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Effect of Fruit Harvest Time and Fermentation Methods on Seed Viability and Seedling Vigour in Oleaginous *Citrullus lanatus*

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Authors' contributions

This work was carried out in collaboration among all authors. Author KBY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MMB and KA managed the analyses of the study. Authors NDK and THK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In an attempt to improve the yields of *Citrullus lanatus*'s *wlêwlê* oilseed cultivar, generally low due to the lack of good germination seeds, the effect of fruit age at harvest and fermentation methods were studied. The present study is a contribution to the improvement the African oleaginous Cucurbits seed production system by optimizing the date of harvest combined to seed fermentation methods through enhancement of seed germination and seedling vigor.
Study Design: Seedling vigor was evaluated on farm trial using five completely randomized blocks represented by seedbeds constituting each, one replication.
Place and Duration of Study: The trials were carried out from July to December 2020, at the

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experimental farm of the Nangui Abrogoua University in the district of Abidjan, southern of Côte d'Ivoire between 5°17' and 5°31' North latitude and between 3°45' and 4°31' West longitude.
Methodology: Fruits from grown plants were harvested at five different times 20, 25, 30, 35 and 40 days after anthesis (DAA)] and seeds fermented following four methods fermentation at ambient air (SFA); fermentation in a plastic bag exposed at ambient air (SFB); fermentation in plastic bag buried in 30 cm depth (SFD) and control or non-fermented (NF)]. Seeds from these 20 treatments (5 harvest time × 4 fermentation methods) were sown for viability tests in laboratory and seedling vigor in the field.
Results: Analysis of variance (ANOVA) showed that delaying harvest time (from 20 to 40 DAA) significantly ($P < .001$) improved fruit weight (from 512.20 to 760.50 g) and its seed content (from 71.75 to 230.70 seeds/fruit), seed size (length: 10.69 to 11.42 mm and width: 5.31 to 5.90 mm) and weight (5.16 to 6.37 g) as well as their viability (from 67.75 to 89.50 %). Moreover, in spite of facilitating seed extraction, all three fermentation methods (SFA, SFB and SFD) significantly improved seed viability and seedling vigor compared to control (NF) seeds. The highest agronomic performance (seed viability and produced seedling vigor), was obtained with fermentation under closed and deep confinement (SFD), followed by the closed confinement (SFB) and then by the open environment (SFA). Positive interaction (harvest time × fermentation method) revealed that the best performance was achieved with later harvested seeds (40 DAA) and closed fermented seeds (SFD) which could provide a better seed quality.
Conclusion: Agronomic performances (viability and vigor) are influenced by both harvest date and fermentation method. Late harvesting of the fruit (40 DAA) and fermentation in a very confined deep soil condition (SFD) ensures the production of high germination quality seeds.

Keywords: Citrullus lanatus; harvest time; fermentation methods viability and vigor.

1. INTRODUCTION

Citrullus lanatus is an important cucurbit crop accounting up for 7% of the worldwide area devoted to vegetable production. This species comprises two types: watermelon type widely studied in America and Europe [1] and oleaginous type called Africa melon or “egusi” frequently cultivated and used in Africa [2]. Watermelon is cultivated for its commercial fruit consumed fresh [3] while Africa melon is produced for its economical edible seeds [4,5].

In sub-Saharan Africa oleaginous types of *C. lanatus* are prized for their seeds reported to be rich in nutrients (60% lipids and 30% proteins) [6,7]. These oleaginous seeds are consumed as thickeners of a traditional soup called “pistachio soup” in Côte d’Ivoire. Furthermore, they represent an important source of income for farmers, mainly for women, in West and Central Africa [5].

Despite the economic, social and nutritional role played by this oleaginous cucurbit, it is classified as a secondary crop. Besides, very little is known about seed production system specific to this plant cultivation in view of optimizing its production. In Côte d'Ivoire, oleaginous *C. lanatus* crop is mostly grown by women, on small areas under the condition of traditional agro-systems using minimal inputs. In general rural farmers harvest fruits of oleaginous cucurbits after complete senescence of the plants. Because of the fruiting extent, all the fruits do not reach maturity before plant senescence, resulting in a mixing of mature and immature seeds at harvest [8]. This can explain the poor seed yield and low germination rate widely reported which confirms the neglect and underestimation of this group of plants. However, cultivation of oleaginous *C. lanatus* constitutes a sure income-generating activity as well as covering the immediate food needs of the family [5].

Because of the high potential of these crops, several institutions and scientific organizations, including the FAO and Bioversity International, now recommend that efforts to increase the productivity and quality of the agricultural system in developing countries be focused on them [9]. The production techniques improvement of any crop requires a good control of the factors related to seed germination [5]. Indeed, seed germination quality strongly influences the future plants yield [10]. Several factors have been recognized as influencing this germination quality. Indeed, seed agronomic quality, i.e. their aptitude to germinate and produce vigorous seedlings [11,12,13] depends mainly on its physiological maturity [14,15,16]. The production of high-quality seed in cucurbits depends on the fruit age at harvest and the extraction procedure [8] as well as fruit pre-storage duration [17]. For the cultivated cucurbit species, fermentation is the main extraction procedure ensuring the separation of seeds from the fruit flesh in which they are strongly encrusted. This process is reported to considerably improve seed nutritive value and germinability [18,8]. However, it is widely demonstrated that fermentation efficiency depends on its occurring conditions [7,15,19,20].

To this end, in previous studies, we showed the existence of a positive correlation between the fruit age and weight at harvest and the effect of fermentation methods on seed viability and seedling vigor of both cultivars (blocky fruited and round fruited) of *Lagenaria siceraria*, another species of oleaginous Cucurbits [17,21] in previous studies. However, little is known about Africa melon type of *Citrullus lanatus* seed production system.

The study globally aims at improving the African oleaginous Cucurbits seed production system, in order to propose to farmers a seed selection strategy that guarantees the crop's successful establishment. Specifically, it will try to determine the optimal fruit harvest time that could guarantee a better seeds germination and seedling vigor quality, and then to determine the best fermentation method that improves these agronomic of the oilseed cultivar (wlêwlê) of *Citrullus lanatus*.

2. MATERIALS AND METHODS

2.1 Plant Material

It composed of mean sized seeds of *Citrullus lanatus*'s *wlêwlê* cultivar (Fig. 1). These seeds were from a local market of Dimbokro (Côte d'Ivoire).

