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An insight into pollen morphology and evaluation of pollen viability, germination and mineral composition of some coconut (Cocos nucifera L.) genotypes

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ABSTRACT

The micro-morphological characteristic of the pollen grain is one of the most important diagnostic traits use for identification and characterization of plants even to the accession levels. In this study, pollen grains characterization of eight different coconut (*Cocos nucifera* L.) accessions were carried out. Morphometric observations and structure analysis of the pollen grains were performed using light and scanning electron microscopy. The variation in length and width observed ranged from 55.06 -71.25 μm and 24.38 -36.27 μm amongst the accessions. The pollen grains exhibited an ellipsoidal to elliptical shape with the exine ornamentation ranging from granulate to reticulate. Pollen viability, germination and pollen tube length were significantly different amongst the tested accessions. While pollen viability varied from 92.35 to 96.5%, germination rate and pollen tube length (after 2 h) ranged from 16.05 - 31.59% and 234.95 μm to 414.96 μm respectively. Pollen mineral composition from different accessions also varied significantly with Malayan Orange Dwarf (MOD) recording higher phosphorus (0.93%), calcium (0.64%) and magnesium (0.38%) content while highest potassium (1.01%), sodium (0.71%), iron (149.35 ppm) zinc (119.59 ppm) and boron (45.05 ppm) content was noticed in San Ramon Tall (SNRT). This investigation highlights the fact that these pollen morphological features could be potentially used for differentiating the selected accessions thereby supporting their conservation and crop improvement studies.

1. Introduction

The coconut (*Cocos nucifera* L.) most popularly known as the ‘Tree of life’ due to its influence in the socioeconomic sector of low to middle income group of the tropics is a monospecific palm of the Genus *Cocos* (Dransfield et al., 2008). This palm belongs to the family Arecaceae, subfamily Arecoideae, tribe Cococeae, and sub tribe Attaleineae. Eventhough the palm doesn’t have close relatives, some degree of affinity towards genera Syagrus, Attaleina, and Parajubaea is observed (Baker et al., 2009). Coconut offers nutritious food, reviving drink, oil for edible and non-edible purposes, fibre of commercial value, shells for industrial and fuel uses, timber and a range of miscellaneous products for both households as well as industrial uses. In the past few years, coconut is increasingly being considered a health food, with tender coconut water, virgin coconut oil and coconut inflorescence sap being encouraged for consumption. The palm is grown in more than 90 countries, comprising primarily coastal areas and island ecosystems. Currently, the coconut production in the world is estimated at 68,833 million nuts from 12.08 million ha and productivity of 5777 nuts ha^{-1} (ICC, 2019). Mainly the world production is concentrated in tropical Asia, with Indonesia, the Philippines, and India, jointly accounting for more than 70% of the total area and production. Worldwide coconut populations have been classified into two main groups: the Pacific group with five sub-groups (Southeast Asia, Micronesia, Polynesia, Melanesia and the Pacific coastline of South and Central America) and the Indo-Atlantic group (Perera et al., 2009). Despite being a monotypic genus, *Cocos nucifera* L. has substantial genetic diversity in its populations. The varieties of coconut could be distinguished based on their qualitative traits such as size, shape, nut colour and pest/disease resistance. In contrast, quantitative traits such as precocity of flowering, bunch, nut numbers and fruits characteristic features are also used to investigate the diversity. Coconut is monoecious, producing both male and female flowers in the same floral bunch and hence highly cross pollinated. Pollen morphology is one of the important tool for the taxonomic study of angiospermic plant groups (Sarkar et al., 2017). Researchers points out the importance of pollen development and morphology in classification and identification of many plant species including Caecalpeniaceae (Ullah et al., 2022), Melliferae (Attique et al., 2022),

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Amaranthaceae (El Ghazali, 2022) and olives (Javady and Arzani, 2001). In palms, such studies were mostly carried out in date palms (Shahsavar and Shahhosseini, 2022). Pollen grains represents haploid phase of life cycle and are an essential component of plant reproduction, modulating gene flow and also offer nutrition for some pollination vectors (Mander et al., 2020).

Studying micro-morphological characteristics of pollen provides valuable information, which ultimately serves in solving controversial taxonomical issues associated with distinguishing species and cultivars (Nikolic and Milatovic, 2016). These studies also helps in understanding the functional role of morphological attributes of pollen grains (Konzmann et al., 2019).

The study of pollen grain provides a clue to the breeding systems and hybridization (Alotaibi et al., 2020). In India, successful pollination and nut set was obtained in double coconut, a rare kind of palm species using pollen obtained from another country (Neema et al., 2020). Pollen characters including exine ornamentation, shape, apertural pattern, pollen symmetry, colpus length, width, and margins used to detect the similarities and dissimilarities between genera and also species of the same genus (Jafari and Karmi, 2007). Bahadur et al. (2022) had pointed out that pollen exine pattern is so genetically stable for the different plant species that it can be used for species or cultivar identification. Several researchers had observed that structural characteristics of the pollen grains of some fruit species and pollen morphology, including size, shape and exine striation patterns may be utilized for species identification (Lanza et al., 1996; Currie et al., 1997; Javady and Arzani, 2001; Arzani et al., 2005; Mert, 2009).

In addition to morphology, knowledge of the viability of pollen is crucial for the genetic improvement of many species (Souza et al., 2015). Pollen viability and vigour decide the quality of pollen (Veluru et al., 2021). Pollen fertility and viability have paramount importance in hybridization programmes. Successful pollination is a prerequisite for fertilization and seed set in most plants. And the insight knowledge of pollen biology, including pollen viability, pollen germination and pollen tube growth, is required for any rational approach to increase productivity (Bolat and Pirlak, 1999; Shivanna, 2019; Acarsoy Bilgin, 2022).

