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RESEARCH

Germination and seedling growth of closely related native and invasive legume trees in Nepal

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Background: This study compares seed germination and seedling growth parameters of native *Senegalia catechu* with its closely related invasive *Leucaena leucocephala* in Nepal. For the comparison of seed germination percentage (GP), mean germination time (MGT), and Timson's index (TI), the seeds of both species were incubated under different light (photoperiod and dark), temperatures (30/20ºC and 25/15ºC) and water stress conditions (−0.1, −0.25, −0.5, −0.75, and −1 MPa). The seedling emergence from different soil depths was also evaluated. The relative growth rate (RGR), root mass fraction (RMF), stem mass fraction, leaf mass fraction (LMF), and root-to-shoot ratio (RSR) of seedlings were also measured.

Results: The seed length and mass of invasive *L. leucocephala* were higher than that of native *S. catechu*. The GP of *S. catechu* was higher at high temperature and photoperiod comparing to *L. leucocephala*. There was no difference in GP between two species under other light and temperature conditions. The MGT of *S. catechu* was shorter than that of *L. leucocephala* at both temperatures. *Senegalia catechu* exhibited higher TI than *L. leucocephala*, particularly at high temperatures. Water stress above −0.5 MPa reduced the GP and TI of both species and it was more pronounced in *S. catechu* than *L. leucocephala*. The seedling emergence percentage of *L. leucocephala* was higher than that of *S. catechu*. Both species exhibited comparable RGR and biomass allocations (RMF, LMF, and RSR). However, *L. leucocephala* had always greater values of shoot height, root length, leaf number and seedling biomass compared to *S. catechu*.

Conclusions: Larger seeds may not always lead to higher seed GP. Some, but not all, seed germination and seedling growth traits can be useful to characterize invasive alien plant species. Invasiveness of *L. leucocephala* could be attributed to relatively high tolerance of seed germination to water stress, capacity to germinate from deeper soil, and larger seedling size compared to the confamilial native species.

Keywords: invasiveness, *Leucaena leucocephala*, relative growth rate, *Senegalia catechu*, Timson's index

Introduction

Seed germination and early growth stages are critical phases in a plant's life cycle that can significantly influence its ability to establish, persist, and interact with its environment. This can have a major effect on the invasive potential of plant species (Gioria and Pyšek 2017). Phylogenetically closely related native and invasive species may differ in life history and growth traits, and such differences in traits can be associated with invasiveness of the successful invaders (Sutherland 2004). Therefore, comparisons of closely related native and invasive species are useful for identifying the characteristics of successful invaders as this approach minimizes biases associated with phylogenetic distance and habitat affinities of the species compared (Gravuer et al. 2008; Kharel et al. 2024). This could also lead to a better understanding of the traits that determine which plant species will be invasive, how invasive these species will be, which habitats they might invade, and how the species can be controlled.

Previous studies analyzed the role of functional traits such as specific leaf area and CO_2 assimilation (Baruch and Goldstein 1999; Grotkopp and Rejmánek 2007), root mass allocation and nitrogen fixation ability (Morris et al. 2011), phenotypic plasticity (Richardson and Pyšek 2006) and reproductive characteristics (van Kleunen et al. 2007) for plant invasiveness. For example, enhanced seed germination and high relative growth rate (RGR) of seedlings are key traits that can contribute to the successful invasion of several alien plant species into new environments (Burns 2004; Kharel et al. 2024; Mandák 2003). Early and rapid germination of seeds allows the plants to establish swiftly, exploiting available resources and creating dense populations thereby displacing the native species. Additionally, these traits enhance plants' resilience and adaptability, and give a competitive edge, facilitating their spread and ecological impact (Hess et al. 2019; Pyšek and Richardson 2007; Wainwright et al. 2012).

Considering the importance of traits for plant invasiveness, it is wise to compare the traits between native and invasive species. The same genera or closely related species exhibit similar traits related to resource use and often show competitive interactions (MacLeod et al. 2000; Mangla and Callaway 2008). Identifying such traits has great potential to know how some of the species become invasive and how they compete with native relatives. Understanding the influence of environmental conditions such as temperature, light, and water stress would be helpful in predicting future invasions and also in developing control and management strategies for invasive alien plants (Fournier et al. 2019).

This study is focused on the characterization of growth performance of two nitrogen fixing tree species (*Leucaena leucocephala* and *Senegalia catechu*) of the family Fabaceae exposing them to different environmental conditions. *Leucaena leucocephala* (native to Central America and the Yucatan Peninsula of Mexico) is a medium-sized tree and a problematic invasive species in many parts of the world including Nepal (Shrestha et al. 2024; Witt et al. 2018). In the introduced region, the species outcompete native vegetation, alters nutrient cycling and soil chemistry, and modifies ecosystem processes (de Sousa Machado et al. 2020). *Senegalia catechu* is a native tree of tropical and subtropical regions in South and South-east Asia, and shares habits and habitats with *L. leucocephala* in Nepal. Understanding germination ecology and seedling development characteristics of both species can provide a reliable insight on the probabilities of further invasions of *L. leucocephala.* The objective of this study is to compare the germination and seedling growth parameters of *S. catechu* and *L. leucocephala,* with the goal of enhancing our capacity to predict invasiveness of *L. leucocephala* in novel habitats.

