Seeds' Germination of Four Traditional Leafy Vegetables in Benin (LFT)

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Summary

Many African traditional leafy vegetables such as Acmella uliginosa, Ceratotheca sesamoides, Justicia tenella *and* Sesamum radiatum *have been under domestication in the rural areas. Experiments were conducted in Benin to test the germination ability of their seeds. The seeds were subjected to 4 treatments and seeds without treatment served as control. The experimental design was a completely randomized block with 3 replications. With a germination rate above 80%, seeds of* J. tenella *did not demonstrate seed dormancy. Similarly, the germination rate of* A. uliginosa *seeds without pappus was above 85% unlike the seeds with pappus whose germination rate was 15%. The germination of the seeds of* S. radiatum *and* C. sesamoides *was very low (< 15%). However, the seeds of* S. radiatum *with low water content (8.77%) and immersed in water for 24 hours gave the best germination rate (50%), whereas under the same conditions with* C. sesamoides *no germination was observed. The seeds of* C. sesamoides *and* S. radiatum *were dormant. Meanwhile, to ensure better germination of* A. uliginosa, *the pappus must be removed from its seeds.*

Résumé

Germination des graines de quatre légumes feuilles traditionnels consommés au Bénin (LFT)

*Plusieurs espèces de légumes feuilles traditionnels sont en cours de domestication au Bénin. Des essais ont été conduits pour tester les conditions de germination des graines d'*Acmella uliginosa, Ceratotheca sesamoides, Justicia tenella *et* Sesamum radiatum. *Les graines ont été soumises à quatre traitements, et le témoin est constitué de graines sans traitement. Le dispositif expérimental est le bloc complètement aléatoire avec 3 répétitions. Avec un taux de germination supérieur à 80%, les graines de* J. tenella *n'ont pas présenté de dormance. Le taux de germination des graines sans aigrette d'*A. uliginosa *a été supérieur à 85% contrairement aux graines avec aigrette (15%). Les taux de germination des graines de* S. radiatum *et de* C. sesamoides *sont restés également très faibles (< 15%). Cependant, les graines de* S. radiatum *à faible teneur en eau (8,77%) et trempées dans l'eau pendant 24 heures ont donné le meilleur taux de germination (50%), par contre, chez* C. sesamoides *aucune germination n'a été notée. Les graines de* C. sesamoides *et de* S. radiatum *ont montré de dormance. Pour assurer davantage la germination d'*A. uliginosa *en pépinière, les graines doivent être débarrassées de leur aigrette.*

Introduction

African traditional leafy vegetables are plant species, wild or cultivated, originated or naturalized in Africa, and whose leaves are used in diet (17). They are very rich in nutrients (11) and play an important role for subsistence and boost the income of the populations in rural areas (1, 17). Apart from nutritional qualities, several species of these traditional leafy vegetables have medicinal properties (8). In Benin, Codjia *et al.* (7) recorded 162 forest plant species consumed by local populations and reported that the leafy vegetables ranked second after the fruits. Adjatin (2) listed over 180 traditional leafy vegetables, including *Acmella uliginosa* (Sw.) Cass. (Asteraceae), *Ceratotheca sesamoides* (Endl.) (Pedaliaceae), *Justicia tenella* (Nees) T. Anderson (Acanthaceae), *Sesamum radiatum* (Schumach. & Thonn.) (Pedaliaceae), commonly consumed in Benin by many ethnic groups rather as wild vegetables than cultivated plants (14).

Unlike the cultivated species, seeds from wild species are generally dormant (15). Some farmers who started the domestication of the four above mentioned vegetables are faced with low germination of seeds including those of *C. sesamoides*, *S. radiatum* and *A. uliginosa*. Therefore, there is a dire need to provide farmers with qualified seeds, since there is no report on the germination of the seeds of these species.

To promote the production of the four leafy vegetables, a vast research program including the farming systems, the conservation, the genetic and biochemical variability, the biology of development and the regime of reproduction, the nutritional quality, the local taxonomy of the 4 species in relation to the socio-economic groups, has been funded by the Scientific Council of the University of Abomey-Calavi

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in Benin. The main objective of the present study was to determine the optimal germination conditions of the four species under domestication in Benin.

Materials and methods

Determination of water content of seeds

The seeds of the four legumes were collected on-farm at Savè (Center of Benin) and multiplied at the International Institute of Tropical Agriculture (IITA) in Abomey-Calavi (6°25 N 2°19 E), Benin for 4 months. At harvest, the water content (w.c.) of the seeds was determined by oven drying at 105 °C for 16 hours (15), and calculated using the following formula:

$$w.c = \frac{100x(iW - fW)}{iW}$$

where *iW* and *fW* were the initial weight and final weight respectively after drying in the oven.

