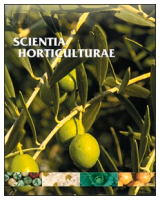
Scientia Horticulturae 314 (2023) 111957

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Pollen morphological study and temperature effect on the pollen germination of cashew (*Anacardium occidentale* L.) varieties

Babli Mog [a,*], G.L. Veena [a], J.D. Adiga [a], K.B. Hebbar [b], Shamsudheen M [a], G.N. Manjesh [a], E. Eradasappa [a], G.S. Mohana [a], V. Thandaiman [a], K. Vanitha [a], Anil Kumar Yadav [c]

[a] *ICAR-Directorate of Cashew Research, Dakshina Kannada, Puttur 574202, Karnataka, India*
[b] *ICAR- Central Plantation Crops Research Institute, Kasaragod, Kerala, 671124, India*
[c] *ICAR-Central Inland Fisheries Research Institute, Regional Centre, Guwahati 781006, Assam, India*

ARTICLE INFO

Keywords:
Climate change
Cashew (*Anacardium occidentale* L.)
Pollen morphology
Pollen germination
Temperature

ABSTRACT

Temperature is one of the main environmental factors that affects plant growth and development. Flowering phenology mainly pollination is highly sensitive to temperature extremes. Thus, pollen tests can be used as a tool to screen temperature tolerant crops. In present study, the *in vitro* pollen germination method was used for screening cashew varieties at different temperatures. The pollens of five cashew varieties comprising three early (VRI-3, Vengurla-4 and Ullal-3), one mid (Bhaskara) and one late (Madakkathara-2) were screened in field (*in vivo*) as well as at controlled temperatures from 5 to 50°C. The morphological features of pollen grains were also studied using Scanning Electron Microscopy (SEM). Significant differences in polar axis (P), equatorial axis (E) and P/E ratio were observed among five cashew varieties. Early varieties had high P and E. Pollen germination under *in vivo* was high for early types with optimum temperature (T_{opt}) of 25°C while it was low in mid and late types with T_{opt} of 30°C. The *in vitro* study further confirmed this finding, pollen germination at 25°C was 75% in early types while it was 57.8 and 50.5% for mid and late types respectively at 30°C. However, mid and late varieties exhibited better tolerance to high temperature beyond 40°C suggesting their wider adaptability to high temperature. The high reducing sugars mainly glucose and fructose in pollens of early varieties may be correlated to high pollen germination in response to temperature. Overall, the varietal differences in pollen behaviour can be used to select cashew varieties for evaluating their adaptability to temperature extremes during flowering across various regions.

1. Introduction

The world is experiencing the havoc of climate change and its implications especially on agricultural production. Rainfall pattern and temperature regimes will become more variable with major effects on agricultural output due to climate change (Lobell et al., 2011). It is predicted that India will experience highest losses in terms of reduction in yield in days to come due to climate change and natural hazards (Gupta et al., 2014). The factors such as over dependence on agriculture, monsoon dependent farming system, absence of adequate cultivable land, higher population, limited technological interventions and also lack of financial support for adaptations to climate change etc. can contribute to more adverse impact of climate change in India. It is also predicted that India will experience increase in average annual temperatures from 24°C to 28°C by the end of 21st century under the climate change scenario. Extremely hot days are also expected to increase with temperature above 35°C, increasing from 5 per year in 2010 to 42 per year in 2100 (Michael Greenstone et al., 2019). The shifts in rainfall pattern and increase in temperature might directly or indirectly cause economic and ecological changes influencing plant growth, incidence of pest and disease occurrence and ultimately crop yield reduction to the tune of 10 to 40% (Maurizio et al., 2022).

About 80% of global agricultural land is rainfed and India stands first in having larger rainfed agricultural lands in the world. India has about 141 million hectares (m ha) of total cultivable area, out of which rainfed area constitutes about 85 m ha. Being rainfed, India's agricultural and horticultural sector come under the purview of climate change due to higher dependency on monsoon rainfall. The changes in onset of monsoon, prolonged dry spell, early monsoon withdrawal etc. are few climate change induced alterations which ultimately affect agricultural

* Corresponding author.
E-mail address: bablicph@gmail.com (B. Mog).

https://doi.org/10.1016/j.scienta.2023.111957
Received 31 October 2022; Received in revised form 17 February 2023; Accepted 26 February 2023
Available online 7 March 2023

production (Lal et al., 2001). Other than variations in rainfall, productivity of rainfed crops also get affected due to changes in temperature (Venkateswarlu et al., 2010). The approaches such as development of high performing varieties with climate resilient traits, introduction of various crops including fruit and plantation crops across agro climatic zones etc. may be considered as important technological interventions.

Cashew (*Anacardium occidentale* L.), a plantation crop of *Anacardiaceae* family, plays significant role for economic improvements of India. It was introduced to India mainly for control of soil erosion and afforestation purpose in coastal degraded lands. However, it has gained its economic importance due to the production of delicious kernels, increasing farmer's income, providing employment opportunities especially to the women engaged in processing and allied sectors and also in contributing significantly to Indian economy as a foreign exchange earner. Therefore, cashew can be introduced as a climate resilient crop to conserve ecology and environment and also to improve yield due to its ability to survive under rainfed conditions.

Cashew is extensively cultivated in 1.12 million hectares in more than 28 countries with annual production of 700000 metric tons (FAO, 2014). Currently, cashew is cultivated in 19 states of India with 1062000 hectare of area and 817000 metric tons of raw cashew nut production (DCR Vision-2050). India contributes around 24% of global cashew production and meets 50% of domestic industries requirement. Hence, cashew industry has been compelled to import rawnuts from other producing countries mainly from African region to meet the own domestic demand. By 2025, Indian cashew production is forecasted around 1000000 metric tons with requirement of 2200000 metric tons of rawnuts by cashew processing industries (Venkatesh Hubbali, 2021). This necessitates the enhancement of rawnut production by 2.5 to 3.0 tons/ha. However, the present productivity is as low as 753 kg/ha. Keeping in view the present scenario, efforts need to be made to increase production either by area expansion or by development of high yielding varieties with stress resilient traits for better performance.

About 600 to 1500 mm annual rainfall, optimum temperature between 22 to 32°C, moderate to well-drained soil etc. are few pre requisite conditions for successful cashew cultivation in a particular region (Rupa, 2010). Cashew is mostly cultivated along west and east coast regions of India. Along west coast, it is spread in Karnataka, Kerala Goa and Maharashtra with annual rainfall of 2000mm and maximum temperature of 30-35°C during day time. Along east coast, cashew is mostly grown in Andhra Pradesh, Orissa, Tamil Nadu and West Bengal where 650 to 1850 mm rainfall is received annually and temperature rises beyond 40°C during summer (Rejani et al., 2013). Majority of cashew growing areas are under rainfed and degraded lands, hilly areas, less fertile soils, coastal areas are mostly available for cashew cultivation in India. Cashew being perennial and plantation crop, has several decades of lifespan. Thus, changes in climatic parameters such as long dry seasons, high annual rainfall and low minimum temperature of coldest months certainly affect climate suitability of cashew growing areas (Roman Gruter et al., 2022).

The fluctuations in climatic parameters affect the phenology of cashew mainly the reproductive phase. Important reproductive phases viz., flowering, fruiting and raw nut yield get affected due to occurrence of severe drought stress during January to May (Yadu Kumar et al., 2010). Cashew also experiences temperature stress during severe drought stress periods. Cashew normally flowers at the end of wet season which makes the flowering mainly its timing and duration more sensitive to changes in temperature (Wunnachit et al., 1992). Temperature beyond 34°C and relative humidity less than 20% are detrimental for cashew flowering which cause yield reduction due to flower drying (Haldanker et al., 2003). Apart from flower drying, fruit drop is also common in cashew when temperature rises beyond 42°C during fruiting (Prasada et al., 2001). In India, cashew varieties are classified as early (December to January), mid (January to March) and late (March to May) flowering types. Among them, mid and late flowering types tend to suffer more as the flowering time coincides with moisture stress periods as well as with high temperature (>34°C) during January to May (Veeraraghavan et al., 1990).

Cashew is an andromonoecious tree where both male and hermaphrodite flowers occur in same inflorescence/panicle (Purseglove et al., 1968). However, both male and hermaphrodite flowers open in diurnal pattern and exhibit different phases of flowering. Male flowers open first in first phase of flowering followed by opening of both male and female flowers in second phase and finally again male flowers opens in final flowering phase (Pavithran et al., 1974). During the transition between male and female flowering phase, pollens are released and transferred from one tree to another for pollination process which influence efficient fruit set and yield. Upon release, pollens undergo several changes in the prevailing environment which makes them more vulnerable to high temperature especially during flowering periods (Kakani et al., 2005). Thus, pollen may lose viability and limit crop productivity under extreme weather (Ledesma et al., 2016). This necessitates the proper understanding of kinetics of pollen functionality mainly viability and germinability under high temperature. Moreover, tolerance to high temperature can also be studied using pollen tests as reported in plantation crop like coconut (Hebbar et al., 2018). Several studies in fruit crops such as Jojoba (Lee et al., 1985), pears (Mellenthin et al,1972), mango (Sukhvibul et al., 2000), almond (Godini et al., 1987), apricot (Pirlak et al., 2002) and also in plantation crop like coconut (Hebbar et al., 2018, 2020) have reported the detrimental effects of high temperature on pollen traits viz., pollen germination and its tube growth. However, till date, no research works on high temperature impact on pollen germination *in vitro* and varietal differences in pollen germination are reported in Indian Cashew.

Therefore, in current climate change scenario, this study was undertaken to evaluate temperature effects on cashew pollen germination. This study, the first basic research, will help in understanding the varietal differences in pollen germination under high temperature. Thus, the study was started with the following goals: (a). to investigate pollen grain morphological characteristics, (b). to optimize pollen germination medium *in vitro* and (c). to study temperature effects on pollen germination of cashew varieties.