Materials and Methods

Test species

Senegalia catechu ***(L.f.) P.J.H. Hurter & Mabb.***

Senegalia catechu, formerly known as *Acacia catechu* (L.f.) Willd., is a moderate-sized deciduous tree native to South and South-east Asia including Nepal (Orwa et al. 2009). It occurs naturally in mixed deciduous forests of lower mountains and hills in subtropical or tropical regions from 100 to 1,500 m above mean sea level (a.s.l.) (Paudel et al. 2011). It prefers sandy or alluvial soils of riverbanks and watersheds. Heartwood of this tree is a valuable source of catechu that contains a high concentration of tannins and is used for dyeing, coloring foods and beverages, treating several ailments as traditional medicines, and production of pharmaceuticals and cosmetics (Ansari et al. 2018; Hakim et al. 2023; Kumari et al. 2022). The plant has been included among the least concern species in the International Union for Conservation of Nature Red List, though, cutting, harvesting, and habitat degradation of the species is contributing decreasing trend of its population (Plummer 2021).

Leucaena leucocephala ***(Lam.) de Wit***

Leucaena leucocephala, native to Central America (Bakewell-Stone 2023), was introduced to Nepal in 1981 as a fodder plant in the agroforestry system (Dhakal et al. 2022). This species was also introduced to several other countries in Africa and Asia as agroforestry species (Bakewell-Stone 2023). It prefers open habitats such as riverine, disturbed and degraded habitats, and other ruderal sites. It has been reported as one of the 100 of the World's worst invasive alien species (Lowe et al. 2000) and its invasion has significant ecological impacts. For example, its monodominant stand can displace native species and its allelopathic effects reduce germination and growth of co-existing other plant species (Mello and Oliveira 2016; Wolfe and Van Bloem 2012). The species is widespread in several South and Southeast Asian countries, and it is currently in the process of naturalization in Nepal, with a high potential to spread into the natural ecosystems (Shrestha et al. 2022, 2024). Self-regeneration of *L. leucocephala* has been observed in the landscapes where *S. catechu* is naturally found in Nepal.

Petri plate assay

Seed collection, seed size and mass

Seeds of both species were collected from Gajuri Rural Municipality of Dhading district, Bagmati Province, Nepal. *Leucaena leccocephala* seeds were collected from a marginal land of agricultural fields (84.83°E, 27.78°N; elevation 505 m a.s.l.) in January 2022. Similarly, seeds of *S.*

catechu were collected from the same landscape (84.84°E, 27.79°N; elevation 435 m a.s.l.) in February 2022. These months (January–February) experience an average temperature of 12.5°C. June is the warmest month in the seed collection site with a mean temperature of 24.7°C. Average annual temperature and precipitation of the seed collection site are 20°C and 2,428 mm, respectively (https://en.climate-data.org).

The seeds were transported to the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Three replicated groups of mature seeds (25 seeds per group) of each species were oven-dried (70°C for 48 hours) to measure seed mass. Size (length and breadth) of randomly selected 20 seeds of each species were measured with the help of a trinocular stereomicroscope. The remaining seeds were stored in air-tight plastic containers with silica gel at 4°C in the refrigerator until use for germination experiments.

Seed germination experiments

A preliminary test on seed germination was conducted, which found that the fresh seeds of *L. leucocephala* did not germinate, whereas the seeds of *S. catechu* germinated. Water imbibition test of the seeds of *L. leucocephala* suggested that it had a physical dormancy due to the impermeable seed coat. Therefore, the physical seed dormancy of *L. leucocephala* was broken by soaking seeds in hot water at 70°C for 10 minutes. Untreated seeds of *S. catechu* were used as this species did not show seed dormancy. Therefore, the experiment compared the germination behavior of seeds of *L. leucocephala* after breaking physical dormancy and the seeds of *S. catechu* without any pre-treatment.

Thirty uniform-sized seeds were sown evenly across the surface of 9-cm-diameter petri plates, each containing 40 g of sieved sand (mesh size 1 mm) as a substrate. The substrate in each petri plate was moistened with 15 mL of distilled water. The plates were sealed with parafilm to prevent moisture loss from the substrate and incubated in the growth chamber (Model: GC-300TLH; Jeio Tech, Daejeon, Korea).

The seeds were incubated under two temperature regimes that were 25/15°C (light/dark), designed as low temperature and 30/20°C, designed as high temperature. This setup aimed to understand how the seeds of both test species will show the response to rising temperatures in the environment and also, the approach is expected helpful to anticipate the effect of future climate change on seed performance. The conditions to evaluate the effect of temperature and light on seed germination were 1) low temperature (25/15°C) and 12-hour photoperiod, 2) low temperature and complete dark, 3) high temperature (30/20°C) and 12-hour photoperiod, 4) high temperature and complete dark.

The petri plates were wrapped with two layers of aluminum foil until harvest in case of the dark treatments. In the photoperiod conditions, the light intensity set in the growth chamber was ca. 55.5 μmol $s^{-1}m^{-2}$ and the relative humidity was 70%. There were five replicated petri plates for each treatment with a total of 150 seeds included in each treatment. Germination was observed daily for 20 days.

Seeds were also incubated under variable water stress conditions (water potentials −0.1, −0.25, −0.5, −0.75, and −1 MPa) to evaluate the effect of water stress on germination. Polyethylene glycol (PEG) solution of −1 MPa was prepared by dissolving 296 g of PEG in distilled water to prepare 1 L of PEG solution (Michel and Kaufmann 1973). Other concentrations were prepared by serial dilution method. The seeds in these treatments were incubated at 25/15°C (light/dark) for 12-hour photoperiod. Petri plate replications and observation frequency were the same as mentioned above.

Pot assay

In this set of experiments, the effect of seed-sowing depth on seedling emergence was evaluated and seedling growth traits were measured. To assess the effect of sowing depth, the seeds of the selected plants were sown in polyethylene pots (12 cm depth) in a greenhouse. As mentioned above, seeds of *L. leucocephala* were subjected to hot water treatment to break dormancy before use in this experiment. The mean temperature was 27°C ± 3°C with the light intensity of ca. 115 ± 68 μmol $s^{-1}m^{-2}$ in the greenhouse throughout the experiment.

