ผลของอุณหภูมิและระยะเวลาเก็บรักษาต่อเปอร์เซ็นต์ความมีชีวิตของกลุ่มเรณูของเอื้องแซะหอม เอื้องสายหลวง เอื้องน้ำครั่งสายสั้น และเอื้องนางลม

Effects of Temperature and Storage Period on Pollinia Viability Percentage of *Dendrobium scabrilingue* Lindl., *D. anosmum* Lindl., *D. parishii* Rchb. f. and *D. peguanum* Lindl.

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Abstract: Orchid pollinia storage can promote the cross pollination when the parental lines have different flowering time. Effects of temperature and storage period on pollinia viability percentage of four fragrant *Dendrobium* species such as *Dendrobium scabrilingue* Lindl., *D. anosmum* Lindl., *D. parishii* Rchb. f. and *D. peguanum* Lindl., were studied at three temperature levels, 4, 6 and 8 °C and fifteen periods, starting from 30-days interval untill 450 days. It was found that pollinia of the four fragrant species kept at 4 °C had significantly viability percentages greater than those kept at 6 and 8 °C. Keeping pollinia of *D. scabrilingue* and *D. anosmum, D. parishii* and *D. peguanum* at 4 °C could be kept for 240, 240, 270 and 210 days, respectively, without significantly losing their viability percentages.

Keywords: Orchid pollinia, storage, fragrant *Dendrobium* species

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บทคัดย่อ: การเก็บรักษากลุ่มเรณูกล้วยไม้สามารถสนับสนุนให้เกิดการผสมข้ามเมื่อต้นพ่อและต้นแม่มีฤดูออกดอกแตกต่างกัน การศึกษาผลของอุณหภูมิและระยะเวลาการเก็บรักษากลุ่มเรณูของกล้วยไม้สกุลหวายพันธุ์แท้กลิ่นหอม จำนวน 4 ชนิด ได้แก่ เอื้องแซะหอม เอื้องสายหลวง เอื้องน้ำครั่งสายสั้น และเอื้องนางลม โดยศึกษาอุณหภูมิในการเก็บรักษา 3 ระดับ คือ 4, 6 และ 8 องศาเซลเซียส และระยะเวลาการเก็บรักษา 15 ระดับ โดยเริ่มจาก 30 วัน เพิ่มขึ้นครั้งละ 30 วัน ถึง 450 วัน พบว่ากลุ่มเรณูของกล้วยไม้หอมทั้ง 4 ชนิด ที่เก็บรักษาที่ 4 องศาเซลเซียส มีเปอร์เซ็นต์ความมีชีวิตมากกว่าเก็บรักษาที่อุณหภูมิ 6 และ 8 องศาเซลเซียสอย่างมีนัยสำคัญทางสถิติ การเก็บรักษากลุ่มเรณูของเอื้องแซะหอม เอื้องสายหลวง เอื้องน้ำครั่งสายสั้น และเอื้องนางลม ไว้ที่อุณหภูมิ 4 องศาเซลเซียส สามารถเก็บไว้ได้นานถึง 240, 240, 270 และ 210 วัน ตามลำดับ โดยเปอร์เซ็นต์ความมีชีวิตยังคงเดิม

