International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 10, Issue 2, pp: 11-18, 2025
https://doi.org/10.55126/ijzab.2025.v10.i02.002 **http://www.ijzab.com**

Research Article

STUDY OF POLLEN REPRODUCTIVE QUALITY FOR *IN VITRO* HAPLOID PRODUCTION IN MAIZE (*Zea Mays* L.) IN CÔTE D'IVOIRE

***[1]SORO Chigata Lohona, [1]YAPO Sopie Edwige-Salomé, [2]KOUADIO Oi Kouadio Samuel, [1]KOUADIO Yatty Justin**

*[1]Jean Lorougnon Guédé University, Faculty of Agroforestry, Department of Genetics, Biology and Plant Physiology, Laboratory for Improvement of Agricultural Production, P.O. Box 150, Daloa, Côte d'Ivoire
[2]Peleforo Gon Coulibaly University, Department of Biological Sciences, P.O. Box 1328, Korhogo, Côte d'Ivoire.

Article History: Received 27th January 2025; Accepted 25th February 2025; Published 31st March 2025

ABSTRACT

Maize is one of the most widely grown cereals in Côte d'Ivoire for both human and animal consumption. However, despite its nutritional and economic importance, its production faces challenges, such as climate change and soil degradation. To create new drought-resistant varieties, double-haploid technology was used in our breeding program using EV8728 maize. This study aimed to assess the reproductive quality of pollen and its ability to germinate *in vitro*. To this end, pollen grains collected from the experimental plot at Lorougnon GUEDE University were stored at 3 ºC and -26 ºC, then cultivated on modified Yu-Pei *in vitro* germination medium. The acetocarmin (1%) test on pollen harvested between 9 h and 11 h and stored for 1 month at 3 °C gave a high viability rate of 98.82% and 98.77%, respectively. *In vitro* germination was optimal on modified Yu-Pei medium at pH 6 supplemented with 25 g/l sucrose. Moreover, incubation in the dark at 30 ºC for 24 hours resulted in the highest pollen germination rate (97%). Pollen grains stored at 3 °C achieved the highest germination rates. Based on these results, the availability of pollen for the implementation of haploid *in vitro* methods in our breeding program is stable.

Keywords: Cereal, Haplo *in vitro* methods, *In vitro* germination, Plant breeding, Preservation, Viability.

INTRODUCTION

Despite the availability of food resources, food security remains a major problem in developing countries. Cereals, particularly maize (*Zea mays* L.), play a central role in ensuring food security. Maize is the most widely grown cereal worldwide, followed by rice and wheat; however, it is the second most consumed cereal after rice (Blassonny, 2013). Its average grain production is approximately 1.2 billion tons from a surface area of 201 million hectares, representing an average yield of approximately 57,547 q/ha (FAOSTAT, 2020).

Maize is the second most widely grown cereal after rice in Côte d' Ivoire. In 2020, the national production reached 1,175,775 tons on 558,406 hectares (FAOSTAT, 2020). It is the most energy-dense cereal owing to its nutritive assets of starch, protein, and minerals (6; 18). Owing to its storage potential, high productivity, and nationwide distribution, maize has been a key component in the development of the Ivorian food trade. Maize cultivation has gradually gained considerable importance in forested areas through the exploitation of topographically elevated areas to complement the cultivation of rice, cassava, yams, and taro (Kouakou *et al.*,2010). Today, maize is an object of agricultural speculation, which is intensifying because of the ever-increasing nutritional and economic stakes involved. Despite its socioeconomic and cultural importance, maize cultivation is subject to several biological and environmental constraints that lead to lower yields. The main biological constraint is striga (*Striga hermontica*) infestation, which can lead to the abandonment and disappearance of certain varieties (Benmahammed *et al*., 2010). In addition to these biological constraints, the main problems associated with maize cultivation are the high sensitivity of cultivars to soil quality and declining rainfall (Benmahammed *et al*., 2010). Maize is a water-

*Corresponding Author: SORO Chigata Lohona, Jean Lorougnon Guédé University, Faculty of Agroforestry, Department of Genetics, Biology and Plant Physiology, Laboratory for Improvement of Agricultural Production,Daloa,Côte d'IvoireEmail:chigatsoro@gmail.com

demanding plant, especially during the two weeks before and after silking (Farahani *et al.*, 2014). Therefore, improving maize production requires better control of cultivar sensitivity to climatic variability and the ability to adapt to agroecological conditions. Given that mutation induction currently occupies a prominent place in plant breeding, this technique could be an interesting tool to improve maize production in Côte d'Ivoire. Numerous studies have demonstrated that double-haploid technology based on haploid induction *in vivo* or *in vitro* can considerably improve the efficiency of maize variety selection. For example, Kleiber *et al*. (2011) confirmed the importance of this approach in improving maize yield and quality. As a result, a research program for haploid maize lines was initiated in Côte d'Ivoire to produce homozygous material that could serve as a progenitor for the creation of homogeneous drought-resistant lines. The aim of this study was to assess the reproductive quality of pollen from the 5th generation of the irradiated maize variety EV8728 using viability tests on pollen harvested at different times of the day and stored at two different temperatures and to identify culture conditions conducive to *in vitro* germination. Controlling the *in vitro* germination capacity of maize pollen is an important step towards the success of maize improvement programs in Côte d'Ivoire.

