ISSN: 2287-688X

Annals of
Plant Sciences

Volume 14, Issue 01 (2025) pp. 6656-6662

Research Article

Occurrence of Viviparous Germination in Berries affects the germination Capacity and Vigour of Seedlings in *Lagenaria siceraria* (Molina) Standley (Cucurbitaceae)

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Abstract

Viviparous germination has been recognized as one of the factors affecting seed viability in orthodox seed species. To better assess the level of dormancy and the influence of the susceptibility of accessions to viviparous germination on the germinative capacity of seeds in *Lagenaria siceraria*, four hundred (400) apparently healthy seeds from two viviparous and two non-viviparous accessions were sown on 5m x 1m beds. The study showed that seeds from viviparous accessions had a low germination index (0.35 ± 0.04; 0.40 ± 0.03) compared with seeds from non-viviparous accessions (0.68 ± 0.02 and 0.59 ± 0.02). Seeds from viviparous accessions also had a shorter germination time (4.82 ± 0.82; 4.90 ± 0.93 days) than those from non-viviparous accessions (8.52 ± 0.88 days; 8.33 ± 0.81 days). However, the best emergence percentages (86.00 ± 3.18% and 87.00 ± 3.53%) and the most vigourous seedlings (90.44 ± 6.04; 80.03 ± 6.50) were obtained with seeds from non-viviparous accessions. These results suggest that seeds from viviparous accessions are less dormant than those from non-viviparous accessions. Also, seed viviparity affects seedling vigour in *L. siceraria*.

Keywords: *Lagenaria siceraria, Cucurbitaceae, Viviparous Germination.*

Introduction

As the most widespread means of plant propagation and distribution, seeds play an important role (Rajjou and Debeaujon, 2008). Most cultivated plants produce seeds that are tolerant to desiccation and are known as orthodox seeds, as opposed to recalcitrant and viviparous seeds that lose their germination capacity if dried and stored (Tweddle, *et al*., 2003). Orthodox seeds can achieve low water content (typically less than 10%), and are also able to retain viability for a species-dependent period of time (Devic & Roscoe, 2016). This is the case for most species in the Cucurbitaceae family (Ellis, 1991; Nerson, 2002). Their seeds undergo a period of metabolic quiescence or dormancy.

Dormancy is a form of arrested seed development and an adaptive trait that helps many organisms survive (Foley, 2001). However, studies by, Kobayachi, *et al*., (2010), Ochi, *et al*., (2013) and N'Gaza, *et al*., (2019) have shown the occurrence of viviparous germination in *Citrullus lanatus*, *Cucumis melo* and *Lagenaria siceraria*, three representatives of the orthodox seeded Cucurbitaceae family.

This unusual germination due to the loss of seed dormancy is thought to be linked to the reduced germinative capacity of the seeds and the poor emergence of seedlings in species with orthodox seeds. Indeed, Barnard and Calitz, (2011) showed that wheat seedlings emergence from viviparous seeds was lower than that from non-viviparous seeds, decreasing from 40% to 10% as the degree of grain vivipary increased. Similarly, studies carried out by Cota-Sánchez, (2018) observed a mortality rate of 83.3% when seedlings from

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DOI: https://doi.org/10.5281/zenodo.14624424

viviparous tomato fruit were removed from the fruit and transplanted. It is therefore important to know the level of seed dormancy and the influence of viviparous germination on seedling vigour in the oilseed cucurbit *Lagenaria siceraria*.

Materials and Methods

Biological Material

The biological material consisted of healthy seeds from highly viviparous (NI153 and NI128) and non-viviparous (NI063 and NI189) accessions selected after two growing cycles for vivipary screening. These accessions were selected on the basis of two criteria. Firstly, they were selected for their contrasting values for the percentage of viviparous fruits and seeds, and secondly for the phenotypic characteristics of their seeds. Highly viviparous accessions are characterized by yellowish seeds with a soft tegument and no cap. The non-viviparous accessions chosen in this study are characterized by whitish seeds with a hard seed coat and no cap, and a soft seed with a dark cap (Figure 1).

Figure 1. Seeds of viviparous and non-viviparous accessions of *Lagenaria siceraria*

a: seeds with soft tegument and without cap of the viviparous accession NI128 ; b: seeds with soft tegument and without cap of the viviparous accession NI153 ; c: seeds with soft tegument and dark cap of the non-viviparous accession NI063 ; d : seeds with hard tegument of the non-viviparous accession NI189.

