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SCREENING OF FIVE MULTIPURPOSE TREE SPECIES FOR WATERLOGGING TOLERANCE USING MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSE AS EFFECTIVE INDICATORS

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Worldwide waterlogging is a matter of great concern affecting 16% of cultivated area in the United States, agricultural lands of Russia, Pakistan, Bangladesh, China and India. This issue affects woody plants distribution as it not only restricts the seed germination but reproductive and vegetative growth, are also influenced that results in mortality of plants. It disturbs the soil physicochemical attributes that ultimately reduces the growth and physiological characteristics of various trees species. Therefore, an experiment was conducted in the research area of Department of Forestry and Range Management, University of Agriculture, Faisalabad to determine the waterlogging tolerance potential and growth of commercially important tree species. Different farm friendly tree species: *Eucalyptus camaldulensis* (Dehnh.), *Populus deltoides* (W. Bartram.), *Dalbergia sissoo* (Roxb.), *Salix tetrasperma* (Roxb.) and *Syzygium cumini* (L.) were selected and their growth (plant height, shoot length, shoot fresh and dry weight, root fresh and dry weight, stem diameter) and physiological attributes (photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll content) were measured under various waterlogging durations (24, 48, 72 and 96 hours) including control (normal irrigation). Results revealed that *E. camaldulensis* exhibited best waterlogging tolerance potential (plant height 83.7cm, shoot length 77.1cm, shoot fresh and dry weight 41.20g & 23.1g, root fresh and dry weight 18.32g & 13.70g, stem diameter 17.3cm, photosynthetic rate 14.62μmolm^{-2}s^{-1}, stomatal conductance 0.10molm^{-2}s^{-1} and chlorophyll content 397.6mgl^{-1} in control conditions) followed by *S. tetrasperma, P. deltoides, S. cumini* and *D. sissoo* for different waterlogging durations. It was concluded from the experiment that *E. camaldulensis* is the most tolerant and *D. sisso* is the most sensitive farm forestry tree species.
Keywords: Abiotic stresses, flooding, tree species, plant growth, tolerance

INTRODUCTION

Worldwide waterlogging is a matter of great concern affecting 16% soils in the United States, agricultural lands of Russia, Pakistan, Bangladesh China and India (Yaduvanshi *et al.*, 2012). Waterlogging is one of the major abiotic stresses that severely limits crop productivity and has become a major problem worldwide (Zhang *et al*., 2016; Jia *et al*., 2019). It is caused by multiple factors such as excessive rainfall, improper irrigation, unleveled land, poor drainage, and heavy soil texture. Flooding adversely affects the plant root function and consequently the shoot growth, with a potential to cause plant death (Arbona *et al*., 2009; Amador *et al*., 2012; Insausti and Gorjón, 2013). When soil is saturated with excessive amount of water and the root zone of the culture cannot be well aerated due to immoderate water content then this condition is known as waterlogging. In this disorder soil becomes unproductive and sterile because of extreme humidity and hypoxic conditions. In order to develop such conditions, it is not always important for the ground-water level to access crop root zone (Pucciariello *et al*., 2019). Sometimes, even capillary forces can supplement this process by joining ground water table with crop root zone, worsening the situation by creating anaerobic conditions (Rasheed *et al.*, 2018).

In Pakistan salinity and waterlogging has affected 25% of irrigated land. Temporary flooding or permanent waterlogging has affected an area of 6.17 mha and 1.16 mha of the land is subjected to the dual menance of salinity and waterlogging issues (Dollinger and Jose, 2018). Oxygen deficiency or hypoxia is a major component of damage under waterlogged conditions (Hodge *et al.*, 2009). In Pakistan 230 mha of agricultural lands are under irrigation and approximately 20% is saline soil which is equivalent to 45 million hectares. About 0.2-0.4% of total cultivated land has been estimated to be affected by waterlogging every year due to improper management practices (Arzani, 2008).