Pollen grains possess nutritionally essential substances, such as carbohydrates, proteins, amino acids, lipids, and minerals (Rzepecka-Stojko et al., 2015). The chemical and mineral composition of pollen differ within and between plant species. They are a good source of minerals such as B, Zn, Se, Fe, Mo, Cu, Mn, Co and Ni (Hassan, 2011).

Eventhough literature search suggests that pollen studies are carried out in a wider scale in other plant species, in coconut, the research works are scarce. Coconut is a cross pollinated crop and a thorough understanding of the pollen used is essential for successful breeding programmes. Hence the present study was carried out to evaluate the micro morphology, viability and mineral content of globally diverse coconut accessions so as to provide critical data for future Crop Improvement programmes.

2. Materials and methods

2.1. Accession attributes and experimental location

Eight different coconut accessions differing in origin, stature, breeding behaviour and tolerance to abiotic stresses were selected for the study (Table 1). These accessions were 20 to 25 years old palm maintained under field gene bank at ICAR-CPCRI (Indian Council of Agricultural Research- Central Plantation Crops Research Institute) under recommended management condition. The field gene bank is geographically located at 12.31°N latitude and 74.51°E longitude with altitude range from 15 to 17 m above mean sea level. The average temperature is 31.5°C during summer and 21.3°C in winter. The mean annual rainfall of the area is 3400 mm, spreading over 132 days with high average annual relative humidity of 88%. Soil is acidic sandy loam with low clay content and pH of 4.4.

Table 1
Details of the coconut genotypes used in the study.

Genotype	Region/Country
Tall	
West Coast Tall (WCT)	South Asia (India)
Laccadive Micro Tall (LMT)	South Asia (India)
San Ramon Tall (SNRT)	South East Asia (Philippines)
Java Tall (JVT)	South East Asia (Java)
Nigerian Tall (NIT)	African region (Nigeria)
Dwarf	
Chowghat Green Dwarf (CGD)	South Asia (India)
Chowghat Orange Dwarf (COD)	South Asia (India)
Malayan Orange Dwarf (MOD)	South East Asia (Malaysia)

2.2. Pollen collection and scanning electron microscopic studies

Coconut inflorescence is a spadix with thick spathe covering the spike. The spathe splits open vertically exposing the spikelets. After 5–6 days, the spikelets are collected and immediately transferred to laboratory in ice boxes. The male flowers are separated and pollen was collected by gently tapping with nylon brush. For obtaining scanning electron micrographs, pollen grains were mounted on aluminium stubs covered with double-sided transparent tape and coated with gold layer (0.02 μm thick) in a sputter coater, EMITECH SC7620 (Quorum Technologies Ltd., UK). The prepared sample were observed at 15 kV with an FEI-Quanta 250 SEM (FEI, Japan) (Brent and Darleen, 1982). Each sample included ten randomly collected pollen grains. Ten randomly collected pollen grains were considered as a sample. For obtaining the group micrographs, three samples per accession were photographed at a magnification of 500x. 3000x for individual pollen and exine ornamentation on pollen surface and colpus edge, 3000x and 10,000x magnification was done. The traits characterised were pollen size and shape (length, width, and length/width ratio), exine ornamentation and aperture characteristics. The first two traits were observed in the lateral view while for the latter, polar view was contemplated. For the size measurement, the length and breadth were expressed as the equatorial diameter (E) and the polar diameter (P) respectively.

2.3. Pollen collection for viability and *in-vitro* germination studies

Six to seven days after complete emergence of inflorescence from leaf axils, the spikelets are collected. Usually, morning time, between 8 and 10 AM is preferred for the collection as the initiation of male flower dehiscence happens during this time. Rainy days are avoided as the atmospheric moisture would affect the viability and storage properties of pollen. Male flowers were stripped off from the spikes and were oven dried at 40 °C for a day. These dried flowers were sieved (mesh size - 0.2 mm) to obtain the pollen.

2.4. Pollen viability and *in-vitro* pollen germination

A pollen sample of approximately 0.02 g was homogenized with 2 μl of 1% aceto carmine and was incubated at 37±1°C for 30 min in a biological incubator. The slides were observed under a compound microscope (Leica camera; DFC 250) at 10x magnification. The pollen grains that stained red with intact walls were considered viable and those that were colourless or stained red with ruptured walls were considered nonviable. Percentage pollen viability was calculated using the formula,

Pollen viability (%) = (No. of stained pollen grains / total number of pollen grains) $\times$ 100

In the study, ability of the pollen grains to germinate in artificial germination media was deliberated. Germination media was prepared as reported by Karun et al. (2006). The media composition was 8% sucrose, 1% agar, 1% gelatine and 0.01% boric acid. An aliquot of 2 ml of the prepared media was spread on a glass slide and pollen was dusted to the media with a nylon brush. The slides were made in triplicates for each accession and replicated thrice. These slides were incubated in a Petri dish lined with moist filter paper for 2 h at 32 ± 2 °C and was then observed under a compound microscope. Pollen grains were considered to have germinated when pollen tube length (PTL) was at least equal to or greater than the pollen grain diameter (Kakani et al., 2002). A minimum of 400–500 pollen grains were counted in 10 randomly selected microscopic fields for the measurement of germination percentage. Photographs of each field were taken using a Leica camera (DFC 250). Pollen tubes attained their maximum length in 2 h and beyond that time tube rupturing was noticed. Hence, the tube length was measured immediately after 2 h incubation for all the treatments using the Leica Q Win software and expressed in a micrometre. Percentage pollen germination was calculated using the formula:

% Pollen germination = (Number of germinated pollen / Total number of pollen) × 100.

