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Propagation of *Treculia africana* Decne Species through Promotion of Seed Germination by Some Physical and Chemical Treatments

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Authors' contributions

This work was carried out in collaboration between both authors. Author MSI designed the study and wrote the study protocol. Author EC managed the analyses of the study and performed the statistical analysis. Both the authors participated in laboratory analysis, the literature searches, wrote the first draft of the manuscript, read and approved the final manuscript.

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ABSTRACT

The benefits, uses and potentials of *Treculia africana* Decne (African breadfruit) have been identified as socio-economically, industrially and environmentally important. However, lack of commercial propagation posses an extinction threat of this endangered species as seeds expressed poor germination under germinating conditions. Consequently this study was carried out in order to ascertain the most effective methods for enhancing seed germination of this economically important plant. The effects of washing the seeds; air-drying the seeds for different time intervals; soaking seeds in H_2O_2 for different time intervals; and germinating in different potassium nitrate concentrations were investigated. The results of all the treatments indicated that for dark germinations, 1 mM potassium nitrate treatment expressed the highest germination (84%), followed by soaking in H_2O_2 for 30 minutes (78%), then 144 hrs air-drying (68%), and washing of seeds (46%) unlike unwashed seeds (28%), while the control for all treatments gave germinations

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that ranged from 2 to 28%. However, the light germinations were lower than the dark germinations with the various treatments expressing the following optimum percentage germinations (KNO_3 – 53%; H_2O_2 – 72%; Air-drying for 72hrs- 28%, washing – 14% and unwashed – 2%) and the controls ranged from 2% to 14%. Light did not promote the seed germinations of *T. africana.* These findings implied that KNO_3, H_2O_2, Air-drying and washing treatments should be employed for the enhancement of seed germination of *T. africana* seeds for the production and propagation of this invaluable and economically viable plant.

Keywords: Seed germination; Treculia africana; washing; air-dry; hydrogen peroxide (H_2O_2); potassium nitrate (KNO_3); dark and light.

1. INTRODUCTION

Germination is a critical first step in the successful establishment of plant species and is influenced by environmental factors. Under natural conditions on the field, seeds are subjected to the vagaries of weather conditions/ treatments- some promotive and others inhibitory. Such treatments include abrasive effect of soil, effect of inorganic salts, presence of soil fauna, alternating temperatures, dessication effects and light regimes.

Treculia africana is a multipurpose tree species commonly known as African breadfruit. It belongs to the family *Moraceae* and grows in the forest zone, particularly the coastal swamp zone [1]. It is widely grown in southern Nigerian for its seeds and it is known by various tribal names in the country. Such names include 'afon' (Yoruba), "barafuta" (Hausa) "Ize" (Bini), "eyo" (Igala), "ediang" (Efik) and "ukwa (Igbo) [2-3]. *T. africana* is also indigenous to other countries like Ghana, Sierra Leone and the West Indies. *T. africana* is a large tree growing up to 30m high with a girth of 4-6 m, with a dense spreading crown and fluted trunk. The habits and habitats have been generously described in various works by [1,4-6] and recently by [7]. The benefits, uses and potentials of *T. africana* has been comprehensively described by [7] and include provision of food, traditional medicine, livestock fodder, wood products, industrial environmental functions, and employment and income generation among others.

Despite these socio-economic attributes, the decline in the population of *T. africana* has become a serious economic challenge; some of the factors promoting the decline of *T. africana* include deforestation, increased population pressure lack of species improvement and non-cultivation of species [7]. The lack of commercial propagation of this species poses a serious threat of erosion of its genetic resources as well as extinction threat. *T. africana* is currently included in the list of endangered species of southern Nigeria [7-8].

To abate total loss or extinction of this economically species, there is need to improve its propagation either by seed, stem cuttings, cropping, tissue culture, budding and shield grafting [9]. The bread fruit is mainly grown from seeds and freshly harvested seeds lose viability in few weeks [10]. The seeds of the breadfruit are enclosed in soft pulp that can decay [11] and the depulped for target purposes (utilization). Often seed ripening and collection does not correspond with the time of seedling production and some species do not produce seeds all year round. [12], thus enhancement of seed germination is therefore vital to secure the supply of seedlings for planting programme. Several authors have reported breaking of dormancy and promotion of germination using different scarification methods and application of nitrogenous compounds for other species [13-20]. However, studies on seed germination of *T africana* have been few and in-between. Dolor [12] noted that no germination was recorded in the seeds of *T. africana* stored beyond two weeks and results showed a progressive reduction in germination with increase in duration of storage up to 5 weeks. Lawal [21] noted that sowing depths influenced seed germination of *T. africana*. Seeds sown on the upper surface of the soil gave higher germination than seeds sown in the deeper depths which may indicate that environmental factors such as alternating temperatures, light, nitrate and water regimes effect its seed germination.

