NaCl stock)

To induce salinity condition, different concentration of NaCl stock were prepared and used, 0mM, 10mM, 20mM, 30mM, 40mM and 50mM NaCl stock were prepared and separately used.

Culture condition

Cow pea Co6 plants grown in continuous light (500 $E/m^2/s^{-1}$) at room temperature (30^oC) and Hoagland's medium were applied twice a day. After Germination plants were exposed to different concentration of NaCl and ozone gas.

Measurement of growth parameter

Growth parameter was analyzed in 15- day's old seedlings. Shoot and root length was measure with an accuracy of 1mm using a meter scale. The fresh weight of the plant was noted immediately after the harvest. Dry weight was estimated after kept the plant sample in hot air oven at 80^oC for 24 hrs before measuring their dry weight.

Estimation of Chrolophyll

Chlorophyll a, b and total chlorophyll were estimated by Arnon (1949) method. Cowpea leaf harvested, washed with tap water and dried. 1.0g of leaves were taken and homogenized with 80% acetone in a blender and the final volume was made up to 10ml. From this 0.5 ml was taken and made up to 5ml with 80% acetone and centrifuged. The supernatant with the chlorophyll pigment was measured at 663 and 645nm.

Estimation of Leaf area

Leaf area was measured using a scanner and the T-scan program. Relative water content was measured according to Radford (1967).

Estimation of Protein

Protein content was estimated by dye binding method of Bradford (1976). 500mg of leaves was homogenized in hot 80% ethanol using mortal with pestle. The homogenate was centrifuged at 2000g for 20 min and the pellet was suspended in 5% TCA in an ice bath for 15 min. To 1ml aliquot add 10% TCA to precipitate the protein and repeat the procedure for twice. The pellet contains protein was suspended in 1ml of 1N NaOH and made up to 10 ml with distilled water from this 50ul of sample added to 950ul of dye solution (10mg of CBB G250 with 10ml of 88% phosphoric acid and 4.7 ml of absolute alcohol dilute to 100ml of water). The tube was incubated for 5 min in room temperature and read the absorbance at 595 nm against the blank.

Estimation of Proline

Proline content was measured by Bates *et al.,* (1973) method using L-Pro as a standard. 0.5g of tissue was homogenized in 10ml of 3% aqueous sulfosalicyclic acid and the homogenate centrifuged at 10,000g for 10 min. The supernatant was incubated with 2ml of acid ninhydrin and glacial acetic acid in the test tube for 1h at 100^0 C and the reaction terminated in ice bath. The reaction mixture was extracted with 4 ml of toluene. Toluene layer was aspirated and the absorbance read at 520nm using toluene as blank. The Proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

=(prolineug ml^{-1} X toluene ml/115.5ug uM^{-1})/ sample g/0.5)
= Prolineu$M\ g^{-1}$ FW of leaf.

Estimation of Ascorbic Acid

Ascorbic acid content was estimated by visual titration method based on the

reduction of 2,6-dichlorophenol dye according to Sridhar *et al.*,(1979). 0.5g of minced leaf was extracted using 0.4% of oxalic acid using mortar and pestle. The extract was filtered through two layer cheese-cloth and centrifuged at 1000g for 20 minutes. The supernatant was round up with aliquot of oxalic acid to proceline dish with the standardized indophenol reagent until the solution becomes pink which should persist for at least 15 second. Calculate the ascorbic acid content of the extract by using the following formula

IX SX D/A X 100/W = mg of ascorbic acid /100g of tissue

I = ml of indophenols reagent used

S= mg of ascorbic acid reacting with 1 ml of reagent

D= volume of the extract in ml

A= the aliquot titrated in ml

W= the weight of the sample in g

Estimation of Total Phenol

Phenol content was measured using Folin-Phenol reagent based on the reaction between phenols and an oxidizing agent phosphomolybdate, which result in blue colour. To 1ml of extract added 1ml of folin-phenol reagent and 2mlof Na_2CO_3Solution heated the mixture for 1 min and cooled under running tap water. Dilute the blue solution to 25ml and measured at 650nm against the blank.

Results and Discussion

Cow pea seedlings showed interesting morphological and physiological changes when they were exposed to different concentration of salinity and ozone stress (Fig.1 and 2)

Growth

Ozone stress caused morphological changes by means of increasing the shoot length at 30 ppb, and 40 ppb ozone increase root length as well as (stimulate secondary root) of seedling

(Table I a). Salinity stress gradually decreased shoot length as well as root length of seedling 50mM NaCl concentration affect 49% and 35% of shoot and roots respectively. (Table I b).