The percentage of pollen germination and pollen tube length data were averaged over all the microscopic fields per slide.

2.5. Determining nutrient content and mineral composition of pollen

Pollen samples were wet digested (Piper, 2019) with a di-acid mixture of HNO_3 and $HClO_4$ in the ratio of 9:4 respectively. Nitrogen content was determined by micro Kjeldahl method (Humphries, 1956). Phosphorus content was analysed by the Vanado-Molybdate yellow colour method (Jackson, 2005) and quantified using UV–VIS spectrophotometer (UV-1601, Shimadzu). Potassium in the digested extracts was determined (Williams and Twine, 1960) using a flame photometer (CL378, Elico). While Calcium and Magnesium content was determined by the versenate titration method (Diehl et al., 1950), sulphur was quantified utilizing turbidimetry method (Chaudry and Cornfield, 1966). Micronutrients (Fe, Mn, Zn and Cu) were analysed using atomic absorption spectrophotometer (ICE-3300, Thermo Fisher Scientific). Boron content was determined using azomethane H reagent (Berger and Troug, 1939).

2.6. Statistical analysis

The data obtained for the selected parameters were subjected to one-way ANOVA and significant differences were evaluated by LSD using the WASP software (Kaplan et al., 2014).

3. Results and discussion

The morphology of pollen grains represents an important diagnostic tool for taxonomists, and breeders to distinguish and identify the possible differences between cultivars within species (Varasteh, 2009).

3.1. Pollen morphology by scanning electron microscopy

While studying pollen morphology, four parameters, namely, pollen size, shape, aperture and exine ornamentation, were taken into consideration. The length (polar diameter) and width (equatorial diameter) exhibited significant differences between the accessions (Table 2) and ranged from 55.09 to 71.25 μm and 24.38 to 36.27 μm respectively. Manthriratna (1965) reported the pollen grains of the coconut variety *typica* measured 65 to 69 μm in length and 28 to 30 μm in width. Coconut pollen length of 24.60 to 32.40 μm and breadth of 44.20–60.10 μm breadth were reported by Rasheed et al. (2016). Our results were in accordance with these values. Javady and Arzani (2001) also reported specific differences including variation in pollen size amongst different cultivars of olive. Pollen from different accessions mainly exhibited elliptical and ellipsoidal shapes (Table 1). While the accessions Nigerian Tall (NIT) and West Coast Tall (WCT) had elliptical shaped pollen, Chowghat Green Dwarf (CGD), Chowghat Orange Dwarf (COD), Laccadive Micro Tall (LMT), San Ramon Tall (SNRT), Malayan Orange Dwarf (MOD) and Java Tall (JVT) presented ellipsoidal pollen grains (Table 2). Manthriratna (1965) stated that coconut pollen grains were ellipsoidal with a prominent furrow running along the length of the grain. He had also stated that this one-furrowed or monocolpate type of grain is characteristic of many monocotyledons and primitive dicotyledons. Haneesa Beevi (2016) reported that there may be other pollen shapes between the elliptical and ellipsoidal forms and there exists both elongate and spheroidal grains. Our results also support this finding. Similarly, Arzani et al. (2005) had reported that the pollen shape varied in eleven studied apricot cultivars. According to their report, the apricot cultivars were easily separated into two groups based on their pollen grain shapes: elliptical-trizono-colpate and obtuse-triangular. Likewise in this study, the selected coconut accessions were grouped into two based on the pollen shape.

Microscopic observations point out the fact that even though pollen aperture is mono-colpate, a certain percentage of trichotomo-colpate pollen grains were observed in WCT. Nair and Sharma (1963) also reported the occurrence of trichotomo-colpate pollen grains in WCT. Pollen grains of all the selected accessions were found to be bilateral and tenuimarginate.

Hillock-like elevations were observed in the exine ornamentation pattern of the colpus edge of the accession SNRT. These elevations

Table 2
Characteristics of pollen grains of eight coconut genotypes.

Genotypes	Length of the pollen (equatorial diameter) (μm)	Width of the pollen (polar diameter) (μm)	Length to width ratio	Pollen shape	Aperture	Exine ornamentation
NIT	55.66	31.75	1.75	Elliptical	Monocolpate	GranulatedPsilate
WCT	55.09	29.82	1.85	Elliptical and trichotornocolpate	Monocolpate	Foveolate
LMT	66.48	30.10	2.21	Ellipsoidal	Monocolpate	Reticulate
SNRT	69.98	29.74	2.35	Ellipsoidal	Monocolpate	Granulatedechinulate
JVT	61.77	24.38	2.53	Ellipsoidal	Monocolpate	Foveolate
CGD	71.25	36.27	1.96	Ellipsoidal	Monocolpate	Reticulate
COD	63.09	29.65	2.13	Ellipsoidal	Monocolpate	Reticulate
MOD	71.22	26.89	2.64	Ellipsoidal	Monocolpate	Foveolate
Mean	64.31	29.82	2.18			
CV	1.51	2.76	3.76			

were covered by a thin veil of granules. This could be considered as a specific feature of some varieties. A similar observation of the surface configuration of the coconut pollen with elevations was made by Beevi (2006) in Niulekha Green Dwarf.

Concerning exine ornamentation, the scanning electron micrographs had revealed a configuration which is far different from the information obtained from previous studies using light microscopes. In the present study, differences in exine pattern with granulate to reticulate ornamentation were observed (Fig. 1). Haneesa Beevi (2016) studied the pollen ornamentation pattern in coconut accessions and reported that the pollen exine is ornamented by reticulate, foveolate, fossulate, rugulate or spinulate forms. Rasheed et al. (2016) had reported that exine in coconut pollen is tectate or semi-tectate.

