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Germination and growth tests of young plants of *Kigelia africana* (Lam.) Benth. (Bignoniaceae) from different climatic origins in Benin

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Abstract

In southern Benin, more than 20 people buy *Kigelia africana* organs a day. *K. africana* is a species classified as vulnerable on the IUCN red list of Benin. In order to contribute to the rational use of the tree because of its importance in Benin, a study of germination and growth tests of young plants of *Kigelia africana* from different climatic origins in Benin was carried out. The two specific objectives of the study were -i- to test the germination of *K. africana* seeds from different climatic origins and -ii- to test the effect of pretreatment of these seeds. Thus 360 seeds were sown in pots. The five following treatments with 12 repetitions were done: T0 treatments: control treatments; T1: Seed soaked in water at room temperature for 24 hours; T2: Seed soaked in water at room temperature for 48 hours; T3: Seed soaked in boiling water for 60 seconds; T4: Seed soaked in boiling water until cool. The seed germination trial conducted showed the existence of significant ($p < 0.05$) differences between provenances in germination rate and seedling growth not only between climatic zones. Hot water inhibited seed germination rate: 0% germination. Seeds from the Sudano-Guinean zone had the best germination rates (75% on average) followed by those from the Sudanian zone (66.67%). The grains of *Kigelia africana* germinate regardless of the climatic environment. They do not require pregermination treatment.

Key words: Germination rate, sausage tree, juvenile growth, germination pretreatment, West Africa

Tests de germination et de croissance de jeunes plants de *Kigelia africana* (Lam.) Benth. (Bignoniacées) de différentes provenances climatiques au Bénin

Résumé

Au Sud-Bénin, plus de 20 personnes achètent par jour les organes de *Kigelia africana*, une espèce classée vulnérable sur la liste rouge UICN du Bénin. Afin de contribuer à l'utilisation rationnelle de l'arbre du fait de son importance au Bénin, une étude de tests de germination et de croissance de jeunes plants de *Kigelia africana* de différentes provenances climatiques au Bénin ont été entreprises. Les objectifs spécifiques étaient -i- de tester la germination des graines de *K. africana* de différentes provenances climatiques et -ii- de tester l'effet de prétraitement de ces graines. Ainsi, 360 graines ont été semées dans des pots. Les cinq traitements suivants avec 12 répétitions ont été réalisés : T0 : traitements témoins ; T1 : Graine trempée dans l'eau à température ambiante pendant 24 h ; T2 : Graine trempée dans l'eau à température ambiante pendant 48 h ; T3 : Graine trempée dans l'eau bouillante pendant 60 secondes ; T4 : Graine trempée dans l'eau bouillante jusqu'à refroidissement. L'essai conduit sur la germination des graines a montré l'existence–de différences significatives ($p < 0,05$) entre les provenances concernant le taux de germination et la croissance des jeunes plantules non seulement entre les zones climatiques. L'eau chaude a inhibé le taux de germination des graines avec 0 % de germination. Les graines de la zone soudano-guinéenne ont eu les meilleurs taux de germination (75 % en moyenne) suivi de celles de la zone soudanienne (66,67 %). Les grains de *Kigelia africana* germent quel que soit le milieu de provenance climatique. Elles ne nécessitent pas un traitement pré germinatif.

Mots clés : Taux de germination, arbre aux saucisses, croissance juvénile, prétraitement germinatif, Afrique de l'Ouest.