Fresh weight/ Dry weight

Fresh weight of the seedling showed significant decrease with increase in concentration of NaCl. Dry weight also decreased by 20% and show gradual reduction in biomass of seedling (Table IIb). Ozone stress increased the weight at 30 ppb (Table II a).

Leaf Area

Leaf area increased by 63% at 30 ppb ozone and above 30 ppb there was a decrease in the leaf area (Table III a). whereas salinity stress showed gradual decrease in leaf area by means of 14%, 30%, 36%, 37% and 44% with increasing concentration of NaCl (10-50mM) (Table III b).

Chlorophyll content

The analysis of chlorophyll content of cowpea seedling showed that salinity and ozone strongly influence the chlorophyll content and composition (Table IV a and IV b). When compared with control cholorophylla was affected more at 10, 20 and 50 ppb. In 30 and 40 ppb of ozone, chlorophyll b was increased. Chlorophyll a/b ratio increased at 10, 20 ppb, whereas it was decreased at 30, 40 and 50 ppb ozone. These

results indicate that chlorophyll a is more sensitive than chlorophyll content with following patterns. Total chlorophyll was affected (62%) at 50 mMNaCl concentration. The chlorophyll a, b content also similarly affected. Chlorophyll b more sensitive than chlorophyll a. chlorophyll a/b ratio increase and decrease when concentration NaCl in increase (Table IV b).

Protein

Salinity drastically increased the protein content of Vigna leaves at all concentration. Ozone stress slowly decreases (10 and 20 ppb) and increase (30, 40 and 50 ppb) the content of protein (Table V). When compared to ozone stress more protein production was observed in cowpea under salinity stress. Chlorophyll- protein ratio decreased in salinity, whereas under ozone stress it decreased up to 20 ppb concentration. At 30 ppb increased chlorophyll-protein ratio. The chlorophyll/ protein ratio indicated that chlorophyll biosynthesis was more sensitive than protein biosynthesis in saline condition. Whereas ozone stress showed reduction in chlorophyll-protein ratio at 10 and 20 ppb and increase in 30 ppb. 40 and 50 ppb also showed decreased in chlorophyll/ protein ratio. This finding suggested that the ozone concentration play a major role in protein and chlorophyll biosynthesis (Table VI a and VI b).

Ascorbic acid

The estimation of ascorbic acid content showed fivefold increase than that of the control under ozone stress (Table VII), whereas under salt stress it showed 2 fold increase, which suggested that ascorbic acid metabolism was more sensitive to ozone than salinity stress.

Total phenol content

The phenolic compound also increased both under ozone and salinity stress. In ozone stress, the phenolic content increased gradually with increase in concentration of ozone. Salinity stress caused induction of the phenolic compounds and it was highest at 50mM of NaCl, (Table VIII). The result showed secondary metabolite production was more under salinity stress when compared to ozone stress.

Proline

Salt stress was known to induce proline accumulation in higher plant (Bhaskaran *et al.*, 1985). We analyzed the accumulation of proline in leaf tissue of seedling at various concentrations of ozone and NaCl. The maximum proline accumulation was observed in the seedling grown under 50mM NaCl concentration two fold when compared with control (Table IX).

Excess amount of salt in the soil adversely affects growth and development, some process such as seed germination growth and vigor vegetative growth, flowering and fruit set are adversely affected by high salt concentration ultimately causing diminished economic yield and also the quality of product (Zhu, 1999).

Though salt stress and dehydration stress shows high degree of similarity with respect to physiological, biochemical and molecular and genetic effect, (Mc Cue and Hanson 1990)there is a strong correlation between increased cellular proline levels and ascorbic acid level to survive both water deficit and the effect of high environmental salinity. It may also serve as an organic nitrogen reserve that can be utilized during recovery, polyamine have recently gained importance in the escape of seedling from the adverse

effect of salinity (Caldevia *et al*., 1999; Lefevre *et al*., 2001).

