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Influence of pre-sowing treatment and substrate on germination and seedling vigour of *Acacia angustissima* (Mill.) Kuntze, *Entandrophragma angolense* (Welw.) C.DC. and *Newtonia camerunensis* Villiers

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Abstract

Seeds of Acacia angustissima (Mill.) Kuntze, Entandrophragma angolense (Welw.) C.DC., and Newtonia camerunensis Villiers were subjected to five pre-sowing treatments (no treatment, soaking in cold water for 24 hours, soaking in hot water for 5 minutes, immersion in 20 % H_2SO_4 for 20 minutes, immersion in 60 % H_2SO_4 for 20 minutes) and sown in three substrates (sand, sawdust, and 1:1 sand:sawdust) in a non-mist propagator for nine weeks. The number of germinating seeds was recorded daily. Seedlings of Entandrophragma angolense were transplanted into polythene bags filled with the three substrates and moved to an outdoor nursery. Treatments were laid out in a slit-plot design with substrate as the whole plot and pre-sowing treatment as the sub-plot. Data on growth were collected twelve weeks after transplant. Germination percentage and growth rate index of Acacia angustissima were highest in hot water and lowest in the H_2SO_4 pre-treatments which did not show significant differences with the untreated control. While germination attributes of Entandrophragma angolense seeds did not respond to pre-sowing and substrate treatments, sand significantly increased seedling height, number of leaves, and leaf area in the species. Seeds of Newtonia camerunensis failed to germinate in any of the treatments. The findings suggest that the hot water scarification treatment could be beneficial for enhancing seed germination in Acacia angustissima and sand is a suitable substrate for early growth of Entandrophragma angolense.

Keywords: Afromontane forest, cold water, germination indices, growth traits, sulfuric acid, hot water.

Introduction

The Western High Plateau that is home to the Cameroonian Highlands forests hosts the largest area of montane habitats in West and west-Central Africa[1]. The forests provide a broad spectrum of services and benefits including water, water catchments, fuel wood, wood for carving, medicine, animals, and ecotourism. Moreover, sacred shrines in some of the forests are of great cultural value to the local people. The western Cameroon Highlands are also of international importance for their endemic birds, amphibians and vascular plants which are amongst the highest in Africa[2]. The area is characterized by fertile volcanic soils and adequate rainfall[3]. Such suitable conditions for farming have attracted a high human population that severely threatens the forest. Over 50% of the forest that once covered most of the landscape has been lost since the 1960s while the forest remnants are under enormous pressure from the high human population density[4]. Populations and ranges of tree species of great ecological and economic significance are declining due to agricultural encroachment, unsustainable harvest of tree barks for medicine, fires established by farmers and graziers, and unsustainable collection of firewood and construction materials[2,5-7].

Acacia angustissima (Mill.) Kuntze (family Fabaceae) is a small tree, 2-7 m high, with a single short trunk[8]. It is an important source of fuel wood, livestock forage, and tannins used in the leather industry. In addition, it improves soil nutrient conditions via atmospheric nitrogen fixation[9]. Unsustainable harvesting, over-grazing, forest fires, and land cultivation have led to notable declines in the distribution and density of the trees.

Entandrophragma angolense (Welw.) C.DC. is a deciduous buttressed tree that attains 60m in height with bole branchless for up to 40 m and 5 m trunk diameter[10]. It belongs to the Meliaceae family. The high market value of its wood and numerous medicinal uses of the bark have led to large-scale extraction of mature individuals, making it threatened in some parts of West and Central Africa[11]. It is categorized vulnerable in The IUCN Red List[12].

Newtonian camerunensis Villiers is a critically endangered tree of the Fabaceae family[12] with less than 50 individuals of seed bearing age remaining[13]. The tree species is endemic to the Western Highlands of Cameroon[14]. It is one of the hardest of woody species in the Western Highlands Forest of Cameroon, an attribute that likely attracted the excessive logging that has contributed in placing the species in its current status.

