International Journal of Agricultural Technology 2022Vol. 18(2):447-458
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The effect of timing and storage temperature on pollen viability and pollen germination in *Zephyranthes* Hybrid

Anuwong, C.*, Teerarak, M. and Saetiew, K.

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Anuwong, C., Teerarak, M. and Saetiew, K. (2022). The effect of timing and storage temperature on pollen viability and pollen germination in *Zephyranthes* Hybrid. International Journal of Agricultural Technology 18(2):447-458.

Abstract The effect of timing and storage temperature on pollen viability and pollen germination in *Zephyranthes* hybrid on eight cultivars i.e. 'Red Candy', 'Bright Eye', 'Golden Mango', 'POS', 'Lookmai', 'Island Breeze', 'Madam Butterfly' and 'Paradee' were studied. The results showed that the low temperature stored pollen maintained a higher germination percentage than the one stored in the room temperature for 4-8 days in which the germination percentage decreased when the storage period increased in all cultivars. Moreover, the interaction between the 2 factors showed that the pollen germination percentage was declined for 4 or 8 days when stored at low temperatures (15.59-27.96% or 10.10-19.25%). Therefore, the optimal time of pollination in *Z.* hybrid should do at 09.00-11.00 a.m. and stored at 5˚C for pollen viability and germination. 'Bright Eye' and 'Island Breeze' could be maintained for 12 days, but 'Golden Mango', 'Red Candy', 'POS', 'Madam Butterfly', 'Lookmai' and 'Paradee' could be maintained only for 8 days.

Keywords: Pollen grains, Stored pollen, Rain lily, Flowering period

Introduction

Zephyranthes spp. is belonged to the Amaryllis family and comprised over 70 species. This genus of plants is native to the Americas. Several species of them have been spread and become naturalized in other countries such as Hawaii, Indonesia, and Thailand (Meerow *et al*., 1999; Chowdhury and Hubstenberger, 2006; Spurrier *et al*., 2015; Pacific Bulb Society, 2021;). Many are values as ornamentals and landscaping with various colors and petal forms (Marta, 2005), such as *Zephyranthes brachyandra*, *Z. candida*, *Z. grandiflora, Z. robusta*, and *Z. rosea* (Dutilh, 2005). The other species were used according to local tradition for defense from various diseases such as viral infections, breast cancer, ear and chest ailments, diabetes mellitus, and tumors (Katoch and Singh, 2015) such as *Z. grandiflora* (Kai *et al*., 2006). The cultivation from seed is easy in this group, but apomictic, cross incompatible, or have widely variable 2n chromosome numbers still was found in some species. Moreover, these

* **Corresponding Author:** Anuwong, C.; **Email:** chamaiporn.an@kmitl.ac.th

plants have non-synchronized flowering and have a relatively short viability period (Chowdhury and Hubstenberger, 2006; Pacific Bulb Society, 2021). Thus, the breeding programs often encounter impediments. The reciprocal crosses may be a difficulty. At that time, plant breeding to enhance the value of ornamental plants cannot be successful in pollinates (Chowdhury and Hubstenberger, 2006).

In vitro, pollen germination is an honorable method for testing pollen viability. Pollen survival is a key factor in successful breeding. It may take as little as a few minutes in self-pollinated plants to hours or days in cross-pollinated plants. The viability of pollen can be determined by staining techniques. Nuclear dyes, such as the acetic carmine, bring the color of the pollen to life. (Mercier, 1995).

Although it is easy to use, however, it may not reflect the actual ability to germinate, for example, some colored pollen grains cannot germinate due to limitations in the pollen tube growth (Galletta, 1983). In bananas, the evaluated pollen viability by acetocarmine staining was investigated that high viability of more than 80% (Soares *et al*., 2016). Determination of pollen viability is very important in artificial pollination, especially related to different species or genus. In vitro pollen germination is the most dependable method, which was the technique used to estimate the viability of pollen, and one of the pollen germination testing methods created by Brewbaker and Kwack (1963, 1964) is widely used and has some modifications (Jayaprakash, 2018). Factors affecting pollen germination in vitro such as species, culture medium, temperature, incubation period, the stage development of flower when collecting pollen, and storage conditions (Stanley and Linskens, 1974; Sharafi, 2011).