Determination of seed viability

In order to check whether the seeds were dormant or not, they were, at harvest, sun dried for 8 hours, and then divided into 3 lots per species. Twenty seeds from each lot were plated per Petri dish containing moistened sterilized Whatman filter paper and incubated at room temperature (15). The germinated seeds were checked daily from day 4 to day 15.

Break of *C. sesamoides* and *S. radiatum* seeds dormancy

In one experiment, the seeds were subjected to four different treatments: water at 100 °C, water at 80 °C, sulfuric acid at 95% and oven drying at 80 °C as suggested by Velempini *et al*. (21) in order to soften seeds tegument. The seeds were immersed for different durations: 30 seconds, 1 minute and 3 minutes. The control consisted of the seeds without any treatment. After 30 seconds, 1 minute and 3 minutes, seeds immersed in hot water were immediately plated in Petri dishes whereas those treated with sulfuric acid were rinsed with running tap water and then plated in Petri dishes. In another experiment, seeds of the same species were divided into lots of seeds obtained immediately at harvest, seeds obtained after one, two, three and eight days of sun drying. Each seed lot was divided into two sub-lots: the first sub-lot was subjected to direct sowing in Petri dishes while the second sub-lot was immersed for 24 hours in tap water at room temperature before plating in Petri dishes. The water-soaking treatment was based on the works of Prins & Maghembe (13), Moussa *et al*. (12) and the habit of the farmers in Benin who are accustomed to soak the seeds in water for one to two nights before planting. The experiment was set up in three replications of each sub-lot and arranged in a completely randomized bloc design.

Determination of the *A. uliginosa* seeds' germination

During the trials, it appeared that, the *A. uliginosa* seeds presented two morphological aspects: some seeds were with papus while some did not have. Then, at harvest, the *A. uliginosa* seeds were split up into seeds with no papus, seeds with papus and exposed to sun-drying for 48 hours. Twenty seeds of each lot were plated in a Petri dish as a replication at room temperature, in a completely randomized bloc design with three replications. The germinated seeds were counted as described above.

Statistical analysis

The number of seeds that had germinated was expressed as percentage of the total number of seeds sown in each treatment of the trials. This percentage was transformed by Arcsin√x as suggested by Snedecor & Cochran (20). The data were transformed by Arcsin√x, and means were compared using the GLM ANOVA Procedure. The Student Newman-Keuls and Kruskal Wallis tests were used to separate the means from the break of the seed dormancy and the germination of *C. sesamoides* and *S. radiatum* seeds at 95% of confidence respectively (4, 9).

Results

Viability of seeds

Table 1 shows the initial germination rate of untreated seeds of the four species of leafy vegetables under domestication. Of the four species, only the seeds of *J. tenella* showed a high germination: 83.3% of seeds sown, followed by *A. uliginosa* whose seeds showed a germination rate relatively low (42.8%). The other two species (Pedaliaceae), *C. sesamoides* and *S. radiatum*, with germination rate of 7.2% and 11.2%, respectively showed the lowest germination rate.

Table 1

Germination rates of untreated leafy vegetables' seeds

Species	Germination (%)
Justicia tenella	83.3 ± 1.8
Acmella uliginosa	42.8 ± 7.5
Sesamum radiatum	11.2 ± 5.3
Ceratotheca sesamoides	7.2 ± 2.5

Effect of different treatments on seed germination

The effect of hot water, sulfuric acid and drying on seed germination of *C. sesamoides* and *S. radiatum* are presented in table 2. The seeds of *C. sesamoides* and *S. radiatum* subjected to hot water at 100 °C and 80 °C or concentrated sulfuric acid at 95% for any of the tested times could not germinate except few seeds of *S. radiatum* treated with hot water at 80 °C for 30 seconds germinated. The seeds dried at 80 °C for 30 seconds, 1 minute or 3 minutes germinated with no significant difference as compared to the control.

Table 2
Germination rate of treated seeds of *C. sesamoides* and *S. radiatum*

Treatments	Germination (%)	
	S. radiatum	*C. sesamoides*
Water 100 °C / 30 sec	0.0 ± 0 c	0.0 ± 0.0 b
Water 100 °C / 1 min	0.0 ± 0 c	0.0 ± 0.0 b
Water 100 °C / 3 min	0.0 ± 0 c	0.0 ± 0.0 b
Water 80 °C / 30 sec	6.7 ± 5,7 b	0.0 ± 0.0 b
Water 80 °C / 1 min	0.0 ± 0 c	0.0 ± 0.0 b
Water 80 °C / 3 min	0.0 ± 0 c	0.0 ± 0.0 b
Sulfuric acid / 30 sec	0.0 ± 0 c	0.0 ± 0.0 b
Sulfuric acid / 1 min	0.0 ± 0 c	0.0 ± 0.0 b
Sulfuric acid / 3 min	0.0 ± 0 c	0.0 ± 0.0 b
Oven drying (80 °C) / 30 sec	15.0 ± 0 a	6.7 ± 2.8 a
Oven drying (80 °C) / 1 min	16.7 ± 2.8 a	5.0 ± 0.0 a
Oven drying (80 °C) / 3 min	13.3 ± 5.7 a	6.7 ± 2.8 a
Control	13.3 ± 2.8 a	5.0 ± 0.0 a

Numbers without a common letter are significantly different at 95% confidence after the Kruskal Wallis test.

