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Physiological and bio chemical response of pigeon pea (*Cajanus Cajan*) to salt stress

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Abstract

The effect of salt stress on some physiological and biochemical parameters in pigeon pea (*Cajanus cajan*) was studied under controlled conditions. The plants were treated with the solutions of 50 mM NaCl, 100 mM NaCl. It was established that the applied doses of both salt concentrations caused stress on the young pigeon pea plants, which found expression in the suppression of growth, and produced changes in photosynthetic pigments, relative water content, biochemical parameters suchas total soluble sugar, total free aminoacids, total protein, nitrate, antioxidants suchas catalase, peroxidase and superoxide dismutase and stress parameters proline and MDA.

Keywords: *Cajanus Cajan,* photosynthetic pigments, biochemical parameters, growth, MDA

1. Introduction

All plants are subjected tovarious biotic and abiotic stresses in natural environments during their lives. One of the biochemical changes occurring when plants are subjected to biotic or abiotic stresses is the production of reactive oxygen species (ROS) are highly reactive and in the absence of any protective mechanism they can disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Allen 1995) [2].

Salinity is one of the major obstacles for increasing production in cropping areas throughout the world, Decreasing the plant productivity and photosynthesis salinity also results in decreased leaf area in all speien. Plant species have been found to be either sensitive or moderately tolerant to stress factors although considerable variability in stress tolerance has been reported among and within plant species (Garg and Noor, 2009) [11]

Salt stress affects the photosynthetic, pigments, chlorophylls and carotenoids (Stepien and Klobus, 2006) [30]. and Reduces soil water potential and availability of water under higher salt concentration in root zone (rhizophere). Reduction of the water content leads to dehydration at cellular level and osmotic stress is observed.

Salinity may affect various metabolic processes such as photosynthesis, protein synthesis, respiration, nitrogen assimilation and phytohormone turnover (Arshi *et al*. 2002) [3]. Salt tolerance in plants is a complex phenomenon, which depends on a number of inter-related factors based on morphological, biochemical and physiological processes (Qasim *et al*. 2003) [28].

increase in Nacl level affect the uptake of many indispensable nutrient through competitive interaction and affected by membrane ion selectivity, These salt stress show dehydration, result in less availability of water to plant cell and cause several agricultural problems in arid and semi- arid regions like India.

The soil salinity affects growth and development of plant at physiological and biochemical level (Munns, 2002) [27]. Which leads to low osmotic potential of soil solution, specific ion effect, nutritional imbalance or a combined effect of all those factors, A primary response of plant exposed to salinity stress decreases plant water potential, leading to plant water use efficiency. The salt tolerant species posses a high capacity to resist salt stress through the bio synthesis and accumulation of compatible solutes. The substance rise the overall osmotic or pressure with in cells to maintain both turgor and the driving gradient for water uptake. These compatible solutes like protein, carbohydrates, amino acid and quaternary ammonium compounds. These compound play a important role as osmotic balancing agents as well as plant cell stabilizers. Reactive Oxygen Species (ROS) under environmental stress cause oxidative stress, such as Superoxide ion, hydrogen peroxide, singlet oxygen, hydroxyl radical ROS are present in all plant as cytotoxic metabolites and are meditators of the stress response. ROS can cause damage to cellular components such as cell membrane, lipids, protein and nuclic acid, disrupting metabolic function and leads to injury or death under environmental stress. Proline level increase in salt stress saline condition (Munns, 2002) [27].

Pigeon Pea (*Cajanus Cajan)*

Pigeon pea (*Cajanus cajan)* commonly known as pigeon pea perennial shrubs or small tree cultivated throught the tropics and subtropics. It is most important species, that seeds are used as food world wide, which is a leguminous plant belonging to the family *Leguminosae-fabaceae*. Pigeon pea is a rich source of protein content, CHO, and certain minerals. The protein content of commonly grown pigeon pea ranges between 17.9 and 24.5g/100g for whole grain samples

Unlike other grain legumes, pigeon pea production is concentrated in developing countries particularly in a few south and southeast Asia and Eastern and southern African countries. It is the preferred pulse crop in dry land areas where it is intercropped or grown in mixed cropping systems with cereals or other short duration annuals. It also improves soil fertility through nitrogen fixation as well as from the leaf fall and recycling of the nutrients.

