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ACCLIMATIZATION STRATEGY OF TWO COMMON TREE SPECIES UNDER WATER STRESS

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Abstract

Due to unpredicted climate change and drought spells, changing towards stress-resistant planting material is dire need in the present scenario. The increasing trend of Agroforestry under arid to semi-arid climatic regions can be proved as an important step to mitigate climate change effects and also can reduce pressure on the existing forest resources. Therefore, the present study was planned to evidence the effect of water stress on two introduced and important tree species of central Punjab i.e. *Eucalyptus camaldulensis* and *Conocarpus erectus* at their early stages of growth. The experiment was conducted under controlled conditions where plants were subjected to two watering (controlled irrigation and high water-deficit) regimes. The biomass production and allocation in different plant organs (leaves, stem and root) along with growth parameters (plant height, stem diameter, no of leaves) were measured during the experiment. Drought negatively impacted plant height and the number of leaves. However, the plant height of *C. erectus* was reduced by (17 %) and the number of leaves by 27% under high water-deficit conditions. Similarly, the minimum reduction was noted in stem fresh biomass (1.7%) and root fresh weight (21.68%) for *C. erectus* under drought conditions. The root biomass production remained unchanged in *Eucalyptus camaldulensis* ($P = 0.079$) but biomass allocation was increased with an increase in soil water deficit ($p<0.001$). The present study revealed that *Conocarpus erectus* showed effective tolerance mechanisms under water stress; therefore, may be recommended for agroforestry under semi-arid regions.

Key words: Drought stress, Woody plants, Biomass production, Agroforestry and sustainable development.

Introduction

One of the major threats to forests and crop productivity is drought stress that prevails around the world (Allen *et al*., 2010). According to one of the documented studies, it has been observed that about 36% of the world's land receives only 50 mm to 150 mm annual precipitation and is categorized as arid to semi-arid lands (Eswaran *et al*., 2001). The remaining 64% of the land is facing severe water stress, particularly during the growing season. In the future, uncertainty prevails about water imbalance areas and time regarding prediction (Trenberth *et al*., 2014).

Prolong stress conditions negatively affect plant health and physiological mechanisms. The most damaging effect of water deficit on both natural and cultivated plants is the reduction in plant biomass productivity. In water stress conditions, leaf growth is more affected than in other parts of the plant. Similarly, plant cell enlargement and growth are important activities that are sensitive to water stress. Healthy leaf growth and development is necessary for healthy plant because leaves are food-producing factories for plant and are directly related to plant growth. As a result, the water shortage has severe negative impacts on overall plant growth and productivity; so, there is a crucial need to cope with it (Zlatev & Lidon, 2012). Against the devastating and injurious level of water stress, most of the tree species grow within a narrow hydraulic range limit (Choat *et al*., 2016). However, if the high temperature and aridity are present at the same pace, then these tree species have to face a long-term reduction in productivity and survival. As a result of global warming, climate models are predicting an increase in many drought spells during the growing seasons (Salinger, 2005).

According to census of 2017, the current population of Pakistan is 207.774 million. So, Pakistan is a thickly populated country with an average annual growth rate of 2.4% (Pakistan Bureau of Statistics, 2018). Pakistan has rare forest resources (4.2 mha). Further, population pressure results in the increasing demand for wood and its products (Rahim & Hasnain, 2010). In Pakistan, unfortunately, forest cover is very low about 0.001 per hectare per capita while about 1.00 ha per capita is in the rest of the world (Hosonuma *et al*., 2012). Anthropogenic activities and deforestation resulted in the gradual reduction of forest cover in Pakistan (Khan *et al*., 1990). Due to water shortage, new plantations in Pakistan is a big challenge. Water shortage affected about 7.8 mha of land in Pakistan but drought-resistant tree species can be planted in these affected areas (Irshad *et al*., 2011).

