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GERMINATION AND EARLY GROWTH PERFORMANCE OF PREKESE, *TETRAPLEURA TETRAPTERA* TO SEED PRETREATMENT METHODS AND PHOSPHORUS FERTILIZER IN THE NURSERY PHASE

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ABSTRACT. Seeds of *Tetrapleura tetraptera* trees have poor germination due to their hard and impervious seed coat. This research was conducted to determine the possibilities of reducing seed dormancy using seed pretreatment via the application of sulphuric acid and hot water and then phosphorus for better seedling growth. Seeds pretreated with sulphuric acid had a significantly ($P<0.05$) increased germination rate (by 60%) compared to seeds pretreated with hot water (40%). The application of phosphorus fertilizer stimulated the early growth of the species. This research provides information for practical use.

Keywords: germination; hot water; phosphorus fertilizer; sulphuric acid; *Tetrapleura tetraptera*.

INTRODUCTION

Tetrapleura tetraptera (Schumach and Thonn) from the Fabaceae family is a leguminous tree species found in the lowland tropical rainforest regions of Africa (Okyere-Agyapong *et al.*, 2019). In Ghana, the plant serves as a remedy for diseases, including malaria, diabetes and hypertension (Abii and Amarachi, 2007; Lekana-Douki *et al.*, 2011; Larbie *et al.*, 2020). The plant contains vital phytochemical elements, such as alkaloids, that are important for the normal functioning of the body (Larbie *et al.*, 2020). *Tetrapleura tetraptera* is usually propagated using the seed; however, only a small percentage of seeds germinate, as many remain dormant due to their hard seed coat, which makes seed germination very difficult (Ibiang *et al.*, 2012). Seed dormancy can be broken by various seed pretreatment methods, such as chemical scarification and hot water treatment, to ensure absorption and uptake of water by the seeds and rapid initiation of the seeds (Usman *et al.*, 2019). Additionally, the seedling phase of *T. tetraptera* is the most vulnerable phase of its life cycle, and plant growth and survival depend on optimum resources of light, water and nutrients, including phosphorus (Amissah *et al.*, 2015; Okyere-Agyapong *et al.*, 2019). Al-Kahtani *et al.* (2017) reported that the application of essential nutrient elements, such as phosphorus, will ensure optimum growth, survival of rhizobia in the soil, nodulation and nitrogen fixation of legumes, including *Tetrapleura tetraptera*.

Despite the nutritional and economic benefits of *Tetrapleura tetraptera*, the plant's potential has remained untapped, with several species on the verge of extinction due to overexploitation, a lack of long-term conservation measures, and the ecological consequences of deforestation (Nya *et al.*, 2000; Jimoh, 2005; Omokhua, 2015; Usman *et al.*, 2019). Al-Kahtani *et al.* (2017) pointed out that phosphorus deficiency and a lack of nodulation could inhibit the initial growth of most legumes, including *Tetrapleura tetraptera*. However, information on the effect of seed pretreatment methods (sulphuric acid and hot water) on the germination and application of phosphorus fertilizer for the early growth performance of *T. tetraptera* (prekese) remains scarce (Ibiang *et al.*, 2012; Maku *et al.*, 2014; Usman *et al.*, 2019). This study provided information on the effect of seed pretreatment methods (sulphuric acid and hot water) on the germination and application of phosphorus fertilizer on the early growth performance of prekese for further recommendation in agroforestry and to ensure large-scale propagation of the species for plantation establishment in Ghana.

MATERIALS AND METHODS

Study Area

Location

The field experiment was situated at the Agroforestry Department demonstration farm at the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. It is located at latitude 6.40°N and longitude 1.37°W in the humid Semi-Deciduous Forest zone of Ghana (*Figure 1*).

Rainfall

It is distinguished by a bimodal pattern of yearly rainfall, with mean values between 1250 and 1500 mm. A minor rainy season that starts in September and lasts until November follows the big wet season, which runs from May through July. In addition, the area endures a long dry season from December through March and a brief one in August.

