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Optimal Pollen Viability and Vigor among varieties of Tagnanan Tall coconut (*Cocos nucifera* L.)

Xi Yun LIEW[1], Mohd Norsazwan GHAZALI[1], Mohd Firdaus ISMAIL[1], Zarina Abd GHAFAR[2], Mohd Syarbani Bin ISNAR[3], Vasudevan VENKATACHALAM[1], Uma Rani SINNIAH[1,*]

[1]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[2]Crops Industry Development Division, Federal Government Administrative Centre, Putrajaya, Malaysia
[3]Crops Industry Development Division, Teluk Bharu Agriculture Center, Hutan Melintang, Perak, Malaysia

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Abstract: Controlled pollination, involving the transfer of pollen from Tagnanan Tall palm (male parent) to the Malayan Yellow or Red dwarf (female parent) is essential for producing MATAG coconut hybrids. However, the pollen from Tagnanan Tall (TAGT), used in hybridization, has been reported to have a germination percentage of less than 40%. This is further complicated by Tagnanan Tall varieties varying in color, TAGT Orange, TAGT Gold, and TAGT Green. TAGT Orange is claimed to have lower pollen viability than TAGT Gold and Green. Hence, a study was conducted to compare inflorescence characteristics, pollen yield, size, viability, and vigor of three Tagnanan tall (TAGT) cultivar. The best-performing cultivar was used to compare pollen viability at different positions on the rachis and rachilla. The results showed that pollen viability of TAGT Gold was 10% higher than TAGT Green and Orange. Pollen viability was influenced by the position of the male flower on the rachis. Pollen collected from the top and middle parts of the rachis (TMRS) had higher pollen vigor and achieved a viability of 61%, whereas mixing pollen from the whole inflorescence only resulted in 39% viability. Therefore, this study which aimed at optimizing pollen quality suggests that pollen should be harvested from the top and middle part of the rachis (TMRS).

Key words: Pollen viability and vigor, pollen germination, coconut inflorescence, male flower, rachis and rachilla

1. Introduction

Coconut (*Cocos nucifera* L.) of the family Arecaceae is cultivated in over 90 countries worldwide (Reddy et al., 2017) and is widely distributed in Asia and along the Pacific Ocean (Omar and Fatah, 2020). The global coconut production was approximately 62 million tons in 2020, with Indonesia being the largest producer, followed by India, the Philippines, Brazil, Sri Lanka, Vietnam, Papua New Guinea, Mexico, Thailand, and Malaysia. Coconuts have been acknowledged for a considerable time as the most beneficial plant variety for human use. Every part of the coconut palm appears to have active economic use; therefore, it is referred to as the 'tree of life'. It is a unique source of various natural products for the development of medicines and provides food to millions of people, especially in the tropical and subtropical regions (DebMandal and Mandal, 2011).

Presently, there has been an increase in the use of coconut-based products and byproducts, such as virgin coconut oil (VCO) and coconut milk, after the revelation of the facts and advantages on the issue of coconut milk and coconut oil myths (Long, 2017; Yon, 2017; Widianingrum et al., 2019). This is expected to drive the market for coconut products in the future, thereby increasing the demand for coconut production. Nevertheless, global food demand is also expected to increase tremendously owing to climate change, and population growth, and will exert more pressure on agricultural land and other resources (Abberton et al., 2016; Razzaq et al., 2021; Singh and Prasad, 2021; van Dijk et al., 2021).

The ultimate solution for higher production and valorization of improved crop plants is the utilization of hybrid crops. Most hybrid crops have significantly increased yield potential and production stability, leading to good insights into the science of hybridization. In coconut hybridization, the combination of two general types of coconuts, Tall (T) and Dwarf (D), is very common. T × D and its reciprocal (D × T) coconut hybrids as well as certain T × T and D × D hybrids have comparable potential. Coconut hybrids are early bearing, intermediate in height, and have a higher yield, that is, the number of nuts, copra weight, copra yield, whole nut weight, husked

* Correspondence: umarani@upm.edu.my

nut weight, oil content, husked nut diameter, and meat thickness (Nampoothiri and Parthasarathy, 2018). To produce hybrids in allogamous species, the crossing of two superior parents is mandatory and is normally performed by controlled pollination of the selected female parent using the desired pollen from a selected male parent with complementary traits. Controlled pollination is important, given that natural hybridization is generally associated with shortcomings such as short-lived pollen, differences in the male and female phases of the parents, unstable flowering stages, and the production of hybrids with doubtful yields (Youmbi et al., 2012; Youmbi et al., 2015).

During hand pollination, the quality of the coconut pollen is one of the major components that plays an important role in determining the success rate of the fruit set. The quality of the coconut pollen is typically determined by its viability and vigor. Pollen viability is measured by observing the pollen germination and pollen tube growth using a suitable growth medium in the laboratory (Karun et al., 2014; Hebbar et al., 2018), whereas pollen vigor refers to the speed of the pollen germination and pollen tube growth (Sulusoglu and Cavusoglu, 2014). Viable pollen is crucial for directed plant breeding, species dispersal, fitness, survival of the next plant generation, and consequently, crop improvement (Impe et al., 2020). Pollen is sensitive to minor fluctuations in atmospheric variables (Hebbar et al., 2018). Several studies found that pollen viability is influenced by cultivars and varieties, and the position of the pollen-containing male flowers on the inflorescence (Nampoothiri, 1970; Ranasinghe et al., 2010; Sunilkumar et al., 2017; Mesnoua et al., 2018).

