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RESEARCH ARTICLE

Water Stress Condition on Early Growth and Biochemical Contents of *Leucaena leucocephala* (Lam.) De Wit.

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Abstract

This study compared the growth performance and biochemical responses of *Leucaena leucocephala* seedling under water stress conditions. Selected sampling seedlings were subjected to different treatments namely; Treatment 1 (watered daily), Treatment 2 (watered 3 d once) and Treatment 3 (watered weekly once) and the growth and biochemical contents were evaluated. Out of three treatments, high growth and development was reported in T1 than treatment T2 and T3. The different treatments were accompanied by the formation of more number of root nodules. These findings would be useful to relate the growth responses and synthesis of biochemical constituents under water stress. Increased root length, root collar, sugar and proline content in the stressed plants may be an adaptation to overcome the water stress conditions.

Keywords: *Leucaena leucocephala,* water stress, treatments, biochemical constituents, growth parameters.

Introduction

Leucaena leucocephala (Lam.) De wit. belongs to the family Mimosaceae. It is a fast growing leguminous tree, native to South Mexico and Central America introduced for fuel and fodder. In India, it has been introduced for agroforestry and afforestation programs. The tree was particularly useful cultivar because of its high yield (Brewbaker *et al.,* 1972; Natarajan *et al.,* 1988). Water stress refers to scarcity or limited supply of water. The major reasons for water scarcity are population growth, increasing urban, industrial demand for water, water pollution and water resource depletion. Water stress adversely affects crop growth and yield in many regions of the world (Teulat *et al.,* 1997). All plants are capable of perceiving and responding to water stress (Bohnert *et al.,* 1995; Bartels and Sunkar, 2005). This study compared the growth performance and biochemical responses of *Leucaena leucocephala* seedling under water stress conditions.

Materials and methods

Study area and seed source: This study was conducted at Botanical garden of VHNSN College, Virudhunagar, Tamil Nadu. *Leucaena leucocephala* seeds were obtained from Srivilliputhur social forestry division, Virudhunagar district, Tamil Nadu.

Nursery experiments: Healthy viable seeds of *Leucaena leucocephala* were surface sterilized with 0.01% mercuric chloride and rinsed several times in distilled water. The seeds were soaked in distilled water for 24 h. Seeds were germinated under laboratory conditions. Germinated seeds were transplanted in polythene bags with outside dimension of 13x21 cm containing soil mixture (2 Clay soil: 1 Sand: 1 Manure).

Selected sampling seedlings were subjected to different treatments namely; Treatment 1 (watered daily), Treatment 2 (watered 3 d once) and Treatment 3 (watered weekly once) and the growth and biochemical contents were evaluated.

Growth parameters and biochemical constituents: Seedling growth namely shoot length, root length, fresh and dry weight of the entire seedlings were measured for 60 d old seedlings. Biochemical contents such as chlorophyll pigment (total chl, chl a and chl b), total protein, total soluble sugar and proline content were evaluated.

Morphological measurements: Seedlings were used to measure root and shoot length, fresh and dry weight measurements. About 6 seedlings were randomly selected. The entire seedlings were removed from the polythene bag and the root region is thoroughly washed in water and the plant is surface drained using the blotting paper. Root length was measured from the root collar region to the root tip region with the help of scale. Shoot length is measured from the root collar region to the terminal bud region and recorded. Fresh weight of the entire seedlings was measured and the plant was kept for drying at 60°C in a hot air oven for a constant weight and the dry weight of the seedlings was measured.

Biochemical studies

Total chlorophyll: Total chlorophyll was extracted from the leaves and estimated by the method of Arnon (1949). About 100 mg of fresh leaf samples were separately taken from control and water stressed plants and macerated with 80% acetone.

The extract was centrifuged and the supernatant was collected and the procedure was repeated till the pellet becomes colorless. The supernatant was pooled individually and OD values were taken using Spectrometer (Spectronic 20; Milton-Roy) at 645 and 663 nm. From the OD values, chlorophyll contents for the seedlings subjected under different treatments were calculated using the following formula:

Chlorophyll 'a' (mg/mL)=(0.0127) x (OD 663) - (0.00269) x (OD 645).

Chlorophyll 'b' (mg/mL) = (0.0229) x (OD 645) - (0.00488) x (OD 663).

Total chlorophyll (mg/mL) = (0.0202) x (OD 645) + (0.00802) x (OD 663).

Total soluble sugar: Total soluble sugar was estimated for the plant growth under experimental conditions. The extraction and assay procedures are followed according to Dubois *et al.* (1956). About 100 mg of fresh leaf tissues were separately taken from control and water stressed plants. Then, the leaf samples were ground well in motor and pestle by adding little quantity of distilled water. The final volume of homogenate was made to 20 mL with distilled water and filtered through a four layer muslin cloth and the filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was collected for further assay. From this extract, 1 mL was taken and to this 1 mL of 5% Conc. H_2SO_4 was added by keeping tubes in an ice bucket undisturbed for 10 min. Again the tube was kept at 35°C for 20 min in water bath. Then, the OD value for the colored component was read at 490 nm and the OD values were used to calculate the soluble sugar content of leaf samples.