The declines in these three tree species has evoked a need to regenerate degraded forest portions of the western Cameroon Highlands. Germination is a critical stage in the life cycle of plants that can determine survival and often controls population dynamics on regeneration sites[15]. While seeds of many forest tree species germinate readily under favourable environmental conditions, those of many others require some form of physical and/or chemical treatment. There were significant improvements in germination of *Afzelia quanzensis*[16] and *Adansia digitata*[17] seeds following treatment with 98% H_2SO_4. In a study that tested the effect of substrate on germination and early growth of *Terminalia ivorensis*[18], sawdust gave the best germination percentage whereas height and root-collar diameter were highest in topsoil. Early growth parameters can provide valuable information on the plant's long-term performance in the field[19]. The important role of pre-sowing treatments in enhancing germination percentage and reducing germination time of seeds of *Entandrophragma angolense*[10] and *Acacia angustissima*[20,21] is also documented. In spite of the difficulty in attaining seed germination in *Newtonia camerunensis*[13], there is no published work on its induction in the species. With the exception of a few studies like Idua and Omonhinmin[22], Radivojevic et al.[23], and Kanmegne et al.[24], efforts on improving germination have generally focused on testing treatment main effects. Noteworthy, however, is that different factors may interact with each other in affecting the process. The interactive effect may not equal the direct sum of individual effects[25]. This study was aimed at examining the combined effects of pre-sowing treatments and substrate son seed germination and seedling vigor of *Acacia angustissima*, *Entandrophragma angolense*, and *Newtonian camerunensis*.

Materials and methods

Treatments: The experiment was comprised of three tree species (*Entandrophragma angolense*, *Acacia angustissima*, and *Newtonian camerunensis*), five pre-sowing treatments (no treatment, soaking in cold water for 24 hours, soaking in hot water for 5 minutes, immersion in 20% H_2SO_4 for 20 minutes, immersion in 60% H_2SO_4 for 20 minutes) and three substrates (sand, sawdust, 1:1 sand:sawdust).

Source of seeds: Seeds of *Acacia angustissima*, *Entandrophragma angolense*, and *Newtonian camerunensis* were collected from the Upland Forest in Tubah Sub-Division (latitude: 4°50' – 5°20'N; longitude: 10°35' – 11°59'E; altitude: 950–1500m above sea level) of the North West Region of Cameroon. The area is characterized by a dry (November to April) and a rainy season (May to October), 1780 - 2290mm mean annual rainfall[26], and 20-22°C / 13-14°C maximum / minimum temperature[27].

Viability test: Viability was tested through the floatation method whereby the seeds were soaked in water in a pale. Those that sank in the water after a few minutes of soaking were collected as viable seeds to be used in the experiment. The seeds that floated were taken to be unviable and discarded.

Experimental design: The study followed a split-plot design for each tree species with substrate as the whole plot and pre-sowing treatment the sub-plot. It was carried out in three non-mist propagators at the National Forestry Development Agency (ANAFOR) Humid Savannah Zone Headquarters in Bamenda, the capital city of the North West Region of Cameroon. The propagator was constructed based the design of Leakey[28] with some modifications. It consisted of a large wooden box (300m long×100m wide×0.25m deep) partitioned into three chambers of equal dimensions and sealed with polythene sheet. A water table was created by placing successive layers of sand, stone, and gravel beneath the substrate in the box. A PVC tube was installed through the substrate into the water table from which the water status of the propagator could be determined and for irrigation. During irrigation, water was allowed to fill up the water table up to the upper limit of the gravel layer so that the substrate was kept moist. A wooden frame was constructed over the box and then the entire set-up was enclosed in polythene sheet to create a permanently humid environment. Access into the propagator was made possible by a shutter that was created through the frame over each chamber. For a more detailed description of the construction of the non-mist propagator, see Ngo[29]. Each of the chambers that represented a substrate type was subdivided into 15 sub-units for the five pre-sowing treatments per tree species.

On 23 April 2018, seeds that had been subjected to the pre-sowing treatments were sown in the substrates that had been saturated with water. The sowing depth for *Acacia angustissima* was 0.5cm. Seeds of *Newtonia camerunensis* were set vertically and covered with a thin layer of substrate. For *Entandrophragma angolense*, sowing was done by burying the embryo completely with the wings out of the substrate and pointing upwards. There were 36 seeds in each species× substrate× pre-sowing treatment divided into three replications. The propagators were situated in a shade house roofed with alternating rows of transparent plastic and corrugated iron roofing sheets.

On 6 June 2018 when there had been no further germination in the last three weeks for any species and treatment, seedlings of *Acacia angustissima* and *Entandrophragma angolense* were transplanted individually into polythene bags filled with the three substrates used for germination. No *Newtonian camerunensis* seed germinated, reason why the species was not transplanted. The polythene bags were moved to an outdoor nursery at ANAFOR and laid out in the same experimental design described previously. Since the experiment was conducted in the rainy season, irrigation was by natural precipitation. There were no fertilizer applications during the entire duration of the experiment that ended on 14 August 2018.

