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Effects of Diverse Pretreatments on Seed Germination in *Cleome gynandra* L. (Capparidaceae) - A Threatened Species, Collected from Different Agro-Ecological Zones in Benin

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Abstract

We analyzed in preliminary survey the behaviour of *Cleome gynandra vis-à-vis* germination characteristics as initial goal in any subsequent program towards its conservation and rehabilitation. To optimize seed germination, the influence of four different chemical treatments (drinkable water, distilled water, potassium nitrate [KNO_3] solutions 2 and 5 gL^{-1}) on the seed germination parameters in three provenances collected in the main agro-climatic zones in Benin was examined. Final germination rate, germination speed and kinetics were analyzed and the data submitted to ANOVA. Kruskal-Wallis non parametric test were used to identify and classify treatments and accessions. Results revealed significant differences between them for all parameters. Germination capacity reached 72.00, 23.66 and 8.00% within Pop 16, Nord 14 and Sud 13, respectively when seeds were treated in KNO_3 5 gL^{-1}. Treatment KNO_3 2 gL^{-1} revealed the weakest germination capacities and no significant difference were noted anymore between Pop 16 and Sud 13 (TG=12.00%). Distilled water permitted to get meaningful differences between populations, and delivered best results compared to tap water and KNO_3 2 gL^{-1}. Little difference was noted for the germination speed (VG=0.17-0.18 seed/day) whatever the treatment. Germination capacity varied very significantly between treatments and accessions. Germination speed varied rather between accessions and less between treatments. Interactions Accession×Treatment were very significant for all estimated parameters indicating a relatively important contribution of the treatments to the phenotypic expression.

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Keywords

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Germination rate (TG)
Germination speed (VG)
Threatened species

Introduction

Diversity is a condition to a balanced biosphere and its capacities towards evolution. Evolution during the last decades has been characterized by a tendency to genetic uniformity of plant resources (Aoudjit Hayet, 2006). Biodiversity conservation contributes among others to master the techniques of reproduction and propagation of the species. Their development hinges indeed upon the generative and vegetative propagation techniques

(Finch-Savage, 2004). Biological diversity is the set of all shapes of a living organism, and is usually subdivided in three levels: genetic, specific and ecosystemic (Aoudjit Hayet, 2006). Benin biodiversity is labelled by great species richness, but also a big variability of landscapes, ecosystems and habitats (Sinsin and Owolabi, 2001). Germination is a physiological phase during which the seed leaves the state of slow to active life (Head et al., 1998). It is defined as a sum of events conducting the dry seed to germinate: it starts with the crucial stage of water absorption by the seed (Othman, 2005) ended by the elongation of the embryonic axis and the emergence of the radicle through the structures enveloping the embryo (Shereena and Nabeesa, 2006).

Desertification, deforestation and genetic erosion are the most important factors responsible for the loss of biodiversity as well as deterioration of soils in the savannah regions. The resulting negative effects can be minimized thanks reforestation programs. However, to reach that objective, collection and seed germination issues must be taken into consideration since any seed germination process is often mainly very difficult for numerous useful species due to the physiological phenomenon of levee of the dormancy. Germination refers merely to a set of changes by which an embryo pushes in a young plant (Bello and Gaba, 2015; Raven et al., 2005). Regardless the genotype as well as the ecological conditions of the natural habitat of plants, three other conditions remain necessary to the germination: humidity, temperature and atmospheric oxygen (Baskin, 2001).

Seeds absorb the water of soil by an imbibition process that entails the swelling and the rupture of their tegument. After that process, hydrolytic enzymes (hydrolases) are activated and decompose in useful compounds the nutriments previously stocked metabolically during seed development. The atmospheric oxygen is provided by the pores of soil, and is used often in the aerobic respiration for energy supply until the beginning of the photosynthesis. Seeds belonging to numerous species how difficulties to germination, and indeed their propagation is affected by dormancy, which results in a weak growth potential (Fredrick et al., 2016; Falemara et al., 2014). Botsheleng et al. (2014) noted that the seeds of most species of trees of the arid and semi-arid regions cannot germinate rapidly whenever they are submitted to ideal conditions to the accomplishment of the physiological phenomenon due to their impervious tegument to water (Fredrick et al., 2016; Aref et al., 2011; Walters et al., 2004). Seed dormancy varies according to species (Botsheleng et al., 2014) and necessary conditions for its levee and germination can therefore be similarly very variable in relationship with the given species or provenance (accession) producing the seed material (Fredrick et al., 2016; Luna et al., 2009). Several studies showed that importance of pretreatments in improving considerably seed germination of various tree species (Hossain et al., 2005, cited by Fredrick et al., 2016). Thus, different treatments have been applied to enhance seed germination success in various species (Botsheleng et al., 2014; Falemara et al., 2014; Iroko et al., 2013; Ibiang et al., 2012; Aref et al., 2011; Hossain et al., 2005; Uniyal et al., 2000; Diallo et al., 1996). Barnes and Fagg (2003) reported in *Faidherbia albida* that the seeds require a pretreatment to stimulate a fast and uniform germination.

In most African countries, the genus *Cleome* is no more considered as an adventitious, but rather an edible plant, or even a source of income for small household. This species gained interest quickly, notably in oriental and southern Africa, where it passed successively from a status of adventitious to one of the most garden vegetables, and then of cultivated plants (Chweya and Mnzava, 1997). Most seeds of *Cleome gynandra* have a water-impervious tegument that causes the phenomenon of dormancy. Then, germination process can spread across months or even years if it could never take place. To propagate the seeds of *Cleome gynandra* in an efficient manner in nursery, it is therefore necessary to apply an ideal pretreatment before the sowing to assure not only a high final germination percentage, but also a fast and uniform germination after sowing. The seeds of *Cleome* are characterized by a weak germination and no explanation to the fact has been provided until to date (Ochuodho, 2005). Stuppy et al. (2010) suggest that the seeds of *Cleome* can possess a physiological dormancy that must be surmounted while imitating the seasonal models of the natural habitat of the species. Physiologically, the mature seeds are either dormant, or not and will germinate once the dormancy is relieved or some optimal conditions are provided (Ochuodho and Modi, 2007). The precocious harvest of the seeds can be desirable for the seed germination of some crops. The fruits of the cat mustache are dehiscent and must be harvested as soon as they are mature before any scattering of seeds on soil (K'Opondo, 2011). Numerous techniques are used to render the seed teguments permeable to water. In Australia, by the last century, boiling or hot water, and sulphuric acid were used efficiently. Germination is triggered in general quickly once the tegument has been made permeable. However, in all techniques in use, there is a danger of lesion of the embryo if the applied treatment is too brutal (FAO, 1992). Also the seed germination rate decreases with conservation time at ambient temperature (Tirlapur and Willemse, 1992).