Agroforestry systems are the best solution to increase forest cover in Pakistan. But because of the water shortage drought, resistant tree species should be introduced for the plantation that results in the successful establishment of this system (Nawaz *et al*., 2018). In Pakistan, to fulfill the wood demand of the community, the agroforestry system is a hope which can reduce the pressure on the existing forest cover along with other benefits (Nawaz *et al*., 2016). To cope with wood deficiency, agroforestry practices are very important as they provide both tangible and intangible benefits including mitigation of microclimate, improvements of degraded soils, and climate services (Paul *et al*., 2017). To fulfill water deficiency, agroforestry systems play a vital role by growing drought-tolerant species (Bauer *et al*., 2013) because against drought stress these species adopt different mechanisms like the accumulation of salts in cells and osmotic adjustment. Morphological adaptations are also associated with different other mechanisms (Brock, 1994).

Throughout the world, *Eucalyptus camaldulensis* is considered one of the important tree species due to its high growth ratio, excellent pulp properties, and superior environmental adaptations but it is considered a high-water consuming tree (Stape *et al*., 2004). Being evergreen species, it absorbs more water at deeper depth compared to other tree species (Baker *et al*., 2002). Besides all these things it is a fact that *Eucalyptus camaldulensis* is growing successfully and adjusted itself to arid to semi-arid areas of Pakistan. Similarly, *Conocarpus erectus*, is considered native to riverine and coastal areas of East Africa and is now found in many countries in the world that fall in arid to semi-arid category like Pakistan and India (Barron & Razaqque, 2012). It can be propagated vegetatively and produces a large amount of biomass and seeds in bulk quantity. This tree is reported to grow well at high temperatures even at 40-45°C (Al-kandari *et al*., 2009). *Conocarpus erectus* adapted itself well in arid to semi-arid regions of Pakistan and growing extensively.

Although some studies have evaluated the drought tolerance mechanisms of many tree species, these mechanisms at the early stages of growth have not been addressed yet in the context of selecting these two tree species for drought-impaired agroforestry systems. The present study was planned to investigate the drought stress tolerance mechanisms of *Conocarpus erectus* and *Eucalyptus camaldulensis by* comparing them on the basis of biomass and morphological parameters. The findings of the present study will help to understand the tolerance potential of these species against water stress conditions and selecting suitable species to be used for agroforestry systems in arid and semi-arid regions of Pakistan.

Material and Method

Experimental site: The present study was conducted in the forest nursery (experimental area) Department of Forestry and Range Management, University of Agriculture Faisalabad, Pakistan during the period of February 2017 to May 2017.

Soil preparation and planting material: The sandy loam soil was collected from the experimental area. The air-dried soil was sieved and mixed thoroughly with peat and sand (1/1 ratio) before putting into plastic pots. Seeds of *E. camaldulensis* and *C. erectus* were obtained from the Punjab Forest Research Institute Gutwala, Faisalabad. Forty polythene bags were filled with soil which was already prepared for the experiment. Three seeds per bag were planted and irrigated daily to facilitate germination activity. After the germination, plants were thinned out and one healthy individual per bag was left to grow under the green net with ambient temperature conditions. During this time, the saplings were irrigated without any restrictions.

Stress application and treatments: After three months of successful vegetative growth, 30 plants per species were selected and shifted in prefilled plastic (peat and sand 1:1 ratio) pots having a weight of 7000 g. All the selected plants for the experiment were healthy, disease-free and same height. To measure the moisture content at field capacity, three cores of soil samples weighing 100 gm were taken randomly and oven-dried at 70°C for 48 hours. The oven-dried soil samples were weighed using electrical balance and the whole process was repeated till constant weight (Hussain *et al*., 2020). The moisture content in 100 gm soil at field capacity (19.05 ml) was used to calculate water contents in 7000 gm soil at 80% and 50% field capacity used for control and treatment respectively (Anjum *et al*., 2017). In other words (1285ml) water at 80% F.C and (803.5ml) water at 50% F.C was calculated for control and treatment respectively for 7000 g soil used in the pots. Five plants per species were randomly selected and harvested to obtain growth data and biomass at the initial stage. The remaining 20 plants (10/species) were randomly assigned for control (80% F.C) and treatment (50% F.C). Irrigation water was supplied to each individual in control and treatment, watered back daily to the respective reference weight throughout the experiment. The experiment was completed in three months (Feb-May) 2017.

