



## The foliar application of salicylic acid improves the growth, physiological and biochemical attributes in three forest tree species under drought stress



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### Abstract

Pakistan's climate is arid to semi-arid and in a climate change scenario where rainfall patterns are expected to be erratic with longer droughts, the survival and growth potential of important forest species is likely to decrease significantly. Therefore, the effect of water stress and the salicylic acid application on the morphological, physiological and behavioural adaptation was tested in three selected tree species *Populus deltoides*, *Dalbergia sissoo* and *Syzygium cumini* in this study. Plants were collected from the UAF forest nursery, and 60 seedlings of uniform size (20 for each species) were selected and randomly assigned to the three treatments (20 under control, 20 under water stress and 20 under drought and salicylic acid). Data on growth, biomass production, biomass partitioning, and physiological and biochemical attributes were collected during the experiment. The results showed that the growth showed a significant effect on the species, as well as the treatment effect, as water stress negatively affected the plant growth. Interestingly, the dry root biomass remained insignificant across the three treatments. Likewise, the percentage allocation of leaf biomass and root biomass allocation remained similar between the three treatments. Therefore, it can be concluded that despite the large differences observed in all species most of the growth occurred in roots under water stress to improve their ability to absorb water.

### 1. Introduction

Drought The agro-food industry obtains raw materials from agriculture. Therefore, the country's goal was to push the agricultural sector forward by producing higher-yielding plants, especially legumes, to put the country on the path of progress, prosperity and wealth. Environmental stress either biotic or abiotic commonly distress plant development and plant growth. Among many devastating factors, drought is a serious environmental factor that restricts plant development and plant growth (Zhu, 2002; Zhu, 2016). Water shortage due to drought is one of the main environmental stressors for plants and is expected to increase under climate change (Chadwick *et al.*, 2016; Jaleel *et al.*, 2009). In Hawaii, climate-induced droughts will last longer with less and less rain overall (Chu *et al.*, 2010; Chen and Chu 2014).

Water, the most important element in life, has become a unique commodity for human production and agriculture. Limited water supply is a major phenomenon that hurts agricultural production around the world. Drought stress affects the normal functioning of organs and plant growth in many ways. This increases the same concentration outside the roots compared to the inner environment of the roots and causes reverse osmosis. As a result, the cell membrane draws water from the cell wall, which can lead to cell death.

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Drought stress hurts plant growth, as evidenced by the size of the leaves and the height of the plants, and the size of the plants as a whole. The apparent decrease in plant growth is caused by a decrease in the swelling or pressure of the fluid throughout the plant due to the water pressure at the cellular level which prevents the expansion and division of cells (Jalil *et al.*, 2009).

Water stress tends to separate interface with roots by absorbing water, creating space in soil, plant and air chain. As the plant continues to lose water due to transpiration, water is withdrawn from the root cells, which leads to the contraction of cell membranes, which leads to a decrease in the integrity of the cell membrane and the death of the living cell. Drought stress suppresses plant photosynthesis by closing the stoma and disrupting chlorophyll and the photosynthetic system. This disturbs the balance between the production of reactive oxygen species (ROS) and antioxidant defense, which leads to the accumulation of ROS, which leads to oxidative stress of proteins, membrane lipids and other cellular components.

It is widely believed that salicylic acid (SA) improves plant defenses against biotic stresses (Lee *et al.*, 2010; Verma *et al.*, 2016). It also plays an additional role in abiotic stresses such as cooling, heat, dehydration, salt, UV rays, and heavy metal stress. (Senaratna *et al.*, 2000; Janda *et al.*, 1999); (Hayata *et al.*, 2010; Chen *et al.*, (2012). In addition, salicylic acid is mainly produced by PAL in several popular varieties (Tsai *et al.*, 2006); (Yuan *et al.*, 2009). Previous literature reported that treatment with salicylic acid increases drought tolerance in some wheat varieties (Kang *et al.*, 2012). Upon drought, a five-fold increase in salicylic acid concentration was reported in the evergreen shrub *Phillyrea angustifolia* (Munné-Bosch and Peñuelas, 2003) and barley roots increased exponentially in the concentration of salicylic acid (Bandurska and Stroiński, 2005). Salicylic acid stimulates the production of reactive oxygen species in the extracellular space, and thus the reactive oxygen species diffuse to the guard cell groups (Mori *et al.*, 2001; Khokon *et al.*, 2011). However, the molecular mechanisms associated with the role of salicylic acid in plant drought resistance are not fully understood.