The pots were filled with a mixture of sand, vermicompost, and cocopeat in a ratio of 7:3:1 by volume. Vermicompost and coco-peat were supplied to enrich the mixture with organic matter and retain the moisture, respectively. Thirty uniform seeds of each species were sown in each pot at the depth of 0 cm (surface), 2 cm, 4 cm, and 6 cm to observe the effect of soil depth on seedling emergence. There were 5 replicated pots for each treatment, with 150 seeds in each depth. Regular watering using an equal volume of water was done to avoid drought and maintain uniform moisture level. The seedlings that emerged were counted every day and the emergence percentage (EP) was calculated. The experiment was terminated 28 days after sowing the seeds.

Additionally, in another set of pots, each pot containing mixture of soil, sand, vermicompost, and cocopeat in the ratio 3:3:3:1, three seeds of each species were sown into every pot (2 cm below the soil surface) to measure seedling growth parameters. After the seedlings had their first true leaf, they were thinned to a single plant in each pot to reduce crowding. Altogether there were 80 pots for each species. Plants were harvested four times to measure the RGR (Pérez-Harguindeguy et al. 2013). Twenty plants of each species were harvested 62 days after sowing and the remaining three harvests were carried out at two-week intervals. During each harvest, the number of leaves and plant height were measured. Roots of harvested plants were washed carefully ensuring no damage, and length of the main axis was measured. Then, the aerial part (stem and leaf, separately) and roots were oven-dried at 80°C for 72 hours to measure dry mass.

Data analyses

As the germination parameters, seed germination percentages (GP), Timson's index (TI), and mean germination time (MGT) were calculated (Baskin and Baskin 2014).

$$\mathrm{GP} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

$$\mathrm{TI} = \Sigma n, \text{ where } n = \text{cumulative daily GP}$$

$$\mathrm{MGT} = \sum_{i=1}^{k} n_i t_i \Big/ \sum_{i=1}^{k} n_i$$

where, t_i = time from the start of the experiment (day), n_i = number of seeds germinated at time t.

Seedling EP was calculated using the formula:

$$\mathrm{EP} = \frac{\text{Number of emerged seedling}}{\text{Total number of seeds sown}} \times 100$$

From seedling harvest data, the stem mass fraction (SMF), root mass fraction (RMF), leaf mass fraction (LMF), and root-to-shoot ratio (RSR) were calculated using the following formulae (Poorter et al. 2012):

$$\mathrm{SMF} = \frac{\text{Stem dry mass (g)}}{\text{Total dry mass of plant (g)}}$$

$$\mathrm{RMF} = \frac{\text{Root dry mass (g)}}{\text{Total dry mass of plant (g)}}$$

$$\mathrm{LMF} = \frac{\text{Leaf dry mass (g)}}{\text{Total dry mass of plant (g)}}$$

$$\mathrm{RSR} = \frac{\text{Root dry mass (g)}}{\text{Stem dry mass + leaf dry mass (g)}}$$

Independent sample t-test was used to compare the mean values of GP, TI, and MGT between two species. The data of GP was arcsine transformed as the data did not meet the assumption of normality. One-way analysis of variance (ANOVA) was used to compare the germination traits among different water stress treatments and seedling growth traits among harvest events. Mean values of seedling growth parameters between species were compared using independent sample t-test. Two-way ANOVA was used to know the effect of interactions of water stress and species on seed germination, and the seed sowing depth and species on seedling emergence. All the statistical analyses were carried out using the software Statistical Package for Social Science (SPSS) version 23 (IBM Co., Armonk, NY, USA).

Results

Seed mass and seed size

The seed mass of invasive *L. leucocephala* was two times higher than that of native *S. catechu* (Table 1). Seeds of *L. leucocephala* were also longer than those of *S. catechu,* with the seed breadth of these two species being nearly equal.

Effect of light and temperature on seed germination

Seeds incubated under both 12-hour photoperiod and complete dark germinated well, with > 50% germination of both *S. catechu* and *L. leucocephala* (Fig. 1). The GP of *S. catechu* was higher at high temperature and 12-hour photoperiod than that of *L. leucocephala* ($p < 0.001$). There was no difference in GP between two species under other light and temperature conditions. Rise in temperature led increased GP in *S. catechu* under both photoperiod and complete dark condition ($p < 0.05$). Nonetheless, in *L. leucocephala,* such an increase in the GP was only evident under complete dark condition (Fig. 1, Table S1).

The MGT of *S. catechu* was shorter than that of *L. leucocephala* at both low and high temperatures ($p < 0.001$, Fig. 2A). In both species, the MGT became shorter when the temperature increased ($p < 0.05$, Fig. 2A, Table S2). The difference in the TI between the two species was temperature dependent; at high-temperature *S. catechu* had a higher TI than that of *L. leucocephala* ($p < 0.001$). Such difference was not observed at low temperatures. As expected, the TI of *S. catechu* was higher at high temperatures compared to low temperature ($p < 0.01$). In contrast, there was no such difference between low and high temperatures in TI of *L. leucocephala* (Fig. 2B, Table S2).

