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Osmolyte accumulation, photosynthetic pigment and growth of *Setaria italica* (L.) P. Beauv. under drought stress

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ABSTRACT

Objective: To investigate morphological and biochemical mechanism between plant and drought stress by the way of plant survival under drought stress. **Methods:** Drought stress was imposed by irrigation daily (Control), 4, 7, 10 and 13 days interval (4 DID, 7 DID, 10 DID and 13 DID). Later they were irrigated at regular intervals for different maturity stage of *Setaria italica* (*S. italica*) (30 DAS, 50 DAS and 70 DAS). **Results:** The results showed the root length and compatible solutes were increased in all treatments when compared to control at the different age of plant maturity. The shoot length and chlorophyll pigment reduced in all treatments when compared to control at increasing plant maturity. **Conclusions:** This study suggestes the reduced shoot length, photosynthetic pigments of chlorophyll and increased root length, compatible solute accumulations made to drought tolerant adaptative mechanism.

1. Introduction

Drought is considered as one of the environmental stress, which decreases crop productivity greatly compared with other environmental stress and an occasional cause of losses of agricultural production in developing countries [1]. Drought or soil water deficit can be chronic in climatic regions with low water availability or random and unpredictable due to changes in weather conditions during the period of plant growth. The effects of drought are expected to increase with climate change and growing water scarcity. Water is an increasingly scarce resource given current and future human population and societal needs, putting an emphasis on sustainable water use [2]. However, Irrigation treatments should be used effectively to increase the sustainability of production in agriculture systems. Rapid growth in different stages of plant, adaptation with tropical regions, relative resistance against dryness, high content of protein in leaves, high leaf area index (LAI), high potential for enough production (in C4 crops) and high water use efficiency are suitable mechanisms for drought tolerance [3]. Thus, an understanding of drought stress and water use in relation to plant growth is of importance for sustainable agriculture.

Water stress not only affects the morphology but also severely affects the physiological metabolism of the plant. Osmotic adjustment in terms of accumulating compatible solutes has been considered as an important physiological adaptation for plant to resist drought [4]. The maintenance of plant water potential during water deficit is essential for continued growth and can be achieved by osmotic adjustment mechanisms resulting from the accumulation of compatible solutes (such as proline, glyciene betaine and organic acids) in the cytoplasm[5,6]. Proline accumulates in many plant species under a broad range of stress conditions such as water shortage, salinity, extreme temperatures, and high light intensity. Proline is considered to be a compatible solute. It protects folded protein structures against denaturation, stabilizes cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source [7]. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing power such as ATP and NADPH. Both the chlorophyll a (CHLa) and (CHLb) are prone to soil drying damages. Drought stress induced changes in the ratio of CHLa and CHLb and carotenoids [8]. The chlorophyll

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content decreased to a significant level at higher water deficits in plants[9].

Millet species need relatively less water than the other crops, because they have short growth season[10]. Millet germination can be used as a potential source of functional food material[11–13], as well as stress millet germination. *Foxtail millet*, *Setaria italica* (L.) P. Beauv. (*S. italica*) is one of the important cereals belongs to Poaceae member. It is widely distributed in China, Europe and Central Asia because it is drought tolerant and well adapted to arid and semiarid regions. To understand the physiological responses of water deficit in foxtail millet, investigation of concurrent effects on soil–plant water relations is essential. In the present study, the effect of water deficit applied at different growth stages of crop was tested.

2. Materials and methods

2.1. Study area

The seeds of *S. italica* were collected from Palai hill in Erode district of Tamilnadu India. The experiments were conducted at the Botanical garden and Stress Physiology Laboratory, Department of Botany, Annamalai University, Tamilnadu, India. The experiment was carried out in a Completely Randomized Block Design (CRBD). The pots were filled with soil containing mixture of red soil, sand and farm yard manure at 1:1:1 ratio. Drought stress imposed in Control, 4 Drought Induced Days (DID), 7 DID, 10 DID and 13 DID For each treatment five replicates were maintained. Treatments were imposed on the plant on 30, 50 and 70 DAS (days after sowing).

