

Comparative study on growth, yield and carbon content in *Pongamia pinnata* under water stress and urea supplementation

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Abstract

The growth, yield, and carbon content of eight-month old seedlings of *Pongamia pinnata* were compared under water and urea supplementation. One set of plants were subjected to water stress condition (WS), whereas the other supplied with 2 g of urea (WS+U) under WS. Both the experimental set ups were exposed to varying treatment levels which include full irrigation (100%, control) followed by 75 % (T1), 50 % (T2), 25 % (T3) and 12.5 % (T4). The growth, leaf area and relative water content were maximum under WS when compared to WS+U ($p < 0.001$). The maximum biomass was produced in the seedlings under WS in control (1.68 g) followed by T1 (1.38 g), T2 (1.53 g), T3 (0.93 g) and T4 (0.73 g). A significant ($p < 0.001$) reduction in biomass production was observed in WS+U in control (1.28 g), T1 (0.66 g), T2 (1.13 g) and T3 (0.44 g). T4 of WS+U showed similar biomass (0.73 g) as that of T4 of WS. Under WS, the highest biomass allocation was recorded in shoots followed by leaves and roots. Similar trend was observed in WS+U. However, the percentage of allocation was more in the roots of WS+U (27.2 %) when compared to WS (22.24 %). The highest amount of carbon content was observed in control plants treated under WS (9.59 g) followed by control plants of WS+U (7.31 g) ($p < 0.001$). The results of the preliminary study clearly indicated that *P. pinnata* seedlings were able to cope-up with water stress conditions without urea application and can perform well in 50 % water availability and is best suited for the plantation programs in the semi-arid ecosystems.

Key words

Water stress, Growth parameters, *Pongamia pinnata*, Semi-arid ecosystem.

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Introduction

Plants take up carbon dioxide from the atmosphere and incorporate it in the form of fixed biomass through photosynthesis during their growth processes, thereby acting as carbon sink. Some of this carbon is emitted back to the atmosphere but what is left-the live and the dead plant parts, above- and below ground, make up organic carbon reservoir (Trumper *et al.*, 2008). Therefore, growing trees can be a potential contributor in reducing the concentration of CO₂ in atmosphere by its accumulation in the form of biomass. As the trees grow, their biomass increases (Mathews *et al.*, 2000) resulting in growth of different parts. The biomass or primary production of the plants depends on their ability to fix carbon from the atmosphere.

This in turn is limited by the supply of water and nutrients to the fixation sites. The process of photosynthesis and thereby the primary productivity of plants are known to decrease under water stress (Gnaana Saraswathi and Paliwal, 2011). Carbon allocation commonly refers to the distribution of carbon among plant organs (e.g., leaves, stems, roots) (Mooney, 1972). The development of efficient resource use in a plant species involves mediation of physiological function and plant structure, the latter being related to carbon reallocation (Gnaana Saraswathi and Paliwal, 2008). Study of primary productivity and carbon allocation patterns at the individual plant as well as ecosystem levels would reveal interactions between ecological and physiological processes (Landsberg, 2003) which in turn determine carbon sequestration. In terms of atmospheric

carbon reduction, growing trees offer the double benefit of direct carbon storage and stability of natural ecosystem with increased primary productivity and recycling of nutrient along with maintenance of climatic conditions by the biogeochemical processes (Chavan and Rasal, 2010). There exist a vast literature on a global scheme for primary production (Friedlingstein *et al.*, 1999; Zhang *et al.*, 2005) and carbon allocation (Gower *et al.*, 2001; Litton *et al.*, 2007) in herbaceous and crop plants whereas, it is limited on woody species under natural growing environment and under nutrient depletion (Wardlaw, 1990). However, studies are scanty in semiarid ecosystem on the primary production and carbon allocation under varying treatment conditions like water and nutrient manipulations (Friend *et al.*, 2004). Studies of this kind would help in carbon sequestration through biomass and carbon allocation management (Feller *et al.*, 1998) as the biomass plays a major role in determining the carbon content which in turn determines the carbon sequestration (Friend *et al.*, 2004). A complete carbon budget is needed for every tree species as it forms the foundation for understanding both wood production (biomass) and carbon sequestration, especially in response to varying environmental conditions (IPCC, 2007). *Pongamia pinnata* is one of the agro-forestry tree species for afforestation programme in the semiarid marginal lands. It is a fast growing leguminous tree species with a potential for high oil seed production and has gained importance in the bio-diesel industry (Scott *et al.*, 2008). The review on this plant so far suggests that the studies have focused mainly on the phytochemical, physio chemical properties and oil content of the seeds (Birajdar *et al.*, 2011). Being a potential agro-forestry tree species, for effective carbon sequestration to check global warming, an extensive research on its physiology and carbon content is needed (Arote and Yeole, 2010). Scott *et al.* (2008) revealed that emphasis is to be given in analyzing carbon sequestration in relation to carbon credit and nitrogen gain of this plant. Despite considerable importance of this species in various sectors especially bio-energy and oil content, the information on carbon content and carbon sequestration is scanty (Rathore *et al.*, 2011). Keeping this in view, present preliminary study was undertaken to examine the growth parameters, primary production (biomass), carbon allocation and carbon content in *P. pinnata* seedlings under water stress condition and urea supplementation under water stress.