Temperature and humidity

The site's mean daily temperature is 25.6°C, with the coldest months from December to February seeing average lows of 20°C and the hottest month, March, seeing average highs of 33°C.

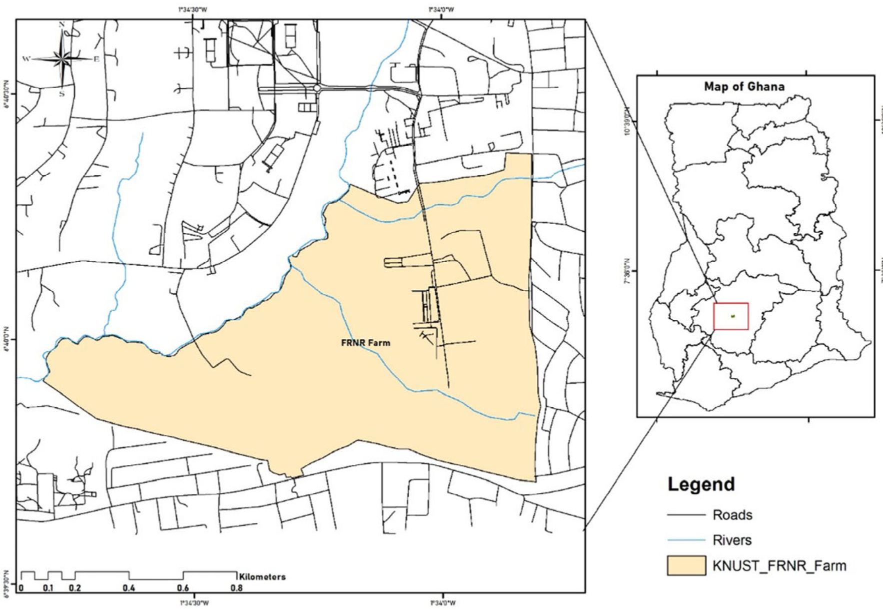

Figure 1 – Map of the Agroforestry Department demonstration farm at the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

The site's average yearly temperature is 26.61°C with 67.6% relative humidity (Adu and Asiamah 1992).

Soil type

According to Neina and Agyarko-Mintah (2022), the soil at the experimental site is well drained and strongly acidic Ferric Acrisol, and the textural class is sandy-loam.

Experimental approach and procedure

Land preparation

The study site was manually cleared of all vegetation and foreign materials with a hoe, cutlass and rake. At the research location, topsoil between 0 and 30 cm deep was removed from the earth's surface and gathered in bags. When filling the polybags, stones and other extraneous elements were manually removed. Two kilograms of earth were placed inside black polybags with holes in them. To avoid root infiltration, a plastic carpet was laid down on the ground where the polybags were positioned.

Sources of material and viability testing

Sulphuric acid was prepared at different concentrations, and tap water was heated to 100°C. Phosphorus fertilizer was weighed into different quantities using an electronic balance and applied at the rates of 50, 37.5, 25 and 0 kg/ha P on the polybags, whereas *Tetrapleura tetraptera* seeds were purchased from the Fumesua location of the Forest Research Institute of Ghana (FORIG). The seeds were put through a viability test using the floating technique; those that floated on water after a short period of soaking were deemed non-viable and thrown away, while those that sank to the bottom of the beaker were collected and deemed viable for use in the study.

Seed pretreatment and sowing

A total of 480 seeds were soaked in a 50% solution of sulphuric acid (H_2SO_4) for 2 minutes and subsequently rinsed in distilled water, while another 480 seeds were soaked in hot (boiling) water for 7 minutes. At planting, two 1-cm deep holes were dug in the soil, and two seeds were placed in each. The seeds were then lightly pressed after being coated with a thin layer of soil to guarantee root anchoring during germination.

