IMPACT OF WATER STRESS ON LIPID PEROXIDATION, ASCORBIC ACID AND ULTRASTRUCTURAL CHANGES IN MESOPHYLL CELLS OF PIGEONPEA (CAJANUS CAJAN (L.) MILLSPAUGH) SEEDLINGS

S. Punyasheela Devi*1 and B. Sujatha2

1Research Scholar Department of Botany, Andhra University, Visakhapatnam, A.P India.
2Associate Professor, Department of Botany, Andhra University, Visakhapatnam, A.P India.

Article Received on 27/07/2015 Article Revised on 18/08/2015 Article Accepted on 09/09/2015

ABSTRACT

Responses of lipid and ascorbic acid content to water stress induced by PEG6000 were studied in two pigeon pea [Cajanus cajan (L.) millspaugh] cultivars. Water stress condition was stimulated in three levels of osmotic stress of 0Mpa (control), -0.3Mpa, -1.1Mpa and -2.3Mpa. Polyethylene glycol-6000 is used for initiation of water stress in seedlings of pigeonpea. The study was performed in two pigeonpea varietie; ICPL-85063(Laxmi) a short duration and short cultivar and ICPL-87119 (Asha) medium duration and tall cultivar were studied. The aim of this study is to understand the levels of both the lipid peroxidation and ascorbic acid. The results depicted higher values in all the treatments when compared to controls in both the cultivars. The activities of lipid peroxidation and ascorbic acid levels were registered lower values in all the treatments when compared to their controls. The level of ascorbic acid content is high in cv ICPL-85063 than in cv ICPL-87119 in response to polyethylene glycol treatments. The cv ICPL-85063 is less sensitive to water stress and polyethylene treatments compared to cv. ICPL-87119. The results from the comparative analyses of the lipid peroxidation and ascorbic acid in short and tall varieties are discussed in terms of their possible involvement in conferring resistance to water stress.

KEYWORDS: Cajanus cajan; drought; pigeonpea; lipid peroxidation; ascorbic acid.
1. INTRODUCTION

Abiotic stress is one of the major problems in rainfed agriculture. Waters stress, cold and extreme temperature are some of the abiotic stresses which severely impair plant growth and productivity.\[24\] Water stress condition became more adverse problem due to the frequent weather vagaries prevails in agricultural crops.\[1\] Plant growth is very much affected by the water stress, as it is an important limiting factor.\[25\] Worldwide approximately one third of the cultivated area is affected by inadequate supplies of water.\[19\] Crop production and food security are the main factors which are affected by the abiotic stress like drought and salinity stress adversely impact the socio economic fabric of many developing countries. According to\[33\] water stress and salinity are the osmotic and toxic affect of salt at the cellular level: Drought is described as a one damaging factor. Day by day water stress is seen in almost every agricultural field in addition to this soil salinity is also another problem\[6\] (Flowers T J 2004). The plants under water stress change their metabolism to overcome the environmental changing conditions. Drought results in the decrease of germination percent and a in reduced seedling growth.\[22\] In the world the most important and severe problem is the drought.\[16\] Abiotic factors like drought salinity, extreme temperatures and oxidative stress are inter connected and directly affect the water relations of a plant on the cellular as well as whole plant level causing specific and unspecific reactions\[2\] and due to these reasons we can observe morphological, biochemical and molecular changes that adversely affect plant growth and productivity.\[31\]

Pigeonpea [Cajanus cajan (L.)Millsp] is an important legume crop cultivated in different climates like tropical and sub tropical regions of the world. This is used for major dietary protein for India and South East Asian countries in addition to its diversified uses like feed, fuel and fertilizer. The pigeonpea is the sixth most important high protein containing food crop and is grown on about 5 million ha in India which accounts for 90 per cent of world production.\[28\]

Polyethylene glycol -6000 (PEG) is used to induce water stress for seedlings in this experiment, as it is a nontoxic with has high molecular weight and can’t pass through plant cell walls.\[4\] The choice of utilization of PEG as stress inducer in plants was previously supported by\[17\] many research workers and stated that it is a non ionic polymer, which is not expected to penetrate into plant tissue.
2. MATERIALS AND METHODS