Introduction

Kigelia africana is one of the woody species of major importance in tropical Africa which is known under the common name of Saucissonnier and is used in several categories of use (Neuenschwander *et al*., 2011; Dassou *et a*l., 2014). The barks, roots, flowers and fruits help in the treatment of rheumatism, haemorrhoids, intestinal worms, female sterility. In southern Benin, more than 20 people buy *Kigelia africana* organs per day (Adomou *et a*l., 2012). The heavy exploitation of these organs makes *K. africana* vulnerable (Lawin *et al*., 2016; Neuenschwander *et al*., 2011). Deleke (2005) and Koukoubou (2008) pointed out that there is a disadvantage when the frequent use of a plant species is not followed by planting and therefore without any attempt at domestication. Neuenschwander *et al*. (2011) recommended as research the regeneration of *Kigelia africana* in Benin. Several studies have been carried out to this effect in Uganda and Nigeria. Miller *et al*. (2008a) reported that in Uganda *Kigelia africana* has a very low germination rate. Rafiu *et al.* (2018) found that the seed of *K. africana* germinates easily. Others have found that it grows best in warm regions, due to its sensitivity to cold (Grace and Davis, 2002). In southern Africa it is said to grow rapidly from seed. In other areas, the germination rate is low (Grace and Davis, 2002). Pritchard and Linington (2002) reported that soaking Kigelia africana seeds in hot or boiling water before sowing would improve its germination. Grace and Davis (2002) confirmed that soaking in hot or boiling water before sowing *K. africana* seeds improve germination.

With the importance recognized to the species today in Benin, the improvement of knowledge on the regeneration of *Kigelia africana* is crucial. The objective of this study is to test the germination and growth of young plants of *Kigelia africana* from different climatic origins in Benin. More specifically, it will involve: (1) testing the germination of *Kigelia africana* seeds from different climatic origins in Benin; (2) test the effect of pretreatment of seeds from different provenances

Study environment and origin of Kigelia africana seeds

The seeds used for the trial came from natural populations of *Kigelia africana* in the Sudanian, Sudano-Guinean and Guinean climatic zones (Figure 1).