2.2 Methods

2.2.1 Production of various aged fruits

The *wlêwlê* cultivar of *Citrullus lanatus* was grown during the small rainfall season (from Agost to October, 2020) in an isolated field at the experimental farm of Nangui Abrogoua (Abidjan, Côte d'Ivoire). To obtain vigorous seedlings, then good yield, field was pre-fertilized with pig manure and regularly maintained by three weedings (Fig. 2) and one insecticidal treatment (Cypercal 50 EC). Approximately 150 pistillate flowers were tagged at anthesis (Fig. 3), and fruits which developed from them were manually harvested by simple rupture of stalk at 20, 25, 30, 35 and 40 days after anthesis (DAA).

Fig. 1. Mean sized seeds of *Citrullus lanatus*'s *wlêwlê* cultivar

Fig. 2. *Citrullus lanatus* creeping plants at bloom beginning on a well weeding plot

Fig. 3. Female flower stalk of *Citrullus lanatus* tagged with a paraffin label on its opening day

2.2.2 Fruits selection, weighing, cutting and seed fermentation methods

After harvest, fruits were randomly selected and individually weighed using the field balance. This allowed their mean weight and standard deviation calculation following harvest times.

Therefore, five samples of 20 fruits each one, corresponding to the five harvest date (20, 25, 30, 35 and 40 DAA), were constituted. For each sample, fruits were split using machete and regrouped into four sub-samples corresponding to four fermentation methods (Fig. 4): 1) Control (unfermented seeds); 2) seeds were fermented by exposing the cut fruits at ambient air of the field (SFA); 3) seeds were fermented by packing the cut fruits in a transparent plastic bag that was exposed in ambient air of the field (SFB); and 4) seeds were fermented by packing the cut fruits in a transparent plastic bag that was hidden 30 cm depth in the soil (SFD). These three fermentation processes are those generally used by rural farmers from Sub-Sahara African and Asia countries [22, 23,19,17]. Fermentation started ten days after harvest for all the selected fruits. During the test, the daily mean temperatures 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) was monitored. Seeds from the control samples were directly extracted manually using aluminum spoons, washed with tap water, and sundried in ambient air until attaining 7 to 6% moisture. After a 10-days fermentation period [17], fruits aspect was noted (Fig. 5). Then, seeds from the three other treatments were manually extracted, abundantly washed with tap water and then sun-dried at ambient air (22-32°C) until constant weight.

Finally 20 different treatments of seeds were performed: 5 fruit ages (20, 25, 30, 35 and 40 DAA) × 4 fermentation methods (Control, SFA, SFB and SFD). The seeds were sorted and sealed in plastic boxes waiting sowing.

2.2.3 Seed viability and seedling vigor tests and data collection

Seed viability was evaluated using the laboratory seed germination test. Seeds were considered as germinated when the emerging radicle reached at least 2 mm in length. The sown seeds were surveyed daily for 14 days [24]. Seed viability was evaluated using fruit weight (FrW), seeds number per fruit (SNF), seed size (length, width and thickness), weight of 100 seeds (W100), germination percentage (GeP), germination speed index (GSI) and germination mean time (GeMT).

Seedling vigor vas evaluated on farm trial using five completely randomized blocks represented by seedbeds constituting each, one replication measuring 6.5 m × 1.2 m. Seedbeds were spaced 50 cm each other. Each seedbed contained the 20 treatments: 5 ages (20, 25, 30, 35 and 40 DAP) × 4 fermentation methods (NF, SFA, SFB and SFD)]. Treatments consisted of 20 sowings spaced 3 cm. Seeds were sown on well-ploughed seedbeds to a depth of 3 cm, with single seed in each hole and the holes spaced 7 cm apart. A total of 2000 seeds were sown. Seedlings were considered emerged when their two cotyledonary leaves were completely opened [25]. Seedling vigor was examined using the following parameters: seedling emergence percentage (EmP), emergence speed index (ESI), shoot length (SSL, measured with a ruler after digging up the emerged seedling), and seedling dry biomass (SDB, measured after drying the seedling to constant weight). The GSI and ESI were calculated on the basis of the procedure used by Maguire [26] 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 seedling on the first count, the second count, and the last count; N1, N2, and Nn are the numbers of days elapsed of the first, second, and last count.

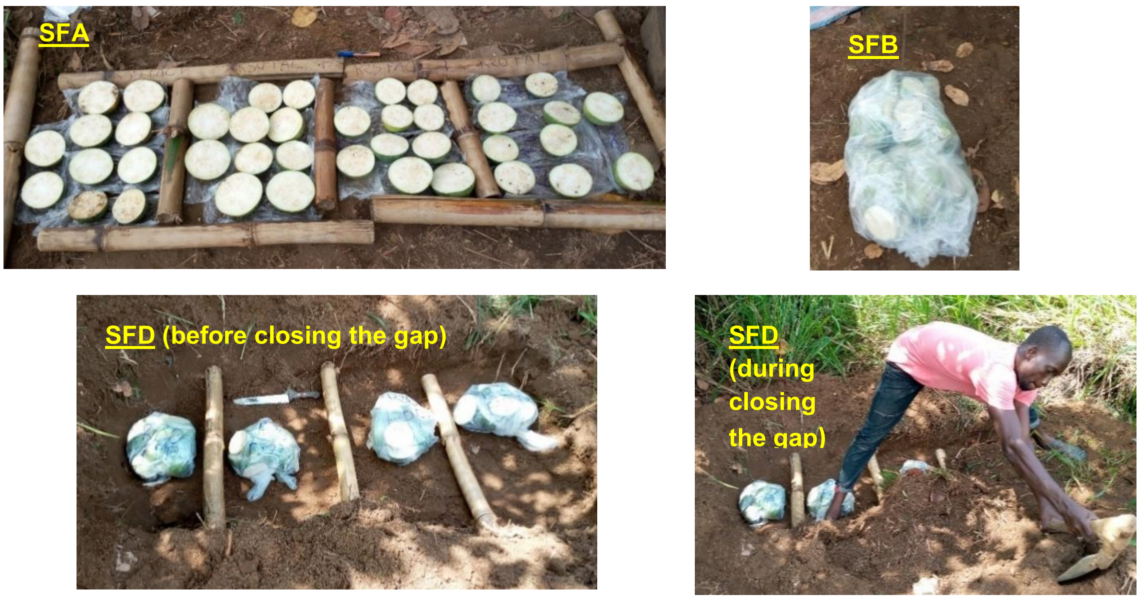

Fig. 4. Different methods of *Citrullus lanatus* seeds fermentation, SFA: fermentation in ambiant air, SFB: fermentation in transparent bag let in ambient air, and SFD: seed fermentation in transparent bag buried 30 cm deeply in the soil