In organisms ranging from bacteria to higher plants, there is a strong correlation between increased cellular proline, ascorbic acid level as the capacity to survive the effect of high environmental salinity. In plants which can withstand (oxidative burst), high proline accumulation was observed in leaves under abiotic stress (Tyagi *et al*., 1999). Although proline can be synthesised from either glutamtate or ornithine, glutamate is proposed to be the primary precursor in saline/ osmotically stressed cell. In this present study we have analyzed the interaction of ozone and salinity on cowpea plant in various morphological and physiological metabolisms.

Table.1a Influence of Ozone on the growth characteristics of Vignaunguiculata (L.) Walp.

Ozone (ppb)	Shoot length (cm)	Root length (cm)
0	10.6 ± 2.97 (100)	6.95 ± 2.40 (100)
10	10.94 ± 3.02 (103)	6.83 ± 2.38 (98)
20	11.46 ± 3.02 (106)	6.90 ± 2.39 (99)
30	11.48 ± 2.98 (108)	7.21 ± 2.50 (103)
40	11.18 ± 2.95 (105)	8.38 ± 2.64 (120)
50	11.12 ± 2.98 (104)	5.88 ± 2.21 (84)

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.1b Influence of salinity on the growth characteristics of Vignaunguiculata (L.) Walp.

NaCl (mM)	Shoot length (cm)	Root length (cm)
0	15.74 ± 1.8 (100)	8.31 ± 2.12 (100)
10	14.06 ± 3.04 (89)	8.08 ± 2.60 (97)
20	13.18 ± 3.23 (83)	6.93 ± 2.34 (83)
30	13.18 ± 3.35 (83)	6.63 ± 2.59 (79)
40	9.12 ± 3.51 (57)	6.63 ± 2.58 (79)
50	8.18 ± 3.18 (51)	5.43 ± 2.44 (65)

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.2a Influence of Ozone on the biomass production and relative water content of Vignaunguiculata (L.) Walp.

Ozone (ppb)	Fresh weight (g)	Dry weight (g)	FW/DW	Relative water content
0	1.410 ± 0.06 (100)	0.178 ± 0.02 (100)	7.92	87.37
10	1.351 ± 0.02 (95)	0.176 ± 0.01 (98)	7.67	86.97
20	1.616 ± 0.02 (114)	0.135 ± 0.09 (75)	11.97	91.64
30	1.761 ± 0.06 (124)	0.215 ± 0.13 (120)	8.190	87.79
40	2.231 ± 0.05 (158)	0.205 ± 0.05 (115)	10.88	90.81
50	2.496 ± 0.04 (177)	0.206 ± 0.09 (115)	12.11	91.74

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.2b Influence of Salinity on the biomass production and relative water content of Vignaunguiculata (L.) Walp.

NaCl(mM)	Fresh weight (g)	Dry weight (g)	FW/DW	Relative water content
0	2.278 ± 0.015 (100)	0.350 ± 0.003 (100)	6.50	84.63
10	2.260 ± 0.014 (99)	0.360 ± 0.006 (102)	6.27	84.07
20	2.317 ± 0.075 (101)	0.331 ± 0.003 (94)	7.0	85.71
30	1.878 ± 0.005 (82)	0.268 ± 0.005 (76)	7.0	85.72
40	1.815 ± 0.001 (79)	0.347 ± 0.004 (99)	5.23	80.88
50	1.551 ± 0.107 (68)	0.280 ± 0.003 (80)	5.53	81.94

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.3a Influence of Ozone on Leaf Area of Vignaunguiculata (L.) Walp.

Ozone (ppb)	Leaf Area (cm^2)	Dry weight (g)	Leaf Area Ratio
0	16.55 ± 0.575 (100)	0.178 ± 0.010 (100)	92.9
10	19.78 ± 0.171 (119)	0.176 ± 0.005 (98)	112.38
20	14.94 ± 2.142 (90)	0.135 ± 0.009 (75)	133.11
30	27.11 ± 2.104 (163)	0.215 ± 0.136 (120)	126.09
40	17.97 ± 1.025 (108)	0.205 ± 0.005 (115)	72.87
50	14.95 ± 2.505 (90)	0.206 ± 0.009 (115)	72.62

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.3b Influence of salinity on Leaf Area of Vignaunguiculata (L.) Walp.