Waterlogging can be of different types such as riverine flood waterlogging, oceanic flood waterlogging, seasonal waterlogging, perennial waterlogging and sub soil waterlogging (LeProvost *et al*., 2012; Zheng *et al*., 2018). This may result in loss of root biomass, leaf necrosis, shedding of leaves, bark damage, eminent susceptibility to insect and fungal pathogens (Koch *et al*., 2004) ultimately leading to plant death (Kreuzwieser *et al*., 2004; Parolin and Wittmann, 2010). During a stressful event, the negative effects caused by flooding on a variety of plant attributes depend on the duration, environmental conditions and level of the flood (Glick, 2005). During floods, plants are exposed to different concentrations of oxygen in the soil also depending on the duration of water saturation, plants can experience hypoxia and anoxia etc. (Parent *et al*., 2008). Permanent flooding interrupts the metabolism, hydraulic conductivity, sweating, respiration and photosynthetic rate of plants (Dollinger and Jose, 2018), however, some species show tolerance to flooding. Tolerant tree species can retain water potential and photosynthetic activity after a period of acclimatization (Rasheed *et al*., 2018).

Forests protect and maintain the environment through ecological (biodiversity maintenance, bio-geo-chemical cycles of water, C and N), financial (natural resources such as timber and energy source) and social functions (Santos *et al*., 2018). Trees are providing environmental services as trend of social forestry is increasing in developed and developing nations (Ashraf and Arfan, 2005; Asif *et al*., 2020). Agroforestry involves interactions of tree components and other elements to create a diverse sustainable system of production (Mosquera-Losada *et al*., 2009). Agroforestry promotes the integration of woody perennials between crops or livestock in the same land management unit, with the purpose of income increasing by the use of multipurpose economically viable trees (Sharma *et al*., 2016). Agroforestry products improve physicochemical properties of soil which opens up a variety of opportunities for crops (Sairm *et al*., 2008; Yousaf *et al*., 2020). Trees can help to ameliorate waterlogged soils by different mechanisms (Ferry *et al*., 2010). Uptake of oxygen is essential for mitochondrial activity continuation; this permits loss of radial oxygen (ROL) from surface of root cells which helps in rhizosphere oxidation (Rasheed *et al*., 2018). For the damaged primary root system, the adventitious roots perform important functions such as incorporation of moisture and nutrients as a substitute (Horchani *et al*., 2008).

Agroforestry can enrich organic carbon, improve nutrient supply and productivity by improving soil microbial dynamics (Dollinger and Jose, 2018). Farm forest contains plants with a high rate of sweat that not only lowers water levels, but can also provide regular yields thus can be more useful than individual forests (Dagar *et al*., 2016). Mutual interaction of trees and agricultural crops help in reduction of erosion, improvement of soil quality and biodiversity as well (Pavlidis and Tsihrintzis, 2018; Pucciariello *et al*., 2019). Farm forestry can be part of the response to climate change in three ways: by adapting to increasing risks and uncertainties, by facilitating energy conversion (in sequestering and storing carbon), and by restoring the earth's multifunctionality to maintain the possession of existing resources (Van Noordwijk, 2018). Various agroforestry systems and practices including agrisiliviculture, silvipastoral, agri-horticulture, horti-silvi-pastoral, agri-horti-silviculture, and agri-silvi-pastoral have been adopted in different regions of the world to combat climate change, desertification and socio-economic challenges. It grows on variety of soils from loam to sands etc. (Ramya *et al*., 2012; Bhattacharya *et al.,* 2014). Different tree species are being used on wide scale in tropical and temperate agroforestry systems as they have great potential to prevent soil erosion by maintaining ecological balance. Trees and crops combinations enhance soil productivity, crop yield, retain more water which ultimately provide food, fuelwood, timber and social benefits to small scale farmers having marginal lands (Khan, 2014).

E. camaldulensis, P. deltoides, D. sissoo, S. tetrasperma and *S. cuminii* are multipurpose and economically important tree species commonly grown in various agroforestry systems. Keeping in view the menace of waterlogging and importance of biological approach to reclaim this issue this study was enacted to assess the waterlogging tolerance potential of mentioned species at sapling stage in term of morphological and physiological response.

