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Salinity-induced changes in the morphology, physiology, and anatomy of seeds and seedlings of smooth narra (*Pterocarpus indicus* Willd. f. *indicus*)

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Abstract. *Manipol MM, Tinio CE, Maldia LSJ, Combalicer MS. 2020. Salinity-induced changes in the morphology, physiology, and anatomy of seeds and seedlings of smooth narra (*Pterocarpus indicus *Willd. f.* indicus*). Biodiversitas 21: 5146-5154.* *Pterocarpus indicus* Willd. forma *indicus* is a commonly planted species for reforestation in the Philippines regardless of the area's condition. Since the species could survive in areas even with harsh conditions, it was hypothesized that the species may also thrive in areas having substantial amount of salt, especially during its early growth. This study assessed the morphological, physiological, and anatomical responses of seeds and seedlings of *P. indicus* Willd. f. *indicus* (Fabaceae), a native species in the Philippines to varying salt (NaCl) concentrations (0-300 mM). Based on the results, *P. indicus* f. *indicus* grew significantly in terms of height, number of leaves, leaf area, root collar diameter (RCD), root nodules, and root-shoot ratio in the control and at 100 mM NaCl. On the other hand, the growth of the species was suppressed at 300 mM NaCl. The physiological characteristics (germination, survival, and photosynthetic rates) were also high in the control compared to the NaCl treatments. As for the seed anatomical characteristics, the radicle cells remained undamaged, with high cell number and longer cells in the control, but showed damaged and reduced cell number and length at 300 mM NaCl. This indicates that *P. indicus* f. *indicus* can only tolerate moderate saline soil conditions. Hence, this result needs to be considered in reforestation efforts of the country using this species.

Keywords: Nitrogen-fixing species, photosynthetic rate, radicle, salt stress, sodium-chloride

Abbreviations: BSWM: Bureau of Soils and Water Management, CFNR: College of Forestry and Natural Resources, DFBS Department of Forest Biological Sciences, MMFR: Mount Makiling Forest Reserve, P_N: net photosynthesis, PAR: photosynthetic active radiation, PPFD: photosynthetic photon flux density, RCD: root collar diameter, UPLB: University of the Philippines Los Baños

INTRODUCTION

Salinity is an environmental stress that limits plant growth and productivity around the world. This problem is most prevalent in India, Pakistan, China, Syria, Iraq, Australia, and the United States (Qadir et al. 2014). Although in the Philippines saline-prone areas are small compared to other countries, there are about 600,000 ha of saline soil sites in the country (BSWM 2018) that need to be managed. Increased soil salinity induces changes in the morphology, physiology, and anatomy of the plants (Poljakoff-Mayber 1975; Huang and Redmann 1995; Vijayan et al. 2008; Rajabpoor et al. 2014; Hasanuzzaman et al. 2018). In many studies, it was reported that the high soil salinity mostly causes anatomical alterations such as reduction in aerenchyma (Naidoo and Mundree 1993), cortex thickness (Dolatabadian et al. 2011), xylem thickness, diameter of the vessels, stomatal index (Akcin et al. 2017), and stomatal density (Jafri and Ahmad 1995). Salinity also causes significant reduction in photosynthetic pigments, some mineral contents, and the level of total carbohydrates (Dawood et al. 2014). Plant roots treated with NaCl were observed shorter and had less secondary roots (Cachorro et al. 1995). In contrast, a significant increase in pith diameter of the stem (Akcin et al. 2017), upper and lower epidermis of leaf, palisade and spongy mesophyll (Boughalleb et al. 2009; Jafri and Ahmad 1995), stomatal size (Jafri and Ahmad 1995), and root-shoot ratio (Acosta-Motos et al. 2015) was observed with increased salinity levels. Other biochemical changes occurred in salt-stressed plants such as an increase in osmoprotectants and activities of antioxidant enzymes (Dawood et al. 2014). This information needs to be considered in selecting salt-tolerant species necessary for sustainable plant productivity (Fall et al. 2016).

