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Research Article
Drought Response of a Tropical Legume (*Pachyrhizus erosus* L.): Physio-Biochemical Adjustments at Seedling Stage

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Abstract

Background and Objective: Introducing crops higher in protein contents than indigenous tubers is one of the ways of combating malnutrition in Nigeria. *Pachyrhizus erosus* tuber contains high protein in addition to its agronomical advantage as a legume crop. Soil water deficit is one of the most critical stresses negatively impacting global food production through its adverse effects on plant growth. The objective of this study was to evaluate the effect of water deficit on *P. erosus* seedlings. **Materials and Methods:** Through the investigation of morphological and physio-biochemical parameters, this study explores the effects of three levels of soil water deficits (25, 50 and 75% of field capacity representing: Slight, moderate and severe water deficit respectively) on *P. erosus* at the seedling stage. **Results:** Results indicated that severe and moderate water deficit reduced the Leaf Relative Water Content (LRWC), Leaf area and increase in the relative growth rate of *P. erosus* seedlings which suggests the adoption of an effective drought adaptive strategy by the plant. Water deficits had no notable effect on proline synthesis but increased Malondialdehyde contents. Severe and moderate water deficit impaired the photosynthetic apparatus of *P. erosus* particularly at the later phase of seedling developments, which was as a result of the reduction in LRWC and area of a leaf of *P. erosus*. **Conclusion:** Despite the negative effects of soil water deficit, *P. erosus* maintained tolerance through a resilient antioxidant mechanism, reduction of LRWC and growth parameters. This will make it survive the seasonal drought period experienced yearly.

Key words: Drought stress, legume, *Pachyrhizus erosus*, antioxidant enzymes, photosynthetic pigments, garden crop

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pachyrhizus erosus also known as yam bean is one of the few tuberous legumes of tropical and subtropical zone and one of the most important crops with multipurpose uses and qualities[1,2]. It belongs to the genus *Pachyrhizus*, which comprises five species[3]. Because of its food value and wide adaptability, *P. erosus* is extensively cultivated, both as a garden crop and in the field on a large scale for export[1]. It is of high nutritive value with its fleshy tuber containing a high amount of carbohydrate and relatively high protein content[4]. *Pachyrhizus erosus* originated from Central America and South America, however, due to its high yield and proven economic potential, it is considered a sustainable crop in tropical countries[5]. It is now cultivated in Benin Republic, China, Indonesia, Thailand, Malaysia and the Philippines[1], including Nigeria. Importantly, its introduction is aimed to achieve a stable supply of nutritional requirements, improved soil resource management and environmental protection in most developing countries.

Although, the nutritional and production capacity of its tuber has been documented[6], among the questions yet to be addressed is how alteration in soil water availability during the year which is common in the semi-arid area may be a constraint to its growth and development.