2.2. Plant growth analysis

The length between shoot tip and point of the root shoot transition region was taken as shoot length. Root length was recorded by measuring below the point of root–shoot transition to the fibrous root and the length of lateral roots was taken as total root length. The shoot and root length are expressed in centimeters per plant.

2.3. Plant pigment

Chlorophyll contents were measured according to Arnon[14] method. 1 g of fresh leaves were extracted with 80 % acetone (v/v) and chlorophyll contents were estimated spectrophotometrically at 645 nm and 663 nm using Hitachi U–2000 spectrophotometer and were expressed in terms of mg chlorophyll presents per gram fresh mass.

2.4. Compatible solutes

2.4.1. Proline

Free proline was assayed followed by ninhydrin method[15]. The plant material (1 g) was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 14 000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of acid ninhydrin and glacial acetic acid, which was boiled at 100 °C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with toluene, and absorbance was read at 520 nm using L–proline as standard.

2.4.2. Glycine betaine

Glycine betaine was estimated by the method of Grieve and Grattan[16]. Briefly, finely ground dried plant tissue (0.5 g) was stirred with 20 cm^3 distilled water for 24 h and filtered. The filtrate was diluted with equal volume of 1 M H_2SO_4, made into aliquots of 0.5 cm^3 in micro centrifuge tubes, cooled over ice for 1 h and to each of these were added 0.2 cm^3 cold KI–I2 reagent. The reactants were gently stirred, stored at 4 °C overnight and centrifuged at 12 000 g for 15 min at 4 °C to get the precipitated per iodide crystals. The crystals were dissolved in 1,2–dichloroethane, and absorbance was measured at 365 nm after 2 h. Glycine betaine dissolved in 1 M H_2SO_4 served as standard.

2.4.3. Free amino acid

Total free amino acids were extracted and estimated by following the method of Moore and Stein[17]. Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 80% boiled ethanol. The extract was centrifuged at 800 g for 15 minutes and the supernatant was made up to 10 mL with 80% ethanol. In 25 mL test tube, ethanol extract was taken and neutralized with 0.1 N NaOH using the methyl red indicator to which ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes, and then 5 mL of diluting solution was added, cooled and made up to 25 mL with distilled water. The absorbance was read at 570 nm.

3. Results

3.1. Effect of drought on growth

The present investigation, drought stress may induce root length growth but reduce shoot length (Table 1). The root length increased by drought stress treatment compared to control for all growth stages of *S. italica*. The highest value of shoot length was recorded in 13 DID, 70 DAS for all the treatments. The present study results find out, the shoot length of *S. italica* was declined by increasing of drought stress. The reduction of shoot growth observed in 13 DID at 70 DAS.

3.2. Effects of drought on pigment

The results showed that the photosynthetic pigment of chlorophyll was decreased by increasing drought stress (Table 2). The chlorophyll was declined from well water (control) condition to severe drought stress (13 DID) and plant pigment was increased by the age of maturity in plant. Accordance our data all treated plants, leaf Chl a/b ratio increased as compared to the control.

Table 1

Effect of drought stress on growth of *S. italica*.

Groups	Root length (cm)			Shoot length (cm)		
	30 DAS	50 DAS	70 DAS	30 DAS	50 DAS	70 DAS
Control	28.35±0.18	37.35±0.24	44.46±0.30	54.53±0.37	71.26±0.79	75.43±0.26
4 DID	32.23±0.12	43.63±0.46	47.40±0.35	50.30±0.20	69.33±0.20	71.33±0.18
7 DID	33.46±0.32	46.38±0.25	50.31±0.25	49.16±0.52	65.71±0.45	68.31±0.24
10 DID	35.63±0.18	47.46±0.30	53.33±0.25	44.28±0.17	61.93±0.72	64.20±0.08
13 DID	37.36±0.16	50.33±1.90	55.50±0.51	39.20±0.06	59.35±0.20	58.50±0.16

Table 2

Effect of drought stress on Chlorophyll pigment of *S. italica*.