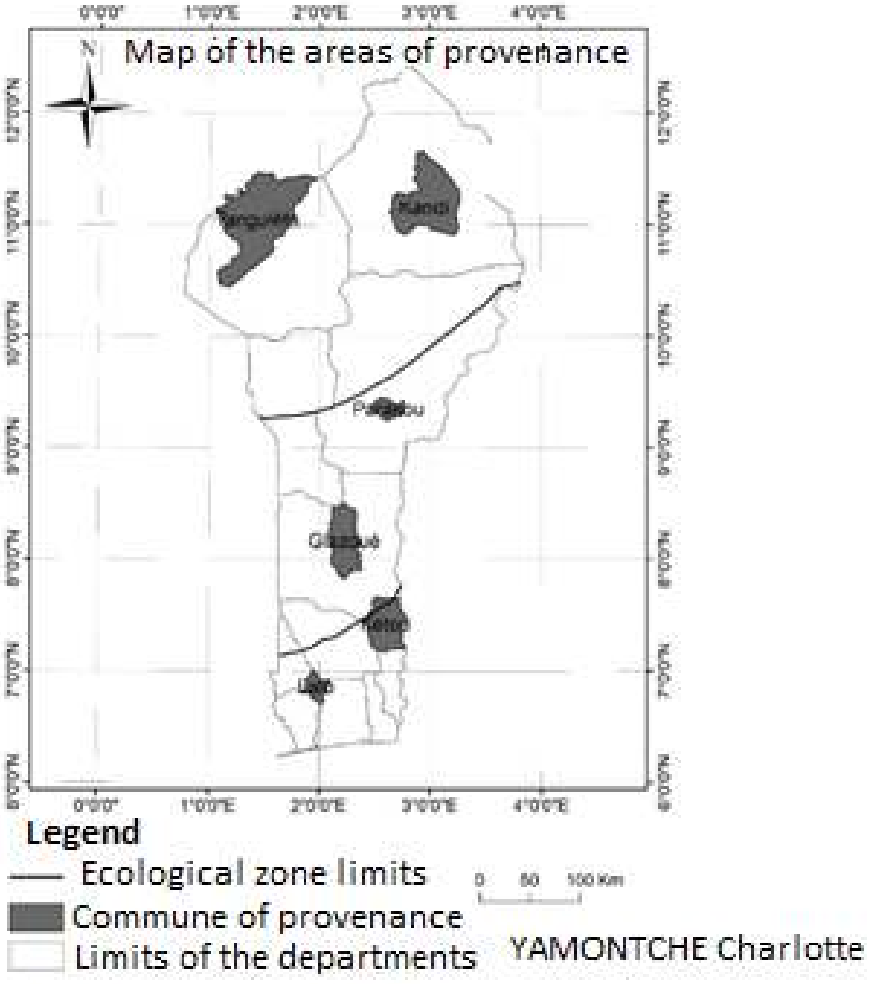

Figure 1. Communes of origin of the seeds

The seeds were extracted from the pulp manually after breaking the fruit with very heavy wood or cutting with a machete. The broken or cut fruits were covered with jute bags for a week so that the pulp decomposed a little to facilitate the extraction of the seeds. The extracted seeds were dried in the open air in a chamber for 3 days. Visually damaged seeds (pierced, atrophied, rotten) are sorted and discarded. Only seeds in good physical condition underwent germination pretreatments and sowing. The tests were carried out in the courtyard of the annexe office of the Forest Studies and Research Laboratory of the University of Parakou.

Materials and methods

Kigelia africana seed germination test

The seeds of *K. africana* were subjected before sowing to the various treatments below:

- T_0 : control treatment
- T_1 : Seed soaked in water at room temperature for 24 hours
- T_2 : Seed soaked in water at room temperature for 48 hours
- T_3 : Seed soaked in boiling water for 60 seconds
- T_4 : Seed soaked in boiling water until cool

Germination experimental device and data collection

The seeds are sown in perforated black polyethylene bags containing compost. A complete random block has been set up. The origin of the seeds (Sudanian zone, Sudano-Guinean zone and Guinean zone) and five germination pretreatments mentioned above, i.e. a total of fifteen (15) treatments (3 x 5) are carried out. Each treatment is repeated 12 times. The experimental unit for data collection is a set of four pots. A total of three hundred and sixty (360) pots are installed.

The pots were watered in the evening on the eve of sowing. Sowing is done very early in the morning without watering. Only one seed is sown per pot. According to Grace and Davis (2002), the seeds should be covered with a thin layer of sand or compost, and kept moist. Thus, the seeding depth respected in the present study is approximately 1 cm. Watering is carried out with a watering can with a capacity of 12 L, twice a day (morning and evening) except in the event of rain. When it rains, watering is suspended for one or two days depending on the intensity of the rain. The trial is conducted in a natural environment on the experimental site of the Forest Studies and Research Laboratory of the University of Parakou. During the conduct of the test, the number of seeds germinated per treatment is counted daily, the number of days before the first germination (the lag time) per treatment as well as the duration of germination is noted. After emergence, 30 plants out of 40 per provenance, i.e. seventy-five percent (75%) are chosen for measurements. They started two (2) months after sowing and are done every week for three (3) months. These measurements relate to the following parameters: the height, the diameter at the collar and the number of leaves. A tape measure was used for the height measurement and a caliper for the diameter measurement at the collar.

Data analysis

Effect of provenance and seed pretreatments on the germination of *Kigelia africana*

To test the effect of seed provenances and seed pretreatments on seed germination, linear mixed-effect models (Pinheiro and Bates, 2000) were established using the lmer function of the lmerTest and lme4 packages. In this model, the treatment is considered as a fixed factor and the block as a random factor. The plan being perfectly balanced, the arithmetic means calculated made it possible to draw the graph showing the evolution of the rate of germination of the seeds over time. The germination rates (Tg) per treatment expressed as the number of seeds germinated (Ngg) relative to the number of seeds sown (Ngs) were given by the following formula: $Tg = \frac{Ngg}{Ngs}\ X\ 100$.