As the earth undergoes an increase of the hydrological cycle, with the intensification of frequency and severity of drought events[7,8] there exists the potential for drought stress to impact the growth and development of crops markedly. Water stress is one of the main factors limiting crop growth and productivity in most regions of the world[9,10]. However, studying the effects of water stress in a plant is challenging because the sensitivity and response time to stress vary among different plant species and are linked to the intensity and length of the stress[9]. Drought events are anticipated to have a potential influence on plant physiological and biochemical processes, thus, adversely affecting plant growth and global crop production[11]. Its negative impacts on plant development are projected to become greater due to the prevalence of longer, more frequent and high intense drought episodes accompanying the present and ongoing climate changes[12,13]. Several studies have reported that drought affects plant growth by stressing some physiological and biochemical processes including leaf respiration, leaf chlorophyll content, leaf water content, plant relative growth rate, among others[14,10]. It has been reported that canopy architecture, such as the leaf areas, the number of leaves and stem length were reduced by water deficit in field-grown potatoes[15]. However, little attention has been paid to the water stress tolerant strategy of non-woody leguminous crops, particularly at the seedling stage. Whereas, plant capacity for enduring environmental stresses begins at the seedling stage. Soil water stress is a recurrent problem during seedling establishment because plants are exposed to high radiation and air movement when transplanted, therefore, the evapotranspiration demand is high when the root system is small. Although no study had documented the sensitivity of *P. erosus* to either water deficit or excess water conditions, like the most tuberous plant, alteration in soil water condition at the seedling stage may affect its growth[16,17]. For example, a study reported that when a potato plant is cultivated in soil with water content below 60-65%, water deficiency issues will be developed[18]. Such issues include darker leaves and wilting due to the loss of internal water pressure in plant cells[16]. However, plants could acclimatize to water stress through coordination of various physiological and biochemical parameters which often serve as a protective mechanism to contest the adverse effects of drought stress at varying stages of plant development[19,20]. This protective mechanism involves the production of many enzymatic and non-enzymatic compounds, which inhibit oxidative damage by scavenging Reactive Oxygen Species (ROS) inside cells[21]. For example, a study reported that the increases in superoxide dismutase and ascorbate peroxidase activities were related to the quick recovery of leaf gas exchange in sugarcane[22]. The synthesis and accumulation of compatible solutes such as proline and soluble sugars is a defense mechanism in plants that utilize osmotic adjustment to regulate cell turgor, growth and gas exchange under drought stress[23]. However, there is a lack of information in the literature on the morphological and physio-biochemical responses of economically proven *P. erosus* to alteration in soil water content at its seedling stages.

In this study, the morphological and physio-biochemical responses of *P. erosus* at a different point in time (weeks) during the seedling stage to different levels of water deficit were examined. This is intending to explore the mechanism by which *P. erosus* could survive drought conditions at this stage. The objectives of this study were to (1) evaluate the effects of water deficit on the morphological and physio-biochemical responses of *P. erosus* at the early stage of development and (2) investigate the degree of water deficit that exerts most the significant negative effect on the morphology and physiology of *P. erosus* seedlings. This plant is expected to play an emergent role in meeting the nutritional, food and economic demand of most developing countries. Therefore, understanding its response to changes in soil water deficit at the seedling stage will play a key role in

designing a proper management strategy to stabilize its yield in the face of future climate change.

MATERIALS AND METHODS

Experimental conditions: The experiment was conducted in a screen house (temperature range 28-32°C, relative humidity range 65-85%, the average daily light intensity of 100200 Lux meter and direct sunlight reduction due to covering was in the range of 4-8%) that was located at Botany Department, Obafemi Awolowo University, Osun State, Nigeria. This research project was conducted from 2017-2018.

Plant materials and growing conditions: Seeds of *Pachyrhizus erosus* used in this experiment were collected from the Department of Crop Production and Protection, Obafemi Awolowo University Ile-Ife, Osun State, Nigeria (Latitude 07° 30'N and Longitude 04° 40'E). Fresh seeds totaling 350 were germinated at the rate of 10 seeds per petri dish in the laboratory. Germinated seeds were transplanted into a total of three hundred experimental pots (11 L) arranged in complete randomized blocked design in the screen house, at the rate of one seedling per bucket. Each experimental pot received adequate watering (80 mL of water, i.e., Field Capacity (FC)) for seven days, for acclimatization. After seven days, a total of 250 pots were selected for the different levels of water treatments based on emerging seedlings with two fully expanded leaves. The pots were further divided into two groups, the first group (125 pots) was used to check the effect of different water regimes on seedling growth (i.e., morphological characters), while the second group (125 pots) was used to study the effect of different water regimes on the physio-biochemical parameters of *P. erosus* seedlings. The samples were harvested weekly until the end of the seedling stage. Different level of water treatments was imposed using four irrigation regimes. Before the treatments, the FC of the soil was determined using the direct gravimetric method[24]. The maximum water retention capacity in 450 g of soil was determined to be 80 mL. Thus, the four irrigation regimes were set as control (T_{100}), in which the soil moisture was maintained at 100% of FC (80 mL), slight deficit irrigation (T_{75})-irrigated at 75% of FC (60 mL), moderate deficit irrigation (T_{50})-irrigated at 50% of FC (40 mL) and severe deficit irrigation (T_{25})-irrigated at 25% of FC (20 mL). Each of the treatments has six replicates.

