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Drought - Induced Accumulation of Soluble Sugars and Proline in Two Pigeon Pea (*Cajanus Cajan* L.Millsp.) Cultivars

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Abstract:
Water deficit induced by polyethylene glycol (PEG) affect physiological and biochemical changes in pigeon pea. Stress was applied with polyethylene glycol (PEG) 6000 and water potentials were: zero (control), -0.3Mpa (PEG50mM), -1.1Mpa (PEG100mM) and -2.3Mpa (150mM).ICPL 85063(lakshmi) and ICPL 87119 (Asha) cultivars of pigeon pea were used in this study. In our experiments, increase in the free proline content during water stress condition suggests that proline is one of the common compatible osmolytes under water stress condition. A higher amount of soluble sugars and a lower amount of starch were found under stress. The maximum amount of proline in ICPL 85063var where as ICPL87119var depicted an opposite trend in accumulation of proline. There is an increase in root proline content than shoot in ICPL 85063. The accumulation of soluble sugars in root and shoot in ICPL 85063 is high when compared to roots and shoots in ICPL 87119var. Starch content decreased in root and shoot of lakshmi var. than asha var.

***Key words**: Drought stress, Pigeon pea, Polyethylene glycol 6000, Proline, Soluble sugars, Starch*

1. Introduction

Plants are subject to various abiotic stresses due to unfavorable environmental conditions that affect their growth, metabolism and yield [1]. Drought is one of the major abiotic stresses which limit the crop production in arid and semi arid tropics like India. Plant resist drought stress by their morphological, physiological and metabolic changes reflected in all of their organs [2].

Pigeon pea (Cajanus Cajan (L) Millspaugh) is one of the major grain legume crops of the tropics and subtropics. It is the most important pulse crop which is cultivated in the gross cropped area (3.58 million ha) under pulse production (2.51 m tones). This accounts for 90% of the world`s pigeon pea production [3].Osmotic Adjustment is affected by accumulation of free amino acids, proline and sugars in the roots and shoots.

Accumulation of proline is a widespread plant response to environmental stress, including low water potential. Proline is an organic osmoprotectant accumulates in a large number of plant species exposed to environmental stresses such as salinity, drought, extreme temperature, UV radiations and heavy metals [4]. Proline acts as a ``compatible solute``, i.e. one that can accumulate to high concentrations in the cell cytoplasm without interfering with cellular structure or metabolism [5, 6]. Later on, in the year 2007 it was suggested that the application of proline successfully improved stress tolerance in plants [7].Proline accumulation is believed to play adaptive roles in plant stress tolerance [8].

PEG is used successfully to decrease the water potential of plants as it doesn`t enter into the root [9]. This neutral polymer is being widely used to impose water stress in plants. Responses of plants to water deficit result in alteration of chlorophyll content and free proline. Plants in response to environmental stresses had synthesis or accumulation of materials and amino acids such as enzymes, proteins, mineral material and amino acid [10] one of the physiological responses, the plants use against drought is proline accumulation (Girousse et., 1996). Accumulation of sugars in different parts of plants is enhanced in response to the variety of environment stresses [11]. A central role of sugars depend not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes [12],action as regulators of gene expression[13]and signal molecules[14,15].

In order to look into drought stress induced biochemical changes and to elucidate adaptive mechanisms at the cellular level, we used different concentrations of polyethyleneglycol6000 as osmoticum to investigate the status of carbohydrate and proline pools in pigeon

pea seedlings grown under normal and high stress. Accumulation of proline has been advocated as a parameter of selection for stress tolerance [5].
The present investigation was aimed to investigate the effect of water deficit imposed by PEG6000, on proline and soluble sugars in pigeon pea. This study would help to understand the responses under drought stress condition and its further improvement of present cultivar.

2. Materials and Methods

Two locally cultivated pigeon pea cultivars were selected and seeds were obtained from Regional Agricultural Research station 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 what man 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°Cfor 6 days under a photoperiod of 18h. The analysis 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 3 times. The concentration of PEG 6000(g/kg of water) for each water stress was determined using the equation of Michel and Kaufmann [16].
Estimation of proline content: free proline content was estimated by following the Bates et al [17].fresh500mg of root shoot samples were homogenized in 5ml of3%(w/v) sulphosalicylic acid using mortar and pestle.2ml of extract was taken test tube and to it 2ml of glacial acetic acid and 2ml of ninhydrin reagent was added .The reaction mixture was boiled in water bath at 100 c for 30 min, after cooling the reaction mixture, 4ml of toluene was added. After thorough mixing, the chromophore containing toluene was separated and absorbance of red colour developed was read at 52onmagainst toluene blank.
Estimation of soluble sugars content: Soluble sugars were determined based on the method of phenol sulphuric acid [18]. 0.5 g fresh weight of roots and shoots was homogenized with deionized water, extract was filtered and extract treated with 5% phenol and 98%sulphuric acid, mixture remained for 1h and then absorbance at 485nmwas determined by spectrophotometer (Biocrom S 2100) contents of soluble sugar were expressed as mg/g FW.
Estimation of starch content: starch content determined using the method of phenol- sulfuric acid [18]. Sediment of extract that filtered in sugar content dried, weighed and boiled with deionized water. Supernatant used for measurement of starch content.

