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Effects of seed fermentation method on seed germination and vigor in the oleaginous gourd *Lagenaria siceraria* (Molina) Standl.

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Supplying high quality seed to rural farmers is the basic requirement for the sustainable development of agriculture in developing countries. The present study was conducted to examine the influence of *Lagenaria siceraria* seed fermentation method on seed germination and vigor. Three seed fermentation methods (fermented in ambient air, plastic bag stored in ambient or in plastic bag buried) were tested on two cultivars during two years. Seed germination and vigor were better when fermentation was conducted in anaerobic, darkness, and at low temperature. Low seed quality was observed in unfermented seed, suggesting the occurrence of postharvest maturity in *L. siceraria*. Seed quality did not vary between cultivars. Regardless of the fermentation process and cultivars used, the best seed and seedling qualities were observed when the amount of rainfall during the experiment period was high, suggesting that wet soil is necessary for an on farm reliable evaluation of seed fermentation method in the bottle gourd *L. siceraria*.

Key words: Cucurbit, *egussi*, maturity, minor crops, oilseed, seedling, viability.

INTRODUCTION

The seeds of the oleaginous *Lagenaria siceraria* are consumed as cake or thickeners of a traditional dish called *egussi* soup in most countries from Western and Central Africa (Bisognin, 2002; Enujiugha and Ayodele-Oni, 2003). This cucurbit is reported to be rich in nutrients (Badifu, 1993), namely protein (36±2.17% of dry content (DC) and fat (45.89±4.73% DC). In addition, it is commonly found in many traditional cropping systems, and it is well adapted to extremely divergent agro-ecosystems and various cropping systems characterized by minimal inputs (Zoro Bi et al., 2005). *L. siceraria* thus represents an excellent plant model for which improved cropping systems implementation can insure the economic prosperity of rural people from tropical Africa where the main

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Abbreviations: SFA, Seeds fermented by exposing the cut fruits in ambient air in the field; **SFB,** seeds fermented by packing the cut fruits into plastic bag that was exposed in ambient air in the field; **SFD,** seeds fermented by packing the cut fruits into a plastic bag that were buried at 30 cm depth; **GSI,** germination speed index; **ESI,** emergence speed index; **GeP,** germination percentage; **EmP,** emergence percentage; **SSL,** seedling shoot length; **SDB,** seedlings dry biomass; **RFC,** round-fruited cultivar.

producers are women.

In spite of *L*. *siceraria* nutritional and agronomic potentials, in depth investigations on the crop are scanty (Williams and Haq, 2002). As for most orphan crops, one of the main constraints the breeders must overcome to ensure a sustainable production is the development of improved seed supply systems (Almekinders et al., 1994). The sustainable production of any crop depends mainly on its seed quality. Indeed, uneven seed germination in a field, particularly in direct-seeded crops can lead to variable density and lack of uniformity in plant growth and phenology (Nerson and Paris, 1988). This may have a negative impact on yield and market value of the crop. In most cucurbit seed production systems, seed fermentation in the juicy endocarp and mesocarp tissues of the fruits (Modgil et al., 2004) is the first step after fruit cutting is employed for various days depending on the species. Seed extraction procedure requires care for seed production in the indigenous cucurbits (Nerson, 2007).

Seeds of *L*. *siceraria* are extracted after a 7-day fermentation period of cut fruit containing a juicy pulp to which they are attached (Zoro Bi et al., 2003). This process is expected to facilitate the seed separation from the surrounding tissues, and increase their nutritive value and germination percentage (Achinewhu and Ryley, 1986). Diverse microorganisms are involved in cucurbits seed fermentation process (Harper and Lynch, 1980; Nelson, 1990; Niemi and Häggman, 2002).