*Syzygium cumini* L. is an enormous evergreen organic fruit tree which is ordinarily developed in the fields of Pakistan. Jaman is the regular name given to this tree in Indo-Pakistan. (Noomrio and Dahot, 1996). It has a wide flexibility, all around developed in the tropical locales and thought to be local to Asia (Steward *et al.*, 1972). The organic product is accounted for to be a decent wellspring of minerals, nutrient C, sugars, phenolic mixes (Gallic corrosive, tannins, flavonoids, anthocyanins) and other antioxidant components (Prabhakaran *et al.*, 2011).

The Poplar (*Populus deltoides*) tree is a fascinating species in Pakistan and was presented in the nation during the late fifties (Sheik, 1988). Its wood is of acceptable quality with white shading, absence of smell, great functionality, even grained, light weight and protection from fragmenting. Its wood is utilized in the arrangement of pressing cases, matches, plywood, particle board, pulp and paper, ice cream sticks toothpicks, sports products, fake appendages, furniture, 'khas' of desert coolers, covering material, fuel and fodder (Siddiqui *et al.*, 1986; Sheik, 1993). Under the World Trade Organization (WTO) poplar wood has been suggested as packing material. After the usage of WTO, its wood request and market potential will improve manifolds. *Dalbergia Sissoo* is an essential colonizer on the new alluvial soils along the riverbanks. It is an enormous deciduous tree growing up to 8 feet in size and 100 feet in height. The pivot of the common yield is around 60 years however in flooded ranches of west Punjab; it is a lot shorter being around 20 years (Bakshi and Singh 1954). It has been generally utilized for afforestation in many pieces of the nation with the exception of the exceptionally hot, cold and wet parcels. The wood is additionally exceptionally valued for warming and cooking because of its high calorific worth. The present experiment was to investigate the effect of foliar application of SA under drought stress tolerance in three forest saplings. Different growth, morphological, physiological and biochemical parameters were investigated under drought stress and after foliar application of SA to assess the tolerance status of plant saplings.

## 2. Materials and Methods

### 2.1 Plant material and growth conditions

A pot experiment was conducted in a greenhouse at Forest Nursery, University of Agriculture Faisalabad, Punjab, Pakistan during the summer, of 2020. Young and healthy seedlings of more than two months of three forest species, i.e., (*Populus deltoides*, *Dalbergia sissoo* and *Syzygium cumini*) were obtained from University Nursery, Faisalabad. Seedlings were transplanted into pots filled with farmyard manure and sandy loam soil in a ratio of 3:1 proportion. Each plastic pot weighs 10 kg after filling. All plastic containers were filled with 14g per capita containing nitrogen, K<sub>2</sub>O, and P<sub>2</sub>O<sub>5</sub>, respectively. The average greenhouse temperature during the day and at night ranged between 18-33 °C during the experiment period, while the relative humidity (RH) was 88% in the morning and 49-83% in the afternoon.

### 2.2 Drought stress treatments

After transplanting the saplings into plastic pots all the plants were grown in the natural environment. Three drought stress levels were maintained throughout the experiment (control 80% medium stress 60% and high stress 40% field capacity). After 15 days of growth under normal conditions, they were subject to different levels of drought stress. A soil moisture meter was used daily to maintain reference soil moisture throughout the experiment.

20 pots were used for each species.

### 2.3 Salicylic acid

Salicylic acid was sprayed on the plants in drought stress conditions to check the difference between different treatments of the experiment. Sodium salicylate (Merck, Darmstadt, Germany) was used to prepare the salicylic acid (SA) solutions by dissolving 0.138 g of SA in 1 L of distilled water, respectively.

## 2.4 Growth Parameters

All the growth parameters were measured during the experiment, stem height (cm) was measured with the help of measuring tape at the collar point of the saplings, and the collar marked all the individuals with the permanent marker. Stem diameter (mm) was determined by using the vainer caliper two readings were marked at the diagonal ends. The number of leaves and branches was counted manually for every individual during the experiment.