The Tagnanan Tall (TAGT) has been among the common male parents used in hybridization since Gemperle and Fremond (1978) first reported its promising potential. Hybrids such as PCA 15-2, PCA 15-4, and PCA 15-9 in the Philippines, and Malayan Yellow Dwarf or Malayan Red Dwarf crosses with TAGT (MATAG) in Malaysia are among the famous hybrids produced using TAGT as the male parent (Rivera et al., 2008). Three TAGT cultivars (TCv) are available and registered under Malaysia's National Crop List, as Tagnanan Green (CN7), Tagnanan Orange (CN8), and Tagnanan Gold (CN9). To support the national food security agenda, the Malaysian government has promoted replanting programs, and MATAG is the most recommended type of coconut. This has resulted in a huge demand for MATAG seedlings from the private sector, individuals, and even public players (Ministry of Finance Malaysia, 2017). Therefore, the production of MATAG must be intensified; hence, it is necessary to optimize the hybridization protocol. Because pollen quality is a contributing factor, there has been revived interest in studying TAGT pollen. Currently, the Commodity Development Centre Teluk Bharu, one of the main TAGT pollen production centers (only two centers produce TAGT pollen) under the Malaysian Department of Agriculture (DOA), uses TAGT Gold and Green as the source of pollen in producing MATAG, whereas TAGT Orange is claimed to have lower pollen quality. However, no detailed reports are available.

Konan et al. (2005) reported that the pollen viability of seven Tall coconut varieties ranged between 39.7%–42.3%. Meanwhile, in their study of in vitro pollen germination of six different Tall and Dwarf coconut varieties, Ranasinghe et al. (2010) recorded a germination percentage of 11.9%–31.6%. In addition, Hebbar et al. (2018) found that the average pollen germination percentage of 12 coconut genotypes belonging to Talls, Dwarfs, and hybrids was 48.5%. For the TAGT cultivar used in the production of the MATAG hybrid program, all male flowers on the inflorescences were used for pollen collection, with approximately 40% germination (Konan et al., 2005). Whereas in vitro pollen germination of other common palms such as *Elaeis guineensis* (oil palm, variety Pisifera) and *Dypsis lutescens* (areca palm) can result in a germination percentage of up to 72% (Youmbi et al., 2015) and 90% (Liu et al., 2013), respectively. It is possible to obtain coconut pollen with a germination percentage of more than 50%; however, this is often not achieved. For example, coconut pollen of the San Ramon variety collected from the top and middle parts of the rachis achieved more than 60% germination (64% for the middle part and 78% for the top part); however, with pollen from the bottom part of the rachis, only 33% germinated (Ranasinghe et al., 2010). Thus, the low pollen viability of TAGT can be improved by understanding the nature and variation of the pollen quality within the inflorescence. According to Nampoothiri (1970) and Ranasinghe et al. (2010), the pollen at the distal end of the rachis is more viable than that at the proximal end. Nonetheless, there are no reports on the viability of coconut pollen at different positions on the rachilla, since the opening of male coconut flowers commences from the apex of the rachilla and extends downward (Niral and Jerard, 2018).

In this study, the aims were to: 1) compare the physical characteristics of inflorescences and the quality of the pollen collected from TAGT Gold, Green, and Orange; 2) determine the influence of the positions of the male flower on the rachis on the quality of the pollen; and 3) determine the influence of the position of the male flower on the rachilla on the quality of the pollen.

2. Materials and methods

2.1. Study site and source of the pollen

The experiment was conducted at the Commodity Development Centre (Pusat Pembangunan Komoditi) in Teluk Bharu, Perak, Malaysia. The soil was classified as

riverine alluvial, with a pH of 5.6. TAGT coconut palms in the Centre were around 11 years old (planted in 2010). A total of 120 inflorescences were collected each year from certified and healthy TCv, namely TAGT Gold, Green, and Orange (Figures 1a–1c) for pollen collection. For this, 40 palms each of TAGT Gold, Green, and Orange were randomly selected. The experiments were conducted in both March 2021, with an average temperature of 28.26 °C and 85.2% humidity, and March 2022, with an average temperature of 28.68 °C and 86.02% humidity, yielding consistent results across both years. Consequently, this paper presents the average values of the results.

2.2. Physical characteristics of the inflorescence

To avoid pollen shedding, TAGT inflorescences were harvested at 8:30 a.m. on the first day after the natural opening of the spathe (Hebbar et al., 2018). The physical characteristics of TAGT inflorescences, including the rachis length (RSL), total number of rachillae (NRA) on the rachis, rachilla length (RAL), and fresh weight of the male flowers (FWMF), were determined. The RSL was measured from the bottom of the first rachilla to the last rachilla at the top of the rachis using a measuring tape (Figure 1d). The rachillae of TAGT Gold, Green, and Orange were divided equally based on the length to the top