NaCl (mM)	Leaf Area (cm^2)	Dry weight (g)	Leaf Area Ratio
0	26.881 ± 1.414 (100)	0.350 ± 0.003 (100)	54.36
10	23.168 ± 4.094 (86)	0.360 ± 0.006 (102)	66.76
20	19.028 ± 2.059 (70)	0.331 ± 0.003 (94)	48.93
30	17.470 ± 3.544 (64)	0.268 ± 0.005 (76)	52.77
40	17.128 ± 7.828 (63)	0.347 ± 0.004 (99)	100.30
50	15.238 ± 3.783 (56)	0.280 ± 0.003 (80)	42.32

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.4a Influence of Ozone on Chlorophyll of the Vignaunguiculata (L.) Walp.

Ozone (ppb)	Chlorophyll a mg g^{-1} FW of leaf	Chlorophyll b mg g^{-1} FW of leaf	Total Chlorophyll mg g^{-1} FW of leaf	Chlorophyll a/b
0	4.66 ± 0.25 (100)	2.35 ± 0.42 (100)	7.01 ± 0.33(100)	1.98
10	4.02 ± 0.03 (86)	1.78 ± 0.05 (75)	5.8 ± 0.04 (82)	2.25
20	2.55 ± 0.20 (54)	1.06 ± 0.03 (45)	3.51 ± 0.11 (50)	2.40
30	4.08 ± 0.38 (87)	2.33 ± 0.05 (99)	6.41 ± 0.21 (91)	1.75
40	3.83 ± 0.11 (82)	2.16 ± 0.005 (91)	5.99 ± 0.05 (85)	1.77
50	2.61 ± 0.02 (56)	1.44 ± 0.018 (61)	4.05 ± 0.01 (57)	1.81

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.4b Influence of Salinity on Chlorophyll of the Vignaunguiculata (L.) Walp.

NaCl (mM)	Chlorophyll a mg g^{-1} FW of leaf	Chlorophyll b mg g^{-1} FW of leaf	Total Chlorophyll mg g^{-1} FW of leaf	Chlorophyll a/b
0	2.01 ± 0.07 (100)	0.97 ± 0.01 (100)	2.98 ± 0.07 (100)	1.49
10	1.87 ± 0.09 (93)	0.66 ± 0.02 (68)	2.53 ± 0.05 (84)	1.26
20	1.79 ± 0.04 (89)	0.77 ± 0.02 (79)	2.56 ± 0.03 (85)	1.28
30	1.12 ± 0.01 (55)	0.59 ± 0.02 (60)	1.71 ± 0.02 (57)	0.85
40	1.07 ± 0.03 (53)	0.49 ± 0.003 (50)	1.56 ± 0.03 (52)	0.78
50	0.79 ± 0.03 (39)	0.37 ± 0.01 (37)	1.16 ± 0.02 (38)	0.58

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.5 Influence of Ozone/Salinity on Protein of the Vignaunguiculata (L.) Walp.

Concentration of Ozone (ppb)/NaCl (mM)	Protein mg g^{-1} of FW of leaf (Ozone Stress)	Protein mg g^{-1} of FW of leaf (NaCl Stress)
0	3.734 ± 0.031 (100)	2.019 ± 0.008 (100)
10	3.715 ± 0.30 (99)	4.190 ± 0.008 (207)
20	3.640 ± 0.08 (99)	5.533 ± 0.113 (274)
30	3.166 ± 0.028 (84)	4.117 ± 0.005 (205)
40	3.127 ± 0.008 (83)	4.156 ± 0.017 (205)
50	3.681 ± 0.02 (98)	4.264 ± 0.017 (211)

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.6a Changes in Chlorophyll-Proteins ratio of Ozone on Vignaunguiculata (L.) Walp.

Ozone (ppb)	Total Chlorophyll mg g^{-1} FW of leaf	Protein mg g^{-1} FW of leaf	Chlorophyll-Proteins ratio
0	7.01 ± 0.33 (100)	3.734 ± 0.031 (100)	1.8773
10	5.80 ± 0.04 (82)	1.560 ± 0.08 (99)	1.5612
20	3.51 ± 0.11 (50)	3.640 ± 0.028 (99)	0.9642
30	6.41 ± 0.21 (91)	3.166 ± 0.028 (84)	2.0246
40	5.99 ± 0.05 (85)	3.127 ± 0.0008 (83)	1.9155
50	4.05 ± 0.01 (57)	8.681 ± 0.02 (98)	1.1005

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.6b Changes in Chlorophyll-Proteins ratio of Ozone on Vignaunguiculata (L.) Walp.