Data collection: Germination: The number of germinating seeds was recorded each day and the following germination parameters were calculated:

$$Germination\ percentage = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown} \times 100$$

$$Germination\ Rate\ Index = [(G_1/1) + (G_2/2) + (G_3/3) + \ldots + (G_x/X)]$$

where, G = germination on each day after sowing; 1, 2, 3,X = corresponding day of germination[30].

Growth: At the end of the experiment, measurements of height and root-collar diameter were performed on one seedling of *Entandrophragma angolense* per treatment and replication. After counting the leaves on the seedling, the leaf area of the most widely expanded leaf was determined by counting the number of squares within a traced outline of the harvested leaf on a graph paper. The fresh weight of the seedling was obtained after rinsing the substrate from the roots. *Acacia angustissima* was excluded from the growth measurements because of the very small size of the seedlings at the time of data collection.

Data analysis: The data were examined for normality and homogeneity of variance. The untransformed data were then subjected to analysis of variance (ANOVA) to test the effect of substrate, pre-sowing treatment, and their interaction on germination and growth of *Acacia angustissima*, *Entandrophragma angolense*, and *Newtonia camerunensis*. When there was a significant main or interactive effect of treatments on a parameter, Scheffe's test was used for multiple means comparison. The statistical tests were performed in Data Desk 6.01 at $p< 0.05$.

Results and discussion

Germination: Unlike in *Entandrophragma angolense* where germination parameters were unaffected by either treatments or their interaction, germination percentage and germination rate index were significantly influenced by pre-sowing treatment in *Acacia angustissima* (Table-1). In contrast, there was no significant effect of substrate or interaction on germination percentage and germination rate index in the latter (Table-1).

Germination percentage was highest in hot water and least in the two H_2SO_4 treatments (Figure-1). However, differences in germination percentage between the H_2SO_4 treatments and untreated control were not statistically significant (Figure-1). Furthermore, there was no significant difference between cold water treatment and the untreated control group for this trait (Figure-1). Germination rate index responded to treatments in a similar manner to germination percentage (Figure-3).

Time to initial germination of *Acacia angustissima* responded to pre-sowing treatment × substrate but not to the individual treatment factors (Table-1). The values were highest for20% H_2SO_4 in sand: sawdust (Figure-2). There were no significant differences in time to initial germination among pre-sowing treatments in any other substrate. With the exception of 20% H_2SO_4 in sand and 60% H_2SO_4 in sawdust, the differences in this parameter between the 20% H_2SO_4 in sand: sawdust and the H_2SO_4 treatments in this and the other substrates were insignificant (Figure-2). Similarly, the statistics did not detect as significant the difference between 20% H_2SO_4in sand: sawdust and the untreated control (Figure-2).

Growth: There was a significant effect of substrate on height, number of leaves, and leaf area but not on root-collar diameter and fresh weight of *Entandrophragma angolense* seedlings (Table-2). In contrast, growth was unaffected by pre-sowing treatment or an interaction (Table-1).

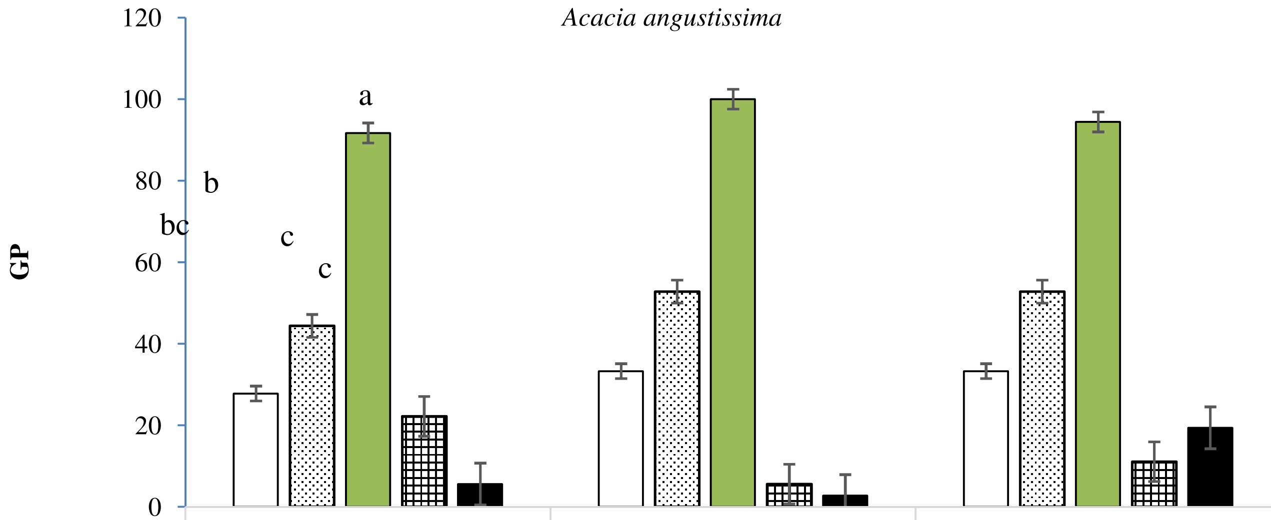
Table-1: ANOVA *p*-values for the effect of pre-sowing treatment, substrate, and their interaction on germination attributes of *Acacia angustissima* and *Entandrophragma angolense*.

