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Cytological Deterioration of Bitter Gourd (*Momordica charantia* L.) Pollen During Storage and Its Impact on Effectiveness for Seed Production

Hamiddah Intan Kusumastuti[A], Eny Widajati[B], Endah Retno Palupi[B]*

[A] Study Program of Seed Science and Technology, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. Jl. Meranti Kampus IPB Dramaga 16680, West Java, Indonesia
[B] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. Jl. Meranti Kampus IPB Dramaga 16680, West Java, Indonesia.

*Corresponding author; email: endah_retno@apps.ipb.ac.id

Abstract

Pollen storage plays an important role in the hybrid seed production of bitter gourd, but a primary challenge is the rapid decline in pollen viability. This research aimed to investigate the mechanisms of bitter gourd pollen deterioration during storage and to assess the effectiveness of stored pollen for hybrid seed production. The study was conducted at the Leuwikopo Research Station, IPB University, from September 2018 to June 2021. Bitter gourd pollen was collected at anthesis and stored in a deep freezer at -21±3ºC. Pollen viability was observed at 0, 2, 4, 6, 8, 10, and 12 weeks after storage (WAS), while pollen ultrastructure was examined at 0, 4, 8, and 12 WAS using a transmission electron microscope. The stored pollen was then used for pollination, observations were made on fruit set, seed set, and seed quality. The viability of bitter gourd pollen declined rapidly following dehydration before storage. The declining pollen viability during storage was due to the degeneration of the intine, followed by mitochondrial deterioration. The cristae became disorganised, leading to the dissolution of the cristae and outer membrane of mitochondria. Storing the pollen in a deep freezer (-21±3ºC) after dehydration did not prevent a further decline in pollen viability. Bitter gourd pollen stored for more than 2 weeks at -21±3ºC is ineffective for seed production. Cucurbit pollen remains viable for only two weeks; thus, fresh pollen is more practical for hybrid seed production. Development of more effective pollen storage methods is necessary to prolong pollen viability and enhance breeding efficiency.

Keywords: intine, mitochondria, pollen viability, seed quality, seed set

Introduction

Research on pollen storage has been of significant interest due to its importance in breeding programs, conservation efforts, and the production of hybrid seeds. Stored pollen enables breeding and hybrid seed production by eliminating the need to plant paternal and maternal lines. This is particularly important in cases where pollen viability declines rapidly after anthesis, causing insufficient pollination (Craeye et al., 2020; Tran et al., 2021; Fayos et al., 2022). Moreover, pollen storage overcomes the constraint related to the asynchronous flowering of the parental lines. Cross-pollination using stored pollen from plants with desirable traits allows for faster development of hybrid varieties (Fayos et al., 2022). Additionally, pollen storage is crucial for genetic preservation, particularly for endangered species, as it enables the long-term preservation of valuable genetic material.

The growing demand for hybrid vegetable seeds underscores the importance of pollen storage. Hybrid seeds are produced by cross-pollinating two pure parental lines (female and male)(Pradeepkumar and Lekshmanan, 2024). By utilising stored pollen provided by the seed industry in participatory hybrid seed production, farmers can optimise land use efficiency (Palupi et al., 2017). This approach enables them to plant only the female lines, resulting in higher yields compared to planting both female and male lines. Pollen for cross-pollination is collected from previously grown male parental lines, then dried, sealed in airtight vials, and stored at low temperatures. Before use, the pollen is removed from storage and tested for viability to ensure effective germination and a successful seed set (Akutsu, 2016).

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Bitter gourd (*Momordica charantia* L./Cucurbitaceae) is a common vegetable in South and Southeast Asia, rich in antioxidants, flavonoids, and other polyphenols that reduce health risks. The pericarp contains cucurbitacin, an active component in anti-hyperglycemic, anti-hyperlipidemic, hepatoprotective, anti-obesity, and anti-cancer compounds (Chen et al., 2015; Ahmad et al., 2016). Due to these benefits, the demand for bitter gourd is continuously increasing.

Bitter gourd varieties can be categorised into two types based on fruit size, color, and surface texture. The first type is characterised by pointed protuberances, a length of 10 to 20 cm, a dark green color, and a very bitter taste. The second type, a more commonly found, has round protuberances, measures 20 to 40 cm in length, is light green, and has milder bitterness (Behera et al., 2010). The latter group consists mainly of hybrid varieties. The Asia-Pacific region, including Indonesia, holds the largest global bitter gourd seed market, valued at approximately $1.2 billion in 2023 (Dataintelo's Bitter Gourd Seeds Market Research Report, 2023). Data on the demand for bitter gourd seeds in Indonesia is limited. A prominent supplier of vegetable seeds in Southeast Asia reported that, although the company supplies both open-pollinated and hybrid seeds, hybrid seeds have become increasingly popular due to their higher yield potential and disease resistance.