The present study points out that the differences in pollen morphology and exine pattern characteristics could be used to separate and identify the selected coconut accessions. All the observed parameters in toto suggests unique pattern for individual accession. The SEM micrographs confirms the correctness in differentiating the selected accessions on the basis of morphological parameters such as pollen size and exine characteristics. The results suggests that pollen characteristics, along with palm morphological characteristics could be considered for describing the accessions.

3.2. *Pollen viability and* in-vitro *germination*

Knowledge on pollen germination and viability is a prerequisite for any hybrid development programmes. Present study points out that the accessions exhibited significant differences in pollen viability. The pollen viability between the selected accessions varied from 92.35 to 96.5% Table 4. While the highest percentage of viable pollen were recorded in LMT, followed by JVT (94.3%), lowest pollen viability was observed in CGD (Fig. 2). Determining the viability of pollen grains is an easy method to increase the efficiency of breeding programs (Tehrani and Lay, 1991; Kester et al., 1994). Comparative studies conducted for pollen viability between seed and micro propagated coconut palms confirmed highest viability of 93.4 ± 8.9% in seed propagated accessions (Armendariz et al., 2006).

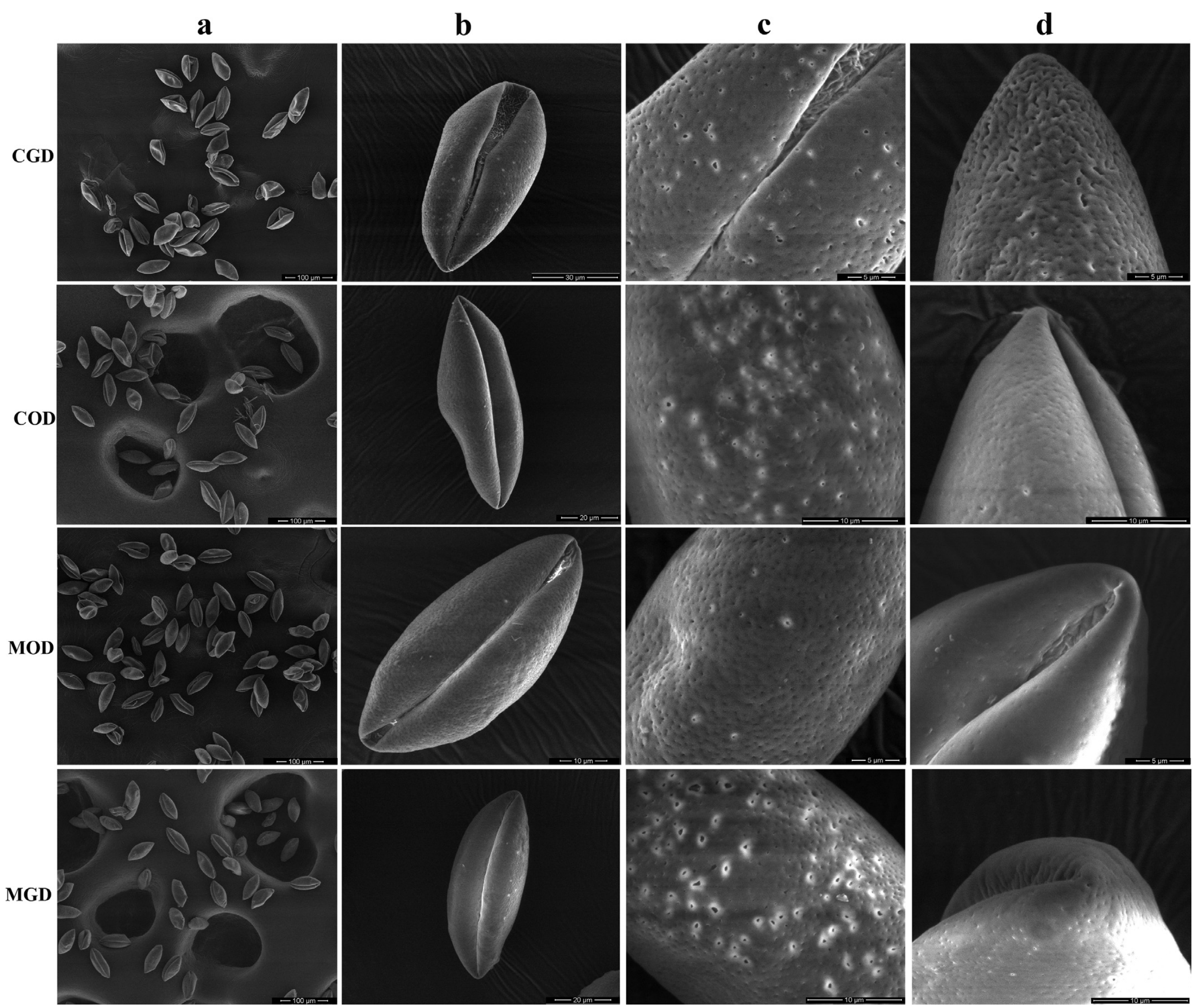

Fig 1. Pollen shape and exine ornamentation pattern in four dwarf coconut genotypes. a. Pollen in group (500x); b. Individual pollen (3000x); c. Exine ornamentation on pollen surface (10,000x); d. Exine ornamentation on colpus edge (10,000x).

