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Drought stress responses in seedlings of three multipurpose agroforestry trees species of central India

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Abstract
The effect of moisture on seedling growth of *Albizia lebbeck* (L) Benth., *Leucaena leucocephala* Lam. and *Dalbergia sissoo* Roxb. was evaluated in central India during 2018. One month old seedlings raised in polyethylene bags containing about 3 kg of garden soil were subjected to three watering frequencies as irrigating daily (control), and withholding irrigation upto 14 days (intermediate stress) and 21 days (severe stress). Observations were made according to different watering frequencies on eight quantitative traits viz. plant height, root length, survival percentage, relative water content, excised leaf water loss, total chlorophyll content, proline content and total biomass. All the traits considered were significantly decreased as stress increased. Under optimum moisture condition *A. lebbeck* and *L. leucocephala* performed well than *D. sissoo.* At intermediate stress *D. sissoo* had maximum reduction of 81.36%, 54.33%, 43.62% and 20.0% for traits like total biomass, root length, total chlorophyll content and survival percent, respectively. In severe stress condition also the pattern was similar as *D. sissoo* had maximum reduction for total biomass (84.75%), root length (63.77%), total chlorophyll content (46.30%) and survival percent (40.0%). While *L. leucocephala* had higher reduction for plant height by 26.46% and 40.03% at intermediate stress and severe stress, respectively and it also had higher reduction for total biomass (84.92%) at severe stress. In contrast, *A. lebbeck* had minimum reduction for the traits like total biomass (60.87%), root length (25.94%), survival percent (no reduction), total chlorophyll content (17.38%) and plant height (17.37%) at intermediate stress. Also the reduction pattern was similar at severe stress for total biomass (63.77%), root length (38.03%), survival percent (no reduction), plant height (32.79%) and total chlorophyll content (27.02%). In both stress levels, *A. lebbeck* had higher relative water content, proline accumulation and lower excised leaf water loss than *L. leucocephala*> *D. sissoo*. Based on physiological and biochemical trait expressions and minimum percent reduction for putative traits the species *A. lebbeck* indicated better drought resistance as compared with *L. leucocephala* > *D. sissoo*. Hence, *A. lebbeck* can be a choice of species for better agroforestry programmes in drought thriving arid and semi-arid regions.

Keywords: Agroforestry, Drought response, Physiological traits, Seedlings, Tree species

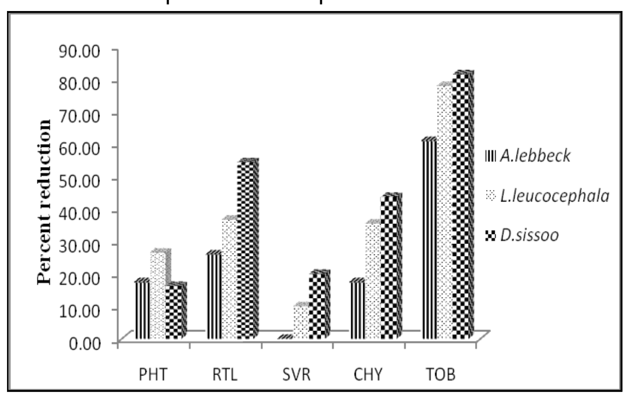
The emphasis on afforestation programme and increasing agroforestry cover in India have been a great impact on demand for production of various multipurpose tree seedlings as quality and healthy planting materials (Rao and Sing, 1985). With global climate change, drought is considered as the main ecological filter for seedling establishment of various multipurpose tree species. In recent years, the negative impacts of climate change on forest trees have intensified, such as heat and drought (Sturrock *et al.,* 2011; La Porta *et al.,* 2008). The most alarming outcome of climate change is the increased number of multipurpose trees seedlings dying off because of drought as it greatly affects the pattern of seed production, germination, survival and seedling development (Khurana and Singh, 2001). Drought also acts as a major limiting factor in agricultural production (Rajarajan and Ganesamurthy, 2014). Besides this, drought stress weakens tree seedlings makes more susceptible to insect and pathogens (McDowell *et al.,* 2008). The information on the physiological response of seedlings to environmental stress should be helpful for better understanding of seedling establishment in plantation to avoid large scale failures in different forestry programmes (Rao, 2005). Also the multipurpose trees production is the major aim of subsistence farmers with most of their farm land (Meena and Nagar, 2018). Hence, it is more important that understanding of what extent do we really know about the seedling responses in respect with drought stress are? Despite some studies showing that morphological responses of tree seedlings to drou-