คำสำคัญ: กลุ่มเรณูกล้วยไม้ การเก็บรักษา กล้วยไม้สกุลหวายพันธุ์แท้กลิ่นหอม

Introduction

Keeping orchid pollinia in a regular refrigerator has been commonly practiced. However, the study on proper storage period and effect of temperature on viability of pollinia has been less mentioned. Pollinia of *Dedrobium phalaenopsis, D. undulatum, D. streblocerас*, and *D.* Jaquelyn Thomas, could be kept at 7 °C for 12 months (Meeyot and Kamemoto, 1969). Similar success with other species of *Dendrobium*, *Vanda*, *Cymbidium* and *Arachnis* was achieved using air-dry storage at 4 to 6 °C for the maximum of 280 days (Shijun, 1984). Pollinia can be also kept in a small tube for 2–12 months in regular refrigerator (Songkhakul, 1983). At 2°C and 85 % relative humidity in refrigerator pollinia of *Dactylorhiza fushsii*, *Orchis morio*, *Orchis maculata* and *Anacamptis pyramidalis* could be kept for 60 days (Prichard and Prendergast, 1989). Pollinia of *D. nobile*, *D.* Lady Hamilton and *Calanthe furcata* germinated well after storing at -79 °C for 957 days. Moreover, pollinia of *D. nobile* stored at -79 °C for 957 days survived in the presence of a chemical cryoprotectant, glycerol and ethanol mixture (Ito, 1965). However, the use of very dry storage is not advisable for all pollen. For example, Gramineae pollen is generally intolerant of desiccation. Moreover, reports on the effect of short-term drying and long-term storage over desiccants in orchid pollinia were conflicting. Desiccation over silica gel reduced pollinia viability (Pritchard and Prendergast, 1989). Some orchids, *Cattleya mossiae* (Curtis and Duncan, 1947), and *D.* Lady Hamilton (Ito, 1965) appeared relatively tolerant to dry condition whereas such conditions were harmful in other dendrobiums and *Oncidium stipitatum* (Meeyot and Kamemoto, 1969).

There are many fragrant *Dendrobium* species in Thailand for example *D. scabrilingue* Lindl., *D. anosmum* Lindl. and *D. parishii* Rchb. f. which are world wide well known (Kamemoto *et al*., 1999). Flowers of different species have different scent, *D. scabrilingue* has a sweet fragrance reminiscent of wallflowers, whereas flowers of *D. anosmum* and *D. parishii* have strong and pleasant scent. Their fragrances have been variously described like rhubarb or raspberries (Baker and Baker, 1996). Another *Dendrobium*, *D. peguanum*, which has small flower with light purple color, has sweet honey liked fragrance (Thaithong, 2000).

It is known that most fragrant *Dendrobium* species has short blooming season, storing pollinia of those plants are needed in order to use the pollinia in interspecific or intergeneric hybridization. Thus, this study was conducted to find the suitable conditions in terms of temperature and storage period for some fragrant *Dendrobium* pollinia.

Materials and methods

Pollinia of four fragrant *Dendrobium* species, *D. scabrilingue*, *D. anosmum*, *D. parishii* and *D. peguanum* (Figure 1), were collected and each pair of pollinia was placed in a sealed plastic tube, total of 135 pollinia for each species. Three levels of temperature, 4, 6 and 8 °C and fifteen different periods; 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 450 days; were employed in this study with 3 x 15 Factorial in CRD, 3 replications in each treatment. Every 30-day, 3 samples were taken from each temperature and species to test for viability, using nutrient solution, 30 g/l sucrose, 50 mg/l boric acid, and 200 mg/l $Ca(NO_3)_2$ (Areevilas, 1994). Pollinia were placed on staining slide and a few drops of the nutrient solution were added to cover all pollinia. Slides were kept in a moist box at room temperature for 36 hours. Viability percentage was scored, by removing those pollinia from the nutrient solution and washed in distilled water and then counted number of pollens that had pollen tube longer than the pollen. One had long pollen tube was counted as germinated pollen, meaning had 100% viability. Statistical analysis was conducted using Duncan's Multiple-Range Test (DMRT) at $P < 0.05$ by Sirichai Statistics Version 6.00 (2001) program (Unsrisong, 2002).