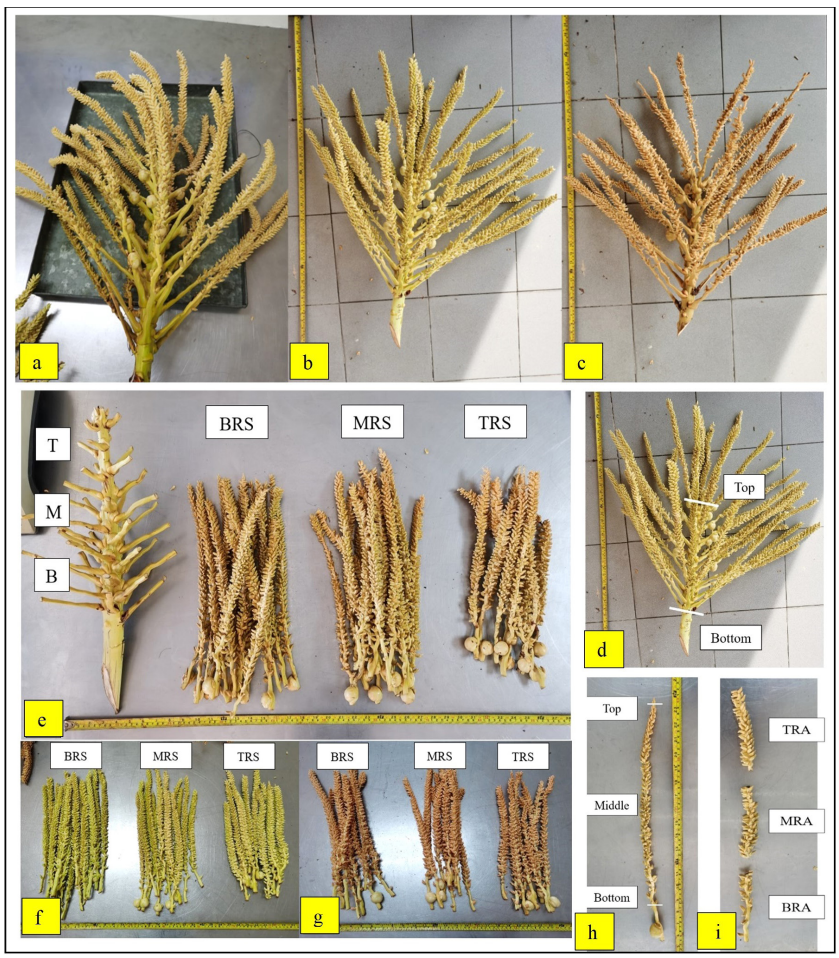

Figure 1. Inflorescence of the TAGT coconut cultivar and the positions of with male flowers on the rachis and rachilla. (a) Inflorescence of TAGT Gold, (b) TAGT Green, (c) TAGT Orange. (d) The RSL was measured from the bottom of the first rachilla to the last rachilla at the top of the rachis. (e) Rachis of TAGT Gold was divided equally into the top (T), middle (M) and bottom (B) parts. Rachillae on the respective parts were cut and divided into the top part of the rachis (TRS), middle part of the rachis (MRS), and bottom part of the rachis (BRS). (f) Rachillae of the TAGT Green and TAGT Orange (g) were also equally divided according to the RAL. (h) The rachilla length (RAL) was measured from the last male flower at the bottom of the rachilla to the bottom end of the male flower at the tip of the rachilla. (i) Rachilla was divided equally into the TRA, MRA, and BRA according to the RAL.

part of the rachis (TRS), middle part of the rachis (MRS), and bottom part of the rachis (BRS), as shown in Figures 1e–1g. The NRA at different positions on the inflorescences was recorded. RAL was measured from the bottom end of the last male flower at the bottom of the rachilla to the bottom end of the male flower at the tip of the rachilla, as shown in Figure 1h. Male flowers were separated from each part of the rachilla, collected in a tray, labelled, and weighed. The FWMF was expressed as g/rachilla.

2.3. Processing of the pollen

The procedures for processing coconut pollen followed the guidelines of DOA Malaysia. Male flowers that were removed from the rachillae were dried for 48 h in a drying room with an average temperature of 35.7 °C (minimum 30.5 °C, maximum 39.8 °C) until all the perianth parts were separated, resulting in the shedding of pollen on the tray. The dried male flowers were gently crushed using a rolling pin to allow more pollen to fall onto the tray. The crushed male flowers were sieved, and the pollen was collected, weighed, and stored in a glass vial.

2.4. Pollen viability and pollen size measurements at different positions on the rachis

To compare the viability of pollen from different positions on the inflorescence, pollen from TAGT Gold, Green, and Orange was divided into parts of the rachis, that is, TRS, MRS, BRS, a mixture of top and middle parts (TMRS), and a mixture of top, middle, and bottom parts (TMBRS). In vitro germination was used to test the TAGT pollen viability as it is more reliable than the staining viability test (Sunilkumar et al., 2011; Mosquera et al., 2021). In vitro germination was conducted using a germination medium consisting of 10% sucrose, 0.3% agar, 0.01% boric acid, and distilled water (Hebbar et al., 2018). All the media components were dissolved in boiling water, poured into 35-mm Petri dishes, and allowed to cool. Pollen was dusted onto the surface of Petri dishes containing 2 mL of germination medium using a watercolor brush. Then, the covered Petri dishes were incubated at ambient temperature (29.5–32 °C) for 2 h as maximum pollen germination of TAGT was observed after this incubation period in preliminary experiment. The Petri dishes were observed under a light microscope equipped with a Dino-Eye AM7025X Edge eyepiece. The germination percentage was obtained using four replicates, accomplished in four random visual areas at 80 × magnification for each replicate, consisting of at least 100 pollen grains each. The pollen grains were considered viable in the presence of pollen tubes. The percentage of pollen viability was calculated using the following formula: % pollen viability = (number of germinated pollen grains / total number of pollen grains) × 100. The pollen tube size and pollen tube length were evaluated using DinoCapture 2.0 (version 1.5.43; AnMo Electronics Corp. Sanchong Dist., New Taipei City, Taiwan) software.

2.5. Pollen viability at different positions on the rachis and rachilla

This experiment was conducted using the TAGT cultivar that performed the best according to the results obtained, as the TAGT pollen had to be sparingly used to produce the MATAG hybrid. The rachis of the best performing TAGT cultivar (TAGT Gold) was divided into different parts, that is, TRS, MRS, and BRS, as mentioned earlier. Rachillae from each part was later divided into the top (TRA), middle (MRA), and bottom parts (BRA), as shown in Figure 1i. Male flowers were processed to obtain pollen, and pollen viability was determined.

2.6. Data analysis

All the experiments were performed as factorial experiments in a completely randomized design with four replicates. Data were subjected to analysis of variance (ANOVA) and where the F value indicated significance, means were compared with the least significance difference (LSD) test at $p \leq 0.05$ using SAS 9.4 (SAS Institute, Cary, NC). Pollen viability (%) was arcsine-transformed, whereas the fresh weight (g/rachilla) of the male flowers were square-root-transformed.