Pterocarpus indicus Willd. f. *indicus* (Smooth Narra) is a native species in the Philippines belonging to the family Fabaceae. It grows up to 35 m height in primary and secondary forests at low and medium altitudes. It usually blooms annually from early March to late September, but most young fruits develop from April to June and mature ones from July to the following year. It has a C_3 photosynthetic pathway, which is common to most trees (Kozlowski and Pallardy 1997). This species is recognized for its hardiness, rapid growth, and a promising species for reforestation due to its nitrogen-fixing ability (Thomson 2006). It is one of the recommended species for the National Greening Program (Executive Order No. 26) of the Philippines which encourages the use of native or indigenous species. However, the success of any planting activity depends on how plants respond to the given site conditions. But there is still a lack of such information available. Few studies have been conducted to determine the effects of environmental factors on *P. indicus* seedlings. These include the studies of Baek and Woo (2010) on the effect of different air pollutant levels to the physiological and biochemical of *P. indicus*, Baek et al. (2018) on the effect of elevated ozone under well-watered and drought conditions, and Lok and Dell (2015) on the effect of phosphorus deficiency on the growth of *P. indicus* seedlings. Other related studies on *P. indicus* seedlings include the nursery seedling grading by Gazal et al. (2004), storage of seeds by Krishnapillay et al. (1994), and seedling growth technique for *Pterocarpus* spp. by Xu et al. (2004).

Several studies have also shown the effect of salinity such as in the study of *Caesalpinia crista* where seed germination and seedling growth were retarded (Patel et al. 2011), in the study of *Trifolium repens* where Mg in tissue was reduced (Cekstere et al. 2015), and in the study of Fabaceae species in Thailand where a slow rate of germination was observed as salinity levels increased (Ku-Or et al. 2020). However, no study has yet been conducted to determine the effect of salinity on the seeds and seedlings of *P. indicus*. Little is known about the mechanisms enabling trees to cope with high salinity for extended periods. Hence, in this study, we examined the effects of different salinity levels on the morphological, physiological, and anatomical structures of *P. indicus* f. *indicus*.

MATERIALS AND METHODS

Two pot experiments were established to determine the effects of varying salinity levels on the morpho-physiology and anatomy of the seeds and seedlings of *P. indicus* f. *indicus*. The seeds were used to determine the germination rate and to characterize the anatomy, while the seedlings were used to evaluate the percentage survival, morphological parameters, and photosynthetic rate.

Study area and pod collection

The experiment was conducted from August 2016 up to March 2017 in the nursery and Microtechnology Laboratory of the DFBS, CFNR, UPLB, Laguna, Philippines. The pods were collected from one of the identified candidate mother trees of *P. indicus* f. *indicus* (Smooth Narra) tagged in MMFR (elevation: 121 m a.s.l.; 14°9’18.5” N and 121°14’7.8” E). The tagged mother tree generally blooms annually and consistently provides a large number of pods. The collected pods contain three or more seeds. The pods were air-dried for two weeks prior to seed extraction. The extracted seeds were then subjected to the floatation test method to select viable seeds (Dayan and Reaviles 1995).

Experimental design and set-up

The experiment was performed using a simple complete randomized design with four treatments, including the control, having three replicates of 50 seeds, and 50 seedlings per replicate. There were 150 seeds and 150 seedlings per treatment, and a total of 600 seeds and seedlings. Garden soil was used as the potting environment for the seeds. The seedlings used were grown from seeds of different selected mother Smooth Narra trees in MMFR. We used only healthy and of uniform size six-month-old seedlings of *P. indicus* f. *indicus* without any deformations such as leaf defoliation and leaf rolling.

This study used four treatments, including the control (distilled water) and three different salt concentration levels (100, 200, and 300 mM). In previous studies on the effects of salinity on plant growth, minimal levels of salt concentration (i.e., 25 mM, 50 mM, 75 mM, 90 mM) did not yield a significant effect on the morphology as well as physiological functions (Glenn et al. 1999). Thus, in this study, the concentration levels were elevated from 100 mM in order to observe potential considerable changes. The salt solutions were prepared by dissolving 58.4 g, 116.9 g, and 175.3 g of sodium chloride (NaCl) in 1 L of water to make the 100, 200, and 300 mM NaCl solution, respectively. Seeds were soaked for 24 hours in each salt concentration and were germinated and examined for the anatomical changes (n = 60). The seedlings were watered (125 mL) twice a week at 08:00 a.m. for about two months. Observations were done every two weeks to assess seedling survival.

Morphological characteristics

The number of leaves, leaf area, height (stem length), number of root nodules, root collar diameter, and root-shoot ratio were measured in the laboratory. The number of leaves and number of root nodules was counted, while the height and RCD were measured using a ruler (cm) and digital vernier caliper (mm), respectively.

