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Auxins' influence on physiological processes adaption of SAL (*Shorea robusta*) seedling cultivated outside their natural habitat (AABR), Chhattisgarh

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Abstract
Exogenous treatment with various auxins (IAA, IBA, and NAA) was used to evaluate physiological parameters on the acclimation pattern of Sal seedlings at 15-day intervals for 10 days. Initially, young (three-leaf) seedlings were taken from the AABR natural forest in Chhattisgarh. These saplings were raised at the departmental nursery in polybags filled with nursery soil. All the auxins used during this experiment were at a concentration of 100 ppm. The seedlings treated with NAA 100 ppm hormone absorbed the most carbon, followed by IAA 100 ppm hormone treatments. IBA 100 ppm hormone treatment resulted in the highest rate of stomatal conductance (1.416 mol H_2O m-2 s-1) and IAA 100 ppm treatment resulted in the lowest rate (0.015 m H_2O m-2 s-1). The rate of evaporation in all auxin hormone treated seedlings was greater than in the control group of saplings. Saplings fed with NAA hormone exhibited the greatest evaporation rate (1.53 mol H_2O m-2 s-1) compared to the control set, followed by IAA and IBA hormone. In terms of sapling growth and establishment, IBA at 100 ppm surpassed other auxins.

Keywords: *Shorea robusta* (Sal), auxins hormone, CO_2 assimilation rate, stomatal conductance rate, adaptability

Introduction

Shorea robusta (Dipterocarpaceae) is a massive deciduous tree that can grow to 50 meters tall. *Shorea robusta* is the most common climax tree, forming both gregarious and pure stands. In India, Sal forests cover 10 million hectares (m ha). Climate and edaphic variables mostly govern distribution. Chhattisgarh has pure natural enclaves of Sal woods. Deforestation has drastically reduced the density of the Sal Forest. According to Chauhan *et al*. (2003) [2], the overall change in Sal Forest is 42.1% of the total wooded area. The main causes of diminishing Sal Forest cover include poor regeneration and delayed development in the early stages. Sal seed is stubborn, and it begins to germinate right before it detaches from the tree. It must promote the growth nature during its early establishing stage, because once established, the seedling can withstand all hard environmental factors. There were no purposeful efforts to promote *Shorea robusta* growth rate throughout the seedling stage.

Plant hormones are involved in a range of activities, including plant growth and development, division of cells, cell elongation and differentiation, and apical dominance of cells (Davies, 2013) [7]. The infusion of a single growth factor, which might be an auxin or a cytokinin, governs the early growth and development of tree seedlings (Su *et al*., 2011) [19]. The synthetic auxins IBA and NAA are well-known and have been proposed for diverse plant growth responses. In general, NAA and IBA hormone have been widely utilized to root shoots (Ludwig-mullar, 2000) [13]. Various doses of either IBA (9.84-29.52 mol L-1) or NAA (10.74-32.22 mol L-1) have been observed for shoot regeneration (Rubluo *et al*., 2002) [18], somatic embryogenesis (Nugent *et al*., 2001) [15], or callus induction (Centeno *et al*., 1999) [1] without the usage of cytokinin. External NAA (32.22 mol L-1) from long-term shoot cultures of Mammillaria senegalensis resulted in 100% shoot induction (Rubluo *et al*., 2002) [18]. Auxins, on the other hand, control some metabolic aspects inside plant tissues and their subsequent translation into unique growth chemicals (Centeno *et al*., 1999) [1].

Sal tree seedling require physiological stimulation during their early stage of development, and during this study, seedlings were given treatments of IAA, IBA, and NAA hormone, and the pattern of induction on CO_2 assimilation, stomatal conductance, and evaporation rate was analyzed, as well as its relationship in sapling establishment outside its natural habitat.