3. Results

3.1. Physical characteristics of the TAGT inflorescence

There was no interaction between different TCv and the position on the rachis (PRs) for the RSL, NRA on the rachis, RAL, and fresh weight of the male flowers (FWMF), as shown in Table 1. Therefore, the results were interpreted based on the main effects (TCv and PRs). The RSL for TAGT Gold, Green, and Orange ranged from 29.00 to 33.38 cm, with around 27–38 rachillae (ranged from 3.57 to 3.72 cm long) on the rachis. The fresh weight of the unprocessed male TAGT flowers (before drying) was approximately 20.0–24.6 g/rachilla. The NRA at the TRS, MRS, and BRS was 10, 11, and 13, respectively. Despite the RAL at different positions on the rachis being different (Figures 1e–1g), no statistical difference was found in the RAL (the TRS was 3.38 cm, while the MRS and BRS were both around 3.80 cm). There was also no significant difference in the FWMFs at different positions on the rachis. Approximately 21.0 g/rachilla of fresh male flowers could be collected from the TRS, whereas both the MRS and BRS could achieve approximately 23.0 g/rachilla. In general, none of the TCv or different positions on the rachis prevailed in terms of the physical characteristics of the inflorescence.

3.2. Pollen yield, pollen size, and pollen performance of TAGT Gold, Green, and Orange at different positions on the rachis

Statistical analyses of the TCv and PRs showed no interaction with the amount of pollen collected, pollen size, pollen viability, or pollen tube length after incubation for 2 h at room temperature (Table 2). By examining the single factors, TAGT Gold, Green, and Orange showed no differences

Table 1. Main and interaction effects of the TAGT cultivars (Gold, Green, and Orange) and the PRs (TRS, MRS, and BRS) on the RSL, NRA, RAL, and FWMF.

Treatment	RSL (cm)	NRA	RAL (cm)	FWMF (g/rachilla)
Cultivar (TCv)				
Gold	33.38 ± 2.56	37 ± 3.07	3.72 ± 0.13	19.98 ± 1.30
Green	33.38 ± 1.14	38 ± 4.27	3.57 ± 0.13	24.60 ± 0.98
Orange	29.00 ± 2.92	27 ± 2.58	3.61 ± 0.90	22.00 ± 1.64
PRs				
TRS	-	10 ± 0.48	3.38 ± 0.10	20.50 ± 1.73
MRS	-	11 ± 0.81	3.75 ± 0.10	22.94 ± 1.29
BRS	-	13 ± 1.24	3.76 ± 0.12	23.14 ± 1.13
Significance level				
TCv	NS	NS	NS	NS
PRs	-	NS	NS	NS
TCv × PRs	-	NS	NS	NS

Results are presented as the mean ± standard error. NS: no significant difference at $p \leq 0.05$ and -:not relevant.

Table 2. Main and interaction effects of the TAGT cultivars (Gold, Green, and Orange) and the PRs (TRS, MRS, BRS, TMRS, and TMBRS) on the amount of pollen obtained (APO), pollen size (PS) after rehydration, pollen viability (PV), and pollen tube length (PTL) after 2 h of incubation. The initial PS (before rehydration) was around 0.035 mm for TAGT Gold, Green, and Orange.

Treatment	APO (g/10 g FW male flower)	PS (mm)	PV (%)	PTL (mm)
Cultivar (TCv)				
Gold	0.75 ± 0.04	0.060 ± 0.001	47.39 ± 2.78a	0.665 ± 0.03
Green	0.57 ± 0.02	0.057 ± 0.001	38.34 ± 2.64b	0.638 ± 0.03
Orange	0.69 ± 0.03	0.057 ± 0.001	37.47 ± 1.61b	0.681 ± 0.03
PRs				
TRS	0.26 ± 0.02a	0.058 ± 0.002	50.61 ± 2.82a	0.732 ± 0.03a
MRS	0.22 ± 0.01ab	0.058 ± 0.001	44.59 ± 2.90ab	0.764 ± 0.03a
BRS	0.19 ± 0.02b	0.059 ± 0.001	23.88 ± 2.95c	0.608 ± 0.04b
TMRS	-	-	49.46 ± 2.28a	-
TMBRS	-	-	37.10 ± 2.23b	-
Significance level				
TCv	NS	NS	*	NS
PRs	*	NS	**	**
TCv × PRs	NS	NS	NS	NS

Results are presented as the mean ± standard error. -: not relevant; NS: no significant difference at $p \leq 0.05$ using the LSD test; *: significant differences at $p \leq 0.05$; **: highly significant differences at $p \leq 0.01$. Means with the same letter within a factor and column are not significant different at $p \leq 0.05$ using the LSD test.

in the amount of pollen collected, size of pollen, or pollen tube length after 2 h of incubation, except for the viability of the pollen. Around 0.6–0.8 g of pollen was collected from 10 g of fresh male flowers (separated from the rachilla) for the TAGT Gold, Green, and Orange. The initial pollen size (before rehydration) for TAGT Gold, Green, and Orange was approximately 0.035 mm. After 2 h of incubation on the germination medium, the pollen size increased to 0.057–0.060 mm. In terms of pollen performance, the viability of the TAGT gold pollen (47.4%) was significantly higher than TAGT Green (38.3%) and Orange (37.5%). However, no difference was observed in the pollen tube length after 2 h of incubation (0.638–0.681 mm) in TAGT Gold, Green, and Orange.

In contrast, pollen collected from different positions on the rachis showed different pollen yields, pollen viability, and pollen tube lengths after 2 h of incubation. A greater amount of pollen was collected from the TRS than from the BRS, although the initial FWMF was not statistically different, as shown in Table 1. However, the amount of pollen collected from the MRS showed no difference compared to the TRS and BRS. The amount of pollen from the TRS was approximately 0.07 g (per 10 g of fresh male flowers) more than the BRS. Meanwhile, pollen collected from the TRS and MRS was significantly more viable (44.6%–50.6%) compared to the 23.9% viability of the pollen collected from the BRS. The mixture of pollen from the TMRS showed approximately 50% viability, whereas the mixture of pollen from the TMBRS showed only 37%. A comparison of the pollen viability based on the microscopic visualization of the different parts of the rachis is shown in Figure 2. Moreover, pollen from the TRS and MRS had higher pollen vigor as the pollen tube length was 0.12–0.16 mm longer than the pollen collected from the BRS after 2 h of incubation on the germination medium.