To determine the root-shoot weight ratio (Rogers et. 2019), oven-dry method was used by separating the root and the shoot of the seedlings. The leaf area was determined by the grid method (Dey et al. 2019) using a graphing paper (for tracing the leaves). In the seed experiment, shape, color, and size were also observed in the laboratory. The formulae used to compute for root-shoot ratio and leaf area are as follows:

$$\text{Root} - \text{Shoot Ratio} = \frac{Dry\ wt.\ of\ roots}{Dry\ wt.\ of\ stem}$$

$$\text{Leaf Area (cm}^2) = \text{Area of 1 grid x Total number of grids}$$

Physiological characteristics

For the survival percentage (Mishra et al. 2015) of seedlings, the following formula was used:

$$\text{Survival percentage} = \frac{Number\ of Seedlings\ Alive}{Number\ of\ Seedlings\ Used}\ x\ 100$$

To determine the photosynthetic rates of the plants subjected to salt stress, the uptake of CO_2, production of O_2, production of carbohydrates, and increase in dry mass of the seedlings were measured using the Portable Photosynthesis System (LI-COR) 6400 (Li-Cor Inc., NE, USA). Firstly, the LI-COR was stabilized at a relative humidity of 63%, CO_2 concentration of 400 ppm, and a temperature of 33.86 °C. When already stabilized, the leaves were inserted in the leaf chamber. The leaves were kept intact on the seedling (n = 60) during the measurement. The third leaf from the shoot of the seedlings, with an approximate leaf area of 4 cm^2, was used in the data collection. The observation period was from 08:00 a.m. until 04:00 p.m.

Seed anatomical characteristics

The transverse sections of the seeds (n=60) were obtained following the procedures of Johansen (1940). In each treatment, the specimens were randomly selected and cut into small pieces by 3 mm without damaging the radicle part. These were fixed in 1:1 ratio of FAA solution (Formalin: Acetic acid: Alcohol) for 2 h. Then, these samples were dehydrated in ethanol series for seven days. After dehydration, the samples were embedded into melted paraffin wax. The samples embedded in the wax were sectioned into thin slices of 10-15 µm using the microtome blade. The sections were stained with Safranin and Fast Green Dye solution. After staining, the specimens were coated with Entelan solution and then air-dried.

For the microscopic examination of the seeds, a compound microscope (Olympus CX 21) was used at the FBS Laboratory of CFNR-UPLB. The magnification used for photomicrographs were 100x and 400x. Identification of the structures of the radicle tissue was observed, while the number of cells and cell sizes were counted and measured, respectively. A total of 5 seeds per replicate (3 replicates) under various treatments (4 treatments) were used to characterize the anatomy. Haupt (1953), Fahn (1967), Bell (2008), and Shipunov (2020) were followed to describe the anatomy of the plant.

Statistical analysis

The one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to test the significance of the mean differences of *P. indicus* f. *indicus* in terms of height, number of leaves, leaf area, RCD, number of root nodules, root-shoot ratio, seed germination rate, and survival rate of the seedlings. All analyses were performed in R Studio version 4.0 (R Studio Team 2015).

RESULTS AND DISCUSSION

Morphological and physiological characteristics

The effects of varying salt concentrations on different morphological parameters of *P. indicus* f. *indicus* seedlings are presented in Table 1. In general, the six morphological parameters, except the leaf area, decreased significantly ($P < 0.05$) as the salt concentration increased. The leaf area (cm^2) showed no significant difference across the varying salt concentration.

Physiological characteristics

The effects of varying salinity of the physiological characteristics of *P. indicus* f. *indicus* seedlings are presented in Figures 1 and 2. It can be readily gleaned in these figures that seed germination, seedling survival, and photosynthetic rates progressively decrease with increasing salinity levels.

Seed germination and seedling survival

Both the mean seed germination and seedling survival percentages of the three salt concentrations were significantly different from the control and generally with each other (Figure 1.A and 1.B).

Photosynthetic rate

The photosynthetic rates of seedlings measured in all the treatments are shown in Figure 2. Increasing photosynthetic rates with PAR increased was observed in the control and also a less deep increase was observed under 100 mM and 200 mM treatments. There were significantly similar photosynthetic rates among the three varying salt concentrations and these were significantly lower than the control ($P < 0.0123$).