Bitter gourd is a monoecious species with separate male (staminate) and female (pistillate) flowers on different nodes. Flowering begins about one month after planting and lasts up to six months. In the tropics, staminate flowers appear 1-2 weeks before pistillate flowers, with ratios of staminate to pistillate flowers varying between 3:1 - 37:1 (Chen et al., 2015; Matsumura et al., 2020; Zurbano et al., 2021; Deyto and Cervancia, 2009). Staminate flowers open between 07:00 and 10:00 h when pollen viability is highest. Pollen viability declines rapidly afterwards, and the flowers abort as early as 13:00 h on the day of anthesis. The stigma of the pistillate flowers remains receptive until the day after the flower opens. Bees, including *Apis cerana indica* Fab, *Apis florea* Fab, *Tetragonula iridipennis* Smith, *Tetragonula cf. biroi*, and *Halictus* sp., are the major pollinators of bitter gourd (Yogapriya et al., 2019; Sumiati et al., 2019; Suhri et al., 2022).

Most Cucurbit seeds available in the Indonesian market are hybrid varieties. A major challenge in the pollen management of cucurbit crops, such as melon, cucumber, and bitter gourd, is the rapid decline in pollen viability (Agustin et al., 2014; Harliani et al., 2014). For instance, the pollen viability of IPB hybrid melon declined from 60% to 24% within 30 days under ultra-freezer conditions (-80 ± 2°C) (Agustin et al., 2014). Using pollen that has been stored for more than six days results in a low seed set. However, Akutsu (2016) reported that bitter gourd pollen stored at -25°C under N_2 gas or vacuum for one year maintained high germination and yielded sufficient fruit set. Williams and Brown (2018) reported that pollen in a hydrated state rapidly loses viability, making dehydration before storage essential. Palupi et al. (2017) found that dehydrating cucumber pollen with $MgCl_2$ for 8 h maintained viability during storage in an ultra-freezer (-79 ± 2°C), and using pollen 56 days after storage yielded a high seed set.

Most Cucurbitaceae pollen grains are larger than 40 µm (Srivastava and Sharma, 2016). *Momordica charantia* pollen has a prolate shape with a reticulate tectum. The pollen wall consists of two layers: the exine (outer layer) and the intine (inner layer), both of which feature tricolporate apertures (Halbritter, 2015). The exine, composed of sexine and nexine, is thicker to protect pollen from adverse conditions (Srivastava and Sharma, 2016). The intine, made of cellulose and pectin, forms the pollen tube during germination to deliver sperm cells (male gametes) to the female gametophyte (embryo sac).

A mature pollen grain is either bicellular (vegetative and generative cells) or tricellular (a vegetative cell and two sperm cells). The generative cell or two sperm cells are enclosed by a plasma membrane and are located within the vegetative cell's cytoplasm, packed with organelles such as vacuoles, chloroplasts, and mitochondria (Albert et al., 2002). Mitochondria generate energy through cellular respiration and synthesise necessary metabolites for cell maintenance. As pollen ages, mitochondrial ultrastructure deteriorates, leading to decreased respiratory capacity and increased reactive oxygen species (ROS), which damage the mitochondrial membrane and cytoplasm (Yin et al., 2016; Wang et al., 2018). This study examines the deterioration of the bitter gourd pollen wall and mitochondria during storage, as well as the effectiveness of stored pollen for seed production.

Materials and Methods

A collection of the Indonesian Vegetable Research Institute (IVEGRI) green-colored, open-pollinated bitter gourd cultivar (local name: paria) was used in this research. The study was conducted at the Leuwikopo Experimental Station, located 250 m above sea level, on the Dramaga Campus of IPB University, Bogor, West Java. Seed multiplication was necessary from September 2018 to June 2019

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due to the limited number of available seeds from IVEGRI. Flowering traits of the bitter gourd were observed, including the onset of staminate and pistillate flowers blooming, the number of staminate and pistillate flowers per plant, and the staminate to pistillate flower ratio at 60 days after planting (DAP. The seeds were then planted in September 2019 for the research. Pollen was collected from staminate flowers, processed, and stored.