Effect of water stress

Water stress up to −0.5 MPa did not have an impact on the GP and TI of both species (Fig. 3). With increasing wa-

Table 1 Mean (± standard error of the mean) seed mass (n = 3) and size (n = 20) of the study species

Seed traits	*Senegalia catechu*	*Leucaena leucocephala*
Mass (g)	0.6 ± 0.03^b	1.2 ± 0.02^a
Seed size		
Length (mm)	5.95 ± 0.80^b	8.06 ± 1.50^a
Breadth (mm)	5.24 ± 0.84^a	5.39 ± 0.08^a

Significant difference in the mean values between two species are indicated by different alphabets in superscript ($p < 0.001$, independent sample t-test).

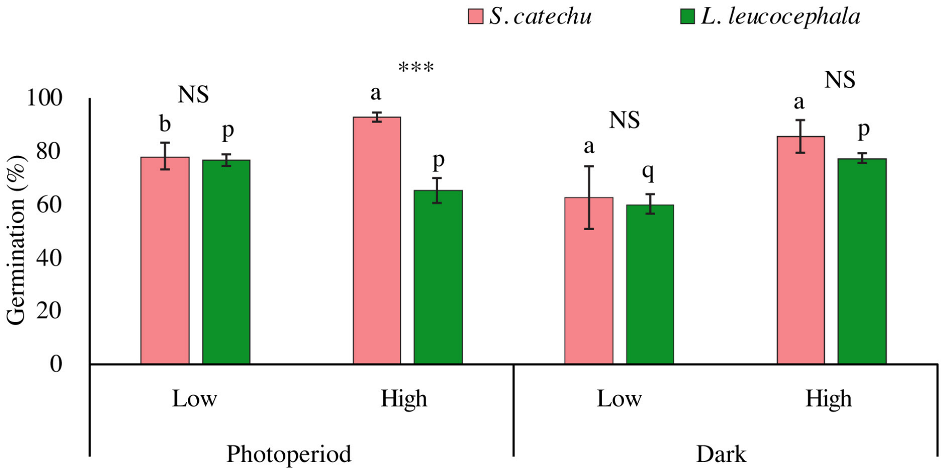

Fig. 1 Effect of light and temperature on germination of *Senegalia catechu* and *Leucaena leucocephala*. Error bars represent ± 1 standard error of the mean. The symbol *** represents significant difference at $p \leq 0.001$ and NS represents no difference between two species within each low and high temperature condition (independent sample t-test). Different alphabets 'a, b' and 'p, q' above error bars represent significant difference between low and high temperature (under photoperiod or dark condition) within *S. catechu* and *L. leucocephala*, respectively ($p \leq 0.05$, independent sample t-test).

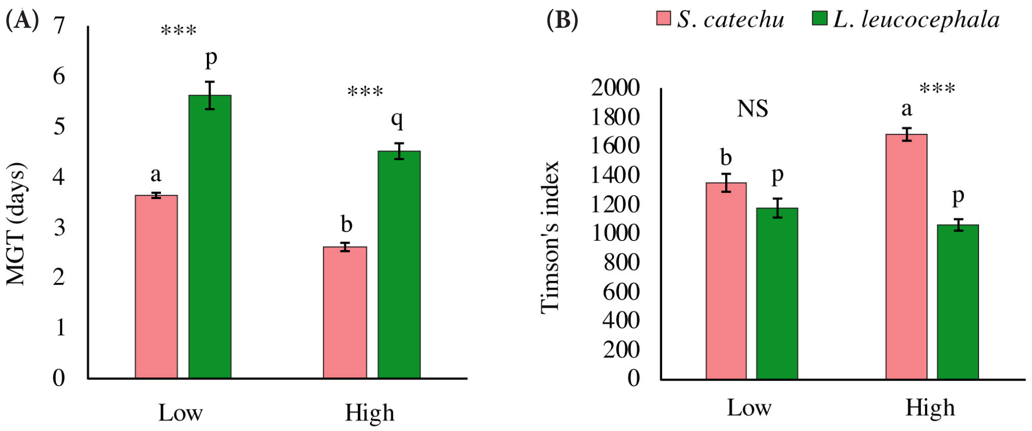

Fig. 2 Effect of temperature on (A) mean germination time (MGT) and (B) Timson's index. Error bars represent ± 1 standard error of the mean. The symbol *** represents significant difference at $p \leq 0.001$ and NS represents no difference between two species (independent sample t-test). Different alphabets 'a, b' and 'p, q' above error bars represents significant difference between low and high temperatures within *Senegalia catechu* and *Leucaena leucocephala*, respectively ($p \leq 0.05$, independent sample t-test).

ter stress (i.e., water potential decreased to −0.75 MPa), sharp decline was observed in GP and TI of both species, with more pronounced change in *S. catechu* than in *L. leucocephala* (Fig. 3A, C). Both GP and TI were higher in *S. catechu* than *L. leucocephala* in the water stress from −0.1 to −0.5 MPa while the result was opposite at −0.75 MPa. Seeds of both species did not germinate at −1.0 MPa (Fig. 3A, C; Tables S3A, C, and S4). Two-way ANOVA revealed that the water potential itself and its interaction with species showed significant effects on GP ($p < 0.001$, Table 2). In case of TI, all the factors (species, water potential, and their interaction) had significant effects on GP ($p < 0.001$, Table 2).

The MGT was always longer in *L. leucocephala* than in *S. catechu* (Fig. 3B). As water stress increased (i.e., water potential declined), the MGT tended to increase in *L. leucocephala* and reached maximum at −0.75 MPa. However, the MGT did not change significantly in *S. catechu* up to −0.75 MPa (Fig. 3B). The MGT differed between species ($p < 0.001$) except at −0.75 MPa (Fig. 3B, Tables S3B and S4), whereas the interaction of species and water potential did not show any effect on MGT ($p > 0.05$, Table 2).