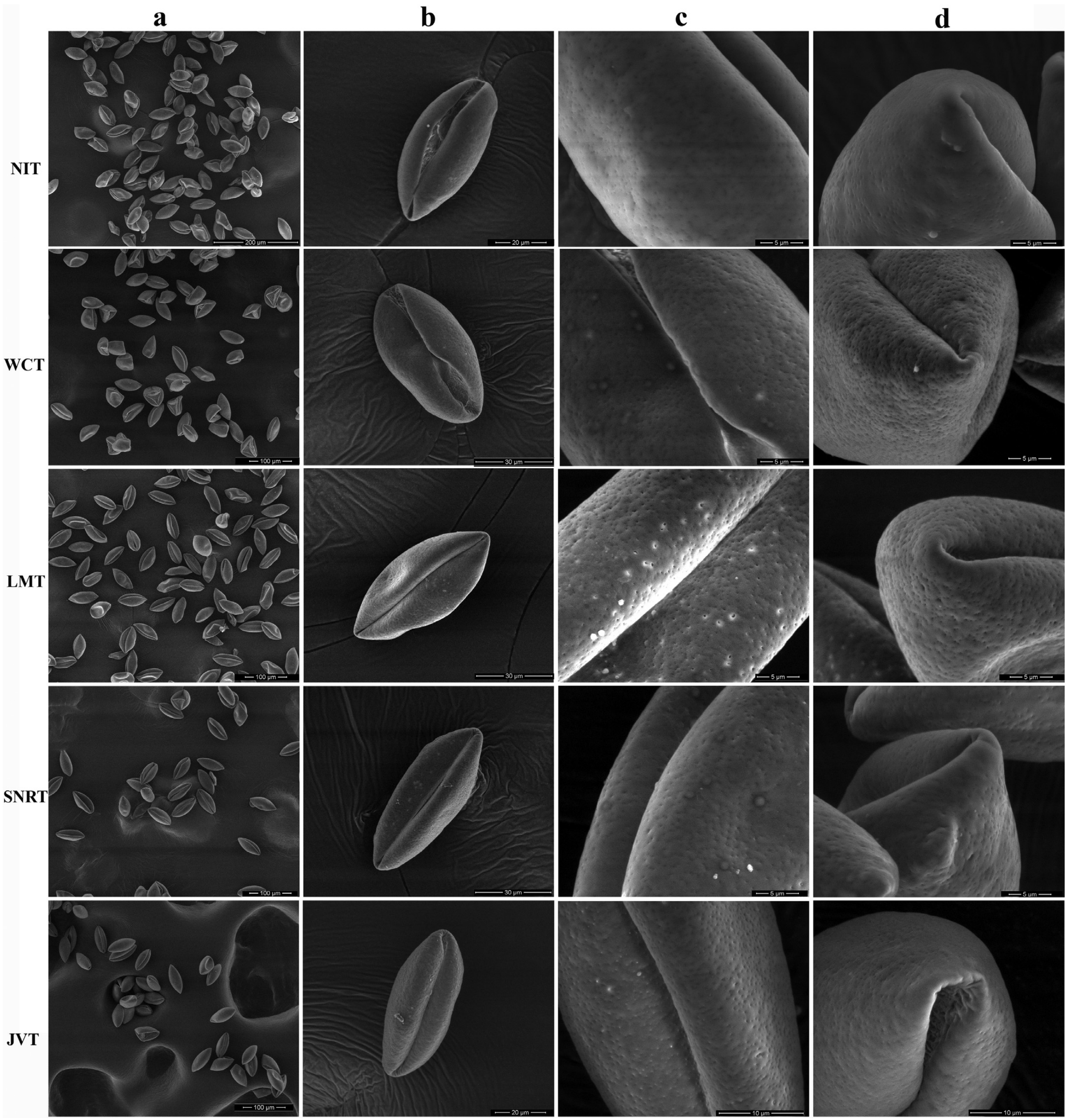

Fig 1. Continued.

The results of current study point out that germination rates and pollen tube growth for the different coconut accessions were asynchronous and heterogeneous. Germination rate and pollen tube growth varied significantly amongst the coconut accessions. In the studied accessions, germination rate after 2 h ranged from 16.05% (CGD) to 31.59% (NIT) (Fig. 3). The average germination observed was 23.75%, which was comparable with previous reports by Ranasinghe et al. (2010), where they obtained about 23% germination in pollen collected during the October–November months. Tall varieties SNRT and WCT and Dwarf varieties CGD and MOD recorded less than 20% pollen germination. Since the pollen were treated uniformly, the observed differences in pollen germination could be attributed to the accession variability. Similar results were reported in sweet cherry, caprifig, and medlar (Tosun and Koyuncu, 2007; Gaaliche et al., 2013; Cavusoglu and Sulusoglu, 2013) Pollen tube length after 2 h of incubation ranged from 234.95 μm to 414.96 μm. Coconut accession COD had the shortest pollen tubes whereas WCT had the longest ones. The differences found in pollen tube length could be due to the genotypic influence of particular accessions. Similar results were observed in chestnut by Beyhan et al. (2009).

In the present study, a highest rate of pollen germination was recorded for NIT while the pollen tube length was longest in WCT.