Post-sowing activities

One week following germination, *Tetrapleura tetraptera* seedlings were thinned out to one (1) seedling per polybag to maintain consistent development and lessen competition for water and nutrients. Watering was done as needed, along with other cultural traditions, such as weeding.

Phosphorus fertilizer application

Two weeks after germination, phosphorus fertilizers were applied to the *Tetrapleura tetraptera* seedlings contained in each polybag as per the treatments (50, 37.5, 25 and 0 kg/ha P), and observations were carried out accordingly.

Experimental design and treatment allocation

Eight treatment combinations were allocated randomly and replicated three times in a completely randomized design (CRD). Seeds were randomly collected from the seed lot of each treatment and sown with two seeds per polybag. A total of 1920 seeds were used for the experiment.

The treatment combinations were as follows:

T1: Hot water + 0 kg/ha P;
T2: Hot water + 25 kg/ha P;
T3: Hot water + 37.5 kg/ha P;
T4: Hot water + 50 kg/ha P;
T5: Sulphuric acid + 0 kg/ha P;
T6: Sulphuric acid + 25 kg/ha P;
T7: Sulphuric acid + 37.5 kg/ha P; and
T8: Sulphuric acid + 50 kg/ha P;

The recommended P rate for legumes is 50 kg/ha (Karanja *et al.*, 2004), where P= phosphorus.

Data collection

Five of the twenty seedlings per treatment were randomly selected, tagged with ribbons, and used for data collection. Data were collected on germination (and thus the germination percentage) for 4 weeks, while height and leaf number were determined within a 2-week interval for 3 months using a metre-rule veneer and a visual count, respectively. Destructive sampling was done at the end of the experiment to visually identify and count the number of nodules, and the above- and belowground biomass (root and shoot dry weight) of *Tetrapleura tetraptera* seedlings were determined using an electronic balance.

Data analysis

Data collected on germination and growth parameters were analysed for differences using analysis of variance (ANOVA) based on STATISTIX 8 software at a 5% level of significance. The Tukey HSD test was employed to compare the means, which were significantly different. The results were presented in tables using Excel.

RESULTS

Germination of *Tetrapleura tetraptera* seeds

The germination (%) of the various seed pretreatment methods (sulphuric acid and hot water) on the seeds of *Tetrapleura tetraptera* is shown in *Table 1*. The germination of *T. tetraptera* seeds was significantly higher in the sulphuric acid pre-treatment compared to the hot water ($p<0.05$).

Height (cm) of *Tetrapleura tetraptera* from 4 to 14 weeks after planting (WAP)

The response of the height (cm) of *Tetrapleura tetraptera* to phosphorus fertilizer over the 14-week growth period is shown in *Table 2*. On a weekly basis, there was a significant difference in height from 4 to 14 WAP, with P-values ranging from 0.0000 to 0.0004.

Number of leaves of *Tetrapleura tetraptera* from 4 to 14 WAP

The effect of seed pretreatment methods and phosphorus fertilizer on the number of leaves of *Tetrapleura tetraptera* from 4 to 14 WAP is illustrated in *Table 3*. There was a significant ($P<0.05$) difference among the treatments on a weekly basis, starting from 4 to 14 WAP. T8 recorded the highest leaf count from 4 to 14 WAP, while T1 had the lowest leaf count from 4 to 14 WAP.

Nodulation, Shoot Dry Weight and Root Dry Weight of *Tetrapleuta tetraptera*

There was a significant ($P<0.05$) difference between the various treatment methods with respect to the number of nodules.

T6 recorded the greatest nodulation (15.9), followed by T4 (15.6), T3 (14.6), T8 (13.5), T2 (12.6), T7 (12.6), T6 (15.9), and T5 (10.6), with the least nodules recorded in T1 and T5 (10.6 each). The root dry weight of the *Tetrapleura tetraptera* plants followed a sequence similar to the number of nodules (*Table 4*).

