

## ***In Vitro* Germination and Viability Testing of Nipa (*Nypa fruticans* Wurmb.) Pollen under Different Storage Conditions**

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**Viability levels of fresh nipa (*Nypa fruticans* Wurmb.) pollen samples were determined using acetocarmine stain to identify viable from non-viable pollen. Regardless of the position of male spikes in the inflorescence, the general viability level was estimated at 97.80% even when pollen samples were subjected to temperature of 40 °C at 12, 24, 36, and 48 h. Pollen samples stored under different temperatures in 75 d revealed that oven-drying (40 °C) achieved 96.4% viability, which is significantly higher than deep freezer (-20 °C). Fresh pollen samples collected in Davao Region without pre-drying showed low initial viability in Bago Aplaya, while those from Hagonoy and Carmen had higher % viability due to exposure to higher temperature during longer transit. *In vitro* germination also showed that pollen broke open by aperture when subjected to different levels of sucrose (2, 4, 6, 8, and 10%) at different duration (1, 2, 3, 4 h). The content of the pollen was released instead of tube growth during this test, which requires further validation.**

Key words: acetocarmine, *in vitro* germination, *Nypa fruticans*, pollen, storage conditions, viability

### INTRODUCTION

Nipa (*Nypa fruticans* Wurmb.), belonging to the palm family Arecaceae, is the only widely distributed palm that is considered as a mangrove-associated species but thrives in an exclusively freshwater environment (Riffle & Craft 2003, Joshi *et al.* 2006). Although nipa minimally constitutes the mangrove vegetation, its distinctive morphology, habitat specificity, and climatic preferences are good indicators of past vegetation and environmental parameters as useful characteristics in tracing primitive

plants (Gee 2001). Economically, several products are obtained from the leaves, inflorescences, and fruits of this palm. Among those versatile uses include medicines, roof thatches, hats, raincoats, and other important commercial products (Hamilton & Murphy 1988). Its sap is used to produce beverage, sugar, and vinegar; it has also been studied as a potential source of biofuel (Rasco 2010).

In spite of these many uses, nipa receives little attention – if any – from the government's plans on rehabilitation of the ecosystems where it thrives. Technical information on nipa regeneration and management is lacking (Joshi *et al.* 2006). There is also a dearth of information regarding

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the breeding and genetics of this palm as *N. fruticans* is reported as the only species of its genus (Joshi *et al.* 2006, Cresti & Tiezzi 2011). The basic understanding of its floral biology is an initial, urgent step in improving and conserving the existing populations of *Nypa*. Effective pollination is a prerequisite for fruit- and seed-set in most plants, and information on pollen biology – including pollen viability and pollen tube growth – is required for any rational approach to increase productivity (Cruzan 1990, Bolat & Pirlak 1999, Shivanna 2003, Abdelgadir *et al.* 2011).

Measuring the viability of pollen would aid in determining the pollen's ability to perform its function as the carrier of sperm cells into the embryo sac. Several methods are done to assess pollen viability depending on the crop or species. These involve the use of various stains, *in vitro* and *in vivo* germination tests, and analysis of final seed set (Shivanna *et al.* 1991, Adhikari & Campbell 1998, Dafni & Firmage 2000).

Besides determining pollen fertility, *in vitro* germination is being used in cytogenetic, physiological, and biochemical studies involving many plant species – in view of the speed and ease of the test (Soares *et al.* 2008). *In vitro* germination test is a method to grow a sample of pollen grains in an appropriated culture medium. Through this technique, it is possible to observe in a microscope the percentage of pollen grains that developed pollen tubes after a certain period. The composition and pH of the medium are among the factors that affect pollen germination (de Franca *et al.* 2009).

In palms, pollen viability was evaluated by staining and developing an optimized germination medium to observe tube growth either in hanging drop or agar plate. The most common stain used among the palms was acetocarmine, although there were instances where 2,3,5-triphenyl tetrazolium chloride (TTC) was also used for oil palm (Armendariz *et al.* 2006, Myint *et al.* 2012, Machado *et al.* 2014, Allahdou *et al.* 2015, Moura *et al.* 2015, Escobar & Dominguez 2018).

Certain parts of plant cells are acidic and have an affinity for the basic dyes. Those molecules in the cytoplasm with basic character have affinities for acid dyes. Acetocarmine in glycerin jelly acts as a basic dye with iron acting as a mordant. The nucleic acids, which are strongly and negatively charged due to the presence of phosphate group in the molecule, readily bind to basic dyes. Therefore, it is used for rapid determinations of the conditions of nuclei and detailed studies of chromosomes (Asghari 2000).

