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*Research Article*

# Effect of drought stress on biochemical contents and proline metabolizing enzymes of *Pennisetum glaucum* L.

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**ABSTRACT**

Drought stress is one of the major environmental problem affecting agricultural production in arid and semi-arid regions of the world. In present study, the effect of drought stress on *Pennisetum glaucum* L. (Pearl millet) plants was studied. The seed were sown in plastic pots from 30 days after sowing (DAS). The plants were treated with 3 DID (Days Interval Droughts), 4 DID, 5 DID and 6DID and 30$^{th}$ to 60$^{th}$ DAS. The plants samples were collected from 40th, 50$^{th}$ and 60$^{th}$ DAS. The biochemical like, protein content decreased to increasing drought stress to a larger extent when compared to control plants and also increased the amino acids and proline content to the larger extent with increasing DID when compared to control. The proline metabolizing enzyme like g-glutamyl kinase activity was increased and decreased proline oxidase activity on drought stressed plants.

## INTRODUCTION

Plant growth and development as well as crop production are highly influenced and sometimes limited by environmental conditions, such as drought, salinity and temperature stresses. Among these, drought stress is the most important environmental constrains to world agricultural production [1]. Water stress not only affects the morphology but also severely affects the physiological metabolism of the plant. In response to various environmental stresses, plants have developed different physiological and biochemical mechanisms to adapt or tolerate stress [2]. Worldwide losses in crop yields from water deficits probably exceed the losses from all other causes combined [3].

Millets are examples of less-utilized crops with adaptation to marginal lands where they can withstand various stress conditions and contribute to sustainable low-input food production [4]. In India, small millets are cultivated in the semi-arid and hilly regions inhabited by traditional farmers. Pearl millet is the principal crop amidst the small millets, occupying 60–70% of the total area under small millet production globally, followed by kodo millet, foxtail millet, pearl millet, proso millet, and barnyard millet. Grains of small millets are extremely resistant to storage pests and can be stored for indefinite periods [5]. Nutrition wise, grains of small millets are rich in micronutrients, particularly calcium and iron. They have high dietary fiber content, rich essential amino acids, and low glycemic index [6].

There is a need to restore the lost interest in millets due to its potential nutritional qualities and health benefits [7]. Hence this study was taken up with an overall objective of knowing the physical and nutritional characters and also to know if variations exist in landraces cultivated in different agro-climatic zones.

## MATERIALS AND METHODS

### *Collection of seeds*

Seeds of *Pennisetum glaucum* L. (Pearl millet) variety were obtained from Krishnagiri Taluk Office, Agri Block. The experiments were conducted at the Botanical Garden and Plant Physiology Laboratory, Post Graduate and Research Department of Botany, Government Arts College for Men, Krishnagiri, Tamil Nadu, India.

The pot culture studies were conducted to measure the growth parameters, biochemical and physiological changes. The seeds were surface sterilized with 0.2% Mercuric chloride solution for five minutes with frequent shaking and thoroughly washed with tap water. The experiment was laid out in a Completely Randomized Block Design (CRBD). In the preliminary experiments of 3, 4, 5, 6, 7, 8 and 9 days interval drought stress were used for experiments. Among these treatments, the dry weight was significantly reduced up to 60% with 6 DID treatments. Hence 3, 4, 5 and 6 days interval drought was used in this experiment.

The Pearl millet 50 seeds were sown and the seedlings were thinned to 25 per pot on 10 days after sowing (DAS). The plants were allowed grow up to 29 DAS in normal condition irrigate alternative days. On 30 to 60 DAS. The control plants were irrigated an alternative day. Mild stress (irrigation once in 3 days) moderate stress (irrigation once in 4days) severe stress (irrigation once in 5 and 6 days) from 30 to 60 DAS. After the drought period all the pots to be irrigated an alternate day up to harvest. Plants were uprooted randomly 40, 50 and 60 DAS, washed carefully and separated into root and shoot for estimating biochemical contents and proine metabolizing enzyme activities are analyzed.

### *Soluble Protein*

Soluble protein was estimated according to Bradford [8] method.

***Free amino acid***

Extraction and estimation of the amino acid content was followed by the method of Moore and Stein [9].