2. Materials and methods

2.1. Details of the experimental location

The experiment was laid out at a well-managed farm of ICAR-Directorate of Cashew Research (ICAR-DCR), Puttur, Karnataka, India. The study area is located in west coast part of Indian subcontinent (12°25N latitude and 75°4N longitude and 90 m above mean sea level). The climate is characterized by tropical hot and humid where temperature reaches as high as 35°C during summer. This area has distinct dry periods of four months starting from January to April. Annual rainfall of study area ranges from 2500 to 3500 mm. The soil is characterized by pH of 4.8 to 5.3, acidic and sandy loam with high organic carbon content. The climatic variables at the experimental site are presented in Table 1.

2.2. Plant materials

This study was conducted on five cashew varieties with distinct agronomical traits which are most widely cultivated across cashew growing regions (Table 2). For this study, 15 to 20 years old cashew trees were selected.

2.3. Pollen collection for pollen grain microscopic characteristics

The collection of pollen samples was carried out from December 2020 to April, 2021. Pollens of early cashew varieties (VRI-3, Ullal-3 and Vengurla-4) were collected in December (flowering season: October to January) at 9.30 am to 11.30 am. Pollens of mid variety (Bhaskara) were

Table 1
Agro meteorological variables at the experimental site.

Month	Temperature (°C)		Humidity (%)		Rainy days (Nos)	Rainfall (mm)
	Max.	Min.	Forenoon average	Afternoon average		
January	25.20	18.65	77.10	46.61	0	0
February	27.10	19.28	79.93	39.59	0	0
March	35.90	24.84	77.16	45.10	0	0
April	34.95	23.70	77.90	48.90	4.00	49.50
May	32.95	23.77	84.06	59.23	7.00	215.90
June	31.12	21.50	90.50	81.60	25.00	523.70
July	29.60	22.13	91.26	80.61	28.00	804.30
August	27.37	22.01	89.39	77.97	23.00	746.90
September	25.03	22.77	80.14	77.21	27.00	614.00
October	25.65	20.16	75.65	70.61	14.00	318.40
November	24.93	19.40	69.27	54.23	6.00	87.30
December	24.19	18.45	77.84	48.39	1.00	19.80

Rainfall and rainy days are monthly total; other parameters are monthly mean values.

collected during March (flowering season: January to March). The pollen collection time of late variety (Madakkathara-2) was also restricted to March (flowering time: March to May). Six healthy cashew trees were selected for pollen grain morphological studies for each cashew variety. Four healthy branches were tagged for each variety. Paper bags were used to cover the tagged branches to keep the flowering panicles with pollens free from contamination. The flower panicles were collected in paper bags. From flower panicles, 50 male flowers were collected in petri dishes and were kept under sunlight for dehiscence. The dehisced 30 anthers were collected in small white paper bags (8.8 × 13.5cm) after removal using forceps and paint brush. The freshly collected anthers with pollens were used for pollen grain microscopic studies.

2.4. Pollen grain microscopic characteristics

The collected pollen grains were studied for morphological traits at Mangalore University, Karnataka using scanning electron microscopy (SEM). The pollen grains were dehydrated, placed onto copper tape and later copper tape containing pollen grains were put on the polished aluminium stub surface. The pollens were kept in vacuum desiccator for 15 minutes for dehydration and dehydrated pollens were spread on stubs and sputtered with gold for 5 minutes. Later, the samples were loaded in SEM and images were taken. Stereo scan SEM was used for studying the morphological traits of pollen grains and photographed at 4000X for whole grain investigation. The morphological features of pollens viz., polar (P) and equatorial (E) axis and P/E ratio were measured from randomly selected five pollens following the protocol as mentioned by Arzani et al. *2005) and Khaleghi et al. (2019).

2.5. Collection of pollen and optimization of growth medium

Before starting the actual research experiment, a few initial trials were conducted in order to optimise pollen collection time and growth medium. Flowering panicles with dehiscing male flowers were collected at different time intervals (timings of 7.30 am, 8.30 am, 9.30 am, 10.30 am, 11.30 am and 12.30 noon) from 4 tagged healthy branches for a period of 10 days in cashew varieties (VRI-3, Bhaskara and Madakkathara-2). Pollen germination percentage of these flowers were recorded in order to identify the optimal pollen collection time for pollen germination studies. The same varieties were also used to optimize pollen growth medium. The same established procedure was later used to evaluate the pollen performance in other cashew varieties. Three slides per cashew variety loaded with pollen grains at each time period were used.

For optimization of growth medium, fresh male flowers were collected in the morning between 9.30 am to 11.30 am in the plastic bags and immediately put in ice box to prevent drying and desiccation. The pollens were collected in the same way as already mentioned for

Table 2
Important agronomical traits and distribution of cashew varieties used in the study.

Variety	Institute of release	Morphological characters	Flowering	Nut and kernel traits	Cashew apple traits	Growing states
Bhaskara	ICAR-Directorate of Cashew Research, Puttur, Karnataka, India	It's a Selection from Goa. It has a medium-spreading canopy with extensive branching.	It has a mid-season flowering habit	Nut weight is 7.38 g and has an average nut count of 136 nuts per kg. Kernel weight is 2.2 g. Shelling percent of 30.6. It has an export grade of W240.	Fruit colour is Pinkish Orange.	Coastal Karnataka
Vengurla-4	Regional Cashew nut Research station, Vengurla, Maharashtra, India	It's a hybrid developed from Midnapur red x vetore-56. It has an open canopy with extensive branching.	Flowers in clusters. It has early season flowering habit	Nut weight is 7.7 g and has an average nut count of 140 nuts per kg. Kernal weight is 1.91g with a shelling percent of 31. It has an export grade of W210.	Fruit colour is red.	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Kerala, Goa, Gujrat and north eastern region.
Madakkathara-2	Cashew Research Station, KAU, Madakkathara, Kerala, India	Selection (Neduvellur). It has an open canopy and intensive branching habit.	Falls under the late season category.	Nut weight is 7.25 g and has an average nut count of 138 nuts per kg. Kernal weight is 1.88 g with a shelling percent of 26. It has an export grade of W210.	Fruit colour is red.	Kerala, Karnataka
Vridhachalam-3 (VRI-3)	Regional research station, Vridhachalam, Tamil Nadu, India	Selection from village Edayanchavadi in Tamil Nadu. It has a compact canopy with intensive branching habit.	Early flowering type. Flowers in clusters.	Nut weight is 7.2 g and has an average nut count of 140 nuts per kg. It has shelling per cent of 29.1 with an export grade of W210.	Fruit in a pear shape, pink to red at maturity.	Tamil Nadu, Karnataka, Kerala and Andhra Pradesh
Ullal-3	Agriculture Research Station, Ullal, Karnataka, India	It's a selection (5/37 Manchery). It has an open canopy with an intensive branching habit.	Early flowering variety	Nut weight is 7.0 g and has an average nut count of 140 nuts per kg. It has shelling per cent of 30. with an export grade of W210.	It has dark red coloured fruits.	Karnataka, North eastern regions

Note: Export grades mean Cashew kernels with specifications based on size, surface colour and wholesomeness for different grades to be exported to countries other than India for trade.

pollen collection for pollen grain microscopic studies. After dehiscence, the pollens were transferred to the petri plates containing different combinations of growth medium for growth medium optimization. At the start of the experiment, the germination medium consisting of 5% and 10% sucrose with 1mg Boric acid+ 3 mg $CaNo_3$ + 2 mg $MgSO_4$ were evaluated. However, no pollen germination was observed due to higher percentage of burst pollen grains. Later, sucrose concentration was raised to 15% in combination with 1mg Boric acid+ 3 mg $CaNo_3$ + 2 mg $MgSO_4$. Apart from sucrose, another medium containing poly ethylene glycol (PEG) was tested. Different concentrations of PEG 6000 at 5%, 10% and 15% with 1mg Boric acid+ 3 mg $CaNo_3$ + 2 mg $MgSO_4$ were evaluated. The *in vitro* pollen germination was recorded by keeping the petri plates at room temperature of 20 to 25°C for 24 hours. The microscopic observation of pollen germination was made after incubating for 24 hours. For the observation, 3 petri dishes for each growth medium were analysed and germination percentage was recorded from 200 pollen grains. This was in conformity with earlier finding by Perez et al. (2019). The germination of pollens (%) was calculated following the method described by Kakani et al. (2002).

2.6. Effects of temperature on pollen germination of cashew pollen grains

A research trial on temperature impacts on pollen germination was carried out in flowering periods of December to April. Fresh flowers were collected from experimental field between 9.30 am to 11.30 am to study the influence of temperature on pollen germination.

Two separate experiments were performed for temperature response studies. One set of experiments dealt with evaluation of temperature impacts on germination of pollens during flowering season in the field (*in vivo*) and another to evaluate the variability of germination of pollens under controlled temperatures (*in vitro*). For the first experiment, flowers were collected on alternate days up to four weeks during peak season of flowering from December to March. The five cashew varieties used in this experiment were early types (VRI-3, Vengurla-4 and Ullal-3), mid type (Bhaskara) and late flowering type (Madakkathara-2). Pollens were collected during January for early types (flowering season being October to January). For mid type, pollens were collected during March (flowering time from January to March). For late type, flowering season starts from March and continues till May. However, under the influence of recent climate change, shift in rainfall was observed in terms of receiving unusual rainfall during April and May. Hence, pollen collection was restricted only to March month for late variety in order to study their responses to high temperature. A data logger was installed in the experimental farm of ICAR-DCR, Puttur for daily monitoring of temperature and other weather variables. For each variety, four slides containing pollen grains were used to measure pollen germination for each week during flowering periods of January and March.