NaCl (mM)	Total Chlorophyll mg g^{-1} FW of leaf	Protein mg g^{-1} FW of leaf	Chlorophyll-Proteins ratio
0	2.0190 ± 07 (100)	2.019 ± 0.008 (100)	1.4759
10	2.533 ± 0.05 (84)	4.190 ± 0.008 (207)	0.6038
20	2.56 ± 0.03 (85)	5.533 ± 0.113 (274)	0.4610
30	1.71 ± 0.02 (57)	4.117 ± 0.005 (205)	0.4153
40	1.56 ± 0.03 (52)	4.156 ± 0.017 (205)	0.3753
50	1.16 ± 0.02 (38)	4.264 ± 0.017 (211)	0.2720

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.7 Influence of Ozone/Salinity on the Ascorbic acid of Vignaunguiculata (L.) Walp.

Concentration of Ozone (ppb) / NaCl (mM)	Ascorbic Acid mg 100 g^{-1} of Fresh weight of leaf (Ozone Stress)	Ascorbic Acid mg 100 g^{-1} of Fresh weight of leaf (NaCl Stress)
0	18.80 ± 0.292 (100)	71.21 ± 0.74 (100)
10	50.7 ± 0.26 (269)	123.8 ± 0.32 (173)
20	61.34 ± 1.44 (326)	136.6 ± 0.02 (191)
30	68.25 ± 0.10 (363)	142.1 ± 0.46 (199)
40	77.73 ± 0.24 (413)	145.3 ± 0.2.38 (204)
50	101.89 ± 0.024 (541)	200.9 ± 1.00 (282)

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

Table.8 Influence of Ozone/Salinity on Total Phenol of Vignaunguiculata (L.) Walp.

Concentration of Ozone (ppb) / NaCl (mM)	Total PhenonlμMg^{-1} Fresh weight of leaf (Ozone Stress)	Total PhenonlμMg^{-1} Fresh weight of leaf (NaCl Stress)
0	480 ± 20.3 (100)	210 ± 1.01 (100)
10	500 ± 0.6.88 (104)	230 ± 16.5 (109)
20	550 ± 7.07 (114)	410 ± 2.13 (195)
30	600 ± 5.63 (125)	410 ± 2.13 (195)
40	600 ± 5.63 (125)	430 ± 1.93 (204)
50	620 ± 8.37 (129)	460 ± 5.26 (219)

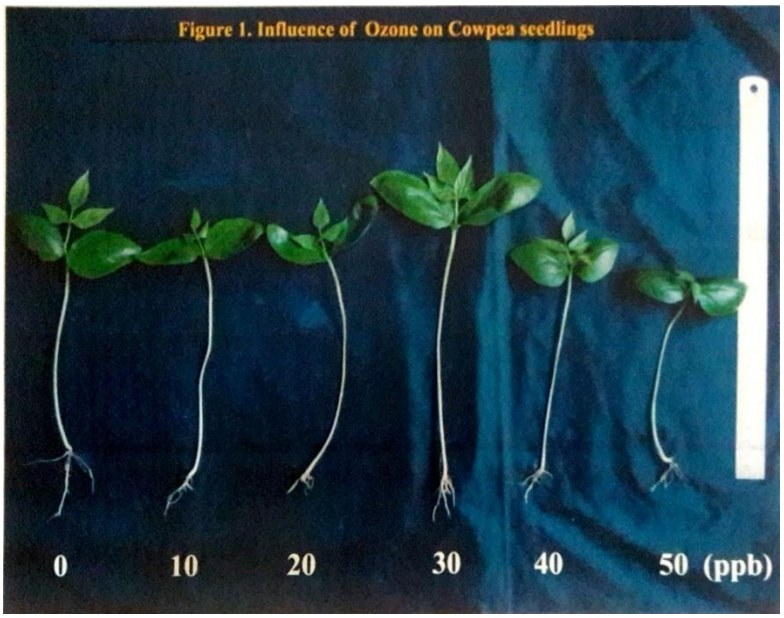
Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5)