Table 1. Effect of three NaCl concentrations on the morphology of *P. indicus* f. *indicus* seedlings. Standard errors are in parentheses

NaCl (mM)	Seedling height (cm)	Leaf number	Leaf area (cm²)	Root collar diameter (mm)	Root nodules	Root-Shoot ratio
0	13.26 (0.10)^{a}	7.55 (0.16)^{a}	2.62 (0.29)^{a}	1.69 (0.06)^{a}	22.67 (1.76)^{a}	0.55 (0.03)^{a}
100	12.90 (0.02)^{b}	5.77 (0.39)^{b}	2.52 (0.22)^{a}	1.60 (0.02)^{ab}	17.33 (4.33)^{ab}	0.51 (0.00)^{a}
200	12.75 (0.05)^{bc}	3.29 (0.67)^{c}	2.46 (0.17)^{a}	1.61 (0.05)^{ab}	9.33 (2.60)^{bc}	0.48 (0.02)^{ab}
300	12.63 (0.02)^{c}	1.50 (0.27)^{d}	2.25 (0.30)^{a}	0.88 (0.45)^{b}	2.67 (1.45)^{c}	0.39 (0.05)^{b}

Note: Different superscripted letters within a column indicate statistically significant differences among the treatments according to Duncan Multiple Range Test (DMRT) ($P < 0.05$)

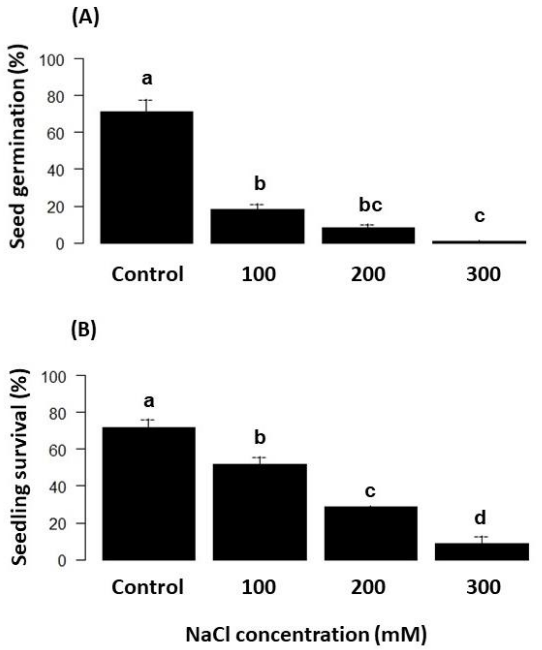

Figure 1. Effect of the varying NaCl concentrations on the (A) seed germination (in %) and (B) seedling survival (in %) of *Pterocarpus indicus* f. *indicus*. Different letters indicate statistically significant differences among the treatments according to DMRT (P<0.05)

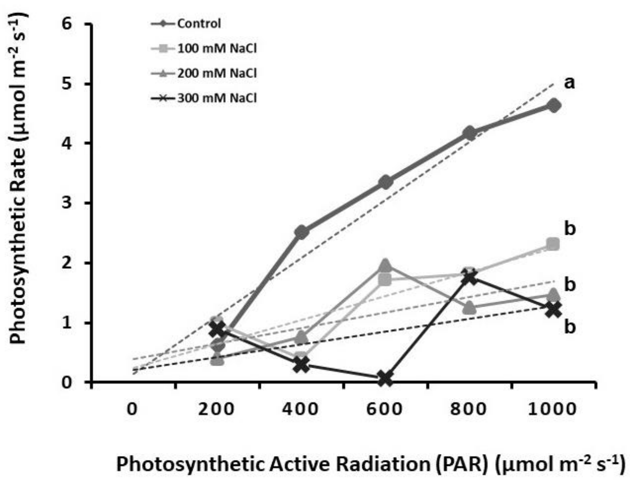

Figure 2. Mean photosynthetic rates of *Pterocarpus indicus* f. *indicus* in all the NaCl concentrations. Significantly different ($P < 0.05$) treatment results bear different letters

Seed anatomical characteristics

The Smooth Narra seeds are flattened, bean-shaped with a size of 6-8 mm long and with a leathery, brittle seed coat. The transverse sections of the seed specifically the radicle is shown in Figure 3. The seed radicle was examined because it is where the primary root occurs. The images shown were viewed under 100x. The figure shows that as NaCl increases the cell structure differs from that of the control. In Figure 3.A (control), the structure of the radicle had no abnormalities. There was no evidences that the cells are damaged. In Figure 3.B (100 mM), the radicle (left) exhibited partly ruined cells and appeared to have tiny black spots in the radicle tissue (right). On the other hand, in Figure 3.C (200 mM) the radicle tissue showed more spatially distributed damaged cells (left), and similar to Figure 3.B, black spots could be observed inside the cells. In Figure 3.D (300 mM), more damaged cells were observed compared to the other treatments applied. Both Figure 3.C and 3.D showed damage cells of the radicle tissue.