In the second experiment, pollen germination *in vitro* was studied under controlled temperatures in growth chamber. The collected pollen grains were transferred to optimized growth medium. Dusting of pollens was done on cover slip lined glass slides containing 2 ml of germination media. The glass slides were later transferred to moist filter paper lined petri plates for control of rupture of pollen grains. The petri plates containing glass slides were incubated in a growth chamber with different ranges of temperature from 5°C to 50°C at 5°C interval for 24 hours. Four slides per cashew variety were used for each temperature treatment.

2.7. Estimation of biochemical parameters

In order to ascertain the factors responsible for influencing the sensitivity of pollen germination to high temperature, biochemical parameters such as total sugar content and sugar profile were analysed in pollens of cashew varieties which were collected during December for early varieties and March for both mid and late varieties. The pollens of five varieties were collected at the 4th week of flowering season when maximum temperature reached 22 to 25C during January and 33 to 35C during March. The anthers with pollen grains collected between 9.30 am to 11.30 am in the morning were brought to the laboratory by placing them in plastic bags and were immediately transferred to petri plates containing moist filter paper. Then, preservation of anthers with pollens was done in 80% ethanol followed by immediate storage at -20°C. The stored anthers with pollen grains (0.5 g for each variety) were carried in ice boxes to Central Lab Facility of ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bangalore, India to estimate biochemical parameters.

Total sugar present in cashew pollens was measured following the protocols described by Du Bois et al. (1956). For estimation of different sugars, pollen grains were extracted using 80% ethanol (5 ml) followed by removal of excess alcohol by pollen extract evaporation. The solvent A (Acetonitrile and water in 80:20 ratio) and solvent B (Acetonitrile and water in 30:70 ratio with 0.1% ammonium hydroxide) was used for dissolving pollen extract in 1:1 ratio. The extract was then purified by filtration in nylon filter paper followed by injection to liquid chromatography- Mass spectrometry (LC-MS/MS) for sugar profiling analysis.

2.8. Statistical analysis

Significant differences among varieties (pollen morphology, germination of pollen, *in vivo* pollen germination in field, pollen germination *in vitro* at controlled temperatures and biochemical parameter) were tested using ANOVA proceeds in SPSS 19.00. Duncan's Multiple Range Test (DMRT) analysis was made to compare means of all the studied parameters. Statistical significance was tested at 5% level of significance.

3. Results

3.1. Morphological characteristics of pollen grains

For cashew pollen morphological characterization, different parameters viz., length of polar (P) and equatorial (E) axis and ratio of P/E were examined in five cashew varieties under scanning electron microscope (Table 3). The P/E ratio was used to study the shapes of pollens. Significant differences in polar and equatorial axis and their P/E ratio were observed among cashew varieties (Table 3). The mean value of polar length ranged from 43.91μm in Madakkathara-2 to 48.53 μm in VRI-3. The values of equatorial line width ranged from 22.49μm in Bhaskara to 27.68μm in Ullal-3. The ratio of P/E ranged from 1.68 (Ullal-3) to 2.03 (Bhaskara). Two different shapes of cashew pollens were observed in this study (Fig. 1). Pollens of four cashew varieties (VRI-3, Ullal-3, Vengurla-4 and Madakkathara-2) shared similar shape of prolate whereas, only one cashew variety, Bhaskara had per prolate type pollen grain (Fig. 1).

Table 3
Morphological characteristics of pollen grains of five cashew varieties.

Varieties	Polar length (μm)	Equatorial width (μm)	P/E	Shape
VRI-3	48.53^{a} ±1.6273	24.45^{a} ±1.46857	1.98^{b} ±0.03055	Prolate
Ullal-3	46.39^{a} ±2.47516	27.68^{a} ±0.98875	1.68c ±0.01528	prolate
Vengurla-4	45.61^{a} ±0.61809	26.23^{a} ±1.83309	1.73^{c} ±0.02082	Prolate
Bhaskara	45.67^{a} ±1.06688	22.49^{a} ±1.2797	2.03^{a} ±0.01	Per prolate
Madakkathara-2	43.91^{a} ±0.59355	23.27^{a} ±1.18327	1.88^{b} ±0.01155	Prolate

Means followed by different letters in a column are statistically significant ($p<0.05$).

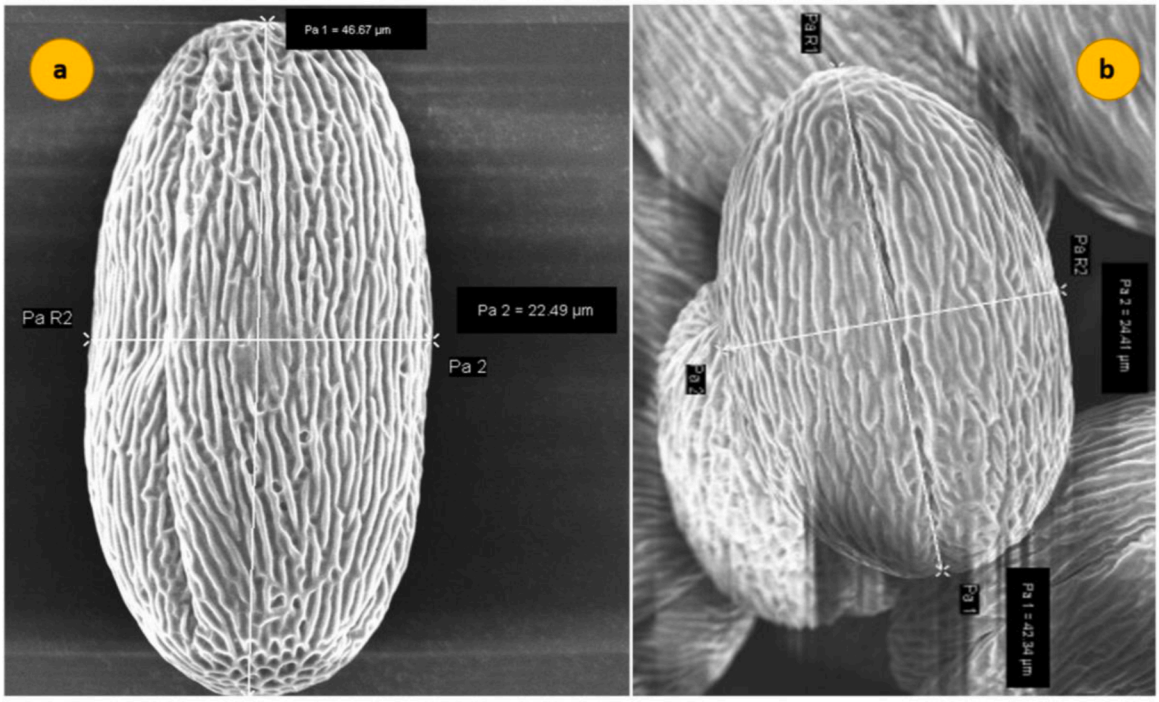


Fig. 1. Pollen shape of cashew varieties photographed under SEM microscope. a. perprolate and b. prolate.

3.2. Pollen germination medium optimization in vitro

The pollen growth medium was optimized in cashew varieties (VRI-3, Bhaskara and Madakkathara-2) based on *in vitro* pollen germination. Cashew varieties exhibited significant changes in pollen germination under different growth media tested (Table 4). The germination medium consisting of 5% and 10% sucrose did not result in any germination (Table 4). However, increased sucrose and PEG concentrations resulted in increased pollen germination. Among the different growth media tested, the growth medium consisting of 15% PEG+ 1mg Boric acid+ 3mg $CaNO_3$+ 2mg $MgSO_4$ resulted in better pollen germination with average pollen germination percentage of 75.28% for all the studied cashew varieties (Table 4).

3.3. Male flower opening pattern and pollen performance in male flower

Cashew produces flowers in large inflorescence which often called as panicle. Cashew panicle is characterized by unique features with occurrence of both hermaphrodite and male flowers together. Cashew produces white to light green colour flowers which turns to pink after dehiscence. Cashew produces identical hermaphrodite and male flowers; however, male flowers are relatively smaller than hermaphrodite ones and also lack pistil in all the studied varieties (Fig. 2**a-c**). In the present study, the male flower opening pattern as well as the pollen collection time was studied. It was observed that significantly high number of male flowers opened between time intervals of 9.30 am to 11.30 am in all the studied varieties (Fig. 3). The pollen germination was high at 9.30 am (49%), 10.30 am (85%) and 11.30 am (65%) compared to those at 7.30 am (2.3%) and 8.30 am (16.6%) in all the studied cashew varieties (Fig. 3).

After the standardization of pollen collection time, pollens of five cashew varieties (VRI-3, Ullal-3, Vengurla-4, Bhaskara and Madakkathara-2) were collected between 9.30 am to 11.30 am to evaluate pollen germination (Fig. 4). Pollen germination showed significant differences among studied cashew varieties ($p<0.05$). Among the varieties, VRI-3 had the highest germination percentage (71.4%) followed by Ullal-3 (69.3%), Vengurla-4 (64.7%), Bhaskara (63.8%) and Madakkathara-2 (61.5%) (Fig. 4).

Table 4
Effect of varying levels of sucrose and polyethylene glycol on the pollen germination of three cashew varieties in a liquid medium.

