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Reproductive biology of two *Lagenaria* (Curcubitaceae) species

Babatunde Olanrewaju Okunlola, Sekinat Okikiola Azeez*, Julius Olaoye Faluyi

Obafemi Awolowo University, Department of Botany, Osun-State, Ile-Ife, Nigeria
*Corresponding author. E-mail: azeezs@oauife.edu.ng; sekinatokiki@gmail.com

Abstract

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The genus *Lagenaria* (Cucurbitaceae) members are underutilised, neglected and on the verge of being threatened to extinction. An investigation of their reproductive biology will serve as the basis for effective strategic planning towards their conservation. The plant materials used for this study were collected from Osun, Ekiti and Niger States in Nigeria. Reproductive parameters, including seed germination, phenology and pollination mechanism, were documented. The highest seed germination rate was observed in the accession of *Lagenaria siceraria,* Ekiti Bowl. The male flowers appeared first, followed by female flowers' emergence 14–28 days later, on the secondary shoots. The female flowers of each accession had unique inferior ovary shapes, which developed into different fruit shapes with distinct seed morphology. This study showed that the members of *Lagenaria* performed better as climbers than as trailers, and the number of fruits produced per accession depends on the size or weight of the fruit. *Chrysomelidae* and *Coryna apicicornis* beetles were the primary pollinators observed. Furthermore, the pollens were predominantly monad, spheroidal and tricolporate, and pollen stainability ranged between 81% and 91% in all the accessions studied. Therefore, the fruit and seed morphology were diagnostic for each accession, and the flowers were pollinated mainly by beetles.

Keywords: conservation, dioecy, germination, monoecy, pollen morphology, pollen stainability, pollination mechanisms.

INTRODUCTION

The genus *Lagenaria* Ser. (Cucurbitaceae) is an economically significant plant group known for its diverse fruit shape throughout the world and various horticultural, social, and cultural usages in Africa. It belongs to the family Curcurbitaceae, the second largest vegetable family after Solanaceae (Morimoto et al., 2005; Chinyere et al., 2009), and it is one of the neglected and underutilised cucurbits (Olvera-Vazquez et al., 2019). Modern musical instruments, plastic, ceramic and steel plates and cutleries have almost completely replaced the locally-used gourds (Morimoto et al., 2004; Yetişir et al., 2008). On the other hand, cucurbits serve as vegetables, and their immature or mature fruits are consumed for their health benefits (Koeyan et al., 2007; Dhiman et al., 2012). Therefore, the production and consumption of these underutilised cucurbits may promote poverty eradication, fight malnourishment among small-scale farmers and protect cucurbit genetic resources. In addition, new sources of income could be established through *ex situ* and *in situ* conservation and improved breeding programme of cucurbits (Olvera-Vazquez et al., 2019). The bottle gourds are multipurpose cucurbits whose usability are determined by their fruit shapes and can serve as selection markers in genetic improvement programme (Mkhize et al., 2021).

Lagenaria is composed of white-flowered gourd plant species, which are characterised by high genetic diversity (Bisognin, 2002). It consists of the annual monoecious species *Lagenaria siceraria* (Mol.) Standl (*Lagenaria vulgaris* Ser.; *Lagenaria leucantha* Dutch) and five perennial dioecious species, namely *Lagenaria breviflora* (Benth) Roberty, *Lagenaria abyssinica* (Hook) C. Jeffrey, *Lagenaria sphaerica* (Sond.) Naudin, *Lagenaria guineensis* (G. Don) C. Jeffrey and *Lagenaria rufa* (Gilg) C. Jeffrey (Zeven & Dewet, 1982). Two dioecious species (*Lagenaria breviflora* and *Lagenaria sphaerica*) and *Lagenaria siceraria* have been reported in Nigeria (Hutchinson & Dalziel, 1958; Chinyere et al., 2009). The monoecious species are cultivated worldwide owing to their economic value as food, medicine, ornaments, household utensils, and musical instruments (Mashilo & Shimelis, 2016; Mkhize et al., 2021). The seed oils in *Lagenaria siceraria* possess acceptable acid and peroxide values with high linoleic acid and low linolenic acid levels making it a potential source of quality edible oils (Emmanuel et al., 2013). Moreover, *Lagenaria siceraria* was reported to be resistant to soil-borne diseases and used as rootstock for watermelon in grafting (Lee, 1994). However, the dioecious species are localised in Africa, Madagascar, and Comoro Island (Khosa & Dhatt, 2015). *Lagenaria breviflora* fruits were reported to be used as abortifacients (Elujoba et al., 1985).

