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Effect of Drought Stress (Mannitol) on Morphological Physiological Activity and Anatomy of Cow Pea Plant(Vigna unguiculata)

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Abstract*: *In this study 21 days old seedlings of Cow pea plant (Vigna unguiculata L) were subject to different drought stress levels (0g, 0.1g and 0.2g mannitol) at germination and early seedling growth stage of plant development. Data were analyzed for growth parameters such as plant height, fresh and dry weight, leaf water content (LWC), and length of radicle and plumule during germination period, and biochemical parameters such as proline content, membrane stability index (MSI), malondealdehyde (MDA) content, chlorophyll content, and antioxidant enzyme activity Catalase (CAT) and Peroxidase (POD). In this study it was seen that the effect of salt stress reduced plant height, fresh and dry weight, LWC, radical and plumule length. Salt stress reduced the biochemical activities and also chlorophyll a, chlorophyll b and total pigment content. The decrease was 0.58,0.11 and 0.69 respectively. The result showed an increase in the activity of CAT enzyme in leaves and root with increasing salt concentration. An increase CAT activity were found with 0.1g, 0.2g mannitol treatment which represented values of relative increasing of 15.2g/L and 17.2 in leaves and 9.2g/L and 11.7g/L in root respectively. There was increase in the activity of POD enzyme in leaves and root with increasing salt concentration. The level of POD activity was found with 0.1g,0.2g NaCl treatment, which represented relative increase 71.14 and 82.34%in leaves.

Keywords: Mannitol, Cow pea plant, MDA, MSI, CAT, POD

I. INTRODUCTION

In this study the 21 days old seedings of Cow pea plant (Vigna unguiculata L) were subject to different mannitol levels (0g, 0.1g and 0.2g NaCl) at germination and early seedling growth stage of plant development. Data were analyzed for growth parameters such as plant height, fresh and dry weight, leaf water content (LWC), and length of radicle and plumule during germination period, and biochemical parameters such as proline content, membrane stability index (MSI), malondealdehyde (MDA) content, chlorophyll content, and antioxidant enzyme activity Catalase (CAT) and Peroxidase (POD). In this study the effect of drought stress reduced plant height, fresh and dry weight, LWC, radical and plumule length.

The intensity of the response to water stress depends on the stress severity and its duration, as well as the plant developmental stage.Moderate stress environment [2] Drought stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange. In plant, cellular activities that are mostly affected by heavy metal pollution including mineral nutrition, photosynthesis, respiration, membrane structure, gene expression and other properties.That is increased activities of antioxidant enzymes are one of the key defense mechanisms which help plants to avert oxidative stress damage.Catalase (CAT), peroxidase (POD) are among the various intricate antioxidant enzymes which carry scavenging of ROS so as to protect cells from the oxidative damage Plant membrane structure. Drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth [19]

II. MATERIALS AND METHODS

The present study is done to analyze the effect of the abiotic environmental stress (drought) response on plants. The plant material selected for the study is Cow pea plant (Vigna unguilata L) considering the ease in the growth of the plant

III. STUDY MATERIAL

The plant material selected for the study is Cow pea plant (Vigna unguilata L) considering the ease in the growth of the plant**.**

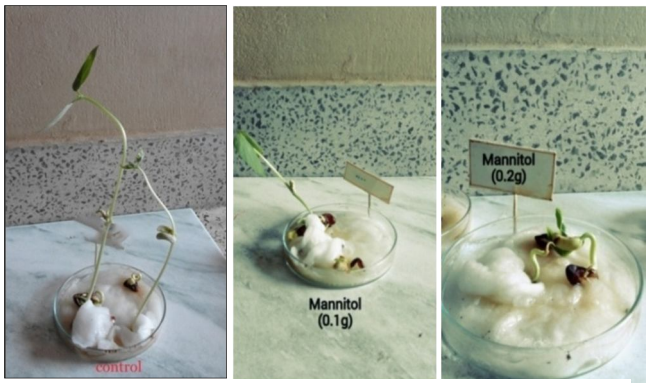

Plate 1 Plate 2 Plate 3

Plate 1, 2 and 3 Study material showing drought stress induced

IV. SAMPLE COLLECTION

Cow pea plant (Vigna unguiculata L) seeds were collected from Kerala Agricultural College, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India.