***Proline***

Proline content was estimated following the standard method [10].

***γ - Glutamylkinase activity***

γ-Glutamyl kinase activity was assayed by standard method [11].

***Proline oxidase activity***

Proline oxidase activity was determined according to the method outlined by Huang and Cavalieri [12].

***Statistical analysis***

Each treatment was analyzed with at least three replicates and a standard deviation (SD) was calculated and data are expressed in mean ±SD of three replicates.

## RESULTS AND DISCUSSION

***Protein Content [Table 1]***

Drought stress caused a decrease in the protein content in all parts of the plants to a larger extent. The water stressed plants reduced quantity of soluble proteins observed in the present experiment can be related to reduced rate of protein biosynthesis and increased breakdown of proteins under water limited environment, similar results also observed in as reported by wheat [13].

***Amino acid content (Table 2)***

Amino acid content increased to a larger extent in all drought stress treatments. The amino acid content has been shown to increase under drought condition in *Radix astragali* [14] and Marsh grasses [15]. The amino acid content increased to higher level immediately after treatment later it declines as day progress. The amino acid content has been shown to increase under drought condition *Arachis hypogaea* [16]. The accumulation of amino acids may be due to the hydrolysis of protein and also accumulated amino acid may be occurring in response to the change in osmotic adjustment of their cellular contents [17]. Amino acids accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species, such as *Radix astragali* [14].

***Proline content***

Drought stress caused a higher accumulation of Proline content in all parts of the plants to a larger extent. The results coincide with reports of previous works. Increased proline accumulation was reported in water stressed sorghum [18]; and in wheat [19]. Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. The similar results were observed in barley [20].

**Table 1. Effect of drought stress on Protein content of Pearl millet (values are expressed in mg/g fresh weight).**

| Days after sowing (DAS) | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | 3DID | 4DID | 5DID | 6DID |
| | **Root** | | | | |
| 40 | 106.37±2.881 | 99.66±2.662 | 90.51±2.468 | 84.87±2.221 | 78.89±2.010 |
| 50 | 111.89±3.239 | 104.87±3.069 | 97.83±3.009 | 91.97±2.070 | 86.21±2.008 |
| 60 | 124.68±3.528 | 113.21±3.213 | 108.17±3.141 | 98.67±3.017 | 87.62±2.808 |
| | **Shoot** | | | | |
| 40 | 67.56±1.803 | 58.44±1.610 | 53.14±1.408 | 47.91±1.203 | 46.19±1.076 |
| 50 | 77.97±1.879 | 69.94±1.693 | 60.19±1.601 | 55.43±1.485 | 51.87±1.371 |
| 60 | 80.44±2.015 | 74.16±1.808 | 69.33±1.936 | 61.51±1.703 | 58.48±1.689 |

**Table 2. Effect of drought stress on amino acid content of Pearl millet (values are expressed in mg/g fresh weight).**

| Days after sowing (DAS) | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | 3DID | 4DID | 5DID | 6DID |
| **Root** | | | | | |
| 40 | 6.184±0.164 | 8.263±0.262 | 9.461±0.303 | 10.766±.344 | 13.221±0.388 |
| 50 | 8.286±0.246 | 9.796±0.305 | 11.426±0.366 | 12.055±0.390 | 14.677±0.398 |
| 60 | 11.144±0.315 | 13.432±0.389 | 15.211±0.413 | 16.047±0.498 | 18.277±0.433 |
| **Shoot** | | | | | |
| 40 | 6.334±0.244 | 11.877±0.381 | 15.331±-0.459 | 16.321±0.466 | 18.678±0.587 |
| 50 | 12.526±0.424 | 14.678±0.523 | 16.088±0.525 | 19.454±0.646 | 22.459±0.746 |
| 60 | 15.261±0.517 | 18.761±0.586 | 22.333±0.735 | 24.541±0.802 | 27.046±0.897 |