Table 1 – Effect of sulphuric acid and hot water on the germination of *Tetrapleura tetraptera*

Treatments	Number of seeds treated and sown	Number of seeds germinated	Germination percentage (%)
T1	120	50	41.7b
T2	120	50	41.7b
T3	120	48	40.0b
T4	120	49	40.0b
T5	120	70	58.3a
T6	120	72	60.0a
T7	120	69	57.5a
T8	120	70	58.3a
P-VALUE			0.0000
TUKEY (HSD)			3.73
CV			2.64
F-RATIO			152
DF			7

CV= Coefficient of variation, DF= Degree of freedom, HSD= Honestly significant difference and T= Treatment. Means with the same superscripts are not significantly different at ($P\leq0.05\%$) using the Tukey HSD test

Table 2 – Effect of phosphorus fertilizer on the height (cm) of *Tetrapleura tetraptera* from 4 to 14 WAP

Treatments	4 WAP (±SeM)	6 WAP (±SeM)	8 WAP (±SeM)	10 WAP (±SeM)	12 WAP (±SeM)	14WAP (±SeM)
T1	6.8±0.3d	15.1±2.2b	18.7±3.4b	24.6±4.3b	30.6±4.7b	36.6±4.7c
T2	12.8±0.5c	24.9±1.3ab	32.4±1.1a	38.5±1.7ab	44.5±1.2ab	49.5±1.2a
T3	15.3±0.8bc	24.2±2.9ab	32.4±1.4a	41.3±3.6a	47.3±3.0a	53.3±3.0a
T4	19.4±1.4ab	27.3±2.1a	35.4±0.8a	43.1±1.6a	48.7±2.0a	52.4±2.0a
T5	6.9±0.3d	15.3±2.2b	21.0±2.8b	24.6±4.3b	30.3±4.0b	34.9±4.0bc
T6	12.8±0.5c	24.7±1.8ab	33.5±1.6a	39.5±0.7ab	45.1±1.0a	48.8±2.0ab
T7	15.4±0.8bc	24.5±2.9ab	34.8±2.5a	41.4±3.5a	47.4±3.0a	53.4±3.0a
T8	19.6±1.3a	27.9±1.8a	36.4±2.7a	39.5±3.4ab	45.5±2.8a	52.8±2.0a
P-VALUES	0.0000	0.0034	0.0001	0.0019	0.0011	0.0004
TUKEY (HSD)	4.14	10.87	10.91	15.43	14.54	14.55
CV	10.76	16.70	12.60	14.91	12.12	10.86

Means with the same superscripts are not significantly different at (P≤0.05%) using the Tukey HSD test

Table 3 – Effect of phosphorus fertilizer on the number of leaves of *Tetrapleura tetraptera* from 4 to 14 WAP

Treatments	4 WAP (±SeM)	6 WAP (±SeM)	8 WAP (±SeM)	10 WAP (±SeM)	12 WAP (±SeM)	14 WAP (±SeM)
T1	18.3±0.9b	24.3±0.9c	30.0±0.6d	35.3±1.2e	45.3±1.2c	50.7±0.9c
T2	21.7±0.3b	27.0±1.2bc	31.0±1.2cd	37.0±1.2cde	47.0±1.2bc	51.7±1.2bc
T3	26.7±0.7a	31.7±1.2ab	36.0±1.0abc	41.7±1.20abcd	51.3±0.9ab	56.7±1.2ab
T4	28.0±1.2a	33.3±1.2a	37.0±1.5ab	43.7±1.33ab	53.3±1.2a	58.3±1.2a
T5	18.7±0.7b	24.7±0.7c	31.0±0.6cd	36.0±1.00de	46.0±1.0c	51.3±0.3bc
T6	22.0±0.0b	27.7±1.2bc	31.7±1.2bcd	37.7±1.45bcde	48.3±0.9abc	52.3±0.9bc
T7	27.3±0.9a	32.3±1.3ab	36.7±1.2abc	42.7±1.67abc	52.0±1.0ab	58.0±1.5a
T8	28.7±1.5a	34.0±1.2a	38.3±1.8a	44.3±1.20a	53.3±1.2a	59.3±1.2a
P-VALUES	0.0000	0.0000	0.0003	0.0003	0.0001	0.0001
TUKEY (HSD)	4.25	5.48	5.84	6.34	5.23	5.42
CV	6.27	6.59	6.07	5.62	3.75	3.49