V. ORPHOLOGICAL PARAMETERS

A. Determination of Plant Height

The plant height (centimeters) was measured with the help of scale at the time of harvest. The length was measured from the point where the root and shoot joins to the end of root for root length and to the top of shoot for shoot length

B. Determination of Fresh And dry Weight of Shoot And Root

After harvesting the seeding, the shoot was cut from root at the point where they joined together. The fresh weight was recorded for each part separately. And the sample was dried in an oven at 70°C up to constant dry weight.

C. Determination of Length of Radicle and Plumule

The radicle and plumule length (centimeter) were measured with the help of a centimeter scale at time of germination. The length was measured from the point where the root tip and shoot joints to the end of root.

D. Determination of leaf Water Content

Leaf water content was estimated according to the method described by [25] Leaf discs were punched from each treated plant and the fresh weight was determined. The same leaf discs were kept on water for 4 hrs and turgid weight recorded. The leaf sample was dried in oven at 85°C for dry weight.

E. Determination of Pigment Content

The chlorophyll content was determined according to [20] Leaf fresh materials (1g) were ground properly in 50ml of 100%acetone then centrifuged for 10min at 2500g, absorbance was read spectrophotometrically at 662,645 and 470nm.Pigment content was estimated following formula;

Chl a = (0.0127xODx663)-(0.00269xODx645)
Chl b= (0.0229xODx645)-(0.00468xODx663)
Total Chl = (0.0202xODx645)+(0.00802xODx663)

F. Biochemical Parameters

The study materials were analyzed for the following biochemical parameters.

1) *Determination of Catalase (CAT):* The enzyme extract of V. *unguiculata* was prepared in phosphate buffer. The homogenate was centrifuged and supernatant was used for enzyme assay. H_2O_2 - phosphate buffer was taken in an experimental cuvette, followed by the rapid addition of 0.1ml of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units recorded at 240 nm in a spectrophotometer (UV-1800 Shimadzu).The enzyme solution containing

H_2O_2 - free phosphate buffer was kept as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05units.

2) *Determination of Peroxidase (POD):* Enzyme extract A (20% homogenate) was prepared in 0.1M Phosphate buffer (pH 6.5) from the various parts of the plant, clarified by centrifugation and supernatant was used for the assay. To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430nm.To the test cuvette, 0.5ml of H_2O_2 was added and was mixed. The change in absorbance was recorded every 30 seconds up to 3minute minutes in a spectrophotometer (UV-1800 Shimadzu). One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

G. Non enzymatic Antioxidant

1) *Determination of Proline Contents:* Dry weight (0.5g) was extracted by homogenization in 3% (w/v) aqueous sulphosalicylic acid. After the 20 min of centrifugation at 3000xg supernatant collected was mixed with acetic acid and ninhydrin. The mixture was boiled for 1 hour and then absorbance was read spectrophotometrically at 520 nm using toluene as blank.
2) *Determination of Malonaldehyde Content:* Fresh leaves were ground in 1% (10 ml/g fresh weight) trichloro acetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 5 minutes. Reaction mixture containing 1.0 ml of supernatant and 4.0 ml of 0.5% (w/v) thiobarbituric acid (TBA) was heated at 95°C for 30 min, cooled on ice bath and centrifuged at 5000 rpm for 5 min for clarification. Absorbance of the supernatant was taken at 532 and 600 nm.
3) *Determination of Membrane Stability Index*

Fresh leaf samples (0.1 g) were taken in test tubes in two sets containing 10 ml of double distilled water. One set was kept in water bath for half an hour at 40°C and the electric conductivity was recorded (C1). Another set was kept in water bath at boiling temperature (100°C) and EC was recorded (C2). MSI was calculated as per the formula: (MSI) = [1 - (C1/C2)] × 100

Anatomy of stem Two to three centimeter long pieces of the material were taken. Thin sections were taken using a razor. The thinnest section of the material was taken with the help of delicate brush. For staining, the sections were left for 3 – 5 minutes in a watch glass with stain. Leave 3-5 minutes. The stained sections were mounded on a watch glass and were viewed under a compound microscope with a camera attached

V. RESULT AND DISCUSSION

Drought stress is one of the major abiotic stresses that severely affect the crop production. In this study the 14 days old seeding of cowpea plant were subjected to short term drought stress in order to observe its effect on chlorophyll content, membrane stability index (MSI), malondealdehyde (M) and antioxidants enzyme activity (CAT and POD) response. The aim of the experiment was to evaluate the change in the content of above parameters upon stress in plants. The 14 days old seedling were subject to drought stress by supplementing Hoagland's solution with different concentration of Mannitol (0.1 and 0.2g/L) drought and the results were obtained as follows