The members of the genus can either be climbers or trailers, characterised by stems with many branches, which can cover a large area depending on the environmental conditions (Mashilo & Shimelis, 2016). Leaves are ovate, orbicular, reniform, or heart-shaped, with shallow or deep lobes and toothed or smooth leaf margins (Yetişir et al., 2008). The leaf apex is blunt or pointed, and tendrils are borne on the axils (Singh, 2008). The monoecious bottle gourd has a high cross-pollinating ability (Tiwari & Ram, 2009). Dioecious and andromonoecious (a plant has both male and bisexual flowers) sex forms exist in bottle gourds' wild accessions and are controlled genetically (Singh et al., 1996). Both male and female flowers open late in the afternoon and occasionally in the morning; however, male flowers have a shorter life span (Sugiyama et al., 2014). The pollen grains of *Lagenaria siceraria* have been reported to be spiny, sticky and not wind-borne, requiring pollinators to move them from male to female flowers (Morimoto et al., 2004). A chromosome count of 2n = 22 has been documented in the genus with normal meiosis of 11 bivalents, metacentric chromosomes, and stable taxon cytology (Morimoto et al., 2005). Chromosome number was reported to have been used on several occasions to describe an organism's important essential genomic characters and lineage. Moreover, the number of chromosomes influences the extent of genetic recombination, a vital attribute of a sexual genetic system (White, 1984; Mayrose & Lysak, 2020). In addition, chromosome numbers of a group of plant species have been suggested to influence the success of inter-breeding among them (Olaoluwa & Azeez, 2022).

Reproductive biology is fundamental in the evolutionary process. It has been employed in investigating the mechanism through which a plant group has become endangered, thereby providing the basis for its protection (Li et al., 2019). Good knowledge of plant reproductive biology is essential in effective strategic planning for plant conservation, sustainable use and subsequent restoration and reintroduction programmes (Ayasse & Arroyo, 2011; Sreekala, 2017; Li et al., 2019).

Despite the sociocultural and economic values of the *Lagenaria*, the members of the genus are vastly, and less attention is being given to their biology in Nigeria, which might lead to the loss of some of the species. This study was carried out to provide baseline information for a future conservation plan for the cultivated and wild accessions of *Lagenaria.* The present study investigated the reproductive biology of some representatives of this genus, which can aid in developing a strategic conservation plan, and identifying some selection markers that may be employed in their genetic improvement.

MATERIALS AND METHODS

Germination, growth, flowering and fruiting

The plant materials used in this study were collected from various geographical locations in Osun, Ekiti and Niger States (Table 1) from 2018 to 2019. One hundred seeds from each accession were planted in small bowls filled with soil and placed in the screen house at the Department of Botany, Obafemi Awolowo University, Ile-Ife. The seeds were monitored daily for 30 days for seedling emergence, and the percentage of seedling emergence was calculated. The seedlings were later transplanted to the field in a Complete Randomized Block design at the Biological Garden, Obafemi Awolowo University, Ile-Ife, Nigeria. Then, the number of days from seedling emergence to first flowering and the number of days to complete the life cycle, which was signalled by the yellowing of leaves, were documented. Qualitative and quantitative parameters (fruit shape, fruit length, fruit width, fruit colour, number of fruits per plant, seed shape, seed colour, seed diameter, number of seeds per fruit and weight of 100 seeds when dry) of fruits and seeds of *Lagenaria* accessions studied were also recorded. One hundred seeds were measured using the side of a ruler calibrated in centimetres. The field study was conducted from 2019 to 2020. Mature dried fruits were harvested from the field and stored for 6–18 months before the seed germination study was repeated between 2020 and 2021.

Pollination mechanism

The time of flower anthesis and time of flower closure were recorded. The activities of putative pollinators visiting the experimental site were monitored, and insect specimens visiting the flowers during this period were captured with a scooping net. The captured insects were identified at the Department of Zoology, Obafemi Awolowo University, Ile-Ife.

Pollen stainability

Fresh pollens from the anthers of *Lagenaria* accessions studied were teased onto microscope slides and stained with cotton blue in lactophenol. One hundred pollen grains were scored at ×400 magnification, and the percentage of stainability was calculated. Intact, well-formed and uniformly stained pollen grains were considered viable. In contrast, those that did not assume intact shape, partially or not stained, were recorded as non-viable according to the method used by Azeez & Faluyi (2018).

Pollen acetolysis

Fresh pollen grains collected from each accession of *Lagenaria* under study were stored in 70% ethanol and later subjected to acetolysis using the technique by Erdtman (1960). Stored pollen grains were crushed using a glass rod, and the suspension was sieved into a clean tube using a fine mesh. The filtrate was centrifuged at 1000 rpm for 5 minutes, and the alcohol was decanted. Glacial acetic acid of 5 cm^3 was added, centrifuged again, and the supernatant was decanted. Acetolysis mixture of 6 cm^3 containing 1 : 9 of concentrated H_2SO_4 and acetic anhydride was added to the residue, boiled and stirred intermittently in a water bath at 100^{o}C. It was then centrifuged, and the acetolysis mixture was decanted. Next, glacial acetic acid of 10 cm^3 was added to the residue, stirred, centrifuged and washed thoroughly with distilled water. The acetolysed pollens were stored in 2 cm^3 of distilled glycerine, later mounted on the slide and covered with a coverslip for examination. One hundred pollen grains were observed, measured, and the pollen photomicrographs were taken at ×400 magnification for proper documentation.