MATERIAL AND METHODS

Plant material

The plant material used consisted of maize grains from the 4th-generation lines of the gamma-irradiated variety EV8728, supplied by the National Agronomic Research Center (CNRA) of Ferkessédougou-Côte d'Ivoire. This variety is tolerant of leaf thinning and root lodging (Akanvou *et al*., 2006)

Study site

The work was carried out in the *in vitro* culture unit of the Laboratory of Biology and Improvement of Plant Production and on the experimental plot of Jean Lorougnon Guédé University, located between 6°54,55'N and 6°26,28'W. The climate is tropical with a ferralitic soil. During the study period, from June 2021 to October 2022, the area was characterized by an average temperature of 27.5 °C with annual rainfall between 1,000 and 1,500 mm Soro e*t al.*, 2015)

Crop preparation, sowing, and maintenance

The field trial was conducted in an 18 m^2 plot (4 m × 4.5 m) using a three-line layout. Each line constituted a replicate containing ten seed pots 0.5 m apart. Seeds from the 4th generation of the EV8728 irradiated maize variety were manually sown to a depth of 3 cm, with two seeds per plot. The seeds were manually watered with 1.5 liters of water per plot for 45 days.

Pollen sampling

From 45 days after sowing (DAS), the panicles were observed daily until 50% of the pollen had been released from the upper half of the spikelets. After 55 DAS, panicles composed of spikelets with anthers containing pollen grains were collected daily using a chisel at 9 h, 10 h, 11 h, 12 h, 14 h, 15 h, 16 h, 17 h, and 18 h for one week. The collected pollen was immediately placed in aluminum foil, sealed, and transported to the laboratory.

Pre-treatment of pollen grain samples

The samples were subdivided into two parts. The first part was directly used in the experiments. The second part of the study was subdivided into two groups. These samples were stored at 3ºC or -26 ºC for 90 days.

Testing pollen grain viability

The viability and non-inucleation of pollen grains were assessed using the method described by (Colas and Mercier, 2000). « A portion of each untreated pollen sample (control) was stained with a drop of 1% acetocarbamate for 5 min ». The pollen viability was assessed using an optical microscope (magnification X400). Every 30 days, a sample of cold-stored pollen (treated pollen) from each collection period was collected for viability assessment, as described above. The number of pollen grains extracted for each test was counted, and the viability rate of pollen from each collection period was calculated using the following formula:

$$Viability\ rate = \frac{Number\ of\ viable\ pollens}{Total\ number\ of\ pollens} X100$$

Culture conditions and in vitro germination assessment of pollen grains

Treated pollen grains with high viability rates were thoroughly washed with tap water. Subsequently, the samples were filtered and soaked for 5 min in sodium hypochlorite containing 3.6% active chlorine under a laminar flow hood. After rinsing three times with sterile distilled water, the sterilized pollen grains were sown using a spatula in Petri dishes containing 20 ml of *in vitro* germination medium. This medium consisted of macroelements of the Yu-Pei (YP) base medium, Genovesi and Collins, (1982) modified with 2 g/l magnesium sulfate ($MgSO_4$), 1 g/l potassium nitrate (KNO_3), and 3 g/l calcium nitrate ($Ca(NO_3)_2$). Different sucrose concentrations (0, 5, 10, 15, 20, 25, and 30% sucrose) were added to this basic medium to assess its influence on *in vitro* pollen grain germination. The pH of the medium was adjusted to 5. The best medium obtained was then used as the basis for evaluating the influence of pH (5; 5.5; 6; 6.5; 7; 7.5; 8; or 8.5 ± 0.1) on pollen grain germination. Culture media were solidified with 10 g/l gelrite.

Petri dishes containing pollen were sealed with Parafilm and incubated in the dark in an oven for 10 days. At this stage, the incubation temperature was assessed from 20 ºC, gradually increasing the temperature by 5 ºC at 48 h intervals. After 48 h of incubation, the presence of pollen

germination structures (pollen tubes) was assessed using a binocular magnifying glass (Zeiss) and an optical microscope (Neo-Tech). The optimal incubation temperature was used to determine the incubation time required for pollen germination (10, 15, 24, 30, or 48 h).