MATERIALS AND METHODS

Planting material and Description of Study Site**:** Saplings of selected tree species; *E. camaidulensis, P. deltoides, S. cuminii, D. sissoo* and *S. tetrasperma* were collected from nursery of the Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan. Plants were transplanted in the earthen pots from polythene bags in the experimental field of Forestry Department (31°25'7.37"N latitude and at 73° 4'44.79"E longitude). After few weeks, plants that were similar in height and vigor were selected for the trial. After transferring to pots plants were subjected to five different treatments on the basis of flooding T_1 = Control, T_2 = 24 hours, T_3 = 48 hours, T_4 = 72 hours and T_5 = 96 hours. These conditions were created artificially by maintaining irrigation durations.

Plants harvesting and measurement of biomass**:** At the termination of experiment plants were harvested. Plant height/shoot length was measured by using a measuring tape right from tip of the shoot to the collar of plant. Numbers of branches produced by each plant were assessed just before harvesting the plants. Plant diameter was measured with the help of a digital calliper at the termination of experiment. Measuring tape was used to measure root length from tip of root to the collar of plant after the removal of soil particles. Shoots and roots of the harvested plants were put into paper bags and sun dried. Then these paper bags containing shoots and roots were oven dried to a constant weight (Yousaf *et al.,* 2020).

Gas exchanges parameters: Plant gas exchange parameters *i.e.* photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll contents were measured before harvesting. The physiological parameters were measured by using IRGA, LCA-4, Analytical Development Company, Hoddesdon, England. (Bashir *et al*., 2018; Asif *et al*., 2020).

Statistical analysis: Experiment was laid out according to Randomized Complete Block Design with factorial arrangements. One pot per plant was considered as an experimental unit. Means were compared by using Tukey's test. Data were statistically analyzed by using SPSS software.

RESULTS

***Morphological parameters*:** Different morphological parameters of mentioned species against different flooding durations are presented in Fig. 1. Comparison of means of recorded morphological attributes revealed significant decline with the increase in flooding durations for all tree species ($p<0.05$). Number of leaves and number of branches