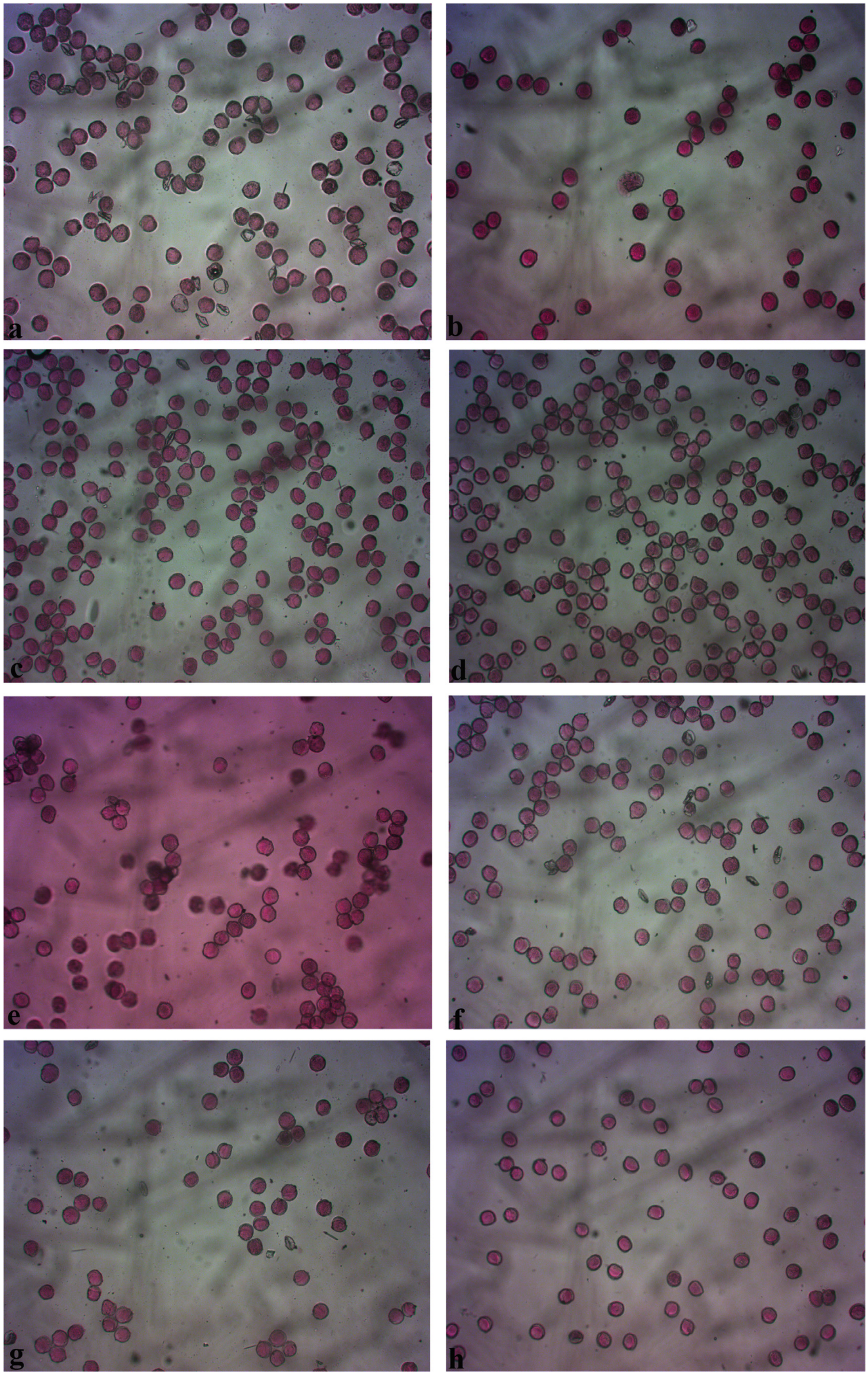

Fig 2. *Invitro* pollen viability of coconut genotypes, a.CGD, b. JVT, c. LMT, d. NIT, e. COD, f. WCT, g. SNRT, h. MOD.