The germination rate or average germination time (MGT) is expressed in relation to the time required to have half of the seeds germinated (Côme, 1970; Dardour, 2014). The lower the speed value and the faster the germination was given by the following formula: $TMG = \frac{(n1*t1)+(n2*t2)+\cdots..(ni*ti)}{N}$, where: $t1$ is the time required for the germination of n1 of seeds; n1 is the cumulative percentage of sprouted seeds whose value is closest to 50% by lower value, n2 is the cumulative percentage of sprouted seeds whose value is closest to 50% by upper value and t2 is the time required to n2 germination of seeds. The different germination parameters (germination rate, latency time, average germination duration and

germination rate) at the end of the test are subjected to the statistical analysis of variance in a linear model with Student's t test under R 3.5.1 software for comparison of means.

Effect of provenance and seed germination pretreatments on the growth of seedlings of *Kigelia africana*

To test the effect of seed provenance and seed germination pretreatments on growth in height and diameter at the collar of seedlings from *K. africana* seeds, linear mixed-effect models on longitudinal data using the LME function of the library NLME (Pinheiro *et al*., 2016) have been established. To test the effect of treatments on the growth of seedlings from germinations, generalized linear mixed effect models (GLMM) were used (Christina Knudson 2018).

In these models, the treatment is considered as a fixed factor and the block as a random factor. The plan being perfectly balanced, the arithmetic means calculated made it possible to draw graphs showing the evolution of the growth parameters (height, collar diameter and number of leaves) of the seedlings over time. All statistical analyzes are performed in R software version 3.5.1 (R Core Team, 2018).

Results

Germination speed and seed germination rates

Seeds from the Sudanian zone had a very fast germination rate unlike seeds from the Guinean zone (Figure 2). As for the treatment of the seeds, the T1 treatment, that is to say the seeds soaked in water at room temperature for 24 hours, had a very fast germination rate compared to the seeds of T0, that was to say the control seeds (Figure 3). Similarly, no seed soaked in hot water gave a germination rate. The combination of provenance and seed treatment showed a low germination rate at ST1 and ST2 but relatively high at GT0 (Figure 4). All the treatments in the Guinean zone (GT0, GT1 and GT2) presented the highest germination speeds. The germination was rapid for seeds from the Sudanian zone and soaked in water at room temperature for one or two days (Figures 2, 3 and 4). Seeds from the unsoaked Guinean zone took more days to germinate. No seed soaked in hot water for 60 minutes or until completely cooled was germinated. The Sudano-Guinean zone had a better rate (over 70%) for all treatment except treatments 3 and 4. The Sudanian zone had the lowest germination rate for all treatments (Figure 5). Seeds soaked in hot water gave a rate of 0% unlike seeds that did not undergo any treatment (T0) regardless of their area of origin.