Except the oven treatments, seeds subjected to the different treatments seemed lose their viability.

The results presented in table 3 show that the water content of those seeds was high at harvest: 14.46% and 16.47% for *S. radiatum* and *C. sesamoides* respectively. The water content of the seeds collapsed drastically after 1 day of sun drying, from 14.46% to 9.68% for *S. radiatum* and from 16.47% to 11.39% for *C. sesamoides*. But, this water content was fairly stable after 3 days of sun drying, 8.8% and 10% for *S. radiatum* and *C. sesamoides* respectively. Then, table 3 shows that immersing in hot water for 24 hours had no effect on seeds of *C. sesamoides* whose germination is very low regardless of their water content. Besides, immersing in water for 24 hours resulted in significant differences in terms of germination rate of *S. radiatum* seeds in relation to their water content. This percentage was inversely proportional to the moisture content of seeds and was higher (50%) when the moisture content was below 8.8%.

Germination of seeds of *A. uliginosa*

After an attentive observation, the seeds with pappus of *A. uliginosa* were separated from the seeds without pappus. When plated and germinated in the Petri dishes, the seeds without pappus of *A. uliginosa* showed a very high germination rate at 86% of seeds sown while those with pappus were negatively affected since only 15% of these seeds germinated. The rate of germination of seeds without pappus of *A. uliginosa* was at least five times higher than the germination rate of seeds with pappus.

Discussion

With germination over 80% without specific treatment, it is obvious that the seeds of *J. tenella* did not show dormancy. Besides, the germination of seeds of *C. sesamoides* and *S. radiatum* was very low (< 15%) without any treatment. Contrary to Bedigian and Adetula (5) observations, our results show that seeds of *C. sesamoides* and *S. radiatum* are dormant. In a previous study, Ashri and Palevitch (3) reported that the seeds of *S. indicum* as *S. radiatum* may show dormancy. Seeds of these two species dried in the sun to reduce moisture content could not yield a high rate of germination, particularly with *C. sesamoides* very low germination rates were recorded. These observations are similar to those of producers who believe with strong conviction that the culture of *C. sesamoides* is impossible.

According to Silvertown (18), there are two types of dormancy: the “endogenous” types are those due to properties of the embryo and the “exogenous” types result from properties of the endosperm or any other tissues of the seed or fruit. Endogenous dormancy may be the result of (i) a physiological inhibiting mechanism in the embryo (“physiological dormancy”), (ii) an undeveloped embryo (the so-called “morphological” dormancy), or (iii) a combination of i and ii (called “morphophysiological” dormancy). The exogenous dormancy types may (i) be caused by seed or fruit coat impermeability to water (“physical”), (ii) due to germination inhibitors (“chemical”), and (iii) caused by woody structures that restrict growth (“mechanical”). Dormancy observed in *C. sesamoides* and *S. radiatum*

Table 3
Germination rates of *C. sesamoides* and *S. radiatum* seeds in relation to seed water content

Treatments	*Sesamum radiatum*		*Ceratotheca sesamoides*	
	Water content	Germination (%)	Water content	Germination (%)
Direct sown	iwc= 14.46 a	15.0 ± 8.6 c	iwc= 16.47 a	3.3 ± 2.8 a
	wc_{1d}= 09.68 ab	20.0 ± 5.0 c	wc_{1d}= 11.39 b	0.0 ± 0.0 a
	wc_{2d}= 09.32 ab	18.3 ± 5.8 c	wc_{2d}= 10.29 b	3.3 ± 2.8 a
	wc_{3d}= 08.86 b	15.0 ± 0.0 c	wc_{3d}= 10.08 b	1.7 ± 2.8 a
	wc_{8d}= 08.77 b	16.7 ± 2.8 c	wc_{8d}= 10.06 b	1.7 ± 2.8 a
Immersed (24 h)	iwc= 14.46 a	30.0 ± 8.7 b	iwc= 16.47 a	0.0 ± 0.0 a
	wc_{1d}= 09.68 ab	33.3 ± 10.4 ab	wc_{1d}= 11.39 b	1.7 ± 2.8 a
	wc_{2d}= 09.32 ab	36.7 ± 5.7 ab	wc_{2d}= 10.29 b	1.7 ± 2.8 a
	wc_{3d}= 08.86 b	51.7 ± 10.4 a	wc_{3d}= 10.08 b	1.7 ± 2.8 a
	wc_{8d}= 08.77 b	50.0 ± 5.0 a	wc_{8d}= 10.06 b	1.0 ± 2.8 a