Experimental conditions: A greenhouse was prepared specially for conducting this experiment. A plastic sheet was used for covering the roof of the greenhouse. The size of the tunnel was 15.5 feet wide and 20.5 feet long. The sides of the greenhouse were kept open on per need basis. The green net was used to save plants from intensive sunlight. This experiment was conducted under an ambient temperature of 30°C. The pots were strictly protected from rainwater, but there was no restriction for air. These conditions were maintained throughout the experiment.

Growth data: Plant height and stem diameter were taken regularly with an interval of 10 days throughout the experiment. Plant height was measured with the help of measuring tape and diameter with a digital Vernier caliper (Hussain *et al*., 2021). Diameter reading was taken from two sides of the stem at the same point and their mean was calculated. The number of leaves was count once a week throughout the experiment.

Biomass production: After 90 days of the experiment, the plants were harvested to obtain biomass. All the individuals from control and treatments were harvested and divided into three plant organs; Leaves, stem, and roots. Each plant organ was tagged properly and fresh weight was recorded using (Electronic Scale JJ3000B, Columbus, OH). Similarly, each plant organ was oven-dried at (70°C) for 48 hours till constant weight to measure the dry weight of each plant organ and total dry biomass. After measuring the dry weight of each plant organ, the water contents W.C of each plant organ were calculated by using the following formula;

$$\text{Moisture contents} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Biomass allocation and root shot ratio for each plant organ of both species were further calculated by using dry biomass. The formula is, biomass allocation = (dry biomass of plant organ/ total dry biomass) × 100 and root shot ratio (R:S ratio) was calculated as a ratio between Root / (leaves +stem) biomass.

Results

Effect of water deficit soil on growth parameters: The present study result showed that plant height and number of leaves were significantly reduced under water deficit conditions in both species. In *E. camaldulensis*, plant height was observed as (76.9 cm) in control and (53.2 cm) in stress conditions. Whereas in *C. erectus* the plant height was noted (42.52 cm) and (35.0 cm) under control and stress conditions as shown in (Fig. 1). Similarly, a minimum number of leaves were observed in *E. camaldulensis* (36.4) compared to *C. erectus* (68.4) under water deficit conditions clearly shown in (Fig. 2). However, no significant changes were observed for stem diameter in both species. In *E. camaldulensis* the stem diameter was recorded (4.21 mm) and (3.3 mm) under well-watered and deficit conditions respectively as indicated in (Fig. 3). Whereas (4.90 mm) and (4.87mm) *C. erectus* under the same scenario. All of the statistical data for the above traits is presented in (Table 1).

Effect of moisture deficit soil on biomass production & allocation: The present study result showed that water deficit conditions reduced fresh biomass significantly in both species. The decrease in maximum fresh biomass for *E. camaldulensis* was observed in leaf biomass (25.29%) and the minimum was recorded in roots (19.81%). In the case of *C. erectus*, the maximum decline (35.86%) in the fresh biomass was recorded in leaf fresh biomass and the minimum (21.68%) was recorded in roots with respect to control conditions. Similar results were found for moisture contents. The minimum decrease in moisture content percentage under treatment for *E. camaldulensis* was found in roots (2.91%). Interestingly, in *C. erectus* the decline in root moisture contents under treatment was minimum (0.98%) compared to *E. camaldulensis*. Meanwhile, a significant decline in leaf and stem dry biomasses of both species was noticed. The root dry biomass of both species interestingly remained similar under treatments as a result root shoot ratio was increased systematically under treatments in both species compared to control conditions. The leaf and stem biomass allocation in both species was decreased under water stress conditions but a significant increase in root biomass allocation in both species was observed under treatment compared to control. The decrease in leaf dry biomass was recorded (39.04%) in *C. erectus* for treatment compared to control whereas in *E. camaldulensis* decrease in leaf dry biomass was observed (24.31 %) for treatment as compared to control as shown in (Fig. 4). Similarly, a decrease in stem dry biomass for *C. erectus* was recorded (8.42%) under treatment and (18.45%) was noted for *E. camaldulensis* as compared to control as indicated in (Fig. 5). Interestingly we observed a little decrease (1.31%) in root dry biomass for *E. camaldulensis* under water deficit conditions compared to control as shown in (Fig. 6). It shows that plants develop a phenomenon in which they increase the development of their root in stress conditions to absorb maximum available moisture contents from surrounding soils.