The images on the right panel of Figure 3 showed the representative images of the radicles of the control and at those of the varying NaCl treatments at the higher magnification. Cells were counted from a sample within a box of 1 cm^2 in the middle of the radicle to eliminate variation. In the control, the number of cells per box ranges from 50 to 100 cells; 30 to 50 in 100 mM; and, at most 30 cells in 200 mM and 300 mM. This clearly showed that the number of cells decreased as the NaCl concentration increased. In the 200 mM and 300 mM NaCl, the cells were no longer intact compared to the control and 100 mM NaCl. The cell sizes were also measured and compared (n=60). In control, the length of the cell varies from 1 to 4 µm; in 100 mM the lengths are less than 1 to 3 µm, and in 200 mM and 300 mM the lengths are less than 1 to 2 µm each.

Discussion

This study showed that *P. indicus* f. *indicus* generally affected both morphologically and physiologically during the early stage of its growth (Table 1). The decrease in the height of seedlings with increasing NaCl concentrations in this study is similar to the results of other salinity-related studies. For instance, there was a significant reduction in height increment of *Pteroceltis tatarinowii* with increasing NaCl concentration 50 days after treatment (Fang et al. 2006). Also, at 150 mM NaCl the growth of *Phaseolus coccineus* and *P. vulgaris* were reduced by 40% (Hassan et al. 2016). In contrast, there are species that can tolerate high saline conditions such as mangroves species. For example, *Avicennia officinalis'* growth was attributed to the increase in NaCl concentration up to 128 mM (Saravanavel et al. 2012). For salt-tolerant species like *Atriplex portulacoides*, a range from 200 mM to 700 mM NaCl can maintain the plants' growth (Benzarti et al. 2014). Munns (1992) concluded that the salts absorbed by plants do not control growth directly, but it influences turgor, photosynthesis, and/or the activity of specific enzymes. According to Kotagiri and Kolluru (2017), plant growth is affected by reduction in the water uptake, lack of nutrients, and accumulation of toxic sodium and chloride ions.