Materials and Methods

The juvenile sapling (three leaves stage) was obtained from the natural forest of AABR, Chhattisgarh and brought to the Department of Forestry, Guru Ghasidas Central University, Bilaspur, Chhattisgarh to grow under nursery conditions. Ten saplings in four groups were kept for each auxin treatment. The nursery with a Soil: Sand: Farmyard Manuare ratio of 1:3 was employed to grow the saplings in polybags. Natural forest soil was used for control set seedlings. The saplings have been watered on a regular basis. The IAA, IBA, and NAA were utilized to compare the physiological adaptation of saplings under different conditions. Exogenously applied 100 ppm concentrations of each auxin to saplings at fifteen-day intervals for eight times.

The growth characteristics were detected with physiological variability after varied auxin treatments. Carbon absorption rate, stomatal conductance, evaporation rate, and internal CO_2 content have all been measured in the leaves of *Shorea robusta*. Finally, data was evaluated to determine its relative physiological adaptability.

The LC Pro Plus (Delta -T USA) instrument was used to assess the various physiological variables.

Result

The effects of Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), and Naphthalene Acetic Acid (NAA) treatments on its development and physiological functions. The IBA treatment stimulated the most sapling development (19.901.37 cm), followed by the IAA treatment (18.20.97 cm). In comparison to the control set, the minimum growth (16.721.05 cm) of Sal seedlings was documented during NAA treatment. The IBA 100 ppm treatment produced the highest average number of leaves (7.2 0.24) per sapling, while the NAA treatment produced the lowest average number of leaves (7.2 0.24) per sapling. The IAA 100 ppm induced the average maximum length of petiole length while the NAA 100 ppm induced the minimum length of petiole length. IBA-treated saplings have shown the better midrib growth in comparison to other auxin-treated saplings followed by control set. (Table.1)

In comparison to the morphological growth of Sal saplings, the NAA-treated saplings demonstrated superior physiological adaptation. NAA 100 ppm treated saplings had the highest rate of CO_2 assimilation (7.64 mol CO_2 m-2 s-1) followed by IAA treated saplings (4.436 mol CO_2 m-2 s-1) (Fig 1-B).

Stomatal conductance was found in *Shorea robusta* saplings, with the highest rate (1.416 mol H_2O m-2 s-1) caused by IBA 100 ppm, followed by NAA 100 ppm, and in the control group of saplings. (Fig 1A)

All auxin-treated saplings exhibited a higher rate of evaporation than untreated saplings. In comparison to the control, the NAA 100 ppm treated saplings had the highest evaporation rate (1.53 mol H_2O m-2 s-1) followed by IAA and IBA treated saplings (Fig 1C).

The internal CO_2 concentration in the leaves was highest in NAA 100 ppm treated saplings (613.22 mol CO_2 m-2 s-1) followed by untreated saplings (Control) (Fig 1D).

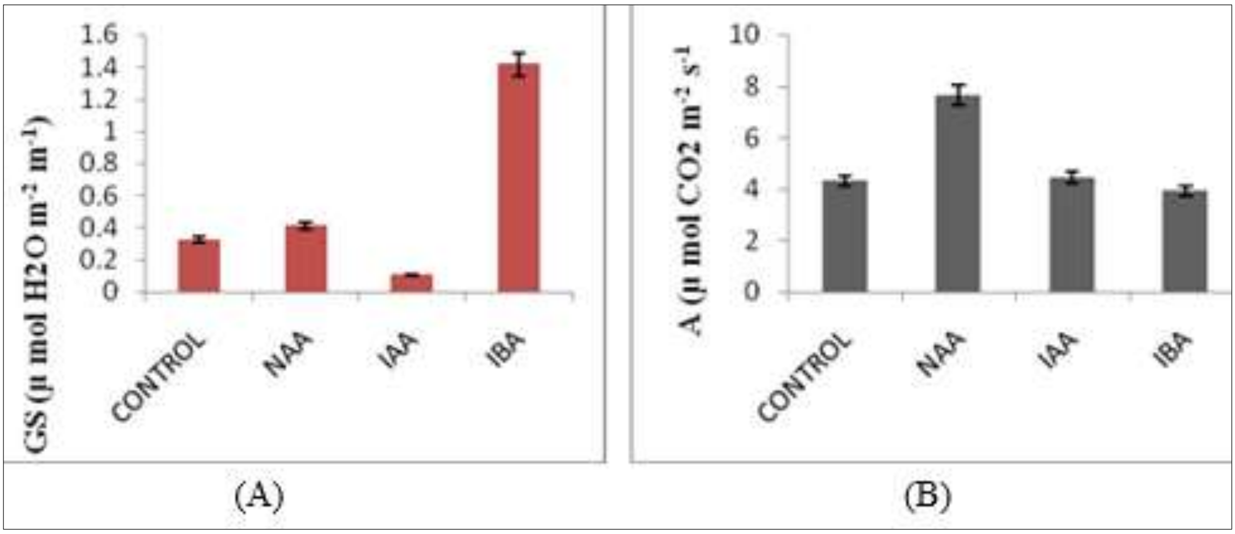
The physiological adaptation of S. robusta saplings outside of the natural forest is improved by NAA treatment. The IBA and IAA treatments also improved seedling physiological adaptability compared to untreated saplings (Control set).