	Pollen germination (%)		
	VRI-3	Bhaskara	M-2
Media 1	0	0	0
Media 2	0	0	0
Media 3	3.27^{d} ±0.51	2.16^{d} ±0.22	1.86^{d} ±0.13
Media 4	10.35^{c} ±0.41	7.56^{c} ±2.01	9.84^{c} ±0.14
Media 5	20.16^{b} ±1.14	14.05^{b} ±0.59	13.32^{b} ±1.23
Media 6	84.83^{a} ±1.73	75.40^{a} ±1.36	65.61^{a} ±1.51

Note: Medium 1: Sucrose 5%+1mg Boric acid+$CaNO_3$ 3 mg+ $MgSO_4$ 2 mg, Medium 2: Sucrose 10%+1mg Boric acid+$CaNO_3$ 3 mg+ $MgSO_4$ 2 mg, Medium 3: Sucrose 15%+1mg Boric acid+$CaNO_3$ 3 mg+ $MgSO_4$ 2 mg, Medium 4: PEG 5%+1mg Boric acid+$CaNO_3$ 3 mg+ $MgSO_4$ 2 mg, Medium 5: PEG 10%+1mg Boric acid+$CaNO_3$ 3 mg+ $MgSO_4$ 2 mg and Medium 6: PEG 15%+1mg Boric acid+$CaNO_3$ 3 mg+ $MgSO_4$ 2 mg. Means followed by different letters in a column are significant ($p<0.05$).

3.4. Changes in pollen germination during flowering seasons

To study the changes in pollen germination during the flowering seasons, pollens of early cashew varieties (VRI-3, Ullal-3 and Vengurla-4) were collected during January whereas pollen collection time was restricted to March for both mid and late cashew varieties (Bhaskara and Madakkathara-2). Minimum, maximum and weekly average temperature were recorded for both January and March (Fig. 5**A**). For January, the minimum and maximum temperature were in the range from 18.6°C to 20.3°C and 22.6°C to 25.0°C for all the four weeks. Significant increase in temperature was recorded at March where minimum and maximum temperature were in the range from 22.0°C to 24.4°C and 33.1°C to 35.9°C (Fig. 5**A**).

Pollen germination of VRI-3, Ullal-3 and Vengurla-4 was also recorded at weekly interval during flowering season of January. The weekly pollen germination showed variable response with mean percentage of germination ranging from 46.2% to 63.4% for VRI-3, from

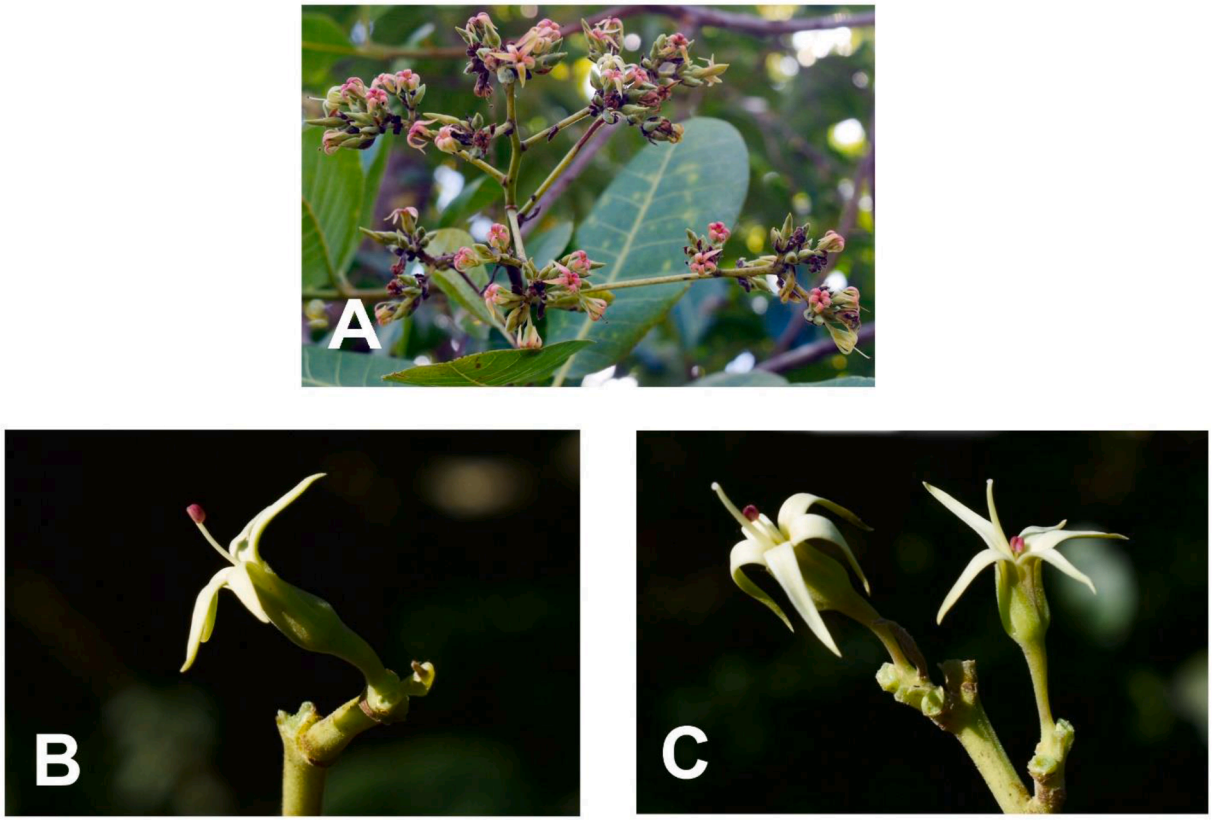


Fig. 2. Cashew flower morphology: A. cashew inflorescence bearing both male and hermaphrodite flowers; B. male flower and C. hermaphrodite flower.

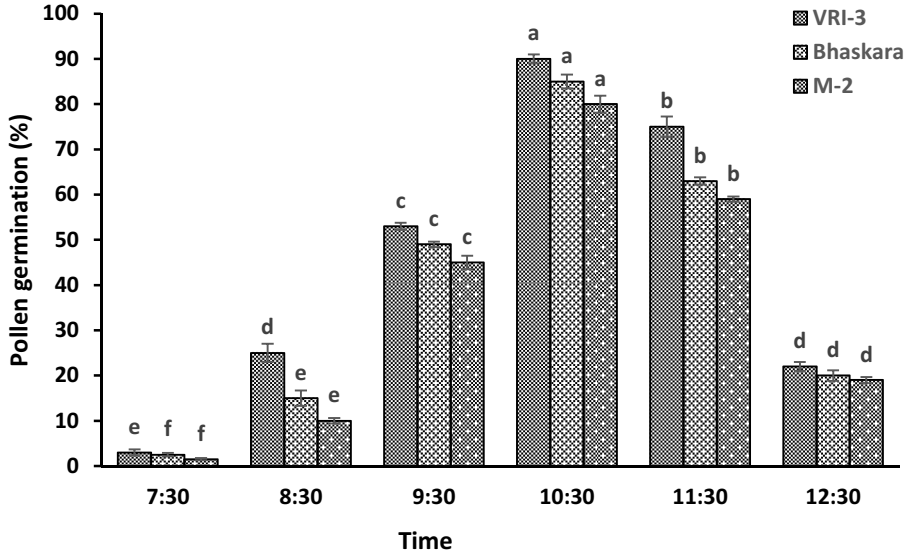


Fig. 3. Germination percentage of fresh pollens from male flowers collected at different time intervals of three cashew varieties. Pollens of VRI-3, Bhaskara and Madakkathara-2 (M-2) were collected at 7.30 am, 8.30 am, 9.30 am, 10.30 am, 11.30 am and 12.30 am. Significant differences among varieties were expressed with different letters ($p<0.05$). Error bars represent ±SE.

40.2% to 58.9% for Ullal-3 and from 37.8% to 50.2% for Vengurla-4 (Fig. 5**B**). Overall, VRI-3 exhibited higher pollen germination (54%) followed by Ullal-3 (49.5%) and Vengurla-4 (44.5%). The average pollen germination of the three varieties was low (41.4%) during first week of January with minimum temperature of 18.6°C. It increased to 46.5% in the second week with increase in minimum temperature to 19.2°C. Similar trend followed in the third week also where pollen germination increased significantly (51.9%) with rise in temperature (minimum and maximum of 19.9°C and 24°C). The highest rate of germination of pollen was observed in the fourth and final week (57.5%) with minimum and maximum temperature of 20.3°C and 25.0°C. Pollen germination was also positively correlated to temperature (minimum, maximum and average temperatures) for early varieties in the present study (Table 5). Pollen germination rate was assessed for Bhaskara and Madakkathara-2 during March (Fig. 5C). The weekly pollen germination was highly variable exhibiting the opposite trend than that of early cashew varieties