Pollen Viability during Storage

Pollen handling was conducted at the Seed Biology and Biophysics Laboratory, Department of Agronomy and Horticulture, IPB University. The staminate flower opened between 06:00 and 07:00 h. A preliminary study measuring pollen viability at two-hour intervals from 08:00 to 16:00 hours revealed that the highest pollen viability, approximately 74%, was recorded at 10:00 hours. Therefore, pollen was collected on the day of anthesis between 7:00 a.m. and 10:00 a.m. The flowers were brought to the laboratory in a cool box. Anthers were removed from the flowers and dried in an air-conditioned room (18-20°C, 60% RH) for 18-24 h (Fariroh et al., 2011). Pollen was extracted from the anthers using a container with a fine cloth as a sieve, and shaken vigorously. The extracted pollen was desiccated using $MgCl_2$ as an absorbent for 8 h at 18-20ºC (Palupi et al., 2017), then placed into 2 ml cryo-vials and stored in a deep freezer (-21±3ºC) (Harliani et al., 2014). The pollen viability was observed immediately after collection and then at 0, 2, 4, 6, 8, 10, and 12 weeks after storage (WAS), with six replications each time. The stored pollen samples were removed from the freezer and left at room temperature for 30 minutes before the germination test or used for pollination. Approximately 200-300 pollen grains were germinated on a glass slide in pollen germination medium (PGM) as described by Fariroh et al. (2011), and incubated for four h at 18-20ºC. Pollen germination was observed using a light microscope (Olympus BX51) at 100x magnification. Germinated pollen was identified by the appearance of a pollen tube at least as long as the pollen diameter.

Cytological Changes during Pollen Storage

Ultrastructural changes related to pollen deterioration were observed at 0, 4, 8, and 12 WAS, with a focus on the integrity of the pollen wall and mitochondrial membranes. Observations on cell ultrastructure were conducted at the Eijkman Institute for Molecular Biology, Jakarta, using a Transmission Electron Microscope (TEM type JEOL 1010). Specimen preparation followed the procedure of the TEM and Histology Laboratory at the Eijkman Institute for Molecular Biology (2013). Pollen was retrieved from the deep freezer, thawed for 30 minutes at room temperature, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 3% sucrose, and further processed at the TEM and Histology Laboratory of the Eijkman Institute for Molecular Biology.

The Effectiveness of Stored Pollen for Seed Production

Fresh and stored pollen (0, 2, 4, 6, 8, 10, and 12 WAS) were used for manual pollination to assess the effectiveness of stored pollen for seed production. The experiment followed a randomised complete block design with one factor: pollen storage period. Each treatment involved three flower samples and was replicated four times. Replications were conducted on different dates when the number of pistillate flowers opened on the same day was insufficient for all replications.

Observations and measurements included fruit set (the proportion of flowers developing into mature fruits), seed set (the proportion of ovules developing into viable seeds per fruit), and non-viable seeds (partly filled and empty seeds) at fruit maturity. The average number of ovules per flower (ovary) was calculated from 12 pistillate flowers, resulting in an average ovule number of 30.3. The pistillate flowers were collected at anthesis, the ovaries were dissected, and the ovules were counted under a light microscope.

Seed germination tests were conducted in plastic boxes (30 cm x 30 cm x 10 cm) using sand sieved through a 0.8 mm mesh (ISTA, 2018). Each treatment used 25 seeds with four replications. Normal seedlings were counted on days 4 and 14 after planting (ISTA, 2018). Observations included germination rate, maximum germination potential, vigor index, germination speed, normal seedlings' dry weight, and seedling growth rate. The analysis of variance was performed using SAS 9.0 with a 95% confidence level, and differences among means were further analysed using Duncan's Multiple Range Test.

Results and Discussion

Staminate and pistillate flowers began to open 30 and 36 days after planting (DAP). Over the following 30 days, approximately 160 staminate flowers and 7 pistillate flowers were recorded, resulting in a staminate to pistillate flowers ratio 23:1 (Table 1). This ratio is consistent with the observation from the previous seed multiplication cycle conducted before the study. Flower sex ratios of Cucurbitaceae are known to vary, ranging from 9:1 to 48:1 (Agustin et

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al., 2014; Anusree et al,. 2015; Ashok et al., 2020).

Pollen Viability during Storage

The viability of fresh pollen declined rapidly from 67.2% to 3.5% following extraction and dehydration in $MgCl_2$ for 8 h (0 WAS - before storage). Therefore, initial pollen viability at the beginning of storage was low (Figure 1). Similar behavior of other members of Cucurbitaceae was also found, such as pumpkin (*Cucurbita pepo* L.) (Nepi et al., 2010), cucumber (*Cucumis sativus* L.) (Fariroh et al., 2011), and melon (*Cucumis melo* L.) (Agustin et al., 2014). The decline in pollen viability could be due to desiccation stress, which leads to structural changes in the plasma membrane during dehydration that alter its permeability. In desiccation-sensitive pollen, the carbohydrates that consist of cytoplasmic polysaccharides and sucrose are absent or in low concentrations; meanwhile, the sucrose is reported to inhibit water loss and protect the membranes for pollen tube development and provide osmotic balance during pollen germination (Liu et al., 2021; Mendez and Acma, 2018). These pollens are classified as desiccation-sensitive pollen or recalcitrant pollen, in which the viability declines sharply during drying (Garcia et al., 2015; Franchi et al., 2011), with characteristics of having high water content at dehiscence, having no furrow, and relatively large size (Pacini and Dolferus, 2019).