Means with the same superscripts are not significantly different at (P≤0.05%) using the Tukey HSD test

Table 4 – Effect of phosphorus fertilizer on the nodulation, dry weight of shoots and dry weight of roots of *Tetrapleura tetraptera* at 14 WAP

Treatments	Nodulation (±SeM)	Weight of dry shoots (g) (±SeM)	Weight of dry roots (g) (±SeM)
T1	10.6±0.08e	1.27±0.07c	0.77±0.03b
T2	12.6±0.12d	3.27±0.09bc	0.77±0.09b
T3	14.6±0.12b	4.70±0.09ab	1.07±0.09ab
T4	15.6±0.12a	5.20±0.35ab	1.20±0.06ab
T5	10.6±0.15e	1.13±0.03c	1.17±0.15ab
T6	15.9±0.15a	6.30±0.06a	1.40±0.06a
T7	12.6±0.09d	5.53±0.49ab	1.27±0.09a
T8	13.5±0.30c	6.17±1.28a	1.30±0.12a
P-VALUES	0.0000	0.0000	0.0007
TUKEY (HSD)	0.75	2.55	0.44
CV	2.00	21.43	14.04

Means with the same superscripts are not significantly different at ($P \leq 0.05\%$) using the Tukey HSD test

Treatment methods did not show a significant difference with regard to shoot dry weight ($P<0.05$).

The maximum dry shoot weight was recorded in T6 (6.3 g), followed by T8 (6.17 g), T7 (5.53 g), T4 (5.2 g), T3 (4.7 g), T2 (3.27 g), and T1 (1.27 g), with the lowest shoot dry weight recorded in T5 (1.13 g). There was a significant ($P<0.05$) difference between the treatments in relation to dry root weight. The highest dry weight of the root was recorded at T6 (1.40 g), followed by T8 (1.30 g), T7 (1.27 g), T4 (1.20 g), T5 (1.17 g), and T3 (1.07 g). The lowest root dry weight was recorded at T1 and T2 (0.77 g each) (*Table 4*).

DISCUSSION

Tetrapluera tetraptera can be propagated through both seed and stem cuttings. In terms of sexual propagation, the seeds exhibited epigeal germination. However, the seeds are dormant because of the presence of a hard seed coat that prevents the entry of air and water (FAO, 1976; Deogratias, 2015). The hard seed coat of *Tetrapleura tetraptera* serves as a protective mechanism, ensuring the survival of the embryo in adverse environmental conditions, such as drought or excessive heat (Lawal, 2016).

However, it also poses a challenge for germination when favourable conditions for growth are present. The impermeable seed coat acts as a physical barrier and hinders the imbibition of water and gas exchange, which is a critical trigger for the initiation of germination processes (Adedire *et al.*, 2015). To overcome hard seed coat dormancy in *Tetrapleura tetraptera* seeds, various treatments can be applied to break or weaken the hard seed coat. Scarification techniques, such as mechanical scarification, acid scarification and hot water treatment, are commonly employed to enhance water absorption and promote germination (Ejimbe *et al.*, 2007; Adeyemi *et al.*, 2019). These treatments create small openings or weaken the seed coat, allowing water to penetrate and facilitating the rehydration of the dormant embryo.

This study demonstrated that the seeds of *Tetrapleura tetraptera* pretreated with sulphuric acid had a higher germination percentage compared to seeds pretreated with hot water. This implies that the application of sulphuric acid is an effective way of rendering the seeds of *Tetrapleura tetraptera* permeable, leading to germination of up to 58.3, 60, 57.5, and 58.3%. This might be attributed to the corrosive ability of sulphuric acid on the hard seed coat of *Tetrapleura tetraptera*, thereby making the seeds permeable to water and air and promoting higher germination.