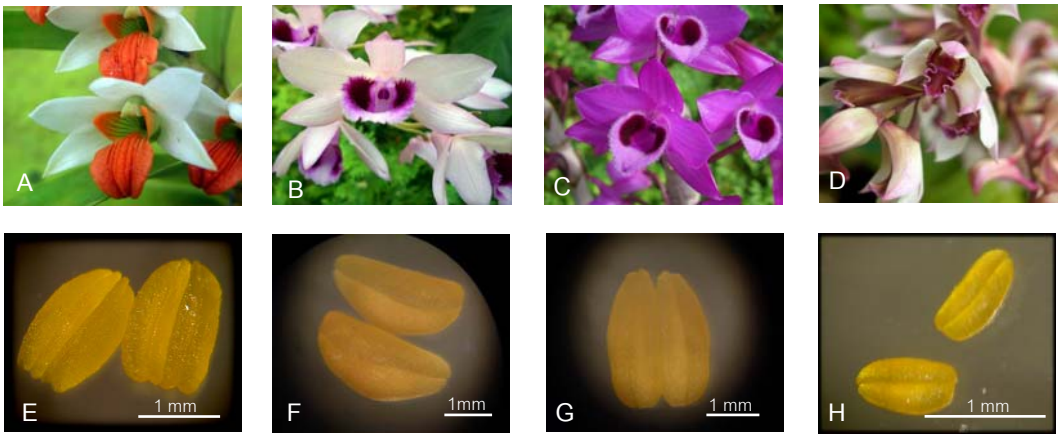

Figure 1 Four fragrant *Dendrobium* species and their pollinia.
(A) *D. scabrilingue*, (B) *D. anosmum*, (C) *D. parishii*, (D) *D. peguanum*, (E) pollinia of *D. scabrilingue*, (F) pollinia of *D. anosmum*, (G) pollinia of *D. parishii* and (H) pollinia of *D. peguanum*.

Results

Effect of storage temperatures

The greatest pollinia viability percentage of *D. scabrilingue*, *D. anosmum, D. parishii* and *D. peguanum* was found when pollinia were kept at 4 °C, 88.56, 92.22, 93.56 and 91.33 %, respectively, and it was significantly greater than those kept at 6 and 8 °C (Table 1, 2, 3 and 4).

Effect of storage periods

Pollinia of *D. scabriligue* and *D. peguanum* could be kept for 180 days (Table 1 and 4) whereas those of *D. anosmum* and *D. parishii* were 240 days (Table 2 and 3) without significantly losing viability percentage, but it was found that viability percentage started to markedly decline from 210 days onwards for *D. scabrilingue* and *D. peguanum* and from 270 days onwards for *D. anosmum* and *D. parishii.* However, after keeping the pollinia for 450 days, the viability percentage was still greater than 60 %.

Effect of interaction between storage temperature and storage period

There was an interaction between temperature and storage period. Pollinia of *D. scabrilingue* could be kept at 4 °C for 240 days, 6 °C for 210 days and 8 °C for 180 days (Table 1) without significantly losing viability percentage, and found that the viability percentage of the pollinia kept at 4 °C started to significantly lose their viability from 270 days onwards, whereas 6 °C from 240 days onwards and 8 °C from 210 days onwards. Pollinia of *D. anosmum* could be kept at 4 and 6 °C for 240 days, and 8 °C for 210 days (Table 2) without significantly losing viability percentage, and found that the viability percentage of the pollinia kept at 4 and 6 °C started to significantly lose their viability from 270 days onwards, whereas at 8 °C from 240 days onwards. Pollinia of *D. parishii* could be kept at 4 °C, for 270 days, 6 and 8 °C for 240 days (Table 3) without significantly losing viability percentage, and found that the viability percentage of the pollinia kept at 4 °C started to significantly lose their viability from 300 days onwards, whereas at 6 and 8 °C from 240 days onwards. Pollinia of *D. peguanum* could be kept at 4 and 6 °C, for 210 days, and 8 °C for 180 days (Table 4) without significantly losing viability percentage, and found that the viability percentage of the pollinia kept at 4 and 6 °C started to significantly lose their viability from 240 days onwards, whereas at 8 °C from 240 days onwards. Of all studied species, after keeping their pollinia at 4, 6 and 8 °C for 450 days the pollinia viability percentage was still greater than 60 %.

Discussion

Keeping pollinia of four fragrant species, *D. scabrilingue, D. anosmum, D. parishii* and *D. peguanum* at three levels of storage temperatures and fifteen storage periods. At 4 ^{0}C pollinia of *D. parishii* could be kept for 270 days whereas *D. anosmum, D. scabrilingue* and *D peguanum* could be kept for 240 days without significantly losing viability percentage but it was found that the viability percentage of *Den. parishii* started to markedly decline from 300 days onwards whereas *D. scabrilingue, D. anosmum* and *D. peguanum* started to markedly decline from 270 days onwards. The result was similar to that of from Shijun (1984) stating that pollinia of some species of *Dendrobium*, *Vanda*, *Cymbidium* and *Arachnis* could be kept air-dry at 4 to 6 °C for 280 days and Meeyot and Kamemoto (1969) reported that the pollinia of

Table 1 Pollinia viability percentage of *Dendrobium scabrilingue* at three levels of storage temperatures and fifteen storage periods.