Thus, in the case of *Cleome gynandra* L., a survey aiming at improving germination efficiency by applying a range of preselected treatments will help to select the most suitable method to obtain the optimal expression of the physiological germination parameters, and so to establish true essential bases for the regeneration of the threatened species in Benin. In this respect, the primordial objective of this survey was to study the effects of the provenance (accession or source of the seeds) and four different treatments, as well as of their possible interactions on the germination of the seeds of *Cleome gynandra* L. in order to identify the most suitable method that will increase the germination of the diverse accessions, and encourage the regeneration of *Cleome* in Benin.

Materials and methods

Study area

The study has been carried out in Benin Republic covering 114763 km^2. It is located between the latitudes 6°15'N and 12° 25'N, the longitudes 0°40'E and 3°45'E, and is constituted of three main climatic zones: the Guinean, Guineo-Sudanese and Sudanese zones (MEPN, 2008). The climate is of type subequatorial in the South, tropical humid of transition in the Middle part and tropical dry in the North of the country (Akoègninou, 2004).

Plant material

A seminal bank of *Cleome gynandra* L. has been established in 2013 and completed in 2014 to 2016 at the Department of Plant Biology of the Faculty of Sciences and Techniques – University Abomey-Calavi, Benin (FAST/UAC). These accessions (seeds) have been harvested in the localities of Dogbo (2013 and 2016), and Tanguiéta (2014 and 2015). The seeds of Dogbo constitute accessions Pop 16 and Sud 13, whereas those of Tanguiéta represent accession Nord 14, respectively. These provenances (accessions) are representative of a wide range of agro-climatic zones of type Guinean subequatorial and Sudanese, respectively. Seed samples have been collected in plastic bags and are stocked in conditioned chambers (16°C) since their harvest. The present study is undertaken on the three accessions of *Cleome gynandra* L. aforementioned: Nord 14, Pop 16 and Sud 13.

Methodology

Experimental protocol

The germination test was carried out under controlled environment in the Laboratory of Botany and Plant ecology of the University Abomey-Calavi, Benin. 100 seeds from each provenance were deposited on Canson filter papers moistened and/or previously dampened in a solution corresponding to a given treatment in Petri dishes. Pretreatments consisted in immersing the seeds in tap, distilled water, and potassium nitrate solution (KNO_3) 2 gL^{-1} and 5 gL^{-1} during 40, 24 and 24 hrs for the three later, respectively. The experimental design is a complete randomized blocks with three repetitions per accession in each treatment. Germinated seeds were counted daily to determine the parameters characterizing the physiological process. Seeds are considered to have germinated whenever the radicle emerges from the tegument. The estimated germination parameters were: initial (TG1), second, third, fourth and fifth dates numbering of germinated seeds (TG2, TG3, TG4 and TG5), germination rates, and germination speed (VG) inversely related to the period of germination (PG); the germination capacity corresponding to the final germination rate, either TG5. The different parameters are given below by the formulas:

$$CG(\%) = \frac{NTGG}{NTG} \times 100$$

$$VG(J^{-1}) = \frac{\sum n}{\sum n.J_n}$$

Where,
CG (%) = germination capacity (percentage of seeds capable to germinate in the conditions of the experimentation until its end); VG (J^{-1}) = germination speed (characterizing the time taken by the seed to germination); NTGG = total number of seeds germinated; NTG = total number of seeds tested; n =

number of seeds germinated in a given day Jn; Jn = number of days from the start of experimentation to a given day.

Statistical analysis

Statistical analysis of the results was achieved by the software JMP version 7 (SAS Institute NC, 2007) and MINITAB 14 (2015). Germination parameters were subjected to one- and two-way analyses of variance at 5%. The Fisher-Snedecor test was applied to analyze the relative importance of the factors Accession and Treatment as well as of their interactions in the observed variability. Parameter means were compared between accessions and treatments by the test of Tukey-Kramer implemented in either JMP or MINITAB 14. The interactions between the various provenances (accessions) were completed using the software MINITAB version 14. Kruskal-Wallis test implemented in MINITAB 14 was used for the comparison of the treatments and accessions studied. This test permitted to identify the best treatments as well as the best accession driving to the higher rates and speed of germination in the neglected species of concern in the current survey.

Results

Kinetics of germination

Average germination rates vary significantly between accessions and treatments applied including the control represented by the tap water (Fig. 1). For the control, the maximum mean of germination rate reached is equal or very close to 6.33, 15.00 and 8.33% for Nord 14, Pop 16 and Sud 13, respectively (Fig. 1). The first accession to reach the maximum of germination with the tap water as pretreatment and in fifteen (15) days is Pop 16 provenance (10.33%), followed by Sud 13 (3%) and Nord 14 (2.67%) in the same period. With distilled water, KNO_3solutions 2 and 5 gL^{-1}, the three accessions undergo some increase of the germination rate as well as its speed (Figs. 1-3). Indeed, the curves displaying germination kinetics of the seeds treated in different solutions indicate values superior to the control. The gap is more accentuated considering the response of Pop 16 seeds.

Otherwise, according to the kinetics curves (Fig. 1), differences in the response to the different treatments are expressed. Nord 14 seeds germinated lower and slower than Pop 16 and Sud 13. Average germination rate remains moderate and varies little during the first fifteen days before showing an exponential acceleration in all accessions, even though results seem better in Pop 16 and Sud 13 compared to Nord 14 (Figs. 1 A-C).