It is an important pulse crop that performs well in poor soils and regions where moisture availability is unreliable or inadequate. In India pigeon pea leaves are used for curing sores, wounds, abdominal tumours, and diabete. Pigeon pea is very tolerant of hot conditions, generally grown in temperature of 18-30.degree C in red soil.

Pigeon pea (Cajanus cajan) is widely grown as a pulse crop in many parts of the Indian subcontinent. It is largely eaten in the form of split pulse as dal, while its tender green pod constitute a very favourite vegetable.

Peroxidase

Peroxidases are a family of isozymes found in all plants; they are home-containing monomeric glycoproteins that utilize either H_2O_2 or O_2 to oxidaze a wide variety of moleculas (Yoshida *et al*. 2002) [33]. Peroxidases that trigger the conversion of H_2O_2 to water and O_2 are part of the enzymatic defense of plant cells. Many physiological functions for peroxidases in plants have been reported as removal of H_2O_2, oxidation of toxic reductants, biosynthesis, defensive responses to wounding, defense against pathogen or insect attack, and some respiratory processes (Gaspar *et al*. 1982) [12].

Proline

It is an α-amino acid, It may have many functions is stress tolerance including osmotic adjustment, protein and membrane, Stabilization, gene induction control of, reactive oxygen species (ROS). Proline accumulation is an important physiological index for plant response to salt stress. Salinity increased markedly the proline content in different salt sensitive and tolerant species/cultivars. Proline accumulation may be caused by increased proteolysis (pr) by decreased protein synthesis.

Soluble sugar

It is an organic compound. It consists of carbon, hydrogen and oxygen. Total soluble sugar (TSS) content is not only the main photosynthate in higher plants, but also the main form of carbohydrate metabolism and temporary storage. Wilcox (2001) [32] indicated that the soluble sugar content plays a very important role in carbohydrate metabolism and has a close relationship with photosynthesis and production. The level of soluble sugar content was a sign of the supply ability of leaves and reflected transformation and ability of grains to use assimilates (Saratha *et al*., 2001) [1].

Protein

It is consisting of one (or) more polypeptides folded into a globular (or) fibrous from. The leaf protein synthesis is most probably dependent upon a carbon supply from the photosynthetic pigments and photo system I and II. In plants decreased protein synthesis was observed in different tissue parts under salt stress condition. Decreased protein content in pigeonpea mature leaves suggests that the protein turnover rate i.e., synthesis/degradation was more toward degradation in these trees.

Total free aminoacids

Accumulation of amino acids has been observed in many studies on plants exposed to abiotic stress (Lugan *et al*., 2010) [23]. This increase might stem from amino acid production and/or from enhanced stress-induced protein breakdown. While the overall accumulation of amino acids upon stress might indicate cell damage in some species (Widodo *et al*., 2009) [31], increased levels of specific amino acids have a beneficial effect during stress acclimation.

Tbars (MDA)

Malondialdehyde is an indicator of oxidative-stress related peroxidation of membrane lipids and its concentration has been shown to correlate with peroxides resulting from stress related membrane damage. The increases in MDA suggest the lack of increases in the activity of enzymes that are known to increase in plants under oxidative stress may be evidence that the stress was mitigated by the normal levels of antioxidant metabolism.

Antioxidant enzymes

Plants have evolved several mechanisms to prevent or alleviate the damage from oxidants. These mechanisms include scavenging the oxidants by natural enzymatic antioxidant system that includes SOD, CAT, POX and GR many of which acts in tandem. The existence of multiple molecular forms of antioxidant enzymes, their location within tissues, cells or organelles and any changes they may undergo in response to various environmental signals or development imply potential roles for these isozymes in the detoxification of ROS.