A. Morphological parameters

The effect of drought stress on plant growth parameters is shown in Table1. Exposure of cow pea plant to various drought (mannitol) conditions reduced fresh and dry weight of plant, plant height, leaf water content, length of plumule and radical during germination period with increasing Mannitol Concentration treatment. Manntol induced reduction in weight was reported to be 1.52g (0.1g mannitol), 0.13g (0.2g mannitol), 0.95g (0.1g Mannitol) and 0.11g (0.2g NaCl) in fresh and dry weight of plant against 2.12 g of fresh weight of control plant and 1.95g of dry weight of control plant. The length of the plumule was measured to be 5.3 cm (0.1g Mannitol), 4.3 cm (0.2g Mannitol) against 15.63 cm (control) and the length of the radicle were 4.2 cm (0.1g Mannitol) and 2.14 cm (0.2g Mannitol), against18.5cm in control plant, respectively at various treatments. Leaf water content (LWC) is 53.5(0.1g Mannitol), 49.7(0.2g Mannitol) against 92.11 in the control plant.

Here, All the morphological parameters measured showed a reduction when compared to the control plant. The reduction in the parameters increased as the Manitole given increased. Mannitol effect on Shoot length and Shoot length decreased gradually with increasing stress. This can be explained by the findings of different researchers [1]. who reported increase accumulation of carbohydrates in roots under drought stress condition. Thus, because of high carbohydrates accumulation at higher stress may have compensated the severe adverse effect of stressDrought stress displayed significant reduction in shoot length at the most drought levels as compared with control (Table I). This reduction in growth might be due to low osmotic potential as well as a decrease in

wall extensibility and cellular expansion [10][3].Reduction of root length under stress conditions may due to an impediment of cell division and elongation leading kinds of tuberization [18]

Table1. Influence of Drought stress on growth parameters of Cowpea plant

SL No:	Treatment	Plant height(cm)	Fresh weight (F.W g)	Dry weight (D.W g)	Leaf water content(LWC %)	Length of radical (cm)	Length of plumule(cm)
01	control	30±1.00	2.12±0.03	1.88±0.06	92.11±2.3	5.63±0.07	8.5±0.141
02	Manitol 01.g	24.5±0.05	0.95±0.03	0.5±0.08	55.3±0.45	4.2±0.03	3.36±0.25
03	Manitol 0.2g	20.3±0.70	0.13±0.004	0.11±0.09	49.78±0.5	3.33±0.15	2.14±0.21

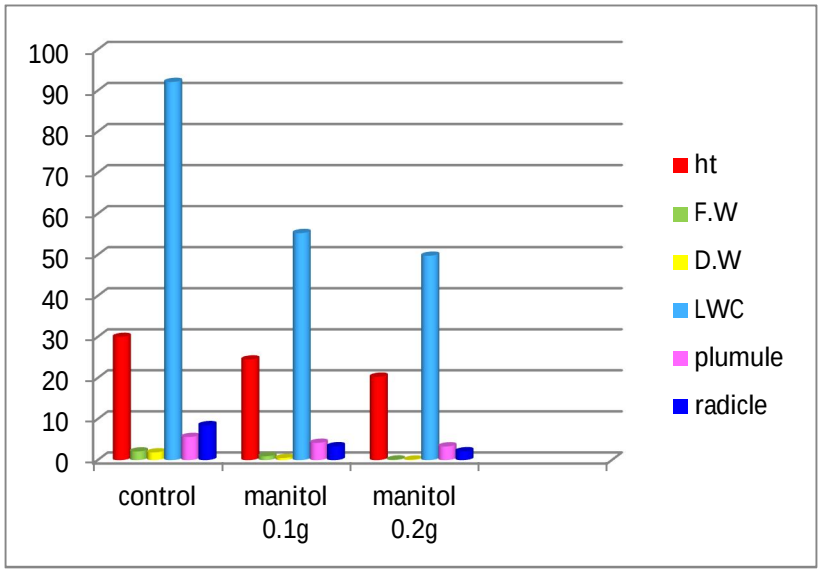

Figure 1.1.The change in the growth parameters

B. Chlorophyll Content

The effect of drought stress on the production of plant pigment is presented in (Table 2). It is seen that drought reduced chlorophyll a, chlorophyll b and total pigment content in the experimental plant. The values obtained with respect to the salt stress given were as follows. Chlorophyll a showed values such as 0.58 (0.1gMannitol) and 0.18(0.2g Mannitol), where the corresponding values for the control plant was 2.14. The content of chlorophyll b was 0.11 (0.1gMannitol) and 0.102(0.2gMannitol) as compared to 0.84 in control and total pigment content showed values 0.69 (0.1gMannitol) and 0.16(0.2gMannitol), when the control plant showed a total chlorophyll content of 2.22mg/g of fresh weight.