Materials and Methods

Site and seed germination: The study site is located in the botanical garden, Holy Cross College, Tiruchirappalli (10° 48' N latitude; 78° 41' E longitude; elevation 2000–3000 feet a m.s.l.), Tamil Nadu, India. The highest temperature ranged between 38–40° C during 2007–2010. The average annual rainfall was about 725 mm. The soil at the experimental site was red, very deep fine with high clay content.

Healthy seeds of *P. pinnata* obtained from Palani Hills Conservation Council, Odukkam Seed Centre, Dindigul, Tamil Nadu were soaked in tap water for 24hr. The seeds were sown in seedbeds and irrigated depending upon the soil moisture. The germinated

seedlings of 2 weeks with four to five leaves were transferred to polythene bags of 30 cm height and 15 cm in diameter filled with soil, natural organic manure (cow dung compost) and silt (2:1:1 ratio). The seedlings were kept under shade for a week and then grown in nursery under field condition for about six months. The seedlings were irrigated in the morning every alternate day.

Experimental design: Two experimental set ups were maintained in six-month old seedlings of *P. pinnata* for two months. One set of the plants was subjected to water stress condition (WS) and the other set was supplied with 2 g of urea (WS+U) under WS supplied once in a month. Based on natural water content of soil in growing season, five soil water gradients were designed including 100 (control), 75 (T1), 50 (T2), 25 (T3) and 12.5 % (T4) as water stress treatments. For each gradient ten plants were maintained. The plants were watered every morning and exposed to sun light. The number of replicates for each treatment (control, T1, T2, T3 and T4) was ten and therefore, for five treatments, fifty plants were maintained.

The germination percentage of the seedlings was calculated by dividing the number of seeds germinated with total number of seeds sown and multiplied with 100. The growth parameters namely the root length, shoot length, height and the leaf area were measured in all the plants under varying treatment conditions.

Relative water content (RWC) was measured in the leaves at the end of the treatment (61st day) by saturated weighing method (Lin and Ehleringer, 1982). The turgid leaf weight was determined after keeping the leaf in distilled water in darkness at 4°C to minimize respiration losses, until they reached a constant weight (full turgid, typically after 12 hr). Leaf dry weight was obtained after keeping the turgid leaf at 30°C in an oven for 24 hr.

Biomass: The biomass estimation was carried out by harvest method (Flombaum and Sala, 2007). Five seedlings from each treatment were uprooted carefully by putting the pot upside down at the time of experiment termination. The fresh weights of the above- and below ground components of the plants were recorded and the samples were kept in the oven at 80°C until the constant weight obtained. Then the dry weight was recorded. The comparative total biomass of the two experimental set ups, at various treatment levels as well in various plant components were determined for the species.

Carbon content analysis: The carbon content of different components of seedlings namely the root, shoot and leaf were estimated by multiplying oven dry biomass with a constant (0.475) as per the method of Magnussen and Reed (2004).

Statistical analysis: The study was conducted in a completely randomized experimental design with five treatment levels in ten replications. All the parameters were subjected to one-way analysis of variance (ANOVA) ($p < 0.001$) and compared using Tukey's test ($P < 5\%$) (Sheskin, 2004).

Results and Discussion

In WS, the maximum soil moisture was observed in control (56%) followed by T1 (48.14%), T2 (34.16%), T3 (19.33%) and minimum in T4 (8.49%). In WS+U, the maximum soil moisture content was observed in control (55.2%) followed by T1 (46.2%), T2 (31.3%), T3 (15.7%) and minimum in T4 (6.32%).