Plant biomass and growth analysis: The number of leaves, Shoot Length (cm), Root Length (cm) and Leaf Area (cm^2) were measured using a measuring tape (for lengths) and a leaf area meter (CI 202, United States), respectively. After removing the plants from the soil, roots were thoroughly washed to remove debris. Samples were oven-dried at 70°C for 24 hrs, to measure biomass and Relative Growth Rate (RGR). The relative growth rate was determined[25]:

$$RGR = \frac{Inw_2 - Inw_1}{t_2 - t_1}$$

where, w_1 is the plant biomass at initial harvest, w_2 is the plant biomass at final harvest, t_1 is the time of initial harvest and t_2 is the time of the final harvest.

Leaf relative water content (LRWC): Fully expanded leaves were randomly collected from each of the replicates. The leaves were cut into discs of uniform size and weighed, i.e., Fresh Weight (FW). They were then immediately floated on distilled water at 25°C in the dark. After 12 hrs, the Turgid Weight (TW) was measured and the discs dried in a Gallenkamp oven at 70°C for 24 hrs to obtain the Dry Weight (DW). The LRWC was calculated by the modified method[26]:

$$LRWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

Determination of proline content: The free proline content was determined[27]. Ground leaf material weighing 0.15 g was added to 5.0 mL aliquot of 3% (w/v) sulfosalicylic acid and boiled in a water bath at 100°C for 10 min. After centrifugation at 2000 g for 5 min, 200 µL aliquot of the supernatant were mixed with 400 µL glacial acetic acid and 600 µL 2.5% ninhydrin and boiled at 100°C for 40 min. The mixture was cooled and thoroughly mixed with 800 µL toluene. The resulting supernatant layer was carefully removed and read at 520 nm. The proline concentration (µg g^{-1} FW) was calculated using L-proline for the standard curve.

Determination of malondialdehyde (MDA) content: Lipid peroxidation was determined by measuring the MDA concentration[28]. Fresh leaves (0.12 g) was ground in 1.2 mL 0.1% (w/v) trichloroacetic acid and then centrifuged at 6000 g for 20 min. Total 0.3 mL 0.5 % (w/v) thiobarbituric acid was added to 0.3 mL of the supernatant and heated at 100°C for 20 min. The mixtures were then subjected to an ice bath and the absorbance was recorded at A_{532}, A_{600} and A_{450}. The interference of soluble sugars in the samples at A_{532} and A_{450} was corrected by subtraction. The MDA content (µmol g^{-1} FW) was calculated according to the formula:

$$MDA = 6.45\times(A_{523}-A_{600})-0.56\times A_{450}$$

where, A_{532}, A_{600} and A_{450} represent the absorbance of the mixture at 532, 600 and 450 nm, respectively.

Estimation of antioxidants enzyme activity (SOD) and catalase (CAT)): To evaluate the activity of antioxidant enzymes, 0.2 g samples were homogenized with 4 mL of a cold solution containing 1% (w/v) polyvinylpolypyrrolidone, 50 mM phosphate buffer (pH 7.8), 1 mM ascorbic acid and 10% glycerol[29]. The homogenate was centrifuged at 6000×g for 30 min and the supernatants were immediately stored in ice until the subsequent assays. The total SOD activity was measured by evaluating the ability to reduce nitro blue tetrazolium[30]. One unit of SOD activity was defined as the amount of enzyme that caused 50 % inhibition of the initial rate of the reaction in the absence of enzyme and expressed as U g^{-1} FW. The CAT activity was determined by the decomposition of H_2O_2[31]. One unit of CAT activity was defined as an increase in 0.01 units at 240 nm and expressed as U g^{-1} FW.