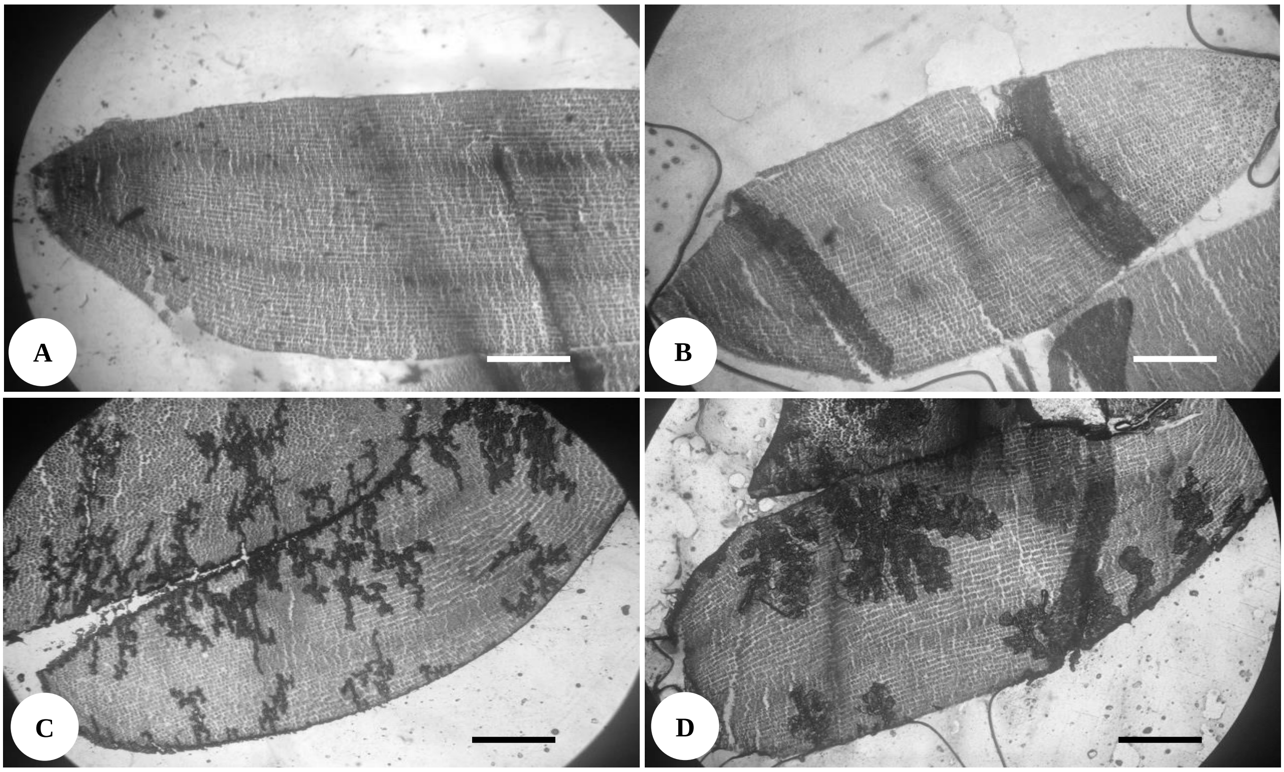

Figure 3. Anatomical characteristics of the seed radicle of *Pterocarpus indicus* forma *indicus* subjected to varying NaCl concentrations A. control (distilled water., B. 100 mM, C. 200 mM, and D. 300 mM viewed under the Olympus compound microscope at 100x magnification; scale bar is 100 µm

Similar to other studies, high salt concentrations inhibit the production of new leaves on *P. indicus* f. *indicus* seedlings and render leaves less turgid causing leaves to wilt easily. In some species like *Physalis peruviana* a reduction in the number of leaves could be already observed in as low as 60 mM NaCl (Miranda et al. 2014). Sohail et al. (2009) similarly observed a reduction (68% and 72%) in the number of leaves of *Ziziphus spina-christi* at 80 and 160 mM NaCl, respectively. When plants cannot generate more leaves, photosynthesis is inhibited and consequently, its productivity. According to Dolatabadian (2012), one probable reason for the low leaf number is the inhibition of the formation of leaf primordia under salinity stress. Since leaf expansion is sensitive (Walker and Bernal 2008), the presence of NaCl is critical as it causes a decrease in leaf growth (Miranda et al. 2014).

Although no statistically significant difference was observed among treatments in terms of leaf area (Table 1), a decreasing tendency with increasing NaCl concentration could be observed. Cramer et al. (1994) and Curtis and Lauchli (1986) observed that the leaf area ratio is significantly reduced in some species under salt stress. However, in *Z. spina-christi* as mentioned previously, there was also a tendency of reduction in the leaf area with increasing salt concentration but not statistically different from the control. According to Munns (2002), with the plant's long-term exposure to NaCl, they develop mechanisms to avoid excessive concentration of ions in the transpiring tissues as the leaves expand. High salt concentrations reduce plant growth (Munns and Tester 2008), and thus, limiting leaf expansion (Cramer 2002). The reduction of specific leaf area indicates that the leaf expansion and carbon allocation among the leaves are altered. Since it is the commonly used parameter to compare the thickness and/or density of leaves, this suggests that leaf area is primarily influenced by the environmental conditions such as availability of moisture, nutrients, and light, and changes in photosynthetic capacity like canopy size (Cunningham et al. 1999; Li et al. 2000; Pinkard and Beadle 1998). The seedlings also exhibited observable injuries in the leaves such as chlorosis and necrosis especially at 200 and 300 mM NaCl. These were also seen in the study of Gupta et al. (2002) devoted to the Indian jujube subjected to 80 and 160 mM NaCl where severe foliar injuries and shedding of affected leaves were observed. According to Sohail et al. (2009), significant decline in the plant height and number of leaves lead to reduction in leaf area with increasing NaCl stress.

Similar to height, a decreasing trend is observed in the RCD of *P. indicus* f. *indicus* with the increasing concentration of NaCl (Table 1). Jimenez-Casas and Zwiazek (2014) observed that *Pinus leiophylla*'s stem diameter growth decreased with increasing NaCl concentration. Also, the RCD of a native dryland agroforestry tree species, *Acacia tortilis*, was severely impacted by salinity (Seid et al. 2016). For *Senegalia senegal*, *Vachellia seyal*, and *Prosopis juliflora*, Fall et al. (2016) observed that salinity reduced significantly the

RCD. In contrast, salinity had no effect on the RCD of *Balanites aegyptiaca* (Seid et al. 2016). In addition, the greenhouse experiment of Duan (2015) demonstrated the effectiveness of N fertilization in improving the growth of seedlings in a saline growth media, resulting in improved RCD growth for both *Pinus contorta* and *Picea glauca* seedlings.