One of the limiting factors of cross-pollination is the timing of flowers and pollen storage method. The use of the stored pollen in a cold room at 4-6 ℃ and pollinated close to noon resulted in seed formation and a high number of pollinations. The use of many pollen parents may help to overcome some barriers of the breeding for both *Zephyrenthes* and *Habaranthus* species and hybrids (Chowdhury and Hubstenberger, 2006). Similar to the herbaceous peony (*Paeonia lactiflora* Pall.) the pollen could be sufficiently 4℃ stored for hand-pollination even the cultivars which have discrepant flowering in a season. The different storage temperature is suitable for different herbaceous peony pollen rather than one year. (Guangcong *et al*., 2019). In addition, the pollen of six species of date palm (*Phoenix dactylifera* L.) cultivars stored at room temperature lose most of their viability between 2 or 3 months of storage. The time passed when the pollen was stored under low temperatures with a storage temperature at −20°C, better than 4°C. (Mohammed *et al*., 2018).

A new variety is generally bred by crossbreeding. The Pollen integrity and flowering mismatch are the important factors affecting reproduction. Some cultivars are forbidden to be parents for propagation

due to flower mismatch problems. Therefore, it is essential to store the pollen, which is accumulated from the male donor parents for later self-pollination. The pollen storage is valuable for breeding programs, genetic conservation, artificial pollination, and self-incompatibility. The longevity of pollen varies greatly with plant species and storage conditions (Mukti, 2014). Pollen is usually sensitive to temperature and loses viability under natural conditions, consequently, pollen preservation is problematic. However, the different storage temperatures, the optimum temperature for pollen germination depends on species and also vary between cultivars (Loupassaki *et al*., 1997). At present, we have various new *Zephyranthes* hybrid occurred by breeding from Thai breeders but the studies of *Zephyranthes* hybrid pollen storage viability are rarely reported in Thailand. Thus, this study aimed to estimate the effect of timing and storage temperature on pollen viability and pollen germination of *Zephyranthes* hybrid. Furthermore, we used the collected timing and the storage conditions to measure the optimal method for short-term pollen storage in breeding a new *Zephyranthes* hybrid in Thailand.

Materials and methods

Plant materials

The eight selected cultivars of *Zephyrenthes* hybrid included, ‘Red Candy’, ‘Bright Eye’, ‘Golden Mango’, ‘POS’, ‘Lookmai’, ‘Island Breeze’ ‘Madam Butterfly’ and ‘Paradee’ (Figure 1).

Figure 1. The eight cultivars flower characteristics of *Zephyrenthes* hybrid

The 6-8 cm flowering size bulb in circumference was planted in an 8 inches (20.32cm) black plastic pot with soil, rice husk ash, and coconut coir ratio 1:1:1. Each bulb was being supplied with chemical fertilizers (16-16-16) at 0.5 grams per month. They were watering every day with a sprinkler system for around 10 minutes each time. The plant was cultivated from April 2019 to April 2020 in the outdoor field at the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The average temperature was 31.0 - 35.0 ℃, the air humidity was about 60 - 63% RH and the light intensity was between 242.65 - 684.34 μmol/s/m^2. The plant started flowering between June-July.

Pollen collection and storage

The experiment was separated into two experiments. The first experiment was an effect of timing on pollen viability and pollen germination of *Zephyranthes* hybrid. The experiment was a completely randomized design (CRD) with three treatments and three replications, each flower per replication. The pollen was collected in different periods during the first anthesis day; 1) 09.00-11.00 a.m., 2) 12.00 a.m.-02.00 p.m., and 3) 03.00-05.00 p.m. The pollen viability was investigated by the 2% acetocarmine staining method. The pollen grains were put on a slide with a droplet of 2% acetocarmine. Then they were spread out and close with the coverslip. After being observed for a few minutes, the consistently dark red stained pollen grains with a round shape were marked as "viable," while abnormally sized and unstained pollen were marked as "non-viable" (Figure 2). For both methods, at least 40 pollen grains per visual field were measured randomly under a compound light microscope at 400x magnifications. The calculation pollen viability percentage was used from the number of viable divided by the number of pollens in all visual fields and multiplied by 100. For the In vitro pollen germination test followed by Brewbaker and Kwack (1963), the germination culture solution contained Boric acid 0.01 g/100 ml, $Ca(NO_3).4H_2O$ 0.03 g/100 ml, $MgSO_4.7H_2O$ 0.02 g/100 ml, KNO_3 0.01 g/100 ml, 10% Sucrose 10g/100 ml and the distilled water. The pollen germination was evaluated after 24 hours. If the length of the pollen tube was equal to or exceeded the pollen diameter after being cultured for 24 hours at 25 ℃, the pollen was distinguished as germinated. The pollen germinated were recorded under a compound light microscope at 400x magnification. The pollen germination percentage was calculated by using the number of germinated pollens divided by the number of pollens in all visual fields and multiplied by 100.