In the light of this, treatments such as washing of seeds, air-drying, soaking in hydrogen peroxide and application of exogeneous potassium nitrate were investigated in the light and dark conditions on the seed germination of *T. africana* species. While laboratory experiments are not exactly true reflection of field conditions, the seeds response allows some deduction regarding possible survival and enhancement of germination of

T. africana by ex-situ methods. In this work germination is simply defined and measured as protrusion of radicle in an environment meeting the normal physiological requirements for germination [13,23-26]. This paper therefore intends to survey and identify some treatment methods that can enhance seed germination of *T. africana* with the view of promoting its sustainability for its potential benefits and economic development.

2. MATERIALS AND METHODS

2.1 Study Location and Source of Material

The fresh fruits of *T. africana* were harvested from the tree, in Oduoha community located in Emohua Local Government Area of Rivers State, Nigeria.

The fruits were depulped after 7 days, allowed for decay process and the seeds removed. The seeds were subsequently subjected to various treatments and germinated under laboratory conditions as described below under germination procedures.

2.2 Viability Test by Floating Method

The viability of the seeds was determined by floating method [26]. The intact seeds were floated in water in a container. The seeds that settled at the bottom of the container were considered viable while those that floated on the surface were considered non-viable. The viable seeds were used for the experiments.

2.3 Pretreatment Methods

Seeds were subjected to the various pretreatment methods and germinated in dark or light conditions as described below under germination procedures.

2.3.1 Unwashed intact seeds

Seeds depulped from the fruit pod covered with slimy substance (untreated) were subjected to the standard germination conditions in both dark and light conditions.

2.3.2 Washing of seeds

Depulped seeds were washed under tap running water and subsequently rinsed in distilled water to remove any slimy covering and germinated in dark or light conditions.

2.3.3 Air-dry storage

Some of the washed batch of seeds were air-dried in dark conditions for 0 hr, 24 hrs, 72 hrs, and 144 hrs at room conditions and subsequently germinated in dark and light conditions.

2.3.4 Hydrogen peroxide (H_2O_2)

Washed seeds were soaked in hydrogen peroxide for different time internals of 0 minute, 10 minutes, 15 minutes, and 30 minutes after which seeds were rinsed in tap running water and subsequently germinated in dark or light conditions.

2.3.5 Potassium nitrate (KNO_3)

Washed seeds were germinated in different concentrations of potassium nitrate (KNO_3), namely 0 mM, 1 mM, 10 mM and 100 mM. These concentrations were prepared by dissolving 0.0 g, 0.101 g, 1.01 g and 10.1 g of KNO_3 respectively in one litre of de-ionized water.

2.4 Germination Procedures

For each treatment described above, four replicates of 20 seeds per replicate were put in petri-dish lined with Whatman's filter paper, watered with 5 ml of distilled water or appropriate solutions and wrapped with aluminium foil for dark incubated seeds or not covered with aluminium foil for light incubation. The seeds were observed daily and moistened as deem necessary. Germination counts were recorded daily, and final count was recorded after 7days of incubation at 25℃ in the dark or light conditions. Germination was scored when the radicle protrudes from the seed coat. For all the different treatments, controls were set-up alongside.

2.5 Statistical Analysis

Data generated in this study were subjected to statistical analysis to determine level of significance using Microsoft Excel 2010.

3. RESULTS

3.1 Germination of Unwashed and Washed Intact Seeds

The mean percentage germinations of unwashed seeds of *T. africana* were 28% and 2% in the dark and light conditions respectively (Fig. 1). The results indicated generally poor germination; however the seeds gave higher germinations in the dark compared to light. Washing of seeds enhanced germination (46%) in the dark and (14%) in light compared to unwashed treatments (Fig. 1). The promotive effect of washing treatment might be ascribed to the removal of the slimy coverings on the seeds. Generally light has no effect in the germination of *T. africana* seeds. Ecologically, the rains aid germination by washing away the slimy coverings on the seeds, thus promoting germinations as observed from this study. The germination percentage of the washed seeds was significantly different form the unwashed ones (Table 1). Also, the dark germinations were significant over the light germinations (p=.05) Table 1.