3.3. Pollen viability of TAGT Gold at different positions on the rachis and rachilla

Figure 3 shows the pollen viability of TAGT Gold at different positions on the rachis and rachilla. TAGT Gold was chosen for this experiment because its pollen performed better than TAGT Green and Orange pollen. A significant interaction was found between the position of the TAGT Gold pollen on different parts of the rachis and rachilla. Up to 68% pollen viability was achieved using the TRS × TRA, TRS × MRA, TRS × BRA, MRS × TRA, and MRS × BRA. In addition to the significant difference found between the TRS × TRA and MRS × MRA, no difference was found between the TRS × TRA, TRS × MRA, TRS × BRA, MRS × TRA, and MRS × BRA (45%–68%). Meanwhile, pollen collected from the BRS × TRA, BRS × MRA, and BRS × BRA had significantly lower pollen viability (13%–22%) than those collected from other parts of the rachis and rachilla. Looking at the graph distribution, pollen at the TRS and MRS was always significantly higher in viability than the pollen at the BRS at every part of the rachilla (TRA, MRA, and BRA). Pollen at the TRS and MRS was approximately two to three times more viable (TRS: 52%–68%; MRS: 45%–59%) than that at the BRS (13%–22%). The viability of the TAGT Gold pollen at different positions on the rachis is shown in Figure 4.

Figure 4 shows the pollen viability of TAGT Gold at the TRS, MRS, BRS, and the TMRS, and the TMBRS. It can be clearly seen that pollen from the TRS (67%) and MRS

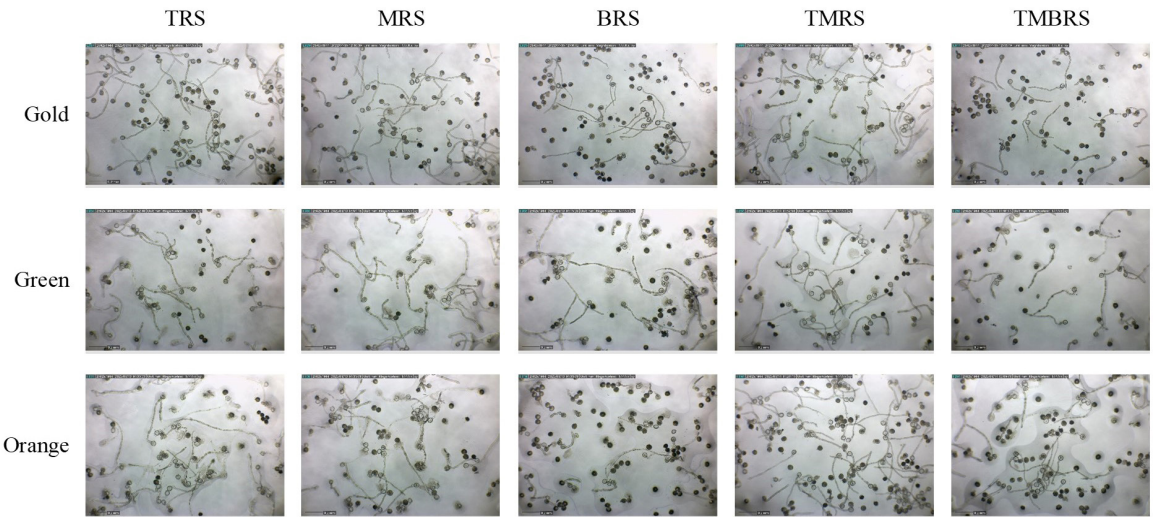

Figure 2. Germination (viability) and pollen tube growth of pollen collected from different TAGT cultivars and positions on the rachis after 2 h of incubation on the germination medium. Pollen with a tail indicates viable pollen. No interaction was found between the TAGT cultivars and the positions on the rachis; however, it can be clearly seen that pollen viability was higher in the TRS and MRS compared to the BRS for TAGT Gold, Green, and Orange. Moreover, the mixture of pollen from the TMRS showed higher viability than that from the TMBRS despite the type of TAGT cultivar.

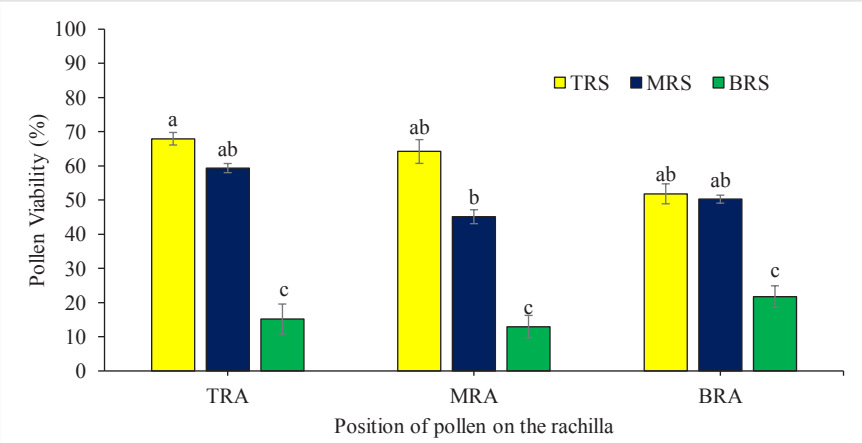

Figure 3. Viability of the TAGT Gold pollen collected from different positions on the rachis and rachilla. B = bottom; RS = rachis; M = middle; T = top; RA = rachilla. Means with the same letter in each source of variation are not significantly different at $p \leq 0.05$. Vertical bars indicate ± standard error.