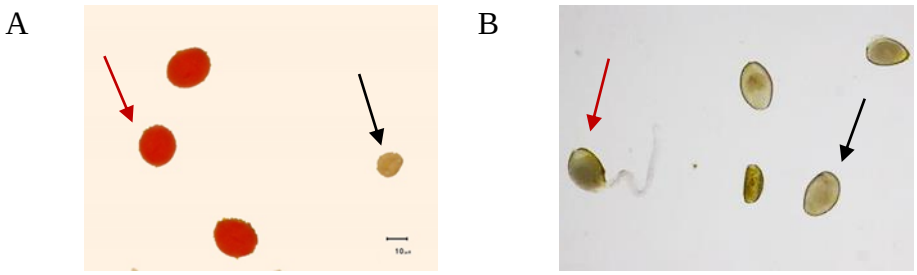

Figure 2. Pollen stained by the 2% acetocarmine staining method (A) and germinated pollen (B) was estimated under a light microscope at 400x magnification. The red shaft represented viable pollen (A) and germinated (B), the black shaft represents no viability (A) and non-germinated (B)

The second experiment was the effect of storage temperature on pollen viability and pollen germination of *Zephyranthes* hybrid. The pollen samples from anthers of 1-day blooming flowers were randomly collected at intervals with the highest viability and germination percentages from the experiment 1. Pollen was kept in a glass vial to prevent contamination and then stored in different storage conditions. The experiment was carried out in two variants, using 2×6 factorial in CRD. The first variant was the storage temperatures (25±2 ℃ and 5±2 ℃), and the second variant was the storage periods (0, 4, 8, 12, 16, and 20 days). Each treatment has four replications and three flowers per each replication. Afterthought, the pollen was randomly cultured in Brewbaker and Kwack's (1963) solution for 24 hours at 25 ℃ and at least 40 pollen grains per visual field were measured randomly under a compound light microscope at 400x magnification. The pollen germination percentage was calculated by using the number of germinated pollens divided by the number of pollens in all visual fields and multiplied by 100.

Data analysis

The collected information is subjected to analysis of variance (ANOVA) in CRD (Ex.1) and 2x6 factorial in CRD (Ex.2) using the Statistix8.0 software program. The means of values were paralleled by the Least Significant Difference Test ($P \leq 0.05$).

Results

The effect of timing on pollen viability and pollen germination of Zephyranthes hybrid

The test of *Zephyranthes* hybrid fresh pollen viability with the 2% acetocarmine staining method revealed that the different periods of pollen

collection comprised of ‘Red Candy’, ‘Bright Eye’, ‘Golden Mango’, ‘Lookmai’, ‘Island Breeze’, ‘Madam Butterfly’ and ‘Paradee’ was not statistically significant (Table 1). Most of them have viability exceeded 60.00% except ‘Paradee’. However, ‘Red Candy’ showed the maximum percentage of pollen viability when they were collected at 09.00-11.00 a.m. (88.06%), while ‘Paradee’ has the lowest when they were collected at 03.00-05.00 p.m. (54.69%). Remarkably, the pollen viability collected at 12.00 a.m.-02.00 p.m. gave lower results than the one at 09.00-11.00 a.m. and 03.00-05.00 p.m. which are ‘Red Candy’, ‘Bright Eye’, ‘Island Breeze’ and ‘Madam Butterfly’. Nevertheless, ‘POS’ was significantly different that showed the highest pollen viability percentage (84.91%) when collected at 09.00-11.00 a.m. and decreased during the daytime, especially in the evening (03.00-05.00 p.m.).

