Effects of Decapitation, Water-Deficit Stress, and Pot Size on Morpho-Anatomy and Physiology of *Pterocarpus indicus*

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ABSTRACT

The interacting effects of stem decapitation, water-deficit stress, and pot size on the growth, morpho-anatomical, and physiological traits of *Pterocarpus indicus* seedlings were analyzed in this study. Changes in root collar diameter (RCD), biomass allocation, number of leaflets (NL), mean leaf area, guard cell size, stomatal aperture size, phloem cap fiber (PCF) thickness, xylem vessel density (XVD), relative leaf water content (RWC), stomatal conductance (g\(_{sw}\)), transpiration rate (E), fluorescence quantum yield, transpiration (E), photosynthesis (P\(_N\)), and electron transport rate (ETR) of decapitated and undecapitated *P. indicus* seedlings in different pot sizes (small, medium, large) and watering regimes (every 2, 7, and 14 days) were analyzed. The decapitation × water-deficit stress × and pot size interaction did not affect growth and morpho-anatomical variables, but they did on most of the physiological traits. Decapitated seedlings watered every 14 days and planted in medium or large pots have lower g\(_{sw}\), P\(_N\), E, and RWC. While the RCD of large-potted and water-stressed (every 14 days) seedlings decreased, allocations to stem and fine roots increased. Moreover, the NL and PCF significantly decreased, while the ETR and XVD significantly increased in decapitated and water-stressed seedlings. Overall, the decapitation-watering interaction caused significant stress to *P. indicus* seedlings.

1. Introduction

Newly transplanted seedlings generally have no vast root systems; hence, they are usually prone to transplant shock due to multiple stresses, including injury from natural disturbance (e.g., stem decapitation) and water-deficit stress. Previous experiments have revealed multiple stresses that can delay the growth and survival of newly transplanted seedlings (Stoneham and Thoday 1985). However, the major causes of seedling death remain understudied. Although attempts have also been made to increase abiotic stress resistance in transplanted seedlings, greater mortality rates, lower shoot growth, and survival could still be observed in many plant species. Moreover, improperly handled plants at the nursery typically do poorly if transplanted in the field (Leakey 2017). This is because seedling quality and performance are influenced mainly by the nursery growth system and cultural practices (Haase et al. 2021). Thus, understanding how seedlings...
respond to multiple stresses during the nursery phase will help us determine the desired growth and survival level suitable to the planting site’s unfavorable environments.

Natural disturbances, such as volcanic eruptions and windstorms, are the major causes of stem damage (decapitation or branch removal), which can influence the forest ecosystem functions and structures by altering the growth and development of the plant (Herbert et al. 1999; Hernandez et al. 2020; Hirsh and Marier 2002). In some studies, decapitation is a good practice for stimulating stem growth by apical dominance (Liu et al. 2017; Pal et al. 2013). The practice of decapitation, coppicing, or cutting of either stem or root has also been explored in several studies to increase the number of foliage, stems, and branches (Pal et al. 2013). Decapitation-pruning interaction displayed a high rooting capacity, and the results varied by crown and pruning intensity (Haines et al. 1993). A simulation experiment also revealed a significant increase in xylem-specific hydraulic conductivity following the decapitation of Betula trees (Tumajer and Treml 2019). However, there is currently a lack of consensus and comprehensive research on how stem decapitation and its interaction with other environmental factors function for tree species. For example, decapitating shoots resulted in slower growth of axillary buds, and the results varied depending on temperature (Yang et al. 2021). The re-sprouting ability of decapitated peach trees was suppressed after applying fresh manure or organic matter (Tsipouridis and Thomidis 2003). Contradictory findings of previous research can be ascribed to variances in species’ life-history traits, stress intensity, and the interacting effects of multiple stresses (e.g., decapitation and water scarcity). Moreover, the existing literature on decapitation research is focused on the impacts of plant growth, with little information on the effects on morpho-anatomy and physiology, particularly in tree species.

Water is the most important resource in seedling establishment and survival in the actual planting sites, particularly during summer, in addition to excessive solar radiation and nutrient-poor soils (Chirino et al. 2011; Jiménez et al. 2007). Seedling establishment (i.e., elongation and emergence) is an important phase of the plant’s life cycle, as seedlings can be exposed to different levels of water-deficit stress (Park et al. 2021), inducing transplant shock (Haase and Rose 1993). In particular, prolonged water stress harms many aspects of plant physiology, resulting in decreased plant growth and development, metabolic disturbance, and death (Hernandez et al. 2023; Jaleel et al. 2008; Pallardy 2010). Unfortunately, knowledge of seedling responses to water-deficit stress, particularly in forest tree species, is still scarce. Previous studies specifically lack emphasis on how drought-induced changes in morpho-anatomical and physiological traits influence seedling growth and development. Although several studies have already been done to observe the complex physiological impacts of water-deficit stress on plants, these studies have not addressed multiple stresses or interaction effects, such as pot size-water stress and/or decapitation-pot size-water stress interaction effects.