Determination of photosynthetic pigment (chlorophyll and carotenoids): Fresh leaves samples (0.1 g) were used to extract chlorophyll a, Chlorophyll b and Carotenoids. The leaves were homogenized with 10 mL acetone and a pinch of sodium bicarbonate and kept in the dark at room temperature for 36 hrs. Absorbance was measured at 470, 646 and 663 nm for chlorophyll a, Chlorophyll b and Carotenoids, respectively. The pigment content was calculated on a fresh weight basis and expressed as mg g^{-1} FW using the following Eq.[32]:

$$\text{Chlorophyll a } C_a\ (\mu g\ mL^{-1}) = 12.25A_{663}-2.79A_{646}$$

$$\text{Chlorophyll b } C_b)\ (\mu g\ mL^{-1}) = 21.50A_{646}-5.10A_{663}$$

$$\text{Total chlorophyll} = 17.76A_{646}+7.34A_{663}$$

$$\text{Total carotenoids } (\mu g\ mL^{-1}) = (1000A_{470}-1.82C_a-85.02C_b)/\ 198$$

where, A_{663}, A_{646}, A_{470} are the absorbance at 663 nm, 646 nm and absorbance at 470 nm.

Statistical analysis: All measurements were replicated six times and presented as mean±Standard Error (SE). The statistical analysis were performed using SAS version 9.1 (SAS Institute, Gary, NC, USA), one-way ANOVA was used to compare the difference among means and Ducan multiple range tests was used to check the level of significance among the means of each water regimes at $p<0.05$.

RESULTS

Morphological response of *P. erosus* to different water regimes: Severe (T_{25}) water deficits significantly lowered the LRWC of *P. erosus* from the first to the last week of the experiment ($p<0.05$) compared to the slight (T_{75}) water deficits and control regime. While Moderate water deficits also significantly reduced the LRWC at the 1st, 2nd and last week of the experiment ($p<0.05$) compared to the T_{75} and control (T_{100}) water regimes (Fig. 1).

There were no significant differences in the growth rate of *P. erosus* between the 1st and the 2nd week of applying different water treatments, until the third and 4rth week, when the growth rate of T_{25} was significantly higher than T_{50}, T_{75} and T_{100} water regimes ($p<0.05$) (Fig. 2). The shoot heights of *P. erosus* seedlings were not significantly affected by the different water regimes (T_{25}, T_{50} and T_{75}) compared to T_{100} water regimes (Fig. 3). The root lengths of T_{25} and T_{50} regimes were observed to be significantly longer than the T_{75} and T_{100} water regimes from the second to the last week ($p<0.05$) (Fig. 4).

The leaf area of *P. erosus* was unaffected by the different water regimes from the 1st to the 3rd week, until the 4th week

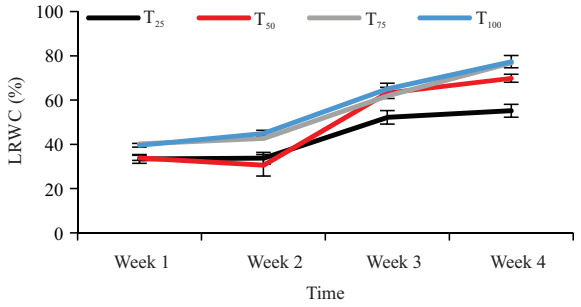

Fig. 1: Leaf relative water content (%) of *P. erosus* seedlings under different irrigation regimes