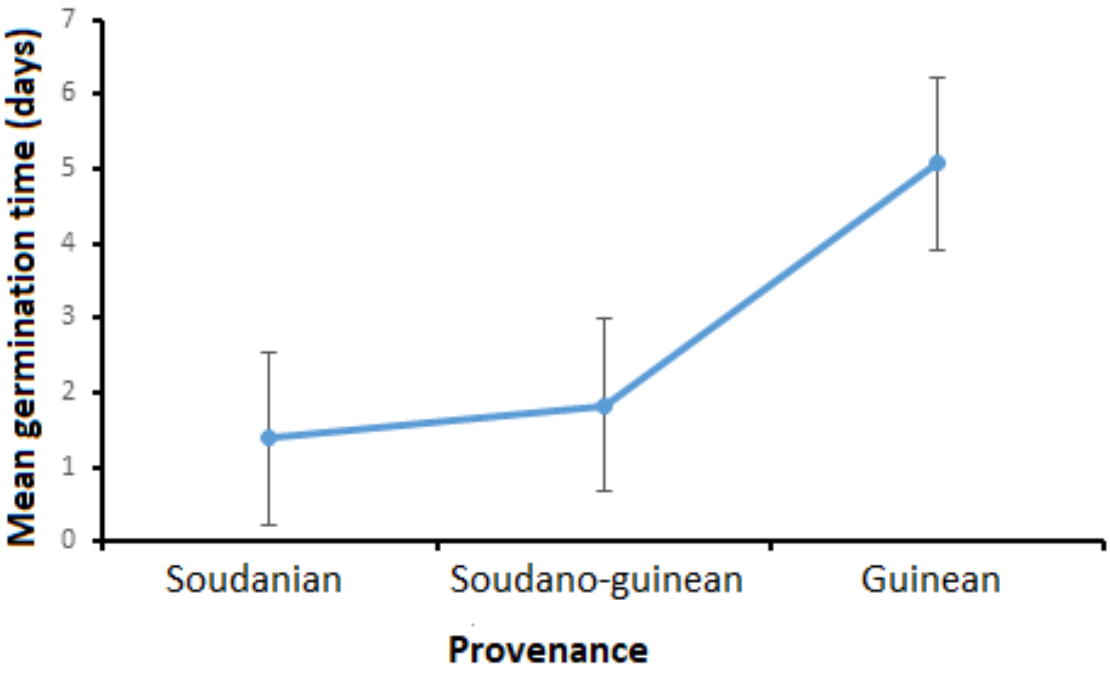

Figure 2. Germination rate according to the origin of the seeds

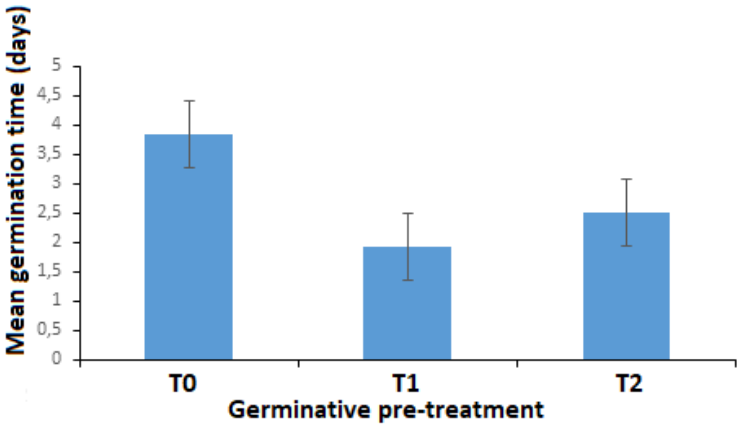

Figure 3. Germination rate following seed treatment

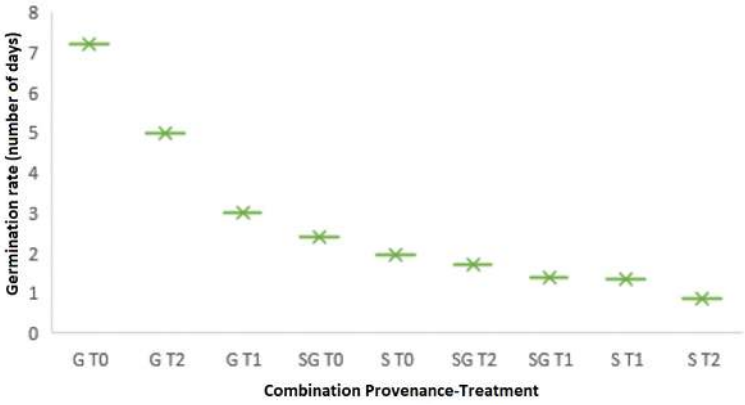

Figure 4. Germination rate according to origin and treatments

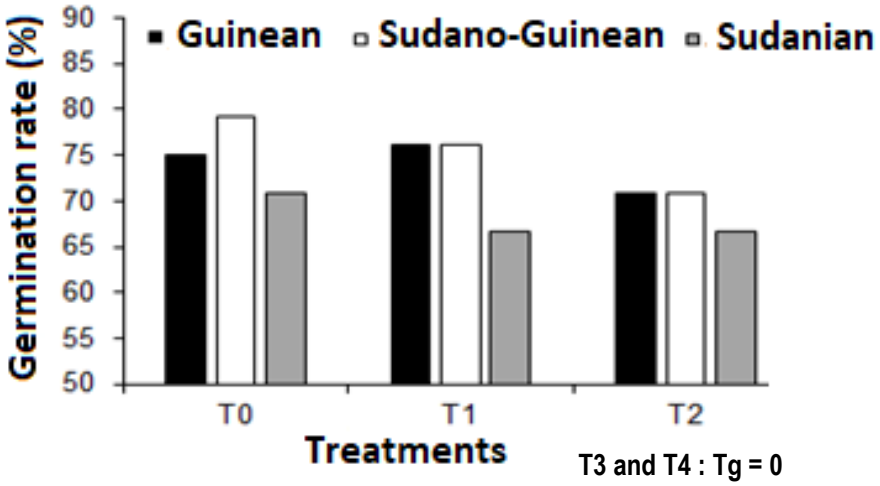

Figure 5. Germination of seeds according to the zones of provenance

Legend: T0: Control treatments; T1: Seed soaked in water at room temperature for 24 hours; T2: Seed soaked in water at room temperature for 48 hours; T3: Seed soaked in hot water for 60 seconds; T4: Seed soaked in hot water until cool; TG: Germination rate.

Effect of provenance and pre-germination treatment on the growth of K. africana seedlings

Height growth

The total height of *K. africana* seedlings showed a very significant difference (P = 0.001) depending on the origin of the seeds (Table 2).

Tableau 2. Growth parameter according to origins and germination pretreatments

Characteristics	Variables	Square sum	df	F	Prob.
Intercept	Height	292,046	1	3,850 x10^3	0,000
	Collar diameter	292,046	1	3,850 x10^3	0,000
	Number of sheets	142339,101	1	4,092 x10^3	0,000
Provenance	Height	786,058	2	6,965	0,001
	Collar diameter	0,111	2	0,735	0,480 (ns)
	Number of sheets	705,443	2	10,141	0,000
Traitement	Height	1332,895	2	11,810	0,000
	Collar diameter	2,774	2	18,285	0,000
	Number of sheets	47,496	2	0,683	0,505 (ns)
Provenance*traitement	Height	439,649	4	1,948	0,100 (ns)
	Collar diameter	0,653	4	2,151	0,073 (ns)
	Number of sheets	266,582	4	1,916	0,106 (ns)

The lowest height growth was noted with seedlings from *K. africana* seeds from the Sudano-Guinean zone and the strongest height growth was observed with seedlings from seeds from the Guinean and Sudanian zones (Figure 6a). The type of seed pretreatment also significantly influenced the height growth of *K. africana* seedlings (P < 0.001), with strong height growth of seedlings from seeds soaked in water at room temperature for 24 h (T1) and 48 h (T2) and poor growth in height of seedlings from seeds that had not undergone any pretreatment (Figure 6b). On the other hand, the combined action of the provenance and the pre-germination treatment of the seeds has no significant effect on the total height of the seedlings of *K. africana*.