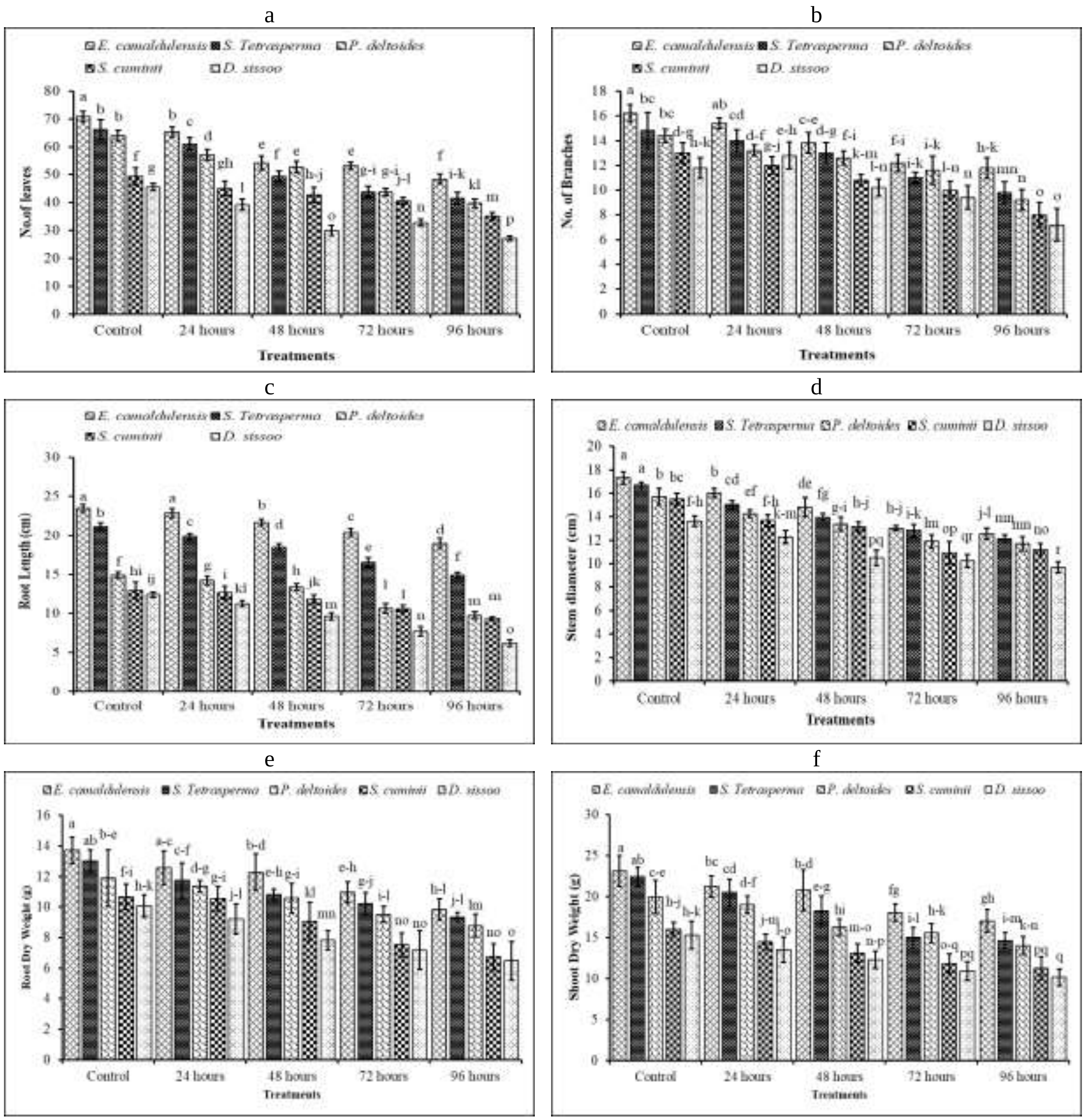

Figure 1. Effect of various Waterlogging durations on the (a) number of leaves, (b) number of branches, (c) Root length, (d) stem diameter, (e) Root dry weight and (f) shoot dry weight of selected agro forestry tree species at sapling stage