3. Results and Discussion

Effects of drought stress on proline content: The plants were subjected to extensive level of water stress of-1.1 MPa and -2.3 MPa induced by polyethylene glycol6000. The results depicted high increase of free proline content in both control and stressed plants of ICPL-85063. It was observed that severe stress (-2.3MPa) severely affect the biochemical parameters as compare to both the cultivars. In water potential -2.3MPa, root and shoot proline content increased in ICPL 85063var than ICPL 87119var as compared to control plants (Fig. 1.)

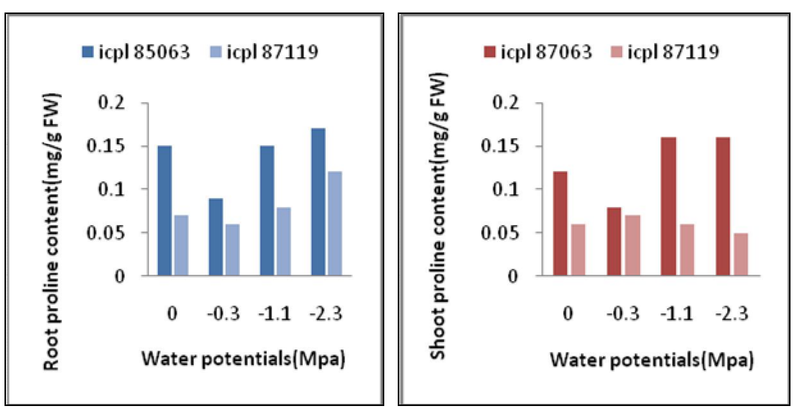

Figure 1: Effect of different water potentials on total proline contents (mg/g FW) in the roots and shoots of two pigeon pea cultivars

Among amino acids, the accumulation of proline is frequently reported in many plants or tissues in response to a variety of abiotic stresses [4]. The accumulation of proline protects the cell under stress by balancing the osmotic strength of cytosol with that of vacuole and the external environment [19] In the present study, when the intensity of drought stress increased the level of proline significantly increased in both the cultivars which could be linked to their ability to perform tissue osmotic adjustment to lower the osmotic potential and protect plants from damages of dehydration. [19].

ICPL 85063 accumulated higher proline under high solute potential, proline acts as a osmoticum and accounted for higher drought tolerance due to greater relative water content and leaf water potential [19]. In this experiment we found that ICPL 85063 var. had increase of proline content higher than ICPL 87119 var. It means that ICPL 85063 var. had higher tolerance than ICPL 87119 var. in severe drought stress. Our present results indicate that proline accumulation might have resulted in more reduction in the decrease in the water potential which results in the increase in yield, similar observations were made in cucumber and Cajanus Cajan [20] usually the magnitude of proline accumulation is relatively dependent on the levels of carbohydrates [4]. Larher et al. [21] mentioned that sucrose was a positive efector of proline accumulation.

Effects of drought stress on soluble sugar and starch contents: There is maximum increase in sugar content in stressed than the control plants in both the cultivars. ICPL 85063 var. showed higher values than ICPL 87119, different PEG treatments to both varieties of pigeon pea seedlings significantly increased total soluble sugar content (Fig. 2), as compare to control, a drastic increase was observed in roots than shoots. (Fig: 2)