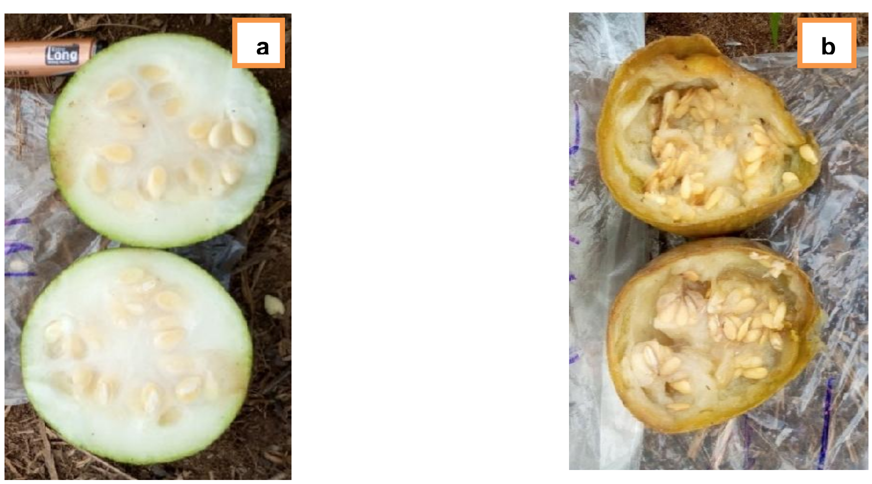

Fig. 5. Fruit pulp aspect before fermentation (a: hard) and after fermentation (b: soft) in *Citrullus lanatus*

2.2.4 Statistical analysis

All data collected in this study were statistically analyzed using SAS statistical software [27]. Percentage data were arcsin-transformed before analysis [28] but untransformed data were used to calculate means to present the results. Analysis of variance with two classification criteria (ANOVA 2) was performed to test individual then combine effect of the studied factors (harvest time and fermentation methods). When the null hypothesis was rejected for each parameter, multiple comparisons using the Least Significant Difference (LSD) were carried out test to separate the means [29]. All the tests (ANOVA and LSD) were performed at $\alpha = 0.05$ significance level.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of harvest time on fruit size, seed viability and seedling vigor

Values of seed viability parameters following fruit harvest time are reported in Table 1.

Effect on fruit and seed viability: ANOVA results of these values revealed that delaying harvest (from 20 to 40 DAA) increased very significantly ($P < .001$) fruit weight (from 512.20 to 760.50 g), and their full seeds content (from 71.75 to 230.70 seeds/ fruit), seed size (length: from 10.69 to 11.42 mm, width: from 5.31 to 5.90 mm) and weight of 100 dried seeds (from 5.16 to 6.37 g). However, seed thickness decreased with delaying (1.54 to 1.36 mm). This delaying harvest also improved seed viability through the increase of germination percentage (from 67.75 to 89.85 %), germination race (from 3.17 to 5.11 seeds/ day) and the shortening of germination mean time (from 5.06 to 4.79 days).

Effect on seedling vigor: Delaying harvest time (from 20 to 40 DPA) increased seedling emergence percentage (64.50 to 89.00 %), emergence speed index (1.66 to 2.66 seeds / day), shoot length (3.24 to 5.21 cm), leaf size (length: 4.23 to 5.83 cm and wide: 2.42 to 3.46 cm), fresh (2.46 to 3.86 g) and dry (0.30 to 0.41 g) weight while decreasing their emergence mean time (8.33 to 7.04 days). Hence, delaying fruit harvest improved seedling vigor produced by the seeds they contained.

3.1.2 Effect of fermentation methods on media temperature, seed viability and seedling vigor

Values of seed viability and seedling vigor parameters following fruit fermentation methods are mentioned in Table 2.

Effect on temperature and seed viability: The ANOVA results showed that temperature of fermentation media significantly ($P < 0.001$) varied following the methods used (conditions) for its process. Indeed opened air fermentation (SFA) tended to maintain the same temperature (29.50°C) as the control (NF: 31.50°C) while close media in plastic bag decreased it in depth (SFD: 25.80°) and increased it in ambient air (SFB: 32.60 °C). Seed viability parameters also varied significantly ($P < 0.001$) according to fermentation methods. Compared to the control (no fermentation) that provided biggest (length: 11.43 cm, wide: 6.09 cm and thickness: 1.75 mm) and heaviest (W100: 6.26 g) seeds, fermentation reduced seed size and weight. Indeed, opened media fermentation (SFA) allowed mean size (length: 11.08 cm, wide: 5.63 cm and thickness: 1.45 mm) and weight (W100: 5.84 g) seeds whereas closed anaerobic media (SFD and SFB) fermentation provided the same smallest (length: 10.94 and 10.93 mm; wide: 5.42 and 5.43 mm, thickness: 1.38 and 1.24 respectively) and lightest (W100: 5.27 and 5.62 g) seeds.

Effect on seedling vigor: Seedling vigor parameters significantly ($P < 0.001$) varied according to the seed fermentation methods and was inversely related to the size and weight of the fermented seeds. The best percentages and speed index with the shortest time of emergence producing the highest and heaviest seedlings carrying the largest leaves were obtained with seeds from the SFB and SFD treatments followed by the SFA treatment, while the lowest values of these parameters were recorded in the control (NF) seeds. Hence fermentation improved seedling vigor in oleaginous *Citrullus lanatus*, the best performance being exhibited by closed anaerobic media (SFD and SFB).

3.1.3 Combined effect of harvest time and fermentation methods on seed viability

The ANOVA results revealed that, among all the *Citrullus lanatus*'seed parameters examined, the combined effect (fruit harvest time × fermentation method) significantly ($P < .001$)

influenced only those of viability through percentage, time and germination speed (Table 3).

Globally, delaying harvest (from 20 to 40 DAA) for unfermented control seeds resulted in an increase of dry seed weight (from 5.41 to 6.12 g), percentage (from 52.00 to 73.00%) and germination speed (from 1.43 to 2.85 seeds/day) while it reduced the mean germination time (from 7.53 to 5.43 days). Seed size (length and width) was not affected (P > 0.05) by harvest date. Therefore, delaying the harvest does not affect seeds size but improves their viability.