2. Materials and Methods

Pigeon pea seeds were collected from Agricultural College in Madurai. The seeds were surface sterilized with mercuric chloride, washed with distilled water and soaked in distilled water for 24 hrs. After sprouting the seeds were sowed in the plastic pots.

Plant Growth Condition and Salinity Treatments

Pigeon pea seedlings were grown under controlled climatic condition temperature of 24-28 °C and stress for 14 days. The plants were watered regularly.

After 4-7 days, uniform seedlings with fully developed trifoliate leaves were transplanted into each of the plastic pots and arranged into three levels of salt stress. The plants were classified into three treatments.

1) Control - Seeds treated in watered regularly.
2) Test 1 - Seeds treated in a solution of 50mm Nacl.
3) Test 2 - Seeds treated in a solution of 100mm Nacl

Treatments were triplicates 3 times and arranged into a randomized complete Block design. At the end of 15th day, the growth parameters and biochemical parameters were measured in the collected seedlings.

Growth Parameters

The growth parameters such as root length shoot length, leaf area index, fresh weight, dry weight and total water content of the seedlings were measured. The shoot and root lengths of the randomly selected seedlings were measured using meter scale. Fresh weight was measured using an electronic balance. The plants were dried in a hot air oven at 80 °C and the dry weights were measured using an electronic balance. Total water content of the plant was calculated by subtracting the dry weight from the fresh weight

Total plant water content = plant fresh weight - plant dry weight/plant fresh weight X100

Biochemical parameters

The biochemical parameters such as chlorophyll and carotenoids (Brougham, 1960) [6] soluble sugars (Jayaraman, 1981) [16], total soluble proteins (Lowry *et al.,* 1951) [22], nitrate (Cataldo et al 1975) [7] total free amino acids (Moore, 1968) [26], proline (Bates *et al.,* 1973) [4], catalase activity (Aebi, 1984) [1], Peroxidase (Chance and Maehly, 1955) [8] TBARS (Heath and Packer, 1968) [15], Superoxide dismutase (Beyer and Fridovich, 1987) [5] were measured both in salinity treated and control plants. All measurements were made on samples in triplicates and data represented mean ± S.D.

Table 1: Effect of salinity (50mM Nacl and 100mM Nacl) on Growth parameters of *Cajanus cajan* The values represent mean ± SD, (n=9).

	Parameters	Control	Treatment 1 (50 Mmnacl)	Treatment 2 (100mmnacl)
1	Fresh shoot length (cm)	12.47 ± 0.09	11 ± 0.33	8.16 ± 0.47
2	Fresh shoot weight (g)	0.05 ± 0.007	0.02 ± 0.004	0.01 ± 0.002
3	Dry shoot length (cm)	9.82 ± 0.29	7.93 ± 0.09	7.11 ± 0.19
4	Dry root weight (g)	0.15 ± 0.007	0.05 ± 0.004	0.03 ± 0.004
5	Fresh root weight (g)	0.17 ± 0.01	0.13 ± 0.006	0.08 ± 0.007
6	Plant dry weight (g)	0.01 ± 0.009	0.009 ± 0.001	0.005 ± 0.004
7	Total plant water content (%)	78.88 ± 1.09	72.66 ± 1.83	64.22 ± 0.92

Table 2: Effect of salinity (50mM Nacl and 100mM Nacl) on photo synthetic pigments of of *Cajanus cajan.* The values represent mean ± SD, (n=9).

S. No.	Parameters	Control	Treatment 1 (50 Mmnacl)	Treatment 2 (100mmnacl)
1	Total Chlorophyll mg/g	0.39 ± 0.01	0.18 ± 0.03	0.16 ± 0.08
2	Chlorophyll A mg/g	0.10 ± 0.009	0.08 ± 0.09	0.05 ± 0.006
3	Chlorophyll B mg/g	0.24 ± 0.006	0.16 ± 0.006	0.14 ± 0.006
4	Carotenoids mg/g	3.72 ± 0.07	2.78 ± 0.09	2.58 ± 0.07

Table 3: Effect of salinity (50mM Nacl and 100mM Nacl) on photo biochemical parameters of *Cajanus Cajan*. The values represent mean ± SD, (n=9).