3.2 Air-dry Storage Treatment

Fig. 2 indicated the effect of air-dry storage at room condition on the germination of seeds of *T. africana*. For dark germinated seeds, results indicated a progressive increase in germinations from 0 hr (control), 24 hours, 72 hrs and 144 hrs with germinations of 28%, 48%, 61% and 68% respectively (Fig. 2) but there is no significant difference with increasing drying time (Table 2). Germinations of light incubated seeds progressively increased from 0hour to 72hours treatments and declined at 144 hours treatment (Fig. 2). Dark germinated seeds showed marked and significant germinations over light germinated seeds (Fig. 2 and Table 2). Also, duration of drying and germinating in the dark have significant effect on the percentage germination of the species studied (Table 2).

Dolor [12] reported a progressive reduction in germination with increase in duration of storage and no germination occurred in *T. africana* seeds beyond two weeks to five weeks of storage duration. The results obtained here showed a progressive increase in percentage germination for 6-days air-dry storage period monitored for 7-days. Critical analysis of germination trend showed slow rate of germination from 3 to 6-days storage period, this may suggest that further storage period may not improve germination. Indeed for light treatment incubated seeds, germination decreased after 6-days air-dry storage duration. The results of air-dry storage reported in this study may be in tandem with [12] findings that freshly harvested *T. africana* seeds lose viability on long storage.

3.3 Hydrogen Peroxide Treatment

Exposure of seeds in hydrogen peroxide for various time intervals of (0 minutes i.e. control, 5 minutes, 10 minutes, 15 minutes, 30 minutes) revealed a progressive increase in percentage germinations for dark and light incubated seeds (Fig. 3). Germinations were 28% dark/2% light for control; 52% dark/36% light for 5minutes; 60% dark/56% light for 10minutes; 66% dark/64% light for 15 minutes and 78% dark/72% light for 30 minutes. The germination trends were similar for dark and light incubations; with significant difference between dark and light incubations/treatments and exposure time. Generally, the results revealed that hydrogen peroxide enhanced germination in both dark and light conditions and is responsible for the significant difference in the germination percentages (Table 3). Hydrogen peroxide appears to oblivate the inhibitory effect of light in seed germination.

Table 1. Analysis of variance (ANOVA) for washed and unwashed seeds of *T. Africana*

Source of variation	SS	df	MS	F	P-value	F crit
Washed and unwashed	1051.25	1	1051.25	12.101	0.003101	4.494
Effect of light and dark (B)	2761.25	1	2761.25	31.784	3.71E-05	4.494
A x B	11.25	1	11.25	0.1295	0.723661	4.494
Error	1390	16	86.875			
Total	5213.75	19				

Table 2. Analysis of variance (ANOVA) for duration of drying on *T. africana* seeds germination

Source of variation	SS	df	MS	F	P-value	F crit
Duration of drying (A)	671.6667	2	335.833	4.404	0.023	3.403
Light and dark (B)	11213.33	1	11213.33	147.060	1.01E-11	4.260
A x B	1271.667	2	635.833	8.339	0.002	3.403
Error	1830	24	76.25			
Total	14986.67	29				