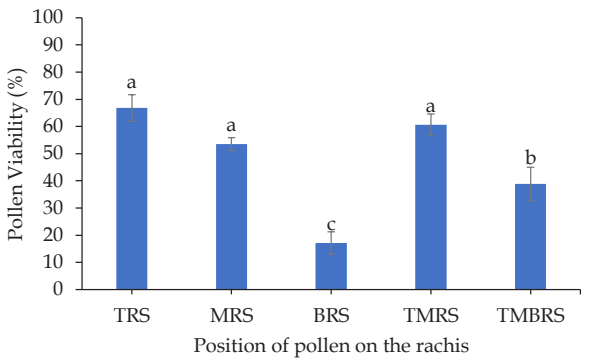

Figure 4. Viability of the TAGT Gold pollen collected from different PRs (TRS, MRS, and BRS) and the mixture of TRS and MRS, and TRS, MRS, and BRS. Means with the same letter are not significantly different at $p \leq 0.05$. Vertical bars indicate ± standard error.

(54%) was more viable than that collected from the BRS (17%). However, mixing the TAGT Gold pollen collected from the TMRS (61%) was approximately 22% higher in viability than mixing the pollen from the TMBRS (39%).

4. Discussion

Information obtained on the physical characteristics of the inflorescence and pollen performance can be used as a reference in the selection of suitable TCv and in determining suitable position on the rachis for pollen collection. Generally, there were no differences in the physical characteristics such as the RSL, rachilla number, RAL, and FWMF in TAGT Gold, Green, and Orange. Meanwhile, other than the differences in pollen viability, the amount of pollen collected, pollen size after 2 h of incubation, and pollen vigor were the same for TAGT Gold, Green, and Orange. Before rehydration, there was no difference in the size of the TAGT pollen among the different TCv. According to Thomas and Josephrajkumar (2013), coconut pollen is spherical when fresh but shrinks rapidly after shedding and becomes ellipsoidal with a longitudinal structure. The pollen immediately becomes hydrated, regaining its spherical shape, and the sutures disappear when placed in water. Generally, the pollen lands on the stigma, absorbs water, and undergoes rehydration. If the pollen is compatible, intense metabolic activity is induced, which triggers the growth of pollen tubes (Bosch and Wang, 2020). Pollen size is also often used as a biological parameter to estimate the viability of mature pollen grains (De Storme et al., 2013).

The present study showed that the viability of pollen collected from the entire inflorescence of TAGT Gold,

Green, and Orange was between 37% and 47%. However, the pollen viability of TAGT Gold was 10% higher than that of TAGT Green and Orange, despite having the same physical characteristics of the inflorescence. This was different from the pollen viability of the different Malayan Dwarf varieties. According to Chaturvedi et al. (2017), the pollen viability of Malayan Green Dwarf, Malayan Orange Dwarf, and Malayan Yellow Dwarf (MYD) was 31%, 30%, and 26% respectively and did not show significant difference. In coconuts, the Talls and the Dwarfs generally differ in their modes of pollination. The Talls are naturally cross-pollinating, and thus, the populations show varying degrees of heterozygosity, whereas the dwarfs are naturally self-pollinating, resulting in predominantly pure lines that are more homogenous and display more similar features (Kamaral et al., 2014). On the other hand, the statement saying 'pollen quality was lower in TAGT Orange' was not true, as TAGT Orange and Green performed the same according to the results obtained. Other than having lower pollen viability than TAGT Gold, TAGT Green and Orange were ideal sources of pollen to produce MATAG. In addition to the selection of TCv as the source of pollen, the color of TAGT is important for determining the colors of the hybrid produced to fulfill market demand. For example, crossing the Malayan Red Dwarf with TAGT Orange will result in MATAG Orange, whereas crossing the MYD with TAGT Orange will result in MATAG Gold.

A significant interaction was found between the position of the pollen on different parts of the rachis and the rachilla. It is recommended to use TAGT Gold pollen collected from the TRS × TRA, TRS × MRA, TRS × BRA, MRS × TRA, MRS × MRA, and MRS × BRA to achieve 68% higher pollen viability compared to the initial 39% when pollen from the whole inflorescence was mixed. Higher pollen viability is important because pollen is strongly related to prezygotic success (Alonso et al., 2013; Arceo-Gómez and Ashman, 2014). For example, high-quality pollen can improve both fruit set and nutritional properties in almonds (Brittain et al., 2014; Klein et al., 2015). Additionally, the results showed that the proportion of viable TAGT pollen differed significantly along the rachis. The amount of TAGT pollen collected from the TRS and MRS was higher than that collected from the BRS. Consequently, the mixing of pollen from the TMRS resulted in 12% more pollen than that with the conventional pollen harvesting method (mixing all the pollen on the inflorescence). This suggests that, if circumstances do not permit the use of TAGT Gold solely as a source of pollen, TAGT pollen should be collected from the TMRS to increase pollen viability. Unlike the pollen of *Butia* (family Arecaceae, palms that produce edible fruits), the viable pollen was not significantly different along the rachis. For instance, *B. odorata* and *B. yatay* showed 56%, 59%, and 56%, and 50%, 47%, and 46% of pollen viability when collected from the apical, medium, and basal parts of the rachis, respectively (Mourelle et al., 2015). According to Perera et al. (2010), flora ontogenesis of coconut was acropetal proceeding from the bottom to top of the rachilla. Interestingly, flower maturation was basipetal, occurring from the top to the bottom. Thus, the difference in pollen viability of TAGT was due to pollen maturity. In addition, the pollen of coconuts must be dispersed at different times so that all the female flowers can be pollinated naturally. Similar results were reported by Nampoothiri (1970) and Ranasinghe et al. (2010), who found that pollen at the distal end of the rachis was more viable than that at the proximal end. In contrast, pollen vigor was also found to be higher in more mature pollen. Higher pollen vigor increases the chance of delivering male gametes into the female gametophytes for fertilization. Thus, pollen from the desired zones of the inflorescence can be used for in vitro germination and fertilization studies. Because the viability of the TAGT pollen was higher when harvested from the TMRS, it is important to choose only male flowers from the selected zones of the inflorescence for controlled pollination purposes.