However, the actual effects of fermentation on the agronomic and nutritional qualities of cucurbit seeds seem to vary greatly, depending mainly on species, fruit age and fermentation duration (Nerson and Paris, 1988; Nerson, 1991; 2002, 2004). Nienhuis and Lower (1981) reported that longer fermentation of cucumber seeds can decrease the germination percent and rate. Examining the relationship between fruit age at harvest and fermentation in melon, cucumber and watermelon, Edwards et al. (1986) and Nerson (1991) noted that germination of seeds from fully-ripe fruits was not affected by fermentation. The authors showed that fermentation increased the germination percent of immature seeds from cucumber, melon, and watermelon, whereas this decreased in squash.

It is worth noting that from the studies addressing the influence of fermentation on cucurbit seed germination, those examining the fermentation methods are scant (Nienhuis and Lower, 1981). Fermentation methods are widely reported to influence microbial diversity and activity (Silva et al., 2000; Nyanga et al., 2007).

In previous investigations, using the oleaginous type of the bottle gourd, we identified the fruit maturity stages at which seed germination and the level of percentage, macronutrients, mineral elements, and vitamins are at their highest values. We report herein results obtained from a study aimed at determining the influence of seed fermentation method on germination and seedling vigor in two cultivars of the oilseed *L*. *siceraria*.

MATERIALS AND METHODS

Plants materials and treatments

Open-pollinated accessions from two edible-seeded *L. siceraria* cultivars, recognizable by the fruit shape (blocky or round) were used. Seeds from the round fruit cultivar are characterized by the presence of a cap on the distal side whereas those from the blocky fruit cultivar lack this cap (Figure 1). Both cultivars were obtained from the cucurbit germplasm of the university of Nangui Abrogoua (Abidjan, Côte d'Ivoire) where they are recorded by the alphanumerical codes NI304 and NI195 for the round- and blocky-fruited cultivar, respectively. The experiments were conducted in two separate years. In Experiment 1, the plants were cultivated during the second growing season of 2006 (from September to December) and seed germination and vigor were evaluated in February, 2007. In Experiment 2, the plants were grown in the same month in 2007 and were evaluated in February 2008 at the experimental station of the University Nangui Abrogoua (Abidjan, Côte d'Ivoire). In each experiment, the harvested fruits were stored at the farm for two weeks before fermentation tests started.

For each cultivar, and year, 40 fruits were selected to constitute four samples of 10 fruits, corresponding to four fermentation treatments: 1) Control (unfermented seeds); 2) seeds were fermented by exposing the cut fruits in ambient air in the field (SFA); 3) seeds were fermented by packing the cut fruits in a transparent plastic bag that was exposed in ambient air in the field (SFB); and 4) seeds were fermented by packing the cut fruits in a transparent plastic bag that was buried 30 cm depth (SFD). Fermentation started two weeks after harvest for all the selected fruits. The three fermentation processes are those in general used by rural farmers from Sub-Sahara African and Asia countries (Okoli 1984; Hopkins et al., 1996; Nerson 2002). All the 40 fruits used in this study were harvested 50 days after anthesis considered elsewhere to be the normal time for seed harvest in the two cultivars examined. During the trials, the daily temperature averages in ambient air (SFA), plastic bag containing cut fruits and exposed in the field (SFB), and plastic bag containing cut fruits and buried 30 cm depth (SFD) were 29, 32 and 26°C, respectively.

Seeds from the control samples were directly extracted, washed with tap water, and sundried in ambient air until attaining 6 to 7% moisture. Seeds from the other treatments were extracted after a 10-day fermentation period (practice widely used by farmers) then washed and dried until 6-7% moisture. After drying, seeds with similar weights (310 ± 76 mg) were selected for germination and vigor evaluation. This mean was determined on the basis of the individual weight of 750 seeds per cultivar.

Experimental design for seed germination and vigor evaluation and data collection

Seed germination and vigor were evaluated in an on farm trial using a completely randomized complete block design with two blocks and five replications (plots of 1 m × 0.5 m). Blocks were spaced by 3 m. Each plot received 20 seeds, resulting into 100 seeds per treatment per block. The seed samples were sown at 3 cm depth and a spacing of 7 x 7 cm. Sowings were done on raised beds (30 cm height mounds). The rainfall, relative humidity, and temperature were respectively 107.19 mm, 93%, and 28°C, in Experiment 1 and 192.28 mm, 87%, and 25°C, in Experiment 2.