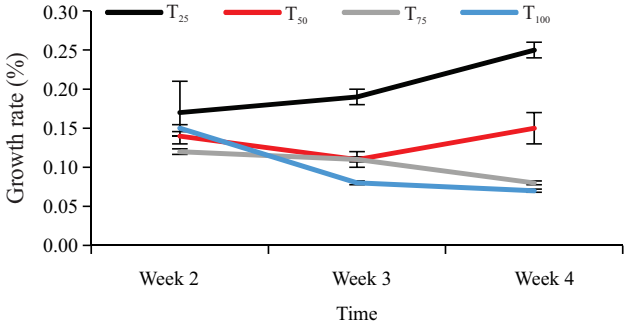

Fig. 2: Relative growth rate of *P. erosus* seedlings under different irrigation regimes

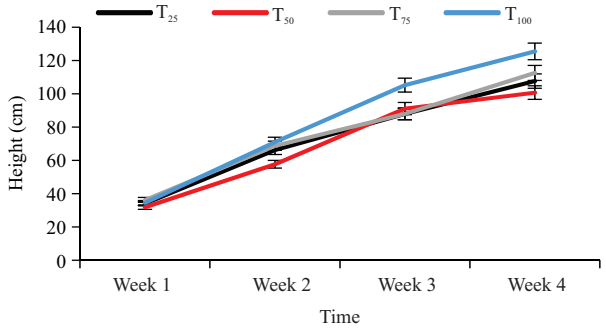

Fig. 3: Shoot height of *P. erosus* seedlings under different irrigation regimes

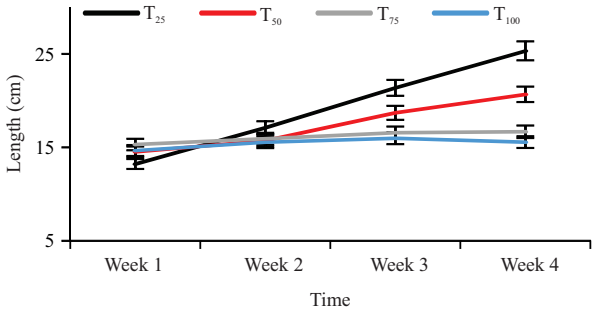

Fig. 4: Root length of *P. erosus* seedlings under different irrigation regimes

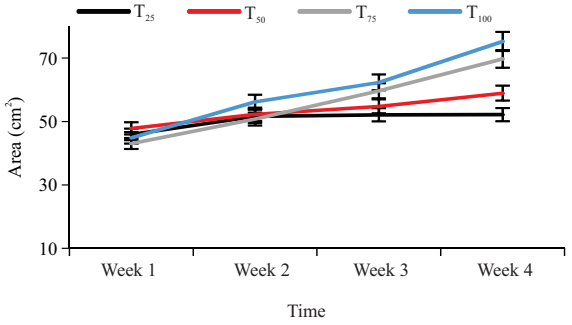

Fig. 5: Leaf area of *P. erosus* seedlings under different irrigation regimes

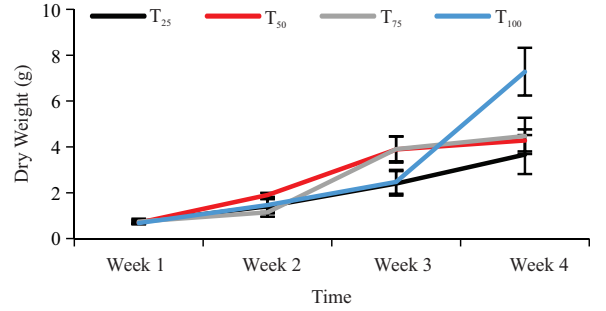

Fig. 6: Total dry biomass of *P. erosus* seedlings under different irrigation regimes

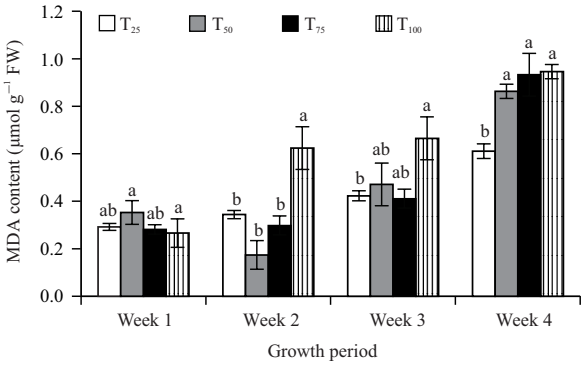

Fig. 7: Changes in lipid peroxidation product (MDA) of *P. erosus* in response to different water regimes