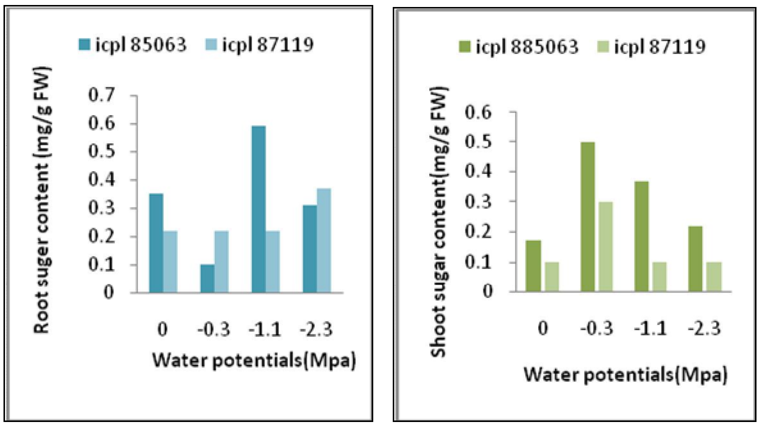

Figure 2: effect of different water potentials on soluble sugar contents (mg/g FW) in roots and shoots of two pigeon pea cultivars

The increase in sugar concentration may be a result from the degradation of starch [22]. Starch may play an important role in accumulation of soluble sugars in cells. Starch depletion in grapevine leaves was noted by Patakas and Noitsakis[23] in response to drought stress ,too. In this study, the concentrations of soluble sugars increased at the same time as a decrease in the starch concentration was observed. It means that the raised soluble sugar fraction was accompanied by a sharp decrease in the starch fraction as the water potential dropped. (Fig: 3).

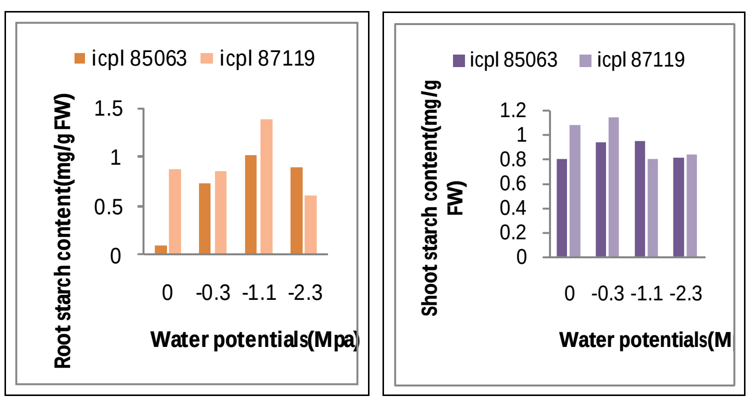

Figure 3: effect of different water potentials on starch contents (mg/g FW) in the roots and shoots of two pigeon pea cultivars

The tolerance mechanism in water –deficit may be associated with accumulation of osmoprotactants such as proline and soluble sugars. The accumulation of soluble sugars is strongly correlated to the acquisition of drought tolerance in plants [24]. In our study the increase of soluble sugar content in roots of ICPL85063var, was higher than ICPL 87119var, similarly there is increase in the sugar content in shoot also. The stressed plants of water potential -2.3MPa showed increased values in sugar content in both the cultivars, but ICPL85063 showed maximum sugar content than the ICPL 87119var.

The accumulation of sugars in response to drought stress is also quite well documented [5, 25, 26].the concentration of soluble sugars increased under drought stress in ICPL 85063cultivar in our study. A complex essential role of soluble sugars in plant metabolism is well known as products of hydrolytic processes, substrates in biosynthesis processes, energy production but also in a sugar sensing and signaling systems. Recently it has been claimed that even sugar flux may be a signal for metabolic regulation [27]. Protection against dehydration by the former sugars was correlated with the increase in shoots and roots soluble sugars may also function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor.

Our present result indicate that a progressive water stress induced PEG-6000 cause significant physiological and biological changes in pigeon pea Studies. With a variety of plants demonstrate that drought induced conversion of hexoses and other carbohydrates, such as sucrose and starch, into sugar alcohols and proline [28]. Earlier reports mentioned that sugars protect the cells during drought by two mechanisms. First, the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydrations. Thus, sugars interact with proteins and membrane through hydrogen –bonding, thereby preventing protein denaturation [29].secondly sugars are a major contributing factor to vitrification, which is a formation of a biological gas in the cytoplasm of dehydrated cells [29,30].

4. Conclusion

Our study suggested that ICPL85063 cultivar had significant higher values at all the stress level in proline and sugar. Under stress conditions, we postulate that the depletion of starch with induced plasmolysis will reduce the volume of cytoplasm accumulation of soluble sugars may be to counter the osmotic stress. Thus under higher solute potential, ICPL85063 cultivar accumulates higher proline content. It is concluded that accumulation of higher values of proline and sugar offer protection against drought in pigeon pea. Accumulation of proline and sugars are one of the potential biochemical indicators in selecting tolerant cultivars.

5. References

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