After two weeks of storage in a deep freezer (-21±3ºC), pollen viability improved substantially to 29.0%, suggesting partial recovery. However, viability steadily decreased over time and was completely lost by 12 WAS (Figure 1). Several researchers have also reported a similar experience (Fariroh et al., 2011; Agustin et al., 2014; Palupi et al., 2017), in which short-term storage improves pollen viability. Thawing the pollen for 30 minutes at room temperature (18-20ºC and relative humidity 60%) allows the pollen's water content to rise slowly, which may improve membrane integrity and, therefore, pollen viability. The protective sugars and other molecules in the cytoplasmic membranes and small vacuoles play a crucial role in the desiccation tolerance of pollen and other desiccation-tolerant cells or tissues (Pacini, 1996; Oliver et al., 2020). Data from this research showed that the longer the storage time, the lower the improvement in viability after thawing.

Cytological Changes During Pollen Storage

Bitter gourd pollen is sensitive to desiccation, and the pollen cell wall plays a role in maintaining water content to sustain its viability. Bitter gourd pollen collected at anthesis had intact cell walls, exine, and intine (Figure 2A). The exine consists of two layers: the outer, thicker sexine, and the inner, thinner nexine

Table 1. Flowering traits of the bitter gourd cultivar

The commencement of the staminate flower blooming	36±6 DAP
The commencement of the pistillate flower blooming	41±6 DAP
Number of staminate flowers per plant (60 DAP)	166±10
Number of pistillate flowers per plant (60 DAP)	7±3
Ratio of staminate to pistillate flowers per plant (60 DAP)	23:1

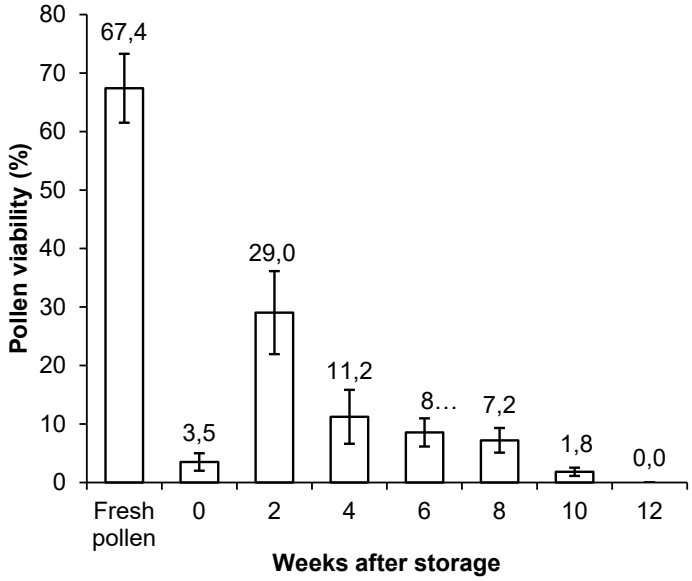

Figure 1. Bitter gourd pollen viability during storage in a deep freezer (-21±3ºC).