S. No.	Parameters	Control	Test 1 (50 Mmnacl)	Test 2 (100mmnacl)
1	Total soluble sugar mg/g FW	15.97 ± 0.14	17.01 ± 0.17	18.57 ± 0.26
2	Nitrate mg/g	7.28 ± 0.18	6.13 ± 0.13	4.97 ± 0.14
3	MDA(TBARS) mol/ml	0.29 ± 0.009	0.44 ± 0.02	0.5 ± 0.01
4	Proline mg/g	22.66 ± 0.47	24.66 ± 0.47	27.55 ± 0.49
5	Protein mg/g	23.92 ± 0.28	21.97 ± 0.11	19.7 ± 0.38
6	Total amino acids mg/g	12.23 ± 0.19	13 ± 0.13	14.33 ± 0.29
7	Catalase u/g	17.33 ± 0.94	15.16 ± 0.70	10.5 ± 0.88
8	SOD u/g	3.41 ± 0.12	1.53 ± 0.08	1.06 ± 0.08
9	Peroxidase u/g	3.0 ±0.11	0.85±0.05	0.06±0.05

According to the results obtained in our study the morphological features and overall growth pattern of the seedlings indicated the effect of salinity stress on 15th day salt stress treated of *Cajanus cajan L.* Salt stress treated plants have decreased shoot length when compared to control plants (fig 9, 10). Salt stress treated

2. Results

In this study, salt stress treated plants had less root fresh weight, fresh shoot weight, fresh root weight dry shoot length, dry root weight, plant dry weight than the untreated plants (both in 100mM Nacl salt stress and 50mM Nacl salt stress treated plants (Table 1))

Total plant water content was decreased in salinity treated plants compared to control (Table 1) The reduction in the pigments total chlorophyll, chl a, chl b, carotenoids were found to be more pronounced in 100mM Nacl treated plants than in 50mM Nacl treatment (Table 2)

The total soluble sugar level was found to be increased in salinity treated plants when compared to control plants. The biochemical parameters such as, protein, and nitrate have all shown a decrease in salinity treated *Cajanus cajan* L when compared with control plants(Table 3) The decrease was more pronounced in 100mM Nacl salt stress treated plants than the 50mM Nacl treated plants.

The total free aminoacid was found to be increased in salinity treated *Cajanus cajan L.* when compared to control plants. The increase was more pronounced in 100mM salt stress treated plants than the 50mM Nacl treated plants (Table 3). The stress parameters such as proline and Malondialdehyde were increased in both salt stress treated plants (Table 3)

Catalase, peroxidase and superoxide dismutase were found to be decreased in salt treated plant compared to untreated *Cajanus Cajan L.* (Table 3). The reduction was found to be high in 100mM Nacl treated plants when compared with 50mM Nacl treated plants.

3. Discussion

The results regarding the changes of the basic growth parameters of pigeon pea plants indicated that the applied salt treatment causes a considerable inhibition of their initial development. They change in accordance with the salt concentration. In both types of salt treatments the higher dose caused a stronger inhibition effect. This tendency was observed with respect to all parameters. Salinity stress would be expected to have an impact on plant growth, where a direct ion toxicity or nutritional imbalance occur (Jenifer and Franklin Janusz, 2002) [17] Various studies reported that increasing salinity is accompanied by significant reductions in root, shoot and leaf biomass, shoot length and increase in root/ shoot ratio in most of plants (Meconi *et al*; 2001; Maggio *et al*; 2007) [24]. In tune with above discussion, in the present study it was emphasized that salinity on stress led to cause dramatic changes in shoot length, root length, and plant biomass.

The observed decrease of Chlorophyll content in the plants grown under saline conditions may be attributed to both of the increased degradation and the inhibited synthesis of that pigment (Garsia-Sanchez *et al*, 2002) [10].