Pot size significantly impacts plant growth, especially when the root environment is under stress, through alteration in plant water use and root system morphological characteristics (Espinoza et al. 2017; Landis et al. 1990). Previous research found that large containers led to larger root systems, whereas small containers limit root growth and development, and these are related to seedlings’ ability to forage water resources under water-limiting conditions (Park et al. 2021; Poorter et al. 2012; Villar-Salvador et al. 2012). The ability of the root system to forage water in a new site is important after transplanting (Rietveld 1989). When roots are enclosed in a container that limits their expansion, the roots become stunted, which could predispose plants to drought stress (Ma et al. 2020). When root mass increases while rooting space is limited, there
might be intense competition for available oxygen (Balliu et al. 2021). While these pot size effects on plant growth are well-known for many herbaceous plants, these effects are not well-documented in forest tree species, which differ widely in functional type, growth form, water use, and root architecture. Moreover, data on how pot size influences the response of seedlings to multiple stresses is relatively lacking.

*Pterocarpus indicus* (narra) is a deciduous nitrogen-fixing tree species that grows best in open areas. In the Philippines, it is widely used for reforestation (Gazal et al. 2004). It can be propagated via seeds and cuttings; seedling stocks are mostly used for reforestation and rehabilitation of denuded lands (Rise 1995). However, establishment trials in degraded forest areas have yielded varied results, with some failing (Orwa et al. 2009). Thus, to understand how narra seedlings respond to multiple stresses typical of denuded lands, we studied the interacting effects of stem decapitation, water-deficit stress, and pot size on the growth, morpho-anatomy, and physiology of narra seedlings.

2. Materials and Methods

2.1. Plant Material and Experimental Design

In March 2022, we collected *P. indicus* seedlings that were physically and physiologically healthy, measuring 4.5–7.5 cm in height and 0.25–0.35 mm in diameter, from a forest floor in Barangay Canaway, Malilipot, Albay. Seedlings were located within 5–10 m radius of large trees, which could be mother trees. *P. indicus* seedlings were growing in association with other dominant species, such as *Weinmannia hutchinsonii*, *Astronia ferruginea*, and *Neoscortechinia nicobarica*. The seedlings were transported from the study site in a storage box with damp soil. Seedlings of similar sizes were individually transplanted into pots containing a sterilized soil mixture. The mixture, composed of coir dust, garden soil, and sand, was combined in equal parts by weight or volume (e.g., 1,000 g), maintaining a 1:1:1 ratio. Such a soil mixture is commonly used as a potting medium for forest tree species in the Philippines, and it effectively nurtures *P. indicus* seedlings. Seedlings underwent an acclimatization period in the greenhouse, where seedlings were made to recover in the nursery and continuously watered for two months.

After a 2-month acclimatization period, a greenhouse experiment on the interacting effects of pot size, stem decapitation, and water stress was conducted from May to October 2022 at the Department of Forest Biological Sciences (DFBS), College of Forestry and Natural Resources (CFNR), University of the Philippines Los Baños (UPLB), Laguna, Philippines. The seedlings were arranged in the greenhouse following the strip plot experimental design. Before any treatment was applied, the development of the earliest terminal bud was monitored until the appearance of the primordium of the first leaf based on the procedures in Chaar et al. (1997). It ensures that the leaves/stems/roots used were only those produced during treatment. Seedlings were subjected to three pot size treatments, i.e., small (5 × 5 × 8 cm³), medium (10 × 10 × 15 cm³), and large (15 × 15 × 18 cm³), three watering treatments, i.e., every two days (control), every seven days, and every fourteen days using 250 ml of tap water per seedling, and two stem decapitation treatments, i.e., undecapitated (control) and decapitated. We used the rounded form for the pot treatments to have uniform root growth distribution and circulation of water and nutrients. For artificial decapitation treatment, approximately 3 cm of apical buds of an orthotropic stem were cut from the seedlings (N = 10). The decapitated and undecapitated seedlings were subjected to three watering regimes.
across pot sizes. A total of 180 wildlings (i.e., 3 pot sizes × 3 watering regimes × 2 decapitation treatments × 10 replicates) were used in this study. Pots were placed in the elevated seedbeds following a 15 cm distance between seedlings and a 0.5 m distance between seedbeds. Dead and inferior-quality seedlings were replaced during the recovery period before the treatment application.