Statistical analysis

The normality of data sets was evaluated by applying the Shapiro-Wilk test, and *MinMaxScaler* (Sklean Library) was used to normalise the data where appropriate. One-way analyses of variance (ANOVA) were employed to determine the significant differences among the means of the fruit, seed and pollen parameters, respectively; the means were separated by the ordinary

Table 1. Lists of *Lagenaria* accessions used in the study and coordinates of the collection sites

Species	Accession code	Voucher numbers	Accession names	Coordinates of collection sites
Lagenaria siceraria	ES	IFE17918	Ede Seere	7.7334° N, 4.4334° E
Lagenaria siceraria	IA	IFE17920	Ife Ado	7.4646° N, 4.5710° E
Lagenaria siceraria	IC	IFE17921	Ife Curved	7.4665° N, 4.7156° E
Lagenaria siceraria	EJ	IFE17919	Ekiti Jolo	7.7554° N, 5.7309° E
Lagenaria siceraria	EB	IFE17917	Ekiti Bowl	7.7554° N, 5.7309° E
Lagenaria siceraria	NA	IFE17916	Niger Ado	9.1175° N, 4.8374° E
Lagenaria breviflora	IB	IFE17922	Ife Breviflora	7.5195° N, 4.5258° E

least square (OLS) method at a 95% level of probability using on *Jupyter Notebook Python version 3.10*. The values measured were presented as Mean ± SE; the means within each row with different superscripts are significantly different at a 95% probability level.

RESULTS

Germination, growth, flowering and fruiting

The seed germination of the accessions studied began on the 6th day after planting in the *Lagenaria siceraria* EB accession. The highest seed germination rate was observed in the IA accession. However, germination started on the 7th day after planting. Then, seed germination continued steadily until a peak of 86% was reached on the 15th day after planting in IA accession. In *Lagenaria breviflora* and *Lagenaria siceraria* (NA, IC, EJ accessions), growth began between 7–10 days and increased gradually before attaining their respective peaks. Creeping of the seedlings started 28 to 42 days after planting and was characterised by the emergence of tendrils and stem elongation. As the growth progressed and the plants trailed on the soil, adventitious roots were produced at the nodes to provide more support.

Male flowers appeared 55–59 days after planting in the seven *Lagenaria* accessions. The blooming of the male flowers occurred earlier and was generally more prolonged than that of female flowers in all the *Lagenaria* accessions studied. Female flowers' appearance began 14–28 days after the emergence of the male flowers on the secondary stems. Both flowers emerged from the nodes. The shape of the ovary was unique to each accession based on its fruit form and could be used to distinguish one accession from the other (Fig. 1 and Fig. 2). The pistillate and staminate flowers had a white corolla and green calyx. The petals in the pistillate flowers were more extensive than the staminate flowers. The sex ratio was dominated by males, with an estimated ratio of male: female ranging from 19: 1 to 23: 1 in all the accessions studied. *Lagenaria breviflora* in the wild was observed to start flowering towards the end of the dry season, and the male flowers were first to emerge too.

All the accessions produced flowers and bore fruits simultaneously except *Lagenaria breviflora,* and few flower abortions were noticed in EJ and EB accessions of *Lagenaria siceraria*. *Lagenaria siceraria* IA accession had the highest flowering and fruit production rate. The fruit production resulted in reduced male flower production, and the mean fruit number produced per each accession was between 1 and 11 per plant. Not more than one fruit was observed per secondary axis in all the accessions except in *Lagenaria siceraria* IA accession, where 2–3 fruits were noted. No new leaves were formed during the fruit growth and maturation period, followed by senescence and death. Furthermore, all the accessions completed their life cycle within five months except *Lagenaria breviflora*, which failed to produce flowers and fruits after eight months of planting (Table 2).

Four different fruit shape categories were noted among the *Lagenaria* accessions studied. The necked shape was observed in *Lagenaria siceraria* NA, IA, EJ and ES accessions; bottled shape in IC accession; flat round in EB accession and oval shape in *Lagenaria breviflora* (Fig. 2). The longest and shortest fruit circumferences were recorded in EB and IA accessions (13.13 ± 0.32 cm and 4.40 ± 0.08, respectively). The fruit rind colour varied among the accessions. It was dark green with creamy patches in *Lagenaria siceraria* EJ accession and *Lagenaria breviflora*; light green in IC accession, while others possessed green colouration. All turned to light brown when mature and dry.

Seeds were oblong and varied in size, with colour ranging from light brown to dark brown. Furrows, ridges and rough seed coat texture were observed in all the accessions except *Lagenaria siceraria* IC accession and *Lagenaria breviflora* whose seeds were smooth (Fig. 3). Frills were observed on the seeds of IC accession. The highest number of seeds per fruit (913) was documented in *Lagenaria breviflora*, and it had the least weight for one hundred seeds, 3.79 ± 0.005 g (Table 2). The sizes of the fruits, as well as that of the seeds among the *Lagenaria* accessions studied, were significantly different. It was observed that the seeds from the stored fruits started losing their viability gradually after six months. After about 18 months, seeds of *Lagenaria siceraria* NA accession and *Lagenaria breviflora* lost their viability.