Coconut (*Cocos nucifera* L.), belonging to the same palm family as *Nypa*, uses acetocarmine stain to measure viable pollens by examining deeply red stained viable nucleus (Moreira & Gurgel 1941, Harley & Baker 2001). The

shape of *Nypa* pollen is reported as spheroidal and large measuring 55–70  $\mu\text{m}$ , with zonosulcate aperture that encircles through the presumed poles with two equal halves having spiny exine ornamentation (Harley & Baker 2001, Chumchim & Khunwasi 2011).

Long-term storage of pollen offers a practical solution for transporting pollen to different areas and scheduling of hybrid seed production by storing without appreciable loss of viability (Shaheen *et al.* 1986). There are several factors that would affect pollen viability based on pollen handling. These include relative humidity, pollen moisture content, equilibrium moisture content (EMC), dehydration and hydration responses, and the temperature at which pollen is handled (Mortazavi *et al.* 2010). Factors such as temperature and relative humidity (RH) have influences on pollen longevity during the storage period (Shivanna *et al.* 1991). Storage of pollen under low temperatures as a means of plant genetic resources conservation has been widely discussed for various species, but the effect of different storage temperatures for *Nypa* have not been studied (Harrington 1970, Baja 1987, Rajasekharan *et al.* 1994, Kozłowski & Pallardy 2002).

Hence, this study aimed to:

1. describe the male phase and viability of *Nypa* pollen using staining and *in vitro* germination methods;
2. examine the effect of different storage temperatures on pollen viability of *N. fruticans*;
3. compare the pollen viability collected from nipa stands in Bago Aplaya, Davao City; Hagonoy, Davao del Sur, and Carmen, Davao de Norte; and
4. establish a storage protocol for pollen of nipa to maintain high pollen viability.

## MATERIALS AND METHODS

### Time and Place of Study

Initial field observations were conducted in Apr 2012 at the nipa stands in Carmen, Davao del Norte to familiarize the development of inflorescence. Collecting and harvesting male inflorescence (staminate rachilla) samples ensued in Oct 2013 to develop handling and viability testing of pollen at the Plant Science Laboratory, University of the Philippines Mindanao. Other pollen sources were eventually expanded to other areas such as Bago Aplaya, Davao City and Hagonoy, Davao del Sur for comparison of viability under different storage conditions.

The sampling site in Bago Aplaya, Davao City was nearest to the coast where nipa stand was regularly inundated by sea water during high tide. In Hagonoy, Davao del Sur, nipa palms were growing along the riverbanks and far

from the coast. Lastly, nipa stands in Carmen, Davao del Norte were considered inland and very far from the coast (Figure 1).

### Pollen Collection

Nipa palms with developing inflorescence were tagged and monitored for male anthesis. The time of anthesis and number of staminate rachillae opened per day for the entire duration of male phase were noted. A series of trials was also conducted to optimize pollen collection.

Preliminary viability testing involved five randomly selected palms with an inflorescence. A staminate rachilla was collected according to each designated region (top, middle, and bottom) due to basipetal maturation of the inflorescence. While in transit, the samples were packed in sterile paper bags to avoid accumulation of moisture. Extraction of pollen was carried out by shaking to dust the pollen grains for collection.

### Pollen Viability Testing

**Staining Method.** The collected nipa pollen samples were hydrated on a slide with a drop of 1:1 glycerin solution to study and familiarize the pollen morphology. The prepared slide was then viewed under the microscope where pollen size was measured using a calibrated ocular micrometer (Figure 2).

The staining method used acetocarmine in order for the viable pollen to stain red (Singh 2009). The stain was

prepared by mixing 55 mL of distilled water and 45 mL of acetic acid and 0.5 g of carmine powder. The mixture was boiled gently for 5 min and shaken well. It was cooled and filtered afterwards.

For each slide assay, the pollen sample collected from a staminate rachilla was examined in nine microscopic fields at the center of each slide. This was replicated by pollen sample collected from staminate rachilla representing top, middle, and bottom region in each of the five inflorescence samples.