The current study found that different auxins (IAA, IBA, and NAA) improve sapling physiological adaptation outside of their natural habitat and can be employed to accelerate the establishment and acclimation of *Shorea robusta* saplings in diverse climatic conditions outside of their forest.

Result

Table 1: Shows the growth pattern of a Sal seedling outside of its native environment as impacted by various auxins.

Hormonal Treatments	Height (in centimeters)	Average no of leaves per seedlings	Petiole length (cm)	Midrib length(cm)	Leaf width (cm)
Control	16.82±1.11	6.77±0.66	1.02±0.03	9.34±0.65	5.14±0.41
IAA	18.7±0.97	6.9±0.34	1.09±0.06	9.95±1.23	5.00±0.23
IBA	20.1±1.37	7.3±0.25	0.98±0.04	11.44±0.89	6.07±0.36
NAA	16.73±1.05	6.10±0.51	0.71±0.03	8.21±1.13	4.05±0.18



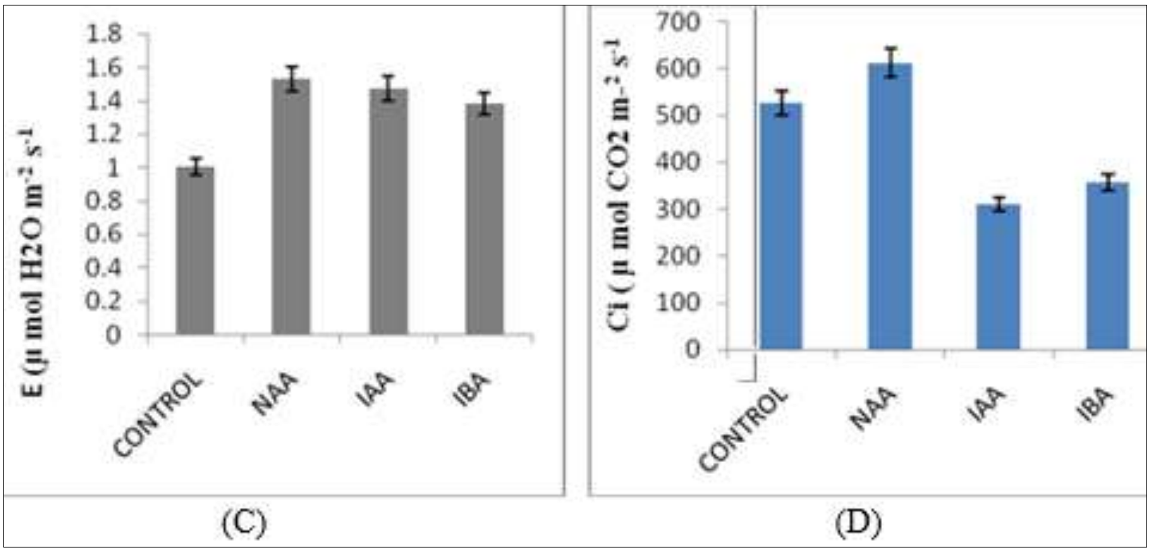


Fig 1: (A, B, C and D) show the Physiological differences in Sal seedlings (A- CO_2 assimilation, Gs- Stomatal conductance, E- Evaporation rate, Ci- Internal Co_2 concentration)