Two locally cultivated pigeonpea cultivars were selected and seeds were obtained from Regional Agricultural Research station, Lam, Guntur, Andhra Pradesh, India. Two cultivars of pigeon pea ICPL87119 (tall) and ICPL85063 (short) were selected for present investigation. The seeds of healthy and uniform size were selected and surface sterilized with 0.05M mercuric chloride for 2min. washed thoroughly with glass distilled water and then soaked in distilled water for 2hrs. The soaked seeds were then spread over plastic trays (approximately 50 seeds per tray) lined with two layered Whatman no 1 filter paper containing different concentrations of polyethylene glycol 6000 representing 0mM, 50mM, 100mM and 150mM. The seeds raised in distilled water were referred to as controls. Ten ml of each test solution was added separately to each tray and the filter papers were replaced on every alternate day during the study period. The seeds of the two cultivars were allowed to germinate at 30±2°C for 6 days under a photoperiod of 18h. The analyses was made in different parts of the seedlings viz., Root and shoot separated prior to the experiment where as for various photosynthetic parameters shoot and leaf is considered. All the experiments were replicated thrice. The concentration of PEG 6000(g/kg of water) for each water stress was determined using the equation of.\[^{[20]}\]

2.1 Lipid peroxidation

The lipid peroxidation of different parts of control and treated 6-day old seedlings were determined as a measure of malondialdehyde formation which is a product of lipid peroxidation. The procedure was a minor modification of the method adopted by\[^{[12]}\] (Heath R L., Packer L., 1968). One g of plant material was macerated in 5 ml of 0.1% TCA. The homogenate was centrifuged at 10,000 x g for 5 min. To one ml of the aliquot of the supernatant 4 ml of 20% TCA containing 0.5% thiobarbutaric acid was added. The mixture was heated at 95°C for 30 min and then quickly cooled on ice bath. The mixture was centrifuged at 10,000 x g for 15 min and the absorbance of the supernatant was measured at 532 nm and the value for the non-specific absorption at 600nm was subtracted. The concentration of malondialdehyde was calculated using extinction coefficient of 155 m M\(^{-1}\) cm\(^{-1}\).

2.2. Ascorbic acid

The determination of Ascorbic acid was carried out by the procedure given by.\[^{[27]}\] Five milliliters of the working standard solution was pipetted out into a100ml conical flask, and
then 10ml of 4% oxalic acid was added and titrated against the dye. End point was the appearance of pink color, which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid. 5ml of plant extract was pipetted out and 10ml of 4% oxalic acid was added and then titrated against the dye.

**Reagents**

(a) **4% Oxalic acid:** 4gms of oxalic acid was dissolved in 100ml of distilled water.

(b) **Dye solution:** 26mg of 2, 6-dichloro-phenol indophenol and 21mg sodium bicarbonate were dissolved in 100ml of volumetric flask made up to 100ml with distilled water.

(c) **Stock standard solution (1mg/ml):** 100mg of ascorbic acid was dissolved in 100ml of 4% oxalic acid solution in a volumetric flask.

(d) **Working standard (10µg/ml):** 10ml of the stock solution was diluted to 100ml with 4% oxalic acid.

**Transmission electron microscopy**

For transmission electron microscope samples were fixed in 3% glutinaldehyde in 0.05M phosphate buffer (pH 7.2) for 24h at 4°C and post fixed in 2% aqueous osmium tetraoxide in the same Buffer for 2h. After post fixation, samples were dehydrated in a graded series of acetone/alcohol, in filtrated and and embedded in Araldite 6005 resin (Glauert and Glauert., 1958; Moller hauer et al., 1959). Semi thin sections 300-500nm) and ultra thin sections (50-70nm) were made with a glass knife on a leica ultra cut (UCT-GA -D/E-1/100) microtome. Semi thin section were mounted on glassed slide stained with toludine blue for light microscopy(Olympus AX-70) and ultra thin sections were mounted on grids and stained with saturated aqueous uranyl acetate and counter stained with4% lead citrate for transmission electron microscopy (Hitachi, H-7500).

3. RESULTS AND DISCUSSION

3.1 Effect of water stress on lipid peroxidation

The formation of malondialdehyde content was considered as a measure of lipid peroxidation. The lipid peroxidation of roots and shoots of the two pigeonpea cultivars increased with increasing concentrations of water stress (table-1). The lipid peroxidation was more active in cv. ICPL-87119 in response to the water stress (fig-1). In the present study, MDA content increased (fig-1) in all the treatment when compared to control in pigeon pea cultivars. In this process, ketones and malondialdehyde are formed, these are the small hydrocarbon fragment’s. Malondialdehyde aggravates the oxidative stress through...
production of lipid derived radicals\cite{21} (Montillet, J.L., S Chamnongpol, 2005). This process results in the decrease of membrane fluidity and increase in the leakiness of the membrane to substances that do not crossed by normal functions and ultimately damage the proteins and enzymes. In this study it is observed that the levels of lipid peroxidation increased in both cultivars of pigeon pea but its level was higher in ICPL-87119 than ICPL-85063. The lower level of lipid peroxidation resulted in correlation higher free radicals scavenging capacity and more efficient protection mechanism of ICPL-85063 against the water stress with lower level of lipid peroxidation. Water stress in *Phaseolus vulgaris*\cite{33} (Zlatev et al., 2006) and tomato\cite{26} were studied previously which showed increased lipid peroxidation, membrane injury index H$_2$O$_2$ and OH- production in leaves.