Moreover for all the harvest times (20, 25, 30, 35 and 40 DAA), regardless of the method used (SFA, SFB and SFD), seed fermentation significantly improved seed viability compared to the control (NF). For example, in fruit harvested at 20 JAF, compared to control (unfermented) seed which germinated in 7.51 days after sowing, fermentation resulted in a reduction of mean germination time to 6.44 (for SFA), to 3.93 days (for SFB) and 3.53 days (SFD). The corresponding germination percentages were also improved from 52.00% (for the control NF) to 61.00, 75.00 and 83% for SFA, SFB and SFD respectively while seed weight decreased from 5.58 g (for NF) to 5.41, 4.91 and 4.61 g. The same tendencies were noted for fruits harvested at 25, 30, 35 and 45 DAA. Consequently, optimal viability is expressed by seeds fermented in very anaerobic plastic medium in depth (SFD), followed by the anaerobic bag let in air (SFB), then fermentation in open air (SFA). Furthermore, the comparison of optimal viability parameters obtained with the SFD treatment showed an increase in the germination percentage (from 83.00 to 99%) and speed (from 4.96 to 6.45 seeds germinated/day), a reduction in 100 dry seeds weight (from 4.61 to 6.82 g) and a shortening of germination mean time (from 3.53 to 3.09 days) with the delay of the harvesting date (from 20 to 40 DAP).

Finally, *C. lanatus* seed viability was strongly improved with both late harvest of heavy fruit (40 JAF) and fermentation in a very deep confined space (SFD).

3.1.4 Combined effect of harvest time and fermentation methods on seedling vigor

The analysis of Table 4 revealed that, among all the seedling vigor parameters examined, the combined effect (fruit harvest time × fermentation method) significantly (P < .001) influenced only emergence percentage and mean time, seedling shoot length and leaf length.

Globally, delaying harvest (from 20 to 40 DAA) for unfermented control seeds resulted in an increase seedling emergence percentage (from 46.00 to 73%), seedling shoot length (from 2.97 to 4.87 cm) while it reduced their mean emergence time (from 10.86 to 8.61 days). However seedling emergence speed, fresh and dried weight, leafs size (length and wide) were not significantly affected (P> 0.05) by harvest date. Therefore, delaying the harvest does not affect seeds size but improves their viability.

Moreover for all the harvest times (20, 25, 30, 35 and 40 JAF), regardless of the methods used (SFA, SFB and SFD), seed fermentation significantly improved seed viability compared to the control (NF). For example, in fruit harvested at 20 JAF, compared to control (unfermented) seed which germinated in 7.51 days after sowing, fermentation resulted in a reduction of mean germination time to 6.44 (for SFA), to 3.93 days (for SFB) and 3.53 days (SFD). The corresponding germination percentages were also improved from 52.00% (for the control NF) to 61.00, 75.00 and 83% for SFA, SFB and SFD respectively while seed weight decreased from 5.58 g (for NF) to 5.41, 4.91 and 4.61 g. The same tendencies were noted for fruits harvested at 25, 30, 35 and 45 DAA. Hence, optimal seedling vigor was expressed by seeds fermented in very anaerobic plastic medium in depth (SFD), followed by the anaerobic bag let in air (SFB), then fermentation in open air (SFA). Furthermore, the comparison of seedling optimal vigor parameters obtained with the SFD treatment showed an increase in the seedling shoot length (from 3.46 to 5.44 cm) and leaf length (from 4.50 to 6.21 cm), and the shortening of emergence mean time (from 7.17 to 5.89 days) with the delay of the harvesting date (from 20 to 40 DAP).

Finally, seedling vigor in *C. lanatus* was partially improved with both late harvest of heavy fruit (40 DAA) and fermentation in a very deep confined space (SFD).

Table 1. Effect of harvest time on fruits, seed viability seedling vigor parameters in *Citrullus lanatus*

Parameters[1]		Fruit harvest time					Statistics[2]	
		20 DAA	**25 DAA**	**30 DAA**	**35 DAA**	**40 DAA**	***F***	***P***
Fruits	Weight (g)	512.20 ± 26.75[c]	571.55 ± 37.00[b]	627.80 ± 35.40[b]	653.75 ± 29.34[b]	760.50 ± 44.68[a]	7.015	< .001
	Seed nb/ fruit	71.75 ± 8.17[e]	125.90 ± 9.56[d]	184.40 ± 16.14[c]	197.90 ± 22.23[b]	230.70 ± 22.37[a]	14.107	< .001
Seeds	Lengh (mm)	10.69 ± 0.11[b]	10.85 ± 0.10[b]	11.24 ± 0.12[a]	11.27 ± 0.10[a]	11.42 ± 0.12[a]	7.480	< .001
	Width (mm)	5.31 ± 0.11[c]	5.59 ± 0.06[b]	5.67 ± 0.13[ab]	5.74 ± 0.11[ab]	5.90 ± 0.10[a]	5.310	< .001
	Thickness (mm)	1.54 ± 0.03[a]	1.55 ± 0.05[a]	1.44 ± 0.06[ab]	1.40 ± 0.05[b]	1.36 ± 0.07[b]	4.302	< 0.003
	W100 (g)	5.16 ± 0.14[c]	5.61 ± 0.28[bc]	5.69 ± 0.19[b]	5.79 ± 0.25[b]	6.37 ± 0.15[a]	5.878	< .001
	GnP (%)	67.75 ± 3.65[b]	74.00 ± 3.81[b]	82.50 ± 3.74[a]	86.00 ± 2.98[a]	89.50 ± 3.20[a]	12.066	< .001
	GMT (d)	5.06 ± 0.14[a]	4.67 ± 0.11[bc]	4.52 ± 0.10[c]	4.79 ± 0.12[b]	3.78 ± 0.06[d]	45.950	< .001
	GSI (Sd/d)	3.17 ± 0.36[d]	3.66 ± 0.38[c]	4.17 ± 0.40[b]	4.28 ± 0.36[b]	5.11 ± 0.35[a]	17.240	< .001
Seedlings	EmP (%)	64.50 ± 3.49[c]	71.50 ± 3.66[c]	78.75 ± 3.81[b]	84.00 ± 3.13[ab]	89.00 ± 3.13[a]	15.273	< .001
	EMT (d)	8.33 ± 0.12[a]	8.38 ± 0.12[a]	8.16 ± 0.11a[b]	8.06 ± 0.13[b]	7.04 ± 0.09[c]	47.760	< .001
	ESI (Sd/d)	1.66 ± 0.12[d]	1.80 ± 0.15[cd]	2.04 ± 0.15b[c]	2.25 ± 0.17[b]	2.66 ± 0.16[a]	19.316	< .001
	SSL (cm)	3.24 ± 0.07[d]	4.02 ± 0.08[c]	3.99 ± 0.08[c]	4.34 ± 0.09[b]	5.21 ± 0.06[a]	83.240	< .001
	LeL (cm)	4.23 ± 0.06[d]	4.62 ± 0.07[c]	4.78 ± 0.06[c]	5.31 ± 0.07[b]	5.80 ± 0.07[a]	84.840	< .001
	LeW (cm)	2.42 ± 0.05[d]	2.51 ± 0.06[cd]	2.86 ± 0.05[c]	3.04 ± 0.06[b]	3.46 ± 0.06[a]	47.690	< .001
	FSW (g)	2.46 ± 0.09[c]	2.71 ± 0.16[c]	2.81 ± 0.17[c]	3.07 ± 0.20[b]	3.86 ± 0.27[a]	11.455	< .001
	DSW (g)	0.30 ± 0.36[c]	0.32 ± 0.38[c]	0.31 ± 0.40[c]	0.36 ± 0.44[b]	0.41 ± 0.52[a]	8.378	< .001