## 2.5 Biomass production

At the end of the experiment, all the saplings were uprooted and divided into their parts leaf, stem and root and fresh weight was measured. Subsequently, all the samples (leaf, stem and root) were packed into paper bags and transferred into an oven (DGH-9202 SERIES electric drying oven) at 74°C for 48 hours till constant weight. Samples were removed from the convection oven and dry weight was measured using a weighing balance.

Total dry weight was measured leaf + stem + root dry weight = total dry weight.

## 2.6 Physiological parameter

Chlorophyll a, b and carotenoid contents were determined by the method demonstrated by (Arnon, 1949). The acetone (80%) extract of the sample was centrifuged at 1500 rpm for 10 minutes.

## 2.7 Biochemical analysis

### 2.7.1 Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)

The H<sub>2</sub>O<sub>2</sub> content was determined according to methods described by Velikova et al. (2000). After homogenizing the fresh leaves with trichloroacetic acid 1%(w/v) and centrifugation at 12000 × g (10min), the absorbance of the reaction solution (KI 1M+ potassium phosphate buffer 10 mM (pH 7.0) + supernatant) was recorded at 320 nm.

### 2.7.2 Antioxidants enzyme

The antioxidant enzyme activity such as catalase (CAT) was measured according to the method described by Bailly et al. (1996). The peroxidase (POD) activity was measured as a method demonstrated by Chance and Maehly (1995).

## 2.8 Statistical Analysis

All the data corresponding to growth attributes, biomass production, chlorophyll a, b, carotenoid contents, and antioxidant activity were analyzed using the two-way ANOVA for species effect (S-effect), treatments (T-effect) and their interaction (S × T effect). Significant differences between treatment means were compared concerning their control using Turkey's honestly (HSD). All means are presented with their standard error (±SE) and all tests were taken significantly at  $p < 0.05$ .

## 3. Results

### 3.1 Effect of drought stress on growth parameters and foliar application of SA

All the growth parameters such as plant height (cm), stem diameter (mm) and number of leaves were significantly decreased in all three species under drought stress treatment respectively (Table 1). The highest decrease was found in *Dalbergia sissoo* followed by *Syzygium cumini* and *Populus deltoids* young saplings under drought stress. In *Populus deltoids* 59.7%, 60% and 54.2% were decreased in plant height, stem diameter and number of leaves was found under drought stress treatment as compared to control. The foliar application of SA significantly improves the growth parameters in all the species respectively (Table.1)

Table 1. All the growth parameters, plant height (cm), stem diameter (mm), number of leaves under drought stress and foliar application of SA. Each value represents the mean (±SE) of species in different treatments. All tests were considered significant at  $p < 0.05$ .

| Species                 | Treatment      | Plant height (cm) | Stem diameter (mm) | No. of leaves |
|-------------------------|----------------|-------------------|--------------------|---------------|
| <i>Populus deltoids</i> | Control        | 18.4 ± 0.56 a     | 6.12 ± 0.05 a      | 78.2 ± 2.33 a |
|                         | Drought stress | 7.41 ± 0.45 c     | 2.42 ± 0.06 c      | 35.8 ± 1.67 c |
|                         | Drought + SA   | 12.6 ± 0.32 b     | 3.12 ± 0.11 b      | 49.6 ± 1.11 b |
| <i>Dalbergia sissoo</i> | Control        | 14.7 ± 0.23 a     | 3.39 ± 0.06 a      | 37.8 ± 2.12 a |
|                         | Drought stress | 5.32 ± 0.19 c     | 1.63 ± 0.12 c      | 21.4 ± 1.67 c |
|                         | Drought + SA   | 9.41 ± 0.18 b     | 2.72 ± 0.11 b      | 29.4 ± 1.32 b |
| <i>Syzygium cumini</i>  | Control        | 16.1 ± 0.33 a     | 3.92 ± 0.21 a      | 56.0 ± 1.45 a |
|                         | Drought stress | 6.71 ± 0.21 c     | 1.54 ± 0.19 c      | 28.1 ± 1.11 c |
|                         | Drought + SA   | 10.3 ± 0.11 b     | 2.67 ± 0.13 b      | 34.6 ± 0.97 b |