G. S		Control	4 DID	7 DID	10 DID	13 DID
Chl a	30 DAS	0.822±0.003	0.734±0.003	0.653±0.002	0.483±0.002	0.315±0.003
	50 DAS	0.972±0.001	0.793±0.002	0.670±0.004	0.454±0.005	0.342±0.003
	70 DAS	0.794±0.002	0.554±0.006	0.533±0.004	0.285±0.002	0.150±0.004
Chl b	30 DAS	0.571±0.001	0.475±0.003	0.325±0.002	0.237±0.002	0.125±0.001
	50 DAS	0.762±0.002	0.535±0.002	0.492±0.003	0.283±0.004	0.155±0.002
	70 DAS	0.674±0.002	0.484±0.002	0.465±0.002	0.235±0.002	0.126±0.002
Chl a/b	30 DAS	1.439±0.014	1.545±0.035	2.009±0.009	2.037±0.027	2.521±0.017
	50 DAS	1.275±0.005	1.482± 0.004	1.361±0.019	1.604±0.016	2.206±0.018
	70 DAS	1.178±0.015	1.144±0.054	1.146±0.037	1.212±0.051	1.196±0.120

3.3. Proline

An osmoprotectant of proline was increased in root and leaf of *S. italica* when compared to control (Figure 1). A high value of proline was recorded at 13 DID of 70 DAS and the highest values being 5.10 mg/g FW in root, and 6.17 mg/g FW in leaves when compared to other treatments.

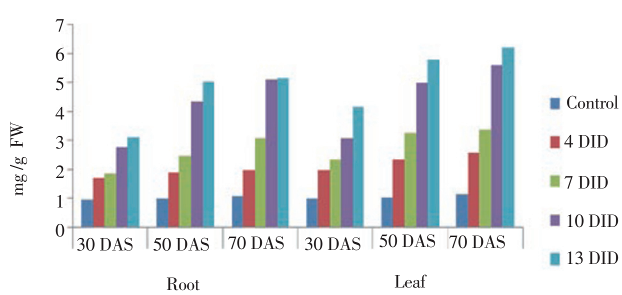

Figure 1. Effect of proline content in *S. italica* under drought stress.

3.4. Glycine betaine

The quaternary ammonium compound of glycine betaine was increased by increasing drought stress. The glycine betaine induced from well water (control) condition to severe drought stress (13 DID) and it was increased by the age of maturity in plant (Figure 2). The amount of accumulation of glycine betaine in leaf tissue was higher compared with that in root tissue.

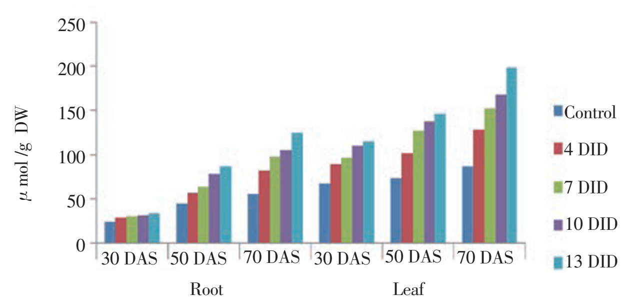

Figure 2. Effect of glycine betaine content in *S.italica* under drought stress.

3.5. Free amino acid

Free amino acids content has been increased under drought condition in all treatment as compared to control at different growth stages of *S. italica* (Figure 3). The highest level of Nitrogen containing compound of amino acid was 389.34 μg/g FW in root and 588.74 μg/g FW in leaves of *S. italica* at 13 DID of 70 DAS.

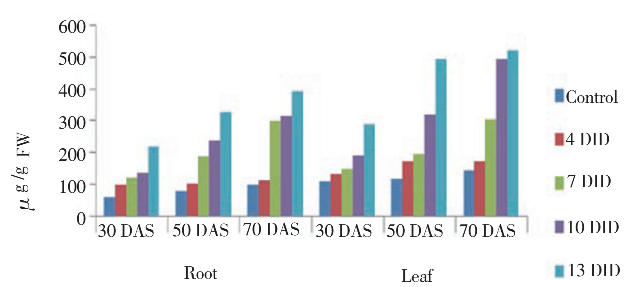

Figure 3. Effect of free amino acid in *S. italica* under drought stress.