Table. 1. Showing the effect of water stress on morphological traits and biomass allocation to different parts of *Eucalyptus camaldulensis* and *Conocarpus erectus.*

	Conocarpus erectus		*Eucalyptus camaldulensis*				
Traits measured	**C**	**T**	**C**	**T**	**S-effect**	**T effect**	**S x T effect**
Plant height (cm)	42.5 (1.04)	35 (0.75)	76.9 (3.93)	53.3 (1.50)	P < 0.001	P < 0.001	P = 0.065
Stem diameter (mm)	4.9 (0.19)	4.87 (0.15)	4.21 (0.23)	3.3 (0.10)	P < 0.001	P = 0.595	P = 0.068
No of leaves	94.4 (6.5)	68.4 (5.02)	42.4 (1.50)	36.4 (6.13)	P < 0.001	P < 0.001	P = 0.689
Leaf fresh biomass (g)	24.62 (1.92)	15.79 (1.16)	8.42 (0.75)	6.29 (0.52)	P < 0.001	P < 0.001	P = 0.062
Stem fresh biomass (g)	7.04 (0.50)	7.92 (0.76)	5.75 (0.84)	4.6 (0.30)	P < 0.001	P = 0.083	P < 0.001
Root fresh biomass (g)	11.39 (0.68)	8.92 (0.73)	5.3 (0.64)	5.79 (0.99)	P < 0.001	P < 0.001	P < 0.001
Stem dry weight (g)	2.85 (0.16)	3.41 (0.29)	2.71 (0.25)	1.56 (0.21)	P = 0.065	P < 0.001	P < 0.001
Leaf dry weight (g)	7.35 (0.38)	4.48 (0.38)	2.92 (0.26)	2.51 (0.24)	P < 0.001	P < 0.001	P = 0.072
Root dry weight (g)	3.41 (0.11)	2.69 (0.27)	1.52 (0.29)	1.5 (0.28)	P < 0.001	P = 0.079	P = 0.059
R:S ratio	0.335 (0.007)	0.341 (0.024)	0.275 (0.052)	0.37 (0.06)	P = 0.098	P < 0.001	P = 0.064
Total biomass (g)	13.6 (0.63)	10.59 (0.84)	7.17 (0.47)	5.57 (0.61)	P < 0.001	P < 0.001	P < 0.001
Leaf W.C (%)	69.8 (1.33)	71.3 (0.85)	64.9 (2.6)	60.1 (1.7)	P < 0.001	P < 0.001	P < 0.001
Stem W.C (%)	59.3 (0.82)	56.6 (0.98)	51.6 (2.7)	66.7 (2.6)	P = 0.061	P < 0.001	P < 0.001
Root W.C (%)	69.8 (0.88)	69.7 (0.89)	74.5 (2.6)	74.3 (0.78)	P < 0.001	P = 0.653	P = 0.064
BA L (%)	53.9 (0.53)	42.3 (1.63)	40.9 (3.24)	45.8 (3.63)	P < 0.001	P < 0.001	P < 0.001
BA S (%)	20.9 (0.56)	32.2 (0.80)	37.9 (2.93)	27.7 (1.68)	P < 0.001	P < 0.001	P < 0.001
BA R (%)	25.1 (0.44)	25.3 (1.36)	21 (3.1)	26.4 (3.26)	P = 0.653	P < 0.001	P = 0.056