[1]nb: number, W100: weight of 100 dried seeds, GnP: germination percentage, GMT: germination mean time et GSI: germination speed index. DAA: days after anthesis, EmP: emergence percentage, EMT: emergence mean time, ESI: emergence speed index, SSL: seedling shoot length, LeL: leaf length, LeW: leaf wide, FSW: fresh seedling weight, DSW: dried seedling weight., Sd: seedlings and d: days

[2]In each row, values with the same superscript letter are not significantly different from each over (ANOVA, P > 0.05)

Table 2. Variation of temperature, seed viability and seedling vigor parameters following fermentation methods in *Citrullus lanatus*

Parameters[1]		Fermentation methods[2]				Statistics[3]	
		NF (Control)	SFA	SFB	SFD	F	P
Fermentation temperature (°C)		31.50 ± 2.00[ab]	29.50 ± 1.50[b]	32.60 ± 1.80[a]	25.80 ± 1.60[c]	41.653	< .001
Seeds	Length (mm)	11.43 ± 0.11[a]	11.08 ± 0.08[b]	10.93 ± 0.10[c]	10.94 ± 0.11[c]	5.410	< .001
	Wide (mm)	6.09 ± 0.09[a]	5.63 ± 0.05[b]	5.43 ± 0.09[c]	5.42 ± 0.10[c]	13.66	< .001
	Thickness (mm)	1.75 ± 0.04[a]	1.45 ± 0.04[b]	1.38 ± 0.03[b]	1.24 ± 0.03[c]	34.303	< .001
	W100 (g)	6.26 ± 0.17[a]	5.84 ± 0.20[b]	5.27 ± 0.15[c]	5.62 ± 0.24[b]	7.517	< .001
	GnP (%)	63.60 ± 2.47[c]	76.20 ± 3.46[b]	88.40 ± 2.67[a]	91.60 ± 1.90[a]	30.840	< .001
	GMT (days)	7.00 ± 0.10[a]	4.75 ± 0.09[b]	3.68 ± 0.06[c]	3.46 ± 0.05[d]	45.350	< .001
	GSI (Sd/d)	1.95 ± 0.11[c]	3.56 ± 0.26[b]	5.20 ± 0.22[a]	5.61 ± 0.16[a]	14.058	< .001
Seedlings	EmP (%)	61.00 ± 2.70[c]	73.80 ± 3.28[b]	85.80 ± 2.56[a]	89.60 ± 2.25[a]	33.482	< .001
	EMT (d)	10.38 ± 0.11[a]	8.01 ± 0.09[b]	7.15 ± 0.07[c]	7.01 ± 0.07[c]	23.960	< .001
	ESI (Sd/d)	1.24 ± 0.07[c]	1.92 ± 0.11[b]	2.50 ± 0.12[a]	2.66 ± 0.10[a]	63.619	< .001
	SSL (cm)	3.72 ± 0.09[c]	4.16 ± 0.09[b]	4.38 ± 0.08[a]	4.39 ± 0.08[a]	20.35	< .001
	LeL (cm)	4.56 ± 0.07[c]	4.88 ± 0.06[b]	5.03 ± 0.06[b]	5.32 ± 0.06[a]	28.460	< .001
	LeW (cm)	2.54 ± 0.05[c]	2.78 ± 0.05[b]	2.99 ± 0.05[b]	3.12 ± 0.06[a]	22.340	< .001
	FSW (g)	2.47 ± 0.16[b]	2.62 ± 0.09[b]	3.40 ± 0.22[a]	3.44 ± 0.18[a]	12.930	< .001
	DSW (g)	0.34 ± 0.02[c]	0.37 ± 0.01[bc]	0.40 ± 0.01[a]	0.41 ± 0.02[a]	3.903	0.011

[1]W100: weight of 100 dried seeds, GnP: germination percentage, GMT: germination mean time et GSI: germination speed index, EmP: emergence percentage, EMT: emergence mean time, ESI: emergence speed index, SSL: seedling shoot length, LeL: leaf length, LeW: leaf wide, FSW: fresh seedling weight, DSW: dried seedling weight, Sd: seedlings and d: days

[2]NF: non fermented seeds, SFA: seed fermentation in ambient air, SFB: seed fermentation in transparent bag let in ambient air, and SFD: seed fermentation in transparent bag buried 30 cm deeply in the soil

[3]In each row, values with the same superscript letter are not significantly different from each over (ANOVA, P > 0.05)

Table 3. Combined effect of fruit harvest time and fermentation method on seed viability parameters in *Citrullus lanatus*