**Table 3. Effect of drought stress on Proline content of Pearl millet (values are expressed in mg/g fresh weight).**

| Days after sowing (DAS) | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | 3DID | 4DID | 5DID | 6DID |
| **Root** | | | | | |
| 40 | 0.632±0.112 | 0.734±0.119 | 0.799±0.121 | 0.866±0.191 | 0.998±0.225 |
| 50 | 0.765 ±0.116 | 0.948±0.226 | 1.108±0.289 | 1.208±0.339 | 1.342±0.380 |
| 60 | 0.833±0.221 | 1.031±0.245 | 1.431±0.340 | 1.645±0.449 | 1.921±0.511 |
| **Shoot** | | | | | |
| 40 | 0.767±0.115 | 0.876±0.120 | 0.987±0.124 | 1.121±0.133 | 1.210±0.166 |
| 50 | 0.864±0.119 | 0.991±0.128 | 1.154±0.129 | 1.245±0.199 | 1.321±0.235 |
| 60 | 0.911±0.228 | 1.188±0.0289 | 1.320±0.230 | 1.439±0.288 | 1.566±0.365 |

**Table 4. Effect of Drought stress induced changes in the glutamyl kinase activities of *Pennisetum glaucum* L.**

| Days after sowing (DAS) | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | 3DID | 4DID | 5DID | 6DID |
| **Root** | | | | | |
| 40 | 1.432±0.050 | 1.608±0.056 | 2.001±0.068 | 2.163±0.071 | 2.387±0.089 |
| 50 | 2.031±0.063 | 2.447±0.078 | 2.639±0.091 | 3.001±0.095 | 3.095±0.100 |
| 60 | 2.677±0.085 | 2.772±0.089 | 3.007±0.109 | 3.308±0.118 | 3.581±0.132 |
| **Shoot** | | | | | |
| 40 | 1.641±0.053 | 1.787±0.059 | 2.013±0.070 | 2.245±0.077 | 2.054±0.082 |
| 50 | 1.866±0.058 | 2.033±0.064 | 2.387±0.080 | 2.648±0.091 | 2.788±0.104 |
| 60 | 2.384±0.085 | 2.487±0.091 | 2.611±0.101 | 3.001±0.114 | 3.314±0.122 |

(values are the mean ± SD of 3 replicates and expressed in U/mg$^{-1}$Protein).

**Table 5. Effect of Drought stress induced changes in the Proline oxidase activity of *Pennisetum glaucum* L.**

| Days after sowing (DAS) | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | 3DID | 4DID | 5DID | 6DID |
| **Root** | | | | | |
| 40 | 0.293±0.005 | 0.286±0.005 | 0.262±0.004 | 0.250±0.005 | 0.241±0.004 |
| 50 | 0.360±0.007 | 0.355±0.007 | 0.341±0.00 | 0.330±0.00 | 0.312±0.006 |
| 60 | 0.414±0.010 | 0.387±0.009 | 0.366±0.008 | 0.355±0.007 | 0.343±0.007 |
| **Shoot** | | | | | |
| 40 | 0.370±0.007 | 0.358±0.007 | 0.343±0.008 | 0.331±0.007 | 0.316±0.008 |
| 50 | 0.414±0.011 | 0.390±0.010 | 0.387±0.010 | 0.342±0.009 | 0.348±0.008 |
| 60 | 0.527±0.014 | 0.500±0.013 | 0.481±0.013 | 0.458±0.011 | 0.430±0.011 |

(values are the mean ± SD of 3 replicates and expressed in U/mg$^{-1}$Protein).

***Proline metabolizing enzymes (Table 5)***

The activity of the proline synthesizing enzyme, g-glutamyl kinase was high in all parts of plants. Drought stress induced an increase in the g-glutamyl kinase activity in drought stressed sorghum [21], and wheat [22].

The level of proline degrading enzyme proline oxidase activity was inhibited in pearl millet under drought stress. A sharp reduction in proline oxidation was observed under water stress in wheat [22] and *Zea mays* [23]. Similar results were reported in tomato [24]. Proline oxidase activity was found inhibited in bhendi species under stress [24].

## CONCLUSION

There was a decrease in protein content in pearl millet plants under drought stress and increased the amino acids and proline content. The proline metabolizing enzyme like g-glutamyl kinase activity was increased and decreased proline oxidase activity on drought stressed plants. These results can be used as a base for further research in abiotic stress effects on the cultivation of this important millet species.

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