Pollen viability was determined by counting stained pollen after a couple of minutes in the test solution (Bolat & Pirlak 1999). Pollen grains that did not stain red were not considered viable. Hence, percentage viability was determined using the formula below:

$$\% \text{ viability} = \frac{\text{number of viable pollen grains per slide}}{\text{total number of pollen grains per slide}} \times 100 \quad (1)$$

**Pollen Germination.** The protocol for *in vitro* germination test was adapted from Lwin (2010) in oil palm (*Elaeis guineensis* Jacq.). The liquid culture medium for this test was a mixture of 50 mL distilled water, 10 g sucrose, 0.01 g boric acid, and was brought to a final volume of 100 mL by adding distilled water as the stock solution. Pollen samples were subjected to different concentrations of the aforementioned stock solution: 2%, 4%, 6%, 8%, and 10%. Five (5) mL of each concentration was dispensed into a

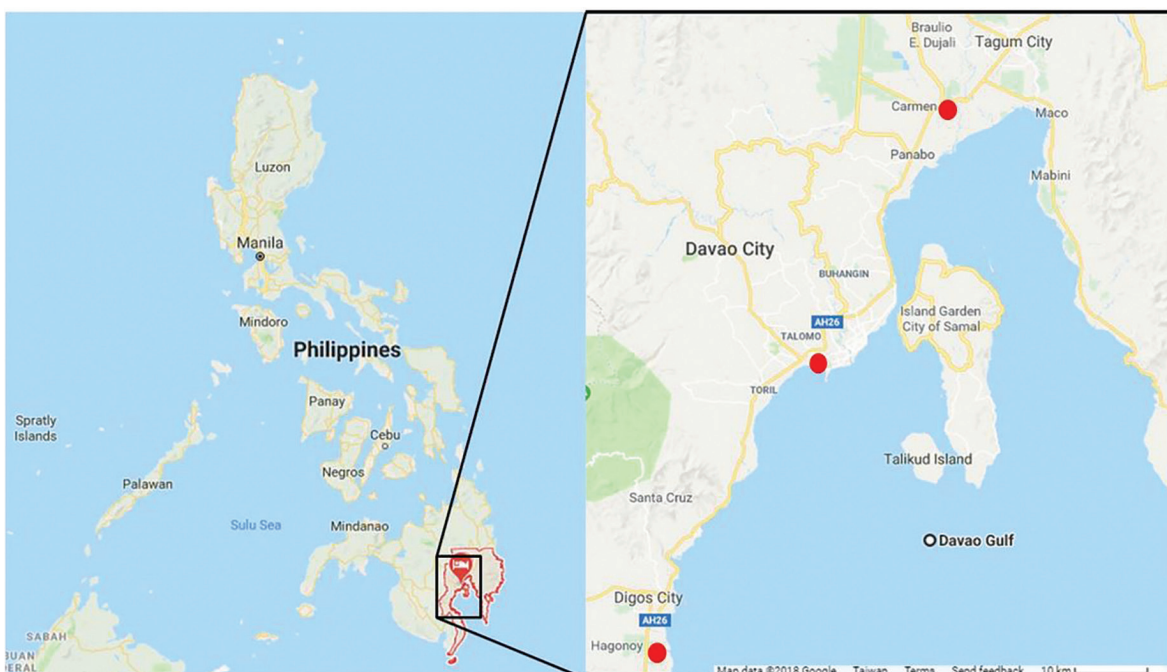
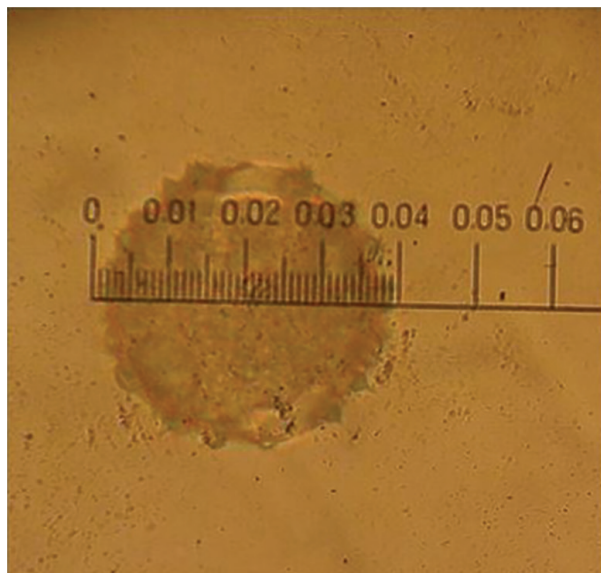


Figure 1. Sampling sites (in red dots) of *Nypa fruticans* pollen in Davao Region, Philippines (Google Maps 2018).



**Figure 2.** *Nypa fruticans* pollen measured on its diameter at the equatorial region (400x magnification).

vial containing sown pollen set aside at room temperature. Germinated pollen grain was regularly checked hourly for the next four hours by following a transect designating the six microscopic fields for counting in triplicate (Lwin 2010, Mortazavi *et al.* 2010) using the following formula:

$$\% \text{ germination} = \frac{\text{number of germinated pollen grains per field of view}}{\text{total number of pollen grains per field of view}} \times 100 \quad (2)$$

Nipa pollen samples were also tested in date palm (*Phoenix dactylifera*) culture medium used by Mortazavi *et al.* (2010) for comparison. A viable pollen grain is expected to germinate and develop pollen tube (Qureshi *et al.* 2009). However, changes and other characteristics unique to the pollen grain of this palm species were also reported.