The effect of NaCl on nodulation has been examined in several investigations (*e.g.*, Ikeda et al. 1992; Delgado et al. 1994; El-Hamdaoui et al. 2003; Kekere 2014). For example, acetylene reduction activity and respiration of the alfalfa nodules decreased to 96% and 84% at 210 mM NaC1, and to 48% and 68% at 420 mM, respectively (Ikeda et al. 1992). Also, when salt was added directly to the incubation mixture of bacteroids, the O_2 uptake of bacteroids isolated from nodules of pea and faba-bean was inhibited (Delgado et al. 1994). Moreover, salinity produced a decrease in B and Ca contents in nodulated roots of *Pisum sativum.* Lastly, it was observed by Kekere (2014) that root nodules of *Arachis hypogaea* declined with increase in NaCl concentration. Similarly to this study, the decreased production of root nodules with increase in NaCl concentration was observed in other Fabaceae species such as *Acacia ehrenbergiana* and *A. tortilis* (Al-Shaharani and Shetta 2011) and *Sesbania rostrata* (Joshua et al. 1995). However, in the study of Borucki and Sujkowska (2008) on *Pisum sativum* the use of 25 mM NaCl stimulated the root nodule formation compared to the control. According to Frankow-Lindberg and Dahlin (2013), the ease in plant activity reduces the nitrogen fixation of plants caused by any stress. The effect of salinity on nitrogen fixation processes can be discussed in terms of total nodule dry weight per plant and the nitrogen-fixing activity of nodule (Reddell and Bowen 1985).

While there is also a tendency of decrease in root-shoot ratio with increase in NaCl concentration in this study, significant reduction was detected only at the 300 mM NaCl concentration (Table 1). Viégas et al. (2001) similarly observed low root-shoot ratio at increase in the NaCl concentration (0, 50, and 100 mol m^{-3}) in *Anacardium occidentale*. Conversely, Àlvarez and Sánchez-Blanco (2014) observed an increased root-shoot ratio and improved the root system in *Callistemon citrinus* plants using saline water. Similarly, Acosta-Motos et al. (2015) observed increase in the root-shoot ratio in *Myrtus communis* plants irrigated for one month with NaCl solution with an EC of 8 dS m^{-1}. The increase or decrease in shoot to root ratio is a common response to salt stress, which is related to factors associated with water stress (osmotic effect) rather than a salt-specific effect (Hsiao and Xu 2000). High salt concentrations result in changing the relationship between the aerial and root parts (Tattini et al. 1995).

Seed germination is the most crucial stage in a plant's life cycle. And when seeds are exposed to saline soils it can be critical to their salt tolerance levels (Khan et al. 2000 as cited by Li 2008). In this study, the increase in concentration of NaCl significantly reduced the germination of *P. indicus* f. *indicus* seeds (Figure 1A). Previous studies in *Cedrela odorata* (Ferreira et al. 2013), *Melaleuca quinquenervia* (Martins et al. 2011), and *P. tatarinowii* (Fang et al. 2006) also showed the adverse effects of NaCl on the speed and percentage of seed germination. Specifically in *P. tatarinowii*, the seed germination was significantly inhibited by NaCl concentration exceeding 17 mM. Seed germination shows that most of the seeds attain their maximum germination under the control experiment and become very sensitive to increase in salinity (Li 2008). Salt stress negatively affects the germination of the seeds, osmotically through reduction of water or ionically by accumulation of Na^+ and Cl^- (Shokohifard et al. 1989), causing an imbalance in the nutrient uptake and making it toxic for the plant (Song et al. 2008).

The results showed that *P. indicus* f. *indicus* seedlings can tolerate certain amount of NaCl as there are still surviving seedlings at the 300 mM NaCl concentration (Figure 1B). However, the percent survival under this treatment was considerably low compared to other treatments. This response is the same as in the numerous plant species affected by salinity stress during germination (*e.g.*, Katembe et al. 1998; Ghazy et al. 2009; Liu et al. 2010; Raddi et al. 2019). In the study of Fang et al. (2006), there was a significant reduction in the seedling survival with increase in NaCl concentration after 50 days of treatment. However, some species like the fast-growing leguminous trees, *Acacia ampliceps* and *A. nilotica*, can tolerate the high salinity level (Abbas et al. 2013). Seeds of halophytes or plants inhabiting highly saline waters have been found to germinate even after prolonged exposure to hypersaline conditions (Saranvanavel et al. 2018). According to Hasanuzzaman et al. (2014), some mangrove species, for example, *Avicennia*, *Aegialitis*, *Aegiceras*, and *Acanthus* possess multicellular salt glands and salt hairs to capture salt from mesophyll cells beneath them where a layer of salt crystals is formed. However, halophytic plants differ in the capacity to recover from exposure to salinity (Keiffer and Ungar 1997). Survival of the plants depends on all the factors available. But depending on the inherent nature of the species, plants may either adapt or die with the stress that causes them to deteriorate (Gull et al. 2019).