Numbers without a common letter are significantly different at 95% confidence after the Student Newman-Keuls test.

is not physical. Their seed coat seemed weak. Indeed, the seeds have completely lost their viability upon contact with hot water at 100 °C for only 30 seconds. However, treatments such as the use of sulfuric acid, hot water or scarification are usually excellent ways to break the dormancy of seeds of many species caused by the hardness of the coat (4, 9, 21). The seeds of leguminous fodder particularly hard seed coat germinated up to 98% when immersed in concentrated sulfuric acid for 15 min (19). Furthermore, *S. radiatum* seed, with water content of 8.8%, immersed in water at room temperature germinated up to 50%. We may then exclude the hypothesis of an endogenous dormancy at least for *S. radiatum*. Although this value is greater than that obtained for the lot that was not immersed in water, this germination percentage is still below the 80% generally required for seeds to be distributed to farmers (15). Nevertheless, the method of immersing seeds in water overnight have been successfully used by producers to trigger germination of seeds of many species including *Leucaena leucocephala*, *Albizzia lebbeck*, *Gliricidia sepium*.

The control values in table 2 are slightly different from those of table 1. In fact, the values of table 2 were obtained from a trial set up one month after the results of table 1. More seeds of *S. radiatum* probably germinated because of the decreasing of the water content while we observed the reversed situation with *C. sesamoides* as indicated by Cervantes *et al*. (6).

However the hypothesis of a negative influence of storage could be eliminated because the seeds submitted to the various tests were not stocked. Light is a factor which ordinary greatly influences the germination of seeds. But in the current experimentation, there was no light during the nights in the laboratory where the tests were carried out. In order terms, the Petri dishes were in closely natural conditions of the plants (18).

Dormancy observed in *C. sesamoides* and *S. radiatum* may be chemical. Especially in the case of *C. sesamoides*, the length of the germination may be an important factor. Indeed, according to Silvertown (18), many annual and biannual plant species seeds may have more than 50 years of longevity in the soil. This hypothesis agrees with the argument of the farmers who think that *C. sesamoides* could stay more than 5 years in the soil before germinating. In, Benin, the farmers actually throw the seeds of *C. sesamoides* in their plots where they wish to have that vegetable many years later.

The position of the seed on the stem could influence positively or negatively the germination (15, 18); thus seeds from the middle (center) of the inflorescence germinate readily than the seeds from the periphery, this is the case for the genus *Bidens*. Also, it has been shown that the dormancy of a seed could come from it mother plant (18).

Therefore, other avenues could be explored to break the dormancy of seeds of *C. sesamoides* and *S. radiatum*. In addition to the methods such as the use of gibberellic acid at low concentrations, potassium nitrate, indole-acetic acid (15), a study on the germination seeds of these two species according to the position of their pod on the inflorescence is necessary (15).

The dormancy observed in *A. uliginosa* could be due to morphological factors caused by immature embryos (morphological dormancy). The presence of the pappus is an indicator of immature embryo which causes dormancy in some species (10, 15, 16). The relatively low germination (42.8%) of seeds of this species obtained from the previous or initial experiment might probably be due to a mixture of mature seeds and immature ones. The separation of the two types of seeds showed that mature seeds, deprived of their pappus, did not show dormancy. However, producers who have started the domestication of this species in their garden harvest very early, henceforth immature seeds especially that the flowers are also sold for consumption (2). Therefore, the seeds reserved for next sowing were a mixture of mature and immature seeds, which results in poor germination. The situation becomes a little complicated because the mature seeds fall to the ground very quickly. Further studies might clarify the best time to harvest these seeds not only to avoid losing seeds that fall on the ground quickly, but to have the mature seeds with good germination. Therefore, this time of mature seed may be very useful for the extension services and development agents during the farmers' trainings.

Conclusion

The results of this study reveal the factors that may influence the dormancy of two species of Pedaliaceae, particularly *S. radiatum*. They also show that the seeds of *J. tenella* did not demonstrate dormancy. The hormones such as gibberellins and indole-acetic acid are to be tested to study their effect on germination capacity of Pedaliaceae species. Furthermore, to produce *A. uliginosa*, the use of seeds without pappus is necessary. Further study is then required to determine the time to harvest the seeds of this species without pappus.

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