Drought stress imposed at the vegetative stage, significantly decreased chlorophyll *a* content, chlorophyll *b* content and total chlorophyll content both at the vegetative and flowering stages.The restricted water supply during the entire vegetative stage had a mild effect on these contents. The lack of effects on the chlorophyll *a*/*b* ratio indicates that chlorophyll *b* is not more sensitive to drought than chlorophyll *a* (Table2). The results are agreement with [10], [7] who described a significant decrease of chlorophyll *a* and *b* caused by water deficit . Decreased or unchanged chlorophyll level during drought stress has been reported in other species, depending on the duration and severity of drought [5] A decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments [12].

Table 2 .Drought stress on pigment system of Vigna unguiculata

SL no	Treatment	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight	Total chlorophyll content (mg/g fresh weight)
01	Control	2.14±0.16	0.84±0.06	2.22±0.07
02	Mannitol (0.1g)	0.58±0.001	0.11±0.009	0.69±0.007
03	Mannitol (0.2g)	0.18±0.002	0.102±0.003	0.160±0.003

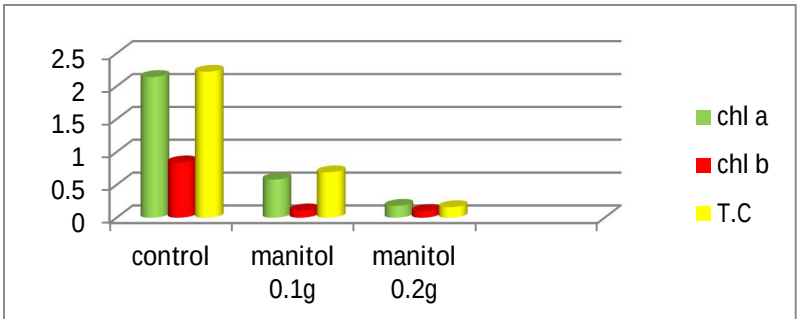

Figure 1.2. Effect of Mannitol treatment on total chlorophyll content

C. Enzymatic antioxidant activity

1) *Catalase (CAT)***:** Drought cause a wide range of responses in plants such as decreased growth, increased osmotic potential and most of important production of ROS due to oxidative stress in the cell. ROS are highly reactive species which readily oxidize protein, lipids, nucleic acid. As reported in many plants both enzymatic and non-enzymatic antioxidant plays an important role in scavenging the ROS. Result of antioxidant content in this study showed that CAT and POD activities increased with salinity stress. Increase in CAT and POD of drought enhancement was 15.2(0.1g mannitol) and 17.2(0.2g mannitol) as compared to 12. 58 in control plant in leaf samples. 9.28 (mannitol0.21), 11.71(mannitol 0.2g) as compared to 7.16 in control plant in root sample. In the drought-tolerant variety,cow pea palnt, CAT activity increased sharply in relative to the control (Table 6). This enzyme activity increased gradually up to the level and higher than the control.

The CAT is one of the highest turnover rates for all enzymes with the potential to directly dismutate H2O2 into H2O and O2 and is indispensible for ROS detoxification in peroxisomes during stress condition [1][9] Tolerance to drought-stress in higher plants correlates to the levels of antioxidant systems and substrates [6][11]. To combat the effects of drought–induced oxidative stress, plants develop a complex mechanism of antioxidant system. Relatively higher activities of ROS-scavenging enzymes have been reported in tolerant phenotypes when compared to susceptible ones, suggesting that the antioxidant system plays an important role in plant tolerance against environmental stress.