**Pollen Storage.** Pollen samples were taken from three staminate rachillae of each region *i.e.*, from top then middle to bottom, or a total of nine for each inflorescence. There were five inflorescence samples per replicaton.

The collected fresh pollen samples were subjected to the following desiccation period:

1. ambient room temperature at 28–30 °C
2. 40 °C for 12 h
3. 40 °C for 24 h
4. 40 °C for 36 h
5. 40 °C for 48 h

These initial observations were adopted from coconut by drying pollen collection at 40 °C as determined by Santos *et al.* (1995). After the required duration of each treatment, pollen samples were determined of % viability to identify the best storage duration. Besides Carmen, Davao de Norte, pollen collection was eventually expanded to other nipa stands in Bago Aplaya, Davao City and Hagonoy, Davao del Sur subjected to different storage temperatures as adopted from Allahdou *et al.* (2015) for longer storage time. Fresh pollen collected from three inflorescence samples were kept separately under the following storage conditions as treatments:

1. air-conditioned (25 °C) as control,
2. deep freezer (-20 °C),
3. freezer (0 °C),
4. chiller (18 °C), and
5. oven-dried (40 °C).

Pollen grains were placed in a separate Eppendorf tube per treatment except for the oven-dried samples, which were stored in a foil stored for 75 d. Viability test was conducted regularly for all the samples in a 15-d interval for the next 75 d *i.e.*, 15, 30, 45, 60, and 75 days. It was assessed by acetocarmine stain observed under HPO (400x). A pollen grain count of 100 was determined per transect as a replicate and repeated three times to obtain the percentage of pollen viability (Firmage & Dafni 2001). Sample pollen grains were measured by their equatorial diameter ( $\mu\text{m}$ ).

### Statistical Analysis

The study was laid out in a two-factor factorial experiment in Completely Randomized Design (CRD) replicated three times to determine the effect of storage temperature on storage duration by assessing pollen viability. Fisher's Least Significant Difference (LSD) was employed for treatment mean comparison using DSAASTAT Ver 1.101.

## RESULTS AND DISCUSSION

### The Male Anthesis

After two months of field observation, it was found out that the beginning of anthesis of the staminate rachilla occurs at dawn. In Carmen, Davao del Norte, the best time to collect staminate rachilla was around 5:00 AM to 6:00 AM (4:00 AM to 5:00 AM in Bago Aplaya, Davao City as observed in a separate set-up) before insects swarmed the dehisced pollen. In fact, considerable loss of pollen can be attributed to foraging insects (Figure 3). In addition, the collection was greatly affected by other environmental factors such as rain and wind.





**Figure 3.** *Nypa fruticans* staminate rachilla few minutes after emergence (a); few hours after emergence, with the presence of insects (b).

On the average, there were one or two staminate rachillae that opened up in the first two days and intensified to five on the sixth day (Figure 4). Eventually, the number dwindled down to two on the 14th day, which covered the entire span of male phase.

### Pollen Viability Assessment

***In Vitro Germination Tests.*** The *in vitro* pollen germination tests of nipa showed that no pollen tube emerged from the viable pollen. Instead, the pollen broke open by the aperture releasing its content (Figure 5). True to all sucrose levels, germinating pollen count increased with time. Particularly, for 8% sucrose, germination significantly increased from 17– 34% after 3 h (Table 1). Generally, however, germinated pollen did not vary across sucrose levels and germination time. Dramatic

changes were observed after about an hour of the set-up by breaking of the aperture. These results were also observed using germination medium for date palm as option to validate results.

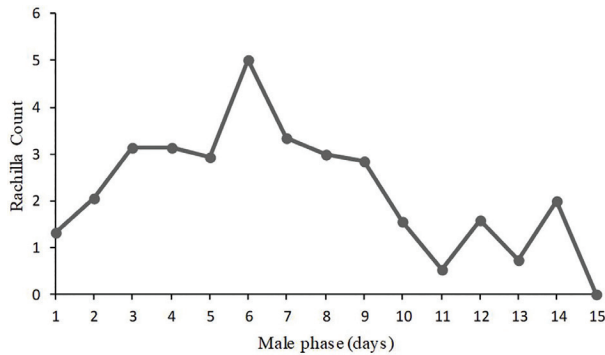
***Acetocarmine Staining.*** In staining technique using acetocarmine, viable pollen stained deep red and no stain at all for non-viable ones (Figure 6). This means that acetocarmine stain can be suitably used to determine viability levels of nipa.