Seed germinability was evaluated using the seed germination percentage (GeP). Seeds were considered as germinated when the cotyledons appeared above the ground level. The seeds sown were surveyed daily for 14 days (ISTA, 1996). Seed vigor was examined using the following parameters: germination speed index (GSI), seedling emergence percentage (EmP), emergence speed index (ESI), shoot length (SSL, measured with a ruler after digging up the

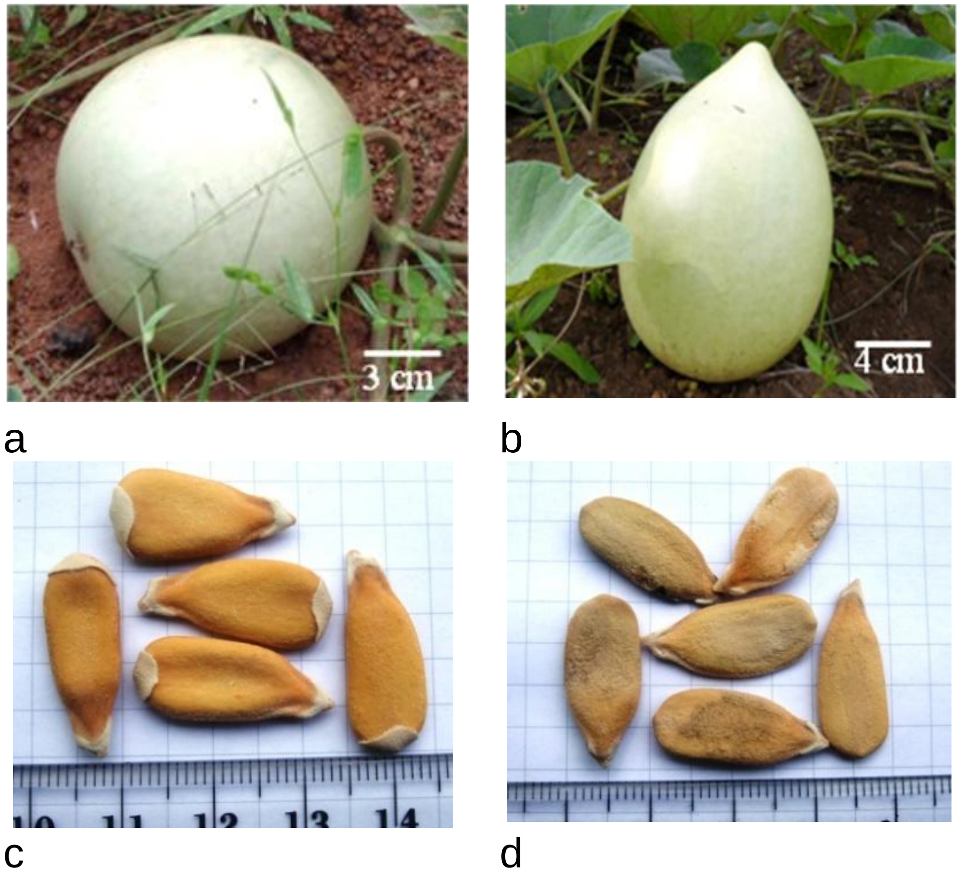

Figure 1. Fruit and seeds of the edible-seeded *Lagenaria siceraria*. a: stem with round fruit; b: stem with blocky fruit; c: seed of the round-fruited cultivar; d: seed of the blocky-fruited cultivar.

emerged seedling), and seedling dry biomass (SDB, measured after drying the seedling to constant weight). A seedling was considered emerged when its two cotyledonary leaves were completely opened (Koffi et al., 2009). The GSI and ESI were calculated on the basis of the procedure used by Maguire (1962) according to the following equation:

$$\text{GSI or ESI} = \frac{X_1}{N_1} + \frac{X_2}{N_2} + \cdots \frac{X_n}{N_n}$$

Where, X_1, X_2, and X_n represent the numbers of germinated seeds or emerged seedlings on the first, second, and last count; N_1, N_2, and N_n corresponding to the numbers of days on the first, second, and last count.