### 3.2 Effect of drought stress on dry biomass production and foliar application of SA.

The dry biomass production such as leaf, stem, root and total dry biomass significantly decreased under drought stress treatment respectively (Table 2). The lowest dry biomass was found in *Dalbergia sissoo* and the highest was found *Populus deltoids* young saplings. A significant difference was found between the drought stress, and control treatments respectively. In *Populus deltoids* saplings the leaf, stem, root and total dry biomass were decreased by (29.0%, 27.9%, 39.5% and 33.2%) under drought stress treatment as compared to control respectively (Table 2). Similarly, in *Syzygium cumini* young saplings leaf, stem, root and total dry biomass were reduced by (42.2%, 40.6%, 32.3% and 37.7%) under drought stress as compared to control. The foliar application of SA significantly improves the leaf, stem root and total dry biomass production in all the species under drought stress environment respectively. In *Dalbergia sissoo* young saplings the leaf, stem, root and total dry biomass increased by (11.6%, 24.0%, 35.7% and 22.7%) under foliar application if SA treatment as compared to drought stress treatment respectively (Table 2).

Table 2. All the growth parameters, leaf dry weight, stem dry weight, root dry weight and total dry weight, under drought stress and foliar application of SA. Each value represents the mean ( $\pm$  SE) of species in different treatments. All tests were considered significant at  $p < 0.05$ .

| Species                 | Treatment      | Leaf dry biomass<br>(g) | Stem dry biomass<br>(g) | Root dry biomass<br>(g) | Total dry biomass<br>(g) |
|-------------------------|----------------|-------------------------|-------------------------|-------------------------|--------------------------|
| <i>Populus deltoids</i> | Control        | 19.6 $\pm$ 0.23         | 8.66 $\pm$ 0.34         | 20.5 $\pm$ 1.11         | 48.7 $\pm$ 2.23          |
|                         | Drought stress | 13.9 $\pm$ 0.19         | 6.24 $\pm$ 0.54         | 12.4 $\pm$ 0.98         | 32.5 $\pm$ 2.11          |
|                         | Drought + SA   | 18.3 $\pm$ 0.21         | 7.45 $\pm$ 0.43         | 16.2 $\pm$ 0.42         | 41.9 $\pm$ 1.98          |
| <i>Dalbergia sissoo</i> | Control        | 13.4 $\pm$ 0.33         | 6.33 $\pm$ 0.27         | 13.4 $\pm$ 0.56         | 33.1 $\pm$ 1.45          |
|                         | Drought stress | 10.3 $\pm$ 0.28         | 3.78 $\pm$ 0.21         | 8.4 $\pm$ 0.34          | 22.4 $\pm$ 1.21          |
|                         | Drought + SA   | 11.5 $\pm$ 0.11         | 4.69 $\pm$ 0.18         | 11.4 $\pm$ 0.23         | 27.5 $\pm$ 1.11          |
| <i>Syzygium cumini</i>  | Control        | 16.5 $\pm$ 0.19         | 5.24 $\pm$ 0.61         | 16.7 $\pm$ 0.45         | 38.4 $\pm$ 1.21          |
|                         | Drought stress | 9.5 $\pm$ 0.11          | 3.11 $\pm$ 0.12         | 11.3 $\pm$ 0.22         | 23.9 $\pm$ 0.98          |
|                         | Drought + SA   | 12.4 $\pm$ 0.14         | 5.32 $\pm$ 0.43         | 13.6 $\pm$ 0.18         | 31.3 $\pm$ 1.09          |

### 3.3 Effect of drought stress on physiological parameters and foliar application of SA.

The total chlorophyll and carotenoid contents significantly decreased under drought stress treatment respectively (Figure 1). The highest decreased was found in *Populus deltoids* young saplings and lowest was found *Syzygium cumini* young saplings respectively. In *Dalbergia sissoo* the total chlorophyll and carotenoids contents were decreased by 23% and 27% under drought stress as compared to the control treatment. Similarly, in the *Populus deltoids* saplings total chlorophyll and carotenoid contents were decreased by 25.0 % and 22.2% under drought stress as compared to control treatment. The foliar application of SA significantly improves the total chlorophyll and carotenoid contents in all the species respectively.