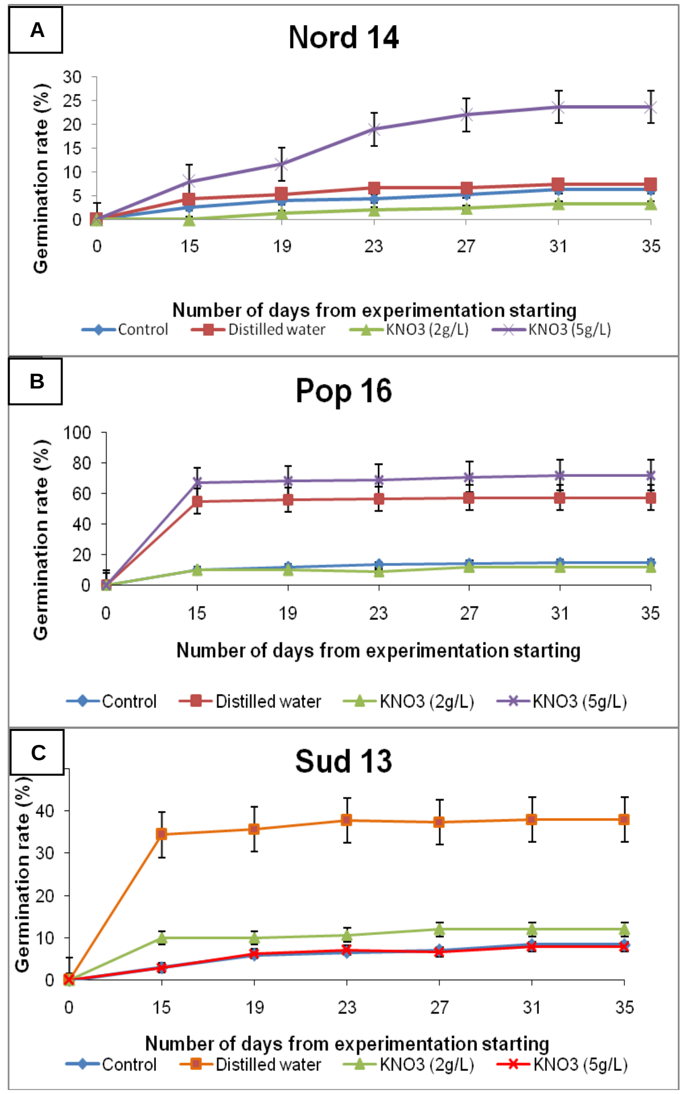

Fig. 1: Seed germination kinetics of three *Cleome gynandra* accessions (Nord 14, Pop 16 and Sud 13) collected in Benin after 35 days experimentation under 4 treatments (Control = tap water; distilled Water; KNO_3 solutions 2 and 5 gL^{-1}). A- Nord 14. B- Pop 16. C- Sud 13.

The highest germination rate (>70%) is recorded in Pop 16, followed by Sud 13 (10-40%), while Nord 14 (5-10% between the control and the solution of KNO_3 to 5 gL^{-1}, respectively) showed smaller trend.

Compared to other treatments, distilled water permitted to get a germination speed significantly more important (about 0.18 germinated seed daily) whatever the accession or provenance considered (Fig. 3). Solution KNO_3 5 gL^{-1} and distilled water permitted to record the best rates and speed of germination in Pop 16 (Fig. 1). However and in a general, seeds of this Pop 16 accession germinate quickly that the two other sources whatever the applied treatment. Nord 14 seeds germinate less that all other and the final germination rate reached never upon 16% in 31 days experimentation (Fig. 3). To sum up, Nord 14 seeds present less germination capability that Sud 13 and Pop 16; the later showing the best results in any case. The treatment in the KNO_3 5 gL^{-1} allowed getting after 31 days in Pop 16 about 80% of germination rate (Fig. 1).

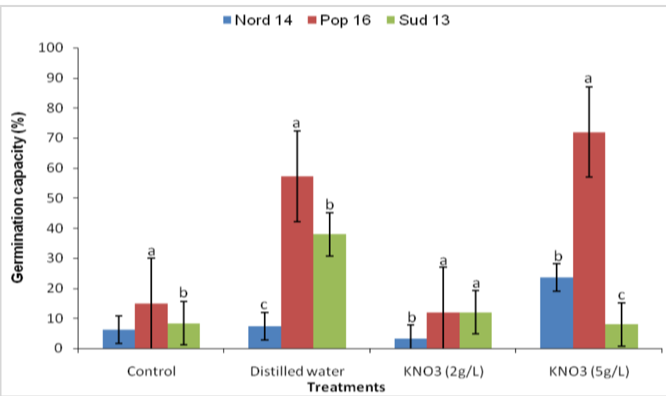

Fig. 2: Seed germination capacity of three *Cleome gynandra* accessions (Nord 14, Pop 16 and Sud 13) collected in Benin after 35 days experimentation under 4 treatments (Control = tap water; distilled Water; KNO_3 solutions 2 and 5 gL^{-1}).

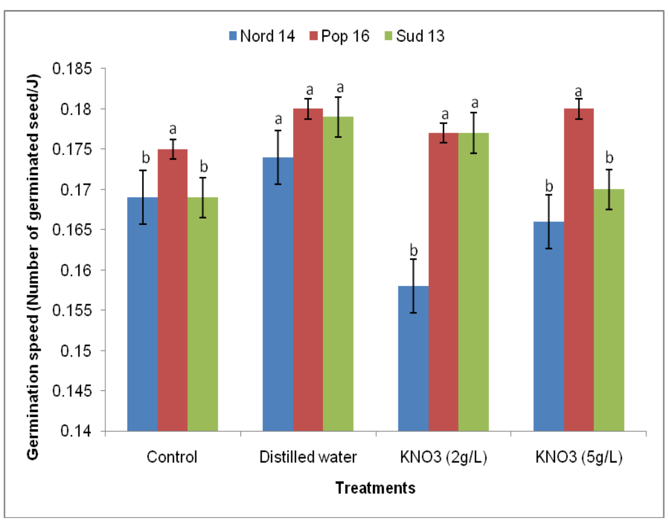

Fig. 3: Seed germination speed comparison of three *Cleome gynandra* accessions (Nord 14, Pop 16 and Sud 13) collected in Benin after 35 days experimentation under 4 treatments (Control = tap water; distilled Water; KNO_3 solutions 2 and 5 gL^{-1}).

Capacity of germination

Results show that the main source of variation of the germination capacity is the accession (Table 1, Figs. 2 and 4). It is followed respectively by the effect of the treatment and the interactions Accession×Treatment. Seeds of Pop 16 react better in relation to the two other accessions. Compared to the two other treatments, KNO_3 solution 5 gL^{-1} and distilled water generate presented significant positive effects on the germination capacity (Fig. 4). Interactions Accession×Treatment showed the same trend in importance regardless the accession considered (Table 2, Fig. 4B). Results out of the statistical analysis presented in Tables 1 and 2 show that the germination rates of all studied accession seeds are affected significantly and positively across all other treatment apart the control represented by the tap water. However, Pop 16 present a germination capacity (80%) higher than Sud 13 and Nord 14, in particular when their seeds are treated in the KNO_3 solution 5 gL^{-1}.

Instead of comparing mean values of the three populations analyzed using an one-way ANOVA, the Kruskal-Wallis test was applied to detect the best treatment and accession with respect to the most common germination parameters: germination capacity and speed. According to this test, H' indicate if the median values of the given factor are equal or not, and allows so their classifying.

The sample medians for the four treatments were 9.0, 40.0, 12.0 and 24.0 for the control, the distilled water, the KNO_3 solutions 2 and 5 gL^{-1}, respectively. The z value for treatment KNO_3 2 gL^{-1} is -1.81, the smallest value of z. This size indicated that the mean rank for this treatment differs least from the mean rank for all observations. Moreover, the mean rank of this treatment (13.0) was lower than the mean of the observation set (18.5), and the z value is negative (z = -1.81). The mean rank for the treatment KNO_3 5 gL^{-1} is higher than in the whole experiment, with a positive z value (z = 1.81) (Table 3).