Table 3. Influence of drought stress of Catalase content on *Vigna unguiculata*

SL no:	Treatment	Catalase(CAT)	
		Leaf	Root
01	Control	12.58±0.106	7.16±0.029
02	Mannitol (0.1g)	15.2±0.028	9.28±0.026
03	Mannitol (0.2g)	17.2±0.251	11.71±0.04

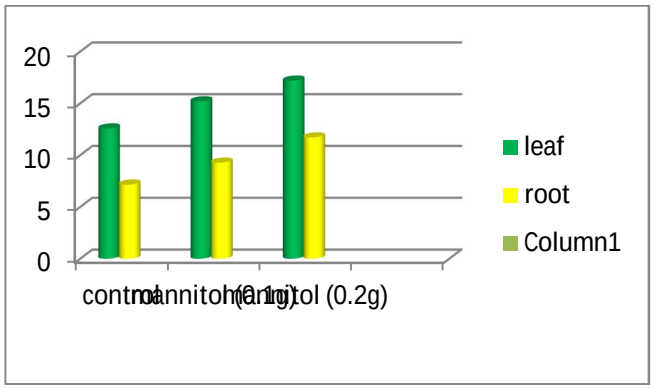

Figure 1.3. Catalase content in drought stress in Cowpea plant (*Vigna unguiculata* L)

2) Peroxidase(POD): Table 7 shows the result of peroxidate enzyme activity in the study plants. The highest levels of peroxidase enzyme (POD) activity in leaves were found with 0.1g and 0.2g mannitol treatment 79.2 and 82.34 in leaf sample.And 75.29(0.1g mannitol),84.2(0.2gmannitol) in root sample.The results of the study showed an increase in the POD activity. POD activity increased with increasing drought stress in all upland rice varieties. Drought induce significant alterations in plant biochemistry and metabolism. Under drought stress, the responses deal with the stimulated production of reactive oxygen species (ROS), (eg singlet oxygen, superoxide radical, hydrogen peroxide, hydroxyl radical [4] that cause membrane injuries, protein degradation, enzyme inactivation and thus induce oxidative stress [22].The main injuries under high temperatures include protein denaturation and increased fluidity of membrane lipids and inactivation of enzymes, reduced synthesis and degradation of proteins, and defaults in membrane integrity [12] Severe cellular injury or death may occur at moderately high temperatures after long-term exposure or within minutes at very high temperatures [13] These injuries may result in reduced ion flux and plant growth, and production of toxic compounds and reactive oxygen species likewise under water deficit. POD also involved in various plant processes, including lignification [12][15].The tolerance of some genotypes to environmental stresses has been associated with higher activities of antioxidant enzymes. For example, the drought-tolerant species of pigeon pea (Cajanus cajan) [21] wheat (Triticum aestivum) [18][19]. and black gram (Phaseolus mungo) had higher activities of SOD, POD and CAT than the drought-sensitive species.

SL No	Treatment	Peroxidase(POD)	
		Leaf	root
01	Control	71.14±0.056	73.2±0.023
02	Mannitol (0.1g)	79.2±0.082	75.2±0.036
03	Mannitol (0.2g)	82.34±0.092	84.2±0.043

Table.4.Peroxidase content in drought stress in Cowpea plant (Vigna unguiculata L)

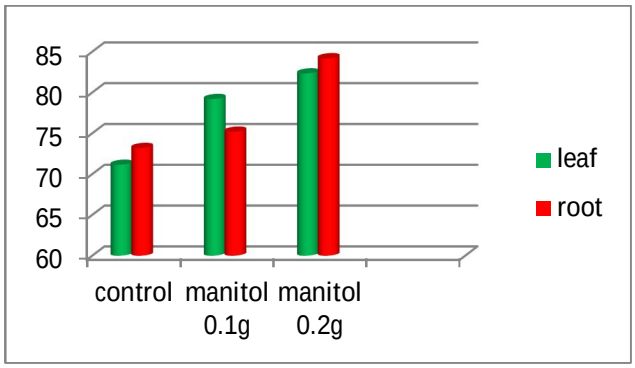

Figure 1.4. Peroxidase activities in stress induced study material

D. Non enzymatic activity

1) *Proline Content :* As seen in Table 5, increase in proline content was conspicuous in salt stressed plant. Manitole drought stress 0.1g, 0.2g induced treatment showed a proline content of 4.86 g and 6.34g respectively, where in the control plant showed 1.93 g only. In this result, There was a significant increase of proline content in drought-tolerant variety cowpea palnt and exhibited highest proline content at all drought levels relative to the control. In drought-sensitive variety, Kusam showed lowest proline content at highest drought level compared to other varieties. Besides, the upland rice varieties, Hita, Sintok, Bertih and Becor showed higher proline content than Nabawan and Tanom at the -8 bar PEG concentration. The increase in proline content due to drought stress was more severe at growing stage. The proline content depends on plant age, leaf age, leaf position or leaf part [8]. Under vegetative stage, drought stress increased proline content about tenfold, this increasing roles as an osmotic compatible and adjust osmotic potential which resulted in drought stress avoidance in chickpea. Prolin accumulation is believed to play adaptive roles in plant stress tolerance Accumulation of proline has been advocated as a parameter of selection for stress tolerance [2].