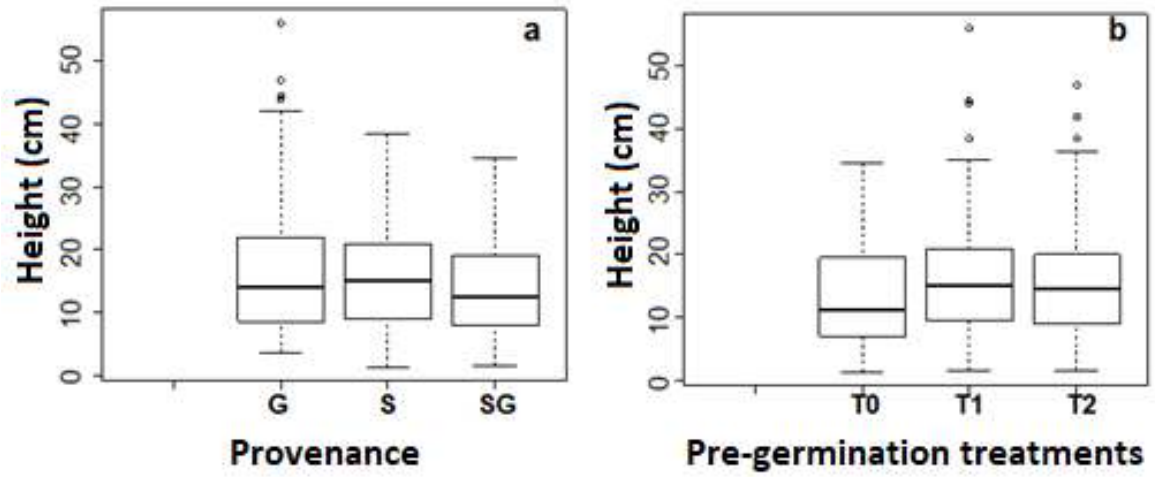

Figure 6. Boxplot diagram of total height values of *K. africana* seedlings for each provenance and pre-germination treatment of seeds

Growth in diameter at the collar

The origin of *K. africana* seeds had no significant influence (P = 0.480) on the collar diameter of seedlings (Table 1). On the other hand, germination pretreatment showed a highly significant influence on the collar diameter of *K. africana* seedlings with a strong growth in diameter for seedlings from seeds soaked in water at room temperature for 24 h (T1) and 48 h (T2) (Figure 7). The combined action of provenance and pre-germination treatment of seeds had no significant effect on the total height of *K. africana* seedlings.

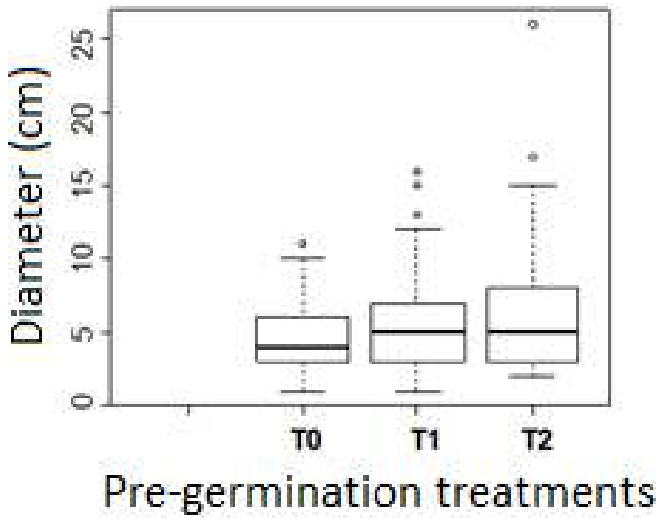

Figure 7. **The origin of *K. africana* seeds had no significant influence (P = 0.480) on the collar diameter of seedlings (Table 1).**

Germination pretreatment showed a highly significant influence on the collar diameter of *K. africana* seedlings with a strong growth in diameter for seedlings from seeds soaked in water at room temperature for 24 h (T1) and 48 h (T2) (Figure 7). The combined action of provenance and pre-germination treatment of seeds has no significant effect on the total height of *K. africana* seedlings.

Number of Leaves

The average number of leaves per seedling of *K. africana* showed a highly significant difference (P < 0.001) depending on the origin of the seeds (Table 1). The lowest average number of leaves per seedling was noted with seedlings grown from seeds of *K. africana* from the Sudano-Guinean zone and the highest average number of leaves per seedling was observed with seedlings grown from seeds of Guinean and Sudanian zones (Figure 8). The pre-germination treatment and its action combined with the provenance had no significant effect on the average number of leaves of *K. africana* seedlings (Table 2).