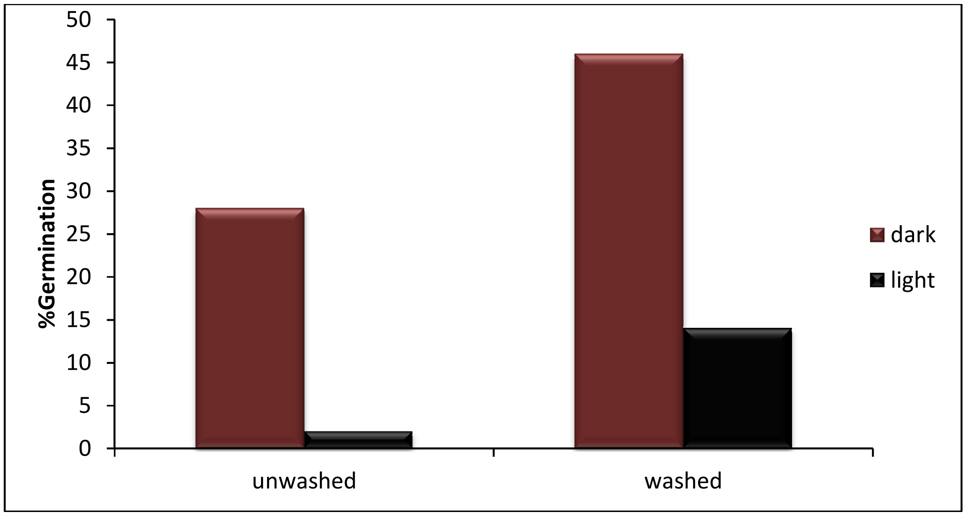

Fig. 1. Percentage germinations of unwashed and washed *T. africana* seeds germinated in the light and dark conditions and incubated at 25℃ for 7 days

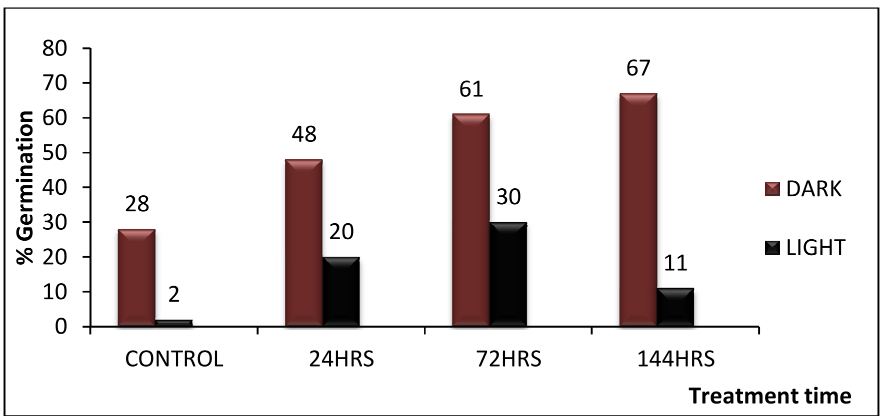

Fig. 2. Percentage germinations of air-dried seeds of *T. africana* germinated in light and dark conditions for different hours and incubated at 25℃ for 7 days

Table 3. Analysis of variance (ANOVA) for the effect of hydrogen peroxide on seeds germination of *T. Africana*

Source of variation	SS	df	MS	F	P-value	F crit
Treatment time (A)	4540	3	1513.33	12.385	1.53E-05	2.901
Light and dark (B)	360	1	360	2.9463	0.095742	4.149
A x B	340	3	113.333	0.9275	0.438687	2.901
Error	3910	32	122.188			
Total	9150	39				

3.4 Potassium Nitrate Treatment (KNO_3)

The application of exogenous potassium nitrate in the seed germination of *T. africana* indicated percentage germinations of 84% dark/53% light incubations in 1 mM treatment; 62% dark/40% light incubations in 10 mM treatment; and 49% dark/29% light incubations in 100 mM treatment. All the treatments gave higher germinations when compared with the control (28% dark/2% light incubations) (Fig. 4). The observed results indicated that germinations increased from the control to 1 mM KNO_3 treatment and gradually decreased to 100 mM. 1 mM KNO_3 treatment gave the highest percentage germinations, while 100 mM gave the lowest germination. Dark germinations were consistently higher than light germinations for all treatments. The trend of germinations is similar for both dark and light incubations. These results revealed that the optimum potassium nitrate concentration for enhancing the seed germination of *T. africana* is about 1 mM and higher concentrations from 10 mM and beyond are inhibitory to germinations. There is significant difference associated with the treatment of the seeds with KNO_3 and incubating in the dark (Table 4).

Studies have shown the propagation and production of seeds of plant with economic, medicinal and ornamental values in large fields in several countries. Thus the need to investigate different methods for the improvement of economically valuable plants is a welcome development and have been done through plant seeds [27-30] and cuttings [31-33].