Generally lipid peroxidation used as an indicator of stress induced oxidative damage of membrane \cite{28} explained that wheat plants showed the weakening of membrane integrity and oxidative damage to lipids in sensitive varieties under field drought conditions. Under water stress, MDA increased and this is due to the production of reactive oxygen species (ROS) which lead to lipid peroxidation,\cite{18} This was previously also reported under polyethylene glycol stress; MDA content was also increased \cite{9} and the increase of MDA content under PEG stress.\cite{23} The present analyses shows that shoots of icpl-85063 (short duration, short cultivar) exhibited lower level of lipid peroxidation when compared to the icpl-87119 cultivar, this indicates the less sensitivity of the cv. Lakshmi(ICPL85063).

**Table 1 Water stress induced changes in Lipid peroxidation and Ascorbic acid in two cultivars of pigeonpea [Cajanus cajan (L.) Millspaugh] seedlings**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>ICPL 85063(cultivar)</th>
<th>ICPL87119(cultivar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lipid peroxidation</td>
<td>Root</td>
<td>1.864$^b$</td>
<td>1.797$^b$</td>
</tr>
<tr>
<td>as MDA equivalents</td>
<td>Shoot</td>
<td>1.204$^b$</td>
<td>1.368$^b$</td>
</tr>
<tr>
<td>(µ Mol per cm)</td>
<td></td>
<td>2.311$^a$</td>
<td>2.145$^a$</td>
</tr>
<tr>
<td>0</td>
<td>-0.3Mpa</td>
<td>2.376$^a$</td>
<td>2.285$^a$</td>
</tr>
<tr>
<td>-1.1Mpa</td>
<td>-2.3Mpa</td>
<td>1.797$^a$</td>
<td>2.160$^c$</td>
</tr>
<tr>
<td>-2.3Mpa</td>
<td></td>
<td>1.943$^b$</td>
<td>2.723$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.332$^a$</td>
<td>2.567$^{bc}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.603$^a$</td>
<td>3.204$^a$</td>
</tr>
<tr>
<td>2. Ascorbic acid</td>
<td>Root</td>
<td>1.480$^d$</td>
<td>2.088$^c$</td>
</tr>
<tr>
<td>(mg/100gm plant)</td>
<td>Shoot</td>
<td>2.156$^c$</td>
<td>2.688$^{bc}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.926$^a$</td>
<td>3.357$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.605$^b$</td>
<td>3.151$^{ab}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.169$^c$</td>
<td>2.261$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.788$^b$</td>
<td>3.285$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.030$^a$</td>
<td>3.237$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.539$^{bc}$</td>
<td>3.048$^a$</td>
</tr>
</tbody>
</table>

The significant values between treatment and control of different parameter of Lipid peroxidation and Ascorbic acid with increasing concentration of polyethylene glycol-6000 of 6-day old seedlings of the different parts of the pigeonpea cultivars ICPL85063 and ICPL87119 were statistically evaluated by two-way ANOVA.
Fig.1 Lipid peroxidation activity of the roots and shoots of 6-day-old seedlings of two pigeon pea cultivars in response to water stress induced by PEG -6000.

3.2 Effect of water stress on ascorbic acid content

The effect of water stress on roots and shoots of the pigeon pea seedlings are shown in fig. 2.between the two cultivars of pigeon pea, the ascorbic acid content was more in icpl-85063 than in ICPL-87119 in response to polyethylene glycol treatments (table-1). The role of ascorbic acid as a key molecule antioxidant involved in abiotic stress has been well described. Water stress leads to generation ROS such as oxide anion radicals hydrogen peroxide and hydroxyl radicals. Reactive oxygen species can damage essential membrane lipid, proteins and nucleic acids.\(^8\) To overcome these damages plants posses an antioxidant system consisting of low molecular weight antioxidants such as β carotene, ascorbic acid (AA), α –tocopherol( α-Toc) and antioxidant enzymes like superoxide dismutase (SOD) catalase (CAT) and peroxidase (pox) an antioxidant molecule which acts as a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen.\(^9\) Pigeonpea cultivar ICPL-85063 registered lower level of lipid peroxidation and higher levels of ascorbic acid content resulted in less oxidative damage confirming that, cv. ICPL-85063 is less sensitive to treatments (fig-2). Further the ascorbic acid of roots and shoots of the two pigeonpea cv showed a significant negative correlation with the increasing concentration of PEG-6000 (table-1).
Fig. 2 Ascorbic acid activity of the roots and shoots of the 6-day-old seedlings of two pigeonpea cultivars in response to water stress induced by PEG-6000.