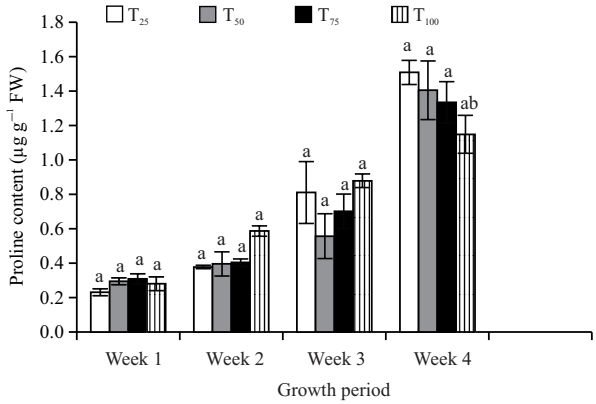

Fig. 8: Effect of different water regimes on osmolyte (proline) production in *P. erosus*

where there was a significant reduction in the leaf area of T_{25} and T_{50} ($p<0.5$) compared with T_{75} and T_{100} (Fig. 5).

The total dry biomass of *P. erosus* was not significantly different in all water regimes from the 1st to the 3rd week. At the 4th week, the total dry biomass of the control regime was significantly higher than the T_{25}, T_{50} and T_{75} water regimes ($p<0.05$) (Fig. 6).

Biochemical parameters: The MDA contents of *P. erosus* in the different water regimes increased across the weeks. Water deficits (T_{25}, T_{50} and T_{75}) lowered the MDA production in the 2nd and 3rd week but at the 4th week, only the T_{25} water regimes were significantly reduced compared to the other water regimes ($p<0.05$) (Fig. 7).

There was no significant difference ($p<0.05$) in the proline accumulation among the different water treatments from the beginning to the end of the experiment (Fig. 8).

SOD activities of *P. erosus* increased gradually from the first to the last week of the experiment. The SOD activities of