The germination percentage of *P. pinnata* seedlings was found to be 96%. Root length of *P. pinnata* seedlings differed significantly both in WS and WS+U experimental set ups as well as in varying treatment levels (control, T1, T2, T3 and T4). In WS, the root length gradually increased as the treatment level proceeded from control (21.3 cm) through T1 (24.0 cm) to T2 (28.99 cm). Thereafter, it declined sharply in T3 (19.68 cm) and T4 (15.54 cm). However, in WS+U there was a gradual reduction in root length as the treatment proceeded from control (21.6 cm) to T1 (20.6 cm), T2 (16.8 cm), T3 (15.32 cm) and T4 (8.01 cm) (Fig. 1a).

Shoot length of *P. pinnata* seedlings showed similar trend as that of root length. However, the treatment levels T3 and T4 in WS and T2 and T3 in WS+U, exhibited nearly similar responses

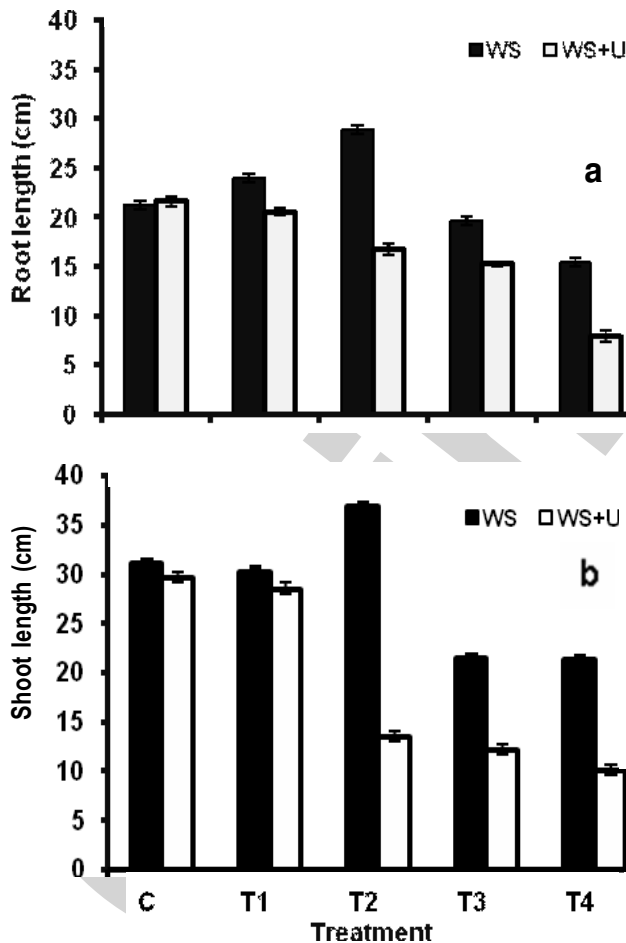


Fig.1: a-b: Root length (a) and shoot length (b) of *P. pinnata* seedlings under water stress (WS) and water stress supplemented with urea (WS+U) conditions. Values are mean of ten replicates ± SE

without any significant differences. In WS, the maximum shoot length of 36.89 cm in T2 and the minimum of 21.52 cm and 21.56 cm were observed in T3 and T4, respectively. Control (31.13 cm) and T1 (30.24 cm) showed almost similar shoot length which was lesser than T2. On contrary, in WS+U, the maximum shoot length was observed in control (29.7 cm), followed by T1 (28.5 cm). However, from T2 onwards there was a gradual reduction in the shoot length in all the treatment levels, which are as follows: T2 (13.5 cm), T3 (12.2 cm) and the minimum in T4 (10.08 cm). In WS+U, the treatment levels T2 and T3 showed more or less similar reduction in shoot length (Fig. 1b).

Height of the seedlings of *P. pinnata* showed a significant ($p < 0.001$) difference in varying treatment levels. In WS, the maximum height of the plant was observed in T2 (65.88 cm) and the minimum in T4 (36.8 cm). The height of the seedlings in other treatments is as follows: control (52.43 cm), T1 (54.26 cm) and T3 (41.2 cm) whereas, in WS+U, the maximum height was observed in control (51.38 cm) followed by T1 (49.1 cm) and the minimum in T4 (18.12 cm). The height of the seedlings in other treatments is as follows: T2 (30.32 cm) and T3 (27.52 cm).