-ght stress, very limited work has been undertaken to understand the physiological and biochemical responses of multipurpose tree species at seedling level in respect with drought tolerance.

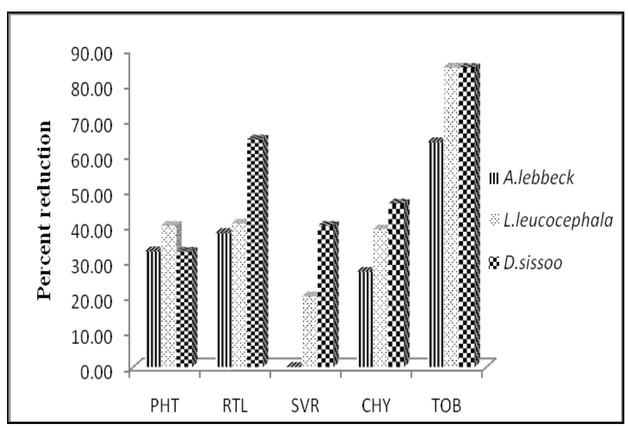
With this context, the seedling growth were investigated under drought stress condition to compare different drought resistance strategy through physiological and biochemical responses and identification of suitable multipurpose tree species for arid and semi-arid environments for afforestation programme and increasing the agroforestry cover.

Three multipurpose tree species considered in this study were; *Albizia lebbeck* (L) Benth., *Leucaena leucocephala* Lam. and *Dalbergia sissoo* Roxb. The experiment was conducted at Tree Improvement Nursery, ICAR-Central Agroforestry Research Institute, Jhansi, India during summer, 2018. One month old three tree species seedlings were raised by sowing seeds in poly bags containing about 3 kg of garden soil. The established seedlings of these species were subjected to regular irrigation served as control, withholding irrigation up to 14 days and 28 days served as intermediate and severe drought stress, respectively. For each species and treatment, 15 bags with one seedling in each were maintained. Observations were taken on seedlings at different intervals as control, intermediate and severe stress condition. The plant height (PHT) measured from the ground level to the tip of the plant on the main stem in centimeters at different intervals. The poly bag was shirked and the roots were obtained for measurement of root length (RTL). Survival percentage (SVR) at the end of stress period as calculated by counting the number of plants of each species that have survived, divide it by the number of plants originally planted of that species and multiply by hundred. Relative water content (RWC) was calculated as (%) using the formula suggested by Barrs and Weatherly (1962). Excised leaf water loss (ELWL) was determined as after removing a leaf of the plant and measuring the decline in fresh weight over a time (McCaig and Romagosa, 1989). The total chlorophyll content (CHY) was estimated as (μg g^{-1} FM) by the method suggested by Lichtenthaler and Buschmann (2001). Quantification of proline (PRL) was carried out according to Bates *et al.* (1973) and expressed as μmol/g. The total dry biomass (TOB) (dried at 60 ± 2 °C) of leaf, stem and roots was estimated by harvesting three seedlings from each treatment at different intervals. Statistical computations were performed with XLSTAT 2017: Addinsoft, Paris, France (2018).