The test statistics (H = 8.69 or 8.74) had a *p*-value of 0.033, when it is adjusted for ties, or 0.034 if it is not, indicating that the null hypothesis can be rejected at levels higher than 0.033 in favor of the alternative hypothesis of the existence of at least one difference among the treatment groups. The treatment KNO_3 5 gL^{-1} appeared the most significant to a relative good seed germination capacity in *Cleome gynandra* collected in different agro-ecological zones of Benin.

Table 1. One-way ANOVA (Treatment or Accession effect) for seed germination capacity (TG5=CG) and speed (VG) in three provenances of *Cleome gynandra* collected in Benin.

Parameter	Source of variation	Degees of freedom	Sum of square	Mean square	F Ratio	Prob> F*
TG5 (CG)	Treatment effect	3	5578	1859	5.51	0.004
	Residuals	32	10808	338	-	-
	Total variance	35	16386	-	-	-
	Accession effect	2	5534.4	2767.2	1581.25	0.000
	Residuals	24	42.0	1.7	-	-
	Total variance	35	16385.9	-	-	-
VG	Treatment effect	3	0.0002793	0.0000931	1.85	0.158
	Residuals	32	0.0016104	0.0000503	-	-
	Total variance	35	0.0018898	-	-	-
	Accession effect	2	0.00077647	0.00038824	22.34	0.000
	Residuals	24	0.00041700	0.00001734	-	-
	Total variance	35	0.00188975	-	-	-

* (Prob<0.05): Significant factor effect according to F-test at 5% threshold.

Table 2: Two-way ANOVA for seed germination capacity (TG5=CG) and speed (VG) in three provenances of *Cleome gynandra* collected in Benin.

Parameter	Source of variation	Degees of freedom	Sum of square	Mean square	F Ratio	Prob> F*
TG5 (CG)	Total variance	35	16385.9	-	-	-
	Accession effect (1)	2	5534.4	2767.2	1581.25	0.000
	Treatment effect (2)	3	5578.3	1859.4	2.13	0.197
	Interaction (1×2)	6	5231.2	871.9	498.21	0.000
	Residuals	24	42.0	1.7	-	-
VG	Total variance	35	0.,00188975	-		
	Accession effect (1)	2	0.00077647	0.00038824	22.34	0.000
	Treatment effect (2)	3	0.00027932	0.00009311	1.34	0.347
	Interaction (1×2)	6	0.00041696	0.00006949	4.00	0.006
	Residuals	24	0.00041700	0.00001737	-	-

* (Prob<0.05): Significant factor effect according to F-test at 5% threshold

Table 3: Kruskal-Wallis non-parametric test of germination parameters (CG and VG) comparisons in three *Cleome gynandra* accessions under 4 treatments.

Parameter	Factor		N*	Median	Average rank	z
TG5 (CG)	Treatment	Control	9	9.000	13.7	-1.59
		Distilled water	9	40.000	23.3	1.59
		KNO_3 $2gL^{-1}$	9	12.000	13.0	-1.81
		KNO_3 $5gL^{-1}$	9	24.000	24.0	1.81
		Overall	36	-	18.5	-
	Accession	Nord 14	12	6.500	10.8	-3.10
		Pop 16	12	36.500	27.1	3.47
		Sud 13	12	10.500	17.6	-0.37
		Overall	36	-	18.5	-
VG	Treatment	Control	9	0.1712	14.3	-1.37
		Distilled water	9	0.1803	25.7	2.36
		KNO_3 $2gL^{-1}$	9	0.1751	17.4	-0.35
		KNO_3 $5gL^{-1}$	9	0.1712	16.6	-0.64
		Overall	36	-	18.5	-
	Accession	Nord 14	12	0.1692	9.7	-3.56
		Pop 16	12	0.1797	26.3	3.15
		Sud 13	12	0.1762	19.5	0.40
		Overall	36	-	18.5	-

(*): Number of observations.

