ノート (Note)

A simple way to store *Calophyllum inophyllum* L. pollen

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Calophyllum inophyllum L. is one of the multi-purpose trees in the coastal regions around Northern Australia, Southeast Asia, and Southern India, and it is planted as not only an efficient shore protector but also an ornamental tree (Orwa et al. 2009). The fruit of *C. inophyllum* is rich in oil that is used for the production of some kinds of medicine and cosmetics, and the quality of its timber is suitable for the manufacture of furniture and boat (Orwa et al. 2009). Forest Tree Breeding Center, Japan has a project that focuses on the selection of better lines of *C. inophyllum* and has established some progeny testing stands (Hanaoka 2012). Controlled crossing is one of the most effective techniques to advance the breeding project. For controlled crossing, vigorous pollens have to been put on appropriate stigmas. However, the time often varies between individuals when their pollens and stigmas mature. It is essential for designed controlled crossings to store the pollen of the plant. It has been reported that *C. inophyllum* flowers twice a year in June and October on Yaeyama Islands, Japan (Kato 2013). The interval between flowering seasons is approximately five months. Therefore, the pollens have to be stored for at least 150 d. The purpose of this study was to develop a storage method for *C. inophyllum* pollen.

In June 2017, freshly opened flowers were collected from three individual trees planted in 2010 in Iriomote Tropical Forest Tree Breeding Technical Garden in Iriomote Island, Japan. The pollens were then collected by holding the flowers upside down over a glassine paper and tapping their anthers with a wooden toothpick in the laboratory. The pollen sample from each tree was wrapped in the paper separately. The pollen samples were then placed under six different storage conditions: in the laboratory (room temperature), a refrigerator (5°C), and a freezer (−20°C) with and without silica gel as desiccant to keep them dry. The samples at room temperature were stored for 2, 4, 10, 18 d. The samples at the other conditions were stored for 150 d and 1 yr. Pollen germination rates of each sample were examined after each storing duration. Before the examination of germination, the samples were placed on a separator shelf above water-saturated filter paper in a sealed box, and were left for 4 h. The pollens were rehydrated in this high-humidity atmosphere. After rehydration, the pollens were placed on 1% solidified agar medium containing 10% sucrose. The pollens on the germination medium were left in a thermostat at 30 °C for 3 h. The conditions of the pollen germination have been determined by our preliminary examination (unpublished data). The experiment was carried out in three replications per tree sample. The pollen grains from each replication were examined under a microscope; a minimum of 100 grains were scored per replication. A pollen grain was scored as germinated if the pollen tube was at least as long as the diameter of the pollen grain. The mean percent germination of pollen from each storage condition was calculated using the germination rate of each replication. The initial pollen germination rate was examined by the same procedure mentioned above without rehydration on the same day of pollen collection. The room temperature was recorded using a thermometer (TR-71U; T&D Corp., Nagano, Japan). Statistical analyses were conducted using the libraries “lme4” and “multcomp” in the statistical package R (version 3.3.3; R Core Team, 2017). We used a generalized liner mixed-effects model with binominal distribution by the glmer function (Bates et al. 2015). In the model, we specified the number of germinated pollens and ungerminated pollens as response variable, storage condition as fixed effects, and the names of individual trees and replications as random effects. The pollen germination rate at each storage condition was compared with the result of the model by Tukey’s all-pair comparison test using the glht function (Hothorn et al. 2008).

The initial pollen germination rates were from 30.7±2.8% to 37.2±7.2% (Table 1). The room temperature ranged from 27 °C to 39 °C from the beginning of the storage period until day 18. The pollens stored at room temperature with or without desiccant lost their germination ability within a few days.

テリハボクの花粉の簡易な貯蔵方法

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The pollens stored at 5 °C without desiccant also lost their viability after 150 d. In contrast, the pollens stored at 5 °C with desiccant and at −20 °C with or without desiccant maintained higher germination rates than that under the other storage conditions. Only the pollens stored at −20 °C with desiccant for 150 d kept the germination rate which was not significantly different from that observed initially ($P < 0.01$). Most of the pollens, irrespective of the storage condition, did not germinate after one year.

The viability of *C. inophyllum* pollen at room temperature decreased in few days in the present study. The pollen should be stored in a freezer (−20 °C) with desiccant immediately after collecting. This method was found to keep the viability of the pollen for 150 d. However, it has been reported that flowering rate of *C. inophyllum* is higher in June than in October in Yaeyama Islands (Kato 2013). The more it flowers, the easier to carry out designed controlled crossings is. Suitable seasons for controlled crossing of *C. inophylum* seem to be June in Yaeyama Islands. Further study on storing vigorous pollens of the plant for 1 yr is needed.

引用文献

Bates, D., Machler, M., Bolker, B. and Walker S. (2015) Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48.

Hanaoka, S. (2012) A review of current research on *Calophyllum inophyllum* L. Forest Tree Breeding, 242, 26-38 (in Japanese).

Hothorn, T., Bretz, F. and Westfall P. (2008) Simultaneous Inference in General Parametric Models. Biometrical Journal, 50(3), 346-363.

Kato, K. (2013) Flowering phenology and pollen germination rate of *Calophyllum inophyllum* L. Annual Report of Forest Tree Breeding Center, Japan, 2013 edition, 65-68 (in Japanese).

Orwa, C., Mutua, A., Kindt. R., Jamnadass, R. and Simons, A. (2009) Agroforestry Database: a tree reference and selection guide version 4.0., World Agroforestry Centre, Kenya, http://www.worldagroforestry.org/treedb2/speciesprofile.php?Spid=2, (accessed 2018-08-24).

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Table 1. Effect of storage condition and duration on in vitro pollen germination of three individuals of *Calophyllum inophyllum* L.

	Name of	Duration						
Condition	individual	0 d	2 d	4 d	10 d	18 d	150d	1 yr
Initial	475	37.1 ±0.1*						
	604	30.7 ±2.8*						
	809	37.2 ±7.2*						
Room temperature	475		0.3±0.4*	0.0±0.0	0.0±0.0	0.0 ±0.0		
	604		2.5±1.0*	0.3±0.4	0.0±0.0	0.0 ±0.0		
	809		2.6±0.9*	0.0±0.0	0.0±0.0	0.0 ±0.0		
Room temperature	475		15.2±4.4	3.2±0.5*	0.2±0.3	0.0 ±0.0		
with silica gel	604		23.9±6.5 ns	3.7±0.7*	0.2±0.3	0.0 ±0.0		
	809		27.7±5.1	10.2±1.6*	0.3±0.4	0.2 ±0.3		
5 °C	475						0.0±0.0	0.0±0.0
	604						0.0±0.0	0.0±0.0
	809						0.7±0.9	0.0±0.0
5 °C	475						29.1±6.2*ns	0.0±0.0
with silica gel	604						15.9±3.3*	0.4±0.5
	809						30.7±3.2*ns	1.8±1.8
-20 °C	475						29.4±7.4 ns	0.0±0.0
	604						25.6±2.3	0.0±0.0
	809						28.9±7.2 ns	0.2±0.2
-20 °C	475						34.7±1.2*ns	1.3±0.9
with silica gel	604						34.8±0.6*ns	8.8±1.5
	809						38.5±4.8*ns	14.6±7.7

Room temperature ranged from 27 to 39 °C from the beginning of storage until day 18. Pollen germination rate (%) is represented as mean ± standard deviation. The values with "*" were significantly different between the individuals in the same storage condition at $P < 0.01$. The values with "ns" were not significantly different from the initial germination rates of the individual at $P < 0.01$.