Statistical analysis of the data

The data obtained were subjected to descriptive analysis using R (Rcmdr) software version 4.3.0, where one- and two-factor analysis of variance (ANOVA) were performed. Where a significant difference was revealed between means when the probability $p < 0.05$, Tukey's HSD test at the 5% threshold was used for classification.

RESULTS AND DISCUSSION

Staining of the pollen grains with acetocarmine revealed viable and non-viable pollen grains (Figure 1). ANOVA revealed that cold-storage time significantly influenced pollen grain viability ($P<0.05$; Table 1). The results showed that pollen grains cryopreserved for one month (M1) had the highest viability rates. For a storage period of 1 month, the viability rate of pollen stored at 3 °C varied from 90.23% to 98.82%, while those stored at -26 °C recorded survival rates ranging from 71.23% to 80.32%, depending on the sampling period. Therefore, these results revealed a decline in pollen viability with increasing cold-storage time. Similarly, pollen viability was significantly influenced by the incubation temperature ($P<0.05$). Pollen grains stored at very low temperatures (-26 °C) recorded the lowest viability rates compared with those stored at 3 °C, irrespective of incubation time and sampling period.

Furthermore, the analysis in Table 1 shows a decrease in the viability of pollen grains collected during the warmer hours of the day (from 12 to 16 h), irrespective of the incubation time and temperature. Thus, for the best viability rates recorded at M1, the viability rate of pollen grains incubated at 3 °C was over 98.77% from 9 h to 11 h. However, this high viability rate decreased, fluctuating between 90.23% and 95.99% between 12 and 16 h, before increasing to approximately 95% between 17 and 18 h.

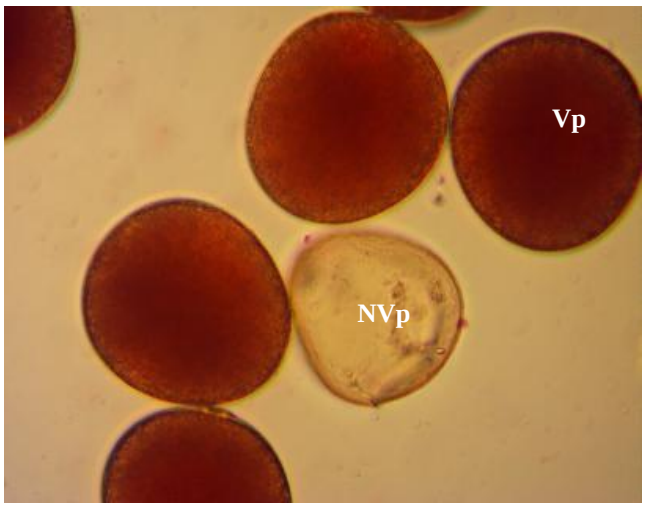

Figure. 1. Microphotograph of low-temperature corn pollen, viable pollen (Vp), non-viable pollen (NVp).

Table 1. Influence of storage time, temperature, and sampling period on pollen viability (%).

Incubation time (Month)	T (° C)	Pollen viability (%) Sampling period 9 h	10 h	11 h	12 h	14 h	15 h	16 h	17 h	18h
M1	3	98.82 ± 0.01^{a}	98.82 ± 0.01^{a}	98.77 ± 0.03^{a}	95.99 ± 0.03^{a}	90.59 ± 0.03^{a}	90.23 ± 0.02^{a}	91.81 ± 0.02^{a}	94.76 ± 0.02^{a}	95.51 ± 0.02^{a}
M2	3	95.01 ± 0.05^{b}	95.01 ± 0.03^{b}	95.01 ± 0.02^{b}	91.53 ± 0.3^{b}	86.13 ± 0.3^{b}	82.87 ± 0.4^{b}	84.45 ± 0.4^{b}	87.40 ± 0.4^{b}	88.15 ± 0.4^{b}
M3	3	93.30 ± 0.1^{c}	93.30 ± 0.1^{c}	86.60 ± 0.1^{c}	77.40 ± 0.3^{c}	72.00 ± 0.3^{c}	74.23 ± 0.01^{c}	75.81 ± 0.01^{c}	78.76 ± 0.01^{c}	79.51 ± 0.01^{c}
M1	-26	80.24 ± 0.1^{e}	80.28 ± 0.1^{e}	80.20 ± 0.1^{e}	80.32 ± 0.2^{e}	74.92 ± 0.2^{e}	71.23 ± 0.01^{d}	72.81 ± 0.01^{d}	75.76 ± 0.01^{d}	76.51 ± 0.01^{d}
M2	-	80.32 ±	80.21 ±	80.10 ±	80.35 ±	74.95 ±	68.15 ±	69.73 ±	72.68 ±	73.43 ±