Pollination mechanism

In the two *Lagenaria* species studied, anthesis occurred during the night in both the male and female

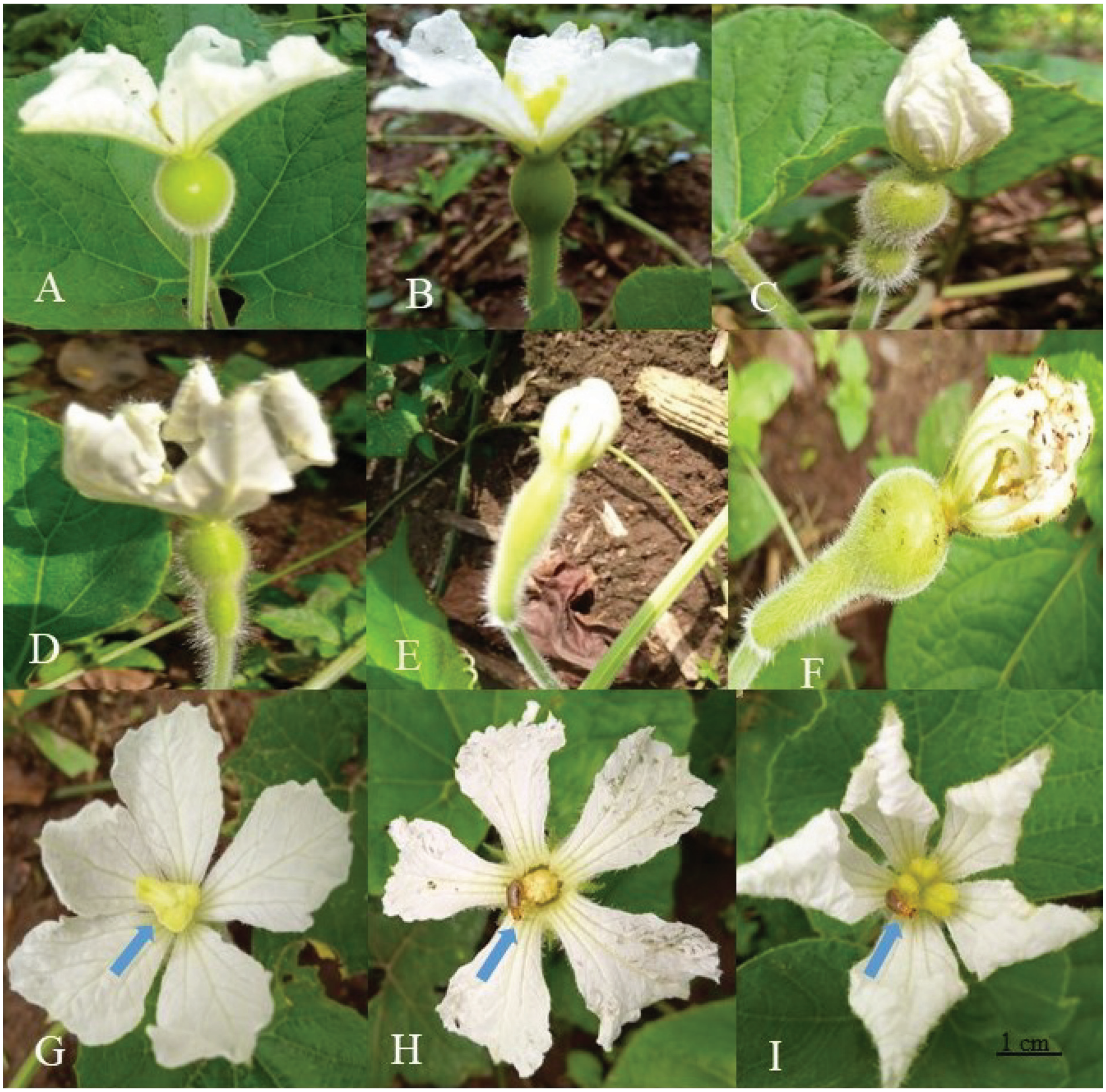

Fig. 1. The floral forms and the pollinators of the *Lagenaria siceraria* accessions studied. A – EB accession, pistillate flower; B – accession EJ, pistillate flower; C – accession IA, pistillate flower; D – accession NA, pistillate flower; E – accession IC, pistillate flower; F – accession ES, pistillate flower; G – the arrangement of the ovules (arrowed) in the pistillate flower; H – *Coryna apicicornis* on staminate flower (arrowed); I – *Coryna apicicornis* on the stigmatic surface (arrowed). Scale bar = 1 cm. For an abbreviation of accessions, see Table 1

flowers; it began between 11:55 pm and 2:30 am, and the flowering took approximately two hours to be fully opened, while anther completely dehiscence within 24 to 48 hours. The flowers close after 8–10 hours, depending on the opening time. The pollinators did not give preference to any of the accessions. The pattern of foraging activities of flower visitors showed that *Chrysomelidae* (cucurbit leaf beetles), *Coryna apicicornis* (blister beetles), dragonflies, honeybees (*Apis mellifera*) and butterflies were the active visitors of *Lagenaria* flowers. *Chrysomelidae* (cucurbit leaf beetles) and *Coryna apicicornis* (blister beetles) were the significant pollinators as they constituted over 80% of potential pollinators (Fig. 1, H and I).