Data statistical analysis

Percentage data were arcsin-transformed before analysis (Little, 1985) but untransformed data were used to calculate means to present the results. Multivariate analysis of variance (MANOVA) appropriate for three-way fixed model was performed using SAS statistical package (SAS, 2004) to check difference between the variable means for each factor tested (fermentation method, cultivar, and time of experiment). For each trait, when the null hypothesis related to the MANOVA was rejected, Duncan's multiple range tests were carried out to identify significant differences among the means of the parameters examined, according to fermentation method, cultivar, experiment, and interactions. All Duncan's multiple range tests were carried out at $\alpha = 0.05$ significance level.

RESULTS

Global effect of analyzed factors

MANOVA revealed significant influence of the fermentation method, cultivar and experiment. Also, all the three double interactions as well as the triple interaction were significant. Nevertheless, for most of the six traits examined, the trends of results related to single effects of fermentation method and cultivar did not change through the two experiments. Thus, data for these two factors were polled over experiments and only the means are presented (Table 1).

Effect of analyzed factors on seed germination and vigor

The results of each analyzed factor (experiment period,

Table 1. MANOVA results of experiment period, fermentation method, cultivar and their different interaction on seed germination and seedling vigor in the oleaginous *Lagenaria siceraria.*

Factor	DF	*F*	*P*
Experiment	1	67.14	<0.001
Cultivar	1	17.03	<0.001
Method	3	14.73	<0.001
Experiment ×Cultivar	3	3.13	<0.001
Cultivar × Method	7	12.27	<0.001
Experiment × Method	3	11.27	<0.001
Experiment × Cultivar × Method	15	18.43	<0.001

fermentation method and cultivar) and their different interactions on seed germination and seedling vigor are shown in Table 2.

Variation of seed germination and vigor with respect to experiment

For both cultivars, the highest values of the six traits examined were obtained during the Experiment 2 (February, 2008). Thus, high proportions of seeds germinated, gave longest and heaviest shoots in February 2008 experiment, regardless of the fermentation process and cultivars used.

Influence of fermentation process on seed germination and vigor in each cultivar

The parameters expressing seed viability (germination percentage and speed index) and vigor (emergence percentage, emergence speed index, shoot length and dry biomass) were significantly ($P < 0.001$) influenced by fermentation method in each cultivar. The highest values of all the six parameters measured were obtained when seeds were fermented after cutting and packing the fruits into plastic bag, and then hidden under ground in 30 cm depth (SFD). These values were followed in decreasing order by treatments SFB, SFA, and the control.

Response of cultivar to seed germination and vigor

Seed germination and seedling emergence percentages did not vary significantly with cultivar (Table 2). Contrarily to this, the speed of seed germination and seedling emergence, as well as the length and biomass showed highly significant variation according cultivars. The highest speeds of seed germination and seedling emergence were observed in the round-fruited cultivar. Accordingly, significantly high shoot length was obtained with the same cultivar. However, the seedling dry biomass was higher in the blocky-fruited cultivar.

Interaction between cultivar and the fermentation method during each experiment

Although there was a significant effect of fermentation method leading to high values with SFD method in each cultivar, the comparison of both cultivars indicated that GeP, EmP, and SSL did not significantly vary from one cultivar to the other during each experiment. However, GSI, ESI and SDB varied significantly according to cultivars (Table 2). Indeed, even for the best fermentation method (SFD), seedling emerged more rapidly (ESI) in round-fruited cultivar (RFC) while the heaviest seedling (SDB) were obtained with blocky-fruited cultivar (BFC). Even if cultivars showed small difference in germination and vigor, fermentation methods have similar effect on them. Independently of these two factors, the weather during experiment has significant effect on germination and vigor.