The analysis of variance was significant (P<0.01) for all the eight quantitative traits considered for three multipurpose agroforestry tree species. The PHT was maximum under control (no stress) in all the species (Table 1). In intermediate stress condition PHT (cm) was higher in *D. sissoo* (20.39), followed by *L. leucocephala* (19.51) and *A. lebbeck* (18.60). At severe stress condition *D. sissoo* (16.68) had maximum PHT, followed by *L. leucocephala* (15.91) and *A. lebbeck* (15.13) (Table 1). This growth reduction at seedling stage might be due to reduced endosperm weight of the planted seed as well as growth of the coleoptile, mesoctyl, radicle, shoot, and root of crop plants (Bayu *et al.,* 2005). Also percent reduction (Fig 1) under intermediate stress for PHT was found higher in *L. leucocephala* (26.46%), followed by *A. lebbeck* (17.37%) and *D. sissoo* (16.19%). Percent reduction under severe drought stress also had similar pattern as *L. leucocephala* (40.03%) > *A. lebbeck* (32.79%)> *D. sissoo* (32.68%). Similarly, Rao and Northup (2008) reported that the reduction in plant height after one year was more than 50% under very high stress in different tree species as compared to no stress condition.



A. Intermediate moisture stress



B. Severe moisture stress

Fig 1. Reduction of growth parameters in seedlings of different tree species under moisture stress gradient (A-B)

Table 1. Drought stress effect on growth related, physiological and biochemical parameters on three different agroforestry tree species

Traits	*A. lebbeck*			*L. leucocephala*			*D. sissoo*		
	No stress	**Intermediate**	**Severe**	**No stress**	**Intermediate**	**Severe**	**No stress**	**Intermediate**	**Severe**
PHT	22.51±0.6^{abc}	18.6±1.9^{bcd}	15.13±1.0^{d}	26.53±2.3^{a}	19.51±1.9^{bcd}	15.91±0.4^{cd}	24.33±1.4^{ab}	20.39±1.6^{abcd}	16.38±0.0^{cd}
RTL	21.51±0.6^{ab}	15.93±0.8^{bcd}	13.33±0.9^{de}	25.13±2.2^{a}	15.93±1.5^{bcd}	14.91±0.4^{cd}	19.51±1.1^{bc}	8.91±0.7^{e}	6.93±0.0^{f}
SVR	100±3.0^{a}	100±5.0^{a}	100±7.0^{a}	100±9.0^{a}	90±9.0^{a}	80±2.4^{ab}	100±6.0^{a}	80±6.4^{ab}	60±0.0^{b}
RWC	*	80.51±2.4^{a}	62.8±3.1^{bc}	*	71.38±6.4^{ab}	48.1±4.8^{cd}	*	60.33±3.6^{bc}	39.13±3.1^{d}
ELWL	*	34±1.0^{c}	42.9±2.1^{bc}	*	47±4.2^{bc}	59.4±5.9^{ab}	*	59±3.5^{ab}	69.3±5.5^{a}
CHY	283.1±8.4^{a}	233.91±11.7^{ab}	206.6±14.4^{b}	279.2±25.1^{a}	180.33±18.0^{b}	170.3±5.1^{bc}	193.5±11.6^{b}	109.1±0.0^{d}	103.9±8.3^{cd}
PRL	*	0.1±0.0^{cd}	0.24±0.0^{a}	*	0.09±0.0^{d}	0.17±0.0^{b}	*	0.04±0.0^{e}	0.12±0.0^{cd}
TOB	1.38±0.1^{a}	0.54±0.0^{b}	0.5±0.0^{b}	1.26±0.1^{a}	0.28±0.0^{c}	0.19±0.0^{c}	0.59±0.0^{b}	0.11±0.0^{c}	0.09±0.0^{c}

PHT: Plant height (cm); RTL: Root length (cm); SVR: Survival percentage (%); RWC: Relative water content (%); ELWL: Excised leaf water loss (%); CHY: Chlorophyll content (µg/g FM); PRL: Proline content ((µmol/g); TOB: Total biomass (g); Values are Mean±SE and different letters indicate significant differences (Duncan test, P<0.01); *Traits only observed for stress condition