4. Discussion

The increases of root length may occur due to stress hormone stimulating the elongation of main root and emergence of lateral roots in response to drought [18]. These results were agreed with Kage *et al* [19] found to be in Cauliflower during drought stress. Under drought condition, cytokinin was response for nutrient deprivation and it might induce root development [20]. Matsuura *et al* [21] reported that total root length increased in sorghum and millet under water stress conditions. The continued growth of roots in drying soil is particularly important to avoid drought [22]. This study result find out, the shoot length of *S. italica* was declined by increasing of drought stress. The decrease in shoot length in response to drought may be either due to decrease in cell elongation resulting from water shortage which led to a decrease in each of cell turgor, cell volume and eventually cell growth and/or due to blocking up of xylem and phloem vessels thus hindering any translocation through [23,24]. Similar results were obtained by Misra and srivastava [25], Choi *et al* [26], Singh *et al* [27]. The reduction of shoot length may protect the loss of water by mechanisms of mitigate drought stress. Such retardation in the content of photosynthetic pigment in response to water stress was attributed to the ultra structural deformation of plastids including the protein membranes forming the thylakoids which in turn causes untying of photo system 2 which captures photons, so its efficiency declined, thus causing declines in electron transfer, ATP and NADPH production and eventually CO_2 fixation processes [28,29]. Photosynthetic pigment degradation exposed to water deficit in rice [30], barley [31], wheat [32]. Increased environmental stresses could also be a cause, which was involved in the oxidation of photosynthetic pigments and the membrane disintegration and damage to chloroplasts [33]. Accordance our data all treated plants, leaf Chl a/b ratio increased as compared to the control because this may due to environmental stress. Chl b was damage more than Chl a, which was involved in conversion from Chl b to Chl a. [34]. During drought stress, the compatible solutes like Proline, Glycine betaine and free amino acid were increased of plants. Under drought, the maintenance of leaf turgor could be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycine betaine, and other solutes in cytoplasm improving water uptake from drying soil. When the water stress increases, osmotic pressure was adjusted by the mechanism of this osmoticum, compatible solute accumulation enhanced in the cytoplasm. Although proline has been considered as compatible osmolyte, its multiple functions in stress adaptation, recovery and signaling were good. In plants, proline is synthesized mainly from glutamate, which is reduced to glutamate-semialdehyde (GSA) by the pyrroline-5-carboxylate synthetase (P5CS) enzyme, and spontaneously converted to pyrroline-5-carboxylate (P5C) [35,36]. These enzymes could be increased due to drought stress. High proline content in wheat and other plants after water stress has been reported by Tatar and Gevrek, Vendruscolo *et al*, Errabii *et al*, Shao *et al* [37-40]. Proline content is also high in drought-tolerant rice varieties [41]. Plants were known to accumulate GB naturally have been reported to grow well under drought and saline environment [42]. The enzyme of choline monooxygenase (CMO) first converts choline into betaine aldehyde and then a NAD^+ dependent enzyme, betaine aldehyde dehydrogenase (BADH) produces glycine betaine found in chloroplast stroma and their activity was increased in response to stress [43]. Amino acid content increased under drought condition in Astragali, Sorghum and wheat [44-46]. The accumulation of amino acids may due to the hydrolysis of protein and adaptative changes of osmotic adjustment of their cellular contents [47].

The root length was induced by drought stress due to the cell development of root because of deep penetration of search water. The shoot length reduced in drought stress and it may be affected cell division and cell enlargement. The accumulation of compatible solutes may have increased due to the osmotic adjustment under drought stress condition and it may prevent the oxidative cell damage. The chlorophyll content decreases in all drought condition due to the reducing water rate in cell, transpiration rate and stomata rate. This is the mechanism of drought tolerance in plants especially *S. italica*. The present investigation concludes with, accumulation of compatible solutes, reduction in pigments, increased root length and reduction in shoot length occur in *S. italica*, it may prevent the oxidative cell death due to the drought stress and mechanism of alternative way to prevent the damage of cell.

Dedare of interest statement

We declare that we have no conflict of interest.

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