Before the staminate flower anthesis, the beetles were observed to invade the flower buds. Immedi-

Fig. 2. Fruit forms of the *Lagenaria siceraria* and *Lagenaria breviflora* accessions studied. A – accession EJ; B – accession IB; C – accession IC; D – accession EB; E – accession IA; F – accession NA; G – accession ES. For an abbreviation of accessions, see Table 1

Table 2. Seed, fruit and reproductive parameters in the *Lagenaria* accessions studied. For an abbreviation of accessions, see Table 1

Parameters	Accessions						
	IA	EB	IC	EJ	NA	ES	IB
Percentage of seed germination for 30 days	86%	77%	63%	83%	53%	70%	73%
Seed length (cm)	1.22 ± 0.02^{d}	1.57 ± 0.01^{b}	1.51 ± 0.03^{c}	1.88 ± 0.02^{a}	1.51 ± 0.01^{c}	1.03 ± 0.01^{e}	1.00 ± 0.01^{e}
Seed diameter (cm)	0.72 ± 0.01^{c}	0.98 ± 0.01^{a}	0.65 ± 0.01^{d}	0.93 ± 0.01^{b}	0.65 ± 0.01^{d}	0.55 ± 0.01^{e}	0.43 ± 0.008^{f}
Seed area (cm^2)	0.88 ± 0.02^{d}	1.54 ± 0.02^{b}	0.98 ± 0.02^{c}	1.75 ± 0.02^{a}	0.98 ± 0.01^{c}	0.56 ± 0.01^{e}	0.43 ± 0.01^{f}
Seed weight (g)	7.67 ± 0.006^{f}	17.63 ± 0.005^{b}	13.10 ± 0.004^{d}	20.43 ± 0.006^{a}	16.47 ± 0.02^{c}	8.47 ± 0.003^{e}	3.79 ± 0.005^{g}
Fruit length (cm)	6.43 ± 0.08^{f}	10.90 ± 0.31^{e}	35.97 ± 1.08^{a}	23.47 ± 0.70^{b}	13.73 ± 0.27^{c}	13.50 ± 0.24^{c}	11.60 ± 0.26^{d}
Fruit circumference (cm)	4.40 ± 0.08^{g}	13.13 ± 0.32^{a}	7.87 ± 0.16^{e}	11.20 ± 0.31^{b}	8.23 ± 0.18^{d}	6.27 ± 0.08^{f}	9.80 ± 0.11^{c}
Number of fruits	10.00 ± 0.67^{a}	3.33 ± 0.37^{c}	2.00 ± 0.28^{d}	2.00 ± 0.13^{d}	4.33 ± 0.33^{b}	4.33 ± 0.30^{b}	–
Number of seed per fruit	44.10 ± 3.43^{g}	173.33 ± 6.92^{c}	182.83 ± 3.75^{b}	91.23 ± 6.31^{e}	131.90 ± 3.63^{d}	71.80 ± 2.07^{f}	913.27 ± 11.61^{a}
Days to first flowering after planting	57	55	55	55	59	55	--
Mean number of fruits produced per accession	11	3	2	1	2	5	--
Number of days spent completing life cycle after planting	142	130	130	125	138	135	--

Fig. 3. Seed morphology in the *Lagenaria* accessions studied**.** A – *Lagenaria breviflora* (IB accession); B – *Lagenaria siceraria* (accession IA), C – accession ES; D – accession NA; E – accession IC; F – accession EJ; G – accession EB. For an abbreviation of accessions, see Table 1

ately after the pollen dehisced, they consumed the pollens within 48 hours, after which they fed on the petals as well. The beetles were attracted to the pistillate flowers, which were surrounded by many staminate flowers by the scent of their nectar. During their feeding, the pollens got attached to their body, and as they were moving around on the ovules, trying to go down into the ovary to get the nectar, pollination took place in the process. The activities of the pollinators were more concentrated on the staminate than on pistillate flowers. When the flower production reduced, the beetles shifted their attention to the leaves. The bees and other flying insects just perched on the flowers and left. Their activities around the flowers and plants were minimal.