Table 1. The pollen viability of *Zephyranthes* hybrid collected at different periods

Pollen collection time	***Zephyranthes* cultivars**							
	Red Candy	**Bright Eye**	**Golden Mango**	**POS**	**Look mai**	**Island Breeze**	**Madam Butterfly**	**Para dee**
09.00-11.00 a.m.	88.06	81.60	72.24	84.91a[1/]	77.63	85.62	78.09	76.62
12.00 a.m.-02.00 p.m.	83.88	73.60	72.95	80.15ab	84.24	81.25	64.52	60.88
03.00-05.00 p.m.	85.05	82.05	73.76	74.29b	81.91	81.83	66.98	54.69
C.V. (%)	3.14	6.55	4.32	4.79	5.53	6.24	11.47	14.10
$LSD_{0.05}$	ns	ns	ns	7.64	ns	ns	ns	ns

[1/] Means within a cultivar followed by the same letters was not different at P<0.05 (LSD test)
ns= non-significant

As the pollen germination of *Zephyranthes* hybrid *In vitro* germination medium collecting in the different periods which are ‘Red Candy’, ‘Lookmai’, ‘Madam Butterfly’ and ‘Paradee’ were not statistically significant (Table 2). The percentages of ‘Red Candy’ pollen germination was between 17.89-22.33%, ‘Lookmai’ 28.12-31.83%, ‘Madam Butterfly’ 31.98-35.43%, and average 27.04-36.82% ‘Paradee’. Besides, the pollen collected at a different time in ‘Bright Eye’, ‘Golden Mango’, ‘POS’ and ‘Island Breeze’ was significantly different. The percentage of germinated pollen in ‘Bright Eye’ and ‘POS’ collected at 09.00-11.00 a.m. was the highest at 26.28% and 54.33%, respectively. The germination of ‘Golden Mango’ was also the highest at 09.00-11.00 a.m. (38.83%) but it was not different from the one collected at 03.00-05.00 p.m. (33.06%). Also, the

highest germination of 'Island Breeze' was found at 09.00-11.00 a.m. (26.58%) and 12.00 a.m.-02.00 p.m. (27.86%). The maximum germination of pollen was found in 'POS' (54.33%) when collected at 09.00-11.00 a.m., and the minimal viability was 'Bright Eye' (14.77%) when collected at 03.00-05.00 p.m. The pollen collection time at 09.00-11.00 a.m. was predominant higher germination than other times in almost eight cultivars.

Table 2. The pollen germination of *Zephyranthes* hybrid collected at different periods

Pollen collection time	*Zephyranthes* cultivars							
	Red Candy	**Bright Eye**	**Golden Mango**	**POS**	**Look mai**	**Island Breeze**	**Madam Butterfly**	**Para dee**
09.00-11.00 a.m.	22.33	26.28a[1/]	38.83a	54.33a	30.61	26.58a	35.43	36.82
12.00 a.m.-02.00 p.m.	18.08	18.59b	25.66b	29.58b	28.12	27.86a	31.98	27.04
03.00-05.00 p.m.	17.89	14.77b	33.06a	27.58b	31.83	16.01b	33.95	31.80
C.V. (%)	22.82	13.04	11.12	10.32	15.95	18.75	8.26	13.61
$LSD_{0.05}$	ns	5.18	7.22	7.66	ns	8.80	ns	ns

[1/] Means within a cultivar followed by the same letters was not different at P<0.05 (LSD test)
ns= non-significant

The effect of storage temperature on pollen germination in Zephyranthes hybrid

There was an effect for *In vitro* review ($p \leq 0.05$) in all variables analyzed (the storage temperature, the storage period) and their interactions. The germinated pollen storage at 5±2 ℃ was significant twice the percentage higher than the one at 25±2 ℃. The storage period decreased the pollen germination percentages by about 5-10% every four days stored and declined nearly zero at 20 days in all cultivars (data not shown).

The interactions between storage temperature x storage period investigated that the germination percentage of most cultivars decreased to zero at later stages of storage and disclaimed the fastest at 25±2 ℃, which was verified by the *In vitro* pollen germination test (Figure 3). The germination percentage of almost all cultivars decreased to 0.00% at 12 or 16 days after storage at 25±2 ℃, except the 'POS' which declined the fastest at eight days. Nevertheless, the pollen storage at 5±2 ℃ was maintained a higher germination percentage than stored at room temperature for 4-8 days. However, the germination percentage decreased when the storage period increased in all cultivars. It declined to zero within 20 days in

‘Red Candy’, ‘POS’, and ‘Madam Butterfly’. At the beginning (0 days) the pollen germination in ‘Red Candy’, ‘Golden Mango’, ‘Island Breeze’, ‘Madam Butterfly’ and ‘Paradee’ have the highest germination percentage at 25.43%, 26.41%, 24.16%, 35.64%, and 28.74%, respectively, then they started to decline to 0% in 16 days after storage at 25±2 ºC. In ‘Bright Eye’, we found that the highest germination percentage was at 0 days (28.18%) in both temperatures and declined to 0% in 20 days after storage at 25±2 ℃. While the highest germination percentage of ‘POS’ at 0 days (33.73%) in both temperatures and declined to 0% in eight days after storage at 25±2 ℃. In ‘Lookmai’ found that the highest germination percentage was at 0 days (36.11%) in both temperatures and declined to 0% in 12 days after storage at 25±2 ℃.