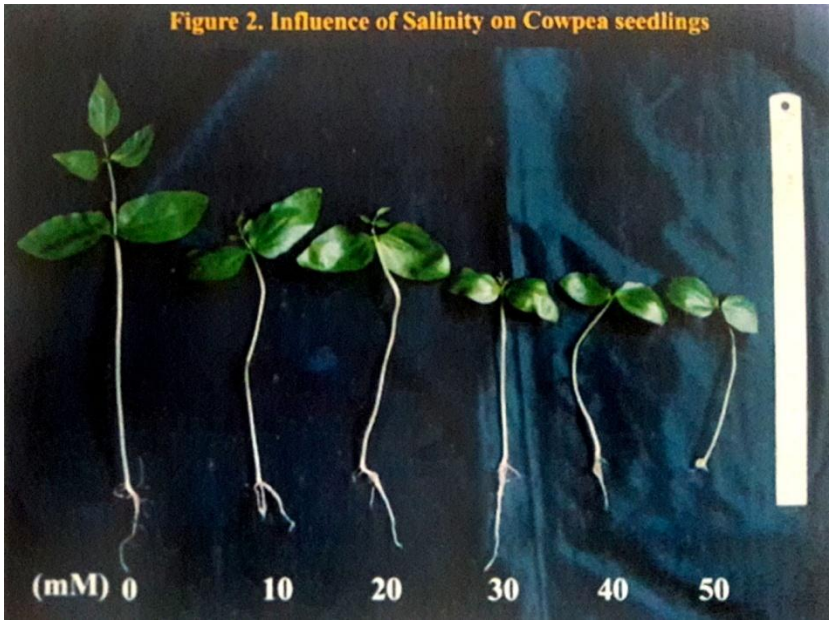
Table.9 Influence of Ozone/Salinity on Proline of the Vignaunguiculata (L.) Walp.

Concentration of salt (mM)/Ozone(ppb)	ProlinenMg^{-1} FW of leaf (Ozone Stress)	ProlinenMg^{-1} FW of leaf (Salt Stress)
0	265 ± 3.26 (100)	540 ± 3.2 (100)
10	355 ± 12.2 (133)	565 ± 3.26 (101)
20	365 ± 4.35 (137)	610 ± 3.92 (122)
30	370 ± 4.77 (139)	705 ± 4.94 (154)
40	375 ± 8.24 (141)	770 ± 5.09 (159)
50	450 ± 5.31 (169)	1420 ± 6.78 (211)

Value in parenthesis indicate percent of the control value are given mean and standard error for replicate container (n=5).

Figures





The analysis of growth characteristic of cowpea shoot and root system has changed gradually with ozone and salinity. This experimental setup used ambient ozone level. Ozone level increases and decreases depending upon the wind velocity. Ambient ozone level of Chennai is 10 to 50 ppb concentration (Pulilkesi *et al*., 2005). Exposure of cowpea plants to various concentration of ozone (10- 50ppb) upto 30 ppb, behind which, decreased the shoot elongation. This resulted that lower concentration of ozone induces the cell elongation during the cell differentiation, and showed that it might be stimulated by gene responsible for Auxin (IAA) gibberilic acid (GA) plant growth regulator (PGR).

The dry matter production in plants primarily depends on photosynthesis. The

dry weight of the ozone treated plant increased 2 fold biomass at 30ppb then the higher concentration. This showed that the biomass production increased at lower concentration of ozone. The relative water content also increases biomass production. This indicated that photosynthesis is not affected by this concentration. Similar results were observed (Agarwal *et al.*, 1993) in biomass content of C*ucumissativus.* Wherein salinity stress gradually decreases the dry weight of the plants which suggest that biomass production decrease by increase in concentration of NaCl.

The leaf area also increased at 30ppb concentration of ozone and above 30ppb it decreased suggesting that this concentration may induce the entire physiological pathway responsible for the leaf formation. The leaf area decreased with increase in salinity in contrast to zone stress. This suggested that cowpea plants are more sensitive to salinity than ozone stress.

Under ozone exposure chlorophyll a content was less when compared to chlorophyll b, Which suggested that chlorophyll a biosynthesis is more affected than chlorophyll b. This reduction of chlorophyll indicated that photosystem I (PS I) is affected more than photosystem (PSII). In higher plants chlorophyll b is exclusively associated with the light harvesting chlorophyll protein complex (LHCP) (Glazer 1983). So it is to be concluded that the ozone stress has not decreased the LHCP content in vigna seedlings. This concluded that there is no reduction in dry matter production of cow pea seedlings in ozone stress whereas salinity stressed plants showed high reduction in biomass. This indicated that chlorophyll biosynthesis is more sensitive in salinity stress then ozone stressed cow pea seedlings.