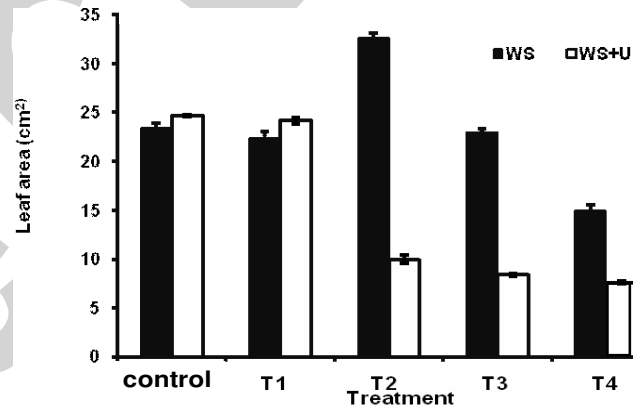


Fig.2: Effect of water stress (WS) and water stress supplemented with urea (WS+U) on leaf area of *P. pinnata* seedlings. Values are mean of ten replicates ± SE

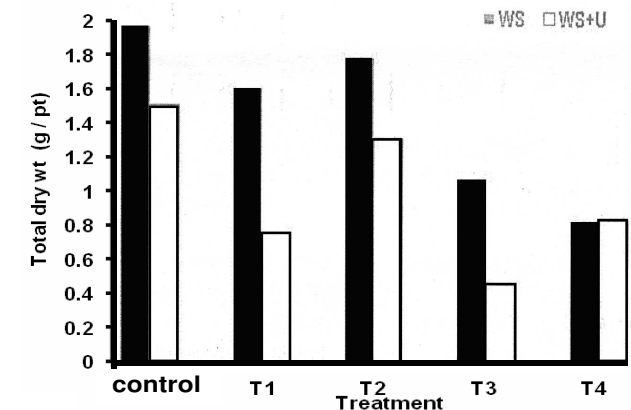


Fig.3: Effect of water stress (WS) and water stress supplemented with urea (WS+U) on total biomass of *P. pinnata* seedlings. Values are mean of five replicates ± SE

WS and WS+U showed a significant ($p < 0.001$) difference in the leaf area of *P. pinnata* seedlings. In WS, the maximum leaf area was observed in T2 (32.66 cm²) followed by control (23.45 cm²), T3 (23.0 cm²) and T1 (22.35 cm²), while minimum leaf area was observed in T4 (15.0 cm²). In WS+U, the maximum leaf area was observed in control (24.6 cm²) and T1 (24.16 cm²) where as declined significantly ($p < 0.001$) in T2 (10 cm²), T3 (8.4 cm²) and T4 (7.6 cm²) respectively (Fig. 2).

The relative water content showed a significant ($p < 0.001$) difference in WS and WS+U. In WS treatment, it ranged from 76.2 % in control to 89.9 % in T2 but with a reduction in T3 (73.9 %) and T4 (68.0 %). In WS+U, the RWC was more in control (78.7 %) than the other treatment levels except T4. Thereafter, it gradually decreased in the following treatments; T1 (63.1 %), T2 (61.1 %) and T3 (59.7 %). However, it showed a characteristic increase in T4 (74.0 %) (Table 1).

Table - 1: Effect of water stress (WS) and water stress supplemented with urea (WS+U) on the relative water content of *P. pinnata* seedlings.

Treatment	Relative water content (%)	
	WS	WS+U
Control	76.18 ± 0.25	78.72 ± 0.9
T1	81.23 ± 0.56	63.11 ± 0.36
T2	89.90 ± 0.97	61.10 ± 0.37
T3	73.89 ± 0.15	59.67 ± 0.39
T4	68.02 ± 0.12	74.82 ± 0.41

Values are mean of ten replicates ± SE.

In WS, the maximum biomass (total d. wt g⁻¹ plant⁻¹) was produced in control (1.68) and T2 (1.53) followed by T1 (1.37) and T3 (0.93) and minimum in T4 (0.74). A significant ($p < 0.001$) decrease in the total biomass was observed in WS+U treatment levels when compared to WS. The maximum biomass was observed in control (1.28) followed by T2 (1.13) and T4 (0.74) whereas, minimum in T3 (0.42) (Fig. 3).