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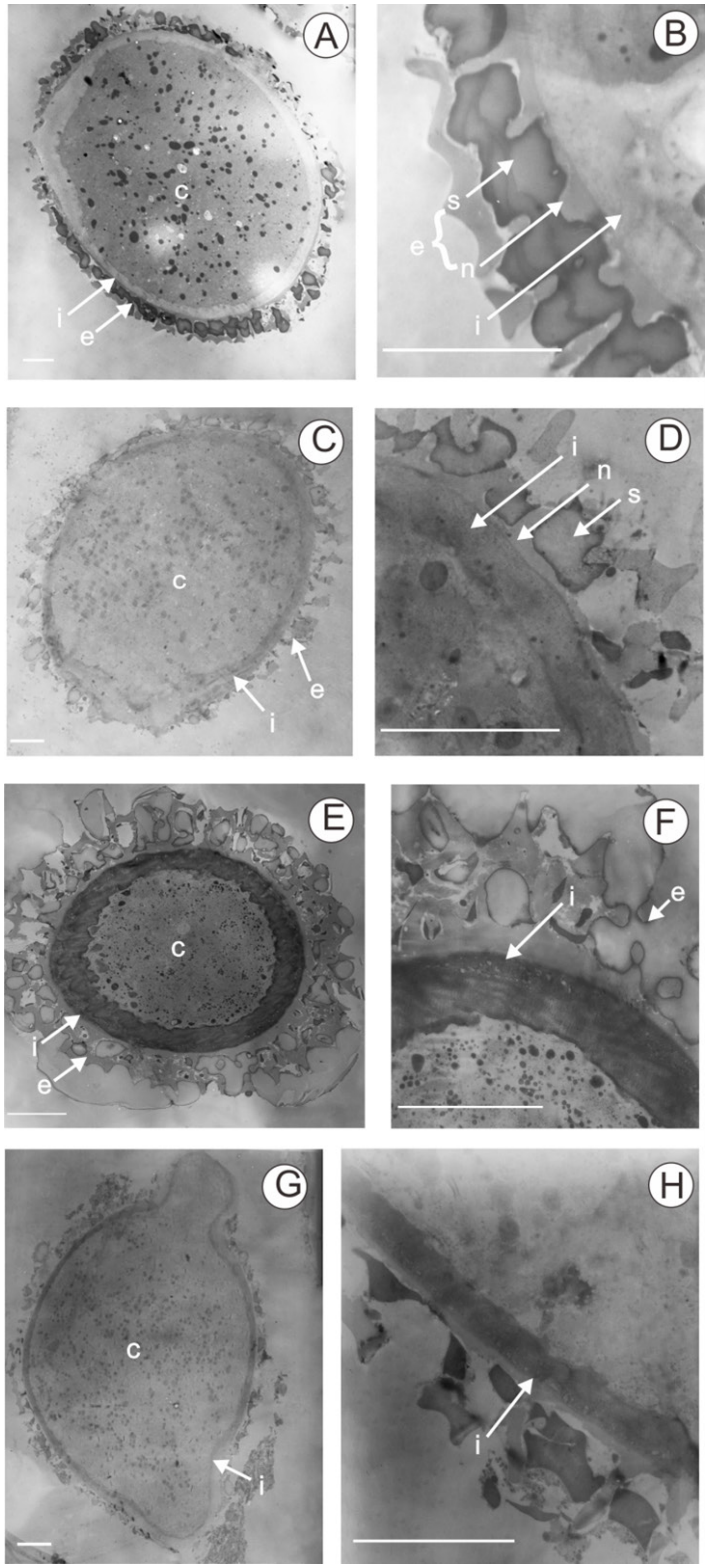

Figure 2. Micrograph of bitter gourd pollen: (A) pollen cell after desiccation before storage (0 WAS) with exine (e) and intine (i); (B) pollen walls at 0 WAS: exine (e) comprises of sexine (s) and nexine (n); and intine (i); (C) pollen at 4 WAS with exine and intine; (D) pollen walls at 4 WAS: sexine was less intact; (E) pollen at 8 WAS with exine, intine, and reduced cytoplasm; (F) pollen walls at 8 WAS: exine and intine were intact; (G) pollen at 12 WAS: intine became thinner (arrow) and protruded; (H) pollen walls at 12 WAS: lysis at deteriorated intine (arrow). Micrograph using TEM (type JEOL 1010, 80.0 kv) with 1200x magnification (A, C, E, G) and 6000x magnification (B, D, F, H). c: cytoplasm, e: exine; i: intine, n: nexine, s: sexine. (A-D: bar = 7 µm; E-H: bar = 5 µm).

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(Figure 2B). Structurally, the pollen walls remained intact at 4 WAS in the deep freezer (-21±3ºC), although the sexine was slightly less intact in some areas (Figure 2D). Some pollen showed reduced cytoplasm at 8 WAS (Figure 2E), with both the exine and intine still intact (Figure 2F). At 12 WAS, pollen exhibited changes in shape, with the intine becoming thinner and protruding beyond the exine in some areas, resembling germinating pollen (Figure 2G). At this stage, lysis was observed where the intine had deteriorated (Figure 2H). By this time, the pollen had lost entirely its viability (Figure 1).

Desiccation-sensitive pollen was to be devoid of furrow (Halbritter, 2015), as was bitter gourd pollen with three colporate sunken apertures. The furrow provides more flexibility during dehydration and rehydration, allowing for variation in shape and volume; therefore, pollen lacking furrows has less tolerance to desiccation (Franchi et al., 2011). On the other hand, the intine represents ordinary plant cell walls (Paccini and Hesse, 2012) and is more susceptible to environmental conditions. During the dehydration and rehydration, the exine of pollen without furrows may fracture, exposing the intine to surrounding conditions. This exposure leads to reduced cytoplasm at 8 WAS (Figure 2E). By 12 WAS, the exine layer had degenerated, as indicated by the protrusion of the intine layer (Figure 2H). The intine layer became thinner at several locations, and lysis was observed. The exine, followed by intine layers, degeneration is considered one of the mechanisms of bitter gourd pollen deterioration.

Outer and inner membranes enclose the mitochondria of fresh pollen. The mitochondrial matrix was evident with the cristae structure (Figure 3A). The cristae function as a specialised compartment to enhance the capacity of ATP production (Ikon and Ryan, 2017). After 4 weeks in storage, the outer membranes of mitochondria (mm) deteriorated (Figure 3B). The absence of cristae in the mitochondria at 8 WAS (Figure 3C) could be due to the dissolution of the cristae. The mitochondria had completely deteriorated at 12 WAS (Figure 3D) when the membranes and the cristae had lost their integrity.