Pollen stainability and morphology

The percentage of pollen stainability observed in this study ranged between 81% and 91%, except in *Lagenaria breviflora*. The highest and lowest stainability percentages were recorded in the *Lagenaria* *siceraria* IC and EJ accessions, respectively (Table 3). The pollen grains observed in all the accessions were sticky, spheroidal, and tricolporate in the polar view and mainly occurred as monads (Fig. 4). Occasionally, dyads were encountered in IA, EB, IC, EJ accessions and triads in EJ accession. At the same time, polyad was only noticed in NA accession. The pollens had oculus and angular aperture. The exine pattern was reticulate, and the pollen surfaces were grooved. The equatorial diameter of the pollen studied ranged between 6.62 μm and 4.75 μm while the pollen axis ranged between 6.65 μm and 4.75 μm. The longest pollen axis and the widest equatorial diameter were observed in the ES accession, while the EJ accession had the least diameter. The polar axes and equatorial diameters of the pollen grains, as well as their ratios analysed, were not significantly different from each other (Table 3).

DISCUSSION

The findings of Chimonyo & Modi (2013) on the seed performance of selected bottle gourd in South Africa showed that seed germination began on the

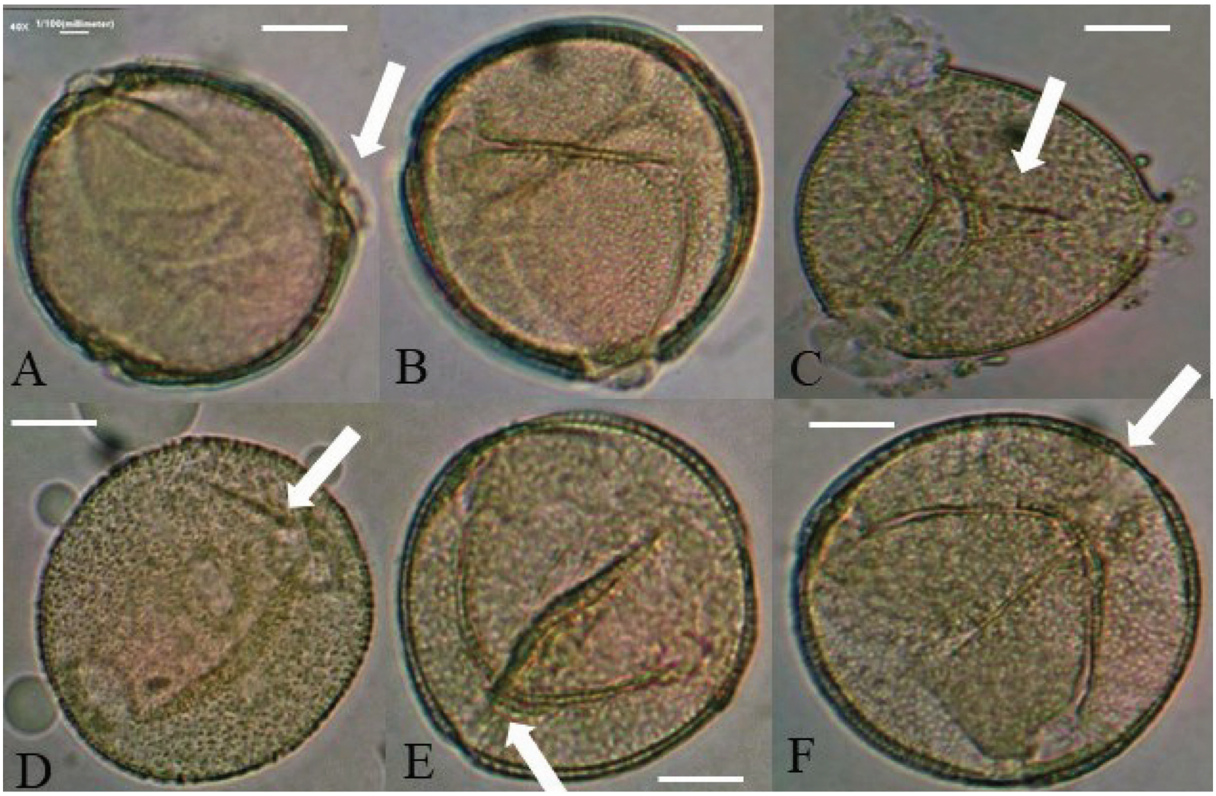

Fig. 4. Pollen grains of *Lagenaria siceraria* accessions studied. A – accession IA, an arrow indicating the oncus, i.e. edge of an aperture; B – accession EB; C – accession IC, an arrow indicating the colpi, i.e. elongated angular apertures; D – accession EJ, an arrow indicating the grooved surface; E – accession NA, an arrow indicating the apocolpi field; F – accession ES, an arrow indicating the reticulate exine. Scale bar – 1 μm. For an abbreviation of accessions, see Table 1

Table 3. Pollen parameters and pollen stainability in the *Lagenaria siceraria* accessions studied. For an abbreviation of accessions, see Table 1