Source	Substrate		Pre-sowing treatment		Substrate × Pre-sowing treatment	
	A. angustissima	*E. angolense*	*A. angustissima*	*E. angolense*	*A. angustissima*	*E. angolense*
GP	0.7848	0.7085	≤0.0001	0.4951	0.8464	0.4163
GT_0	0.1513	0.8013	0.0814	0.5132	0.0125	0.2028
GRI	0.9150	0.5619	≤0.0001	0.8043	0.7289	0.9065

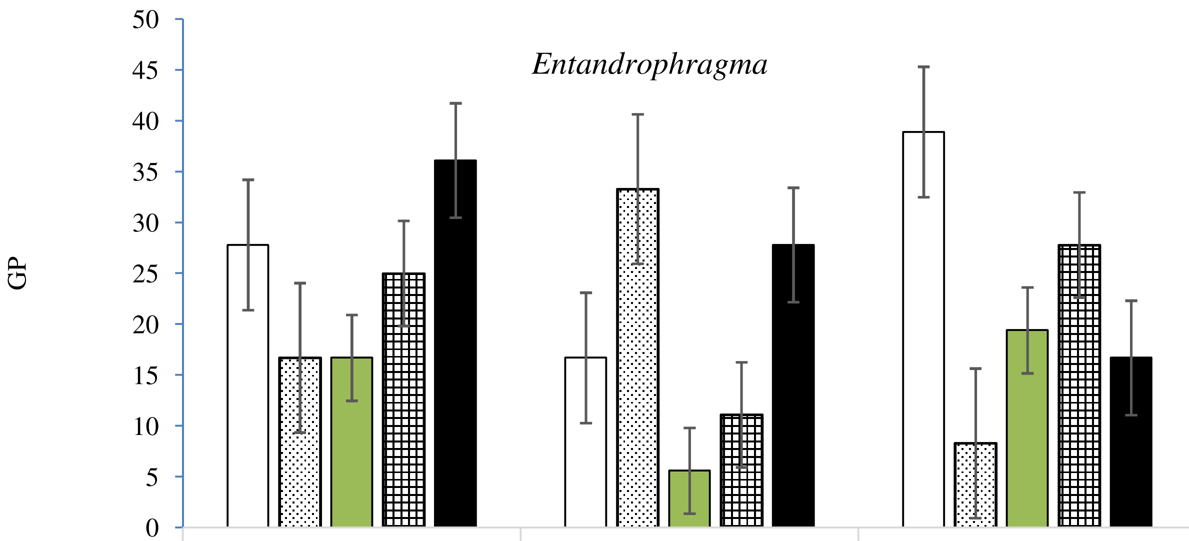
GP–germination percentage, GT_0 – time to initial germination, and GRI – germination rate index.

Table-2: ANOVA *p*-values for the effect of pre-sowing treatment, substrate, and their interaction on growth parameters of *Entandrophragma angolense* seedlings.

Source	Pre-sowing treatment	Substrate	Pre-sowing treatment × Substrate
Height	0.1874	0.0007	0.7150
Root-collar diameter	0.0546	0.7285	0.1218
Number of leaves	0.8673	0.0186	0.0961
Fresh weight	0.6179	0.5582	0.1142
Leaf area	0.0649	0.0248	0.1211