Fig 3A&B: Ultrastructure of mesophyll cells 6-day old pigeonpea cv. ICPL8506 (control, ×2895) and ICPL87119 (control, ×3474).
Fig4(C-H): Ultrastructure of mesophyll cells 6-day old pigeonpea cv.ICP85063 and ICPL87119; C (50mM, PEG-6000,X1930) D(50Mm,PEG-6000,X4825) E(100Mm,PEG-6000,X5790) F( 100Mm,PEG-6000,X4825)G(150mM,PEG-6000,x11580)H(150mM,PEG-6000,x2316). cell wall (cw), vacuole(v), chloroplast (ch)

As illustrated in Figure. 3A and B leaf material from control plant was characterized by well defined cell structure. All of the cellular components like cell wall (cw), protoplasm(pm), nucleus (n), nucleolus(nl), nuclear membrane (nm), chloroplast (Ch), and mitochondria(m)were normal in appearance. Chloroplast structure was normal with nucleus, nuclear membrane and well developed mitochondria.

At the chloroplast level, the starch content was reduced in the leaves of water stressed plants when compared to the control plants which is shown in the transmission electron microscopy pictures (Fig.4C, D).
When compared to the treated leaves the chlorophyll content of pigeon pea (Cajanus cajan (L.) Millspaugh) displayed a damaged cell as the water stress increased. The osmotic stress of -0.3Mpa, (Fig4.C, D,) of the two cultivars showed the major damages in the chloroplast. The water stress affected the cell contained a chloroplast, which had begun to lose its integrity, the thylakoids were dilated and the nucleus (n) was degenerated. The loss of integrity was observed at -1.1Mpa of (Fig4E) osmotic potential which shows fragmented nucleus, degradation and disruption of the nuclear envelope. Chloroplast and mitochondria were disturbed. Thylakoid arrays disassembled.

Grana thylakoids were poorly developed starch granules vanished and chloroplast showed vacuolation in (Fig4G). Water stress caused significant changes in the grana and stroma lamellae, palisade cell walls, number and size of chloroplast and the structure of mitochondria. Water stress results in a reduction in chloroplast structures this observation is compatible with the decrease in photosynthesis characteristic of water stressed plants. Stressed cells showed larger central vacuoles, disrupt chloroplast structure and mitochondria with internal vesicle which is abnormally shaped is observed. Water deficiency by accelerating differentiation brings about smaller cell size. Water deficiency may bring out smaller size cells by accelerating differentiation. Thylakoids disruption and swelling is the resultant of the water stress it was reported in wheat plants, it has also been found in bean. The chloroplast of spongy parenchyma shows disrupted chloroplast (Fig4 F).Severe water stress (-2.3Mpa) induced through polyethylene glycol-6000 resulted in a plasmolysis, cell wall is being separated from protoplasm (Fig4H) and also shrunken chloroplast, some small round electron dense bodies called plastoglobules were observed.

**CONCLUSION**

The present study showed that water stress caused oxidative damage to plants through excessive ROS generation. ICPL-87119 showed the susceptibility to water deficit stress by showing the increased MDA content. The antioxidant ascorbic acid has an important function of reducing harmful effects of oxidative stress and improves the growth of plant under water stress condition. From the results it is clear that ICPL-85063 shows better performance than the ICPL-87119. The cultivar may recommend for rain fed areas of its cultivation in subtropical regions.
REFERENCES


2. Beck E H, Fetiting S


10. GAUSMAN HW, PS BAUR, MP PORTERFIELD, R CARDENAS Effects of salt treatments in cotton plants(Gossypium hirsutum L.). Leaf mesophyll cell micro structure. Argon J, 1972; 64: 133-136


17. Kawasaki T, Akiba T, Moritsgu M. Effects of high concentrations of sodium chloride and polyethylene glycol on the growth and ion absorption in plants. I. Water culture experiment in a greenhouse, 1983; 75: 75-85