2.2. Growth and Morpho-Anatomical Traits Measurement

The seedlings’ root collar diameter (RCD), mean leaf area, number of leaflets (NL), and biomass allocations were measured during the initial and final weeks of the experiment. Using a digital caliper, the RCD of each seedling was measured. Plants were harvested after six months of treatment application to measure biomass allocation and were separated into leaves, stems, branches/twigs, and fine and coarse roots. The roots were gently cleansed with flowing tap water and air-dried. The oven-drying method was then used to determine the biomass allocations at 65°C for 48 hours.

Ten fully grown and healthy leaves attached to an orthotropic branch were randomly collected from each pot for the measurement of morpho-anatomical traits. All leaves were taken in the morning (i.e., 8:00 to 10:00 AM) with similar internodal orientations on twigs. Leaf samples were packed in plastic bags and stored in a cold storage box for further analysis in the laboratory. The mean leaf area (MLA) was determined using the grid counting method, where leaves were traced on the 1 cm grid paper. The NL was determined by counting all leaflets in a compound leaf of each seedling across treatments.

For anatomical analysis, young leaf and stem samples (approximately 1 mm and 2 mm) were cut from the middle of the leaves and fixed in microcentrifuge tubes for several weeks containing a fixative solution. The anatomical analysis was done using a freehand technique following the procedures of Hernandez et al. (2022). Samples were dehydrated in a graded series of ethanol solutions (50, 65, 95, and 100%) at room temperature for one month. The guard cell size, stomatal aperture size, phloem cap fiber (PCF) thickness, and xylem vessel density (XVD) of decapitated and undecapitated P. indicus seedlings in different pot sizes and watering regimes were determined and analyzed in the final week. The abaxial epidermal peels were collected from each leaf using colorless nail polish for stomatal aperture size and guard cell size measurement. Approximately 10–15 stomatal aperture and pairs of guard cells from each leaf sample were measured using digital image processing (i.e., ImageJ). The PCF thickness (µm) and the XVD in the young stem of the seedlings were measured using the same image processing software.

2.3. Physiological Traits Measurement

In this study, the physiology of all the seedlings, such as leaf relative water content (RWC), stomatal conductance (gsw), transpiration rate (E), fluorescence quantum yield (ΦF), and electron transport rate (ETR), were measured. Ten leaves were collected to determine RWC following the procedures of Jiménez et al. (2007). Leaves were weighed to obtain the fresh mass (FM), petioles were immersed in water overnight, reweighed to obtain turgid mass (TM), and oven-dried at 65°C for 48 h. The RWC was then computed as (FM – DM)/(TM – DM) × 100 (Diaz-Perez et al. 1995). The gsw, E, ΦF, and ETR were determined using a handheld LI-600 Porometer/Fluorometer (LI-COR, Nebraska, USA). A portable photosynthesis system (LI-6400T, Li-Cor Inc., USA) was used to measure the photosynthesis rate (PN, µmol CO2 m⁻² s⁻¹). PN was measured under an artificial
irradiance of 1000 µmol m$^{-2}$ s$^{-1}$ with a temperature of 26°C and chamber relative humidity of 50–70%. Measurements were done between 9:00 AM and 12:00 PM (PST) in the same healthy and fully expanded or sun-exposed leaves (two leaves per seedling) attached to an orthotropic branch (approximately 4–5 nodes).

2.4. Statistical Analysis

Using the “Shapiro.test”, the normal distribution of the data was initially assessed. The effects of the treatment on the growth, morpho-anatomy, and physiology of *P. indicus* seedlings were tested using a three-way ANOVA. Using Tukey’s HSD post hoc test, means were compared. All calculations were performed using R statistical software (version R-3.5.1) at a significance level of $\alpha = 0.05$.

3. Results and Discussion

3.1. Interacting Effects of Stem Decapitation, Pot Size, and Watering Regimes on the Growth of *Pterocarpus indicus* Seedlings

In Fig. 1, the RCD, MLA, and NL of both undecapitated and decapitated seedlings were, in general, significantly lowest at 14 days water regime across pot sizes. However, there was a clear diminishing trend in the MLA and NL values of the decapitated seedlings as the water regime and pot size increased. The interacting effects of pot size and watering regime in decapitated seedlings were significant only for RCD. However, the interactions among decapitation, pot size, and watering had no significant effects on the RCD, NL, and MLA growth of both undecapitated and decapitated *P. indicus* seedlings (Table 1).