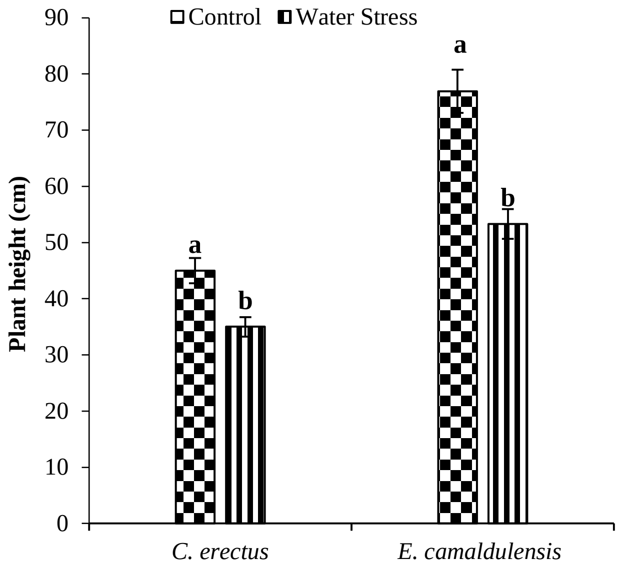

Fig. 1. Effect of drought stress on plant height.

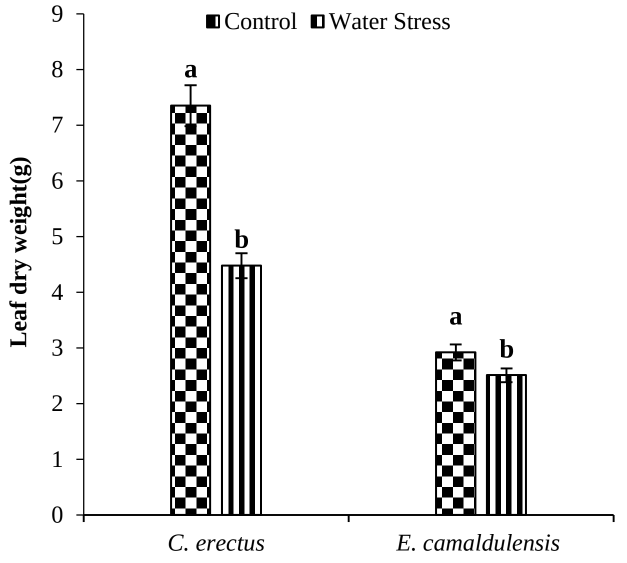

Fig. 4. Effect of drought stress on leaf dry weight.

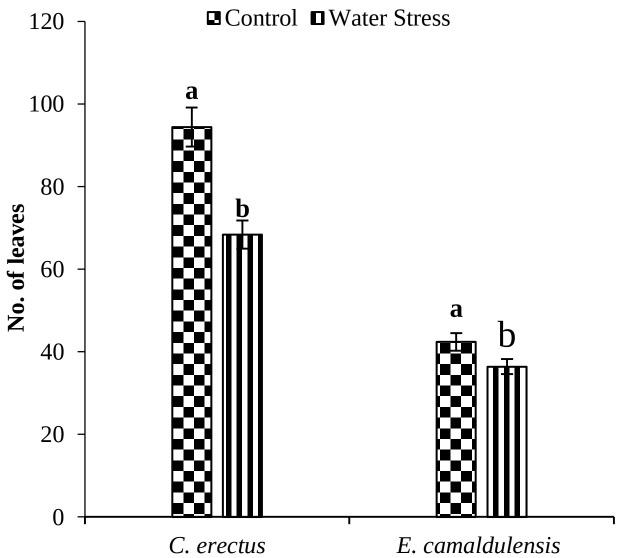

Fig. 2. Effect of drought stress on No. of leaves.