Effect of depth on seedling emergence

In contrast to the results of the petri dish experiments, the seedling EP of *L. leucocephala* was higher than that of *S. catechu* in the pots at all seed-sowing depths. In *L. leucocephala,* the EP was nearly the same up to 4 cm depth but it declined significantly at 6 cm depth (Fig. 4). In *S. catechu*, the EP was nearly 50% when seeds were sown on the surface. The EP declined sharply to about 10% at a depth of 2 cm, with no seedling emergence observed at depths greater than 2 cm (Fig. 4). All the factors (species, seed-sowing depth, and their interaction) had significant effects on seedling emergence ($p < 0.001$, Table 2).

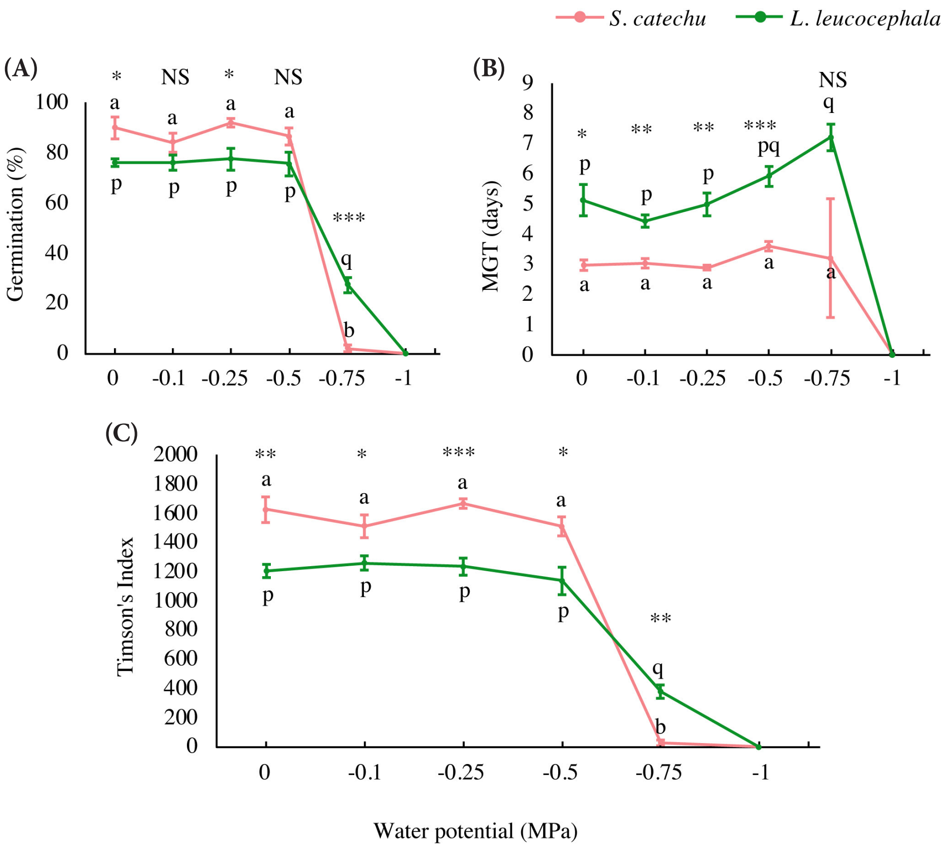

Fig. 3 Effect of waters stress on (A) germination percentage, (B) mean germination time (MGT), and (C) Timson's index at low temperature and 12-hour photoperiod condition. Error bars represent ± 1 standard error of the mean. The symbols *, **, *** represent significant difference at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively and NS represents no differences between two species (independent sample t-test). Different alphabets 'a, b' and 'p, q' represent significant difference among different water potentials within *Senegalia catechu* and *Leucaena leucocephala*, respectively ($p \leq 0.05$, one-way ANOVA).

Table 2 Results of two-way ANOVA on germination parameters and seedling emergence

	df	F	*p*-value
Germination percent			
Species	1	2.11	0.154
Water potential	4	122.72	< 0.001^a
Species × water potential	4	17.80	< 0.001^a
Mean germination time			
Species	1	30.30	< 0.001^a
Water potential	4	1.66	0.179
Species × water potential	4	1.01	0.413
Timson's index			
Species	1	33.10	< 0.001^a
Water potential	4	152.14	< 0.001^a
Species × water potential	4	14.33	< 0.001^a
Emergence percent			
Species	1	51.03	< 0.001^a
Depth	3	17.20	< 0.001^a
Species × depth	3	14.49	0.001^a

^{a}Significant values.

Biomass allocation and relative growth rate

The RMF, LMF, and RSR did not show differences between *L. leucocephala* and *S. catechu* when harvested 90 days after sowing while the SMF was higher in *L. leucocephala* ($p < 0.05$) (Fig. 5). The RGR of *L. leucocephala* (48 mg/g/day) and *S. catechu* (46 mg/g/day) were nearly equal.

Seedling morphology and biomass

Shoot height, root length, seedling biomass, and number of leaves in each seedling of invasive *L. leucocephala* were always higher than that of the native species (Fig. 6). The shoot height increased with increasing days after sowing in both species, with pronounced increase in *L. leucocephala* compared to *S. catechu* (Fig. 6A). An increase in root length of *S. catechu* was also observed in the subsequent harvests, whereas such growth was not observed in *L. leucocephala* (Fig. 6B). Leaf number also increased in successive harvest in both species (Fig. 6C). Total seedling biomass increased in the successive harvests and the difference between species was very high (Fig. 6D). Depending on the harvest events, seedling biomass of the invasive *L. leucocephala* was 14 to 20 times higher than that of the native *S. catechu*.