Regarding biomass allocation, no significant difference was found among undecapitated seedlings across pot sizes and watering regimes (Fig. 2). A significant difference in biomass was observed in decapitated seedlings watered every 14 days. Specifically, stem (aboveground and fine root (belowground) biomass significantly increases as pot size increases. The interactions of the three variables had no significant effect on biomass allocations of undecapitated *P. indicus* seedlings across all treatments (Table 1).

The results indicated that pot size-watering interaction significantly affects the plant growth of decapitated *P. indicus* seedlings. A significant decrease in RCD of large-potted decapitated seedlings watered every 14 days can be attributed to the interacting effects of the large volume of soil and low water supply on the ability of the seedlings to absorb nutrients from the soil. This result could explain the significant increase in fine root biomass under such conditions as they need to forage into large pots amid a decreased water supply. Since stressed seedlings are planted in large pots, they may have shifted the carbon allocation to increasing the belowground system to enhance foraging traits by increasing fine root biomass instead of increasing the RCD. The increase in fine root biomass may have improved the root surface area in contact with the soil solution and the overall uptake of water. The main route for plants to absorb water and nutrients is through fine roots, particularly those with a diameter of about 2 mm (Hendrick and Pregitzer 1996). An increase in coarse roots, on the other hand, is significant in improving anchorage (Ramalingam et al. 2017; Sorgonà et al. 2018). Hence, the observed decrease in coarse root biomass in all decapitated large-potted narra seedlings watered every 14 days indicates a trade-off between coarse roots’ anchoring function and fine roots’ water absorption function. Our results partially agree with those of the
previous studies, i.e., plant growth of trees (Poorter et al. 2012; Salisu et al. 2018) and herbs (Al-Menaie et al. 2012; Oagile et al. 2016) increased with increasing pot size.

![Graphs showing RCD, mean leaf area, and no. of leaflets of decapitated and undecapitated P. indicus seedlings in different pot sizes (S, M, L) and watering regimes (every 2, 7, and 14 days)](image)

**Fig. 1.** RCD, mean leaf area, and no. of leaflets of decapitated and undecapitated *P. indicus* seedlings in different pot sizes (S, M, L) and watering regimes (every 2, 7, and 14 days) (Note: Lowercase letters indicate statistical significance between treatments at $\alpha = 0.05$ (Three-way ANOVA)).

This tendency supports the recorded significant decrease in RWC in all decapitated potted seedlings watered every 14 days (**Table 1**). This considerable drop in RWC can signal a critical seedling water status or cellular water deficiency. In addition, the observed lower transpiration rates ($E$) and stomatal conductance ($g_{sw}$) may explain the potential rise in water consumption in large-potted seedlings watered every 14 days.
We could also deduce that the increase in the belowground system is linked to a considerable increase in stem biomass allocation of decapitated, large-potted, and water-stressed seedlings. The observed higher xylem vessel density in decapitated, large-potted, and water-stressed seedlings (Fig. 3) supports such a result. Increased xylem vessel or tracheid diameter and/or density improves water transport efficiency (Hernandez and Park 2022; Pittermann 2010) and maintains hydraulic conductance by reducing the effects of path length (Kim et al. 2014) as stem and root length increase over time. Herrera et al. (2021) reported a contrasting pattern, i.e., xylem vessels were larger in small pots than large ones, which resulted in a highly significant increase in the theoretical hydraulic conductance. The difference in the results can be ascribed to many factors, including species traits, plant organs, and treatment imposition.

**Fig. 2.** Biomass allocations of decapitated and undecapitated *P. indicus* seedlings in different pot sizes (S, M, L) and watering regimes (every 2, 7, and 14 days) (Note: Different uppercase and lowercase letters indicate statistical significance between treatments for total biomass (aboveground and belowground) and plant organs, respectively (Three-way ANOVA, $\alpha = 0.05$)).

### 3.2. Interacting Effects of Stem Decapitation, Pot Size, and Watering Regimes on Morpho-Anatomical Traits of *P. indicus* Seedlings

Among the anatomical traits observed (Fig. 3–Fig. 6), the xylem density had the most striking increase in decapitated seedlings watered every 14 days, regardless of pot sizes (Fig. 3–Fig. 6). Guard cells and PCF thickness somehow showed a slight significant size reduction in decapitated seedlings watered every 14 days, regardless of pot sizes (Fig. 3–Fig. 5). Surprisingly, there was a significant increase in the stomatal aperture size in both decapitated and undecapitated seedlings watered every 14 days, regardless of pot sizes (Fig. 3).
The interaction between decapitation and watering significantly impacted PCF thickness (Fig. 3 to Fig. 5b) and XVD (Fig. 3 to Fig. 6b). The PCF thickness of decapitated *P. indicus* seedlings watered every 14 days was significantly lower than that of undecapitated and well-watered seedlings. A reverse pattern was observed in XVD, wherein we found no significant interacting effects of decapitation × pot size × watering on any of the morpho-anatomical traits studied (Table 1 and Fig. 3 to Fig. 6). However, the decapitation × pot size interaction significantly influenced seedling guard cell size (Table 1 and Fig. 3).