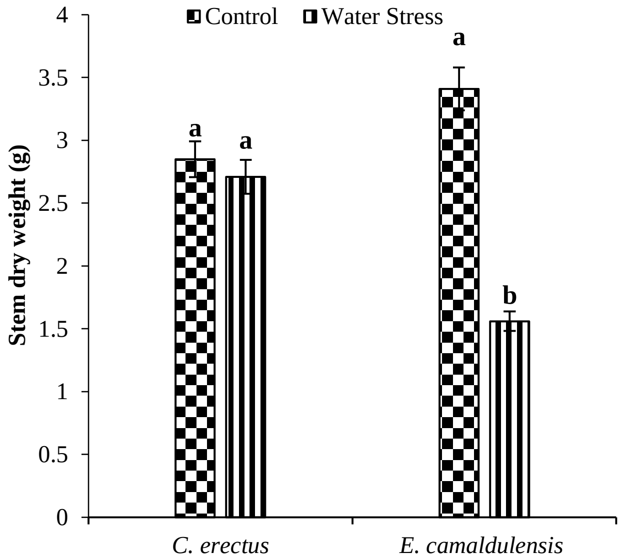

Fig. 5. Effect of drought stress on stem dry weight.

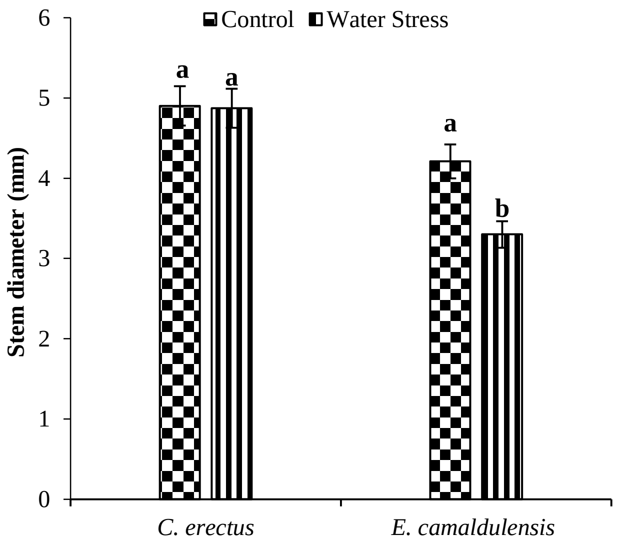

Fig. 3. Effect of drought stress on Stem diameter.

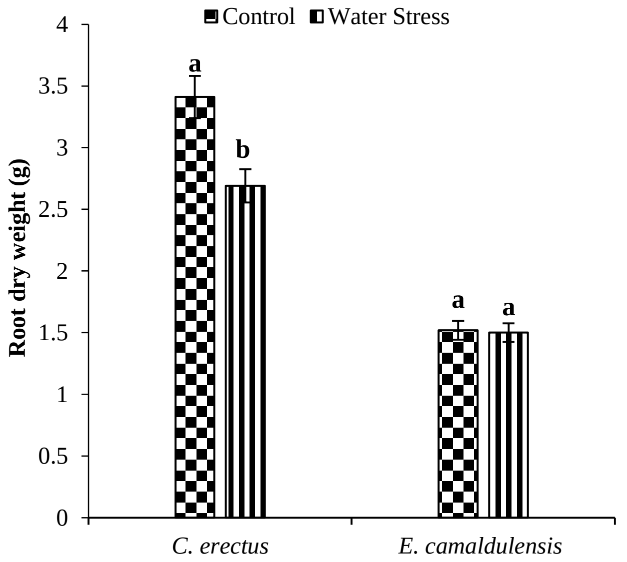

Fig. 6. Effect of drought stress on root dry weight.