5. Conclusions

This study clearly showed that none of the TCv prevailed in terms of the physical characteristics of the inflorescence, the amount of pollen collected, pollen size, or pollen vigor. Thus, the selection of suitable TCv cannot be based on these characteristics. However, the pollen viability of TAGT Gold was 10% higher than those of TAGT Green and Orange. Nevertheless, TAGT Green and Orange are also recommended for use as a source of pollen in producing coconut hybrids, if pollen is collected from the recommended zones on the inflorescence. Based on this study, pollen from TAGT at different positions on the rachilla was not the key to determining pollen viability, but the position of the pollen on the rachis influenced pollen viability. Pollen collected from the TMRS was always significantly more viable than that collected from the BRS. Mixing pollen from the TMRS increased pollen viability by 13%–22% to achieve 50%–61% viability compared to 37%–39% when pollen from the whole inflorescence was mixed. This detailed study on TAGT pollen provides a precise reference for the selection of suitable TCv and for determining the suitable pollen PRs and rachilla as the source of pollen in producing coconut hybrids.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

Abberton M, Batley J, Bently A, Bryant J, Cai H et al. (2016). Global agricultural intensification during climate change: a role for genomics. Plant Biotechnology Journal 14 (4): 1095-1098. https://doi.org/10.1111/pbi.12467

Alonso C, Navarro-Fernandez CM, Arceo-Gomez G, Meindl GA, Parra-Tabla V et al. (2013). Among-species differences in pollen quality and quantity limitation: implications for endemics in biodiverse hotspots. Annals of Botany 112 (7): 1461-1469. https://doi.org/10.1093/aob/mct213

Arceo-Gómez G, Asman, TL (2014). Co-flowering community context influences female fitness and alters the adaptive value of flower longevity in *Mimulus guttatus*. The American Naturalist 183 (2): E50-63. https://doi.org/10.1086/674358

Bosch M, Wang L (2020). Pollen-stigma interactions in Brassicaceae: complex communication event regulating pollen hydration. Journal of Experimental of Botany 71: 2465-2458. https://doi.org/10.1093/jxb/eraa117

Brittain C, Kremen C, Garber A, Klein AM (2014). Pollination and plant resources change the nutritional quality of almonds for human health. PLoS One 9 (2): e90082. https://doi.org/10.1371/journal.pone.0090082

Chaturvedi VK, Hebbar KB, Chandran KP, Tomas RJ, Shareefa M, Nampoothiri CK, Sivadasan J (2017). Influence of temperature, germination duration and cultivar on in vitro pollen germination and pollen tube growth in coconut (*Cocos nucifera* L.). International Journal of Advanced Research 5 (5): 544-551. https://doi.org/10.21474/IJAR01/4144

DebMandal M, Mandal S (2011). Coconut (*Cocos nucifera* L.: Arecaceae): in health promotion and disease prevention. Asian Pacific Journal of Tropical Medicine 4 (3): 241-247. http://doi.org/10.1016/S1995-7645(11)60078-3

De Storme N, Zamariola L, Mau M, Sharbel TF, Geelen D (2013). Volume-based pollen size analysis: an advanced method to assess somatic and gametophytic ploidy in flowering plants. Plant Reproduction 26 (2): 65-81. https://doi.org/10.1007/s00497-012-0209-0

Gemperle FR, Fremond YL (1978). Tagnanan coconut population: a promising breeding material. In: Proceedings of the International Conference on Cocoa and Coconuts; Kuala Lumpur, Malaysia. pp. 500-507.

Hebbar KB, Rose HM, Nair AR, Kannan S, Niral V et al. (2018). Difference in in vitro pollen germination and pollen tube growth in coconut (*Cocos nucifera* L.) cultivars in response to high temperature stress. Environmental and Experimental Botany 153: 35-44. https://doi.org/10.1016/j.envexpbot.2018.04.014

Impe D, Reitz J, Kopnick C, Rolletschek H, Borner A et al. (2020). Assessment of pollen viability for wheat. Frontiers in Plant Science PMC6987437. https://doi.org/10.3389/fpls.2019.01588

Karun A, Sjini KK, Niral V, Amarnth CH, Remya P et al. (2014). Coconut (*Cocos nucifera* L.) pollen cryopreservation. Cryo Letters 35 (5): 407-417.

Kamaral LCJ, Perera SACN, Perera KLNS, Dassanayaka PN (2014). Genetic diversity of the Sri Lanka yellow dwarf coconut form as revealed by microsatellite markers. Tropical Agricultural Research 26 (1): 131-139. https://doi.org/10.4038/tar.v26i1.8078

Klein AM, Hendrix SD, Clough Y, Scofield A, Kremen C (2015). Interacting effects of pollination, water and nutrients on fruit tree performance. Plant Biology 17 (1): 201-208. https://doi.org/10.1111/plb.12180

Konan JL, Bourdeix R, Batugal P (2005). Production and provision of hybrid seednuts. In Batugal P, Benigno D, Oliver J (editors). Coconut hybrids for smallholders, project reports and related papers of the multilocation trials to identify suitable coconut hybrids and varieties for Africa, Latin America and the Caribbean. Amsterdam: Common Fund for Commodities, pp. 12-25.

Liu L, Huang L, Li Y (2013). Influence of boric acid and sucrose on the germination and growth of areca pollen. American Journal of Plant Sciences 4: 1669-1674. http://dx.doi.org/10.4236/ajps.2013.48202

Long K (2017). Debunking the Coconut Milk and Coconut Oil Myths. ScientiaMARDI Agricultural Transformation, vol. 010. Serdang, Malaysia: MARDI, pp. 2.