DISCUSSION

Techniques used to improve seedling establishment are widely documented (Taylor and Harman, 1990; Copeland and McDonald, 2001). One of the main factors commonly considered to improve seed quality in the continuously flowering fleshy-fruited crops such as cucurbits is seed fermentation (Nerson and Paris, 1988). Seed fermentation efficiency is widely demonstrated but seems to be linked to conditions (that is, method) in which it takes place (Nienhuis and Lower, 1981; Nerson and Paris, 1988; Demir and Samit, 2001; Nerson, 2002; 2007). That is the case in our study.

Seed quality response to fermentation method has been examined in the oleaginous bottle gourd with respect to cultivar and month of experiment. The results show that individual and combined effect of these three factors (fermentation method, cultivar and experiment period) influenced seed germination and vigor. The analysis of these results showed that whatever was extracted in the procedure, seeds obtained from each method germinated and produced at least vigorous seedlings in both cultivars. This proved that seeds used during this study were mature, but compared to control

Table 2. Interaction effect of time of experiment, cultivar and seed fermentation method on the seed germination and seedling vigor in oleaginous *Lagenaria siceraria*.

Experiment	Cultivar	Fermentation method[1]	Parameters[2]					
			GSI[3]	ESI	GeP (%)	EmP (%)	SSL (mm)	SDB (mg)
Experiment 1 (February 2007)	Round-fruited	SFD	3.64 ± 0.05^{d}	2.12 ± 0.05^{bcd}	81.00 ±2.56^{bcde}	79.50 ± 2.63^{bcd}	89.66 ± 1.10^{b}	5.48 ±0.11^{d}
		SFB	3.08 ± 0.04^{e}	1.93 ± 0.05^{d}	74.50 ±2.41^{defg}	72.50 ± 2.01^{def}	87.05 ±0.99^{cd}	4.76 ± 0.11^{f}
		SFA	2.78 ± 0.07^{efg}	1.52 ± 0.08^{f}	67.00 ± 3.35^{g}	65.50± 3.91^{fg}	85.43 ± 1.22^{d}	4.80 ± 0.12^{ef}
		Control	1.95 ± 0.04^{h}	1.35 ± 0.10^{f}	54.50 ± 1.74^{h}	51.00 ± 2.08^{hi}	81.68 ± 1.34^{ef}	4.33 ± 0.14^{g}
	Blocky-fruited	SFD	3.74 ± 0.14^{c}	1.94 ± 0.09^{d}	86.50 ± 2.48^{abc}	84.50 ± 2.52^{abc}	73.22 ± 0.68^{h}	6.76 ± 0.10^{b}
		SFB	3.15 ± 0.12^{d}	1.57 ± 0.09^{f}	79.00 ± 4.52^{cdef}	77.00 ± 4.29^{cde}	64.83 ± 0.86^{i}	5.16 ± 0.09^{de}
		SFA	2.16 ± 0.18^{h}	1.50 ± 0.09^{f}	71.00 ± 4.82^{fg}	69.00 ± 4.64^{efg}	60.38 ± 0.84^{j}	4.69 ±0.10^{f}
		Control	1.97 ± 0.12^{h}	1.06 ± 0.12^{g}	49.00 ± 5.04^{h}	44.00 ± 4.33^{i}	57.67 ± 1.00^{j}	4.58 ± 0.12^{fg}
Experiment 2 (February 2008)	Round-fruited	SFD	4.71 ± 0.11^{a}	2.44 ± 0.07^{a}	95.00 ± 3.16^{a}	92.50 ± 2.81^{a}	84.62 ± 1.05^{de}	7.59 ± 0.20^{a}
		SFB	4.27 ± 0.18^{ab}	2.26 ± 0.09^{abc}	90.83 ± 3.00^{ab}	88.33 ± 3.57^{ab}	76.35 ± 1.08^{g}	5.79±0.12^{c}
		SFA	4.63 ± 0.15^{a}	2.43 ± 0.07^{a}	85.83 ± 1.54^{abc}	83.33 ± 1.67^{abc}	72.38 ± 1.17^{h}	5.45 ± 0.20^{de}
		Control	2.92 ± 0.15^{efg}	1.86 ± 0.11^{de}	68.33 ± 3.80^{g}	59.17± 3.52^{gh}	66.85 ± 1.09^{i}	4.60 ± 0.20^{fg}
	Blocky-fruited	SFD	4.30 ± 0.19^{ab}	1.99 ± 0.12^{cd}	93.33 ± 3.07^{a}	90.83 ± 2.39^{a}	93.04 ± 0.79^{a}	6.88 ± 0.14^{b}
		SFB	4.12 ± 0.22^{bc}	2.44 ± 0.09^{a}	86.67± 3.33^{abc}	85.00 ± 4.08^{abc}	89.10 ± 0.92^{bc}	6.33 ± 0.17^{b}
		SFA	3.72 ± 0.24^{cd}	2.31 ± 0.12^{ab}	85.00 ±1.83^{abcd}	82.50 ±2.14^{abcd}	85.36 ± 0.83^{d}	5.46 ± 0.06^{de}
		Control	2.60 ± 0.18^{g}	1.57 ± 0.08^{ef}	72.50 ± 5.59^{efg}	66.67± 6.15^{efg}	80.72 ± 0.97^{f}	5.07 ± 0.11^{e}
Statistic test results	*F*		5.49	3.51	4.65	4.01	4.48	2.49
	P		<0.001	0.003	<0.001	<0.001	<0.001	0.017