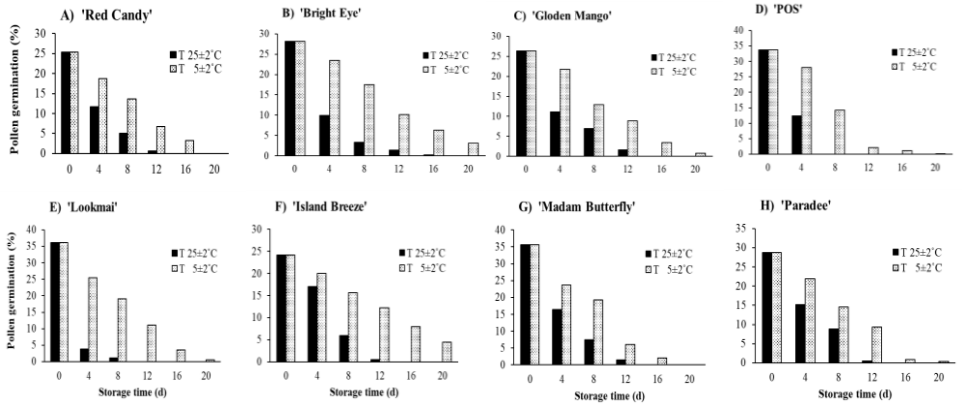

Figure 3. The pollen germination of *Zephyranthes* hybrid when stored in different temperatures (25±2 ℃ and 5±2 ℃) and periods (0,4,8,12,16,20 days)

Discussion

When the non-synchronized flowering between species or cultivars naturally occurs, the determination of the viability and storage of pollen is a practical method for breeding ornamentals (Naiara *et al*., 2019). Hence, the timing of pollination is crucial for the success of cross-pollination breeding. Although in this study, the different time was not significant on pollen viability and pollen germination in some cultivars e.g., the time between 09.00-11.00 a.m. showed higher pollen viability and pollen germination than some other’s time. Normally, the plant starts flowering around 10.00-12.00 a.m., thereby, it could be the time between 09.00-11.00 a.m. that they started flower opening which makes the pollen fresher and more active than the afternoon time. The results showed that the viability and the germination percentage were slightly decreased from the morning to the evening, especially in ‘POS’. Several factors can affect the pollen viability, for example, the handle of pollen in the collection, the flowering maturation

stage, and the external environments such as temperature and air humidity (Martins *et al*., 2017). Besides, the period of the day of pollen collection also induced in *Zephyrenthes* hybrid pollen viability. The pollen grains collected at 09.00-11.00 a.m., when temperature and relative humidities were approximately 31 ℃ and 63%; respectively, were more viable than the pollen collected at 12.00 a.m.-02.00 p.m. and 03.00-05.00 p.m. when temperature ranged between 35.0 and 32.5 ℃ and relative humidity ranged between 60% and 61%, respectively (data not shown). At 12.00 a.m.-02.00 p.m., the mean pollen viability was at the lowest. The most beneficial climatic conditions for pollen viability occurred between 09.00-11.00a.m., when there was no surplus humidity in *Zephyrenthes* hybrid flower, along with the temperature was gentle. These results are related to those acquired by Ferreira *et al*. (2007), who examined three different collection time points (09:00h, 14:00h, and 16:00h) and noticed the highest germination rate at 09:00h and the lowest at 16:00h. High temperatures, usually recognized from noon to late afternoon, can lessen the In vitro viability of the pollen grains. Kakani *et al*. (2005) stated that the high temperatures during anthesis might give onto failure in pollination or fertilization, which also develops in reduced viability. The optimum temperature for the growth of pollen tubes is in the range of 25-30 ℃. If this temperature exceeds, it is harmful to the soft tissues of the pistil and the stamens (Maheshwari, 1950).