	26	0.2^{e}	0.3^{e}	0.3^{e}	0.2^{e}	0.2^{e}	1^{e}	1^{e}	0.1^{e}	1^{e}
	-	76.74 ±	76.81 ±	76.67 ±	73.99 ±	68.59 ±	65.22 ±	66.80 ±	69.75 ±	70.50 ±
M3	26	0.2^{d}	0.03^{d}	0.3^{d}	0.03^{d}	0.03^{d}	0.01^{f}	0.01^{f}	0.01^{f}	0.01^{f}
(P< 0.05)		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Mean values within a column followed by the same letters are not significantly different at p ≤ 0.05 by Tukey's HSD test; ± standard error; M1 (Month 1); M2 (Month 2); M3 (Month 3)

Different germination media were used to induce pollen tube formation (Figure. 2). The rate of pollen grain germination was influenced by the sucrose concentration (Figure. 3). The pollen germination rates ranged from 15% to 97%. A sucrose concentration of 25 g/l in the culture medium resulting in the highest germination rate (97%). Consequently, 25 g/l sucrose was used for further experiments.

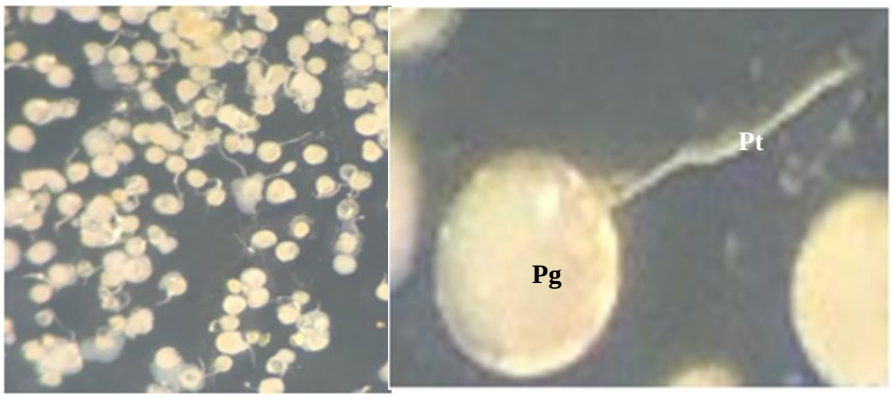

Figure 2. *In vitro* germination of maize pollen grains, pollen grain (Pg), pollen tube (Pt).

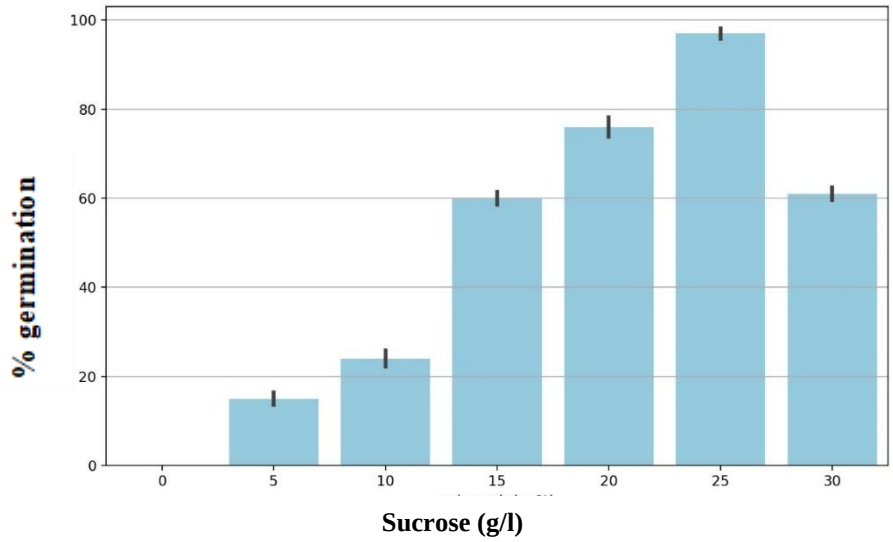

Figure 3. Influence of sucrose concentration on *in vitro* pollen grain germination in maize.

We next tested the effect of different pH levels on the germination of pollen grains. Germination was significantly influenced (P< 0.05) by changes in pH (Fig. 4). The highest germination rate (97%) was recorded in a medium with pH 6, whereas the lowest germination rate (24%) was recorded in the pH 8 medium.