Table 5. Influence of drought stress on proline content of Vigna unguiculata

SL no	Treatment	Proline content
01	Control	1.93±0.23
02	Mannitol (0.1g)	4.86±0.42
03	Mannitol (0.2g)	6.34±0.40

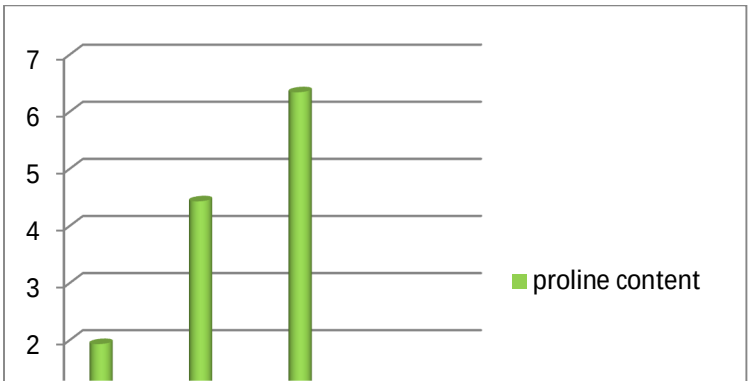

Figure 1.5. Effects of drought treatment on proline content

2) *Malodealdehyde (MDA):* In given table 4, Lipid peroxidation measured in terms of MDA content was reported to increase by 8.1 (0.1gmannitol) and 10.10(0.2g mannitoll) treated cow pea plant. The control plant showed only 4.12 g of MDA. The lipid peroxidation level, as indicated by MDA accumulation, increased significantly under the drought stress.

To determine whether growth reduction in cowpea plant is associate with drought stress induced oxidative damage, MDA were measured.The significant increase in MDA content which which was higher under severe stress than mild stress.Oxidative damage is one of the main cause of growth reduction under drought stress conditions[17]One of the main damages inflicted by oxidative stress is lipid peroxidation,Which is often used to assess oxidative stress damage in plants[20] [17].peroxidation of membrane lipid usually weakness the membranes causing minerals and other important cellular metabolites to leak out of the cells, consequently

leading to the cell [16]. The beneficial roles of in drought affected wheat plants were observed as those treatments reduced oxidative stress (decreased H2O2 and MDA levels), compared to untreated drought affected plants.

Table.6. Influence of drought stress Malondialdehyde (MDA)

SL no	Treatment	Malondialdehyde (MDA) g
01	Control	4.12±0.42
02	Mannitol (0.1g)	8.1±0.026
03	Mannitol (0.2g)	10.10±0.16

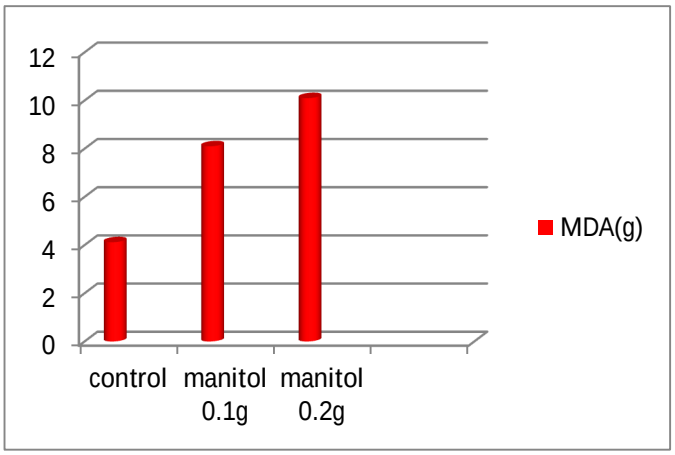

Figure 1.6. Effects of drought treatment on Malondialdehyde (MDA)

3) *Membrane Stability Index (MSI):* The results of MSI are given in table 3. It can be seen that both level of salinity stress induced reduced MSI in comparison to control. Salinity induced reduction is reported to be 71.06% (0.1gMannitol) and 52.4% (0.2gMannitol).