The finding is in agreement with previous studies by Asiyire *et al.* (2008), Lacerna *et al.* (2004), Onyekwelu (1990) and Sam (2019), who reported sulphuric acid as an effective way of breaking the hard seed coats of plants.

However, this study disagrees with Missanjo *et al.* (2014), who found that *Acacia polyacantha* seeds pretreated with hot water were more effective in breaking the hard seed coat, resulting in a greater germination percentage compared to seeds treated with concentrated sulphuric acid.

Furthermore, Sera *et al.* (2023) demonstrated that using non-thermal plasma as a seed priming method significantly improved seed germination potential compared to untreated seeds.

In addition to nitrogen, phosphorus (P) is one of the most crucial microelements for plant growth and to produce their best yields, adequate P fertilization is necessary (Taliman *et al.*, 2019). Prathap *et al.* (2022) emphasized that when seeds develop, the phosphorus that plants have received is transferred from the roots and leaves to the seeds, where phytic acid is synthesized.

The significant increase in growth parameters demonstrated by *Tetrapleura tetraptera* with phosphorus application throughout the experiment demonstrated that there is better growth performance in *Tetrapleura tetraptera* when phosphorus is added to improve soil nutrient content. The phosphorus applied was absorbed by *Tetrapleura tetraptera* plants in the initial phase and used for root development, while the residual nutrient was not sufficient for subsequent crop growth support (Yu *et al.*, 2022).

In addition, the substantial influence of P on *Tetrapleura tetraptera* diameter was possible because the applied P caused cell expansion during the latter stages of the plant's growth, hence the evidence of a significant improvement in diameter (Ebeid *et al.*, 2015). The effect of P levels on the number of leaves showed that the P applied may have aided cell expansion and the production of photosynthates for vegetative leaf growth. This is in line with the findings of Nakayama *et al.* (2022), who claimed that all leaf cells divide at the beginning of leaf development just after the leaf primordium emerges from the shoot apical meristem. The cells near the tip of the leaf eventually stop dividing and begin to expand, while the cells at the base continue to divide to support future leaf growth.

Additionally, the application of P influenced nodulation, the weight of the dry shoot, and the weight of the dry root of the *Tetrapleura tetraptera* plant. P fertilizer dosages enhanced the nodulation, dry shoot weight, and dry root weight; the P level in T6 (H_2SO_4 + 25 kg P) had the highest nodule formation and root dry weight. This could have happened if the cotyledons or endosperm had been present, and the embryo would have grown at the expense of the nutrients it received (Ali *et al.*, 2019).

According to Míguez-Montero *et al.* (2020), these results demonstrated that phosphorus may induce an increase in nodulation and the weights of the dry shoots and roots of the tested tree and contribute to making it possible for the plant to be established in the field.

CONCLUSIONS

In conclusion, the results showed that the seeds of *Tetrapleura tetraptera* pretreated with sulphuric acid significantly increased the germination rate (60%) compared to seeds pretreated with hot water (40%). The application of P at T4 (hot water + 50 kg/ha P) had the highest height. T8 (H_2SO_4 + 50 kg/ha P) had the highest leaf number, and T6 (H_2SO_4 + 25 kg P) had the greatest number of nodules (nodulation) and dry shoot and dry root weights of *T. tetraptera* seedlings.

This study recommends that the seeds of *Tetrapleura tetraptera* to be pretreated with sulphuric acid to break their hard seed coats to decrease dormancy and improve germination during planting. Phosphorus fertilizer can be applied during the early stages of growth to improve its growth performance because the tested species exhibited a prudent demand for P during the early growth stages.

Furthermore, since the current study only focused on the number of nodules without looking at active and inactive nodules, further research could be conducted to increase the number of minutes the seeds spent in the respective seed pretreatments and to identify the number of active and inactive nodules.

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