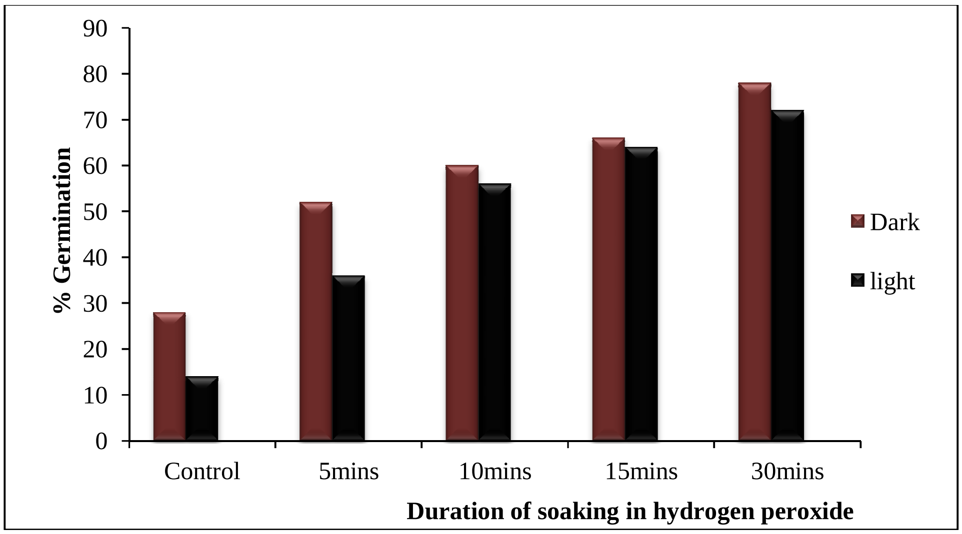

Fig. 3. Percentage germinations of seeds of *T. africana* soaked in Hydrogen peroxide and incubated at 25℃ in light and dark conditions for 7 days

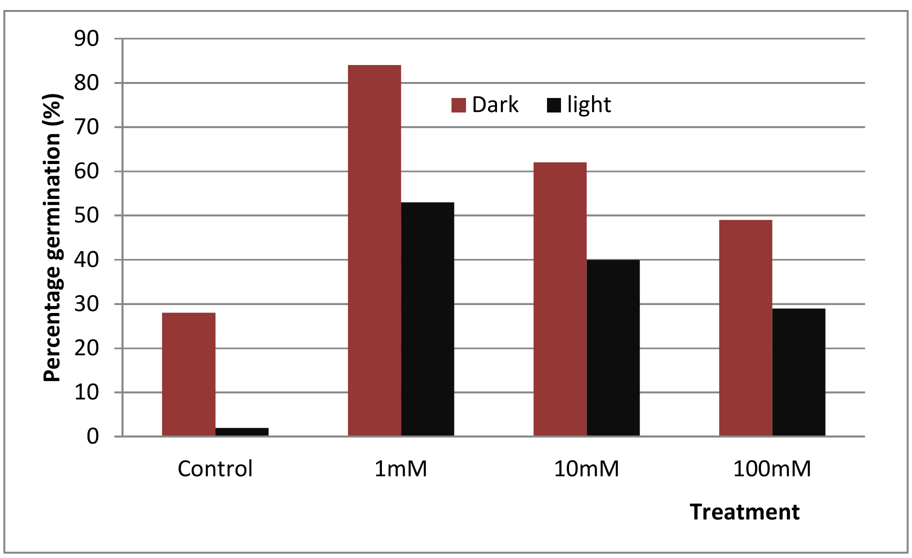

Fig. 4. Percentage germination of seeds of *T. africana* treated with potassium nitrate and incubated at 25℃ in light and dark condition for 7 days

Table 4. Analysis of variance (ANOVA) for the effect of potassium nitrate on seeds germination of *T. africana*

Source of variation	SS	df	MS	F	P-value	F. crit
Concentration (A)	4385	2	2192.5	20.717	5.93E-06	3.403
Effect of light and dark (B)	4320	1	4320	40.819	1.32E-06	4.260
A x B	185	2	92.5	0.8740	0.430	3.403
Error	2540	24	105.833			
Total	11430	29				

4. DISCUSSION

4.1 Washing Treatment

Washing treatment significantly promoted the seed germination of *T. africana*, indicating that the slimy coverings on the seeds might inhibit germination. It is also important that seeds are sown in wet soils and during rainy season to enhance germination. Okeyo and Ochnodho [34] noted that there is improved germination in washed seeds of *Zanthoxylum gilleti* when compared to the unwashed seeds.