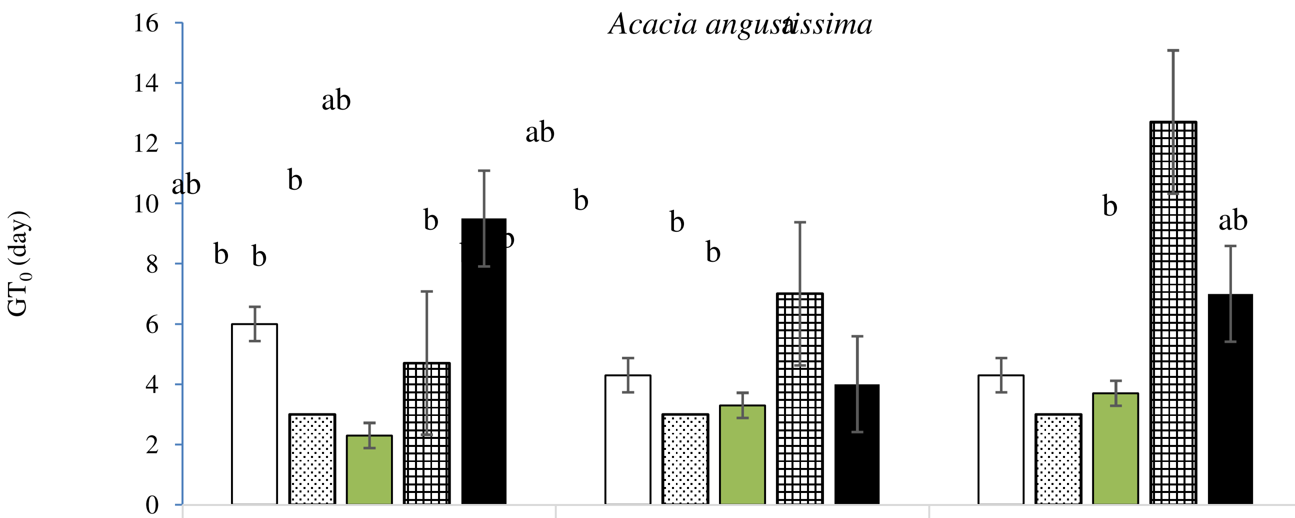
	Sand	Sawdust	Sand+Sawdust
Control	27.8	33.3	33.3
Cold water	44.4	52.8	52.8
Hot water	91.7	100	94.4
20% H2SO4	22.2	5.6	11.1
60% H2SO4	5.6	2.8	19.4



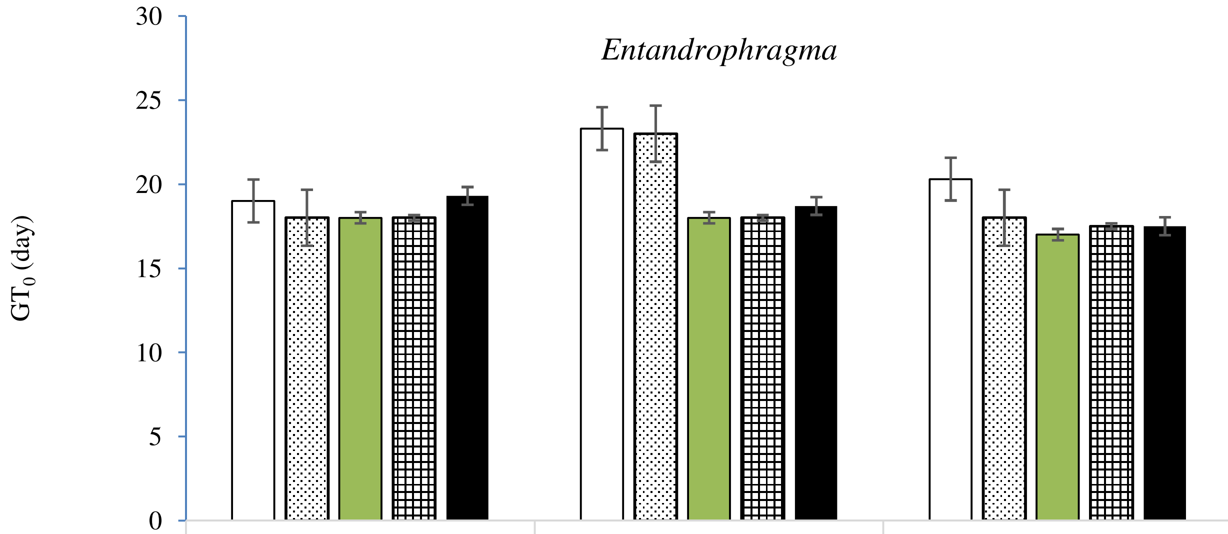
	Sand	Sawdust	Sand+Sawdust
Control	27.8	16.7	38.9
Cold water	16.7	33.3	8.3
Hot water	16.7	5.6	19.4
20% H2SO4	25	11.1	27.8
60% H2SO4	36.1	27.8	16.7

Substrate

Figure-1: Effects of different pre-sowing treatments and substrates on seed germination percentage (GP) of *Acacia angustissima* and *Entanndrophragma angolense*. Means underneath the same letter are not significantly different.