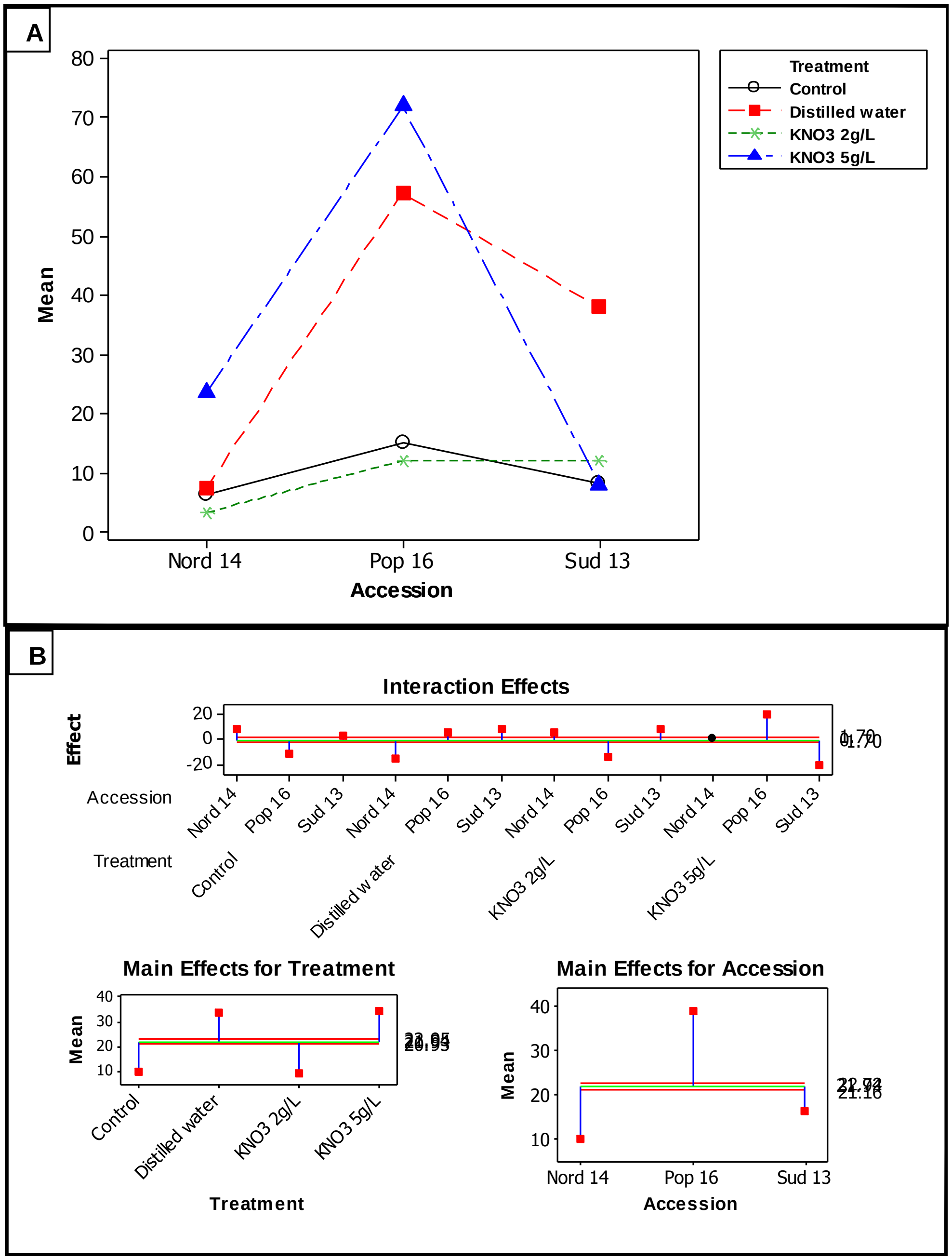

Fig. 4: Seed final germination rate comparison (A), interaction plots and treatment main effects (B) in three *Cleome gynandra* accessions (Nord 14, Pop 16 and Sud 13)collected in Benin after 35 days experimentation under 4 treatments (Control = tap water; distilled Water; KNO_3 solutions 2 and 5 gL^{-1}).

The sample medians for the three accessions were 6.5, 36.5 and 10.5 for Nord 14, Pop 16 and Sud 13, respectively as delivered by the Kruskal-Wallis test. The z value for Sud 13 is -0.37, the smallest absolute value of z. This size shows that the mean rank for this accession is the one that differs least from the mean rank across all observations. Furthermore, the mean rank of this provenance (17.6) was lower compared to the average over the whole study (18.5), and the z value is negative (z = -0.37). Pop 16 mean rank is higher, with a positive z value (z = 3.47) (Table 3). The test statistics (H = 14.56 or 14.64) had a *p*-value of 0.001 when it is adjusted or not, showing that the null hypothesis can be rejected at levels superior to 0.001 in favour of the alternative hypothesis of the existence of at least a difference among the groups of accessions. The germination capacity Pop 16 proved to be significantly the best whatever the treatment in *Cleome gynandra*.

Speed versus period of germination

Tables 1 and 2 indicate the results out of the analysis of variance for the germination period in *Cleome gynandra* seeds submitted to the above mentioned treatments. The same tendencies as for the germination capacity are revealed. Hence, similar observations are made while referring to the effects of the different factors evolved, as well as with regard to their interactions (Table 2). For all accessions, the seed germination period was longer when the seeds are not treated with the tap water.

Statistical analysis indicated that the effect of the treatments presented in Fig. 3 resulted in the significant reduction of the germination period regardless the genotype examined. However, Pop 16showed a better tendency than Sud 13 which was greater than Nord 14 (Figs. 3 and 5).

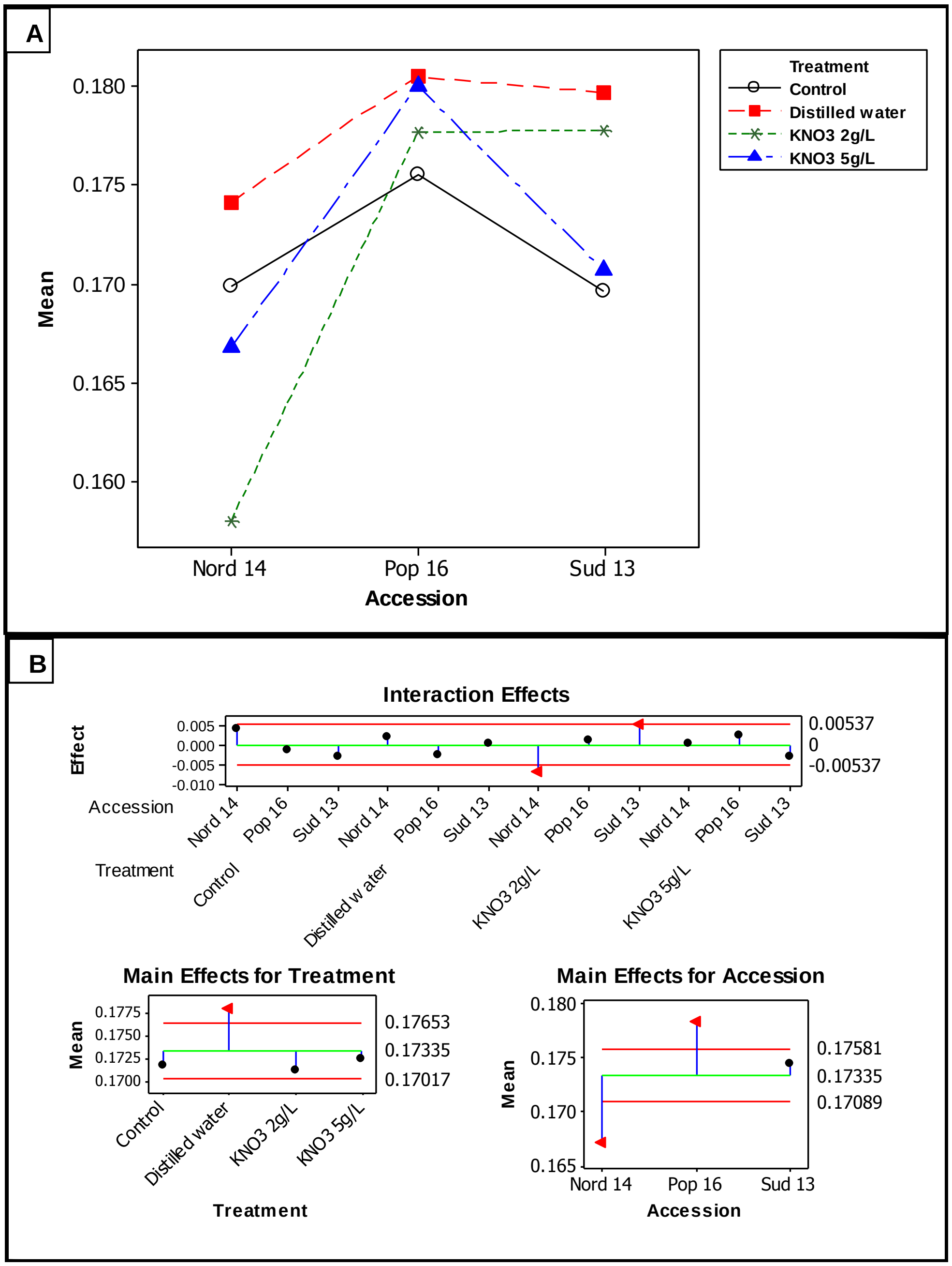

Fig. 5: Seed germination speed comparison (A), interaction plots and treatment main effects (B) in three *Cleome gynandra* accessions (Nord 14, Pop 16 and Sud 13) collected in Benin after 35 days experimentation under 4 treatments (Control = tap water; distilled Water; KNO_3 solutions 2 and 5 gL^{-1}).