Treatments		**Seed parameters**[3]					
Harvest time	**Fermentation methods**[2]	**Length (mm)**	**Wide (mm)**	**W100 (g)**	**GnP (%)**	**GMT (j)**	**GSI (Sd/d)**
20 DAA[1]	NF	11.11 ± 0.33^{a}	5.75 ± 0.18^{a}	5.58 ± 0.15^{de}	52.00 ± 3.74^{k}	7.51 ± 0.21^{ab}	1.43 ± 0.11^{h}
	SFA	10.75 ± 0.14^{a}	5.56 ± 0.04^{a}	5.41 ± 0.13^{cd}	61.00 ± 5.09^{ij}	6.44 ± 0.32^{c}	2.12 ± 0.33^{gh}
	SFB	10.47 ± 0.15^{a}	4.98 ± 0.08^{a}	4.91 ± 0.14^{gh}	75.00 ± 6.51^{ef}	3.93 ± 0.15^{fg}	4.16 ± 0.46^{de}
	SFD	10.46 ± 0.12^{a}	4.94 ± 0.29^{a}	4.61 ± 0.12^{h}	83.00 ± 5.14^{cd}	3.53 ± 0.10^{gh}	4.96 ± 0.30^{cd}
25 DAA	NF	11.30 ± 0.18^{a}	5.69 ± 0.20^{a}	6.68 ± 0.60^{ab}	54.00 ± 5.78^{jk}	7.81 ± 0.25^{a}	1.45 ± 0.14^{h}
	SFA	10.78 ± 0.20^{a}	5.57 ± 0.02^{a}	5.74 ± 0.66^{bc}	69.00 ± 4.30^{gh}	5.14 ± 0.15^{d}	2.86 ± 0.14^{fg}
	SFB	10.69 ± 0.16^{a}	5.56 ± 0.17^{a}	4.98 ± 0.10^{gh}	84.00 ± 4.30^{bc}	3.58 ± 0.10^{gh}	4.92 ± 0.18^{cd}
	SFD	10.65 ± 0.21^{a}	5.52 ± 0.09^{a}	5.06 ± 0.29^{gh}	89.00 ± 4.30^{abc}	3.44 ± 0.11^{hi}	5.44 ± 0.44^{bc}
30 DAA	NF	11.57 ± 0.31^{a}	6.34 ± 0.18^{a}	6.68 ± 0.37^{ab}	68.00 ± 3.00^{hi}	7.11 ± 0.18^{b}	1.99 ± 0.13^{gh}
	SFA	11.22 ± 0.17^{a}	5.50 ± 0.10^{a}	5.23 ± 0.06^{f}	79.00 ± 8.86^{de}	4.62 ± 0.12^{e}	3.61 ± 0.50^{ef}
	SFB	11.03 ± 0.26^{a}	5.34 ± 0.37^{a}	5.30 ± 0.12^{e}	91.00 ± 7.81^{ab}	3.52 ± 0.10^{gh}	5.41 ± 0.59^{bc}
	SFD	11.14 ± 0.23^{a}	5.49 ± 0.15^{a}	5.58 ± 0.14^{cd}	92.00 ± 4.63^{ab}	3.52 ± 0.15^{gh}	5.68 ± 0.45^{ab}
35 DAA	NF	11.56 ± 0.31^{a}	6.20 ± 0.14^{a}	6.41 ± 0.04^{ab}	71.00 ± 4.30^{fg}	7.49 ± 0.25^{ab}	2.04 ± 0.08^{gh}
	SFA	11.25 ± 0.16^{a}	5.67 ± 0.15^{a}	6.25 ± 0.41^{ab}	84.00 ± 6.59^{bc}	4.38 ± 0.17^{ef}	4.25 ± 0.49^{de}
	SFB	11.15 ± 0.12^{a}	5.60 ± 0.18^{a}	4.84 ± 0.32^{gh}	93.00 ± 2.54^{ab}	4.35 ± 0.23^{ef}	5.04 ± 0.49^{cd}
	SFD	11.11 ± 0.15^{a}	5.51 ± 0.31^{a}	5.68 ± 0.62^{cd}	96.00 ± 2.44^{a}	3.58 ± 0.15^{gh}	5.79 ± 0.29^{ab}
40 DAA	NF	11.61 ± 0.19^{a}	6.45 ± 0.14^{a}	6.12 ± 0.04^{ab}	73.00 ± 3.39^{fg}	5.43 ± 0.15^{d}	2.85 ± 0.16^{fg}
	SFA	11.41 ± 0.17^{a}	5.84 ± 0.20^{a}	6.31 ± 0.52^{ab}	88.00 ± 8.45^{abc}	3.77 ± 0.12^{gh}	4.96 ± 0.52^{cd}
	SFB	11.33 ± 0.23^{a}	5.66 ± 0.08^{a}	6.22 ± 0.03^{ab}	98.00 ± 1.00^{a}	3.27 ± 0.03^{ij}	6.18 ± 0.07^{ab}
	SFD	11.34 ± 0.37^{a}	5.6 ± 0.21^{a}	6.82 ± 0.29^{ab}	99.00 ± 1.22^{a}	3.09 ± 0.07^{j}	6.45 ± 0.13^{a}
Statistics[4]	*F*	0.130	0.930	14.962	17.253	11.282	21.011
	P	0.999	0.521	< .001	< .001	< .001	< .001

[1]*W100: weight of 100 dried seeds, GnP: germination percentage, GMT: germination mean time et GSI: germination speed index. DAA: days after anthesis, EmP: emergence percentage, EMT: emergence mean time, ESI: emergence speed index, SSL: seedling shoot length, LeL: leaf length, LeW: leaf wide, FSW: fresh seedling weight, DSW: dried seedling weight.*

[2]*NF: non fermented seeds (control), SFA: seed fermentation in ambient air, SFB: seed fermentation in transparent bag let in ambient air, and SFD: seed fermentation in transparent bag buried 30 cm deeply in the soil*

[3]*In each column, values with the same superscript letter are not significantly different from each over (ANOVA, P > 0.05)*

Table 4. Combined effect of fruit harvest time and fermentation method on seedling vigor parameters in *Citrullus lanatus*