Mesnoua M, Roumani M, Salem A (2018). The effect of pollen storage temperature on pollen viability, fruit set and fruit quality of six date palm cultivars. Scientia Horticulturae 236: 279-283. https://doi.org/10.1016/j.scienta.2018.03.053

Ministry of Finance Malaysia (2017). 2018 Budget. https://www.mof.gov.my/arkib/budget/2018/bs18.pdf. Accessed on 30 March 2021.

Mosquera DJC, Salinas DGC, Moreno GAL (2021). Pollen viability and germination in *Elaeis oleifera*, *Elaeis guineensis* and their interspecific hybrid. Pesquisa Agropecuária Tropical 51: e68076. https://doi.org/10.1590/1983-40632021v5168076

Mourelle D, Gaiero P, Speroni G, Millán C, Gutiérrez L et al. (2015). Comparative pollen morphology and viability among endangered species of *Butia* (Arecaceae) and its implications for species delimitation and conservation. Palynology 2015: 1-12. https://dx.doi.org/10.1080/01916122.2014.999955

Nampoothiri KUK (1970). Pollen studies in coconut (*Cocos nucifera*) with special reference to a sampling procedure. Indian Journal of Agricultural Science 19: 457-460.

Nampoothiri KUK, Parthasarathy VA (2018). Varietal improvement. In Nampoothiri KUK, Krishnakumar V, Thampan PK, Nair MA (editors). The Coconut Palm (*Cocos nucifera* L.) – Research and Development Perspective. Singapore: Springer Nature Singapore Pte Ltd, pp. 113-156.

Niral V, Jerard BA (2018). Botany, Origin and Genetic Resources in Coconut. In Nampoothiri KUK, Krishnakumar V, Thampan PK, Nair MA (editors). The Coconut Palm (Cocos nucifera L.) – Research and Development Perspective. Singapore: Springer Nature Singapore Pte Ltd, pp. 57-111.

Omar Z, Fatah FA (2020). Unravelling the factors affecting agriculture profitability enterprise: evidence from coconut smallholder production. Accounting 6 (2020): 493-500. https://doi.org/10.5267/j.ac.2020.4.009

Perera PIP, Hocher V, Weerakoon LK, Yakandawala DMD, Fernando SC et al. (2010). Early inflorescence and floral development in *Cocos nucifera* L. (Arecaceae: Arecoideae). South African Journal of Botany 76 (3): 482-492. https://doi.org/10.1016/j.sajb.2010.03.006

Ranasinghe CS, Waidyarathana KP, Pradeep APC, Meneriptiya MSK (2010). Approach to screen coconut varieties for high temperature tolerance by *in-vitro* pollen germination. Cocos 19: 1-11. https://doi.org/10.4038/cocos.v19i1.4748

Razzaq A, Kaur P, Akhter N, Wani SH, Saleem F. (2021). Next-generation breeding strategies for climate ready crops. Frontiers in Plant Science 12: 620420. https://doi.org/10.3389/fpls.2021.620420

Reddy KV, Kumar P, Rao SVR (2017). Economic analysis of coconut in West Godavari district of Andhra Pradesh. IOSR Journal of Business and Management 19 (6): 68-72. https://doi.org/10.9790/487X-1906066872

Rivera RL, Santos GA, Rivera SM, Emmanuel EE, Baylon GB (2008). Development of synthetic variety of coconut: PCA Syn Var 001 I. Status and prospects. Cord 24 (1): 90-112. https://doi.org/10.37833/cord.v24i1.161

Singh RK, Prasad M (2021). Big genomic data analysis leads to more accurate trait prediction in hybrid breeding for yield enhancement in crop plants. Plant Cell Reports 40: 2009-2011. https://doi.org/10.1007/s00299-021-02761-x

Sulusoglu M, Cavusoglu A (2014). In vitro pollen viability and pollen germination in cherry Laurel (*Prunus laurocerasus* L.). The Scientific World Journal 2014: 657123. https://doi.org/10.1155/2014/657123

Sunilkumar K, Mathur RK, Babu DSS (2011). Efficacy of dyes and media on pollen viability and germinability in oil palm (*Elaeis guineensis* Jacq.). International Journal of Oil Palm Research 8 (1): 9-12.

Sunilkumar K, Mathur RK, Babu DSS (2017). Differential pollen longevity in Dura and Pisifera oil palm (*Elaeis guineensis*) fruit types at storage temperatures. Indian Journal of Agricultural Sciences 87 (7): 893-898.

Thomas RJ, Josephrajkumar A (2013). Flowering and pollination biology in coconut. Journal of Plantation Crops 41 (2): 109-117.

van Dijk M, Morley T, Rau ML, Saghai Y (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period of 2010-2050. Nature Food 2: 494-501. https://doi.org/10.1038/s43016-021-00322-9

Widianingrum DC, Noviandi CT, Salasia SIO (2019). Antibacterial and immonumodulator activities of virgin coconut oil (VCO) against *Staphylococcus aureus*. Heliyon 5 (10): e02612. https://doi.org/10.1016/j.heliyon.2019.e02612

Yon RM (2017). Revival of the Coconut Industry. ScientiaMARDI Agricultural Transformation, vol. 010. Serdang, Malaysia: MARDI, pp. 1.

Youmbi E, Tabi K, Ebongue N, Frank G, Tonfack, LB et al. (2015). Oil palm (*Elaeis guineensis* Jacq.) improvement: pollen assessment for better conservation and germination. Journal of Oil Palm Research 27 (3): 212-219.

Youmbi E, Tonfack LB, Mbogning JBD, Nkongmeneck BA (2012). Effect of storage conditions on pollen grains viability and pollen tubes elongation of four *Cola* species (*Malvaceae*). Research & Reviews in Biosciences 6 (1): 35-40.