The protein content of ozone stressed plants gradually decreased but salinity increased the protein content suggesting the accumulation of protien is more in salinity and less in ozone.

The capacity to accumulate proline is being taken as criteria for the development of salt tolerant plants(Iyer and caplan, 1998),for which attempts have been made to clone the genes of proline biosynthetic pathway in non-halophytes to enhance their salt tolerance. The increased accumulation of proline in the various tissues of Vigna seedlings showed their expected response of a non halophytic plant of salinity. Thus the differential accumulation in proline in leaves is an important observation made in this study. In conclusion exposure of the cowpea plant to ozone and salinity altered the morphology and physiology of the plant system enabling the plant to promote several biosynthetic pathways resulting in high secondary metabolite yield.

References

Agrawal, R. 1993. Mining association rules between sets of items in large databases. In proceedings, ACM SIG-MOD conference on management of data. 207-216.

Arnon, D.I. 194, copper enzymes in isolated chloroplast. Polyuphenol oxidase in beta vulgaries. *Plant Physiol*. 24: 1-15.

Bradford, M.M. 1976. A dye binding assay for protein. *Anal. Biochem.,* 72: 248-254.

Bhaskaran, S., Smith, R.H., and Newton, R.J. 1985. Physiological changes in cultured sorghum cells in response to water stress. *Plant Physiol.*, 79, 266-269.

Cushman, M., Kanamathareddy, S. 1990. *Tetrahedron*, 46, 1491–1498.

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

Darrell, T., Sclaroff, S., and Pentland, A. 1990. Segmentation by minimal description". In *Proceedings of IEEE International Conference on Computer Vision*, pages 112--116.

Flower, T.J. 1977. The mechanism of salt tolerance in halophytes. *Annual review of plant physiol*., 28, 89-121.

Iyer, S., Caplan, A. 1998. Products of proline catabolism can induce osmotically regulated genes in rice. *Plant Physiol*. 116, 203.

Krupa, S.V. and Manning, W.J. 1988. Atmospheric ozone: formation and effects on vegetation. *Environ. Pollu.*, 50: 101– 137.

Mccue, K.F., Hanson, A.D. 1990. Drought and salt tolerance: towards understanding and application; *Trends Biotech.*, 358 – 362.

Pulikesi, M., Baskaralingam, P. 2006. Air quality monitoring in Chennai, India, in the summer of 2005. *J. Hazardous Materials*, 136(3): 589–596.

Rao, M.V., Koch, J.R., and Davis, K.R. 2000. A tool for probing programmed cell death in plants. *Plant Mol. Biol*., 44(3): 345-358.

Sandermann, H., jr 1998. An abiotic elicitor of plant defense reaction. *Trends Plant Sci.*, 3: 47-50.

Sharma, Y.K., Davis, K.R. 1997. The effects of ozone on antioxidant responses in plants. *Free Radic. Biol. Med.*, 23(3):480-8.

Tyagi, A., Rajalakshmi, M., Antony Jeyaraj, D., Sharma, R.S. and Bajaj, J.S. 1999c. Effects of long-term administration of testosterone enanthate on glucose metabolism in rhesus monkeys. *Contraception*. 59, 333- 337.

Wyn Jones, R.G., Storey, R., Leigh, R.A., Ahmad, N., Pollard, A. 1977. A hypothesis on cytoplasmic osmoregulation. In "Regulation of Cell Membrane Activities in Plants" (E Marre, O Cifferieds), Elsevier, Amsterdam, pp. 121-136.

Zhu, B.C., Su, J., Chang, M.C., Venma D.P.S., Fan, Y.L and Wu, R. 1999. Over expression of d- proline-5-carboxylate synthetase gene and analysis of tolerance to water and salt stress in transgenic rice. *Plant Sci.*, 139: 41-48.

How to cite this article:

Manickam Akila, Ravanappan Srinivasan Ramya, Selvakumar Dharmaraj, Balaji Poojashree and Shibi Nivetha. 2016. Effect of Salt and Ozone Stress on Cowpea (*Vigna unguiculata* (L) WalP.) Secondary Metabolite Production. *Int.J.Curr.Res.Aca.Rev.* Special Issue-3: 51-66.