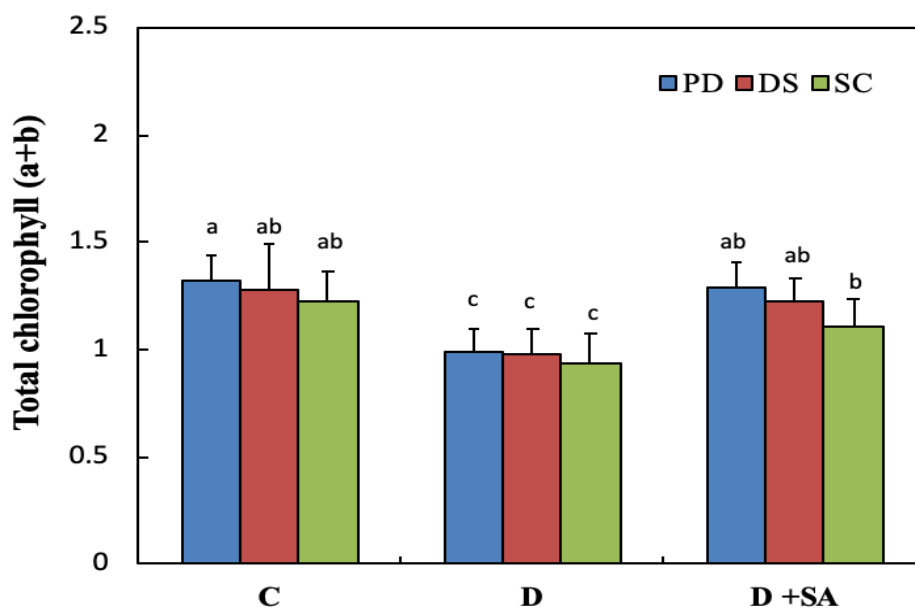


Figure 1: Effect of drought stress and foliar application of SA on total chl content

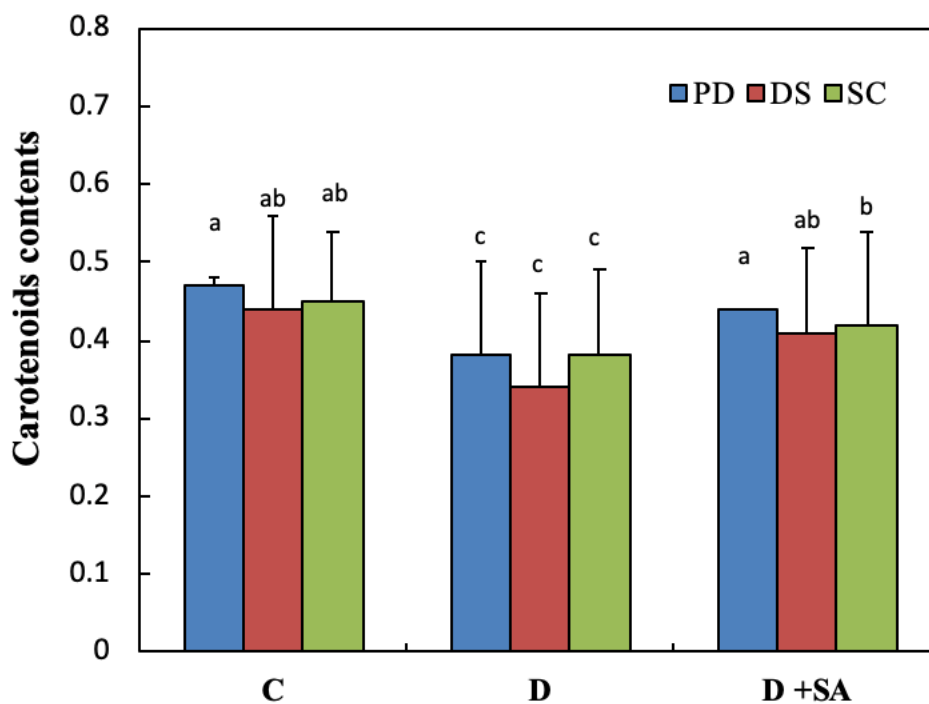


Figure 2: Effect of drought stress and foliar application of SA on carotenoid content

### 3.4 Effect of drought stress on antioxidants enzyme and foliar application of SA.

The antioxidant enzymes such as catalase (CAT) and peroxidase (POD) were increased under drought stress treatment in all the species. The highest value was found in *S. cumini* seedlings and the lowest value was observed in *Populus deltoides* young saplings. The foliar application of SA improves the catalase (CAT) and peroxidase (POD) under drought stress treatments in all the species respectively.