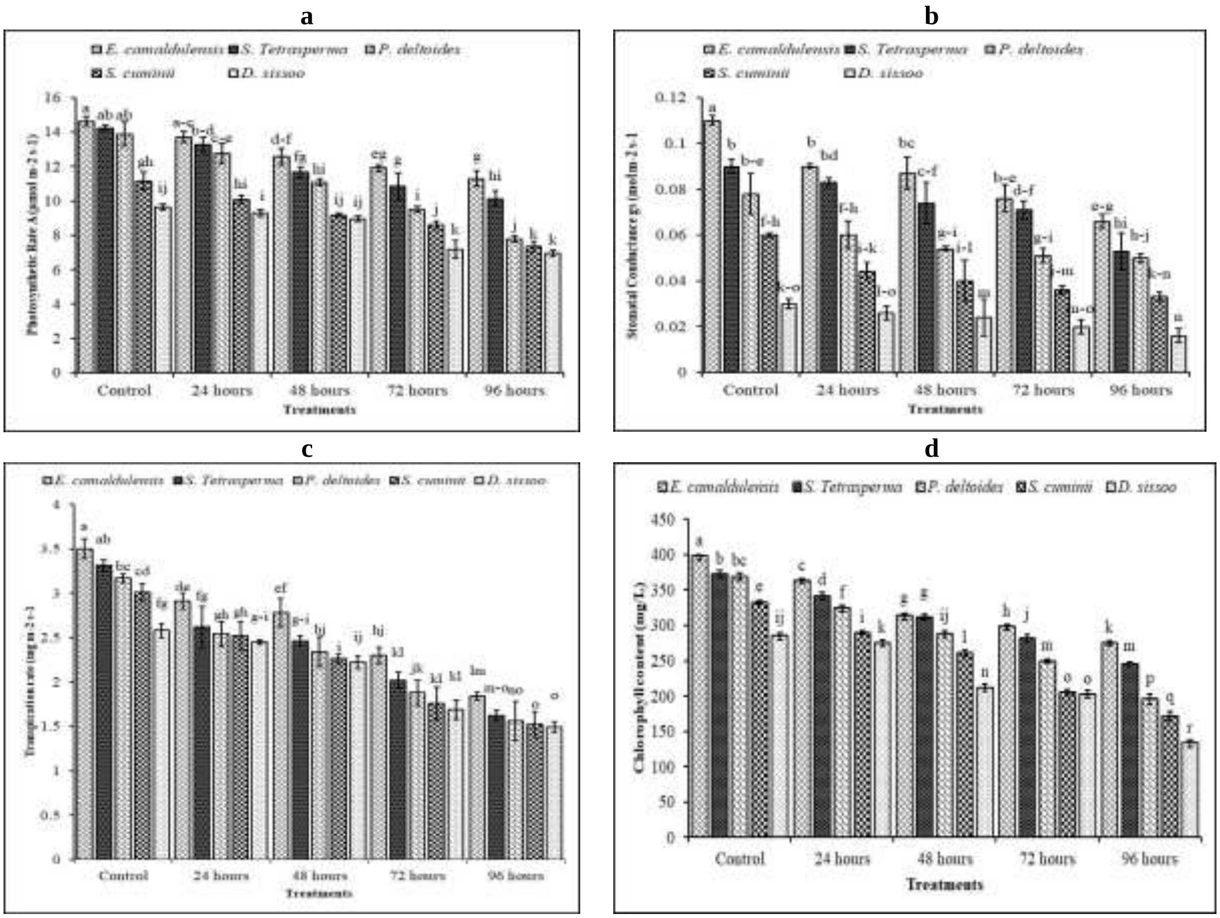

Figure 2. Effect of various waterlogging durations on (a) Photosynthetic rate, (b) Stomatal conductance, (c) Transpiration rate and (d) Chlorophyll contents of selected agro forestry tree species at sapling stage. Values are means with letters reported significant differences (*p*< 0.05).

of all the tree species at different waterlogging durations was found to be in range from 27.2-70.8 (Fig. 1a), 7.2– 6.2 (Fig. 1b) respectively. Root length of all the tree species at different waterlogging durations observed to be in range from 6.16–23.5cm (Fig 1c), shoot length also behaved likewise. Stem diameter of all the tree species in different treatments was measured in range from 9.68–17.3cm (Fig. 1d). Root dry weight and shoot dry weight of all the tree species at different waterlogging durations were found in range from 6.48–13.70 (Fig 1e), 10.14–23.1g (Fig 1f) respectively. Results revealed the rank of tree species regarding waterlogging tolerance potential as *E. camaldulensis* > *S. tetraasperma* > *P. deltoides* > *S. cuminii* > *D. sisso*, while for the waterlogging durations was control > 24 hours > 48 hours > 72 hours > 96 hours.

Physiological parameters**:** Fig. 2 depicts the results of different physiological parameters and chlorophyll contents for mentioned species against different flooding durations. Photosynthetic rate of all the tree species at different waterlogging durations was found to be in range from 6.96–14.62µmol m^{-2} s^{-1} (Fig 2a). Stomatal conductance of all the tree species at different waterlogging durations was observed to be in range from 0.016–0.10mol m^{-2} s^{-1} (Fig. 2b). Transpiration rate of all the tree species at different waterlogging durations was noticed in range from 1.49–3.50mol m^{-2} s^{-1} (Fig. 2c). Chlorophyll content of all the tree species at different waterlogging durations was found to be in range from 133–397.6mg/L (Fig. 2d). Results revealed the rank of tree species regarding waterlogging tolerance potential as *E. camaldulensis* > *S. tetrasperma* > *P. deltoides* > *S. cuminii* > *D. sisso*, while for the waterlogging durations was control > 24 hours > 48 hours > 72 hours > 96 hours.