Storage period (day)	Pollinia viability (%)[1/]			Average[2/]
	4 ^{0}C	6 ^{0}C	8 ^{0}C	
30	100.00 a	100.00 a	100.00 a	100.00 a
60	100.00 a	100.00 a	100.00 a	100.00 a
90	100.00 a	100.00 a	100.00 a	100.00 a
120	100.00 a	100.00 a	100.00 a	100.00 a
150	100.00 a	100.00 a	100.00 a	100.00 a
180	100.00 a	100.00 a	100.00 a	100.00 a
210	100.00 a	96.67 ab	95.00 b	97.22 b
240	96.66 ab	95.00 b	88.33 c	93.33 c
270	86.67 cd	86.67 cd	81.67 fg	85.00 d
300	85.00 de	83.33 ef	78.33 h	82.22 e
330	80.00 gh	78.33 h	73.33 i	77.22 f
360	73.33 i	73.33 i	68.33 jk	71.11 g
390	71.67 i	70.00 ij	65.00 lm	68.88 h
420	68.33 jk	65.00 lm	61.66 n	65.00 i
450	66.66 kl	63.33 mn	61.66 n	63.89 i
Average[3/]	88.56 a	87.84 b	84.88 c	

[1/]Means followed by different letters were significantly different at P <0.05 by DMRT.

[2/]Means followed by different letters of the same column were significantly different at P <0.05 by DMRT.

[3/]Means followed by different letters of the same row were significantly different at P <0.05 by DMRT.

Table 2 Pollinia viability percentage of *Dendrobium anosmum* at three levels of storage temperatures and fifteen storage periods.

Storage period (day)	Pollinia viability (%)[1/]			Average[2/]
	4 ^{0}C	6 ^{0}C	8 ^{0}C	
30	100.00 a	100.00 a	100.00 a	100.00 a
60	100.00 a	100.00 a	100.00 a	100.00 a
90	100.00 a	100.00 a	100.00 a	100.00 a
120	100.00 a	100.00 a	100.00 a	100.00 a
150	100.00 a	100.00 a	100.00 a	100.00 a
180	100.00 a	100.00 a	100.00 a	100.00 a
210	100.00 a	100.00 a	100.00 a	100.00 a
240	100.00 a	100.00 a	96.67 b	98.89 a
270	93.33 c	90.00 d	90.00 d	91.11 b

Table 2 (Cont.)

Storage period (day)	Pollinia viability (%)[1/]			Average[2/]
	4 ^{0}C	6 ^{0}C	8 ^{0}C	
300	90.00 d	90.00 d	90.00 d	90.00 b
330	90.00 d	83.33 e	83.33 e	85.56 c
360	80.00 f	80.00 f	80.00 f	80.00 d
390	80.00 f	76.67 g	75.00 gh	77.22 e
420	76.67 g	73.33 hi	73.33 hi	74.44 f
450	73.33 hi	71.67 i	71.67 i	72.22 f
Average[3/]	92.22 a	91.00 b	90.67 b	

[1/]Means followed by different letters were significantly different at P <0.05 by DMRT.

[2/]Means followed by different letters of the same column were significantly different at P <0.05 by DMRT.

[3/]Means followed by different letters of the same row were significantly different at P <0.05 by DMRT.

Table 3 Pollinia viability percentage of *Dendrobium parishii* at three levels of storage temperatures and fifteen storage periods.