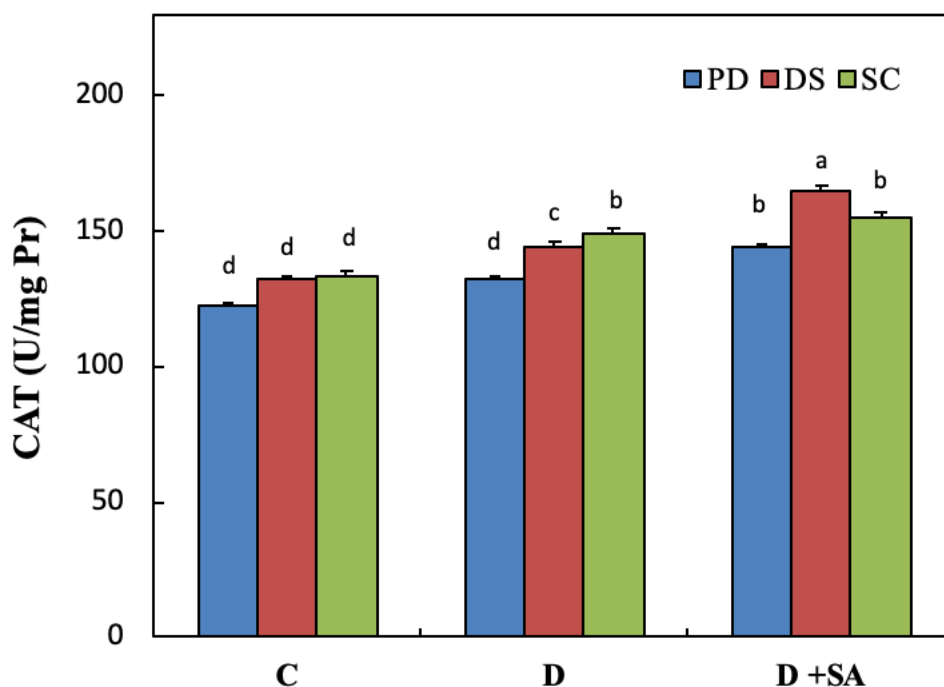


Figure 3: Effect of drought stress and foliar application of SA on CAT activity

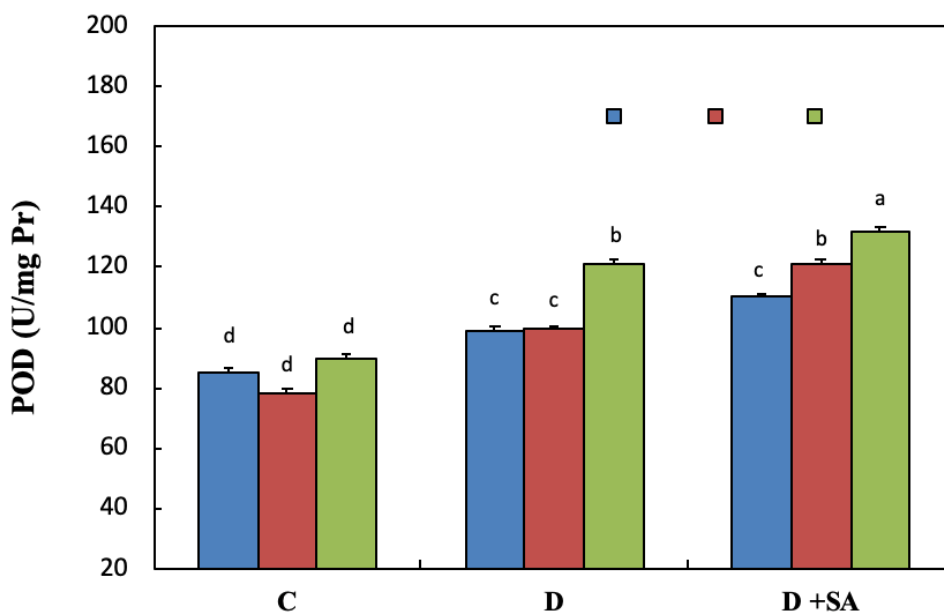


Figure 4: Effect of drought stress and foliar application of SA on POD activity

## 4. Discussion

### 4.1: Effect of drought stress on growth behavior

Water stress has a profound impact on plant growth and development, and our study corroborates this understanding. The significant reduction in growth observed under water stress conditions in *Populus deltoides*, *Dalbergia sissoo*, and *Syzygium cumini* suggests that these species are highly sensitive to water availability. This aligns with the findings of Hussain et al. (2019), who reported

that reduced water availability leads to decreased plant height, stem diameter, and leaf area in various tree species. In our experiment, the reduction in growth parameters under water stress can be attributed to the limited water uptake, leading to reduced photosynthetic activity and overall biomass production.

Despite the adverse effects of water stress on overall plant growth, an interesting observation was that the dry root biomass remained statistically insignificant across the treatments. This suggests that under water stress conditions, the selected tree species allocate more resources toward root development rather than shoot or leaf biomass. This root allocation strategy is a survival mechanism, enhancing the plant's ability to access deeper soil moisture and nutrients, which is crucial during prolonged drought periods (Chaves *et al.*, 2003). Our findings are consistent with the work of Joshi *et al.* (2018), who found that root biomass allocation increases under water-deficit conditions in many tree species as a drought adaptive strategy. The application of salicylic acid (SA) in the treatment regime revealed some interesting physiological adaptations. Salicylic acid is known for its role in enhancing plant stress tolerance by modulating various biochemical and physiological processes. In our study, the SA treatment resulted in better growth performance compared to the water-stressed plants without SA application. This suggests that SA helps mitigate the negative effects of water stress, possibly by enhancing antioxidant activity and osmotic adjustment, which are critical for maintaining cell turgor and function under drought conditions (Khan *et al.*, 2015).