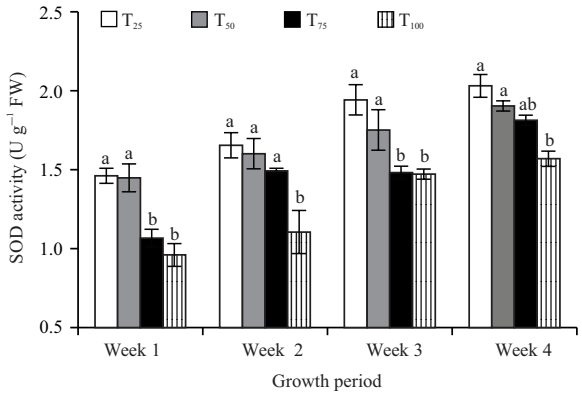

Fig. 9: Effect of different water regimes on SOD activity of *P. erosus*

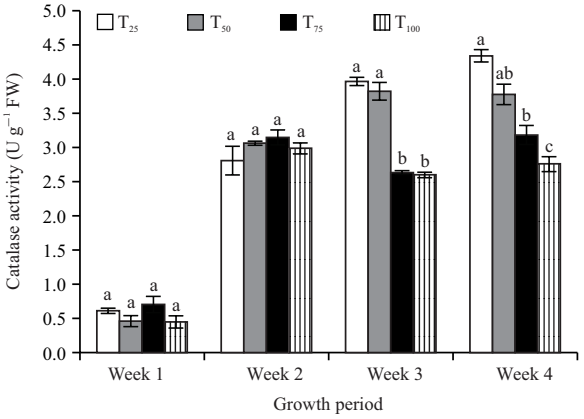

Fig. 10: Effect of different water regimes on CAT activity of *P. erosus*

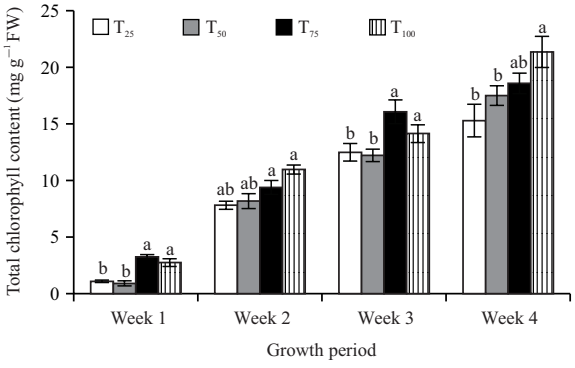

Fig. 11: Changes in total chlorophyll contents of *P. erosus* in response to different water regimes

T_{25} and T_{50} water regimes were significantly higher ($p<0.05$) than T_{100} from the 1st to the 4th week and significantly higher ($p<0.05$) than T_{75} at the 1st and 3rd week of the experiment (Fig. 9).

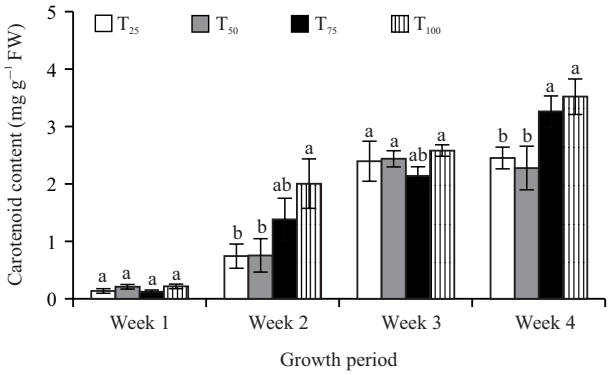

Fig. 12: Effect of different water regimes on *P. erosus* carotenoids content

There were no significant differences ($p<0.05$) in the CAT activities of *P. erosus* seedlings at week 1 and week 2. CAT activities were significantly higher in T_{25} and T_{50} than T_{75} and T_{100} at the 3rd and 4th week of the experiment (Fig. 10).

The total chlorophyll contents of *P. erosus* seedlings in the T_{25} and T_{50} regimes were significantly lower ($p<0.05$) than T_{75} and T_{100} water regimes from the first to the last week of the experiment (Fig. 11).

The carotenoids content of *P. erosus* seedlings in T_{25} and T_{50} were significantly lower ($p<0.05$) in the 2nd and the 4th week of the experiment (Fig. 12).

DISCUSSION

In this study, LRWC and leaf area of *Pachyrhizus erosus* seedlings was significantly impaired by severe and moderate water deficit than those of the slight water deficit and control, which indicates a high sensitivity of the seedlings to soil dehydration, particularly at the 3rd and 4th week of imposing water deficit. A similar observation has been reported in several previous studies on different plant species[10,33,34]. For instance, while Khanna *et al.*[33] reported a reduction in LRWC of chickpeas[34], recorded a decrease in LRWC of *Matricaria chamomilla* L. and[10] observed a decrease in LRWC of three different varieties of *Capsicum* spp. studied. The decrease in LRWC of *P. erosus* seedlings by severe and moderate water deficit could be that the water storage tissue in the plant leaf was unable to hold a greater fraction of water and thus resulted in a lower turgor potential[35]. *P. erosus* reduction in LRWC suggests its inability to tolerate drought during the seedling developmental phase. However, the reduced leaf area suggests the adoption of an effective drought adaptive strategy by *P. erosus* to alleviate the evaporative losses and water demands of the root system.