Discussion

This study has provided insights into the germination and seedling growth patterns of confamilial native *S. catechu* and invasive *L. leucocephala* in Nepal. A trait characterization of closely related native and invasive plant species could offer a valuable insight into the competitive ability of an invasive member and potential for further invasions. Understanding the seed germination ecology and seedling development characteristics of plants is crucial as early developmental traits significantly impact plants' es-

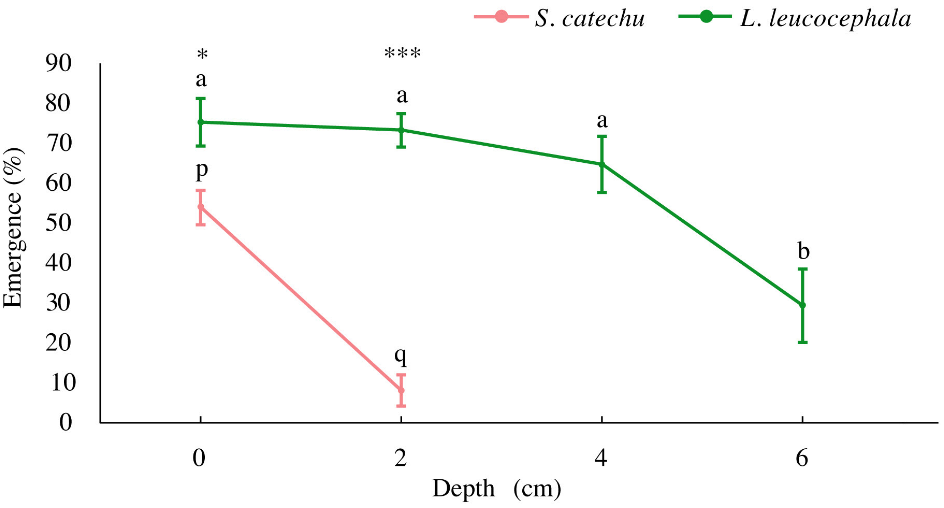

Fig. 4 Seedling emergence percent at different seed sowing depths. Error bars represent ± 1 standard error of the mean. The symbols * and *** represents significant difference at $p \leq 0.05$ and $p \leq 0.001$, respectively, between two species at each depth (independent sample t-test). Different alphabets 'a, b' and 'p, q' represent significant difference among soil depths within *Senegalia catechu* and *Leucaena leucocephala*, respectively ($p \leq 0.05$, one-way ANOVA).

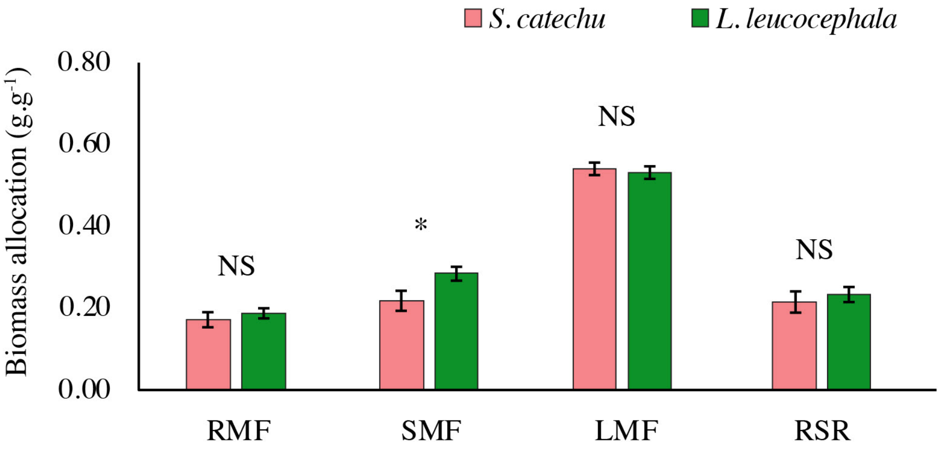

Fig. 5 Biomass allocation in seedlings at 90 days after seed sowing. Error bars represent ± 1 standard error of the mean. The symbol * represents significant difference at $p \leq 0.05$ and NS represents no difference between two species (independent sample t-test). RMF: root mass fraction; SMF: shoot mass fraction; LMF: leaf mass fraction; RSR: root-to-shoot ratio. *S. catechu*: *Senegalia catechu*; *L. leucocephala*: *Leucaena leucocephala*.

tablishment and survival (Larson et al. 2020). This knowledge can provide broad insight into the abilities of the invasive plants to colonize and thrive in new environments.

The results showed that *L. leucophala* bears larger seeds with high biomass compared to *S. catechu* (Table 1). These variations may have direct implications to seed germination and seedling survival. In particular, larger seeds have greater nutrient reserves as well as larger embryonic tissue which is positively correlated with seedling survival (Moles and Westoby 2004; Saeed and Shaukat 2000).

In spite of a large difference in seed mass, the seed GP of these two species were mostly similar except a high GP of *S. catechu* at high temperature and photoperiod conditions. The results suggest that the larger seeds may not always lead to higher seed GP. It can be expected that the larger seeds with higher nutrient content could be more prone to microbial infection when soaked with water leading to death of embryos before germination (Pringle et al. 2007).

The results also suggest that the native *S. catechu* may be benefited more by warming than the invasive *L. leucocephala*, at least in term of temperature-induced enhancement of germination. However, we incubated seeds under only two temperature regimes that differed only 5°C, and these temperature regimes are close to the optimum temperatures for germination of *S. catechu* (Todaria et al. 2004) and *L. leucocephala* (Dhanda and Chauhan 2022). To fully understand the impacts of warming on germination behaviors, experiments under wider temperature regimes including extremes with several intermediate temperature treatments are needed (e.g., Gareca et al. 2012).