DISCUSSION

It is obvious from the findings of current study that various waterlogging durations significantly affected physiological, morphological, and growth attributes of the various agro forestry tree species. Different tree species behaved differently for the studied attributes under waterlogging stress. *E. Camaldulensis* was the top ranked species regarding waterlogging tolerance potential followed by *S. tetraasperma*, *P. Deltoides*, *S. cuminii* and *D. sisso* in different treatments. Waterlogging affects negatively plant development and growth, due to poor oxygen level in root zone (Horchani *et al.,* 2009), which normally slower down the photosynthetic activity, closure of stomata and other biochemical attributes (Ashraf *et al.*, 2011).

In terms of morphological parameters like root and shoot length, number of leaves, diameter of stem, plant height, shoot and root weight computed greater in normal soils as compared to waterlogged soil for all tree species. This study is in accordance with previous studies (Ashraf and Arfan, 2005, Horchani *et al.*, 2009). The results of present research are similar to those explained by Yordanova *et al.* (2005). This was may be due to the poor supply of oxygen to the roots of said plants (Horchani *et al.,* 2009). The researchers investigated that plants didn't perform well under waterlogging stress as stunted growth was observed in most of the cases. In present study, *E. camaldulensis* had greater values for photosynthetic rate (11.3-14.62µmol m^{-2} s^{-1}), stomatal conductance (0.066-0.11mol m^{-2} s^{-1}), transpiration rate (1.84-3.5mg m^{-2} s^{-1}) and chlorophyll contents (397.6mgl^{-1}) for all treatments when compared with other tree species. Similar results of different species were also observed by Sairam *et al.* (2008). Yordanova and Popova (2001) observed that the stress of waterlogging limits the activity of ribulose bisphosphate carboxylase (RuBPC) and glycollate oxidase in barley plants that ultimately reduced the chlorophyll content of crop plants or trees.

The first indication of waterlogging stress is the reduction in stomatal conductance (Horchani *et al.,* 2008). Similarly, Ashraf *et al.* (2011) noted a decline in net photosynthesis in cotton. Ashraf *et al.* (2011) stated that reduction in leaf area, chlorophyll content and leaf senescence are those factors which are responsible for decreased photosynthetic rates under waterlogging conditions.

The roots and shoots of plants not exposed to flooding were larger than those exposed to uninterrupted flooding. The growth of the seedlings is associated with an increased physiological activity; therefore, plants with well-developed roots during early development could be more competitive (Castro *et al*., 2008). The results indicate that waterlogging has negatively affected the growth of trees species. It could be due to the lack of essential nutrients. Water extraction causes a deficiency of vital nutrients such as nitrogen, phosphorus, potassium, magnesium and calcium. Thus, the deficiency of essential nutrients impedes the overall growth of tree species, resulting in poor plant growth. It is notable that a metabolic acclimatization occurs in plants under short-term waterlogging or at the beginning of waterlogging, involving the production of anaerobic stress proteins that help them to tolerate hypoxia (Irfan *et al.,* 2010). Long-term waterlogging, however, results in oxidative stress (Sairam *et al.,* 2008), metabolic imbalance which negatively affects the growth of plant structures and cellular processes during the maturation process of seed and seedling's physiological efficiency. Similarly, Koch *et al.* (2004) reported that waterlogging increases organic acid production, which may inhibit plant growth, tillering, and absorption of nutrients at higher concentrations. Waterlogging during the vegetative growth phase has a negative impact on plant growth and potential for seed yield, similarly Cho and Yamakawa (2006) reported that the duration of waterlogging is also negatively associated with plant productivity which are in accordance with the findings of current study.

Conclusion**:** It was concluded that waterlogging has a negative effect on the morphological and physiological attributes of the various farm tree. Moreover *E. camaldulensis* was found to be highly tolerant against waterlogging followed by *S. tetrasperma, P. deltoids* and *S. cumini* whereas *D. sissoo* was found to be least effective. Nonetheless, the response to waterlogging may be genotype-dependent; therefore, additional studies will include different tree species of the same family to better understand the effect of waterlogging on plant productivity.

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