The sample median values recorded for the four treatments were 0.171, 0.180, 0.175 and 0.171 seed germinated per day for the control, distilled water, the KNO_3 solutions 2 and 5 gL^{-1}, respectively. The z value for treatment KNO_3 2 gL^{-1} is -0.35, the smallest absolute value of z. This dimension shows that the mean rank for this treatment differs least from the overall mean. The mean rank of this treatment (17.4) was revealed lower to the overall mean rank (18.5), and the value of z is negative (z = -0.35). The same tendency has been observed thus that with the capacity of germination. The mean rank for the distilled water was here higher, with a positive z value (z = 2.36) (Table 3).

The test statistics (H = 5.97) had a *p*-value 0.113 when it is adjusted or not, indicating that the null hypothesis can be rejected at levels superior to 0.113 in favour of the alternative hypothesis of the existence of at least a difference among the groups of treatments. The probability being superior to 0.05, the differences between the four treatments are not therefore significant. Distilled water appeared to be however more efficient to obtain better results in *Cleome gynandra* from different agro-ecological zones of Benin.

The calculation of the sample medians for the three accessions delivered 0.169, 0.179 and 0.176 seed germinated per day for Nord 14, Pop 16 and Sud 13, respectively. The z value for Sud 13 is 0.40, the smallest absolute value of z. This size is showing that the mean rank for this accession is the one that differs least from the overall mean rank. Sud 13 mean rank (19.5) was lower compared to the general average (18.5), and the z value is rather positive (z = +0.40). Pop 16 mean rank (26.3) was higher, with a positive z value (z = 3.15) (Table 3).

The Kruskal-Wallis test statistics (H = 15.18) had a *p*-value of 0.001 when she/it is adjusted or not, showing that the null hypothesis can be rejected at levels superior to 0.001 in favour of the alternative hypothesis of the existence of at least a difference among the groups of accessions. The probability is distinctly lower than 0.05. Thus, differences between the three accessions are indeed very significant. Pop 16 germination speed seemed better whatever the treatment.

Discussion

Seed germination requires the mobilization of reserves accumulated during its maturation. Degradation of those reserves brings the necessary energy to the young plant growth. This mobilization is the consequence of: (i) the hydrolytic activities that release the nutriments stocked in the reserve, and (ii) the mechanisms of their transportation toward the embryo (Mihoub et al., 2005). Depending upon the species, these reserves can be majorly of carbohydrate, lipidic and/or protein nature (Khemiri et al., 2004). Respiration, hydrolysis of the reserves and many enzymatic activities stay under the dependence of the temperature. Indeed, all variation of the incubation temperature can affect in addition to the activity of enzymes, some indispensable processes in the control of germination such as the membrane permeability and the extensibility of the cell pecto-cellulosic cock (Bewley and Black, 1994; Gul and Weber, 1999). Hawker and Jenner (1993) suggested that the high temperatures inhibit the germination of the seeds while limiting the availability of energy and hydrolysats, resulting from a delay and an inhibition of the synthesis and/or the activity of the hydrolytic enzymes. Similarly, low temperatures entail a disruption and a delay of coordination at the time of the mobilization of the reserves (Nykiforuk and Johnson-Flanagan, 1994).

Seed tegument hardiness is an important factor that affects germination (Aref et al., 2011). Seed dormancy is known in numerous tropical tree species (Amusa, 2011). Uniyal et al. (2000) noted that the response of seeds to pretreatment is specific and no type of treatment may be considered as universally efficient. Aref et al. (2011) showed that all treatments applied to *Faidherbia albida* had significantly (p<0.05) affected germination parameters examined (germination percentage, average time and capacity) in all studied provenances. *Cleome gynandra* L. is one of the numerous multipurpose species in the tropics, particularly in Benin. However, it remains to date threatened because of its weak natural regeneration (Essou, *pers. comm.*). Many woody species produce seeds that germinate thereafter with a lot of difficulties (Niang et al., 2015). Seed germination issues in numerous species are bound among others to the physiological phenomenon named dormancy which appeared in various types. That phenomenon allows seeds to remain in slow life during a variable period of time according to the species or its taxonomical subdivisions (Aoudjit Hayet, 2006). Germination would be only possible if the dormancy is eliminated. Many studies on the seed germination process within the plant species have been undertaken by several authors. Thus, Niang et al. (2015) reported significant differences of the germination rate between various provenances of baobab in Senegal as seeds were pretreated in sulphuric acid extract. Ahoton et al. (2009) showed various tendencies on the seed germination rate and speed of *Prosopis africana* collected in Benin when submitted to four treatments having implied hot water, sulphuric acid, tap water and/or their combinations. Of these different works, germination rates of 90-100% were observed (Fredrick et al., 2016 in *Faidherbia albida*; Ahoton et al., 2009 in *Prosopis africana*; Moussa et al., 1998 in *Hyphaenae thebaica*; Njoukam, 1997 in *Canarium schweinfurthii*).

In the present survey, freshly harvested seeds of *Cleome gynandra* L. from three different agro-ecological zones in Benin have been subjected to germinate at the laboratory in Petri dishes filled with Canson paper moistened by tap (Control), distilled water, and after the pretreatment in KNO_3 solutions of to 2 and 5 gL^{-1} for 24 hrs followed by incubation on humid paper. Seed germination parameters have been estimated in 35 trial days. The capacity of germination remained generally weak (less than 40%), except in the Pop 16 population where it reached 72%. Pop 16 seeds germinated also more rapidly during the first 20 trial days reaching about 58% germination rate whatever the applied treatment (Fig. 1B). Those of Nord 14 and Sud 13 presented a weak germination rate from the beginning to the end of the experimentation (Figs. 1A and 1C). The germination speed stayed relatively weak generally even though it reached more than 0.18 seed germinated daily in Pop 16 by the end of the experimentation with the treatment KNO_3 5 gL^{-1}. These results show that there is a high genotypic variability for the physiological parameters of germination in *Cleome gynandra* as the recent survey of Fredrick et al. (2016) demonstrated in *Faidherbia albida*. Salt solutions and nitrogenous compounds have often been used to break the seed dormancy (Vandelook et al., 2008; Alboresi et al., 2005; Pérez-Fernandez and Rodriguez-Echeverria, 2003; Bell et al., 1999; Hendricks and Taylorson, 1972; Mayer and Polkjaoff-Mayber, 1963). In the present study, we used 2 and 5 gL^{-1} of KNO_3. Results with the solution 2 gL^{-1} were not significantly different from those gotten at the control (Figs. 1-5). Results obtained by soaking the seeds in KNO_3 solution 5 gL^{-1} for 24 hrs were significant and permitted to get the most elevated germination rate (72%) (Fig. 4). These results remain in contrast to those presented by Zharare (2012) who reported that KNO_3 and K_2SO_4 are generally inefficient to unlock dormancies and to accelerate any germination process. Factorial tests combining the four treatments used are in progress. Moreover, further collections (seeds) of *Cleome gynandra* genetic resources in Benin Republic are continuing with regards to mastering the seed physiology of the species, indispensable pledge for its preservation by accelerating a better regeneration. Pretreatments as applied by different authors (Fredrick et al., 2016; Bello and Gada, 2015; Niang et al., 2015; Ahoton et al., 2009) combined with the methodology used in the present report are in progress and will permit to surround the various types of dormancy, and then to enhance the possibilities of their levee towards raising germination capacity and speed in *Cleome gynandra*. This may be encouraging for a better regeneration of the species and therefore its conservation. Seed source plays also an important role as factor as the treatment. This observation is in conformity with the findings of Uniyal et al. (2000), who noted the meaningful importance of seed source in the germination of *Grewia oppositifolia*.