The results indicated that the low temperature stored pollen maintained a higher germination percentage than the one stored in the room temperature for 4-8 days, and the germination percentage decreased when the storage period increased in all cultivars. The depletion in germination capacity of the pollen grains stored at room temperature may be caused by the inactivation of crucial germination enzymes and substrates (Youmbi *et al*., 2012). The enzymes will easily spread into the surrounding medium. Enzyme activity, which reduces respiratory substrates, brings about the pollen viability to decline during storage (Gandadikusumah *et al*., 2017). Similarly, the results in Amaryllis exhibited a rapid loss of viability at 25 ℃ storage temperature, and the temperature of -20 ℃ succeeded in the top responses for the defense of the absoluteness, and the preservation of the viability for individual Amaryllis cultivars (Naiara *et al*., 2019). The pollen grain germination of different Chinese chinquapin (*Castanea henryi*) cultivars remarkably reduced at all four storage temperatures (room temperature at 25 °C, 4°C, −20°C, and −80°C). The pollen viability was 14.4% after only 24 days of storage at room temperature. The germination rate was 13.3% after 90 days of storage at 4 ℃, and 14.5% after 180 days at −20°C (Xiong *et al*., 2020). The germination rate of 'Baniluzi' was 1.5% after 4 days at room temperature (Zheng *et al*., 2004). The germination rate of 'YLZ01' was 2.12% after 10 days at room temperature (Lai *et al*., 2017). The interaction between 2 factors resulted in the declined of the pollen germination percentage for 4 or 8 days when stored at low temperatures

which were similar to Phalaenopsis, in which the pollen can be kept at 4 ℃ for up to 40 weeks for temporary-term purposes, while they lost its viability after 4 weeks at room temperature (Yuan *et al*., 2018). Metz *et al*. (2000) noticed that the pollen of *Hylocereus* stored at 4 ℃ for 3 or 9 months exhibited only 60–70% fruit set after pollination. The pollen longevity has been discovered to be expanded by storing at lower temperatures (4, −20, and −80°C) in other plant species, for example, in Almond (Martínez-Gómez *et al*., 2002), Cherimoya (Lora *et al*., 2006), Mango (Dutta *et al*., 2013), Hazel (Novara *et al*., 2017), Date Palm (Maryam *et al*., 2017), and several others. These discoveries encourage the outcome acquired by other researchers, who indicated that the pollen viability was higher at lower temperatures, and advised that the loss of pollen structural integrity is possibly one of the principal reasons for the pollen viability reduction at lower temperatures (Youmbi *et al*., 2012). Other clarified methods also use low-temperatures for the conservation of pollen grains but they can apply only for brief periods of storage (days, weeks, months) such as freezer storage (-10 to -20 ℃) and refrigerator (5-10 ℃), as the research in Citrus species (Ali and Perveen, 2014), Castor (Vargas *et al*., 2009), Olives (Zambon *et al*., 2018), Roses (Giovannini *et al*., 2015) and Kiwi (Borghezan *et al*., 2011).

The optimal time for pollination in *Zephyrenthes* hybrid should be at 09.00-11.00 a.m. and it should be stored at 5±2 ˚C for the preservation of pollen viability and germination. It is useful for hand-pollination between cultivars that have non-synchronized flowering in a season. The pollen germination for ‘Bright Eye’ and ‘Island Breeze’ could be maintained for 12 days, nevertheless, ‘Golden Mango’, ‘Red Candy’, ‘POS’, ‘Madam Butterfly’, ‘Lookmai’ and ‘Paradee’ could be maintained only for 8 days.

Acknowledgments

We would like to thank the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang for partially financially supported.

References

Ali, S. and Perveen, A. (2014). In vitro pollen germination of five citrus species. Pakistan Journal of Botany, 46:951-956.

Borghezan, M., Clauman, A. D., Steinmacher, D. A., Guerra, M. P. and Orth, A. I. (2011). In vitro viability and preservation of pollen grain of kiwi (*Actinidia chinensis* var. *deliciosa* (A. Chev.) A. Chev). Crop Breeding and Applied Biotechnology, 11:338-344.

Brewbaker, J. L. and Kwack, B. H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany, 50:859-865.

Brewbaker, J. L. and Kwack, B. H. (1964). The calcium ion and substances influencing pollen growth. In: Linskens HF ed. Pollen Physiology and Fertilization. Amsterdam: Elsevier North Holland, pp.145-151.