4.2 Air-drying Treatment

Air-dry storage at room temperature progressively enhanced germination of *T. africana* seeds from 0 minute (28%) to 144 hrs (68%) in the dark conditions. Again, like washing treatment, light, incubated seeds gave poor germinations when compared to dark incubated seeds. Germinations recorded for the air-drying treatment were higher than those of washing treatment indicting that air-dry treatment is more effective in improving the germination of *T. africana* seeds. Dolor [12] noted reduction in germination of *T. africana* seeds after two to five weeks storage period, indeed no germination occurred beyond two weeks. For *T. africana,* it may be that seeds lose viability during long storage period [12], the storage period for this study did not exceed 6-days. These findings suggest that seeds of *T. africana* can be considered recalcitrant and seeds rapidly lose viability during long storage and supports [35].

Ecologically, the effect of high temperature in the soil might act to produce drying effect in the soil and this may impose or release dormancy and enhance germination. This effect is regarded as after-ripening and may be considered as any change which occurs in the seeds during storage as a result of which conditions under which dormancy can be broken and germination expressed are widened [22]. Drying has been reported to break dormancy, especially at elevated temperatures for other species [36-37]. However, in some cases, drying could also impose dormancy, e.g. in lettuce seeds [38].

4.3 Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) resulted in progressive increase in percentage germination with increase time of soaking both in dark and light conditions (Fig. 3). Light incubated hydrogen peroxide treated seeds gave the highest germinations when compared to other treatments germinated in light, it may be that be that H_2O_2 oblivates the inhibitory effect of light or there is a synergy between H_2O_2 and light. H_2O_2 has been reported to increase germination of pea seeds [39]; in cereals [40]; *Zinna elegans* (Jacq) [41]; and *Poa pratensis* [42]. The mechanism of action might be purely due to chemical scarification of the seed coat because it is a strong oxidizing agent. Alternatively, it may be physiological, H_2O_2 acts through being spared by inhibition of catalase action by the action of nitrite and nitrate [43] or and by being hydrogen acceptors. However, H_2O_2 may also stimulate germination by increasing oxygen tension or promoting oxidation of any inhibitory substance on the seed coat.

4.4 Potassium Nitrite (KNO_3)

Incubation in KNO_3 enhanced germinations both in the dark and light conditions when compared to controls. Dark germinations were higher compared to their respective light germinations. Also germination decreased with increase in KNO_3 concentrations and 1 mM concentration recorded the highest percentage germination while 100 mM gave the lowest germination in both dark and light conditions. The results observed in this study agree with the findings from other studies that KNO_3 effectively improved seed germination in *Calotropis* *persicaria* [43]; pigweed seeds and lettuce sativa [44-46], *Medicago* species [47]; *Gmelina arborea* [48]; *Prunus avium* [49]; barley and rice [50]. KNO_3 promotes seed germination in varied and wide variety of species, however was ineffective in seeds of *Senna obtusifolia* [13].

The mode of action by which potassium nitrate promote germination is not fully understood, Hendricks and Taylorson [44-46] suggested that the chemical function by irreversible inhibition of catalase activity and promoting pentose phosphate (pp) pathway, and also reduction of nitrate to nitrite provides ions which might be coupled to cyanide-resistant respiratory activity (alternate pathway). However, Esashi et al. [51] noted that in non-dormant unimbibed Cocklebur seeds KNO_3 inhibited catalase actions, but was ineffective in stimulating germination. Furthermore, Junttila and Nilsen [52] observed that respiratory intensity is not correlated with germination in *Phalaris anudinacea*. The findings with KNO_3 revealed that for enhance propagation of *T. africana* plants, freshly harvested seeds should be sown in soil media with low concentration of KNO_3.

Generally, the light results indicated that light did not enhance seed germination of *T. africana* species and may be considered inhibitory to germination in contrast to the promontory effects of light reported for other species [19,53-54] or in combination with other treatments like nitrate, chilling [17-18]. However, for H_2O_2 treatments, percentage germinations in the light were comparable to dark germinations unlike other treatments (washing, air-drying and KNO_3 treatments). It could be argued that H_2O_2 nullified the inhibitory effect and/or mechanism of action as earlier discussed, and thus promoted seed germination. The effect of light on the seed germination of *T. africana* needs an indepth investigation, using R/FR irradiation treatment.

5. CONCLUSION

The challenges of poor seed germination of *T. africana* can be improved with treatments such as washing the seeds, air-drying the seed for short duration, soaking seeds in H_2O_2 for short duration and germination seeds in low concentration of 1 mM KNO_3 as identified in this study. These identified efforts, hopefully, shall reverse the decline and enhance the production of *T. africana* plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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