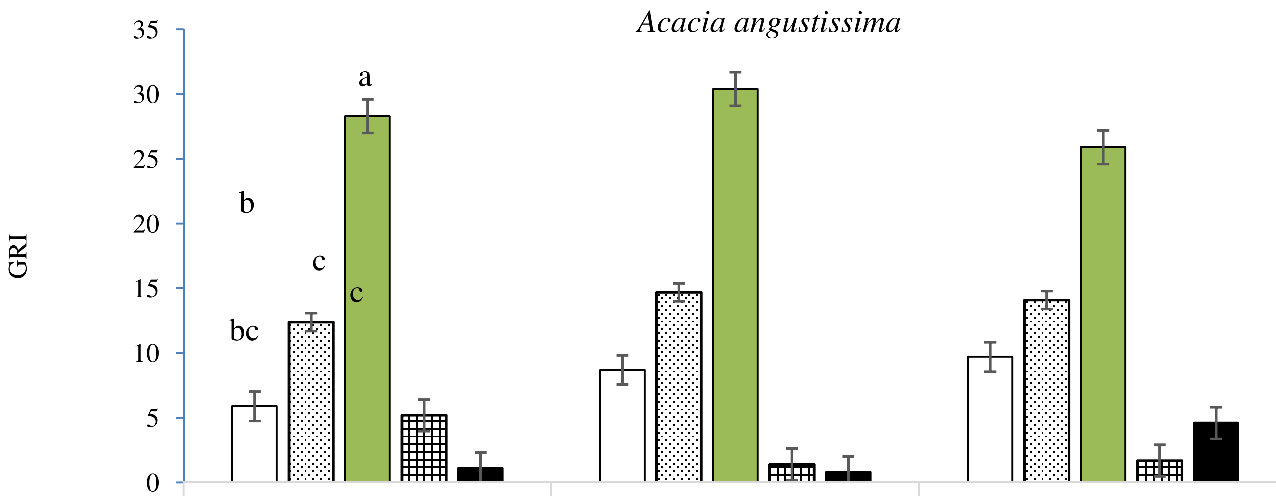
	Sand	Sawdust	Sand+Sawdust
Control	6	4.3	4.3
Cold water	3	3	3
Hot water	2.3	3.3	3.7
20% H2SO4	4.7	7	12.7
60% H2SO4	9.5	4	7



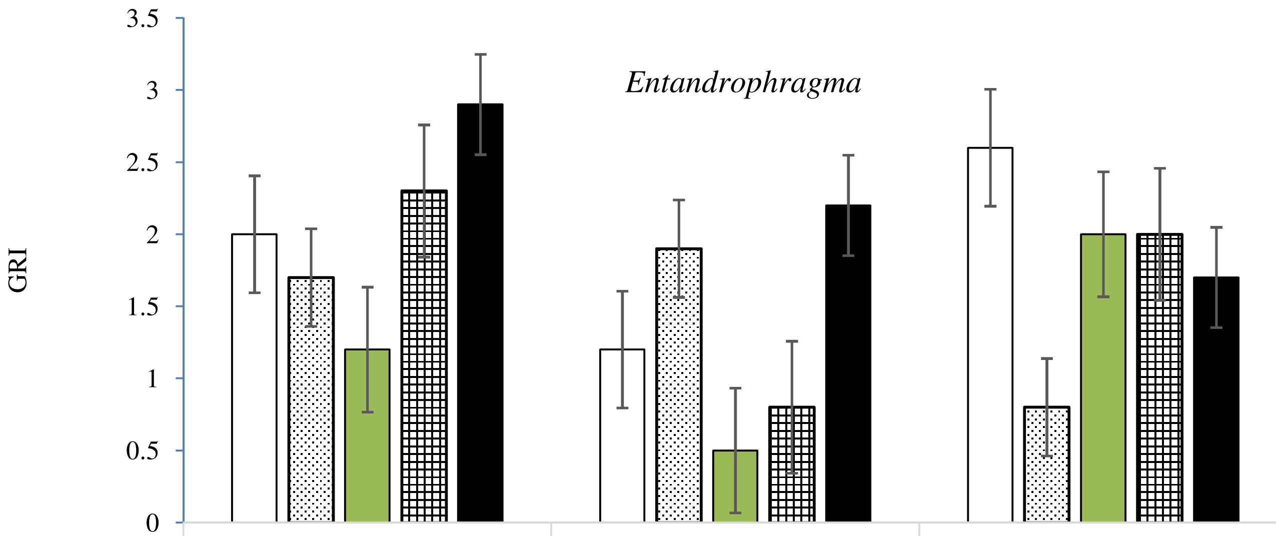
	Sand	Sawdust	Sand+Sawdust
Control	19	23.3	20.3
Cold water	18	23	18
Hot water	18	18	17
20% H2SO4	18	18	17.5
60% H2SO4	19.3	18.7	17.5

Substrate

Figure-2: Effects of different pre-sowing treatments and substrates on time to initial seed germination (GT_0) of *Acacia angustissima* and *Entanndrophragma angolense*. Means underneath the same letter are not significantly different.