***Pollen Viability During Storage.*** Fresh pollen samples from Carmen stand when subjected to initial air-drying had 100% viability based on staining technique. Viability levels consistently decreased when monitored in a 12-h regular interval oven-dried in 48-h duration. Based on acetocarmine test, the viability of collected pollen samples

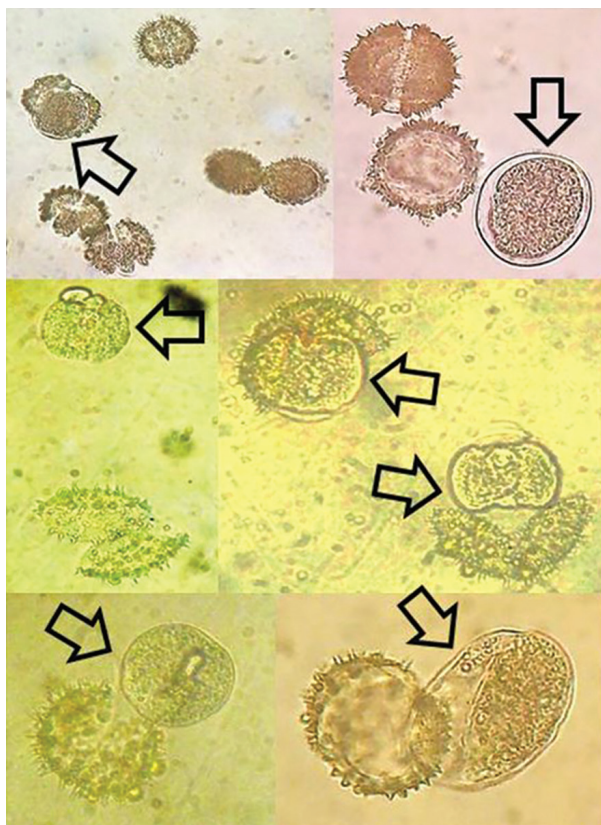
**Table 1.** Percent germinated pollen under *in vitro* germination of *Nypa fruticans* pollen.

Germination Duration (h)	% Germinated Pollen Sucrose Concentration (%) <sup>z</sup>					Mean
	2	4	6	8	10	
1	29.30 <sup>abc</sup>	20.53 <sup>cde</sup>	27.14 <sup>a-d</sup>	16.91 <sup>e</sup>	23.46 <sup>b-e</sup>	23.47AB
2	20.56 <sup>cde</sup>	19.74 <sup>cde</sup>	24.19 <sup>b-e</sup>	16.82 <sup>de</sup>	26.33 <sup>a-e</sup>	21.53B
3	39.91 <sup>ab</sup>	24.68 <sup>b-e</sup>	26.19 <sup>a-e</sup>	34.35 <sup>ab</sup>	25.99 <sup>a-e</sup>	30.22AB
4	42.69 <sup>a</sup>	30.58 <sup>abc</sup>	35.71 <sup>ab</sup>	42.41 <sup>a</sup>	34.48 <sup>ab</sup>	37.17A
Mean	33.12	23.88	28.31	27.62	27.56	28.10

<sup>z</sup>treatment means having the same letter(s) are not significant at  $\alpha=0.05$  using LSD with data transformed using  $\log_{10}$ .



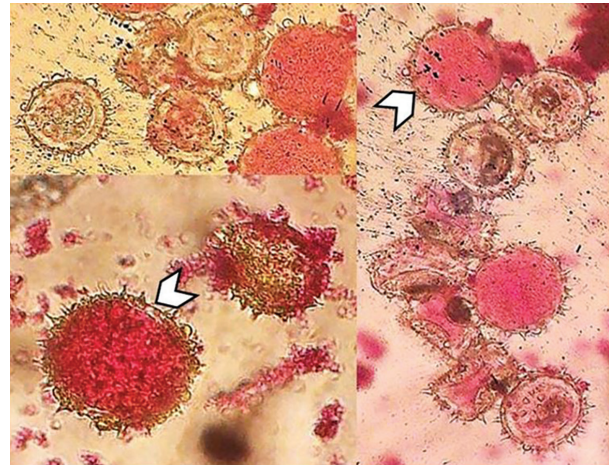
**Figure 4.** The number of staminate rachillae produced every day for the duration of the *Nypa fruticans* male phase.