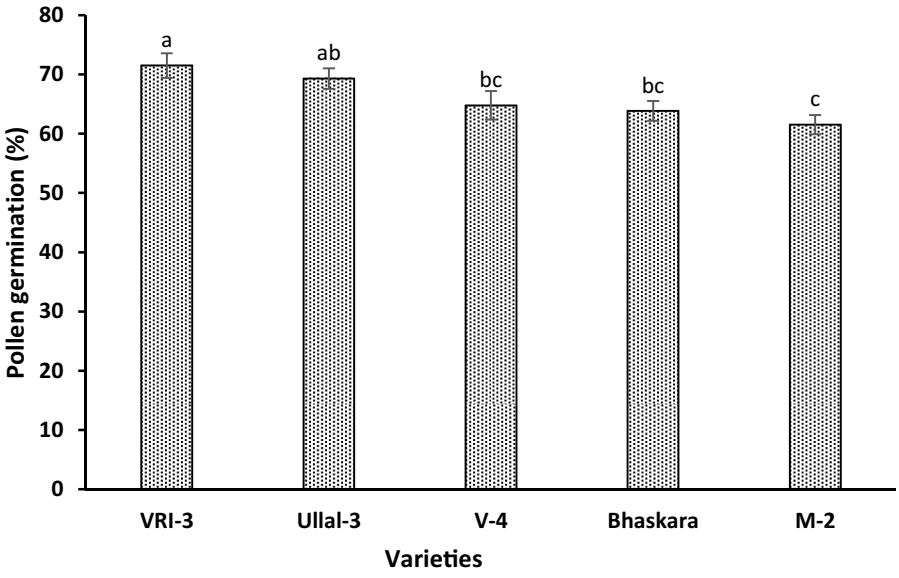


Fig. 4. Pollen germination in male flowers of five cashew varieties. Pollens of VRI-3, Ullal-3, Vengurla-4 (V-4), Bhaskara and Madakkathara-2 (M-2) were collected between 9.30 am to 11.30 am to evaluate pollen performance in male flowers in terms of pollen germination. Significant differences in pollen germination were expressed with different letters ($p<0.05$). Error bars represent ±SE. Note: Initially, flowering panicles with dehiscing male flowers were collected at different time intervals (timings of 7.30 am, 8.30 am, 9.30 am, 10.30 am, 11.30 am and 12.30 noon) for a period of 10 days in cashew varieties (VRI-3, Bhaskara and Madakkathara-2). Pollen germination percentage of these flowers were recorded in order to identify the optimal pollen collection time for pollen germination studies. The pollen germination was high at 9.30 am (49%), 10.30 am (85%) and 11.30 am (65%) compared to those at 7.30 am (2.3%) and 8.30 am (16.6%) in all the studied cashew varieties. After the standardization of pollen collection time, pollens of five cashew varieties (VRI-3, Ullal-3, Vengurla-4, Bhaskara and Madakkathara-2) were collected between 9.30 am to 11.30 am to evaluate pollen performance in terms of in vitro pollen germination. Mean Pollen germination values of each variety were presented in Fig. 4.

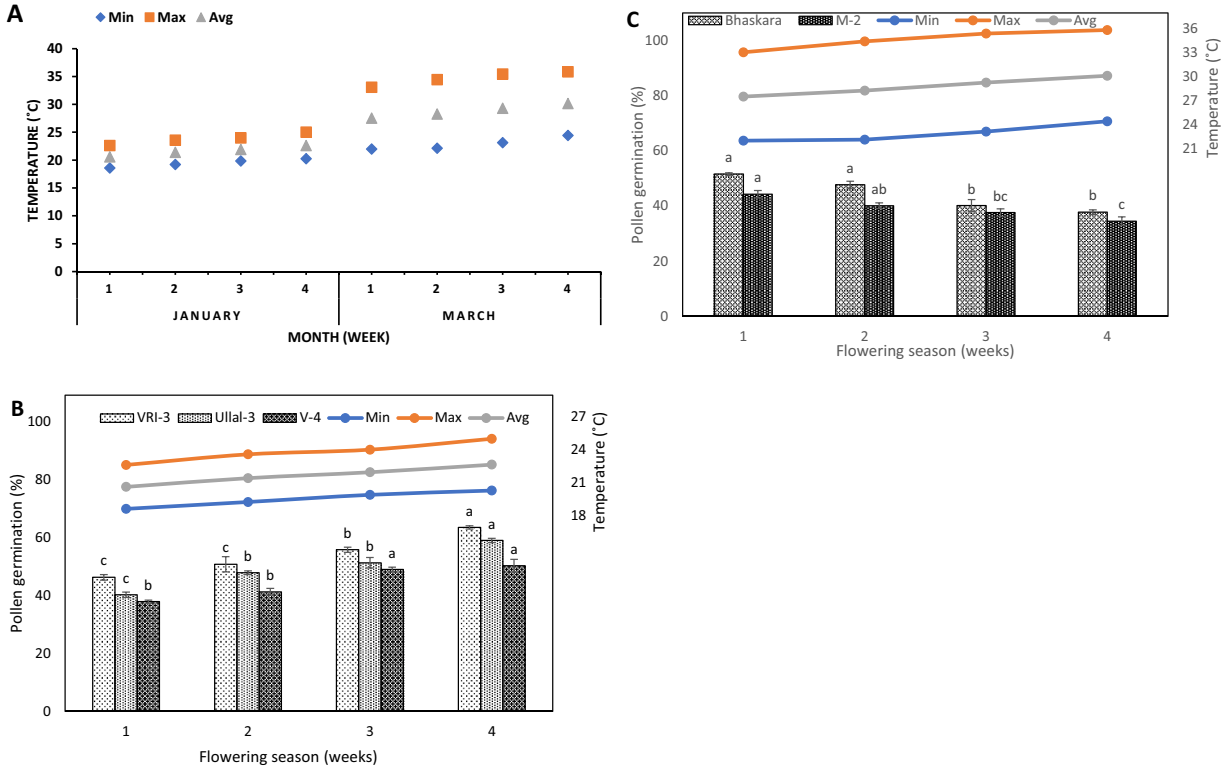


Fig. 5. Germination percentage of five cashew varieties during flowering season. A. Trend of minimum, maximum and average temperature measured during flowering seasons of January and March, B. Pollen germination recorded for VRI-3, Ullal-3 and Vengurla-4 (V-4) during flowering season of January and C. Pollen germination recorded for Bhaskara and Madakkathara-2 during flowering season of March. Pollens of early cashew varieties (VRI-3, Ullal-3 and Vengurla-4) and mid and late varieties (Bhaskara and madakkathara-2) were collected during flowering seasons of January and March. Changes in percentage pollen germination for early, mid and late cashew varieties were recorded at weekly interval. Differences in pollen germination for each week were expressed with different letters ($p<0.05$). Error bars represent ±SE.

studied. Among the four flowering weeks studied in Bhaskara and Madakkathara-2, the pollen germination was highest (47.9%) during the first week of March followed by a noticeable reduction in the subsequent weeks while it was only 36.1% during final week of March. Bhaskara had higher germination rate (44.3%) compared to Madakkathara-2 (39.1%) for all the four weeks of flowering season. The correlation between germination of pollen and temperature exhibited differential response for mid and late varieties unlike the early varieties. No correlation was observed between minimum temperature and pollen germination whereas, maximum and average temperatures were significantly and negatively correlated with pollen germination (Table 5).

Table 5
Relationship among pollen germination and minimum, maximum and weekly temperatures recorded for early varieties (VRI-3, Ullal-3 and Vengurla-4), mid variety (Bhaskara) and late variety (Madakkathara-2) during flowering seasons.

Pollen germination/ Variety	Minimum temp	Max temp	Avg. temp.
VRI-3	0.974*	0.985*	0.987*
Ullal-3	0.975*	0.993**	0.997**
Vengurla-4	0.986*	0.92*	0.95*
Bhaskar	-0.923	-0.981*	-0.989*
Madakkathara-2	-0.914	-0.99**	-0.991**

Note: * and **, correlation is significant at 0.05 and 0.01 level, respectively.

3.5. In vitro pollen germination under controlled condition

Wide pollen germination variability was observed among the studied cashew varieties at different controlled temperatures (Table 6 and Fig. 6). At 5°C temperature, pollen germination was not seen. At 10°C, early varieties (VRI-3, Ullal-3 and Vengurla-4) showed germination rate of 4.3%, mid variety (Bhaskara) 1% and no germination for late variety (Madakkathara-2). In early varieties, the germination was significantly ($p<0.05$) high at 20°C (59.3%), 25°C (75.2%) and 30°C (33.7%). Further increase in temperature reduced the germination rate to 21.3% at 35°C, 4.4% at 40°C and no germination above 40°C for early cashew varieties. For mid variety Bhaskara also, the germination was significantly high at 25°C (45.6%), 30°C (57.8%) and 35°C (38.9%). However, it could exhibit relatively higher pollen germination (13.2%) at 40°C compared to early varieties, yet, percentage of pollen germination was significantly affected at temperature more than 45°C. Madakkathara-2 also followed the similar pattern as that of Bhaskara, where, the maximum pollen germination was noticed between 25°C to 35°C and very poor pollen germination beyond 45°C.

3.6. Biochemical parameter

Total sugar content was estimated and sugar profiling was also carried out in collected pollen grains and the results are presented in Table 7. The content of total sugar ranged from 79.9 mg/g to 134.3 mg/g in all the studied cashew varieties. Significant differences in total sugar content were observed among early, mid and late cashew varieties. The average total sugar content was 129.8 mg/g for VRI-3, Vengurla-4 and Ullal-3 (early varieties) followed by 118.3 mg/g for Bhaskara (mid variety) and 79.9 mg/g for Madakkathara-2 (late variety). Among the varieties studied, total sugar content was higher in VRI-3 (134.3 mg/g) and lower in Madakkathara-2 (79.9 mg/g). Based on the sugar profiling data, glucose and fructose were the main soluble carbohydrates found in cashew pollens in the present study. Relatively smaller amount of sucrose was also detected in the cashew pollens. Other sugar types were also detected in minute quantity in the pollens (< 1mg/g). Higher fructose and glucose content was recorded in VRI-3 (54.90 mg/g) and Ullal-3 (37.34 mg/g) and lower in Madakkathara-2 (38.05 mg/g and 24.24 mg/g) among all the studied cashew varieties. Sucrose content ranged from 2.55 mg/g in Madakkathara-2 to 4.95 mg/g in Ullal-3.

Table 6
Pollen germination of five cashew varieties in response to controlled temperatures.