Cleome gynandra provenances collected in different agro-ecological areas differed from one another in the expression of the germination parameters indicating that the source of seeds and therefore the genotype plays an important role in the seed response whenever they are submitted to a particular treatment. These results are in agreement with those of Fredrick et al. (2016) at *Faidherbia albida* indicating that conditions necessary to allow the easy passage of seeds from latent life to germination can be very variable between species, but also and particularly within a given species as the seeds originated in various ecologically different places and might be potentially diverse genotypically.

Conclusion

The objective of this survey was to study the effect of different treatments on the seed germination of *Cleome gynandra* collected in different agro-climatic zones (Guinean and Sudanese climates) from Benin in order to determine the most suitable treatment to improve and increase any expression of the germination parameters. The study revealed the existence of variation of germination rates, capacity and speed between the provenances and the treatments. Interactions Accession×Treatment were important and significant. Distilled water and KNO_3 5 gL^{-1} were the most efficient treatments conducting to better results. Pop 16 originated in the Guinean subequatorial climate delivered best results compared to the two other studied accessions.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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References

Ahoton, L.E., Adjakpa, J.B., M'po, M.I., Akpo E.L., 2009. Effet des prétraitements des semences sur la germination de *Prosopis africana* (Guill., Perrot. et Rich.) Taub., (Césalpiniacées). Tropicultura. 27(4), 233-238.

Akoègninou, A., 2004. Recherches botanique et écologiques sur les forêts actuelles du Bénin. Thése de doctorat d'état, Université de Cocody-Abidjan (Côte d'Ivoire).

Alboresi, A., Gestin, C., Leydecker, M.T., Bedu, M., Meyer, C., Truong, H.N., 2005. Nitrate, une dormance des graines signal de relaxation dans *Arabidopsis*. Plant Cell Environ. 28, 500-512.

Amusa, T.O., 2011. Effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex pers). J. Hortic. For. 3(4), 96-103.

Aoudjit Hayet, M., 2006. Etude de la germination des grains du *Pistacia atlantica* Desf. (pistachier de l'Atlas) et essai de multiplication de *Pistacia vera* L. (pistachier vrai) en pépinière selon deux types de greffes Ecusson et « Chip budding ». Mémoire de Magistère en Sciences Agronomiques, Institut National Agronomique El Harrach. 93p.

Aref, I.M., Ali, H., Atta, E., Al Shahrani, T., Ismail, A., 2011. Effects of seed pretreatment and seed source on germination of five *Acacia* spp. Afr. J. Biotechnol. 10(71), 15901-15910.

Barnes, R.D., Fagg, C.W., 2003. *Faidherbia albida*: Monograph and Annotated Bibliography. Oxford Forestry Institute, Tropical Forestry Papers, No.41.

Baskin, C., 2001. Seeds Ecology, Biography and Evolution of Dormancy and Germination. Chapter 6, Academic Press, London, UK, 2001.

Bell, D.T., King, L.A., Plummer, J., 1999. Ecophysiological effects of lightquality and nitrate on seed germination in speciesfrom Western Australia. Aust. J. Ecol., 24, 2-10.

Bello, A.G., Gada, Z.Y., 2015. Germination and early growth assessment of *Tamarindus indica* L. in Sokoto State, Nigeria. Int. J. For. Res. 2015, Article ID 634108. 5p.

Bewley, J.D., Black, M., 1994. Seeds-Physiology of Development and Germination. 2nd Edn. Plenum Press, New York.

Botsheleng, B., Mathowa, T., Mojeremane, W., 2014. Effects of pre-treatments methods on the germination of Pod mahogany (*Afzelia quanzensis*) and mukusi (*Baikiaea plurijuga*) seeds. Int. J. Innov. Res. Sci. Eng. Technol. 3(1), 8108-8113.

Chweya, J.A., Mnzava, N.A. 1997. Cat's whiskers. *Cleome gynandra* L. Promoting the conservation and use of underutilized and neglected crops. 11. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International, Plant Genetic Resources Institute, Rome, Italy.

Diallo, I., Danthu, P., Sambou, B., Dione, D., Goudiaby, A.S., Poulsen, K., 1996. Effects of different pretreatments on the germination of *Faidherbia albida* (Del.) A. Chev. seeds. Int. Tree Crops J. 9(1), 31-36.

Falemara, B.C., Chomini, M.S., Thlama, D.M., Udenkwere, M., 2014. Pre-germination and dormancy response of *Adansonia digitata* L. seeds to pre-treatment techniques and growth media. Eur. J. Bot. Plant Sci. Pathol. 2(1), 13-23.

FAO, 1992. Effet des contraintes hydrique et saline sur la germination de quelques Acacias africains. Projet National de Semences Forestières du Sénégal (PRONASEF FAO Pays –Bas, GPC 1 SEN 1039 1NET) et supporté par l'Union Européenne (Programme STDIII, Contrat CT 92-0047).

Finch-Savage, W.E., 2004. The use of population-based threshold models to describe and predict the effects of seedbed environment on germination and seedling emergence of crops. In: Hand Book of Seed Physiology: Applications to Agriculture (Eds.: Benech-Arnold, R.L., Sánchez, R.A.). Food Products Press, New York, London & Oxford. 501p.

Fredrick, C., Muthuri, C., Ngamau, K., Sinclair, F., 2016. Provenance and pretreatment effect on seed germination of six provenances of *Faidherbia albida* (Delile) A. Chev. Agroforest. Syst. DOI 10.1007/s10457-016-9974-3.

Gul, B., Weber, D.J., 1999. Effects of salinity, light and temperature on germination in *Allenrolfea* *occidentalis*. Can. J. Bot. 77, 240-246.

Hawker, J.S., Jenner, C.F., 1993. High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. Aust. J. Plant Physiol. 20, 197-209.