Teatments		Parameters of seedling vigor[2]							
Harvest time	**Fermentation method**	**EmP (%)**	**EMT (d)**	**ESI (Sd/d)**	**SSL (cm)**	**LeL: (cm)**	**LeW (cm)**	**FSW (g)**	**DSW (g)**
20 DAA[1]	NF	46.00 ± 2.91^{i}	10.86 ± 0.24^{a}	0.97 ± 0.11^{a}	2.97 ± 0.19^{f}	3.68 ± 0.12^{i}	2.06 ± 0.11^{a}	2.05 ± 0.13^{a}	0.29 ± 0.03^{a}
	SFA	60.00 ± 4.74^{g}	8.40 ± 0.21^{d}	1.47 ± 0.15^{a}	3.11 ± 0.13^{f}	4.33 ± 0.10^{g}	2.47 ± 0.09^{a}	2.62 ± 0.08^{a}	0.37 ± 0.01^{a}
	SFB	72.00 ± 3.00^{e}	7.98 ± 0.22^{e}	1.89 ± 0.14^{a}	3.44 ± 0.12^{e}	4.40 ± 0.14^{g}	2.58 ± 0.11^{a}	2.56 ± 0.27^{a}	0.33 ± 0.03^{a}
	SFD	80.00 ± 5.24^{c}	7.13 ± 0.15^{h}	2.31 ± 0.13^{a}	3.46 ± 0.13^{e}	4.50 ± 0.10^{f}	2.58 ± 0.12^{a}	2.61 ± 0.14^{a}	0.33 ± 0.01^{a}
25 DAA	NF	53.00 ± 4.63^{h}	11.13 ± 0.21^{a}	0.97 ± 0.10^{a}	3.81 ± 0.17^{d}	4.11 ± 0.13^{h}	2.36 ± 0.18^{a}	2.12 ± 0.19^{a}	0.33 ± 0.04^{a}
	SFA	67.00 ± 4.89^{f}	8.97 ± 0.22^{c}	1.55 ± 0.08^{a}	3.91 ± 0.14^{d}	4.55 ± 0.15^{e}	2.45 ± 0.11^{a}	2.26 ± 0.22^{a}	0.32 ± 0.02^{a}
	SFB	81.00 ± 4.30^{b}	7.17 ± 0.12^{h}	2.30 ± 0.09^{a}	4.18 ± 0.12^{c}	4.92 ± 0.12^{c}	2.54 ± 0.09^{a}	3.59 ± 0.10^{a}	0.40 ± 0.02^{a}
	SFD	85.00 ± 5.70^{a}	7.37 ± 0.16^{g}	2.38 ± 0.28^{a}	4.18 ± 0.20^{c}	4.91 ± 0.13^{c}	2.68 ± 0.09^{a}	2.85 ± 0.30^{a}	0.36 ± 0.02^{a}
30 DAA	NF	64.00 ± 5.09^{g}	10.51 ± 0.19^{b}	1.24 ± 0.07^{a}	3.39 ± 0.16^{e}	4.32 ± 0.13^{g}	2.52 ± 0.09^{a}	2.18 ± 0.22^{a}	0.29 ± 0.02^{a}
	SFA	74.00 ± 7.64^{d}	8.40 ± 0.21^{d}	1.83 ± 0.21^{a}	4.12 ± 0.20^{c}	4.68 ± 0.12 ^{e}	2.65 ± 0.09^{a}	2.55 ± 0.27^{a}	0.34 ± 0.04^{a}
	SFB	87.00 ± 7.17^{a}	7.14 ± 0.13^{h}	2.49 ± 0.19^{a}	4.22 ± 0.14^{c}	5.06 ± 0.11^{c}	3.13 ± 0.13^{a}	3.34 ± 0.39^{a}	0.40 ± 0.04^{a}
	SFD	90.00 ± 5.70^{a}	7.27 ± 0.19^{h}	2.60 ± 0.25^{a}	4.25 ± 0.13^{c}	5.06 ± 0.11^{c}	3.16 ± 0.10^{a}	3.19 ± 0.26^{a}	0.39 ± 0.02^{a}
35 DAA	NF	69.00 ± 5.09^{f}	11.21 ± 0.24^{a}	1.27 ± 0.09^{a}	3.55 ± 0.14^{e}	5.24 ± 0.15^{b}	2.72 ± 0.11^{a}	2.67 ± 0.31^{a}	0.37 ± 0.03^{a}
	SFA	81.00 ± 6.00^{b}	7.48 ± 0.19^{f}	2.28 ± 0.22^{a}	4.42 ± 0.18^{c}	5.28 ± 0.13^{b}	3.02 ± 0.13^{a}	2.71 ± 0.21^{a}	0.37 ± 0.02^{a}
	SFB	91.00 ± 4.30^{a}	7.84 ± 0.17^{f}	2.42 ± 0.23^{a}	4.64 ± 0.18^{b}	4.78 ± 0.11^{d}	3.08 ± 0.10^{a}	2.84 ± 0.40^{a}	0.36 ± 0.02^{a}
	SFD	95.00 ± 2.23^{a}	6.47 ± 0.15^{j}	3.05 ± 0.18^{a}	4.76 ± 0.14^{b}	5.95 ± 0.12^{a}	3.35 ± 0.13^{a}	4.08 ± 0.39^{a}	0.49 ± 0.05^{a}
40 DAA	NF	73.00± 3.39^{dc}	8.61 ± 0.23^{c}	1.77 ± 0.15^{a}	4.87 ± 0.15^{b}	5.45 ± 0.19^{b}	3.03 ± 0.10^{a}	3.31 ± 0.59^{a}	0.43 ± 0.07^{a}
	SFA	87.00 ± 8.15^{b}	7.18 ± 0.16^{h}	2.50 ± 0.27^{a}	5.25 ± 0.11^{a}	5.54 ± 0.14^{b}	3.30 ± 0.11^{a}	2.98 ± 0.21^{a}	0.43 ± 0.01^{a}
	SFB	97.00 ± 1.21^{a}	5.89 ± 0.09^{k}	2.97 ± 0.20^{a}	5.44 ± 0.10^{a}	6.01 ± 0.13^{a}	3.64 ± 0.13^{a}	4.89 ± 0.54^{a}	0.50 ± 0.04^{a}
	SFD	98.00 ± 1.22^{a}	6.88 ± 0.15^{i}	3.39 ± 0.11^{a}	5.29 ± 0.14^{a}	6.21 ± 0.13^{a}	3.84 ± 0.15^{a}	4.25 ± 0.40^{a}	0.50 ± 0.04^{a}
Statistics[3]	F	20.162	10.700	1.086	1.280	3.730	0.910	1.825	0.057
	P	< .001	< .001	0.383	< .001	< .001	0.533	0.935	0.517

[1]*DAA: days after anthesis, EmP: emergence percentage, EMT: emergence mean time, ESI: emergence speed index, SSL: seedling shoot length, LeL: leaf length, LeW: leaf wide, FSW: fresh seedling weight, DSW: dried seedling weight, Sd: seedlings and d: days*

[2]*NF: non fermented seeds, SFA: seed fermentation in ambient air, SFB: seed fermentation in transparent bag let in ambient air, and SFD: seed fermentation in transparent bag buried 30 cm deeply in the soil*

[3]*In each column, values with the same superscript letter are not significantly different from each over (ANOVA, P > 0.05)*

4. DISCUSSION

The agricultural productivity of any crop depends mainly on the quality of sown seeds [10]. This quality is related not only to fruit maturity at harvest but also to post-harvest treatments like fermentation [8]. The performance of *Citrullus lanatus* seeds, extracted from fruits harvested at different times and fermented following different methods was evaluated in this study through their aptitude to germinate and produce vigorous seedlings.