Storage period (day)	Pollinia viability (%)[1/]			Average[2/]
	4 ^{0}C	6 ^{0}C	8 ^{0}C	
30	100.00 a	100.00 a	100.00 a	100.00 a
60	100.00 a	100.00 a	100.00 a	100.00 a
90	100.00 a	100.00 a	100.00 a	100.00 a
120	100.00 a	100.00 a	100.00 a	100.00 a
150	100.00 a	100.00 a	100.00 a	100.00 a
180	100.00 a	100.00 a	100.00 a	100.00 a
210	100.00 a	100.00 a	100.00 a	100.00 a
240	100.00 a	100.00 a	100.00 a	100.00 a
270	100.00 a	95.00 b	93.33 b	96.11 b
300	90.00 c	90.00 c	90.00 c	90.00 c
330	90.00 c	88.33 c	88.33 c	88.88 c
360	88.33 c	81.67 d	80.00 de	83.33 d
390	80.00 de	78.33 ef	76.67 fg	78.33 e
420	80.00 de	76.67 fg	73.33 hi	76.67 f
450	76.67 fg	73.33 hi	71.67 i	73.33 g
Average[3/]	93.56 a	92.22 b	91.56 c	

[1/]Means followed by different letters were significantly different at P <0.05 by DMRT.

[2/]Means followed by different letters of the same column were significantly different at P <0.05 by DMRT.

[3/]Means followed by different letters of the same row were significantly different at P <0.05 by DMRT.

Table 4 Pollinia viability percentage of *Dendrobium peguanum* at three levels of storage temperatures and fifteen storage periods.

Storage period (day)	Pollinia viability (%)[1/]			Average[2/]
	4 ^{0}C	6 ^{0}C	8 ^{0}C	
30	100.00 a	100.00 a	100.00 a	100.00 a
60	100.00 a	100.00 a	100.00 a	100.00 a
90	100.00 a	100.00 a	100.00 a	100.00 a
120	100.00 a	100.00 a	100.00 a	100.00 a
150	100.00 a	100.00 a	100.00 a	100.00 a
180	100.00 a	100.00 a	100.00 a	100.00 a
210	96.67 a	96.66 a	93.33 b	95.56 b
240	93.33 b	90.00 c	86.66d	90.00 c
270	90.00 c	86.67 d	80.00 f	85.55 d
300	90.00 c	83.33 e	76.67 g	83.33 e
330	86.67 d	80.00 f	73.33 h	80.00 f
360	83.33 e	73.33 h	70.00 hi	75.56 g
390	80.00 f	70.00 i	66.66 ij	72.22 h
420	76.67 g	70.00 i	66.33 jk	70.00 h
450	73.33 h	66.66 j	60.00 k	66.67 i
Average[3/]	91.33 a	87.78 b	84.89 c	

[1/]Means followed by different letters were significantly different at P <0.05 by DMRT.

[2/]Means followed by different letters of the same column were significantly different at P <0.05 by DMRT.

[3/]Means followed by different letters of the same row were significantly different at P <0.05 by DMRT.

D. phalaenopsis, D. undulatum, D. strebloceras, and *D.* Jaquelyn Thomas, could be kept in air-dry storage at 7 °C for 12 months. Pollinia viability percentages were decreased when storage periods and temperature increased. Storing pollinia of *D. scabrilingue, D. anosmum, D. parishii,* and *D. peguanum* at 4 °C could extend pollinia viability better than those kept at 6 and 8 °C. It showed that species could play an important role on pollinia viability. Each species requires different temperature and storage period. However, after keeping at 4, 6 and 8 °C for 450 days, the pollinia viability percentages of *D. scabrilingue, D. anosmum, D. parishii,* and *D. peguanum* were still greater than 60 % which have high levels viability percentage for orchid pollination. It was supported by the report of Songkhakul (1983) stated that orchid pollinia could be stored in a small tube for 2–12 months in a regular refrigerator. Thus, the results of this study, help to assure orchid breeders and growers to keep pollinia of some fragrant *Dendrobium* in regular refrigerator in drug shelf where temperature is around 4–8 °C up to 450 days for interspecific or intergeneric hybridization.

Conclusion

Keeping pollinia of four fragrant species, *D. scabrilingue, D. anosmum, D. parishii,* and *D. peguanum* at 4 °C gave the best result. They could be kept for longer period when compared with higher temperature, 6 and 8 °C. However, pollinia viability percentage at 8 °C was still greater than 60 % after storing for 450 days which was good enough to use for hybridization. Moreover, the results of this study are practicable, orchid breeders and growers can keep pollinia of some fragrant *Dendrobium* in regular refrigerator in drug shelf where temperature is around 4–8 °C up to 450 days for interspecific or intergeneric hybridization.

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