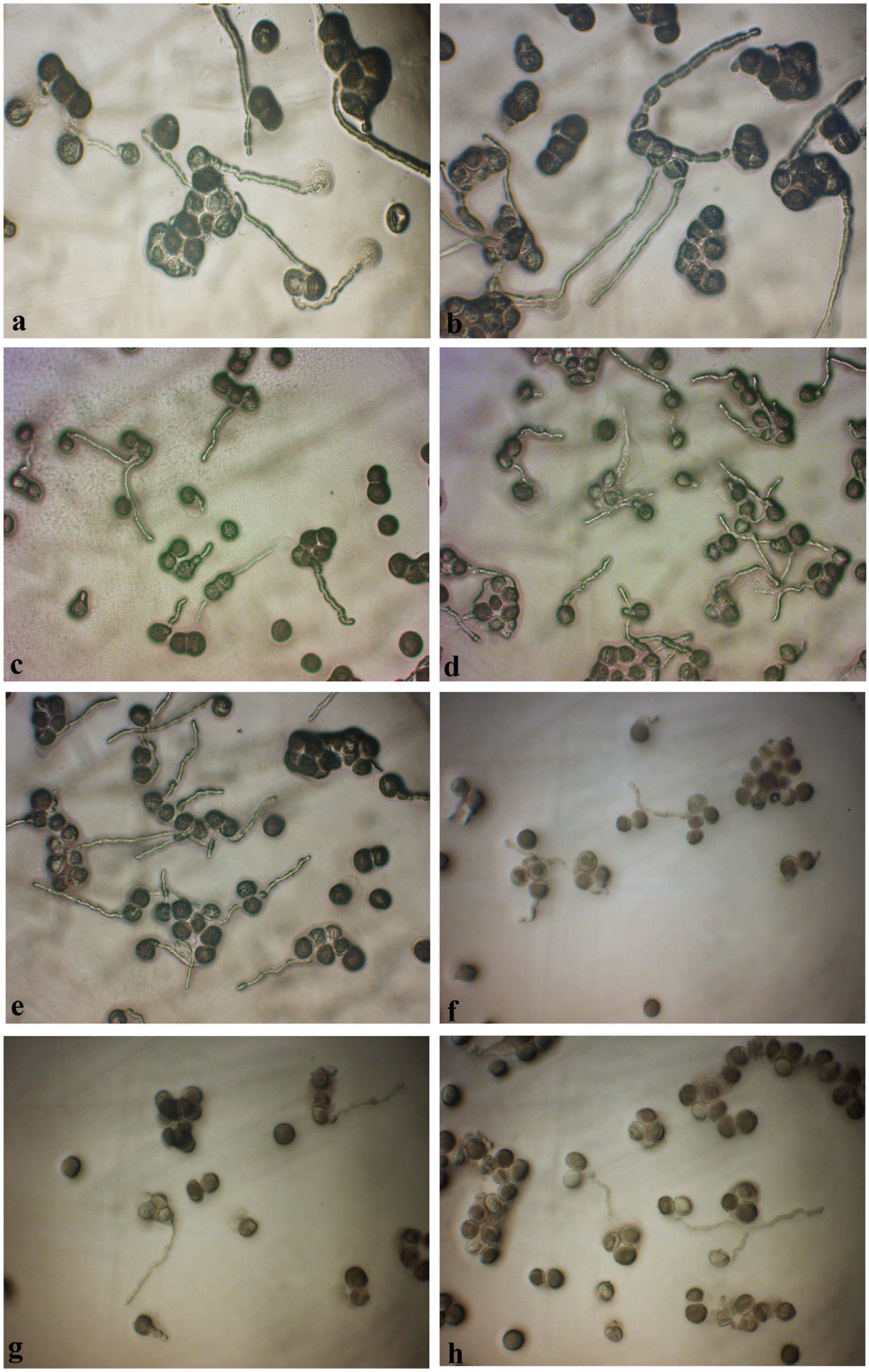

Fig 3. Invitro pollen germination and pollen tube growth of coconut genotypes, a.CGD, b. WCT, c.JVT, d.LMT, e. NIT, f. COD, g. MOD, h.SNRT.

Table 3
Mineral content of the pollen grains of eight coconut genotypes.

Accessions	N(%)	P(%)	K(%)	Na(%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
MOD	5.43 ^{c}	0.87 ^{b}	0.81 ^{bc}	0.47 ^{f}	0.44 ^{e}	0.26 ^{c}	0.31 ^{c}	95.85 ^{f}	249.01 ^{g}	92.68 ^{d}	14.02 ^{e}	27.95 ^{c}
SNRT	5.47 ^{bc}	0.82 ^{d}	1.01 ^{a}	0.71 ^{a}	0.60 ^{b}	0.36 ^{b}	0.29 ^{cd}	149.35 ^{a}	250.58 ^{g}	119.59 ^{a}	32.50 ^{b}	45.05 ^{a}
COD	5.64 ^{ab}	0.88 ^{b}	0.98 ^{a}	0.62 ^{c}	0.60 ^{b}	0.26 ^{c}	0.44 ^{b}	126.89 ^{b}	440.80 ^{e}	90.01 ^{e}	26.47 ^{c}	13.35 ^{g}
CGD	5.52 ^{abc}	0.81 ^{de}	1.04 ^{a}	0.56 ^{d}	0.48 ^{d}	0.24 ^{d}	0.49 ^{b}	98.34 ^{e}	334.21 ^{f}	82.82 ^{f}	13.63 ^{e}	20.95 ^{e}
WCT	5.43 ^{c}	0.94 ^{a}	0.86 ^{b}	0.47 ^{f}	0.56 ^{c}	0.24 ^{d}	0.54 ^{a}	114.20 ^{d}	664.20 ^{c}	100.85 ^{b}	49.38 ^{a}	15.00 ^{f}
NIT	4.32 ^{d}	0.93 ^{a}	0.99 ^{a}	0.65 ^{b}	0.64 ^{a}	0.38 ^{a}	0.27 ^{d}	124.24 ^{c}	700.65 ^{b}	78.76 ^{g}	9.33 ^{f}	37.20 ^{b}
LMT	5.66^{a}	0.85 ^{c}	0.78 ^{c}	0.47 ^{f}	0.44 ^{e}	0.26 ^{c}	0.33 ^{c}	72.44 ^{h}	512.25 ^{d}	94.43 ^{c}	14.08 ^{e}	24.25 ^{d}
JVT	5.37 ^{c}	0.80 ^{e}	0.87 ^{b}	0.50 ^{e}	0.56 ^{c}	0.26 ^{c}	0.30 ^{cd}	85.64 ^{g}	975.15 ^{a}	102.12 ^{b}	20.80 ^{d}	25.60 ^{d}
Mean	5.35	0.86	0.92	0.56	0.54	0.29	0.37	108.37	515.86	95.16	22.53	26.17
CV	2.01	1.21	4.42	1.92	1.92	3.78	6.12	1.00	1.1	1.2	4.7	2.85

Even though in some accessions with high pollen germination, longer pollen tubes were observed (Table 4), it is not mandatory that accessions with the highest germination rate should have the longest tube length. Sharafi and Bahmani (2011) noticed the same in genus prunus and these differences could be related to the genetic differences amongst the accessions. In palms, namely date palm, variable pollen germination percentage were reported by Ibrahim et al. (2013) and Ismaiel (2014). Similarly, variability induced by genotype on pollen tube length and germination in different cotton accessions were reported by Kakani et al. (2005).

There is evidence that the staining method overestimates pollen germination percentages, whereas the in-*vitro* tests underestimate them (Galletta, 1983). According to Scorza and Sherman (1995), reactions to staining materials may not correlate well with *in-vitro* pollen germination or with fertilisation abilities. The results obtained in the present study are in agreement with that statement, given that our pollen germination data indicated a significantly lower rate than that observed using staining materials. Similar results were reported in different guava accessions (Coser et al., 2012) where *in-vitro* germination percentage of pollen were low when compared to percent pollen viability based on staining procedures. However, the staining techniques are extremely attractive due to their simplicity and ease of use.

Pollen germination and tube growth are the most important characteristics that decide pollen quality, which ultimately determines the success of fertilization. Lower germination and tube growth may lead to low fruit set due to the degradation of ovule before pollen tube reaches the ovary. Thus, the studies of pollen morphology and vigour (viability, *in-vitro* germination and tube length) offer a detailed characterization of the accession, which can differentiate identical accessions thereby supporting conservation and genetic improvement.