The decreased in the Nitrate reductase (NR) activity in the stressed plant may be considered a biochemical adaptation to conserve energy by stopping nitrate assimilation. The reason that seems most appropriate to explain the SA mediated elevation in the activity of nitrate reductase is that it corrects the stress mediated damage to the plasma membrane, as evident from an increase in the membrane stability index The membrane correction/stabilization could have facilitated the increased uptake of nutrients including that of nitrate, which act as an inducer of NR [14]. The increase in the uptake of various nutrients including NO3 and activation of NR, under normal condition is well established[13]. which strongly support the present findings. Also, [22][21] cleared that drought significantly decreased MSI in different wheat cultivars. Moreover, the amount of electrolyte leakage from the leaves of poplar plants was reported to increase under water stress conditions.

SL No	Treatment	Membrane stability(MSI)%
01**0.1**	Control	87.12±2.11
020.2	Mannitol (0.1g)	52.4±2.43
00.3	Mannitol (0.2g)	44.5±0.07

Table 7. Influence of drought stress on Membrane Stability index (MSI)

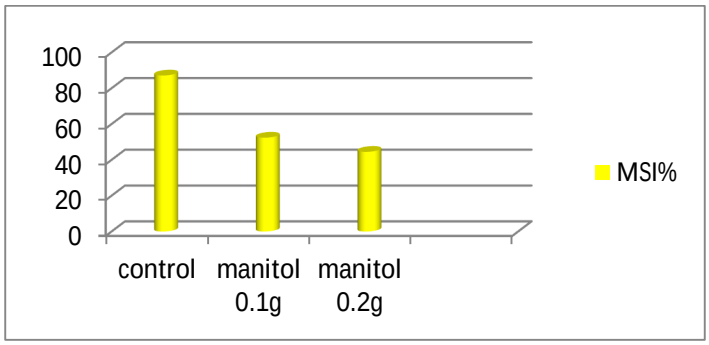

Figure 1.7 Effects of drought treatment on Membrane Stability (MSI%)

E. Anatomy of stem

The cross section (anatomy) of stem of Vigna unguiculata was analyzed to assess the effect of various mannitol concentrations and the anatomical adaptation of this plant to be acclimatized under drought stress. There was significant alteration in anatomical feature of stem of cow pea seedings imposed to various level of drought . Transverse section of stem V. unguiculata showed decrease in thickness of upper epidermal layer at 0.1g mannitol and 0.2g mannitol compared to control. The thickness of cortex layers of stem was reduced by different concentration level. The thickness of hypodermal layer and pith area of stem were increased by 0.1g mannitole, 0.2g mannitol treated plants. The pith cell diameter increased significantly in stem of mannitol treated seedling as compared to control. There was no significant change observed in the xylem vessels diameter of the stem of cow pea plant.

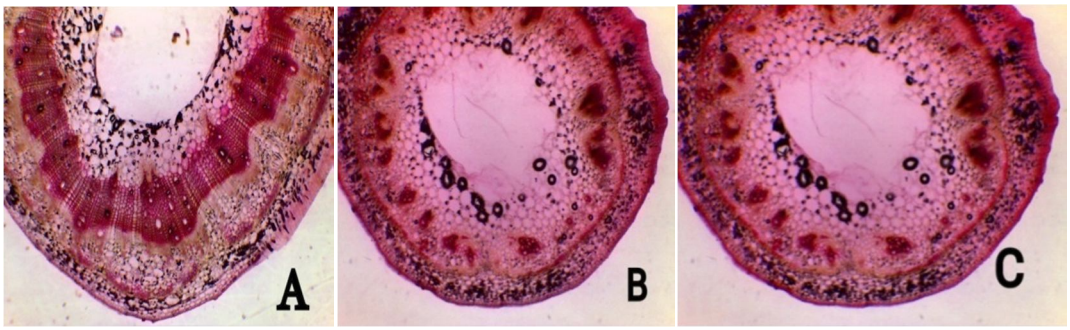

Figure (A) Control(0g NaCl) Figure (B) 0.1g/L mannitol Figure(C) 0.2g/L mannitol

Plate 4: Figures show effects of drought on stem anatomy (10x magnification)

VI. CONCLUSION

Present study of morphological and physiological and anatomical changes in the Cow pea plant (*Vigna unguiculata*). The study indicate decreased plant growth, fresh and dry weight of plant, and increase in the Proline content, Malodialdehyde (MDA) as well as activity of antioxidant enzymes (CAT and POD). The study concludes that environmental stress such as salt greatly influence the activity of both Catalase and Peroxidase. Drought cause oxidative stress which affect the biochemical and enzymatic component in plant cell drought(mannitol) stress.