**Fig. 3.** Guard cell size, stomatal aperture size, PCF thickness, and XVD of decapitated and undecapitated *Pterocarpus indicus* seedlings in different pot sizes (S, M, and L) and watering regimes (every 2, 7, and 14 days) (Note: Lowercase letters indicate statistical significance between treatments at $\alpha = 0.05$ (Three-way ANOVA)).
The decapitation-watering interaction significantly affected the NL, PCF, ETR, and XVD (Table 2). Although stem decapitation stimulated seedling coppicing ability as evidenced by increased stem biomass (i.e., higher stem density per seedling), water stress may have prevented seedlings from developing leaves to prevent excessive water loss via transpiration and carbon consumption via photosynthesis. Water stress may have negatively affected the leaf mitotic activity or cell division in the meristematic zone of the seedlings, ultimately influencing the development of vegetative organs and, thus, carbon allocation to leaves. Several studies found
similar results, i.e., altered cell division due to drought stress reduced leaf growth and development (Avramova et al. 2015; Nelissen et al. 2018).

**Fig. 5.** Phloem cap fibers of undecapitated and decapitated *P. indicus* seedlings in different pot sizes and watering regimes.
Fig. 6. Xylem vessels of undecapitated and decapitated Pterocarpus indicus seedlings in different pot sizes and watering regimes.

We found a lower PCF thickness in decapitated seedlings watered every 14 days, and this further explains the observed lower leaf production. Leaf photosynthesis is highly correlated with CO₂-diffusion capacities, controlled by leaf morpho-anatomical traits and environmental factors (Huang et al. 2022; Ye et al. 2022; Zhang et al. 2020). Thus, a lower PCF thickness could indicate
a lower production of glucose molecules during photosynthesis, as PCF’s main role is to provide mechanical strength to food-transporting tissues, such as the phloem. Because there was not much glucose to transport that required mechanical support, decapitated *P. indicus* seedlings planted under every 14 days watering regime may not have invested in establishing more phloem fibers for long-distance loading. Alternatively, decapitation-water stress interaction may have negatively affected the phloem transport mechanisms, eventually altering carbon allocation to leaves.

### 3.3. Interacting Effects of Stem Decapitation, Pot Size, and Watering Regimes on the Physiology of *P. indicus* Seedlings

Significant interaction effects of decapitation × pot size × watering on *g*ₚₛ, *E*, *P*ₙ, and Φᵢ were observed in this study (Table 1). The decapitated and water-stressed seedlings planted in medium to large pots generally had lower *g*ₚₛ, *E*, and *P*ₙ compared to undecapitated and well-watered seedlings planted in small pots (Table 2). Regarding Φᵢ, the undecapitated and well-watered seedlings in small pots had a higher Φᵢ than other seedlings. The RWC was significantly affected by pot size, watering, and their interaction, with well-watered and small-potted seedlings having significantly higher RWC (Table 2). Finally, we discovered that the decapitation watering interaction significantly affected the electron ETR. A higher ETR is commonly observed in photosynthesis or photorespiration of plants exposed to different levels of drought stress (Zivcak et al. 2013).