At varying levels of NaCl, the *P. indicus* f. *indicus* seedlings exhibited varying photosynthetic rates. Seedlings subjected to NaCl concentrations tend to increase to a certain photosynthetic active radiation (PAR), but later display fluctuating pattern as shown in Figure 2. Light responses of photosynthesis showed that net photosynthetic rate (P_N) values were continuously raised with the increase of photosynthetic photon flux density (PPFD) at all salinity levels and plants treated with 600 mmol L^{-1} salinity suffered from photoinhibition with the lowest P_N values (Qin et al. 2013). This negative response from the increased NaCl concentrations was also true in *P. tatarinowii* (Fang et al. 2006). Under salinity, when there is a reduction in photosynthetic rate, photo-oxidative damage to the photosynthetic machinery may happen (Mustafa et al. 2014). Romero-Aranda et al. (1998) studied anatomical disturbances produced by chloride salts (including NaCl) in both sensitivity (*Carrizo citrange*) and tolerant (*Cleopatra mandarin*) citrus varieties. The salt-induced declines in net photosynthesis (P_N) were linked to changes in the leaf anatomical properties such as the increase in leaf thickness and the lower area/volume ratio of mesophyll cells. Salinity also increased the succulence of leaves and reduced the intercellular air spaces, the surface/volume ratio of cells, and tissue density. More than 80% of the angiosperms are C_3 plants wherein the photosynthetic CO_2 assimilation (μmol m^{-2} s^{-1}) follows the trend that at the maximum rate of photosynthesis as the saturation point, any further increase in photosynthetic active radiation (PAR) beyond this point will no longer result in more light fixation (Taiz and Zeiger 2003).

Figure 3 shows that at 100 mM NaCl the cells of the radicle tissue of *P. indicus* f. *indicus* started to show damages or disruptions of cells. This was also evident in the anatomical modifications of the radicle and hypocotyl of cotton induced by NaCl where the radicle growth decreased under 100 meq L^{-1} and 250 meq L^{-1} (Casenave et al. 1999). Given that there are distinguishable changes in the cell tissues of the radicle as the NaCl concentration increases, the assumption is that the ability of the radicle to become the primary root of the plant is inhibited (Koning 1994). Moreover, according to Bliss et al. (1984), salinity induces changes in the lipid composition of some plants, which serves as an important structural modification to achieve membrane compartmentation by separating the salts from sensitive cellular processes. As a result, the amount of NaCl or loss of water can cause some changes in the cell membrane.

In terms of morphological characteristics, the height and number of leaves showed significant changes in 200 mM and 300 mM NaCl. Root collar diameter and number of nodules showed significant changes in 100 mM, 200 mM, and 300 mM NaCl. Root-shoot ratio only showed significant changes in 300 mM NaCl, and leaf area did not show significant changes in all NaCl concentrations applied. In terms of physiological characteristics, germination and survival rates showed significant changes in 100 mM to 300 mM NaCl while photosynthetic rate did not show significant changes to the NaCl concentrations. And in terms of anatomy, the radicle tissue showed immediate change in its cells under the 100 mM NaCl concentration. It can be concurred that using the NaCl concentrations from 100 mM to 300 mM, the species can thrive in more than 100 mM but less than 200 mM NaCl concentrations. In this study, the best parameters to measure the salt sensitivity of *P. indicus* are height, number of leaves, root collar diameter, root-shoot ratio, and anatomy of the seed as these showed significant changes after salt application and will determine if the species can really survive in saline conditions. The observed parameters are similar to the studies of Fang et al. (2006) for the height, Miranda et al. (2014) for the number of leaves, Jimenez-Casas and Zwiazek (2014) for root collar diameter, Viégas et al. (2001) for the roo-shoot ratio, and Casenave et al. (1999) for the radicle growth.

Therefore, it is concluded in this study that *P. indicus* f. *indicus* seeds and seedlings can only tolerate up to moderate level of saline condition. The results of this study are crucial in using *P. indicus* as a reforestation species. The species has limitations if planted in areas of high saline soil. Although *P. indicus* can only tolerate an appreciable amount of salts, its role as a nitrogen-fixing species will help facilitate the conversion of free nitrogen in the soil to ammonia, which the plant can utilize for its development.

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