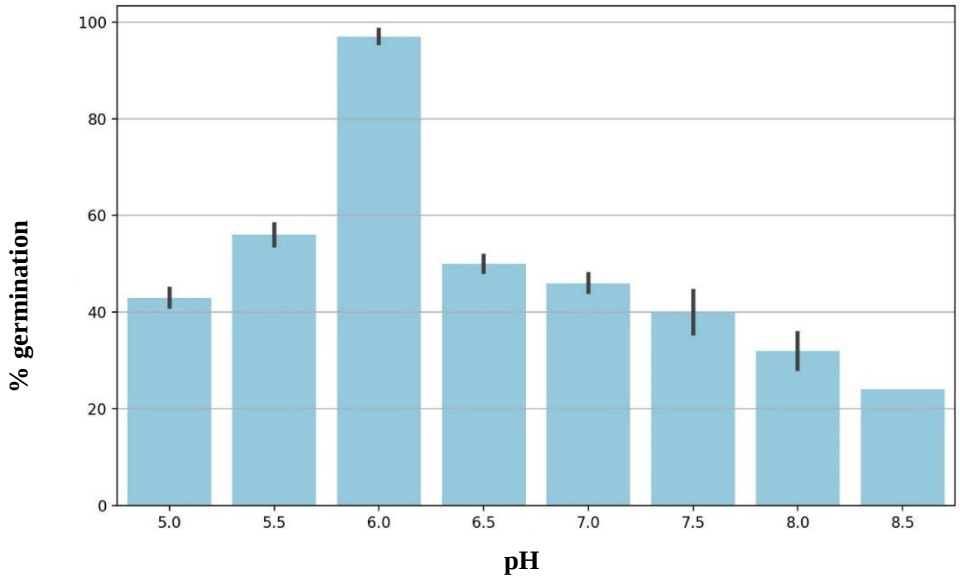

Figure 4. Effect of pH on *in vitro* germination of maize pollen grains.

Incubation trials with germination media at different temperatures further optimized the *in vitro* germination of pollen grains (Figure. 5). The germination rate of pollen grains increased steadily from 46% to 96% as the incubation temperature increased from 20 °C to 30 °C. The best germination rate (96%) was recorded at 30 °C. Beyond this optimum temperature, a decrease in germination rate was observed, reaching its lowest value (15%) at 40 °C.

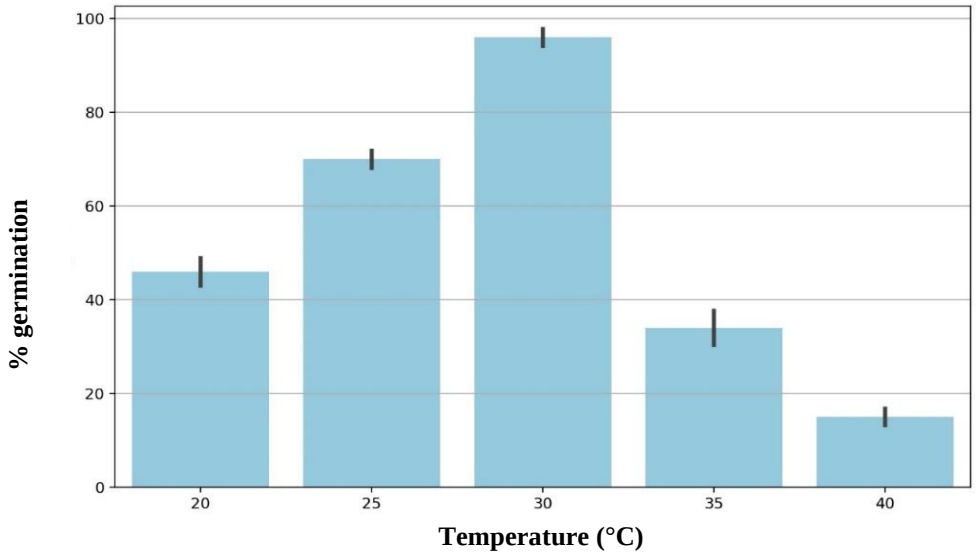

Figure 5. Influence of incubation temperature on *in vitro* germination of pollen grains.

The results showed that incubation time significantly influenced pollen germination (Figure 6). The 24 h incubation period was the most effective in optimizing *in vitro* pollen grain germination, achieving a rate of 97%.