Chowdhury, M. R. and Hubstenberger, J. (2006). Evaluation of cross pollination of *Zephyranthes* and *Habranthus* species and hybrids. Journal of the Arkansas Academy of Science, 60:113-118.

Dutilh, J. H. A. (2005). Ornamental bulbous plants of Brazil. Acta Horticulturae 683:37-42.

Dutta, S. K, Srivastav, M., Chaudhary, R., Lal, K., Patil, P., Singh, S. K. and Singh, A. K. (2013). Low temperature storage of mango (*Mangifera inidica* L.) pollen. Scientia Horticulturae, 161:193-197.

Ferreira, C. A. Édila, V. R. V. P., Patrícia, O. A., Vinícius, A., Tanismare, T. A. S. and Deisy, L. C. (2007). Conservação e determinação da viabilidade de grão de pólen de milho. Revista Brasileira de Milho e Sorgo (Impresso), 6:159-173.

Galletta, G. J. (1983). Pollen and Seed Management. In: Moore JN and Janick J eds. Methods in fruit breeding. West Lafayette, Purdue University Press, pp.23-35.

Gandadikusumah, V., Wawangningrum, H. and Rahayu, S. (2017). Pollen viability of *Aeschyanathus tricolor* Hook. Journal of Tropical Life Science, 7:53-60.

Giovannini, A., Macovei, A., Donà, M., Valassi, A., Caser, M., Mansuino, A., Ghione, G. G., Carbonera, D., Scariot, V. and Balestrazzi, A. (2015). Polen grain preservation at low temperature in valuable commercial rose cultivar. Acta Horticulturae, 1064:63-66.

Guangcong, D., Jinguang, X., Chengrong, G., Jie, L., Qian, L., Jie, D., Mengwen, L. and Xia, S. (2019). Effect of low storage temperature on pollen viability of fifteen herbaceous peonies. Biotechnology Reports, 21:e00309 https://doi.org/10.1016/j.btre.2019.e00309

Jayaprakash, P. (2018). Pollen Germination in vitro. In: Pollination in Plants, Phatlane William Mokwala, Intech Open, doi: 10.5772/intechopen.75360. Available from: https://www.intechopen.com/chapters/60074.

Kai, G., Lu, Y., Qian, Z. Y. O., Luo, N., Zhou, G. and Tang, K. (2006). Molecular characterization and expression analysis of a gene encoding mannose-binding lectin from bulb of *Zephyranthes grandiflora*. Biologia, Bratislava, 61:671-677.

Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, P. V. V., Reddy, V. R. and Zhao, D. (2005). Differences in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Annals of Botany, 96:59-67.

Katoch, D. and Singh, B. (2015). Phytochemistry and pharmacology of genus *Zephyranthes*. Medicinal & Aromatic Plants, 4:212 doi: 10.4172/2167-0412.1000212.

Lai, J. S., Gong, B. C., Jiang, X. B., Zhang, J. M., Hu, Q. S., Wu, C. L. and Wu, Q. (2017). Study on germination rate and viability of pollen for *Castanea henryi*. Journal of the Southwest For. Univ. 37:42-48 (in Chinese with English abstract).

Lora, J., Pérez de Oteyza, M. A., Fuentetaja, P. and Hormaza, J. I. (2006). Low temperature storage and in vitro germination of cherimoya (*Annona cherimola* Mill.) pollen. Scientia Horticulturae, 108:91-94.

Loupassaki, M., Vasilakakis, M. and Androulakis, I. (1997). Effect of pre-incubation humidity and temperature treatment on the in vitro germination of avocado pollen grains. Euphytica, 94:247-251.

Maheshwari, P. (1950). An Introduction to the Embryology of Angiosperms. McGraw-Hill. New York.

Marta, F. (2005). Breeding of Rain lilies. Bulbs: Bulletin of the International Bulb Society, 7:25-32.

Martínez-Gómez, P., Gradziel, T. M., Ortega, E. and Dicenta, F. (2002). Low temperature storage of almond pollen. HortScience, 37:691-692.

Martins, E. S., Davide L. M. C., Miranda, G. J., Barizon, J. O., Junior, F. A. S., Carvalho, R. P. and Gonçalves, M. C. (2017). In vitro pollen viability of maize cultivars at different times of collection. Ciência Rural, Santa Maria, 47:e20151077, https://doi.org/10.1590/0103-8478cr20151077.