Light is one of the regulating factors for seed germination. The seeds of both *S. catechu* and *L. leucocephala* were found to be non-photoblastic as the percentage of seed germination in both species was > 50% in photoperiod as well as under complete dark conditions. No absolute requirement of light for germination has been also reported previously for *S. catechu* (Khera and Singh 2005), *L. leucocephala* (Obiazi 2015), and other members of Fabaceae (e.g., Kharel et al. 2024).

We have also measured other parameters of germination such as MGT and TI. The MGT is a measure of the time it takes for the maximum seed germination, focusing on the day at which most seeds have germinated while the TI is the measure of a germination speed (Baskin and Baskin 2014). Shorter MGT and higher TI in native *S. catechu* indicate that this native species may colonize the habitat rel-

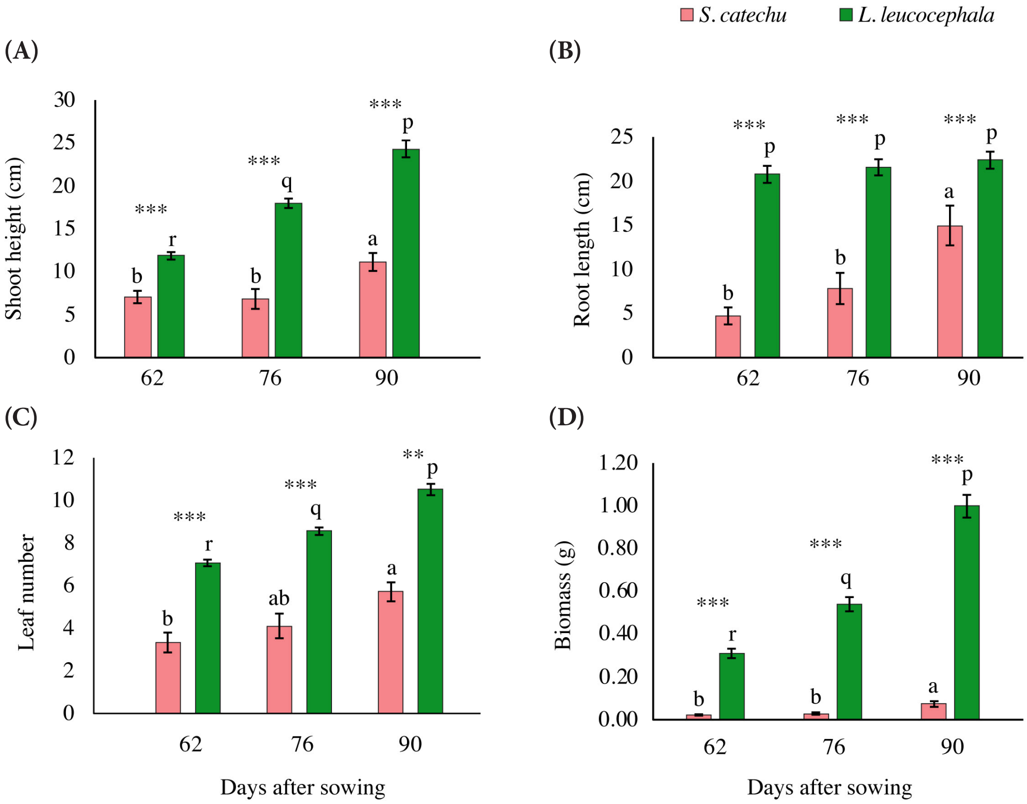

Fig. 6 Seedling (A) shoot height, (B) root length, (C) leaf number, and (D) total biomass of *Senegalia catechu* and *Leucaena leucocephala* at three successive harvests. Error bars represent ± 1 standard error of the mean. The symbols ** and *** represent significant difference at $p \leq 0.01$ and $p \leq 0.001$, and NS represents no difference between two species (independent sample t-test). Different alphabets 'a, b' and 'p–r' represent significant difference among the days after seed sowing within *S. catechu* and *L. leucocephala*, respectively ($p \leq 0.05$, one-way ANOVA).

atively faster than *L. leucocephala*. However, interactions between these two species in natural habitat may also depend on other factors. For example, as discussed below, seedling emergence may fail when small seeds of *S. catechu* are buried at a more depth (> 2 cm). There may also be a possibility of harming germination and growth behavior of native species by allelopathy of *L. leucocephala*. Allelopathic effect of *L. leucocephala* on native species is pronounced (Kato-Noguchi and Kurniadie 2022) though its effects on *S. catechu* have yet to be evaluated.

Water stress is one of the factors that can either slow down or prevent seed germination depending on the intensity and duration of the stress (Ebrahimi and Eslami 2012). A few previous studies have indicated that seeds of invasive species possess the ability to germinate under moderate water stress conditions (Chauhan and Johnson 2008, 2009). Various methods are available to induce water stress during seed gerdmination. We utilized PEG solutions to establish water potential gradient while comparing the responses of *L. leucocephala* and *S. catechu.* Although the exact establishment of moisture potential gradient is complex, the PEG assay is one of the best options as the PEG dilution can create a controlled approximation of such gradients (Muscolo et al. 2014). Increasing the concentrations of PEG induces water deficit conditions by lowering water potential, thereby leading dehydration stress in plants or cells which simulates natural drought conditions (Zhang et al. 2018).