Parameters	Accessions					
	IA	EB	IC	EJ	NA	ES
Polar axis (PA) (μm)	*4.90 ± 0.01 [a]	6.05 ± 0.01[a]	5.55 ± 0.01[a]	4.75 ± 0.01[a]	5.73 ± 0.01[a]	6.65 ± 0.05[a]
Equitorial diameter (ED) (μm)	4.75 ± 0.01[a]	6.10 ± 0.00[a]	5.55 ± 0.001[a]	4.75 ± 0.002[a]	5.67 ± 0.00[a]	6.62 ± 0.00[a]
PA/ED	1.04 ± 0.01[a]	0.99 ± 0.001[a]	1.01 ± 0.001[a]	1.00 ± 0.01[a]	1.00 ± 0.00[a]	0.99 ± 0.00[a]
Pollen stainability (%)	89	88	91	81	85	90

fifth day, and a peak of 84% was achieved on the 12th day after planting. However, in this present study, germination started on the sixth day after planting in *Lagenaria siceraria* accession EB. A peak of 86% was recorded in accession IA, the 15th day after planting. The minor variation observed in the two reports might be due to the different geographical locations of the studied area. The loss of seed viability observed after six months of storage in the present study is in line with the findings of Yao et al. (2020) in *Lagenaria siceraria*, which further confirms the necessity for a strategic conservation plan for the members of the genus *Lagenaria.*

Flower initiation was observed between 55 and 59 days in the accessions studied, while Morimoto et al. (2004) have reported 77 and 131 days for the early and late flowering varieties, respectively in Kenya. In the present study, the accessions of the two species can then be categorised as early flowering varieties. The difference in the time of flower initiation in these two locations might have been due to different morphotypes of *Lagenaria siceraria* investigated as well as geographical locations. The female flowers emerged 2–4 weeks after the emergence of male flowers in the present study; this report is not entirely different from the findings (1–4 weeks) of Morimoto et al. (2004). Early emergence of male flowers attracted pollinators in large numbers, thereby ensuring the pollination of the few female flowers produced, which might not be unconnected with the enormous cost of fruit production as the plant cannot afford to waste such resources, being a protandrous monoecious species (Delesalle & Mooreside, 1995). Moreover, Morimoto et al. (2004) have stated that the difference in the timing of initiation of male and female flowers in these *Lagenaria* species was considered a mechanism that encouraged cross-pollination. More so, Cheng (1990) has opined that cross-pollination can occur among different morphotypes of *Lagenaria siceraria* and between the species and *Lagenaria sphaerica*, a wild relative. Additionally, the early appearance of male flowers has been reported in some species of *Cleome* L. and *Peucedanum multivittatum* Maxim. (Olaoluwa & Azeez, 2022), and it was stated that this phenomenon ensures the availability of abundant pollen grains for successful pollination.

The male flower had three fused stamens, while three stigmatic lobes were observed in the pistillate flower of the two species studied. This agrees with the reports of Khosa & Dhatt (2015). The female flowers are borne on the vines' secondary axils; the fruit formation tendency is high in female flowers, which are close to the main vine. This might be because these flowers have immediate access to photosynthetic products compared to those far from the main vine. Five petals and sepals were documented in the pistillate and staminate flowers. The presence of larger petals in the pistillate flowers, as opposed to the smaller ones in the staminate flowers and variation in the shape of the ovary agrees with the report of Khosa & Dhatt (2015). The occurrence of larger pistillate flowers would confer an advantage on them by providing more expansive landing surface areas for the pollinators, thereby ensuring pollination. Similarly, Khosa & Dhatt (2015) have observed tricarpellary, syncarpous and unilocular ovaries in the female flowers of *Lagenaria* species. The pistillate and staminate flowers studied opened during the night. However, Sugiyama et al. (2014) have reported that flower openings occur late in the afternoon and sometimes during the night. In the present study, it took approximately 120 minutes for the flowers to be fully opened, and anther dehiscence occurred between 24 to 48 hours. However, Morimoto et al. (2005) have shown that it takes almost 60 to 90 minutes for the flowers to be fully opened, and the pollen is only viable for 48 hours (Sugiyama et al., 2014). Morimoto et al. (2004) have reported an estimated male: female flower ratio of 26:1 in Kenya, which is not significantly different from 23:1, which was observed in this study. This showed that the findings on some reproductive parameters in this study were not entirely different from previous studies (Morimoto et al., 2004, 2005; Sugiyama et al., 2014; Khosa & Dhatt, 2015), and differences noted may be attributed to different climatic conditions in areas where these studies have been carried out.

The number of fruits harvested from the accessions ranged from 1 to 11 per stand. However, about 18 fruits were harvested in *Lagenaria siceraria* accession EJ that was observed growing around the Department of Botany's screen house. This plant found its way to climb a small tree, *Annona muricata* L. Similarly, *Lagenaria siceraria* accession NA at its collection site in the North Central Region of the country (Nigeria) climbed a medium size tree and produced numerous fruits. The fruit shape might slightly change due to the location of the fruits along the vines or when encountering an obstruction. Moreover, Morimoto et al. (2004) have reported a harvest, which ranged between 1 and 20 fruits per plant under optimum environmental conditions in Kenya. Also, as documented in the present study, Morimoto et al. (2004) have reported that accessions with bigger fruits produced fewer fruits, while those with small fruits produced more. This might be because higher nutrient allocation is required towards producing fruits with higher weight or bigger size. Thus, the higher the weight of the fruit, the higher the production cost, resulting in fewer fruits. Furthermore, fruit development was accompanied by a reduction in male flower production, which implies that resources are being directed towards fruit production at the expense of flower production.