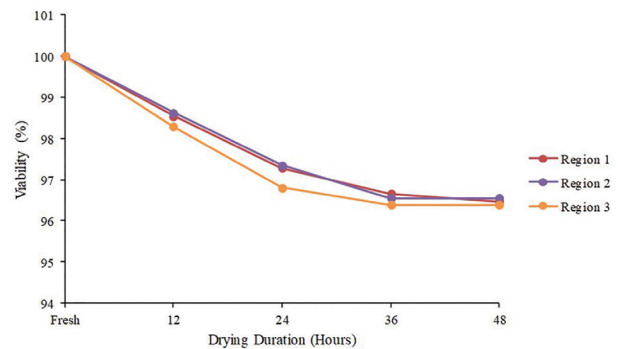
**Figure 5.** *Nypa fruticans* pollen under *in vitro* germination media dehiscing content by aperture and undergoing cell division (arrow) (400x magnification).

significantly decreased after 12-h storage. However, general viability levels decreased only slightly under extended storage. This staining method measured high viability level across inflorescence regions at 97.80% (Figure 7).

In terms of pollen storage under different temperature regimes, the viability level of oven-dried (40°C) pollen from Bago Aplaya was significantly higher compared to



**Figure 6.** *Nypa fruticans* pollen in acetocarmine stain at 400x with viable pollen stained red (arrowhead).



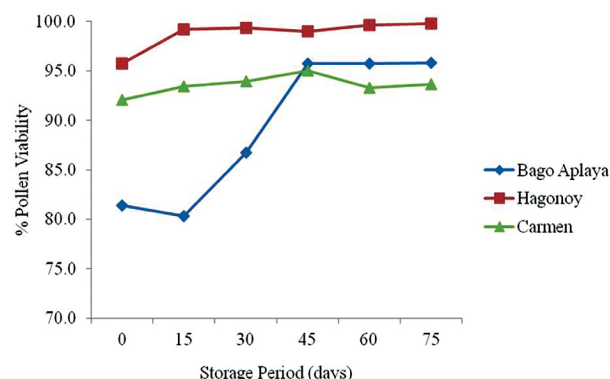
**Figure 7.** The viability of *Nypa fruticans* pollen using acetocarmine stain across three regions at five drying durations.

deep-freezer (-20 °C) across storage duration. Generally, the former produced 94.6% viable pollen but similar to pollen stored under air-conditioned (25 °C), chiller (18 °C), and freezer (0 °C). Pollen sample subjected to deep-freezer measured mean viability of 82.8% after 75 d.

Across storage durations, viability increased significantly after 45 d of storage that stabilized even as long as 75 d. Because the distance of Bago Aplaya to the laboratory was closer compared to other areas, the transit was shorter. Since samples were not subjected to initial drying, the viability of pollen would take a longer time to become stable during storage. However, viability levels in the other two sites were consistently stable early on.

Carmen is fairly far from Davao City. Although pollen viability from this site did not vary across storage temperatures and duration, oven-dried pollen nevertheless had viability levels that significantly improved after 30 d of storage (Figure 8). Stability of viability level was observed to occur earlier compared to Bago Aplaya as





**Figure 8.** Effect of sampling areas on mean % pollen viability of *Nypa fruticans* across storage conditions.

transit would take longer besides time for processing in the laboratory. The sample under deep-freezer was already noticed as stable even at the beginning of testing.

Hagonoy, Davao del Sur was the farthest of the three sampling areas. Pollen viability significantly increased at the shortest time *i.e.*, after 15 d of storage. This remained stable even at 75 d. There were no significant differences observed across storage temperatures at 75 d. In general, samples from Hagonoy had the highest viability level (98.6%) compared to Carmen (93.6%) and Bago Aplaya (89.2%).

### Pollen Diameter

Table 2 shows the mean equatorial diameter of nipa pollen from Carmen stand measured almost 34  $\mu\text{m}$ . While there were significant changes between initial and final diameter, pollen samples subjected to the chiller (18  $^{\circ}\text{C}$ ) and oven-drying (40  $^{\circ}\text{C}$ ) were least affected. This means that under these conditions viable pollen samples maintained sizes during storage. In fact, air-conditioned room storage was statistically comparable to oven-dried pollen by attaining a final size of 36.6  $\mu\text{m}$ .

**Table 2.** Effects of different storage conditions on *Nypa fruticans* pollen equatorial diameter ( $\mu\text{m}$ ) in Carmen after 100 days of storage (n=10)<sup>z</sup>.