	Sand	Sawdust	Sand+Sawdust
Control	5.9	8.7	9.7
Cold water	12.4	14.7	14.1
Hot water	28.3	30.4	25.9
20% H_2SO_4	5.2	1.4	1.7
60% H_2SO_4	1.1	0.8	4.6



	Sand	Sawdust	Sand+Sawdust
Control	2	1.2	2.6
Cold water	1.7	1.9	0.8
Hot water	1.2	0.5	2
20% H_2SO_4	2.3	0.8	2
60% H_2SO_4	2.9	2.2	1.7

Substrate

Figure-3: Effects of different pre-sowing treatments and substrates on germination rate index (GRI) of *Acacia angustissima* and *Entanndrophragma angolense*. Means underneath the same letter are not significantly different.

Height was significantly higher in sand than the other two substrates (Table-3). For number of leaves and leaf area, values displayed an increasing trend from sawdust to sand. However, the differences in number of leaves and leaf area between sand: sawdust and either sawdust or sand were statistically insignificant (Table-3).

Table-3: Effect of pre-sowing treatment and substrate on growth parameters (mean±SE) of *Entandrophragma angolense* seedlings.

Parameter	Pre-sowing treatment	Substrate			Pre-treatment main effect
		Sand	Sawdust	Sand+Sawdust	
H (cm)	Control	12.17±0.58	10.30±0.30	11.07±0.15	11.23±0.34
	Cold water	14.20±0.91	10.07±1.74	11.41±1.86	12.13±1.50
	Hot water	13.57±0.54	12.43±0.92	12.23±0.15	13.00±0.54
	20% H_2SO_4	12.80±0.61	11.73±0.50	10.83±1.09	12.27±0.73
	60% H_2SO_4	13.77±0.15	11.70±0.42	11.20±0.35	12.73±0.31
	Substrate main effect	13.30±0.56^{a}	11.25±0.78^{b}	11.35±0.72^{b}	
RCD (cm)	Control	0.40±0.01	0.40±0.00	0.38±0.01	0.39±0.01
	Cold water	0.42±0.02	0.46±0.01	0.30±0.03	0.39±0.02
	Hot water	0.36±0.02	0.35±0.01	0.39±0.02	0.36±0.02
	20% H_2SO_4	0.39±0.05	0.39±0.02	0.40±0.02	0.39±0.03
	60% H_2SO_4	0.41±0.04	0.39±0.02	0.43±0.01	0.41±0.02
	Substrate main effect	0.39±0.03	0.39±0.01	0.38±0.02	
NL	Control	3.00±0.00	2.33±0.33	2.67±0.33	2.67±0.22
	Cold water	3.33±0.33	2.00±0.00	3.33±0.33	2.89±0.22
	Hot water	3.33±0.33	2.00±0.00	3.33±0.33	2.89±0.22
	20% H_2SO_4	2.67±0.47	3.00±0.00	2.67±0.33	2.78±0.27
	60% H_2SO_4	2.67±0.47	2.67±0.67	2.67±0.33	2.67±0.49
	Substrate main effect	3.00±0.32^{a}	2.40±0.20^{b}	2.93±0.33^{ab}	
FW (g)	Control	3.57±0.39	2.19±0.06	3.09±0.20	2.95±0.22
	Cold water	3.85±0.15	2.62±0.75	2.41±0.81	2.96±0.57
	Hot water	2.48±0.28	2.71±0.16	3.52±0.23	2.90±0.22
	20% H_2SO_4	2.22±0.33	3.40±0.22	2.37±0.38	2.66±0.31
	60% H_2SO_4	2.74±0.66	3.06±0.13	3.12±0.74	2.97±0.51
	Substrate main effect	2.97±0.36	2.79±0.26	2.90±0.47	
LA (cm^2)	Control	40.33±1.20	14.83±0.33	32.50±7.47	29.22±3.00
	Cold water	40.17±3.48	24.17±11.61	17.56±0.00	27.29±5.03
	Hot water	36.33±1.64	30.67±6.29	42.50±2.02	36.50±3.32
	20% H_2SO_4	34.50±6.06	35.42±6.14	23.83±9.94	31.25±7.38
	60% H_2SO_4	41.00±5.00	35.08±7.15	40.67±1.09	38.92±4.41
	Substrate main effect	38.47±3.48^{a}	28.03±6.30^{b}	31.41±4.10^{ab}	

H - height, RCD – root-collar diameter, NL - number of leaves, FW - fresh weight, and LA - leaf area.