In the present study, *Lagenaria siceraria* accession NA produced an average of two fruits in Southwestern Nigeria, where the study was carried out; a rainforest region, whereas numerous fruits were produced by the parent plant in North Central Region of the country; a guinea savanna zone. The North cultivars of *Lagenaria siceraria* have been reported to find it challenging to adapt to the different climatic conditions in the southern part of the country (Emmanuel et al., 2013). The high fruit production observed in the accessions (*Lagenaria siceraria* NA and EJ accessions) that were able to climb the trees for support may be attributed to the better access to sunlight and more exposure to pollinators. This shows that *Lagenaria* is naturally a climber rather than a trailer. Moreover, the shade was discovered to have negatively affected the growth and performance of these accessions at the beginning of this study, which led to the relocation of the experimental site to a more open field. Gianoli (2015) has stated that the vine, through external support, grows vertically, thereby enhancing the acquisition of light, ensuring better performance and fitness compared to its prostrate counterpart. Furthermore, finding support by a climber stimulates changes in its growth form, plant morphology and biomass resource allocation (Gianoli, 2015). The climbing accessions did not need to produce additional adventitious roots; tendrils were sufficient to provide necessary attachment and (or) support. Hence the resources that might have been directed to adventitious root production would have been channelled to the production of more fruits.

The fruit shape and seed morphology documented were unique and diagnostic for each *Lagenaria* accession studied. In addition, frills present on *Lagenaria siceraria* accession IC could be used to delimit this accession. It could be deduced from this study that the fruit size does not determine the size and weight of the seeds. This is because the weight of 100 seeds of *Lagenaria siceraria* accession IA with the smallest fruit size and the least number of seeds per fruit was higher than that of *Lagenaria breviflora* with the highest number of seeds per fruit and bigger fruit. The oblong seed shape observed in this study is similar to the report of Achigan-Dako et al. (2008). However, from the present study, the seed's diameter ranged from 0.43 ± 0.008 cm to 0.98 ± 0.01 cm, whereas, Mladenovic et al. (2005) have reported a range of 0.5 to 1.2 cm from their study in Serbia. The difference in the two reports was not unexpected because the fruit and seed sizes in the genus *Lagenaria* greatly vary.

The potential primary pollinators observed during this study were *Chrysomelidae* (cucurbit leaf beetles) and C*oryna apicicornis* (blister beetles). At the same time, Morimoto et al. (2004) have documented hawkmoths (*Hyles lineata* and *Manduca quinquemaculata*) as the primary pollinators in Kenya. The staminate and pistillate flowers rewarded these beetles with pollens and nectar . The different pollinators reported in these separate studies could be attributed to the other components of biodiversity in the two diverse locations. Furthermore, the honeybee can influence cross-pollination within the same community since they can cover a wide area during nectar collection, though their activities in the current study were minimal. Based on the random movement of some pollinators within the study area, there was a high probability of cross-pollination between the accessions studied. However, no intermediate fruit shape was observed from the accessions studied.

Tricolporate spheroidal pollens were observed in this study, and they occurred mainly as monad with oculus and angular apertures. This agrees with the findings of Halbritter (2016), who has documented tricolporate spheroidal pollens in *Lagenaria siceraria.* Besides monads, dyad, triad and polyad pollen grains were noticed in the present study; the occurrence of aggregate and compound pollens has an advantage over monads, which ensures that fertilisation is achieved. Azeez et al. (2014) have reported germination of up to four pollen tubes from some tetrad pollens in *Monodora tenuifolia* Benth. The pollens observed during this study were sticky and did not have spines; thus, they were not wind-borne and could only be transported through insects when they moved from one flower to another. This contradicts the findings of Morimoto et al. (2004), who have reported spiny pollen in *Lagenaria siceraria*.

This present study showed that fruit shape and seed morphology were diagnostic for each accession studied. The production of staminate flowers before the pistillate flowers ensured that abundant pollens were available for fertilisation of the ovules. Furthermore, it could be deduced from this study that the higher the weight and/or size of the fruit, the higher the cost of production; hence, more small fruits were produced compared to the giant fruits. The study revealed that the members of *Lagenaria* are natural climbers, as they performed better in this state compared to trailers. The spheroidal tricolporate pollen was observed in all the *Lagenaria* accessions studied. Therefore, the reproductive parameters such as germination, flowering and fruiting periods, pollination mechanism and plant performance, as well as diagnostic traits documented in the present study, can be employed by the breeders in the selection and breeding programmes as well as genetic improvement of other vegetable crops in the family Cucurbitaceae.

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SOA https://orcid.org/0000-0003-1324-7136