**Table 1.** P-values obtained in testing the effects of stem decapitation (decapitated and undecapitated), pot size (small, medium, and large), and watering regimes (every 2 days, every 7 days, and every 14 days) on growth, morpho-anatomical traits, and physiological traits *P. indicus* using the three-way ANOVA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Decapitation (D)</th>
<th>Pot size (P)</th>
<th>Watering (W)</th>
<th>D × P</th>
<th>D × W</th>
<th>P × W</th>
<th>D × P × W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GROWTH</strong></td>
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<tr>
<td>RCD (mm)</td>
<td>0.880</td>
<td>0.843</td>
<td>&lt;0.001</td>
<td>0.082</td>
<td>0.189</td>
<td>&lt;0.001</td>
<td>0.177</td>
</tr>
<tr>
<td>No. of leaflets</td>
<td>&lt;0.001</td>
<td>0.018</td>
<td>&lt;0.001</td>
<td>0.221</td>
<td>0.003</td>
<td>0.082</td>
<td>0.101</td>
</tr>
<tr>
<td>Mean leaf area (cm²)</td>
<td>0.815</td>
<td>0.097</td>
<td>&lt;0.001</td>
<td>0.511</td>
<td>0.744</td>
<td>0.334</td>
<td>0.728</td>
</tr>
<tr>
<td>Fine root biomass (g)</td>
<td>0.231</td>
<td>0.002</td>
<td>0.984</td>
<td>0.199</td>
<td>0.003</td>
<td>0.320</td>
<td>0.163</td>
</tr>
<tr>
<td>Coarse root biomass (g)</td>
<td>0.127</td>
<td>0.558</td>
<td>0.115</td>
<td>0.577</td>
<td>0.141</td>
<td>0.501</td>
<td>0.463</td>
</tr>
<tr>
<td>Leaf biomass (g)</td>
<td>0.523</td>
<td>0.037</td>
<td>0.127</td>
<td>0.172</td>
<td>0.017</td>
<td>0.389</td>
<td>0.352</td>
</tr>
<tr>
<td>Twig biomass (g)</td>
<td>0.115</td>
<td>0.005</td>
<td>0.465</td>
<td>0.923</td>
<td>0.004</td>
<td>0.818</td>
<td>0.215</td>
</tr>
<tr>
<td>Stem root biomass (g)</td>
<td>0.076</td>
<td>0.794</td>
<td>0.061</td>
<td>0.583</td>
<td>0.030</td>
<td>0.548</td>
<td>0.915</td>
</tr>
<tr>
<td>Total biomass (g)</td>
<td>0.115</td>
<td>0.043</td>
<td>0.032</td>
<td>0.923</td>
<td>0.004</td>
<td>0.818</td>
<td>0.258</td>
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<tr>
<td><strong>MORPHO-ANATOMY</strong></td>
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<tr>
<td>Guard cell size (µm)</td>
<td>0.628</td>
<td>0.539</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.995</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Stomatal aperture size (µm)</td>
<td>0.086</td>
<td>0.458</td>
<td>&lt;0.001</td>
<td>0.057</td>
<td>0.937</td>
<td>0.991</td>
<td>0.998</td>
</tr>
<tr>
<td>Phloem cap fibers thickness (µm)</td>
<td><strong>0.031</strong></td>
<td>0.409</td>
<td><strong>0.008</strong></td>
<td><strong>0.018</strong></td>
<td>&lt;0.001</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Xylem vessel density (count seedling⁻¹)</td>
<td>&lt;0.001</td>
<td>0.586</td>
<td>&lt;0.001</td>
<td>0.689</td>
<td>&lt;0.001</td>
<td>0.253</td>
<td>0.419</td>
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<tr>
<td><strong>PHYSIOLOGY</strong></td>
<td></td>
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<tr>
<td>Relative leaf water content (%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.693</td>
<td>0.061</td>
<td>&lt;0.001</td>
<td>0.719</td>
</tr>
<tr>
<td>Stomatal conductance (mol H₂O m⁻² s⁻¹)</td>
<td>0.198</td>
<td>0.742</td>
<td>0.864</td>
<td>0.511</td>
<td>0.574</td>
<td>0.943</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Transpiration rate (mmol H₂O m⁻² s⁻¹)</td>
<td>0.546</td>
<td>0.813</td>
<td>0.704</td>
<td>0.499</td>
<td>0.630</td>
<td>0.976</td>
<td>0.006</td>
</tr>
<tr>
<td>Photosynthesis (µmol CO₂ m⁻² s⁻¹)</td>
<td>0.332</td>
<td>0.412</td>
<td>0.126</td>
<td>0.154</td>
<td>0.165</td>
<td>0.842</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Fluorescence quantum yield</td>
<td><strong>0.054</strong></td>
<td>0.056</td>
<td>0.463</td>
<td>0.222</td>
<td>0.156</td>
<td>0.538</td>
<td><strong>0.023</strong></td>
</tr>
<tr>
<td>Electron transport rate (µmol electrons m⁻² s⁻¹)</td>
<td><strong>0.001</strong></td>
<td><strong>0.035</strong></td>
<td>&lt;0.001</td>
<td>0.759</td>
<td>&lt;0.001</td>
<td>0.683</td>
<td>0.809</td>
</tr>
</tbody>
</table>

Note: Significant effects are written in bold.
Table 2. Physiological traits of decapitated and undecapitated *P. indicus* seedlings in different pot sizes and watering regimes. Lowercase letters indicate statistical significance between treatments at $\alpha = 0.05$ (Three-way ANOVA).