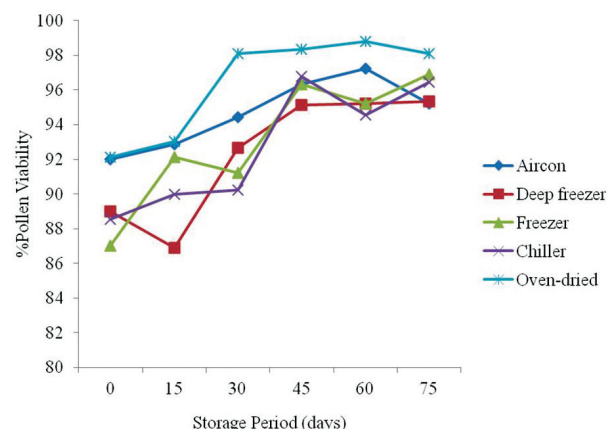
Storage temperature ( $^{\circ}\text{C}$ )	Days in storage		
	0	100	Mean
Aircon (25)	33.37 <sup>bc</sup>	36.60 <sup>a</sup>	34.98 <sup>A</sup>
Deep freezer (-20)	33.37 <sup>bc</sup>	29.13 <sup>d</sup>	31.25 <sup>B</sup>
Freezer (0)	35.69 <sup>a</sup>	32.45 <sup>c</sup>	34.07 <sup>A</sup>
Chiller (18)	34.86 <sup>ab</sup>	33.28 <sup>bc</sup>	34.07 <sup>A</sup>
Oven-dried (40)	35.44 <sup>a</sup>	34.78 <sup>ab</sup>	35.11 <sup>A</sup>
Mean	34.54 <sup>A</sup>	33.25 <sup>B</sup>	33.90

<sup>z</sup>treatment means having the same letter(s) are not significant at  $\alpha = 0.05$  LSD.

Generally, pollen sample under deep-freezer (-20  $^{\circ}\text{C}$ ) had significantly smaller size (31.25  $\mu\text{m}$ ) compared to other treatments. Final diameter was determined after 100 days of storage.

### Indications of Pollen Viability for Nipa

Several trials were done in an attempt to achieve proper handling protocol for collection, transport, storage, and assessment of pollen. Initial procedure for collecting pollen was conducted by bagging the entire inflorescence. This was adopted from coconut as reported by Santos *et al.* (1995). However, it turned out to be inappropriate as male anthesis occurred basipetally *i.e.*, maturation began from top to bottom of the inflorescence progressively with concomitant elongation of the lower lateral branches. Also, nipa inflorescences emitted heat during anthesis just as reported by Henderson (1986). The bagged inflorescence was dampened by the accumulation of moisture inside that made the pollen clumped. Thus, bagging was abandoned as it was tedious and impractical leaving some damage to staminate rachillae and fast deterioration of pollen grains.



**Figure 9.** Effect of storage conditions on mean % pollen viability of *Nypa fruticans* across areas.

In coconut, inflorescence can be forced open to harvest the male florets for pollen processing (Santos *et al.* 1995). Nipa requires timing during male anthesis to collect pollen as pre-anthesis obtained empty spikes. In this case, pollen dehiscence only upon opening.

As to viability testing, acetocarmine actively stained the nipa pollen and showed sharp differences between viable from non-viable ones. This test can discriminately identify stained viable from unstained non-viable pollen.

In nipa, *in vitro* pollen germination showed that the content emerged by the aperture was suspected to be the sperm nucleus. However, no pollen tube growth was observed. Adopted from an oil palm media, the sucrose in the

medium is considered to be the maintenance of osmotic pressure and for providing nutrition to germinating pollen (Nair 1985, Soni *et al.* 2010). If the sucrose enrichments (2, 4, 6, 8, and 10%) were still considered hypoosmotic levels then higher concentrations will be tested in the future. Likely pollen grains burst because of water uptake. There are no reports yet of plant species that conduct double fertilization without corresponding pollen tube growth.

Viability is defined as having the capacity to live, grow, germinate, or develop, but if the conditions are not suitable, pollen may not actually germinate. Many pollen grains can germinate in water or aqueous solutions of sucrose with no additives. However, the pollen of some species such as trinucleated pollen grains needs special substrates for germination (Beyhan & Serdar 2008). The composition of the germination medium can dramatically affect pollen metabolism (Taylor & Hepler 1997). The rate of pollen germination *in vitro* depends largely on finding the optimal medium (Beyhan & Serdar 2008). Hence, further trials in developing techniques to evaluate nipa pollen and finding optimal culture medium ideal for the pollen germination is necessary.

During initial storage, viability tests revealed that keeping the pollen at 40 °C would maintain its longevity within 2 d. However, the slight decrease of viability after 12–24 h can be attributed to the preparation of pollen. Samples collected as fresh pollen for the test may have been dehisced early on while the succeeding samples may still have developmental maturation by desiccation. From an evolutionary viewpoint, developmental arrest in plants is a mechanism that allows survival in vegetative or reproductive structures, also called as ‘desiccation and survival in plants’ (Footitt & Cohn 2001) or ‘drying without dying’ according to Black & Pritchard (2002).