Temperature (°C)	Pollen gemination (%)				
	VRI-3	Ullal-3	Vengurla-4	Bhaskara	Madakkathara-2
5	0	0	0	0	0
10	5.23^{f} ±0.399	4.12^{e} ±0.540	3.67^{f} ±0.588	1^{g} ±0.064	0
15	20.2^{e} ±1.276	18.1^{d} ±1.332	12.5^{e} ±0.559	5.65^{f} ±0.493	3.32^{f} ±0.383
20	68.4^{b} ±0.517	59.78^{b} ±2.672	49.98^{b} ±0.783	28.98^{d} ±0.647	20.34^{d} ±0.721
25	81.32^{a} ±1.863	74.56^{a} ±0.342	69.89^{a} ±0.569	45.64^{b} ±1.823	35.34^{b} ±0.502
30	38.92^{c} ±1.710	32.32^{c} ±1.232	29.98^{c} ±0.656	57.86^{a} ±0.441	50.45^{a} ±2.158
35	25.21^{d} ±1.106	20.12^{d} ±1.084	18.78^{d} ±0.976	38.93^{c} ±0.919	31.23^{c} ±1.240
40	6.72^{f} ±0.327	4.23^{e} ±0.287	2.43^{f} ±0.356	13.23^{e} ±1.263	12.12^{e} ±1.037
45	0	0	0	1^{g} ±0.179	1.5^{fg} ±0.566
50	0	0	0	0	0

Means followed by different letters in a column are statistically significant ($p<0.05$).

4. Discussion

4.1. Morphology of pollen

Pollen morphological studies are very important for understanding the taxonomy, phylogeny and palaeobotanical history of plants. Moreover, determination of inter specific and intraspecific relationship between fruit tree species mainly depends on pollen morphological studies which influence the pollination, fertilization and ultimately fruit development processes (Javady et al., 2001, Khaleghi et al., 2019). The identification and differentiation of plant classifications and taxonomy of closely related and unknown taxa also depend on pollen morphological data (Quamar et al., 2017, Rui Dong et al., 2020). Pollen size, shape, exine pattern, length and width of colpus and structure of wall etc. are the major morphological and structural features for the identification of plant species (Bahadur et al., 2018, Mass et al., 1977). Amidst various tools to study pollen morphology, scanning electron microscopy (SEM) studies are most widely used tool for pollen morphological studies including for taxonomic purposes as well as for identification of a variety or a cultivar (Lanza et al., 1996, Mortazavi et al., 2010).

So far, no studies on pollen morphological traits are reported in Indian cashew varieties. So, the present study holds good to understand the palynological characteristics of cashew. In this study, significant differences in pollen morphological characteristics were found among all the studied cashew varieties. The size of cashew pollen grains was medium consisting of 46.0μm polar length and 24.8μm equatorial width in elliptical or prolate shape. Studies in several fruit crops viz., pear (Westwood et al., 1978), apricot (Arzani et al., 2005, Thakur et al., 1970, Dezhong et al., 1995) and plum (Gilani et al., 2010) also reported almost similar size of pollen grains. The present pollen morphological features such as polar axis length and equatorial axis width data were also in consistent with studies reported by Kahraman et al. (2013) where almost similar size was reported in Vicia species. The ratio of P/E was significantly different among the varieties which was recorded using SEM images of cashew pollen grains. The values of P/E ratio were 1.98,1.68, 1.73, 2.03 and 1.88 for VRI-3, Ullal-3, Vengurla-4, Bhaskara and Madakkathara-2. Similar data was also quoted by Rui Dong et al. (2020). The exine ornamentation was thick, striate to reticulate type and was separated by grooves. Small perforation was also observed on the outer surface of exine.

Pollen size is an important trait influencing pollination of plant species. Plants with larger pollens have several advantages such as faster germinability and higher viability due to presence of more energy reserves than those with smaller pollens (Britnie et al., 2016). Pollen size also positively correlate with pistil length, length of style and pollen tube growth which influences pollinator attractiveness (Mazer et al., 2016). In this study, the size of cashew pollen was medium and VRI-3 exhibited higher polar length (48.53μm) while equatorial width was higher in Ullal-3 (27.68 μm). This clearly indicates the varietal differences in pollen size. Over all, early varieties (VRI-3, Ullal-3 and Vengurla-4) had bigger pollens (polar and equatorial length of 46.8 μm and 26.1 μm) than mid variety, Bhaskara (45.67 μm and 22.49 μm) and late variety, Madakkathara-2 (43.91 μm and 23.27 μm). Though it was not studied,

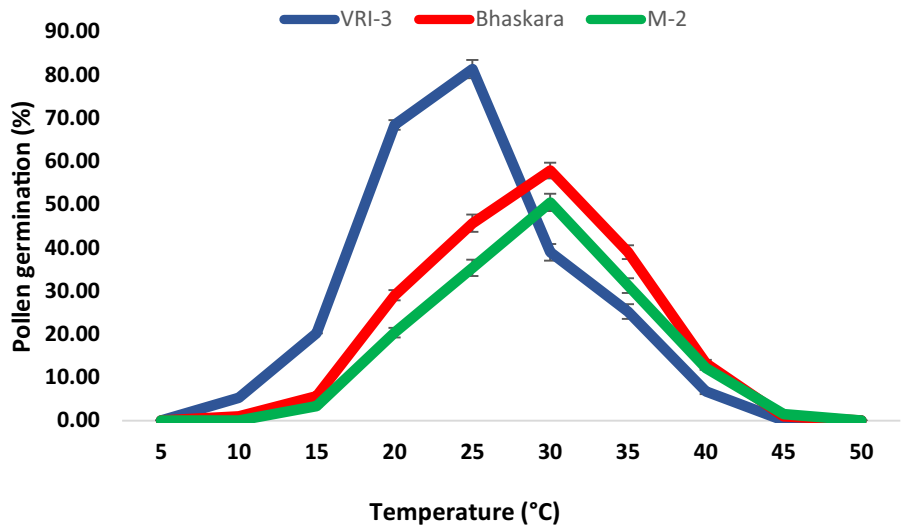


Fig. 6. *In vitro* pollen germination of cashew varieties in response to temperature throughout the experimental period. Varieties with variation in pollen germination (VRI-3: early; Bhaskara: mid and Madakkathara-2: late) are presented for clarity. Error bars indicate ±SD.

Table 7
Total sugar content and sugar profiling in pollens of five cashew varieties collected during flowering seasons.

Parameters	Madakkathara-2	Vengurla-4	Ullal-3	Bhaskara	VRI-3
Total sugar	79.9^{e} ±0.557	129.3^{b} ±0.625	125.8^{c} ±0.503	118.3^{d} ±1.320	134.3^{a} ±1.331
Ribose	0.818^{d} ±0.016	0.557^{e} ±0.021	2.94^{a} ±0.063	1.545^{b} ±0.009	1.357^{c} ±0.038
Arabinose	0.105^{e} ±0.001	0.289^{c} ±0.002	0.409^{a} ±0.003	0.313^{b} ±0.006	0.206^{d} ±0.002
Xylose	0.172^{d} ±0.002	0.056^{e} ±0.002	0.281^{b} ±0.008	0.356^{a} ±0.008	0.195^{c} ±0.004
Rhamnose	0.014^{d} ±0.001	0.019^{c} ±0.001	0.036^{a} ±0.001	0.027^{b} ±0.001	0.019^{c} ±0.001
Fucose	0.038^{c} ±0.001	0.045^{b} ±0.002	0.015^{d} ±0.001	0.065^{a} ±0.002	0.035^{c} ±0.001
Fructose	38.059^{e} ±0.176	51.308^{b} ±0.016	49.363^{c} ±0.068	47.549^{d} ±0.339	54.903^{a} ±0.371
Glucose	24.243^{e} ±0.148	26.603^{d} ±0.023	37.343^{a} ±0.206	33.003^{b} ±0.009	27.691^{c} ±0.124
Mannose	1.589^{b} ±0.054	1.437^{bc} ±0.042	1.271^{c} ±0.035	1.8^{a} ±0.028	1.442^{bc} ±0.084
Galactose	0.192^{d} ±0.009	0.144^{d} ±0.003	0.783^{a} ±0.039	0.499^{b} ±0.039	0.337^{c} ±0.005
Inositol	1.291^{ab} ±0.014	1.345^{a} ±0.018	1.239^{b} ±0.021	1.105^{c} ±0.025	1.268^{b} ±0.031
Sorbitol	0.836^{c} ±0.011	0.249^{e} ±0.021	0.966^{b} ±0.029	0.702^{d} ±0.012	1.098^{a} ±0.049
Sucrose	2.555^{e} ±0.028	3.423^{c} ±0.006	4.955^{a} ±0.002	2.648^{d} ±0.002	4.253^{b} ±0.011
Maltose	0.032^{e} ±0.002	0.042^{d} ±0.001	0.052^{c} ±0.001	0.236^{a} ±0.006	0.132^{b} ±0.002
Trehalose	0.004^{e} ±0.001	0.01^{c} ±0.001	0.008^{d} ±0.001	0.019^{a} ±0.001	0.012^{b} ±0.001
Lactose	0.021^{d} ±0.001	0.015^{e} ±0.001	0.05^{b} ±0.001	0.054^{a} ±0.001	0.03^{c} ±0.001

All values are expressed as mg per g pollen. Means followed by different letters in a row are statistically significant ($p<0.05$).

pollen size is found to play significant role in regulating dessication tolerance of pollen grains. Few studies indicated positive correlation between pollen size and desiccation pressure where pollen size increased under intense desiccation stress (Maciej et al., 2011; Nogue et al., 2022). While others reported that pollen grains tend to be smaller in size in high desiccation tolerant species and much bigger in less desiccation tolerant ones existing in dry forest conditions (Nogue et al., 2022). They further observed that pollen grains with high desiccation tolerance survived longer under low moisture conditions enabling better dispersal and crossing between different species. Low desiccation tolerant pollen grains survived for few hours after anther opening and germinated immediately whenever moisture was available and also exhibited death in absence of moisture. These make pollen more susceptible to desiccation causing early pollen death and more narrow pollination window (Franchi, 2002; Pacini et al., 2006). These findings clearly support the present data in cashew where mid and late varieties produced smaller pollen grains which might be one of the adaptive mechanisms for desiccation tolerance.