Head, R.M., Jones, R.I., Bailey-Watts, A.E., 1998. Akinete germination and recruitment of planctonic Cyanobacteria from lake sediments. Verh. Int. Ver. Limnol. 26, 1711-1715.

Hendricks, S.B., Taylorson, R.B., 1972. Interactions of light and a temperature shift on seed germination. Plant Physiol. 49(2), 127-130.

Hossain, M.A., Arefin, M.K., Khan, B.M., Rahman, M.A., 2005. Effects of seed treatments on germination and seedling growth attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. Res. J. Agric. Biol. Sci. 1(2), 135-141.

Ibiang, Y.B., Ita, E.E., Ekanem, B.E., Edu, N.E., 2012. Effect of different pretreatment protocols on seed germination of *Tetrapleura tetraptera* (Schum and Thonn). J. Environ. Sci. Toxicol. Food Technol. 2(3), 25-29.

Iroko, O.A., Asinwa, I.O., Kareem, A.A., Kasim-Ibrahim, F., 2013. Pretreatment effects on seed germination of *Vitellaria paradoxa* (Gaertn) Hepper. J. Agric. Sci. 3(4), 121-125.

K'Opondo, F.B.O., 2011. Morphological characterization of selected spider plant (*Cleome gynandra* L.) types from western Kenya. Ann. Biol. Res. 2(2), 54-64.

Khemiri, H, Belguith, H, Jridi, T, Ben El Arbi, M., Ben Hamida, J., 2004. Caractérisation biochimique d'une amylase active au cours du processus germinatif des graines de colza (*Brassica napus* L.). Enzymologie et métabolisme, pp.: 146-149. Congrès International de Biochimie. Marrakech.

Luna, T., Wilkinson, K., Dumroese, R.K., 2009. Seed germination and sowing options. In: Nursery Manual for Native Plants: A Guide for Tribal Nurseries—Vol. 1: Nursery Management (Eds.: Dumroese, R.K., Luna, T., Landis, T.D.). Agriculture Handbook, Vol 730. Department of Agriculture Forest Service, Washington DC. pp.133-151.

Mayer, A.M., Polkjaoff-Mayber, A., 1963. La Germination des Graines. Macmillan, New York, USA.

MEPN (Ministère de l'Environnement et de la Protection de la Nature), 2008. Programme d'action national d'adaptation aux changements climatiques du Bénin. Convention-cadre des Nation Unies sur les changements climatiques, Cotonou, Bénin, 81p.

Mihoub, A., Chaoui, A., El Ferjani, E., 2005. Changements biochimiques induits par le cadmium et le cuivre au cours de la germination des graines de petit pois (*Pisum sativum* L.). C. R. Biologies. 328, 33-41.

MINITAB, 2015. Release 14. Minitab Inc., USA. ISBN 0-925636-48-7.

Moussa, H., Margolis, H.A., Dube, P.A., Odongo, J., 1998. Factors affecting the germination of doum palm (*Hyphaene thebaica* Mart) seeds from semi-arid zone of Niger, West Africa. Forest Ecol. Manag. 104, 27-41.

MS/INRSP/DMT, 2004. Politique National de Médicine Traditionnelle, Bamako. 21p.

Niang, M., Diouf, M., Ndiaye Samba, S.A., Ndoye, O., Cissé, N., Van Damme, P., 2015. Difference in germination rate of baobab (*Adansonia digitata* L.) provenances constrasting in their seed morphometrics when pretreated with concentrated sulphuric acid. Afr. J. Agric. Res. 10(2), 1412-1420.

Njoukam, R., 1997. Germination des semences et croissance de l'aielé *Canarium schweinfurthii* Engl. en plantation. In: Actes du 2ème séminaire international sur la valorisation du safoutier et autres oléagineux non conventionnels (Eds. : Kapseu, C., Kayem, G.J.). Presses Universitaires de Yaoundé. pp.45-54.

Nykiforuk, C.L., Johnson Flanagan, A.M., 1994. Germination and early seedling development under low temperature in canola. Crop Sci. 34, 1047-1054.

Ochuodho, J.O., 2005. Temperature and light requirements for the germination of *Cleome gynandra* L. South Afr. J. Plant Soil. 22, 227-242.

Ochuodho, J.O., Modi, A.T., 2007. Light-induced transient dormancy in *Cleome gynandra* seeds Afr. J. Agric. Res. 2. 587-891.

Othman, Y., 2005. Evaluation of barley cultivars grown in Jordan for salt tolerance. Thesis, Jordan University of Science and Technology, Jordan.

Pérez-Fernández, M.A., Rodríguez-Echeverría, S., 2003. Effect of smoke, charred wood and nitrogenous compounds on seed germination of ten species from woodland in Central-Western Spain. J. Chem. Ecol. 29, 237-251.

Raven, P.H., Ray, F.E., Susan, E.E., 2005. Biology of Plants. 7th Edn. Freeman W.H., New York, NY, USA.

SAS Institute, 2007. JMP User Guide, Release 7. SAS Institute Inc., Cary, NC, USA.

Shereena, J., Nabeesa, S., 2006. Effect of temperature on protein profile of *Pisum sativum* L. seeds during germination. J. Biol. Sci. 6, 1153-1155.

Sinsin, B., Owolabi, L., 2001. Monographie Nationale de la Diversité Biologique. Rapport de Synthèse. MEHU/PNUD, Cotonou, Bénin.

Stuppy, W., Kessler, R.. Kew, R.B.G., 2010. *Cleome gynandra*, Atlas numérique économique. Plants, Etats-Unis. 144p.

Tirlapur, U. K., Willemse, M. T. M., 1992. Changes in calcium and calmodulin levels during microsporogenesis, pollen development and germination in *Gasteria verrucosa* (Mill) H. Dunal. Sex. Plant Reprod. 5, 214-223.

Uniyal, A.K., Bhatt, B.P., Todaria, N.P., 2000. Provenance characteristics and pretreatment effects on seed germination of *Grewia oppositifolia* Roxb.—A promising agroforestry tree crop of Garhwal Himalaya, India. Int. Tree Crops J. 10(3), 203-213.

Vandelook, F., Van de Moer, D., Van Assche, J.A., 2008. Environmental signals for seed germination reflect habitat adaptations in four temperate Caryophyllaceae. Funct. Ecol. 22, 470-478.

Walters, M., Midgley, J.J., Somers, M.J., 2004. Effects of fire and fire intensity on the germination and establishment of *Acacia karroo*, *Acacia nilotica*, *Acacia luederitzii* and *Dichrostachys cinerea* in the field. BMC Ecol. 4, 1-13.

Zharare, G.E., 2012. Differential requirements for breaking seed dormancy in biotypes of *Cleome gynandra* L. and two *Amaranthus* species. J. Agric. Res. 7(36), 5049-5059.

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