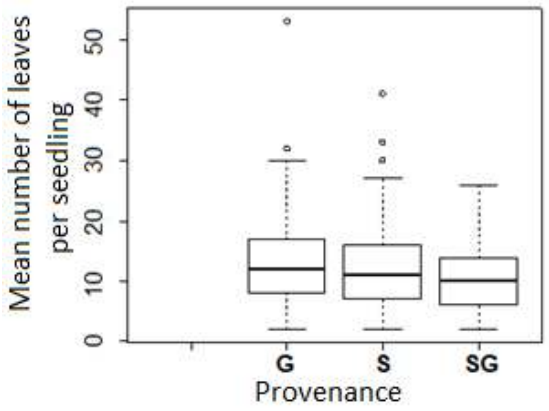

Figure 8. **Boxplot diagram of the values of the average number of leaves per seedling of K. africana for each provenance of the seeds G = Guinean zone; S = Sudanian zone; SG = Sudano-Guinean zone**

Discussion

Kigelia africana seed germination

The seeds of *K. africana* from all climatic zones combined, that is to say the Guinean, Sudano-Guinean and Sudanian zones germinated with a rate of at least 60% which corresponds to the Sudanian zone and nearly 80% which corresponds to the Sudano-Guinean zone. These results corroborate those of Rafiu *et al* (2018) in Nigeria, i.e. 77% for *Kigelia africana*. On the other hand, the results are contrary to those of Meunier *et al*. (2010) and Meunier *et al*. (2008) in Uganda who found unsatisfactory results, i.e. a very low germination rate for this same species. The results of Meunier *et al*. (2010) and Meunier *et al*. (2008) may be due to relative humidity (Guinean zone) where the test was conducted, on the one hand, or the origin of the seeds from a Guinean zone (wet zone) on the other hand. Indeed Guan *et al*. (2020) and Grace and Davis (2002) found that humidity influences germination rate especially for *Kigelia africana.*

Considering the different pre-germination treatments, seeds soaked in boiling water for 60 seconds or in boiling water until completely cooled have a rate of zero (0%). The results of boiling water soaking are contrary to those of Pritchard and Linington (2002) who suggest that boiling water soaking improves germination of *K. africana*. Seeds that have not undergone any germination treatment has the best germination rate.

The germination rate is fast, that is to say low (2 days) for the Sudanian zone, unlike the Guinean zone which is higher (5 days). This speed is much higher than that observed in cashew (16 days), which is a tree like *Kigelia africana* but with larger seeds than *K. africana* (Touré *et al*., 2018).

Growth of Kigelia africana

The average growth observed is 16.11cm for the three climatic zones. The Sudanian zone has the highest height (17 cm). Bolanle–Ojo *et al*. (2014) found in Nigeria 14.30 cm maximum height for this same species for 3 months. Similarly, in the same country Rafiu *et a*l. (2018) found about 14 cm in height for 12 3-month-old *K. africana* plants smoked with poultry droppings. 13.97 cm is the height of a cashew tree plant in Benin in its 90th day (Toué *et al*., 2018). This seems paradoxical because it is the exotic plants that are called rapid growth and a native plant has a better growth in height in a few days.

The average number of leaves is 11 with the Guinean zone having the highest number (12). For this same species and for 3 months in Nigeria gave a more improved result (about 14 leaves) for plants in sunlight (Bolanle-Ojo *et al*., 2014). Rafiu *et al*. (2018) found almost 20 leaves per plant of *K. africana* smoked with beef dung and/or poultry droppings in Nigeria.

Conclusion

The seeds of *Kigelia africana* from the three climatic zones namely Sudanian, Sudano-Guinean and Guinean germinate very well in Benin. Seeds without pre-germination treatment have a higher germination rate than seeds that have undergone pretreatment. No seed soaked in boiling water germinated. The growth parameters, that is to say the height and the diameter, are significantly depending on the origin. In perspective, it will be a question of testing the effect of the duration of conservation of the seeds for germination of these.

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