Apart from pollen size, presence of apertures (furrows and pores) in pollen grains happens to be one of the traits affecting desiccation tolerance (Nogue et al., 2022). The presence of furrows in pollen grains of high desiccation tolerance helps in controlling changes in pollen volume during dehydration and rehydration processes (Tweddle et al., 2003). In the present study, thick furrows and pores were observed on the exine ornamentation of pollen grains. In cross sectional or longitudinal view, inward folding was also noticed on exine. These pollen traits viz., smaller size and presence of furrows and pores might also have contributed in conferring tolerance to desiccation in mid and late varieties due to harmomegathic efficiency allowing variation in pollen volume with changing moisture content (Franchi et al., 2011).

Mid and late varieties also exhibited lower pollen germination compared to early varieties in the present study. These might be due to their high desiccation tolerance traits in pollen grains where initially pollens exhibit low metabolic activity in dehydrated state followed by rehydration to start germination process making germination process slower (Andrew, 2012). However, this transition may be advantageous for mid and late varieties as pollen grains can remain alive for longer time in prevailing environments. There are studies indicating the possible role of high desiccation tolerant pollens as pollinator attractants for their nutritionally dense food reserves due to their smaller proportion of water (Franchi et al., 2002). Studies on morphological features of pollens of cashew varieties are not available till date which makes the present study more valuable and useful to investigate pollen morphological features of other cashew varieties to understand the pollination kinetics for enhanced yield.

4.2. *Optimization of a pollen germination medium in vitro*

A successful germination of pollen and its tube growth of any plant species depend on interactions of several compounds as well as

modifications in the concentrations of such compounds for better response. Among the several osmoregulatory and nutritive compounds, sucrose and poly ethylene glycol (PEG) play key role in osmo- regulation (Shivanna et al., 1995) for increased germination of pollen and controlling rupture of pollen grain by regulating permeability of plasma membrane (Subbaiah, 1984; Shivanna et al., 1995). In addition, varying concentrations of other mineral salts such as boron, calcium, magnesium etc. also influence pollen germination and pollen tube growth (Feijo et al., 1995). The growth medium for cashew pollen was already developed by Subbaiah (1984) with 50 to 60 % pollen germination. In this study, the growth medium was optimized with an aim of increasing germination of pollen. The concentrations of sucrose, PEG and other salts were changed suitably in the present study. The earlier cashew medium developed by Subbaiah (1984) consisted of different combinations of sucrose and PEG with boron and calcium and increased germination was observed with combined effect of both sucrose (20%) and PEG (30%). Growth compounds viz., sucrose, PEG, boric acid and other mineral salts (calcium nitrate and magnesium sulphate) were included in the optimized growth medium.

Out of all the different combinations tested, the growth medium consisting of 15% PEG+1mg boric acid+3mg calcium nitrate+2mg magnesium sulphate resulted in maximum pollen germination (75.28%). These data clearly indicate significance of PEG as osmotic compound for enhanced pollen germination compared to sucrose. Usually, plant cells take up sucrose more readily in large quantities which might affect germinating pollens by disturbing osmotic relations (Goode et al., 1965). In case of PEG, the phenomena are different. Usually, PEG of high molecular weight is utilized in the growth medium which facilitate little entry into the cell for the maintenance of cell metabolism and proper regulation of osmotic relations (Handa et al., 1982; Janes, 1974). Zhang et al. (1982) also reported similar works in petunia species where PEG 4000 resulted in better germination compared to sucrose medium.

4.3. Ideal time of pollen collection

Small and large stamens of hermaphrodite and male flowers produce pollens of four distinct types in cashew. Cashew is andromonoecious having sticky pollen and even longer stamen of the hermaphrodite flower is shorter than style, thus making self-pollination difficult and hence favouring cross-pollination by insects. Several studies showed that fruit set in cashew is mainly influenced by the activity of pollinators (Freitas and Paxton, 1996; Reddi, 1993). Flies (Roubik, 1995), moths (Kevan, 1975) and bees (Bhattacharya, 2004; Freitas and Paxton, 1998; Heard, 1990) have been recorded as cashew pollinators worldwide. Among them, bees are considered as important pollinators of cashew in India (Sundararaju, 2011). A total of thirteen bee species belonging to *Apidae* and *Halictidae* were recorded as pollinators of cashew flowers in Karnataka. Within bees of *Apidae*, the highest species abundance was recorded for *Braunsapis picitarsis* (20%) followed by *Apis cerana indica* (16.7%). Bees of *Halictidae* contributed to 24.4% of bee abundance, among which *Pseudapis oxybeloides* was most abundant (17.6%). Peak bee activity was recorded between 11.00 and 13.00 h for most of the bees. The peak foraging period of the bees were between 11.00 am and 1.00 pm (Vanitha and Raviprasad, 2019). Peak anthesis of the cashew flowers occurs between 9.00 and 11.00 h (Rao and Hassan, 1957) and more than 85% open during forenoon hours. It is important to note that peak foraging period of pollinators occurs when maximum flowers remain open, which is very much advantageous for effective pollination in cashew.

Cashew pollen falling by gravity on receptive stigma is not a factor because cashew pollen grains are sticky and hence circumstances of falling by gravity is ruled out. Non-dehisced anthers are pinkish in colour and when bees visit these anthers pollen grains stick to their body parts which helps in pollination when they visit bisexual flowers. Furthermore, greyish pollen dust formed from the dehisced anthers after getting enough sunlight is also not able to fall by gravity as it forms ball of sticky mass. To effect cross pollination, the male flowers with dehisced anther heads having greyish mass of pollen dust have to be plucked and their anther heads need to be rubbed or brushed over the stigma. Cashew trees produce four types of pollen from the large and small stamens of the hermaphrodite and male flower (Wunnachit et al., 1992). They are hermaphrodite large (HL), hermaphrodite small (HS), male large (ML) and male small (MS). Despite early suggestions that cashew is wind pollinated (Haarer, 1954; Aiyadurai and Koyamu, 1957; Rao and Hassan, 1957), later studies through bagging of panicles (to exclude insects and wind as pollinating agent) and caging / bagging with nylon mosquito nets (allow access to wind but exclude insects) have proven that wind does not have any role and insects play a key role in pollination of cashew (Reddi, 1991; Frietas and Paxton, 1996).

There are several contradictory reports on production of pollens by hermaphrodite and male flowers in other crops. Wunnachit et al. (1992) reported that hermaphrodite and male flowers produce pollens with no difference in pollen number in cashew. On the other hand, workers viz., Perez et al. (2019) and Gehrke-Velez et al. (2011) reported production of more pollens by hermaphrodite flowers than by male ones in mango. The regulatory roles of both male and hermaphrodite flowers in cashew were reported by Wunnachit et al. (1992). They opined that the metabolites viz., sugar and amino acid content was high in pollens produced from hermaphrodite flowers compared to male flowers and were highly nutritious. Thus, pollens from hermaphrodite flowers serve as food reserves to attract insect pollinators and has limited effect on fertilization. On the other hand, male flowers produce pollens with higher fertility and also act as an important source to provide pollens for pollination and fruit set. Similar results were also reported in Cassia species where large anthers from male flowers produced pollens for pollination whereas, both small and large anthers from hermaphrodite flowers produced pollens for bee consumption (Dulberger, 1981 b). There are several studies exhibiting the role of hermaphrodite flowers in proper fruit set and enhanced nut yield in cashew, however, Wunnachit et al. (1992) gave their opinion that selection of cashew varieties with more hermaphrodite flowers may not always result in higher yield. Therefore, male flowers may also be taken into consideration while selecting varieties as male flowers are very efficient for increased pollen germination and better penetration of ovule. These clearly indicate the significant role of male flowers in crop development and production. Therefore, *in vitro* pollen germination studies were restricted to only male flowers in the present study.

Cashew panicle bears both hermaphrodite and male flowers together and thereby exhibit andromonoecy trait (Purseglove, 1968). A typical monochasial cyme arrangement is observed in cashew panicle where each cyme consists of a hermaphrodite flower in terminal position and two male flowers in lateral positions (Copeland, 1961). Both cashew flower types show differential opening patterns. During flowering, male flowers open early in the morning followed by hermaphrodite flowers several hours later. However, it varies with different growing areas and flower location in the canopy. For instance, male flowers open between 6 am to 2 pm in India (Damodaran et al., 1965), 6 am to 6 pm in Tanzania (Northwood, 1966) and 5 am to mid-day in Thailand (Wunnachit,1992) reaching peak in two to four hours after the opening. Studies by Damodaran et al (1965) and Northwood (1966) reported that flower opening and anther dehiscence also depend on the flower location in a panicle where flower opens early and anther dehiscence starts and finishes earlier in the canopy facing towards sun compared to the panicle on the shaded side of the tree. The time period between 9.30 am to 11.30 am corresponded to most of male flowers opening in this study. Thus, pollen collection time was restricted to 9.30 am to 11.30 am to study pollen performance in terms of pollen germination in studied cashew varieties. Similar studies were also reported in perennial crops like coconut where pollens of male flowers exhibited higher pollen germination at the time interval between 8.30 am to 10.30 am (Hebbar et al., 2018; Menon et al., 1958).

4.4. Temperature response on pollen germination during flowering seasons (in vivo)

The plant life cycles are governed by several developmental stages such as vegetative and reproductive traits, responsiveness towards surrounding environments etc. Changes in climatic variables influence reproductive traits development with most significant effect on phenology of flowering (Menzel et al., 2006; Mohammed et al., 2018). Flowering phenology is controlled by several climatic parameters, yet, sensitivities to increase in temperature is most widely studied. Usually, plants are categorized as early, mid and late types based on their season of flowering and in turn exhibit differential phenological development for inducing changes in their reproductive development. Increase in temperature affects the phenological stages of mid and late flowering plants most compared to early types (Meng et al., 2016).