Chaanine (2019) reported that mitochondrial deterioration begins with membrane fragmentation, followed by cristae disorganisation, indicated by the widened space between them. Cristae are folds of the inner mitochondrial membrane that increase the surface area, facilitating ATP production. Disorganised cristae indicate dysfunctional mitochondria. The next stage is the dissolution of cristae in one area of the mitochondria, followed by the dissolution of all cristae and the mitochondrial inner and outer membranes. At this stage, adjacent mitochondria also begin to deteriorate. At 4 WAS, the cristae have been disorganized (Figure 3B). At 8 WAS, the entire cristae structure within the mitochondria was absent, indicating a more advanced stage of mitochondrial deterioration, following the rupture of the inner and outer membranes. (Figure 3C). The mitochondria had deteriorated entirely, with the membranes and cristae losing their integrity at 12 WAS (Figure 3D).

The Effectiveness of Stored Pollen for Seed Production

Pollen viability declined rapidly during storage (Table 2). Pollen viability declined from 58% to about 19% within 2 WAS and completely lost its viability by 12 WAS. The rapid decline of pollen viability following anthesis also occurs in zucchini (Craeye et al. 2020). Using pollen at 2 WAS did not reduce fruit set, but it did reduce seed set, as indicated by the number of filled seeds per fruit. Tran et al. (2021) reported the percentage of fruit set and seed set of *Momordica cochinchinensis* Spreng. (Cucurbitaceae) from pollen at 2 WAS (86.7% and 80.4% respectively) was not significantly different from fresh pollen (96.7% and 86.3%), contrary to this study, in which the seed set was lower than that of fresh pollen. Nagar et al. (2023) reported that luffa pollen viability was negatively correlated with temperature during storage at 25, 4, and -20°C. However, cryopreserved pollen stored at -196°C for two months was comparable to fresh pollen in terms of germination, fruit set, and seed set.

Seed production from pollen stored up to 4 weeks after sowing waless than half of that from fresh pollen. The data indicated that pollen processing and storage used in this research would not be efficient for supporting seed production. The effectiveness of bitter gourd pollen for seed production after being stored at (-21±3)ºC was only up to 2 weeks after storage, much shorter than *Momordica cochinchinensis* Spreng for eight weeks (Tran et al., 2021). Additionally, pollen drying techniques following extraction from anthers needed to be improved to maintain high pollen viability before storage. Franchi et al. (2011) reported that recalcitrant pollen can survive desiccation when the water content decreases slowly to approximately 30% of the water content of fresh pollen.

Partly filled and empty seeds increase as pollen viability decreases (Table 2). Low-viability pollen germinates more slowly, reaching the ovule later than highly viable pollen. Consequently, late-set seeds tend to be smaller and develop into partly filled seeds. Additionally, less vigorous pollen may result in abnormal endosperm development, or the endosperm may fail to develop entirely. Since the

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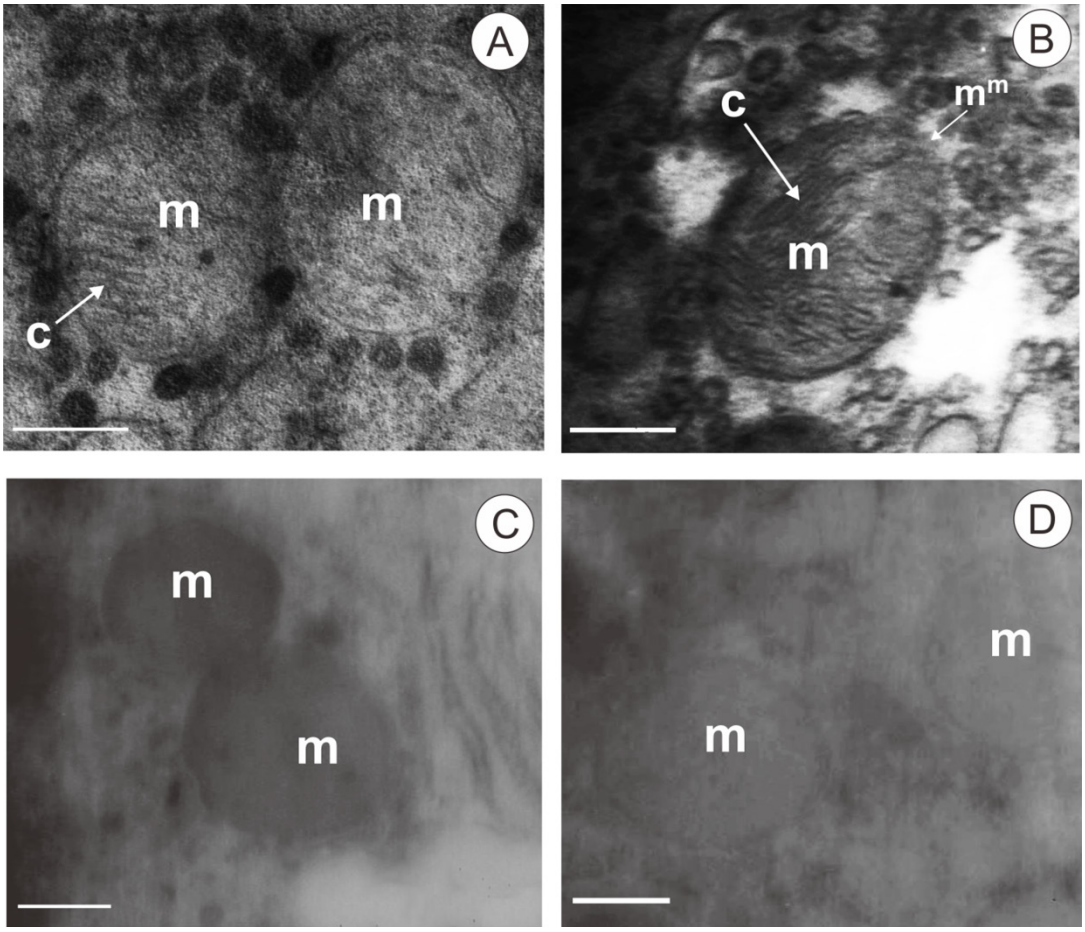