Discussion

The present research discovered that Naphthalene Acetic Acid (NAA), Indole Acetic Acid (IAA), and Indole Butyric Acid (IBA) all have different impacts on the development and morphological characteristics of *Shorea robusta* seedlings. This implies that availability of exogenous auxin limits shoot elongation in *Shorea robusta*. IAA and NAA have been shown to have a variety of impacts on the development of Pinus sylvestris and *Picea glauca* (Little & Macdonold, 2003) [12]. Indole Butyric Acid (IBA) stimulated shoot length, midrib length, and average number of leaves more than the control group. It also shows that the auxin hormone group has a significant impact on the Sal growth properties. IBA stimulation of growth might be ascribed to the well-known fact that IBA is required for root stimulation and development because they alter basic processes such as cell division and elongation (George *et al*., 2008; Nakhooda *et al*., 2014) [9, 14]. Hartmann *et al*., 1997 [10] discovered that IBA concentration was more effective than other treatments in increasing root number, root length, and shoot development in tree species. Furthermore, IBA may have stimulated enzyme activities in plants that regulate several biochemical pathways such as protein, carbohydrates, nitrogen, and phenolics (Das *et al*., 1997; Woodward and Bartel, 2005; Gasper *et al*., 1997) [6, 20, 8]. The current study's findings show that, among diverse auxin groups, the administration of NAA and IBA can be useful in the growth of plants.

There are numerous studies that demonstrate the impact of auxins on higher plant physiological and biochemical processes. Auxin's physiological impact on plants can help with acclimation, which can increase their establishment at a young age. Early development is one of the main problems that *Shorea robusta* needs to deal with. The species with excellent physiological flexibility and environmental reaction will be able to overcome the early growth challenges. Exogenous production of IAA has been found to promote plant drought tolerance (Rotino *et al*., 1997) [17]. In the current study, the administration of Napthalene Acetic Acid (NAA), Indole Acetic Acid (IAA), and Indole Butyric Acid (IBA) significantly impacted photosynthetic activity. Bidwell and Wendy (1996) [21] observed that supplementing bean (Phaseolus vulgaris) with Indole Acetic Acid (IAA) increased the rate of CO_2 absorption. Czerpak *et al*. (2004) [3] discovered that NAA increased the photosynthetic rate of *Wolffia arrhiza* to 124.5% while PAA increased it to 127.2%. Auxins stimulate photosynthetic activity through an increase in chlorophyll content and activation of cellular redox mechanisms (Dao *et al*., 2018) [5]. Plants may also create additional pigments, monosaccharides, and soluble proteins with the aid of auxins (Pitrowska-Niczyporuk and Bajguz, 2014) [16] Moreover, it has been shown that auxins play a crucial role in the transmission of cell-cell positional information., This finally brings it into physiological homeostasis about the external environment (Le Bail *et al*., 2010) [11]. The current work demonstrates that exogenous auxin administration can aid in physiological acclimatization of Sal saplings outside of their natural habitat. Furthermore, it may be a good solution to its early establishment issues.

Conclusion

Shorea robusta is a climax tree that grows in gregarious and pure stands. Natural regeneration occurs through seeds; however, it is hampered by their limited viability period because its seeds are refractory and lose viability after a week of dropping to the ground, *S. robusta* has a difficult time establishing itself. In this study, the effect of IBA, IAA, and NAA hormone on seedling growth, the assimilation of CO_2, stomatal conductance, evaporation rate, and internal CO_2 concentration were also investigated, as well as its establishment outside its natural habitat (AABR- Achanakmar Amarkantak Biosphere Reserve, Chhattisgarh). In the current investigation, several auxin treatments increased CO_2 assimilation rate and stomatal conductance towards its assimilation point. All three auxins group hormone (IBA, IAA, and NAA) increased the rate of evaporation, which in turn enhanced the rate of water absorption and transportation. Sal seedlings outperformed untreated seedling in terms of acclimatization and auxin establishment treatment.

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