3.3. *Mineral composition of the pollen*

The mineral composition of pollen grains is shown in Table 3. The obtained results revealed that palm pollen grains constitute a rich source of mineral elements. Pollen grains of coconut accessions contain macronutrients and N in the highest concentrations, followed by K, P, Ca, Mg and Na. Considering the micronutrients, Mn presented in the highest concentrations, followed by Fe, Zn, B and Cu. The mineral composition in pollen differs with the accessions evaluated. Differences in the mineral content of the pollen could be associated with the high mineral content of the soil horizons in areas wherever this accession grows. But, the capability to accumulate elements within the pollen is additionally associated with the genetic makeup of the accessions (Samir and Mohamed 2019). The nutrient status indicates the probability of the participation of the pollen grains in a successful fertilization event. Male reproductive success is tightly associated with plant reproduction and survival requiring proper pollen grain development and adequate pollen tube growth. In the present study, NIT recorded higher Ca (0.64%) and Mg (0.38%) content whereas SNRT exhibited higher K (1.01%), Na (0.71%), Fe (149.35 ppm), Zn (119.59 ppm) and B (45.05 ppm) content. Higher P (0.94%), S (0.54%) and Cu (49.38 ppm) content was found in WCT. LMT recorded higher N content (5.66%) and JVT recorded higher Mn content (975.15 ppm). Pollen germination requires calcium and plays a role in determining the direction of pollen tube growth. Calcium has an essential signalling, physiological, and regulatory role during sexual reproduction in flowering plants. Calcium establishes the polarity of the pollen tube and forms a basis for growth (Ge et al., 2007). Boron is also an important element which is essential for the germination and the growth of pollen tubes, minimizing the sensitivity to variations in the germination medium, affecting membrane permeability and stiffening the pollen tube (Bhojwani and Bhatnagar, 1974). Several researchers have studied and reported the role of boron for germination of pollen in the stigmatic secretions (Gaugh and Ouggar, 1953) or in the germination medium (Leduc et al., 1990; Mucciforaet al., 2003; Mortazavi et al., 2010) in coconut and other plant species.

Yang Jie et al. (2009) investigated the role of Zn in pollen germination and pollen tube growth in *Paris polyphylla* var. *stenophylla* through tissue culture and reported that when the concentration of Zn was high, the pollen germination and pollen tube growth would be inhibited. For the other hand, if the concentration of Zn was low, it did not influence the pollen germination and the growth of the pollen tube. When the concentration of Zn was within a certain range, the pollen tube growth would be enhanced. Sharma et al. (1991) reported Mn deficiency reduced the *in-vitro* germination of pollen grains significantly. Sabrine et al. (2010) stated that the higher copper concentrations (10.5 g/ml and 50 g/ml) rendered a significant reduction *in-vitro* pollen germination whereas copper deficiency induced nearly complete sterility of the pollen formed and inhibited all grain production. Temporary Cu deficiencies significantly reduced the viability rate and the number of proline-rich pollen grains without affecting pollen grain production (Azouaou et al., 1993). Deficiency of micronutrients can lead to pollen ultra-structural changes (Cu, Zn), decrease pollen productivity and pollen grain size (Mn, Fe, Zn, Mo),

Table 4
Viability percentage of pollen, germination percent of pollen and pollen tube length in coconut genotypes.

Genotypes	Pollen tube length (μm)	Pollen germination percent (%)	Pollen viability (%)
COD	234.95 ^{c}	30.40 (33.39)^{a}	92.5 ^{d}
MOD	370.45 ^{ab}	17.67 (24.77)^{bc}	94.0 ^{bcd}
SNRT	302.97 ^{bc}	17.26 (23.56)^{c}	93.0 ^{cd}
CGD	357.45 ^{ab}	16.05 (23.16)^{c}	92.35 ^{d}
WCT	414.96^{a}	17.61 (24.67)^{bc}	95.0 ^{ab}
JVT	350.85 ^{ab}	30.42 (33.44)^{a}	94.3^{bc}
LMT	328.17 ^{ab}	29.00 (32.31)^{ab}	96.5 ^{a}
NIT	395.66 ^{a}	31.59 (34.10)^{a}	93.6
CD(0.05%)	91.99	7.70	1.73
CV	18.30	18.39	1.07

and impact viability and pollen tube growth (Mn, Cu, Zn, B) as well as lower starch content in pollen grains (Mo, Cu). Several researchers have studied the effect of macro and microelements on pollen germination (Davarynejad et al., 1995). The results indicated that the pollen grains characterized by high content of mineral elements (Zn, Fe, Mn, Cu and Mg reflect greatly on fertility and yield. Quality nuts are obtained when such pollen grains are used for pollination programmes as these elements have role in specific metabolic processes. The higher content of mineral components especially zinc and iron in coconut pollen was reported by Kavitha et al. (2021). Thus, the results of this study contribute to the characterization of the selected coconut accessions based on pollen micro morphology as well as mineral composition, thereby supporting the conservation and genetic improvement with substantial gains in both sectors.

4. Conclusion

Pollen characteristics are of importance as far as the general description of accessions or varieties are concerned. The method could be useful for initial varietal identification studies due to its economical and simple nature as compared to other complicated methods. This information is expected to be useful for plant breeders or for gene bank curators who may needs general information regarding coconut pollen. The results obtained in the present study contribute to the coconut genetic breeding programme. Our results also improve the understanding and facilitate the identification of the accessions by evaluating the existing viability of pollen in the germplasm, for subsequent utilisation in controlled hybridizations.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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