The physiological measurements, including stomatal conductance, chlorophyll content, and relative water content (RWC), exhibited significant differences among the treatments. Water-stressed plants showed reduced stomatal conductance and chlorophyll content, indicating a decrease in photosynthetic efficiency. This is in line with the findings of Farooq *et al.* (2009), who reported that water stress leads to stomatal closure and reduced chlorophyll content to minimize water loss and oxidative damage. However, the SA-treated plants maintained higher RWC and chlorophyll content, suggesting that SA application helps in sustaining photosynthetic activity and water retention under drought conditions. The interaction effect of species and treatment was significant, indicating that different species respond differently to water stress and SA application. *Populus deltoides* showed the highest sensitivity to water stress with a notable reduction in growth and physiological attributes, whereas *Syzygium cumini* exhibited relatively better tolerance. This interspecies variation in drought response highlights the importance of species selection for reforestation and afforestation programs in arid and semi-arid regions. Species with better water stress tolerance and positive response to SA application, like *Syzygium cumini*, should be prioritized for planting in drought-prone areas (Ahmed *et al.*, 2018). The findings of this study have practical implications for forest management and conservation strategies in the face of climate change. Understanding the differential response of tree species to water stress and the mitigating role of SA can aid in developing targeted strategies for enhancing forest resilience. Future research should focus on long-term field studies to validate the efficacy of SA under natural conditions and explore its potential in other tree species. Additionally, molecular studies could provide insights into the underlying genetic mechanisms of drought tolerance and SA-induced stress mitigation.

## Conclusion

The study provides valuable insights into the adaptive mechanisms of these tree species under water stress conditions and the beneficial role of salicylic acid in enhancing drought tolerance. These findings can inform reforestation and afforestation strategies, suggesting that species with better drought tolerance and positive response to salicylic acid, such as *Syzygium cumini*, should be prioritized for planting in drought-prone areas. Further research, particularly long-term field studies and molecular investigations is recommended to deepen our understanding of these adaptive mechanisms and to validate the efficacy of salicylic acid under natural conditions.

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