Figure 3. Micrographs of mitochondria: (A) fresh pollen (0 WAS), mitochondria with intact membranes and cristae; (B) pollen at 4 WAS, the outer membrane of mitochondria had deteriorated (m^m) with disorganized cristae; (C) pollen at 8 WAS, mitochondria without cristae; and (D) pollen at 12 WAS, mitochondria with no cristae. Micrograph using TEM (type JEOL 1010, 80.0 kV) at 12000x magnification. c: cristae; m: mitochondria; m^m: deteriorated membrane of mitochondria. Scale bar = 200 nm.

Table 2. Fruit set, seed set (viable seeds), and non-viable seeds following pollination with fresh and stored pollen in a deep freezer (-21±3ºC)

Pollen storage (WAS)	Pollen viability (%)	Fruit set (%)	Viable seed per pod (%)*	Non-viable seed	
				Partly filled (%)**	Empty (%)**
0 (fresh)	58.0^{a}	100.0^{a}	26.8^{a} (88.4)	3.3 (10.9)	0.2^{c} (0.7)
2	19.1^{b}	83.4^{ab}	16.7^{b} (55.1)	1.4 (4.6)	12.1^{b} (39.9)
4	8.8^{c}	66.7^{bc}	11.8^{c} (38.9)	2.4 (7.9)	16.1^{b} (53.1)
6	7.4^{d}	41.7^{cd}	3.8^{d} (12.5)	1.4 (4.6)	25.1^{a} (82.8)
8	5.4^{d}	41.7^{cd}	1.6^{d} (5.3)	4.4 (14.5)	24.3^{a} (80.2)
10	0.5^{e}	33.3^{de}	0.6^{d} (2.0)	4.5 (14.9)	25.2^{a} (83.1)
12	0e	8.3^{e}	0^{d}	1.3 (4.3)	29.1^{a} (95.7)
CV (%)	26.7	30.8	34.2	23.2	16.9

Notes: *) The number of ovules per flower (30.3) was based on the average number of ovules of 12 ovaries (n=12) and was used for calculating the percentage of viable and non-viable seeds; **) the values in brackets are the percentages. Values in the same column followed by the same letters are not significantly different based on the DMRT at α=0.05; WAS = week after storage.

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endosperm functions as a nurturing tissue during embryo development, its lack of development leads to failure of embryo development or being aborted early, resulting in empty seeds (Fang et al., 2012).

Seed viability and vigor resulting from fresh pollen not significantly different from those of 2 WAS pollen, as indicated by germination rate, vigor index, speed of germination, seedling dry weight, and seedling growth rate. However, the seed quality yielded from pollen at 4 WAS was significantly lower than that from fresh pollen (Table 3). Pollen at ≥ 4 WAS resulted in lower seed quality and should not be used for seed production. The longer the pollen was stored, the lower the quality of the yielded seeds. This data strengthens the notion that storing bitter gourd pollen at (-21±3)ºC would only be effective in ensuring the availability of pollen for a short term.

A simple linear regression described the linear relationship between pollen viability and seed set. The coefficient of determination (R^2 = 0.7483) indicates a close relationship between seed set variability and pollen viability (Figure 4).