Total soluble protein: Total soluble protein content was estimated by the method of Lowry *et al*. (1951). About 100 g of fresh leaves was taken from control and water stressed plants. Then, the leaves were macerated using 10 mL of 20% ice cold TCA using mortar and pestle and centrifuged at 5000 rpm. The supernatant was discarded, and to the pellet, 5 mL of 0.1N NaOH was added and centrifuged at 6000 rpm for 5 min. The supernatant was collected and made up to 5 mL using 0.1N NaOH and the protein was estimated by the method of Lowry *et al*. (1951).

Proline content: About 100 mg of leaves from control and water stressed plants were separately homogenized in 10 mL of 3% sulphosalicylic acid using mortar and pestle and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the proline content was estimated according to the method of Bates *et al.* (1973).

Data analysis: Data on the growth parameters were collected from 6 individual sampling for each treatment given for each seedling. The data were computed for finding out the mean values and their respective standard deviation values.

Results and discussion

In the present study, *Leucaena leucocephala* seedlings were grown in containers under nursery conditions with different levels of water stress. Results on water stress effects on morphological growth and biochemical contents are illustrated.

Root length and shoot length: The extent and the pattern of root development are closely related to the ability of the plant to absorb water and minerals, hence the root growth is considered as great importance in water stress condition. In the present study, increased root length is seen in moderate and heavy water stress treatments. The root length is an important trend against water stress condition. Seedlings with no water stress (control) attained less root length when compared to all other treatments. Maximum root length was reported in T3 and T2 (Plate 1a,b and c; Table 1). Generally during water stress conditions, shoot length decreases and the degree of reduction in shoot length depends on intensity and duration of stress. Water stress decreases cell division; cell elongation and cell enlargement due to low turgor pressure which might have ultimately lead to reduction in the plant height under scarcity of water. In the present study, decrease in shoot length in T2 and increase in T3 was noted. Shoot length of the seedlings are adversely affected with the heavy water stress (T3). Whereas, moderate water stressed (T2) plants were observed with medium decrease than to the control treatment which shows the greater shoot length growth (Table 1).

Plate 1. Root length and shoot length of *L. leucocephala* seedlings under different water stress conditions (T1, T2 & T3) in the nursery.

Table 1. Root length and shoot length of *Leucaena leucocephala* during water stress.

Treatment	Root length (cm/plant)	Shoot length (cm/plant)
T1	26.5	22
T2	30	18
T3	34.2	8.9

Table 2. Early growth parameters of *L. leucocephala* under different water stress conditions (T1, T2 & T3).

Treatment	Root collar dia (mm/plant)	No. of nodule/plant	Total dry weight (g/plant)
T1	2.4	15	1.4
T2	3	10	0.9
T3	3.6	6	0.4

Table 3. Biochemical contents of *L. leucocephala* under different water stress conditions (T1, T2 & T3).

Treatment	Chl a (mg/mL)	Chl b (mg/mL)	Total chl (mg/mL)	Protein content (mg/g fresh wt.)	Soluble sugar (mg/g fresh wt.)	Proline content (mg/g fresh wt.)
T1	0.25	1.96	2.21	28.64	11.59	11.66
T2	0.18	1.5	1.68	25.57	17.76	16.58
T3	0.11	1.14	1.25	24.34	48.80	20

Root collar diameter: Unlike the shoot length, diameter measurement at the root collar region showed a reversed trend (Table 2). During the sampling period, more or less equal value is observed for the plants under T1 as well as moderate water stress (T2) treatments. Whereas in the seedlings, subjected under heavy water stress condition (T3) showed the greatest diameter increment.

Seedling dry biomass: A greater reduction was observed with the seedling dry biomass when it was subjected under heavy water stress condition (Table 2). Seedling grown with control produced the greater amount of dry biomass, among all the other treatments. Meanwhile, seedlings under T2 produced moderate amount of biomass production.

Biochemical contents: Total chlorophyll pigments and protein were found to be more in the seedlings grown under T1. In the heavily water stressed plants, these contents were considerably lesser. Total sugar and proline content was found to be higher in the plants with heavy water stress (T3) than T2 and T1 plants (Table 3).

Conclusion

The findings showed that increasing water stress significantly reduced the shoot length, nodule number, dry weight, total chlorophyll contents and protein content. The experiment showed that protein accumulated lowest in T3 than T2. To conclude, decreased protein content in the stressed plants may be an adaptation to overcome the water stress conditions. Protein content is determined not only based on water stress but also with the type of soil. The reduced proline oxidase may be the reason for increasing proline accumulation. Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress. Proline may protect protein structure and membranes from damage, and reduce enzyme denaturation.

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