The results showed that both factors considered individually or simultaneously, significantly influenced the seed viability and seedling vigor in this oleaginous species.

Precocious seeds (20 DAA) showed, at least, viability. This relatively high viability (67.75%) indicates that physiological maturity of the first seeds began before 20 DAA. In addition, delaying harvest from 20 to 40 days after anthesis (DAA) significantly increased fruit weight and seed content. As a result, fruits late harvested at 40 DAA were the heaviest and contained more seeds than those harvested sooner at 35 DAA followed respectively by 30, 25 and 20 DAA. We also [17] reported an increase in fruit size and seed number with delaying harvest (from 30 to 50 DAA) in both oleaginous cultiavrs of *Lagenaria siceraria*. Delaying harvest time could have allowed the immature seeds, at harvest, to complete their physiological maturation [8]. Indeed, seeds from these late harvested fruits (40 JAF) certainly had more time to accumulate their nutrient reserves and reach a high physiological maturity. According to Amiba et al. [30], the heavier the fruit, the more storage of nutrients devoted to the successful growth of its seeds. Our results also showed that this fruit delaying harvest in *C. lantus* (from 20 to 40 DAA), significantly improved seed viability through the increase of germination percentage (from 69.75 to 89.50%). Nerson and Paris [8] reported similar seeds viability of 75% in watermelon (*Citrullus lanatus*) which was also improved to 100% with the delaying harvest time from 28 to 49 DAA. According to Valantin et al. [31], seeds from the heavier fruits are not only more numerous but also accumulate enough nutrients to better germinate. In addition, fruits harvested at 40 JAF provided more viable seeds than those from fruits harvested at 35 DAA followed respectively by 30, 25 and 20 DAA.

Although the *C. lanatus* seeds viability was improved with delaying harvest, our study also showed that it depended on post-harvest treatments, especially fermentation. Indeed, compared to the control (unfermented seed); viability of all fermented seeds was significantly improved. It means that, apart from facilitating extraction of seeds firmly encrusted in the fruit pulp, fermentation improves their germination and vigor [18,8,32]. This can partially explain why peasants always ferment cucurbits seeds before extracting them [20]. Several researchers reported that fermentation is a process which allows the physiological maturation of immature seeds, at harvest, to complete [8,33]. This completion increased the rate and percentage of germination [18]. Moreover, comparison of our fermentation methods to each other showed that seeds fermented in closed media (SFB and SFD methods) exhibit better germination and vigor than those fermented at ambient air (SFA). This difference of seed viability and seedling vigor in this *C. lanatus* cultivar tended to prove that although seeds fermentation improves their quality, its efficiency depends on its occurring conditions [8,15,19,20]. In addition, SFA method being the easiest to apply they are widely used by rural farmers [23,19]. We already showed that our close media fermentation methods (SFB and SFD) are more useful than the peasants one (SFA) in seeds quality improvement of *L. siceraria* [21]. The efficiency of SFB and SFD methods in seed germination and vigor improvement appeared to be related to fermentation in anaerobic, darkness, and relatively low temperature (26°C) conditions (that is, SFD). High performance of the SFD treatment seed quality improving might be due to single or combined effects of darkness and the relatively low temperature [34,15,35]. Several studies proved that, for each species, speed and result of the fermentation process largely depend on the temperature and its application duration [15]. 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 [36,19]. Darkness could have favored the metabolic process and/or the development of microorganisms involved in seed fermentation [37,38]. Finally anaerobic medium, water saturation, and reduced air conditions seemed to favor the proliferation and the activity of microorganisms involved in fermentation [39,40]. Proliferation of beneficial microorganisms on seeds during the fermentation process might promote seedling establishment or provide seed borne diseases control [41,42]. The beneficial effect of fermentation on these seeds viability could also be attributed to the lifting of a germination inhibitor (cucurbitacin) during this process [8,43].

Our study also revealed that the interaction (harvest time × fermentation methods) affected seeds viability and the vigor of the produced seedlings. Indeed *C. lanatus* seed agronomic performance (seed viability and produced seedling vigor), increased both with delaying harvest time (from 20 to 40 DAA) and fermentation under closed and deep confinement (SFD), followed by the closed confinement (SFB) and then by the open environment (SFA). The highest agronomic performance was achieved with later harvested seeds (40 DAA) and closed fermented seeds (SFD). This result could be explained by two complementary hypothesizes. First, seeds from the heavier fruits were not only more numerous but also accumulated enough nutrients to better germinate. Second, during these seeds fermentation, anaerobic medium, water saturation and reduced air conditions with SFD could have favored the proliferation and activity of microorganisms involved in the success of this process [39,40]. These beneficial microorganisms might promote seedling establishment or provide seed borne diseases control [41,42]. The lifting of a germination inhibitor (cucurbitacin) with this fermentation method could also explain this result [8,43].

Usually, seed viability and seedling vigor are correlated [12]. In this study, we noted that the most vigorous seedlings were produced by the most viable seeds. The fact that the first germinated seedlings are also the first to emerge can explain this positive correlation between seed viability and seedlings vigor. According to Hamman et al. [43], poor emergence is generally not due to a decrease of viability but rather to a decrease of seedlings growth: what prevents them from emergence on the ground surface. Moreover, we already observed a similar positive correlation seeds viability and seedling vigor in both cultivars (RFC and BFC) of *Lagenaria siceraria* [17].

5. CONCLUSION

Fruits harvested tardily (40 DAA) provided seeds with the highest viability and the most vigorous seedlings than those harvested precociously (20 DAA). Delaying harvest time allows the immature seeds, at harvest, to complete their physiological maturation by accumulating nutrients that increases their size and weight, cons fruit weight. Compared to control (NF), all the fermented seeds (SFA, SFB and SFD) showed good germination. It indicates that fermentation, a part from facilitating seed extraction, improves their viability. However, the higher agronomic performance (seed viability and seedling vigor), was obtained with fermentation under closed and deep confinement (SFD), followed by the closed confinement (SFB) and then by the open environment (SFA). Positive interaction (harvest time × fermentation method) revealed that the best performance was achieved with later harvested seeds (40 DAA) and closed fermented seeds (SFD).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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