Maryam, A., Jaskani, M. J. and Naqvi, S. A. (2017). Storage and viability assessment of date palm pollen. Methods Molecular Biology, 1638:3-13.

Meerow, A. W., Fay, M. F., Guy, C. L., Li, Q. B., Zaman, F. Q. and Chase, M. W. (1999). Systematics of Amaryllidaceae based on cladistic analysis of plastid RBCL and TML-F Sequences of data. American Journal of Botany, 86:1325-1345.

Mercier, S. (1995). The role of pollen bank in the tree genetic improvement program in Quebéc (Canada). Grana, 34:367-370.

Metz, C., Nerd, A. and Mizrahi, Y. (2000). Viability of pollen of two fruit crop cacti of the genus *Hylocereus* is afected by temperature and duration of storage. HortScience, 35:22-24.

Mohammed, M., Messaoud, R. and Ahmed, S. (2018). The effect of pollen storage temperatures on pollen viability, fruit set and fruit quality of six date palm cultivars Scientia Horticulturae, 236:279-283.

Mukti, G. (2014). Pollen storage and viability. International Journal of Botany and Research, 4:1-18.

Naiara, V. A., Camila, Y. N. S. and Jean, C. C. (2019). Characterization of cultivars and low-temperature pollen grain storage in amaryllis (*Hippeastrum* sp.). Revista Ceres, Viçosa, 66:451-459.

Novara, C., Ascari, L., La Morgia, V., Reale, L., Genre, A. and Siniscalco, C. (2017). Viability and germinability in long term storage of *Corylus avellana* pollen. Scientia Horticulturae, 214:295-303.

Pacific Bulb Society (2021). Zephyranthes. Monitors on Photographs and Information: Zephyrenthes. Retrieved from https://www.pacificbulbsociety.org/pbswiki/index.php/zephyranthes

Sharafi, Y. (2011). An investigation on the pollen germination and tube growth in some *Prunus persica* genotypes and cultivars. African Journal of Microbiology Research, 5:2003-2007.

Soares, T. L., Souza, E. H., Costa, M. A. P. C., Silva, S. O., and Santos-Serejo, J. A. (2016). Viability of pollen grains of tetraploid banana. Bragantia, Campinas 75(2): 145-151.

Spurrier, M. A., Gerald, L. S., Raymond, O. F. and Adolfo, E. S. (2015). A New Species of *Zephyranthes* (Amaryllidaceae) from Mexico. Novon: A Journal for Botanical Nomenclature, 24:289-295.

Stanley, R. G. and Liskens, H. F. (1974). Pollen: Biology, Biochemistry, Management. New York, Springer-Verlag. 310 p.

Vargas, D. P., Souza, S., Silva, D. and Bobrowski, V. L. (2009). Pollen grain analysis of some cultivars of castor-oil plant (*Ricinus communis* L., Euphorbiaceae): conservation and viability: Arquivos do Instituto Biológico, 76:115-120.

Xiong, H., Deyi, Y., Zhi-Yu, D., Genhua, N. and Feng, Z. (2020). Effects of storage temperature and duration on pollen grain viability and pollen-tube elongation in chinese chinqapin (*Castanea henryi* skan). Bangladesh Journal of Botany, 49:297-304.

Youmbi, E., Tonfack, L., Mbogning, J. and Nkongmeneck, B. (2012). Effect of storage conditions on pollen grains viability and pollen tubes elongation of four Cola species (Malvaceae). Research & Reviews in BioSciences, 6:35-40.

Yuan, S. C., Chin, S. W., Lee, C. Y. and Chen, F. C. (2018). Phalaenopsis pollinia storage at sub-zero temperature and its pollen viability assessment. Botanical Studies, 59:1-8.

Zambon, C. R., Silva, L. F. O., Pio, R., Bianchini, F. G. and Oliveira, A. F. (2018). Storage of pollen and properties of olive stigma for breeding purposes. Revista Ciência Agronômica, 49:291-297.

Zheng, C. L., Yu, X. Q., Wang, X. F. and She, W. Q. (2004). A study on the viability of the pollen of *Castanea henryi* Rehd et Wils. Acta Agriculturae Universitatis Jiangxiensis, 26:200-202 (in Chinese with English abstract).

(Received: 23 September 2021, accepted: 10 February 2022)