[1] Control: unfermented seeds; SFA: seeds fermented by exposing the cut fruits in ambient air in the field; SFB: seeds fermented by packing the cut fruits into plastic bag that was exposed in ambient air in the field; SFD: seeds fermented by packing the cut fruits into a plastic bag that were buried at 30 cm depth.
[2] GSI: germination speed index; ESI: emergence speed index; GeP: germination percentage; EmP: emergence percentage; SSL: seedling shoot length; SDB: Seedlings dry biomass.
[3] In each column, values with the same superscript letter are not significantly different from each other ($P < 0.05$) using Duncan's multiple range test.
NB: Rainfall in the Experiment 1 was low (107.19 mm) compared to this obtained in the Experiment 2 (192.28 mm).

method (unfermented seeds), all the fermented seeds (SFA, SFB and SFD) showed better germination and vigor. It means that, apart from facilitating extraction of seeds firmly encrusted in the fruit pulp, fermentation improves their germination and vigor (Edwards et al., 1986; Nerson and Paris, 1988; Taylor and Harman 1990). This can partially explain why peasants always ferment cucurbits seeds before extracting them (Nerson, 2007). Comparison of our fermentation methods to each other showed that seeds obtained from closed media (that is, SFB and SFD methods) exhibit better germination and vigor than those fermented at ambient air (SFA).

This difference of seed germinability and vigor observed in *L. siceraria* both cultivars tended to prove that although fermentation improves seed quality, its efficiency depends on condition in which it takes (Nienhuis and Lower, 1981; Nerson and Paris, 1988; Demir and Samit, 2001; Nerson, 2002, 2007). In addition, SFA method being the easiest to apply and widely used by peasant (Okoli 1984; Hopkins et al., 1996; Nerson, 2002) we can assumed that our fermentation methods (SFB and SFD) are more useful than the peasants one (SFA) in seeds quality improvement of this species. Moreover, comparison of our closed media methods (SFB and SFD) revealed that best germination and vigor were obtained with seed fermented in anaerobic, darkness, and relatively low temperature (26°C) conditions (that is, SFD). High performance of the SFD treatment in *L. siceraria* seed quality improving might be due to single or combined effects of darkness and the relatively low temperature (Nienhuis and Lower, 1981; Demir and Samit, 2001; Woo and Song, 2010). Several studies proved that, for each species, speed and result of the fermentation process largely depend on the temperature and its application duration (Demir and Samit, 2001). For our oleaginous *L. siceraria*, good quality of seed was obtained after incubating at 26°C in dark as reported (24-27°C) in tomatoes (Nienhuis and Lower, 1981; Nerson and Paris, 1988; Nerson, 1991; 2002). Darkness could have favored the metabolic process and/or the development of microorganisms involved in seed fermentation (Madigan et al., 1980; Teramoto et al., 1993). Anaerobic medium, water saturation, and reduced air conditions seemed to favor the proliferation and the activity of microorganisms involved in fermentation (Nienhuis and Lower, 1981; Nerson and Paris, 1988; Silva et al., 2008; Stringini et al., 2009). Beneficial microorganisms developed on seeds during the fermentation process might promote seedling establishment or provide seed borne diseases control (Beaulieu et al., 2004; Bennett and Whipps, 2008). Indeed not all of the various microorganisms proliferating during cucurbit crops seed fermentation (Leben, 1981; Bankole, 1993) have deleterious effects on their germinability. Another hypothesis of the beneficial effect of fermentation on *L. siceraria* mature seeds germinability could also be attributed to cucurbitacin (germination inhibitor) lifting during the process (Nerson and Paris, 1988; Nerson, 1991; 2002; Martin and Blackburn, 2003).