The above- and below ground components of *P. pinnata* seedlings responded significantly ($p < 0.001$) in allocating the biomass depending on the various irrigation levels and fertilization. In general, in WS experiment, the highest biomass allocation was recorded in shoots followed by leaves and roots. Higher biomass allocation in roots was observed in T3 (24.2%) followed by T4 (22.8%) than in control (20.9), T1 (20.38%) and T2 (20.91%), which showed similar responses. The maximum biomass allocation in shoots was observed in control (45.7%) and T2 (45.1%) followed by T4 (44.02%) and the minimum allocation was recorded in T3 (42.61%) and T1 (42.6%). However, the overall biomass allocation in shoots did not vary much between different treatment levels. Leaves of *P. pinnata* seedlings showed remarkably more allocation of biomass than roots. The biomass allocation to the leaves showed similar trend in the all treatment levels ranging from control to T4 (33.42%) except for a slight increase in T2 (35.02%).

WS+U experiment also showed similar trend as that of WS in biomass allocation in various treatment levels. However, the percentage of biomass allocation in roots was found to be higher in WS+U than WS. The highest allocation of biomass in roots was observed in T1 (33.5%) followed by T4 (27.9%), T2 (26.5%), control (25.3%) and the lowest in T3 (22.7%). The maximum allocation of biomass in shoots was recorded in T3 (49.3%) followed by T4 (42.2%), control (40.4%), T1 (40.2%) and minimum in T2 (39.8%). More allocation of biomass in leaves was observed in control (34.3%) followed by T2 (33.6%), T4 (29.8%), T3 (27.9%) than in T1 (26.3%) (Table 2).

Table-2: Biomass allocation of *P. pinnata* under varying levels of water stress (WS) and water stress supplied with urea (WS+U) conditions.

Treatment	Biomass allocation (%)					
	Root		Shoot		Leaves	
	WS	WS+U	WS	WS+U	WS	WS+U
Control	20.9	25.27	45.72	40.40	33.37	34.32
T1	20.38	33.53	42.58	40.18	35.02	26.28
T2	20.91	26.54	45.09	39.82	33.98	33.62
T3	24.19	22.74	42.61	49.28	33.19	27.96
T4	22.82	27.98	44.02	42.14	33.15	29.87

The highest amount of carbon content was observed in the seedlings of *P. pinnata* under WS. Carbon content (g plant yr⁻¹) was more in shoots followed by leaves than in roots. The carbon content was gradually reduced as the stress treatment proceeded. In WS experiment, the maximum total carbon content was recorded in control (9.59) followed by T1 (9.84) and T2 (9.72). The carbon content of T3 was 5.32 and the minimum was in T4 (4.19). The total carbon content as well as the carbon content of the individual components decreased considerably in WS+U when compared to WS. The total carbon content was maximum in control (7.31) followed by T2 (6.44), T1 (3.77) and T4 (4.21). The minimum carbon content was recorded in T3 (2.41) (Table 3).

Table-3: Effect of water stress (WS) and water stress supplemented with urea (WS+U) on the carbon content of *P. pinnata* seedlings.

Treatment	Carbon content (g plant ⁻¹ yr ⁻¹)							
	Root		Shoot		Leaves		Total	
	WS	WS+U	WS	WS+U	WS	WS+U	WS	WS+U
Control	2.00	1.84	4.38	2.95	3.20	2.50	9.59	7.30
T1	1.75	1.26	3.34	1.51	2.74	0.99	7.84	3.77
T2	1.82	1.71	3.93	2.56	2.96	2.166	9.72	6.441
T3	1.28	0.54	2.26	1.18	1.76	0.67	5.32	2.40
T4	0.95	1.17	1.84	1.77	1.39	1.25	4.19	4.20

Seedling height and leaf area provide a measure of photosynthetic and transpiration traits and are indicators of seedling quality (Ohashi *et al.*, 2009). When compared WS and WS+U experiments for the root, shoot length and plant height in the seedlings

of *P. pinnata*, urea supplementation during water stress conditions (WS+U) did not effectively increase the root, shoot length and the height of the seedlings until the final measurement period. This was due to low-optimal availability of water coupled with injury in uptake of nutrients due to water stress (Axelsson and Axelsson, 1986).

A significant increase in leaf area in WS+U seedlings in control and T1 conditions indicate the effective nitrogen utilization in the well watered condition followed by 75 % irrigation, (Gomez *et al.*, 2008). As the treatment level proceeded towards more severe water stress, the leaf area of *P. pinnata* seedlings showed a remarkable reduction as a mechanism of drought avoidance, a similar response observed from previous study of Litton *et al.* (2007).