<table>
<thead>
<tr>
<th>Decapitation</th>
<th>Pot size</th>
<th>Watering regime</th>
<th>RWC (%)</th>
<th>$g_{w}$ (mol H$_2$O m$^{-2}$s$^{-1}$)</th>
<th>E (mmol H$_2$O m$^{-2}$s$^{-1}$)</th>
<th>$\Phi_F$</th>
<th>ETR ($\mu$mol electrons m$^{-2}$s$^{-1}$)</th>
<th>P$_{N}$ ($\mu$mol CO$_2$m$^{-2}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undecapitated</td>
<td>S</td>
<td>Every 2 days</td>
<td>88.86</td>
<td>0.057 (0.02)$^a$</td>
<td>0.84 (0.25)$^a$</td>
<td>0.49 (0.15)$^b$</td>
<td>7.17 (2.24)$^c$</td>
<td>1.11 (2.24)$^a$</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>89.81</td>
<td>0.055 (0.02)$^a$</td>
<td>0.72 (0.22)$^a$</td>
<td>0.69 (0.02)$^{ab}$</td>
<td>9.55 (0.43)$^{de}$</td>
<td>0.55 (0.43)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>90.09</td>
<td>0.049 (0.01)$^a$</td>
<td>0.65 (0.13)$^{ab}$</td>
<td>0.73 (0.01)$^a$</td>
<td>9.74 (0.16)$^{de}$</td>
<td>0.44 (0.16)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Every 7 days</td>
<td>75.12</td>
<td>0.041 (0.02)$^a$</td>
<td>0.63 (0.27)$^{ab}$</td>
<td>0.64 (0.04)$^{ab}$</td>
<td>10.05 (1.13)$^{de}$</td>
<td>0.45 (1.13)$^a$</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>82.05</td>
<td>0.061 (0.01)$^a$</td>
<td>0.85 (0.17)$^a$</td>
<td>0.72 (0.01)$^a$</td>
<td>11.39 (0.98)$^{de}$</td>
<td>1.03 (0.98)$^a$</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>92.16</td>
<td>0.064 (0.02)$^a$</td>
<td>0.51 (0.12)$^{ab}$</td>
<td>0.74 (0.01)$^a$</td>
<td>12.59 (1.58)$^{de}$</td>
<td>0.89 (1.58)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Every 14 days</td>
<td>74.75</td>
<td>0.046 (0.02)$^{ab}$</td>
<td>0.41 (0.14)$^b$</td>
<td>0.72 (0.01)$^a$</td>
<td>15.00 (1.30)$^{abcd}$</td>
<td>0.90 (1.30)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>71.67</td>
<td>0.027 (0.02)$^b$</td>
<td>0.38 (0.15)$^b$</td>
<td>0.67 (0.05)$^b$</td>
<td>14.81 (1.63)$^{bcd}$</td>
<td>0.71 (1.63)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>71.90</td>
<td>0.019 (0.02)$^c$</td>
<td>1.42 (0.78)$^e$</td>
<td>0.67 (0.06)$^{ab}$</td>
<td>14.49 (1.07)$^{bcd}$</td>
<td>0.49 (1.07)$^{ab}$</td>
</tr>
<tr>
<td>Decapitated</td>
<td>S</td>
<td>Every 2 days</td>
<td>91.63</td>
<td>0.023 (0.01)$^a$</td>
<td>0.37 (0.16)$^b$</td>
<td>0.75 (0.00)$^a$</td>
<td>9.27 (0.28)$^{de}$</td>
<td>1.27 (0.28)$^a$</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>94.94</td>
<td>0.031 (0.02)$^b$</td>
<td>0.47 (0.17)$^b$</td>
<td>0.72 (0.02)$^a$</td>
<td>10.16 (0.52)$^{de}$</td>
<td>1.26 (0.52)$^a$</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>92.26</td>
<td>0.043 (0.02)$^b$</td>
<td>0.67 (0.28)$^{ab}$</td>
<td>0.74 (0.00)$^a$</td>
<td>10.90 (0.46)$^{de}$</td>
<td>1.20 (0.46)$^a$</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Every 7 days</td>
<td>82.79</td>
<td>0.047 (0.01)$^a$</td>
<td>0.73 (0.19)$^a$</td>
<td>0.72 (0.01)$^a$</td>
<td>8.53 (0.55)$^{de}$</td>
<td>0.83 (0.55)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>88.91</td>
<td>0.026 (0.01)$^b$</td>
<td>0.46 (0.13)$^b$</td>
<td>0.74 (0.02)$^a$</td>
<td>9.73 (0.50)$^{de}$</td>
<td>0.43 (0.50)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>97.36</td>
<td>0.021 (0.01)$^c$</td>
<td>0.79 (0.18)$^{b}$</td>
<td>0.71 (0.03)$^{ab}$</td>
<td>12.89 (2.31)$^{de}$</td>
<td>0.19 (2.31)$^c$</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Every 14 days</td>
<td>38.42</td>
<td>0.014 (0.01)$^c$</td>
<td>1.02 (0.13)$^a$</td>
<td>0.64 (0.01)$^c$</td>
<td>19.45 (1.74)$^{abc}$</td>
<td>0.45 (1.74)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>39.04</td>
<td>0.017 (0.01)$^c$</td>
<td>0.92 (0.20)$^a$</td>
<td>0.72 (0.01)$^a$</td>
<td>22.11 (2.22)$^a$</td>
<td>0.15 (2.22)$^c$</td>
</tr>
</tbody>
</table>
The results can be attributed to several factors, such as leaf temperature, light, phloem transport mechanisms, enzymes, CO$_2$, and leaf anatomical structures. For example, water stress significantly inhibited the linear electron transport in leaves depending on leaf temperature, although residual electron transport was higher in water-stressed leaves than in control (Loreto and Marco 1995). The ETR in decapitated and water-stressed $P$. indicus seedlings may be higher. However, the leaf exterior and interior environments may not have been suitable for establishing a proton gradient for ATP and NADPH production to complete the photosynthetic process. During the experimental period, water stress-induced modifications in the photosynthetic reaction centers could have also occurred (Zong et al. 2014), influencing the plant leaf’s photosynthetic machinery. This tendency can be seen from the observed lower $g_{sw}$, which leaf morpho-anatomical traits can influence in decapitated and water-stressed seedlings. Generally, a decrease in $g_{sw}$ results in photosynthesis limitation (Noormets et al. 2001).