In the present study, the total chlorophyll content of pigeon pea leaves was decreased with the increasing concentration of Nacl. Maximum reduction in the total chlorophyll content was observed in 50mM and 100mM Nacl.

The reduction in chlorophyll content under stress at various developmental stages may be attributed to deterioration of plants. The breakdown of chlorophyll and inhibition of photosynthesis through stomatal closure decreases biomass. There is also a reason for decreased stress induced by Nacl produce reactive oxygen species (ROS) which leads to chlorophyll destruction with decreasing chlorophyll content due to changing green colour of the leaf into yellow.

The decreased chlorophyll and carotenoids could probably be a result from the mineral deficiency. It was established that carotenoid decreased to a lesser extent than chlorophyll. Free radical accumulation in leaves adversely affected chlorophyll content.

The pigeon pea plant synthesized greater amounts of free aminoacids particularly at the highest salt treatment. This increase might stem from amino acid production and/or from enhanced stress-induced protein breakdown. While the overall accumulation of amino acids upon stress might indicate cell damage in some species (Widodo *et al.*, 2009) [31],

Increased soluble sugar in salt stress treated plants observed in our study might be accumulation of sugars in plant occurs as a consequence for osmotic adjustment under salt stress. Considering the evidences on plant soluble protein response to salinity, there is a marked difference between the control and treated plants was observed in our study. A decrease of total protein concentration in salt-stressed plants in comparison with control plants was reported earlier. (Morant – Avicce *et al*.1998) [25].

Plants are often exposed to a variety of environmental stresses that are known to generate reactive oxygen species and create oxidative stress. The effect of salinity has been reported to decrease in antioxidant enzyme activities and alterations in the level of antioxidant molecules. In the present study, we had attempts to address changes in the status of antioxidant enzymes such as catalase, peroxidase and superoxide dismutase due to salinity stress. Our results demonstrate that salinity stress in pigeonpea is linked to complex changes observed in antioxidant enzymes

The decline in catalase activity is regarded as a general response to many stresses. The reduction of catalase activity is due to inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stressful conditions. The ability of pigeon pea plant to overcome oxidative stress relies on induction of superoxide dismutase activity and subsequently upregulation of antioxidant enzymes. However, the cultivars of pigeon pea showed a significant decrease in superoxide dismutase activity Ws+Cd and this may be related to low potential to remove O_2^- under the combination of stresses. H_2O_2, which results from the action of superoxide dismutase, is toxic to cells. Therefore, it is important that H_2O_2 be scavenged rapidly by the antioxidative defense system to water and oxygen. (Guo *et al*., 2006) [13].

It was suggested that the heavy metal induced decrease in catalase activity can be attributed to inhibition of the synthesis of catalase and other oxidase proteins. The decline in CAT activity is regarded as a general response to many stresses. (Jung *et al*., 2004, Liu *et al*., 2008) [18, 20]. It was suggested that proline accumulation may be caused by increased proteolysis or by decreased protein synthesis. The higher concentration of proline under salt stress observed in our study is favorable to plants as proline participate to osmotic potential of leaf and thus to osmotic adjustment (Hasegawa *et al*., 2000) [14]. Remarkable increase in MDA (TBARS) observed in our study might be due to salinity induced increase in lipid peroxidation as reported earlier (Keutgen and Pawelzik, 2008) [19]

4. Conclusion

Cajanus cajan is an attractive crop because of its adaptability to different cropping systems and short growing cycle. The results in our study have shown the salinity induced changes in biochemical status and morphological characters of pigeon pea *Cajanus cajan*). The reduction in these metabolic changes was found to be more pronounced in 100mM Nacl treatment than the 50mM Nacl. Salinity stress may lead to significant reduction in crop yield. We can improve stress tolerance in pigeon pea and it is very essential to identify the salt tolerant cultivars to improve the production to a possible extent to meet out the protein needs of our increasing population

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