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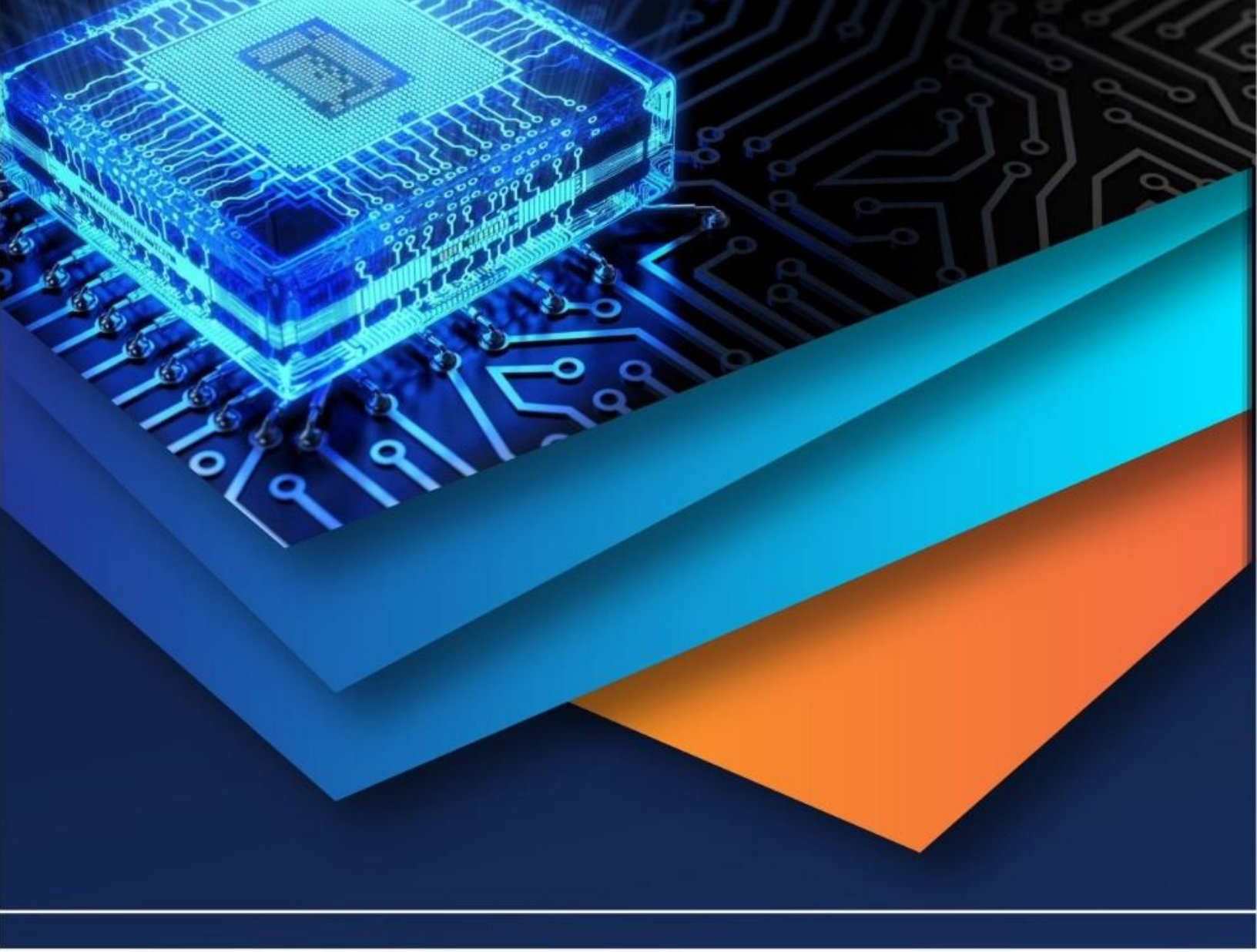

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