While the interactions between decapitation, pot size, and watering did not affect growth and morpho-anatomical variables, the decapitated and water-stressed seedlings grown in medium and/or large pots exhibited lower $g_{sw}$, $P_N$, $E$, and RWC than other seedlings. This result implies that interacting multiple abiotic stresses can negatively trigger the complex physiological responses in $P$. indicus seedlings. Although either decapitation or water stress inhibited seedling development, results imply that large-potted seedlings’ survival may be improved when exposed to abiotic stresses by lowering $g_{sw}$, $P_N$, and $E$ while retaining high ETR. Rising CO$_2$ concentrations, which may have resulted from high ETR in leaves, can cause a decrease in stomatal conductance, resulting in a decreased rate of transpiration and improved plant water use efficiency (Ainsworth and Rogers 2007). Moreover, the high ETR can be attributed to the observed high stomatal aperture size in decapitated and water-stressed seedlings in medium and large pots. Such a high ETR may have provided sufficient ATP to fuel ion exchanges during the stomatal opening for osmoregulation or stomatal aperture regulation and photosynthesis via chloroplast-containing guard cells (Lawson 2008; Shimazaki and Zeiger 1985). Thus, CO$_2$ levels may have been fully used up by photosynthesis that possibly took place in the guard cells, triggering the opening of the stomatal aperture of $P$. indicus seedlings for replenishment.

4. Conclusions

Through a greenhouse experiment, we investigated the interacting effects of decapitation, pot size, and water stress on the growth, morpho-anatomy, and physiology of $P$. indicus seedlings. While the interactions of the three factors had no significant impact on growth and morpho-anatomical traits, they did on most physiological traits of the seedlings. Medium and large-potted, decapitated, and water-stressed seedlings had lower $g_{sw}$, $E$, $P_N$, and RWC and higher ETR than those in control. We also detected a significant effect of pot size-watering interaction on plant growth (i.e., RCD, biomass allocation, particularly to stem, fine root, and coarse root) of decapitated $P$. indicus seedlings and decapitation-water stress interaction on some of the morpho-anatomical and physiological traits (i.e., no. of leaflets, PCF, XVD, and ETR). Overall, we found that the response on growth, morpho-anatomy, and physiology of $P$. indicus seedlings to multiple stresses (decapitation and water-deficit stress) can be improved by planting the seedlings in medium and/or large pots during initial growth in the nursery. Although the responses might differ in the field, planting the seedlings in medium or large containers could be a potential nursery practice for improving the survival of the seedlings against multiple abiotic stresses. However,
large-scale field experiments are recommended to elucidate the responses of the seedlings, particularly the newly transplanted ones, to multiple stresses.

Acknowledgments

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References