The differences in the initial viability levels of the samples were likely influenced by the proximity of the area to the laboratory where samples were examined. Bago Aplaya is the closest area where pollen samples were still likely developing that required ‘season’ of drying to make them viable. Carmen, which is closer than Hagonoy, had their respective samples already subjected to drying at least one day during transit. The pollen grains may have already reached optimum level of desiccation during observation making them ‘mature’ and hence increased the viability levels of the samples. In many instances, anthers are always subjected to drying prior to pollen collection such as coconut male florets at 40 °C oven-drying (Santos *et al.* 1995), while litchi anthers were under air-blowing electric dryer at 35 °C (Wang *et al.* 2015) until completely dried. Transporting staminate rachillae to long distances was believed to simulate pretreatment of drying once they were separated from the inflorescence.

Pollen longevity has long been associated with relative humidity and temperature. Longer viability is associated with lower internal moisture content (MC), which is brought about by equilibration at lower level of relative humidity in the atmosphere. Apparently, ageing is much faster when MC is high. There are physiological changes that occur during imbibition due to lipid breakdown causing excessive loss of endogenous solutes. In general, longevity is doubled in every 2% reduction of endogenous MC by keeping the temperature constant (Hoekstra 1995).

Final viability levels may have also affected by the location of the stands. Hoekstra (1995) attributed release of bad-quality pollen to spells of drought, high temperature, and even low temperature. Those stands near the coast, as in the case of Bago Aplaya, must have experienced considerable stresses from the regular cycle of heat and wind compared to Carmen and Hagonoy. The nipa stands in Carmen were found in the floodplains of the inland, whereas those in Hagonoy were growing in the upper intertidal zone, a natural habitat of this palm species.

As shown in Figure 8, Hagonoy stands had the highest viability levels among the other sites. These differences were assumed to be influenced by environmental factors that were experienced by pollen samples and their collection procedure. Reports on pollen viability reviewed by Stanley and Linskens (1974) indicated that those environmental factors include relative humidity, temperature, atmospheric composition, and oxygen pressure. Moreover, pollen viability as mentioned by Bots and Mariani (2005) may be affected at different stages of development and most direct interaction between pollen grains and the environment occurs after release from the anther, including the pollen collection time. As expected, most of the factors that influence pollen viability do so at this stage.

The ability of nipa to attain high viability under higher temperatures is best explained by its demographic location and pollen morphology. As mentioned by Gee (2001), nipa is confined in hot tropical regions. The resistance of nipa pollen at a high temperature is an adaptive mechanism. Thus, although it appears that heat stress can affect pollen viability, it has limited consequences on mature pollen after dehiscence and temperatures required to sort an effect on pollen must be much higher than usually encountered in natural environments (Rao *et al.* 1992). The tolerance of nipa pollen to a wide range of temperature without shrinking may indicate the high levels of viability during storage. The mean equatorial pollen diameter of 34 µm falls within the range as reported by Chumchim and Khunwasi (2011) in Thailand, where the equatorial diameter ranged 31.0–42.5 µm while the polar diameter measured 32.2–48.0 µm.



## CONCLUSION

Viability testing of nipa (*Nypa fruticans* Wurmb.) pollen by acetocarmine method was found sufficient to effectively discriminate viable pollen by staining deep red compared to non-viable unstained pollen. Percent viability levels of pollen were estimated at 98% where oven-drying significantly influenced pollen viability of nipa rather than the position of male spikes in the inflorescence. Generally, pollen samples subjected to oven-drying at 40 °C were found significantly higher compared to the deep freezer (-20 °C).

The requirement for higher temperature was clearly seen when using fresh pollen samples as a pretreatment to maximize the viability potentials on this palm. The initial low viability levels of pollen from Bago Aplaya, Davao City can be attributed to insufficient drying as this area is closer to the laboratory compared to the other two sites (Carmen, Davao del Norte and Hagonoy, Davao del Sur), which may have exposed the samples to higher temperature while in transit. Even during storage, oven-dried (40 °C) pollen grains had maintained sizes at almost 35 µm after 100 d. This indicates that nipa pollen storage does not require sub-zero temperature to maintain longevity. Indeed, desiccation of nipa pollen is necessary to achieve higher viability.

On *in vitro* germination testing, the oil palm medium with varying sucrose levels of up to 10% did not show pollen germination characterized by pollen tube growth. Instead the pollen broke open by the aperture and released its content. This has to be further validated by working out the levels of sucrose for future testing. Also, the challenges for bagging the staminate rachillae are needed to be looked into in order to provide a true measurement of viability using mature pollen sampled at different times.

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