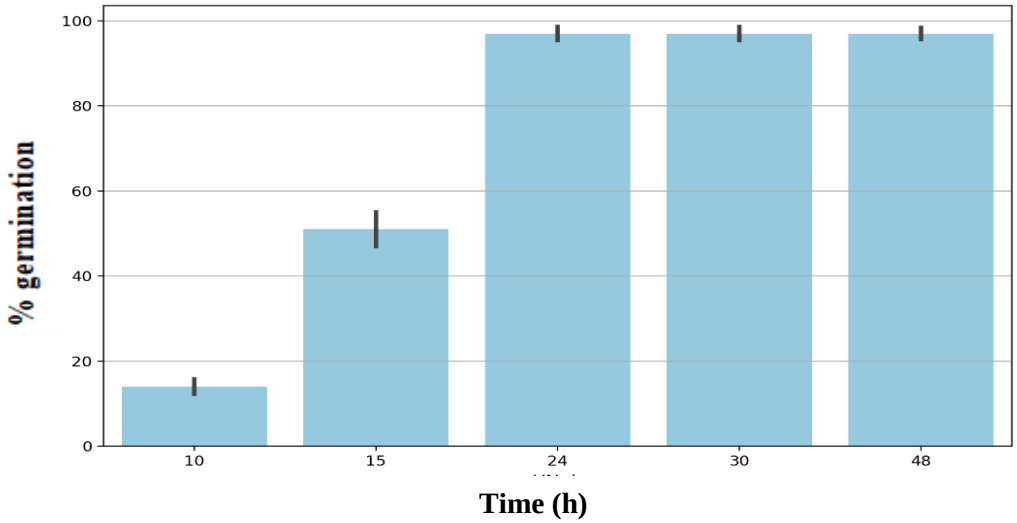

Figure 6. Pollen germination capacity as a function of incubation time.

The aim of pollen conservation is to maintain, over a long period of time, the ability to fertilize and produce viable offspring (Charrier,1990). To this end, knowledge of the collection time, temperature, and storage time is essential to maintain pollen viability. In our study, variations in pollen viability were observed over a three-month period. The results showed a high viability rate for pollen grains collected between 9 h and 11 h, irrespective of storage time and temperature. In contrast, pollen collected after 11 h exhibited a reduced viability rate, especially during warmer hours (12 to 18 h). These results suggest that pollen collected in the morning is better suited for fertilization. This indicates that the pollen had reached morphological maturity; they are cytodifferentiated, resulting in good fertility. Fonseca and Westgate,(2005) reported that the viability rate of a pollen sample is positively correlated with the average water content. The latter decreased progressively with daily ambient temperature. Over the course of the day, the ambient air temperature increased progressively, leading to pollen dehydration and a drop in pollen viability between 12 and 18 h. Our results are consistent with those of Veiga *et al*. (2012) who reported that corn pollen collected in the morning had higher germination rates than pollen grains collected in the afternoon. They also observed a decrease in the water content and viability of pollen grains over the course of the day, leading to a reduction in fertilization opportunities.

In our study, an assessment of the influence of temperature on the ability of pollen grains to maintain fertility and productivity resulted in a viability rate of 98% at 3 °C and 80% at -26 °C after one month of incubation. The best temperature for maintaining corn pollen grain viability was therefore 3 °C. This could be explained by the fact that at this temperature, cytoplasmic contents are not frozen; therefore, the enzymatic reactions required for cell function are still active, as molecular mobility in the cytoplasm is not inhibited. On the other hand, at -26 °C, freezing the cytoplasmic content inhibits certain reactions and leads to the formation of ice crystals capable of damaging cells. Fabienne *et al*. (2000) showed that a storage temperature between 3 and 8 °C is one of the best storage temperatures for black spruce. Buitink et al. (2000) also showed that low temperatures reduced molecular mobility in the cytoplasm, which may be a determining factor for longevity. Similarly, Bramlett and Matthews, (1991) reported that pollen preservation is delicate below -20 °C.

The results obtained show that pollen stored at 3 °C and -26 °C maintains its viability for a limited time. According to Hanna and Towill, (1995), pollen of many species can be stored at temperatures between 4 °C and -20 °C in the short term. However, dry pollen stored between 4 °C and -20 °C remains viable for a few days to a year, which is sufficient for use in breeding programs. Indeed, under natural conditions, the lifespan of a pollen grain varies from a few hours to 24 h for grasses, such as maize or wheat, to several weeks depending on the ecological conditions. In summary, the viability of maize pollen depends on several parameters. According to Buitink *et al*. (2000), successful pollen storage depends on the long-term monitoring of stored pollen. Water content and storage temperature affect the longevity of stored pollen. In addition, regardless of the storage temperature, the time of pollen collection significantly affected the viability rate.

Pollen germination is crucial for the success of breeding programs. To this end, the best combination identified for this study was the basic *in vitro* germination medium enriched with 25% sucrose and adjusted to pH 6, under an incubation temperature of 30 °C for 24 h. Medium containing 25% sucrose induced 97% pollen germination. This high rate of pollen germination is in line with the results obtained by Younbi et al. (2009) for pollen from *Pachypodium lameiri, Catharanthus roseus*. The authors

indicated that sugar is necessary for pollen grain germination and that its concentration varies between species of the same family and between taxa belonging to different families. Sugar plays an osmotic role in the culture medium but also serves as a nutrient for pollen tube growth (Youmbi *et al.,* 2009).