Discussion

Among other abiotic stresses, water stress is known as an important limiting factor for plants at the initial stage of plant growth and development (Jaleel *et al*., 2008). The elongation and expansion process of plant growth is badly affected due to soil water deficit conditions as reported by (Shao *et al*., 2008). The findings of Possen *et al*., (2011) gave strength to our study results, whose results on birch (*Betula pendula*) and aspen (*Populus tremula* L.) showed that biomass of plant organs was reduced significantly under soil water deficit conditions. In another similar study, it was observed that when the biomass of aerial parts of a plant started decreasing under water stress conditions ultimately reducing the cell elongation process due to a reduction in the meristematic activity which is governed by cell turgor pressure (Muller *et al*., 2011). Our study results are in line with the recent findings of Hussain *et al*., (2020) who found that the water stress conditions significantly reduced the plant height and biomass of *E. camaldulensis* in the early stage of growth and development. Plant height and number of leaves and branches are sensitive to water stress, they are reduced when face stress. Present study results also correlated with the recent finding on *E. camaldulensis* that the number of leaves and height was decreased under water stress conditions (Hussain *et al*., 2020). The same results were also found in a study on other species which showed a major decrease in growth parameters under soil water deficit conditions (Rasheed & Delagrange 2016). Another study shows similar results as it is concluded that in water stress conditions plants show a serious decline in height, number of leaves and biomass production (Sirousmehr *et al*., 2014). Plants growing in less water conditions show a decline in total leaf area which results in a reduction in surface area for transpiration and it ultimately increases water use efficiency of the plant (Aguirrezabal *et al*., 2006). Similar results were observed by (Cheng & Cheng, 2015) who stated that with the increase of irrigation interval, the plant height was also significantly decreased. Our results showed contrast with (Haworth *et al*., 2017) who stated that significant differences were not observed in the mean height of *Arundo donax*.

There were no significant changes observed in root biomass in both species under water deficit conditions. Our results are evidence of a morphological adaptation in plant species that help them to survive in a scant water environment. This kind of adaptive strategy has been reported in a recent study on *Z. jujube* (Sabir *et al*., 2020). The adaptability of both species in terms of the extensive root system may be concerned with their ability to uptake and transport water in harsh environmental conditions. It has been previously reported that under low moisture conditions shoot development is more sensitive than root developments in plants (Raza *et al*., 2007). Present study results are in support of this hypothesis, by showing a significant increase in root biomass allocation percentage for both species under treatment effect. However, *C. erectus* performed better than *E. camaldulensis*. A higher increase in root biomass allocation percentage in *C. erectus* was observed as compared to *E. camaldulensis*. Thus, it can be concluded that in *C. erectus* the increased root allocation to root development under water deficit conditions ensures a sufficient supply of water for normal plant functioning.

Conclusions and Recommendations

In the present study, the overall % growth and morphology of both species *C. erectus* and *E. camaldulensis* were significantly affected by soil water deficit conditions. Both species reduced their number of leaves but *E. camaldulensis* performed better than *C. erectus* under water deficit conditions. Similarly, overall biomass production was significantly reduced under soil water deficit conditions in both species but *E. camaldulensis* reduce less biomass under low water availability as compared to *C. erectus*. There were no significant effects of low moisture contents on root biomass in species, resultantly root biomass allocation and root-shoot ratio increased. It can be concluded that due to the fast-growing and high environment adaptability nature *E. camaldulensis* show better response under water deficit conditions at the early growth stage. At the same time, the *C. erectus* high root growth may be considered as a long-term strategy of this species to counter water stress conditions. Pakistan is facing severe water shortages; therefore, it is recommended that less water consuming and drought-tolerant tree species such as *C. erectus* and *E. camaldulensis* should be recommended for arid to semi-arid climates. However, further research is needed to evaluate their physiological response under water deficit conditions.

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