Other avoidance mechanisms operative under drought condition is increased in the relative growth rate of the plant. In this study, severe water deficit "speed-up" the growth rate of *P. erosus* seedling at the later phase of the experiment. This revealed that the plant is sensitive to soil water deficit particularly at the later phase of seedling development as indicated by the overall reduction in leaf area, however, it has good adaptive mechanisms by maintaining stable shoot height and unhindered plant total dry biomass to survive drought stress. This is also evident by the extended root system adopted by *P. erosus* in severely and moderately droughted plants.

Drought-induced overproduction of reactive oxygen species has been reported to increase the content of malondialdehyde[36,37] and thus, considered as an indicator of oxidative damage[38]. Contrary to common observations[39-41], the malondialdehyde contents of *P. erosus* seedlings were significantly decreased by severe, moderate and slight water deficit, especially in the 2nd and 4th week of imposing drought. However, the MDA contents increase across the experimental period. Perhaps, the high protein and moisture content in the seed of *P. erosus* serves as the inherent qualities that protected the cells during the seedling development from oxidative damage and rapidly rebuilt it after the adversity alleviated. Moreover, plants do employ a very specialized enzyme-catalyzed antioxidant defensive system for ROS scavenging to avoid injuries caused by ROS under water deficit.

Plant synthesize a variety of solutes (organic and inorganic) that maintain water uptake and cell turgor[37]. The production of osmolytes is one of the strategies by which plants stabilize membranes and maintain protein conformation at low leaf water potentials. The synthesis and accumulation of osmolytes proline vary among plant species as well as among different cultivars of the same species[42]. Proline production in response to drought differs in *P. erosus* compared to previous studies[43,37] but similar to the findings of Pirzad *et al.*[34]. Previous studies conducted on different plant species showed a varied response of proline accumulation under water deficit. For example, it was suggested that proline concentration significantly decreases in water-stressed *Fargesi arufa*[44], however, other research found that proline accumulation significantly increased in sorghum[45]. In this study, however, proline content was not significantly different among the treatments within each of the seedling developmental period, which could be due to the possibility of its accumulation to result from both induction of proline biosynthesis and/or inhibition of its oxidation[39]. It can also be inferred that proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte[42].

This study reveals the ability of *P. erosus* to withstand severe and moderate water deficit by increasing the production of antioxidants (CAT and SOD) to scavenge ROS, decrease damage caused by oxidative stress and improved other physiological processes.

In this study, severe and moderate water deficit impaired the photosynthetic apparatus (total chlorophyll and carotenoids) of *P. erosus* particularly at the later phase of seedling development. Another function of carotenoids apart from being an accessory pigment in photosynthesis is its role as a precursor in the biosynthesis of phytohormones[46] and drought has been reported to negatively affect carotenoids synthesis[46,47].

Leaf chlorophyll contents are susceptible to soil dehydration and therefore, considered as the leading cause of photosynthesis inactivation[37]. The decrease in leaf relative water content has been reported as one of the factors that induce stomatal closure and thus a parallel decrease in photosynthesis[48]. Reduction in LRWC and area of a leaf of a *P. erosus* under water deficit give rise to a decrease in photosynthetic rate, which was evident, in a reduction of photosynthetic pigments.

CONCLUSION

Water deficit affects the seedling growth and metabolism of *P. erosus* as indicated by decreased leaf area, LRWC and photosynthetic pigments. An increase in CAT and SOD, suggest high sensitivity of *P. erosus* seedlings to moderate and severe soil dehydration. However, the reduced leaf area and extensive root length suggest the adoption of an active drought adaptive strategy by *P. erosus* seedlings to alleviate the evaporative losses and water demands of the root system. *P. erosus* seedlings will therefore survive a prolonged drought period occurring in the country.

SIGNIFICANCE STATEMENT

This study discovered that *Pachyrizus erosus* seedlings can survive seasonal drought in arid regions by building strong antioxidant enzyme mechanisms and also reduce the effect of water loss through a reduction in leaf area. This report will help many researchers to understand how legumes respond to water shortages in the environment.

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