The RTL was maximum under control (no stress) in all tree species considered (Table 1). Among the species under intermediate stress, *L. leucocephala* (15.93 cm) and *A. lebbeck* (15.93 cm) had higher RTL as compared with *D. sissoo* (8.91 cm). In severe stress condition, *L. leucocephala* (14.91 cm) had higher RTL, followed by *A.lebbeck* (13.33 cm) and *D. sissoo* (6.93 cm). The reduction of RTL (Fig 1) under intermediate stress condition was found higher in *D. sissoo* (54.33%) followed by *L. leucocephala* (36.61%) and *A. lebbeck* (25.94%). Similar trend was also observed in the case of severe stress (Fig 1) as *D. sissoo* (64.48%) had higher RTL reduction percent, followed by *L. leucocephala* (40.67%) and *A. lebbeck* (38.03%). In our study, drought stress significantly affected the RTL of three species considered. Similarly, Meier and Leuschner (2008) found that the drought caused shortening of fine roots and consequently reduced specific root length. In our study, *A.lebbeck* had higher root length in both stress condition as compared with other species, which might be due to higher water uptake capacity. In addition, the species had high potential of root length and absorptive root under drought stress which attributed to a greater ability to maintain high and relatively stable xylem water potential (ΨW) during drought stress condition (Comas *et al.,* 2013; Armas *et al.,* 2010). In contrast, species with shallow roots were only able to take advantage of available surface water (Torres *et al.,* 2002).

The SVR percentage was maximum under control (no stress) than the drought stressed in all three species considered (Table 1). However, in intermediate stress condition among the species, it was higher in *A.lebbeck* (100%), followed by *L. leucocephala* (90%) and *D. sissoo* (80%). The similar trend was also observed in severe stress condition as *A.lebbeck* (100%) had higher SVR percent, followed by *L. leucocephala* (80%) and *D. sissoo* (60%). McDowell (2011) suggested that survival capacity is the most critical parameter for predicting tree response to a changing climate. In our study, *A.lebbeck* had higher survival capacity under drought stress condition, which might be due to greater responses of physiological functions in respect with stress conditions. Further, McDowell *et al.* (2008) suggested that plant survival and mortality responses to drought depend on both hydraulic failure and carbon starvation arises from how the plant's xylem responds to dry soil conditions.

The RWC was estimated for intermediate and severe stress condition (Table 1). Among the species under intermediate stress *A. lebbeck* had higher RWC (80.51%), followed by *L. leucocephala* (71.38%) and *D. sissoo* (60.33%). In severe stress condition also, the pattern was similar as *A. lebbeck* had higher RWC (62.8%) followed by *L. leucocephala* (48.1%) and *D. sissoo* (39.13%). From this study, it could be well understood that as the intensity of stress increases the plant water status had been found greatly reduced. McCutchan and Shackel (1992) compared the relative sensitivity of plant and soil-based measures of water availability for prune stress. In our study, *A. lebbeck* had greater RWC in both stress conditions indicated that it maintains better tissue water content and transport of photosynthetic assimilates across the plant system as compared with other species (McDowell *et al.,* 2008).

The ELWL was estimated for intermediate and severe stress condition (Table 1). Among the species under intermediate stress *A.lebbeck* had lower ELWL (34%), followed by *L. leucocephala* (47%) and *D. sissoo* (59%). In severe stress condition also the pattern was similar as *A. lebbek* (42.9%) had lower ELWL followed by *L. leucocephala* (59.4%) and *D. sissoo* (69.3%). A.lebbek had less ELWL than other species; which indicated it conserved maximum water in the plant system as compared with other species and that could be the possible reason for its better growth performance. Similarly, a number of physiological traits like ELWL and RWC which conferred drought resistance to wheat were also identified by Malik (1995) earlier. Based on ELWL performance, seedlings could be selected as drought tolerant, since many workers suggested that ELWL as an early stage selection criteria for drought tolerance (Salim *et al.,* 1969; Clarke and McCaig, 1982; Malik, 1995).