pH also plays a significant role in optimizing the germination medium. In this study, pH 6 was used to optimize the *in vitro* germination of pollen grains. This pH differs from that used by Youmbi *et al.* (2009) (pH 5.3), who focused on the germination of *Darcryode edulis* pollen. According to these authors, pH is a factor that stimulates pollen germination, the value of which depends on the species. Thus, pollen germination depends on the specific pH, above or below which germination may be inhibited or weakened. pH is involved in regulating the biochemical processes required for pollen germination, such as cell wall degradation, pollen tube growth, and energy metabolism (Munzuroglu *et al.*,2003). The high germination rates showed that pH6 effectively interacted with the composition of the medium (nutrients), making it more favorable for pollen germination. In addition, the incubation temperature of the culture medium played an important role in activating pollen grain metabolism. Indeed, optimal temperature activates enzymatic reactions and metabolic processes involved in pollen tube germination (Liu *et al*., 2023). These observations confirmed the high germination rates observed between 25 ºC and 30 ºC during the experiment. High temperature (30 ºC) intensively promoted the activation of enzymatic and metabolic processes in pollen grains, resulting in a maximum germination rate of 97%. Our results are consistent with those of (14), who showed that pollen viability and germination are highly sensitive to heat stress and are greatly affected by high temperatures for long durations or extreme temperatures for short durations.

Pollen germination rates varied widely. Most pollen germinated easily after 24 h; however, germination below 24 h was poor. In addition, an incubation time between 10 and 15 h is not conducive to germination because the germinative power of pollen enters dormancy. These results are consistent with those of (Verdeil & Pannetiero 1990). These authors showed that the stimulation of pollen germination after several months depended on rehydration with gaseous water or a semi-solid culture medium. According to these authors, pollen rehydration (a slow-living organ of dissemination) enables a metabolic response in which gas exchange (respiration) plays an important role. In our study, we found that metabolic processes, including cellular respiration and protein synthesis, required 24 hours for activation. After this period, these processes actively resume, enabling pollen ejection from the pollen tube.

CONCLUSION

A study of androgenesis in maize (*Zea mays* L.) pollen from EV8728 suggested that pollen reproductive quality and viability depend on the storage method and harvesting period. Variations in pollen storage temperature significantly affected pollen viability. A temperature of 3 °C induced the highest viability rates. In addition to the influence of temperature variation, a storage period of one month and a daily pollen collection period of 9 h to 11 h favored pollen viability rates. In contrast, pollen collected between 11 h and 16 h had the lowest viability rates. This study of corn pollen grains highlights the characteristics that make them susceptible to preservation. These results are corroborated by the fact that 97% of the germinative potential was retained after 1 month of storage at 3 °C. The germinative potential of the preserved pollen was tested on a medium containing 2 g/l magnesium sulfate, 1 g/L potassium nitrate, 3 g/l calcium nitrate, and 10 g/l gelrite with 25% sucrose at pH 6. Incubation was performed in the dark at 30 °C for 24 h. This study enabled us to identify the correct harvesting period and storage temperature, which will make it possible to create a pollen bank of different varieties for future work. The creation of this bank will enable research activities to be conducted to select homogeneous and stable elite varieties. It would also be interesting to store pollen at more varied temperatures to find the optimum storage temperature range and to increase the storage time to determine the maximum duration of pollen grain viability.

ETHICS APPROVAL

Not applicable

FUNDING

This project IVC5040 was funded by the IAEA and the Government of Côte d'Ivoire.

ACKNOWLEDGEMENTS

The authors would like to thank the International Atomic Energy Agency (IAEA) for their financial support of project IVC5040 and the coordinator Professor KOUADIO Yatty for their contribution to the success of this activity.

REFERENCES

Akanvou L, Akanvou R, Toto K. (2006). Effets des variétés de maïs et de légumineuses dans la lutte contre *Striga hermontica* en zone de savane en Côte d'Ivoire. *Agronomie Africaine.* 18, 13-21. https://doi.org/10.4314/aga.v18i1.1675

Benmahammed A, Kribaa M, Bouzerzour H, Djekoun A. (2010). Assessment of stress tolerance in barley (*Hordeum vulgare* L.) advanced breeding lines under semi arid conditions of the eastern high plateaus of Algeria. *Euphytica* 172, 383–394. https://doi.org/10.1007/s10681-009-0046-x.

Blassonny, R. (2013). Ivoire grain. http://www.ivoiregion.net/index.php/lanouvelle/726-germination-le-maïs-veut-damer-le-pion-au-coton. Access on 26/10/2023.

Bramlett DL, Matthews FR. (1991). Storing loblolly pine pollen. *Southern Journal of Applied Forestry*. 15, 153–157. https://doi.org/10.1093/sjaf/15.3.153.

Buitink J, Leprince O, Hemminga MA. Hoekstra FA. (2000). The effects of moisture and temperature on the ageing kinetics of pollen : Interpretation based on cytoplasmic mobility. *Plant Cell Environment*. 23, 967-974. https://doi.org/10.1046/j.1365-3040.2000.00601.x

Charcosset M, Gallais N. (2009). Théorie de la sélection en amélioration des plantes. Conférence Internationale sur les Moyens Alternatifs de Protection des Cultures, Torino (Italie) pp 68-71.