Our results show that GP, MGT, and TI are greatly reduced at lower water potentials (−0.75 and −1 MPa). The GP and TI in *L. leucocephala* were lower, while MGT was higher than *S. catechu* in the water potential ≥ −0.5 MPa (Fig. 3). However, a higher GP of *L. leucocephala* at water potential (−0.75 MPa) compared to *S. catechu* suggests that *L. leucocephala* has greater potential to recruit higher number of seedlings in drier areas.

Despite having similar GP of two species in the petri plate experiments, a large difference in seedling emergence in the pot experiment could be attributed to a large difference in seed mass. In particular, seedlings emerged above soil surface from the larger seeds of *L. leucocephala* when sown as deep as 6 cm. For small seeds of *S. catechu*, the seedlings failed to emerge when sown below 2 cm. It is highly likely that the seedling growth below the soil surface mainly depends on the food reserve available in the seeds as photosynthesis would not be possible due to the lack of light below the soil surface (Singh et al. 2017). As such, deep-sown larger seeds may support seedling elongation until they emerge above the soil to initiate photosynthesis while the food reserve of deep-sown smaller seeds may exhaust before seedlings emerge above the soil surface, with a subsequent death of seedlings. As seeds of both species were capable of germination in the absence of light, we have ruled out the possibility of the lack of germination of the deep-sown small seeds of *S. catechu* in the present study. The results also suggest that at frequently disturbed sites where seed burial at deeper depth is highly likely, seedling emergence of the invasive *L. leucocephala* may be higher than that of native *S. catechu* from soil seed bank. Such a difference provides a competitive advantage to the invasive alien plants and the seedling emergence is strongly related to plant survival and invasion (Rejmánek and Richardson 1996).

Biomass allocation affects the subsequent capture rate of resources and reproduction (Feng et al. 2007). Therefore, experiments on biomass allocation are considered useful for knowing invasive plants' growth, reproduction, and competitive ability. SMF, RMF, and LMF together may contribute to resource acquisition, photosynthetic efficacy and stress resilience in plants and higher values of such traits are expected in invasive as compared to native species (Matzek 2012). However, in the present study, native and invasive species did not differ in three (i.e., RMF, LMF, and RSR) of the four parameters derived to characterize biomass allocation. In contrast, growth performance of the invasive *L. leucocephala* was better than that of native *S. catechu* in terms of the measured morphological traits (seedling height, root length, leaf number) and seedling biomass. Difference in germination time between two species was not so large in petri dish (MGTs were 4 and 6 days, respectively for *S. catechu* and *L. leucocephala*) and pot experiments. However, the large difference in seedling biomass might be associated with smaller seed mass and lower initial seedling dry biomass of native *S. catechu* compared to the invasive *L. leucocephala*.

Additionally, the longer root systems of *L. leucocephala* seedlings may improve access to water and nutrients from deeper soil which obviously enhances nutrient uptake leading to rapid growth and survival (Morris et al. 2011). Similarly, increased shoot height enables the plants to capture more sunlight and additionally, the leaf abundance are linked with higher photosynthetic capacities (Wang et al. 2021). These characteristics suggest that the *L. leucocephala* is better suited than native species to utilizing light and nutrient resources. Besides the above-mentioned traits of the invasive plant, it can be expected that a high RGR could be a characteristic of invasiveness (Dawson et al. 2011; Grotkopp et al. 2010). However, our data suggests that invasive species do not always exhibit higher RGR than closely related native. Lack of difference in growth traits between native and invasive is not uncommon in the literature (Daehler 2003). Hence, in the pair of species we studied, the RGR does not appear to be a suitable growth trait to explain the invasiveness of *L. leucocephala*.

Conclusions

This study offers understandings into the germination and seedling growth traits of confamilial native *S. catechu* and invasive *L. leucocephala* in Nepal. Larger seeds of *L. leucophala* could potentially have positive impacts on seedling survival and plant growth. Although the larger seeds may not always lead to higher seed germination, such larger seeds may germinate when buried at deeper soil depth compared to the smaller seeds. Additionally, native *S. catechu* may benefit more from warming than the invasive *L. leucocephala*. However, the invasive *L. leucocephala* appears to be more droughts tolerant in germination than native *S. catechu*. Despite no significant difference in the RGR between native and invasive species, a greater seedling biomass, longer root systems, increased shoot height, and leaf abundance of *L. leucocephala* suggest that the invasive species is better suited than the native *S. catechu* to utilize light and nutrient resources. Overall, our results suggest that the usefulness of germination and seedling growth traits for the prediction of species' invasiveness is highly species-specific.

Supplementary Information

Supplementary information accompanies this paper at https://doi.org/10.5141/jee.24.034.

Table S1. Independent sample t-test statistics in germination percentage. **Table S2.** Independent sample t-test statistics in mean germination time and Timson's index. **Table S3.** T-test statistics in germination parameters between *Senegalia catechu* and *Leucaena leucocephala* in different water potentials. **Table S4.** One-way ANOVA statistics in germination parameters within species among different water potentials.

Abbreviations

GP: Germination percentage
MGT: Mean germination time
TI: Timson's index
RGR: Relative growth rate
RMF: Root mass fraction
SMF: Stem mass fraction
LMF: Leaf mass fraction
RSR: Root-to-shoot ratio
SLA: Specific leaf area
PEG: Polyethylene glycol
EP: Emergence percentage
ANOVA: One-way analysis of variance

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Authors' contribution

BBS, LBT, and Anjana Devkota conceptualized research and experimental design; Anuj Dangol performed the experiments with support from AS, HA, and NK; Anuj Dangol analyzed data and wrote the first draft with supports from AS, HA, and NK; LBT and BBS jointly supervised the research; LBT, BBS and Anjana Devkota revised the manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable requests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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