The CHY (µg/g FM) was maximum under control (no stress) in the three species studied (Table 1). Among the species under intermediate stress *A. lebbeck* (233.91) had higher CHY, followed by *L. leucocephala* (180.33) and *D. sissoo* (109.1). In severe stress condition also the pattern was similar as *A. lebbeck* (206.6) had higher CHY than *L. leucocephala* (170.3) and *D. sissoo* (103.9). The reduction in CHY was found higher in *D. sissoo* (43.62%) followed by *L. leucocephala* (35.41%) and *A. lebbeck* (17.38%) under intermediate drought stress condition (Fig 1). In case of severe stress condition, the pattern was also similar as *D. sissoo* (46.30%) had higher percent reduction followed by *L. leucocephala* (39.00%) and *A. lebbeck* (27.02%) (Fig 1). Similarly drought stress significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content of three cultivars of chickpea (Mafakheri *et al.,* 2010). *A. lebbeck* had less reduction (27.02%) compared to other species indicated that its ability to tolerate drought stress through efficient physiological functions like avoiding photo-oxidation, chlorophyll degradation and impaired chlorophyll biosynthesis (Smirnoff, 1993). Those crops, which used to maintain higher chlorophyll content even under stress conditions, are usually considered as drought tolerant (AL-Hamdani and Barger, 2003).

The proline content (μmol/g) was estimated for intermediate and severe stress condition (Table 1). Under intermediate stress condition, *A. lebbeck* had higher proline content (0.1), followed by *L. leucocephala* (0.09) and *D. sissoo* (0.04). In severe stress condition also the pattern was similar as *A. lebbeck* had higher proline content (0.24) followed by *L. leucocephala* (0.17) and *D. sissoo* (0.12). From this study, it could be well understood that as the intensity of stress increases the proline accumulation also increased (Table 1). Proline and soluble sugars overproduction in plants are indeed biochemical responses to drought stress tolerance (Ahmed *et al.,* 2009). In our study, higher proline accumulation was found in *A.lebbeck* in response to drought stress tolerance when compared to other species. Further, this proline act as osmolytes to maintain water in the cytoplasm and prevent protein denaturation and cell membrane damage, and induce stability in the structure of enzymatic proteins, thereby preserving their activity (Hessini *et al.,* 2009).

The total dry biomass was maximum under control (no stress) in all the tree species (Table 1). Under intermediate stress, *A. lebbeck* (0.54 g) had higher total dry biomass, followed by *L. leucocephala* (0.28 g) and *D. sissoo* (0.11 g). In severe stress condition also the pattern was similar to intermediate stress, as *A. lebbeck* (0.50 g) had higher biomass followed by *L. leucocephala* (0.19 g) and *D. sissoo* (0.09 g). The reduction in total dry biomass was found higher in *D. sissoo* (81.36%) followed by *L. leucocephala* (77.78%) and *A. lebbeck* (60.87%) under intermediate drought stress condition (Fig 1). In severe stress condition the reduction pattern was like *L. leucocephala* (84.92%) > *D. sissoo* (84.75%) > *A. lebbeck* (63.77%) (Fig 1). The reduced total biomass under drought stress might be due to poor photosynthesis performance accompanied with decreased leaf water potential. In the present study, the values of reduction were very high, indicating that these species are more sensitive to water stress with respect to total biomass production at seedling stages. Reduction in total seedling biomass yield under water stress conditions was also reported earlier by Rao and Northup (2008) where seedling height and dry biomass decreased at very high stress in five tree species. Seedling height of *Quercus brantii* from Melasyah and Chegeni provenances was declined by 14% and 34%, respectively when subjected to severe drought stress (Jafarnia *et al.,* 2018).

In comparison with seedling responses of three multipurpose species to drought stress, *A. lebbeck* performed relatively better than other two species. As this species showed greater survival capacity, higher maintenance of plant water status, higher accumulation of osmolyte as proline, higher total chlorophyll content and higher root length system for more water uptake. Expression of overall these putative indicator traits under water stress by *A. lebbeck* seedlings have made this species well suited for semi-arid or rainfed environments and needs to be exploited for maintaining good tree density as well avoiding seedling mortality in afforestation as well as agroforestry programmes.

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