Methods

Sowing of Seeds

The seeds were sown in the field in a completely randomized block design. The seeds were sown on a 5 m x 1 m plot. The bed consisted of 20 elementary plots, each measuring 20 cm × 15 cm (Figure 2). The individual plots were arranged in two rows. Each elementary plot represented one accession. Each accession was repeated five times on the experimental plot. On each elementary plot, 20 seedling points spaced 5 cm apart were arranged in four rows and five columns. Pockets 3 cm deep were dug using a measuring stick. The seedlings were sown by hand and a single seed was planted in each pot. A total of 100 seeds of the four accessions were sown.

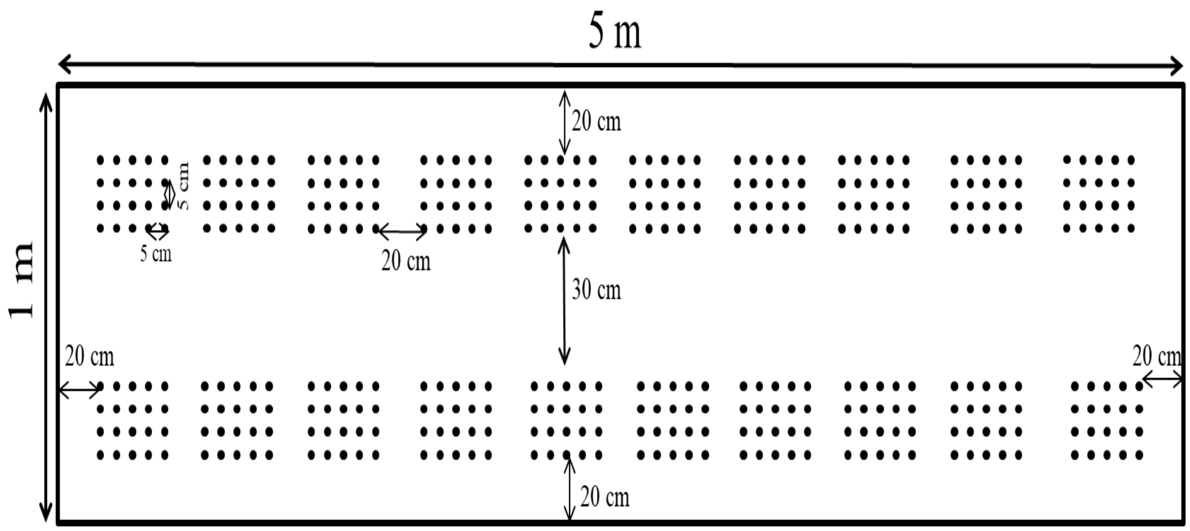

Figure 2: Experimental disign for studying the influence of viviparity of accessions on the germinative capacity of *Lagenaria siceraria* seeds

Parameters Measured
In this study, four parameters were used to assess seed viability. These were mean germination time (GeTi), germination percentage (GePe), germination index (GeIn) and germination speed (GeSp). Seedling vigour was assessed in this study through emergence time (EmTi), emergence percentage (EmPe), emergence speed (EmSp), seedling stem length (SsLe), fresh seedling weight (FsWe) and seedling biomass (SeBi).

- Germination percentage is defined as the ratio between the number of seeds that germinate and the total number of seeds sown, multiplied by 100. The emergence percentage is the ratio between the number of seedlings that emerge and the total number of seeds sown, multiplied by 100.

- Germination speed is the sum total of the ratios between the number (*n)* of seeds germinated on day (*i*) and the number of days (ji) that have elapsed since sowing. The speeds were determined using the following formulae (Al-Maskri, *et al*., 2004):

$$GeSp = \sum_{i-1}^{k} \frac{ni}{ji}$$

Where k = total number of experimental days

- Emergence speed is the sum total of the ratios between the number n of seedlings emerging on day i and the number of days ji that have elapsed since sowing. Speeds were determined using the following formulae (Al-Maskri, *et al*., 2004):

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Where k = total number of experimental days

- The germination index is a measure of seed dormancy. The indices were calculated using the following equation (Walker-Simmons, 1987):

$$GeIn = \frac{d \times n1 + (d-1) \times n2 + (d-2) \times n3 + \cdots + (1 \times nd)}{d \times N}$$

Where n1, n2, n3..., nd are the number of seeds that germinated on the 1st, 2nd and subsequent days until the last day. N is the total number of seeds sown and d is the total number of days taken for all the seeds to germinate. The maximum germination index representing non-dormant seeds is 1.0 and the minimum representing completely dormant seeds is 0.0.

Germination and Emergence Criteria
Seeds were considered germinated (Figure 3a) when the cotyledonary leaves appeared on the soil surface (Zoro Bi, *et al*., 2003). Seedlings were considered to have emerged when the cotyledonary leaves had opened out and the first true leaf was about 1 cm long (Figure 3b).