Charrier A. (1990). Pollen et ressources génétiques, *Bulletin de la Société Botanique de France*. 137, 101-104. https://doi.org/10.1080/01811789.1990.10827005.

Colas F, Mercier S. (2000). Évaluation du maintien de la viabilité des pollens utilises dans le programme amélioration des arbres. Mémoire de recherche forestière n° 135, 98p. Memoire135.pdf (gouv.qc.ca)

FAOSTAT. (2020). Agricultural production, cropprimarydatabase. Food and agricultural organization of the United Nations, Rome. http://faostat.fao.org/faostat/. Access on 20/05/2022.

Farahani H, Wagiran R, Hamid MN. (2014). Humidity sensors principle, mechanism, and fabrication technologies: A comprehensive review. *Sensors*, 14, 7881-7939. https://doi.org/10.3390/s140507881 .

Fonseca AE, Westgate ME. (2005). Relationship between desiccation and viability of maize pollen. *Field Crops Research.* 94, 114-125. https://doi.org/10.1016/j.fcr.2004.12.001

Genovesi AD, Collins GB. (1982). *In vitro* production of haploid plants of corn via anther culture. *Crop Science*. 22, 1137-1144. https://doi.org/10.2135/cropsci1982.0011183X002200060013x.

Hanna WW, Towill LE. (1995). Long-term pollen storage. *Plant Breeding Reviews*. 13, 179-207. https://doi.org/10.1002/9780470650059.ch5

Kouakou CK, Akanvou L, Konan YA, Mahyao A. (2010). Stratégies paysannes de maintien et de gestion de la biodiversité du maïs (*Zea mays* L.) dans le département de Katiola, Côte d'Ivoire. *Journal of Applied Biosciences.* 33, 2100-2109. https://m.elewa.org/JABS/2010/33/9.pdf.

Liu L, Ziyan L, Hu H, Baiyu Q, Yangfan L, Xiongkui H, Yajia L. (2023). Effect of pre-germination temperature regime on pollen germination and fruit set in pear, *Pyrus bretschneiderilia*. Horticulturae. 9, 1151. https://doi.org/10.3390/horticulturae9101151.

Munzuroglu O, Obek E, Geckil H. (2003). Effects of simulated acid rain on the pollen germination and pollen tube growth of apple (*Malus sylvestris* miller cv. Golden). *Acta Biologica Hungarica*. 54, 95-103. https://doi.org/10.1556/ABiol.54.2003.1.10.

Naveed S, Aslam M, Maqbool MA, Bano S, Zaman QU, Ahmad RM. (2014). Physiology of high temperature stress tolerance at reproductive stages in maize. *Journal of Animal and Plant Sciences*, 24, 1141-1145. https://www.researchgate.net/publication/269985726.

Nuss ET, Tanumihardjorefe SA. (2011). Quality protein maize for Africa: Closing the Protein Inadequacy Gap in Vulnerable Populations. *Advance Nutrition*, 2, 217-224. Doi: 10.3945/an.110.000182.

Kleiber D, Prigge V, Melchinger AE, Burkard F, San Vicente F, Palomino G, Gordillo GA. (2011). Haploid Fertility in Temperate and Tropical Maize Germplasm. *Crop Science.* 52, 623-630. https://doi.org/10.2135/cropsci2011.07.0395.

Soro D, Ayolie K, Ferdinand GBZ, Ferdinand YY, Hippolitte KK, Sydiky B, Pascal TA, Yatty Kouadio Y. (2015). Impact of organic fertilization on maize (*Zea mays* L.) production in a ferralitic soil of Centre – West Côte d'Ivoire. *Journal of Experimental Biology and Agricultural Sciences.* 3, 556-565. http://dx.doi.org/10.18006/2015.3(6).556.565.

Veiga P, De OA, Von Pinho RG, Von Pinho EV, De R, Veiga A.D, De Olivira KC, Diniz RP. (2012). Meios de cultura para germinação de grãos de pólen de milho/ Culture media for germination of pollen grains of maize. *Revista. Agrarian*. 5, 206-211. DOI:10.30612/agrarian.v5i17.1054.

Verdeil JL, Pannetiero C. (1990). Optimisation des conditions de germination in vitro du pollen de cocotier (*Cocos nucifera* L.) pour la mise au point d'un test de viabilité. Oléagineux, 45, 175-181. https://agritrop.cirad.fr/441175/1/ID441175.pdf.

Youmbi E, Cerceau-larrival MT, Verhille AM, Carbonnier-Jarreau MC. (2009). Morphologie et germination *in vitro* du pollen de *Dacryodes edulis* (Burseraceae)/ Détermination des facteurs contrôlant la germination. *Grana*, 37, 87-89. https://doi.org/10.1080/00173139809362648.