RWC is considered as a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for identifying legumes with contrasting differences in dehydration tolerance (Litton *et al.*, 2007). In the treatment study, RWC of WS and WS+U was more than 55 % in all the treatments. This clearly indicates that *P. pinnata* have certain adaptability and adjustment capacity on drought / water stress which was similar to the results observed by Yuwan *et al.* (2007).

Water stress and nutrient supply induced variation plays a major role not only on partitioning the photosynthate allocation pattern but also influencing the total biomass production and stem wood yield (Stape *et al.*, 2008). Our study indicates with increased water stress and urea availability, plant biomass allocation shifts from below ground to above ground, i.e., from root to shoot. Similar results were obtained in the previous study of Gower *et al.* (2001). Water stress along with nutrient does not always result in shifts in biomass distribution to roots. Substantial nutrient-induced growth reductions are commonly required before such shifts are evident. An additional source of uncertainty is the change in root:shoot ratio that occurs as a function of plant size rather than nutrient addition per se. (Friend *et al.*, 2004). Our results are in agreement with the studies undertaken by Coleman *et al.* (2004) in *Populus deltoides*. The carbon allocation in rapidly growing *P. pinnata* seedlings shifts from below- to above ground as urea availability couples with water stress conditions. This shift was independent of seedlings development evident from studies on soybean seedlings under stress (Ohashi *et al.*, 2009). Our results are contradictory to studies by Sileshi *et al.* (2007), in that, the biomass allocation during water stress (WS) condition was more in shoots than in roots but it was reverse in WS+U experiment. This may be due to improper mobilization effect due to water stress. Tree seedlings growing under water-stress or nutrient stress are known to show higher root to shoot ratios because plants shift relatively more assimilates to the roots in drought-prone and nutrient-prone environment (Axelsson and Axelsson 1986). But the shift from roots to shoots and then to leaves may be attributed to low resource availability which changes accordingly (Litton *et al.*, 2007).

The carbon content of vegetation is surprisingly constant across a wide variety of species. The amount of carbon stored in

dry wood is approximately 50% by weight. Most of the information for carbon estimation described in the literature suggests that carbon constitutes between 45 to 50 % of dry matter (Schlesinger, 2001). *P. pinnata* seedlings under WS produced increased total dry biomass and carbon content. The carbon content of WS was more than that of WS+U treated seedlings. The total biomass and the carbon content of control of WS+U were similar to that of the control of WS. This may be due to the perfect combination of the fertilization at the time of transplantation which would have lead to the proper nutrient cycling, thereby higher biomass production. This is evident from the studies by Stape *et al.* (2008) in *Eucalyptus* species where the productivity was increased on fully irrigated seedlings supplied with urea. The study also revealed that the plant strongly responded by producing higher biomass under fertilization at moderate or optimum level of water.

There was a general pattern observed in our study, where the growth parameters, biomass allocation and the present carbon content showed a typical trend of gradual reduction as the treatment proceed from control to T4. However, there was a sudden raise in the values of all these parameters in T2, the optimum condition in WS which was not observed in WS+U. In WS, the increase in root, shoot length, leaf area, relative water content, biomass allocation and carbon content in T2 may be attributed to the selective optimal growing conditions as well as the photosynthetic capacity which reflect on the leaf phenology and physiological performance at that particular optimum condition (Litton *et al.*, 2007).

WS+U showed a remarkable decline in all these parameters except in RWC, where the RWC increased in T4, which may be attributed to the drought avoidance mechanism of the plants during severe stress conditions (Litton *et al.*, 2007). Similarly, significant increase in the root dry weight in T4 conditions also indicates that the plants can cope up with severe water stress condition with the help of little fertilizer.

Total biomass and carbon content showed a significant increase in T2, followed by C, which can be attributed to the optimum growth in T2 in root, shoot length and leaf area, which could mobilized more photosynthates thereby, increasing the total biomass as well the carbon content (Friend *et al.*, 2004). Similar results were obtained in our previous studies also, where *Cassia siamea* showed higher biomass production under drought condition than *A. lebbek* (Gnaana Saraswathi and Paliwal, 2011).

Our study reveals that the seedlings of *P. pinnata* have the ability to with stand water stress conditions with drought avoidance strategy. Supplementation of urea under well watered condition showed better effect than urea supplemented under severe water stress conditions. This plant species can be grown in the marginal lands of limited water availability for plantation to improve the soil fertility and to sequester carbon.

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