Figure 3: *Lagenaria siceraria* seedlings at the germination (a) and emergence (b) stages

Statistical Analysis of Data

The influence of viviparity on seed germination and seedling vigour was assessed using multivariate analysis of variance (MANOVA). The aim of this analysis was to reveal significant differences between accessions for all the parameters measured. When a significant difference was observed between accessions, a one-factor analysis of variance (ANOVA) was performed for each parameter. Where there was a significant difference between parameters, comparisons of means were made using the Gupta test. This test was used to classify the different accessions into two groups for each parameter. These were accessions with high values for germination, seedling vigour and yield, and accessions with low values for these parameters. The data were analyzed using SAS software.

Results and Discussion

Results

Influence of Vivipary on Seed Viability

The MANOVA showed a significant difference (F = 107.81; *P* < 0.001) between accessions. The results of the analysis of variance (ANOVA) reported in Table 1 showed that all the traits analysed were significantly (P < 0.001) influenced by seed viviparity except for germination percentage (PoGe). This test revealed that the shortest germination times were obtained with seeds from viviparous accessions NI53 (4.82 ± 0.82 days) and NI128 (4.90 ± 0.93 days) while the longest times were obtained with seeds from non-viviparous accessions NI189 (8.52 ± 0.88 days) and NI063 (8.33 ± 0.81 days). Seeds from viviparous accessions NI153 and NI128 showed the best germination index (0.68 ± 0.02 and 0.59 ± 0.02 respectively). The lowest germination indexes were observed in the non-viviparous accessions NI189 and NI063 with indices of 0.35 ± 0.04 and 0.40 ± 0.03 respectively. Similarly, the best emergence times, germination speeds and emergence speeds were also obtained with seeds from viviparous accessions NI53 and NI128. These results suggest that seeds from viviparous accessions germinate earlier than non-viviparous accessions.

Table 1: Mean values (± standard deviations) of viability parameters in *Lagenaria siceraria*, as a function of vivipary

Accessions		Parameters			
		GeTi (D)	GeSp (Se/D)	GePe (%)	GeIn
Non viviparous	NI189	8.52 ± 0.88[b]	2.26 ± 0.10[b]	89.00 ± 11.25	0.35 ± 0.04[b]
	NI063	8.33 ± 0.81[b]	2.20 ± 0.08[b]	89.88 ± 13.41	0.40 ± 0.03[b]
Viviparous	NI128	4.90 ± 0.93[a]	3.76 ± 0.22[a]	90.10 ± 12.05	0.59 ± 0.02[a]
	NI153	4.82 ± 0.82[a]	3,78 ± 0,32[a]	90,78 ± 13,33	0,68 ± 0,02[a]
Statistics	*F*	248.18	85.78	5.10	463.95
	P	< 0.001	< 0.001	0.111	< 0.001

For each variable, values marked with the letter 'a' are better and values marked with the letter 'b' are worse. TeGi: germination time; GeSp: germination speed; GePe: germination percentage, GeIn: germination index.

Influence of Viviparity on Seedling Growth

Regarding seedling vigour, the highest percentages of emergence were observed in the non-viviparous accessions NI189 (86.00 ± 3.18%) and NI063 (87.00 ± 3.53%). Similarly, non-viviparous accessions showed the best emergence rates compared with viviparous accessions. The longest (90.44 ± 6.04 mm) and most vigourous seedlings, with fresh and dry biomass of 17.45 ± 1.92 and 2.56 ± 0.16 respectively, were also observed in the non-viviparous accession NI189 (Tables 2 and 3). These results suggest that seeds from viviparous accessions have less vigourous seedlings, whereas seeds from non-viviparous accessions have good seedling vigour.

Tableau 2: Mean values (± standard deviations) of seedling vigour parameters in *Lagenaria siceraria*, as a function of vivipary

Accessions		Parameters			
		EmTi (D)	EmSp (Se/D)	EmPe (%)	SsLe (mm)
Non viviparous	NI189	12.79 ± 0.91[b]	1.64 ± 0.13[a]	86.00 ± 3.18[a]	90.44 ± 6.04[a]
	NI063	13.26 ± 0.85[b]	1.63 ± 0.13[a]	87.00 ± 3.53[a]	80.03 ± 6.50[a]
Viviparous	NI128	11.78 ± 0.82[a]	1.43 ± 0.02[b]	69.00 ± 3.37[b]	66.57 ± 4.91[b]
	NI153	11.86 ± 0.96[a]	1.31 ± 0.05[b]	65.00 ± 3.75[b]	57.76 ± 2.54[b]
Statistics	*F*	55.30	15.52	37.75	55.62
	P	< 0.001	< 0.001	0.010	< 0.001

For each variable, values marked 'a' are better and values marked 'b' are worse. EmTi: emergence time, EmSp: emergence speed; EmPe: Emergence percentage, SsLe: seedling stems length.