Germination: The data highlight hot water scarification as the most effective treatment for improvement of seed germination properties of *Acacia angustissima*. The beneficial role of this pre-sowing treatment on germination of *Acacia sp.*[31,32], *Cassia fistula*[33], *Iliamna remota*[34], *Afzelia quanzensis*, *Baikiaea plurijuga*[16], and *Albizia zigia*[35] has been demonstrated. For trees which exhibit external seed dormancy like *Acacia*, the hot water disrupts the seed coat enhancing the seed uptake of water and oxygen for germination to proceed[36].Since the number of days to start of germination tended to be reduced and germination speed was highest following the immersion of seeds in hot water, it seems reasonable to conclude that this pre-treatment will not only augment germination percentage but also ensure early colonization of a regeneration site. Hot water scarification has become common practice for elevating the rate, percentage, and uniformity of germination or seedling emergence in many tree species. On the other hand, a delay in germination as observed in the acid treatment in sand: saw dust can reduce the competitive advantage that is associated with early emergence and establishment[37,38]. The inability of H_2SO_4 to improve germination attributes of *Acacia anguatissima* here and *Thrinax morrisii*[39], *Calotropis persica*[15], and *Astralagus podolobus*[40] demonstrates the destructive effect of inappropriate duration of exposure to this treatment on the embryo. Soaking seeds in concentrated acids can induce seed germination but increasing the duration of seed contact leads to a decline of germination percentage[24,41]. This study's findings suggest that none of the treatments is suitable for improving seed germination in *Entandrophragma angolense* and *Newtonia camerunensis*, supporting the conclusion of Dewiret al.[39] and Tavili[40] that responses to germination treatments are species-dependent.

Growth: According to the finding of Purwantoro[42] on *Aganope heptaphylla*, substrate is not a limiting factor for seed germination but rather of seedling growth after germination. This view is supported by the results of the present study where there were greater values of *Entandrophragma angolense* seedling height, number of leaves, and leaf area in sand than sawdust. The number of leaves and leaf size are indices of light attenuation and photosynthesis for further growth[43]. The substrate effect on the seedling traits may be explained by differences in substrate physical properties which determine the supply of water and air for metabolism and plant growth[44]. Although the bulk density and labour requirement of sawdust are lower than those of sand, there is a yield penalty when the former is used because air-filled porosity is low[45]. We believe that air, and not water, was the growth-limiting resource in sawdust since all the substrates were subjected to favorable moisture conditions. Such was not the case in the sand medium where the availability of air is not a problem.

Another physical attribute that might have led to the substrate-related differences in growth is the thermal conductivity of the media. Sand warms up more rapidly than sawdust[46] and even a slight temperature change in substrate can induce dramatic changes in plant physiological processes and growth[47,48]. Beneath the optimum for a given plant species, an increase in substrate temperature usually results in greater root growth, permeability and water uptake with the outcome that shoot water potential, stomatal conductance to carbon dioxide, and photosynthetic carbon gain are augmented[49]. On the other hand, the temperature elevation in sand may result in a loss of additional carbon gained by the seedlings via respiration or root exudation, explaining the unresponsiveness of biomass to substrate observed in this study. There is often an exponential increase in root respiration with temperature[50]. As much as 52% of the total carbon taken up per day in photosynthesis can be expended in root respiration[51,52].

An important consideration in nursery production of reforestation material is the potential for survival after out-planting. There is a positive relationship between root-collar diameter and survival rate[53]. Long-term survival of *Entandrophragma angolense* could not be determined from the data presented here because of the lack of treatment effect on root-collar diameter. However, other morphological traits like root mass[54] and number of first-order lateral roots[55]and some physiological variables like root growth potential[56,57] and photosynthetic capacity[19] are demonstrated predictors of survival. To conclude on the suitability of the substrates for field survival of *Entandrophragma angolense*, further studies are required to generate data on parameters of the sort.

Conclusion

It is concluded that hot water scarification can be beneficial for enhancing seed germination in *Acacia angustissima* and sand is a suitable substrate for early growth of *Entandrophragma angolense*. Since *Newtonia camerunensis* did not respond to the treatments tested in this study, it is necessary that attempts be made to induce seed germination in the species with other pre-sowing treatments.

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