In our study, seed germination and seedling emergence percentages did not vary significantly between both cultivars following seed fermentation method. Even if studies examining differences between crop plant cultivars for seed germination and vigor following fermentation procedure are scant, investigations on the variation of seed germination among cultivars in a plant species are extensive in literature (Silvertown, 1984; Ellison, 2001; Cisse and Ejeta, 2003). Difference between both cultivars of *L. siceraria* could be due to a genetic control of seed germination in several crop plants (Ecker et al., 1994; Sadeghian and Khodaii, 1998; Dias et al., 2011).

Contrarily to germination and emergence percentages, significant variations were noted between cultivars for the speed of seed germination and seedling emergence, as well as the seedling length and biomass; the best performances being observed in the round-fruited cultivar (RFC). This difference between both cultivars for the seedling vigor could be attributed to the difference in their agronomical performances, rather than fermentation method or seed reserves. In fact, the seeds used in this study were of similar weights (310 ± 76 mg), suggesting their similar contents in reserves contrarily to several plant species for which a large amount of seed reserves is used to form a certain size of stem and the necessary number of leaves to set the plant up for photosynthesis during germination and early stages of seedling growth (Wanasundara et al., 1999; Ichie et al., 2001; Kolb and Joly, 2010). Furthermore, we noted from seed regeneration trials that compared to the blocky fruit cultivar (BFC), the RFC usually shows more vigorous vegetative growth and flowers about two weeks earlier.

Independently of the fermentation process and cultivar used, the best seed and seedling qualities were observed in Experiment 2 (February 2008). Such differences could be explained by the difference in rainfall amounts between both experiment periods. Indeed, the rainfall amount in February 2008 (192.28 mm) was about twice that of February 2007 (107.19 mm). It has been proven for several plant species that seed reserves are easily mobilized and seedlings grow more quickly when soil is wet (Bouaziz and Hicks, 1990; Evans and Etherington 1990; Bochet et al., 2007). Although the bottle gourd is well adapted to water deficit, appropriately warm and wet soils are necessary during its germination and early stages of growth (Zoro Bi et al., 2003; Olson et al., 2009). It thus appeared that regardless of cultivar, wet soil is necessary for an on farm reliable evaluation of seed fermentation method in the bottle gourd *L. siceraria*.

The interaction effect of cultivar and seed fermentation method was significant for three vigor parameters (GSI, ESI, and SDB). However, the trends in seed germinability with respect to cultivars and fermentation methods were not quite contrasted, the best values of the parameters analyzed being obtained when seeds were fermented after cutting and packing the fruits into plastic bag, and then hidden under ground at 30 cm depth (SFD). A similar trend could be noted from the examination of the triple interaction effects on seed germination and vigor. Indeed, except for the seedling shoot length (SSL), the highest values of parameters analyzed were obtained in Experiment 2, regardless of the cultivars and treatments. The fermentation method thus appeared as the main factor to assess in order to produce best quality seed in the oleaginous bottle gourd. However, since high rainfall amount enhanced seed germination and vigor, wet soil is also necessary for an on farm reliable evaluation of seed or for crop establishment in the bottle gourd *L. siceraria*.

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