Linear regression analysis demonstrated a positive correlation between pollen viability and seed set (r=0.8650), with the model, with the model indicating that a pollen viability of 50% would result in approximately 80% seed set. This finding is valuable for establishing a minimum viability threshold for pollen used in seed production – a standard that has yet to be clearly defined. However, maintaining pollen viability at or above 50% during storage remains a constraint. To meet this benchmark, initial pollen viability must be at least 50% before storage. Notably, a previous study reported that cucumber pollen dried for 8 hours retained its viability for up to 56 days when stored in an ultra-freezer at -79±2°C, and the resulting seed set and seed quality were comparable to those achieved using fresh pollen (Palupi et al., 2017). These results suggest that optimized drying

Table 3. The quality of bitter gourd seed produced from pollinating with fresh and stored pollen

Storage period (WAS)	Germination rate (%)	Maximum germination potential (%)	Vigour index (%)	Germination speed (%/day)	Seedling dry weight (g)	Seedling growth rate (g per seedling)
Fresh pollen	85.0±8.9^{a}	88.0±11.8^{a}	20.0±3.3^{a}	8.8±1.4^{a}	2.098±0.239^{a}	0.119±0.017^{a}
2	62.0±5.2^{a}	84.0±7.3^{a}	14.0±13.3^{ab}	7.6±1.2^{a}	1.946±0.376^{a}	0.112±0.013^{ab}
4	40.0±15.0^{b}	48.0±11.3^{b}	3.0±2.0^{bc}	2.7±1.4^{b}	0.828±0.347^{b}	0.108±0.006^{ab}
6	22.0±12.0^{b}	30.0±24.8^{b}	0^{c}	2.0±1.5^{bc}	0.567±0.381^{bc}	0.096±0.072^{abc}
8	1.0±2.0^{c}	2.0±2.3^{c}	0^{c}	0.1±0.2^{c}	0.028±0.057^{c}	0.028±0.057^{bcd}
10	1.0±2.0^{c}	2.0±2.3^{c}	0^{c}	0.1±0.2^{c}	0.017±0.033^{c}	0.017±0.033^{cd}
12	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{d}
StDev (%)	8.3	11.6	5.2	1.0	0.3	0.1

Notes: Values followed by the same letters in the same column are not significantly different based on the Tukey test at α= 0.05; WAS = week after storage

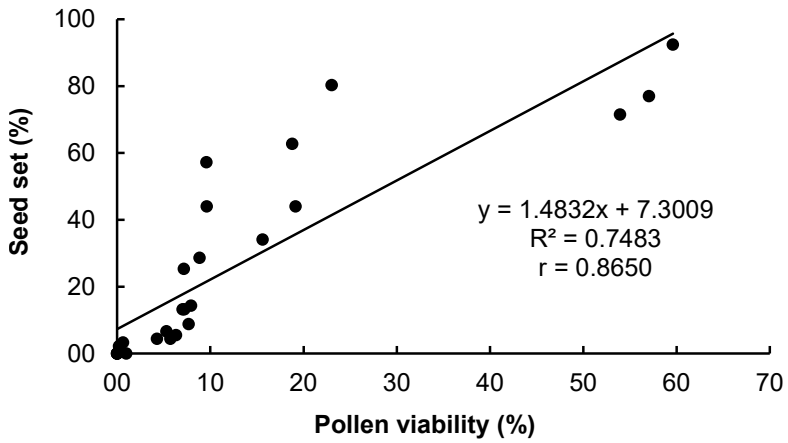

Figure 4. Linear regression between pollen viability and seed set of bitter gourds

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and ultra-low-temperature storage may be feasible for preserving pollen viability over extended periods.

This study highlights the cytological and mitochondrial deterioration of bitter gourd (*Momordica charantia* L.) pollen during storage, linking structural damage, particularly mitochondrial disintegration and cytoplasmic reduction, to a loss of viability. This cellular-level understanding clarifies why pollen storage remains a significant challenge to produce cucurbit hybrid seeds. In practice, the findings emphasize the importance of using fresh pollen whenever possible or carefully managing pollen storage when time is limited. Viability testing is recommended to ensure only high-quality pollen is used for pollination. Future research should focus on identifying protective treatments for preserving mitochondrial and membrane integrity during storage. Improved preservation methods are needed to prolong pollen storage for reliable hybrid seed production and enhance breeding efficiency.

Conclusions

The viability of bitter gourd pollen declined rapidly after dehydration, prior to storage. The decline in pollen viability during storage begins with the degeneration of the intine, followed by mitochondrial deterioration, disorganization of the cristae, and dissolution of the cristae and the outer membrane of the mitochondria, ultimately leading to complete loss of viability. Storing the pollen in a deep freezer (-21±3ºC) after dehydration did not prevent a further decline in pollen viability. Bitter gourd pollen stored for more than 2 weeks at -21±3ºC is ineffective for pollination. Development of more effective pollen storage methods is necessary to prolong pollen viability and enhance breeding efficiency.

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