Tableau 3: Mean values (± standard deviations) of seedling fresh weight and biomass in *Lagenaria siceraria*, as a function of vivipary

Accessions		Parameters	
		FsWe (mg)	SeBi (mg)
Non viviparous	NI189	17.45 ± 1.92[a]	2.56 ± 0.16[a]
	NI063	15.12 ± 0.66[a]	2,48 ± 0,22[a]
Viviparous	NI128	11.56 ± 1.06[b]	1.25 ± 0.09[b]
	NI153	9.81 ± 0.57[b]	1.26 ± 0.07[b]
Statistics	*F*	726.02	780.89
	P	< 0.001	< 0.001

For each variable, the values marked with the letter 'a' are higher and the values marked with the letter 'b' are lower. FsWe: fresh seedling weight, SeBi: Seedling biomass

Discussion

The use of good quality seed is the first step towards good crop production. Seed quality is measured by germination capacity and seedling vigour (Bewley, 1997). The present study on the influence of susceptibility to viviparity on the agronomic quality of seeds showed that seeds from viviparous and non-viviparous accessions had the same germination percentages. These same results were reported by Barnard and Calitz, (2011) in wheat (*Triticum aestivum* L.). In fact, these researchers reported that the germination percentages of viviparous and non-viviparous seeds did not differ when the latter were sown without being stored. However, after storage, the germination percentage of viviparous seeds fell significantly compared with that of non-viviparous seeds.

Although they had the same germination percentages, seeds from viviparous accessions germinated faster than those from non-viviparous accessions. The better germination rates observed in viviparous accessions are thought to be due to the degree of dormancy of the embryos in their seeds. Indeed, the germination index, which is a measure of seed dormancy, is higher for viviparous accessions NI153 (0.68) and NI128 (0.59) and lower for non-viviparous accessions NI189 (0.35) and NI063 (0.40). According to Ali-Rachedi, *et al*., (2004), embryos with a high level of dormancy and therefore a low germination index are able to maintain high concentrations of ABA during the first hours of imbibition under optimal culture conditions, thus delaying their germination. The higher germination rates observed in viviparous accessions could also be due to the initiation of seed germination. According to Cota-Sánchez and Abreu (2007), around 40% of mature, non-viviparous seeds from viviparous fruit germinate in petri dishes 24 hours after extraction from the fruit, suggesting that the viviparity process has begun in these seeds. In orthodox seeds, the process of pre-germination or viviparity could be interrupted before the radicle emerges by desiccation, when the seeds removed from the fruit remain in unfavourable conditions. In this case, the germinative capacity of the seeds is maintained and when conditions become favourable again, germination is faster (Gualano & Benech-Arnold, 2009; Baek & Chung, 2014).

Despite the better germination rates and high germination rates observed in seeds from viviparous accessions, they had a low emergence rate. Of the 90% of seeds that germinated on average, only 59% and 51% reached the seedling stage in viviparous accessions NI128 and NI153 respectively. The viviparous accessions also had less vigourous seedlings than the non-viviparous accessions. This low seedling vigour also had an impact on plant growth and yield. These results are similar to those obtained by Barnard and Calitz, (2011) who showed that grain viviparity firstly reduces the number of plants per hectare, then the number of spikes per hectare and finally the weight of 1000 grains in wheat. The poor growth and low yields observed in viviparous seeds are thought to be the result of poor accumulation of reserves stored in the seeds due to the initiation of viviparity and the resumption of seed germination. Indeed, the efficiency of reserve mobilization during germination and seedling establishment in orthodox seed species depends on the extent of reserve accumulation during seed maturation. However, viviparity will break the dormancy of maturing seeds imposed by ABA and interrupt the process of maturation and accumulation of reserves in the seeds (Farnsworth, 2000). This low accumulation of reserves due to the absence or low level of seed dormancy will reduce the energy required for seedling growth. This explains the poor growth and reduced yield observed in viviparous berry seeds.

Conclusion

Seed quality is one of the essential factors in plant production. It is determined by the germination capacity and vigour of the seeds. The study carried out on the influence of viviparity on seed quality showed that seeds from viviparous accessions have a high germination rate and a high germination index compared with seeds from the berries of non-viviparous accessions. However, seeds from non-viviparous accessions have good vegetative growth.

Acknowledgments
We are grateful to the research group on minor crops at Nangui Abrogoua University.

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Source of support: Nil;
Conflict of interest: Nil.

Cite this article as:

N'Gaza, A.L.F., Doubi, B.T.S. and Zoro, B.I.A. "Occurrence of Viviparous Germination in Berries affects the germination Capacity and Vigour of Seedlings in *Lagenaria siceraria* (Molina) Standley (Cucurbitaceae)." *Annals of Plant Sciences*.14.01 (2025): pp. 6656-6662.