In cashew, early, mid and late varieties exhibit differential response of flower opening in terms of initiation of flowering and flowering duration. For early varieties, initiation of flowering starts in November to December and continues up to January. For mid variety, flowering initiation starts in December to January and continues up to March while for late variety, flowering initiation starts in January to February and continues up to April. Due to flowering seasons, phenology of mid and late varieties coincides with increase in temperature. In the present study, five varieties viz., VRI-3, Ullal-3, Vengurla-4, Bhaskara and Madakkathara-2 were used for temperature response studies due to their differential flower opening patterns. VRI-3 is early and long duration variety with flowering duration of 75-80 days. Both Vengurla-4 and Ullal-3 are early and short duration varieties with flowering duration of 70 to 75 days and 65-75 days. Bhaskara is mid variety with mid duration of 75-80 days. Madakkathara-2 is late variety with short duration up to 75 days (Nayak, 2014).

The germination of pollens was quantified in early, mid and late varieties during their flowering seasons in this study. The results indicated more sensitivities of mid and late varieties to increase in temperature. Significant increase in pollen germination was recorded for early varieties at the end of flowering seasons which correlated with increase in temperature during January. There are several studies in mango which support the present results (Perez et al., 2019; Issarakraisila et al., 1992). Variable responses in terms of pollen germination were also observed for mid and late varieties. Unlike early varieties, pollen germination was reduced significantly during the final flowering week with increase in temperature in March. Further, studies by Suonan et al. (2017) confirms the findings of this present study. Under the climate change scenario, it is also predicted that temperature will increase more invariably especially during summer which will ultimately worsen the conditions of those plant species that flowers towards summer. Therefore, the phenology and functioning of mid and late flowering varieties are likely to get affected more than to early flowering types (Takkis et al., 2018). The present results also support the fact. The mid and late flowering plant species might face the high temperature impacts on their phenology in terms of changes in flowering pattern as well as yield due to depletion of available soil moisture, changes in species distribution, reduction in crop production, disturbance in nutrient balance and recycling (Richardson et al., 2013).

Severe moisture stress exists in cashew during January to May. Due to depletion of available soil moisture during extended drought periods, cashew also experiences high temperature stress affecting yield. One of such incidence of extended drought periods and rise in temperature was reported in cashew plantations of Kerala during 1983 to 1986 where cashew yield reduced to the extent of 50% due to inflorescence drying and incidence of inflorescence thrips (Veeraragharan et al., 1990). Veeraragharan et al. (1990) also reported that mid and late varieties tend to suffer more as the flowering time coincides with moisture stress periods and high temperature (>34°C) during January to May. All these studies positively support the present study where pollens of Bhaskara and Madakkathara-2 exhibited low germination during the final flowering week of March with maximum temperature above 35°C.

In the present study, low temperature induced reduction in pollen germination was also observed among five varieties other than high temperature induced responses. Low temperature induced reduction in pollen germination was more evident in case of early varieties especially during initial weeks of flowering seasons in January. In contrast, pollen germination increased during the initial flowering weeks with no effect of low temperature in mid and late varieties. This may be due to their differential flowering seasons. Early cashew varieties flower during peak seasons of December to January where average minimum temperature ranges between 15-18°C. Mid and late cashew varieties flower during January to March and March to May where average minimum temperature ranges between 20.3 to 25.0°C. Thus, mid and late varieties might partially escape from low temperature under *in vivo* conditions. However, to validate the same, germination of pollen was recorded under *in vitro* conditions in growth chamber at ten constant temperatures (5 to 50°C). Mid and late varieties exhibited significant reduction in pollen germination at low temperature which are discussed subsequently. Several workers viz., Sukhvibul et al. (2000), Huang et al. (2010), Gaurang et al. (2015) and Perez et al. (2019) also reported the same.

4.5. Pollen germination under controlled temperatures

After studying the differential responses of pollens during flowering seasons, pollen germination was tested *in vitro* at 5°C to 50°C temperatures and varietal differences for pollen germination was estimated. Pollen germination reduced significantly in all the varieties in response to low temperature. At 10°C, the germination percentage were 4.3% for early varieties, 1% for mid variety and no germination for late cashew variety. Pollen germination was maximum at optimum temperature ranges between 20°C to 30°C for early varieties and between 25°C to 35°C for mid and late varieties. Several studies in mango (Perez et al., 2019, Sukhvibul et al., 2000; Huang et al., 2010), avocado (Alcaraz et al., 2011), lychee (Stern et al., 1998) and longan (Pham et al., 2015) support the results as reported in the present study. The present study also reports the fact that beyond the optimum range of temperature, pollen germination reduced as temperature increased. At 40°C, pollen germination was significantly affected in early varieties (VRI-3, Ullal-3 and Vengurla-4) to the tune of 4.4% whereas, relatively better germination was observed for mid variety (Bhaskara) and late variety (Madakkathara-2) to the extent of 13.2% and 12.1%. Although, germination was negligible at 45°C where no germination was recorded for early varieties, yet some germination was recorded for mid variety (1%) and late variety (1.5%). It may be further indicated that mid and late varieties exhibit better tolerance to high temperature than early varieties. These clearly reflect the adaptation strategies of different varieties to different flowering dates. Thus, mid variety, Bhaskara and late variety, Madakkathara-2 can be introduced as high temperature tolerant varieties and pollens of such varieties may be used in breeding programme to transfer the heat tolerance trait into sensitive varieties for enhanced tolerance for their multiplications in extreme environments. Similar studies were also reported in cotton (Garay et al., 1988) and tomato (Peet et al., 1997). In cashew, tea mosquito bug (TMB) is major insect pest causing considerable yield loss up to 50-60%. However, studies have shown the lesser incidence of TMB during summer when high temperature exists (Sree Kumar et al., 2011). These are the reasons that Bhaskara and Madakkathara-2 escape from TMB damage due to their flowering dates during hot summer. These also clearly support the benefit of using Bhaskara and Madakkathara-2 as temperature tolerant varieties for enhanced yield.

Positive correlation between germination of pollen and final fruit set is reported in several annual crops such as groundnut, bell pepper etc. under high temperature (Prasad et al., 1999; Aloni et al., 2001). The same aspect needs further validation in cashew too where Bhaskara and Madakkathara-2 needs thorough screening in field conditions under high temperature to evaluate fruit set and nut yield. Currently, studies

on this line are ongoing under field for evaluation of high temperature effect on fruit set and yield of cashew varieties.

4.6. Sugar profiling in cashew pollens

Important plant metabolites mainly sugar accumulation was estimated in pollens of five cashew varieties which were collected during final flowering week of January and March when maximum temperature reached 25°C and 35°C. Based on sugar profiling, glucose and fructose were found to be the most abundant reducing sugars in all the studied varieties in the present study. However, total sugar content as well as reducing sugars were higher in early varieties (VRI-3, Vengurla-4 and Ullal-3) in contrast to mid variety (Bhaskara) and late variety (Madakkathara-2).

Generally, sucrose is collected by the pollens during pollen development which further undergoes hydrolysis by invertase enzyme to convert to glucose and fructose for their utilization at the onset of germination (Aloni et al., 2001). The presence of more glucose and fructose content in pollens acts as an important source of energy for germination. The maintenance of higher glucose and fructose content might have contributed in higher pollen germination in early varieties at higher temperature during final flowering season of January (minimum and maximum temperature ranging from 18°C to 20°C and 22°C to 25°C) as well as at controlled temperature conditions (15°C to 30°C). Reduced pollen germination in mid and late varieties might be attributed to reduced sugar content thus affecting the inversion process (sucrose hydrolysed to glucose and fructose). Studies on metabolite profiling of mango pollens also support the present results (Shivashankara et al., 2019). The reduced total and reducing sugar content in both mid and late varieties may also be attributed to utilization of total sugar during pollen germination as reported in coconut (Hebbar et al., 2018) and also due to impact of high temperature (reaching above 35°C during March) on available carbohydrate utilization in pollens thus hindering the pollen germination process (Pressman et al., 2002).

5. Conclusions

The research findings obtained in this study have served to understand the pollen morphology as well as to develop an optimized pollen germination medium *in vitro* for cashew for better understanding of pollen biology studies of the same. The findings of this study also imply differential varietal responses to temperature for pollen germination. The optimum temperature (T_{opt}) for pollen germination was 25°C for early varieties and 30°C for mid and late varieties. However, mid and late varieties exhibited better tolerance to high temperature beyond 40°C under controlled conditions. Therefore, mid variety (Bhaskara) and late variety (Madakkathara-2) may be more tolerant to high temperature stress during flowering. Targeted breeding using such temperature tolerant varieties and incorporation of high temperature tolerance during flowering will be an important adaptative strategy in regions that are vulnerable to climate change and climate variability. However, as in other field and horticultural crops, the usefulness of *in vitro* pollen germination techniques needs to be further explored to screen large germplasm of cashew to high temperature.

Competing interests

The Authors declare no competing financial interests.

CRediT authorship contribution statement

Babli Mog: Conceptualization, Methodology, Writing – original draft. **G.L. Veena:** Investigation. **J.D. Adiga:** Funding acquisition, Supervision. **K.B. Hebbar:** Writing – review & editing. **Shamsudheen M:** Software. **G.N. Manjesh:** Project administration. **E. Eradasappa:** Resources. **G.S. Mohana:** Visualization. **V. Thandaiman:** Data curation. **K. Vanitha:** Validation. **Anil Kumar Yadav:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Authors thank Indian Council of Agricultural Research, New Delhi for the financial support to carry out this research. Authors also thank Director, ICAR-Directorate of Cashew Research, Puttur for providing all the facilities for this research. The authors thank Sophisticated Analytical Instrument Facility, ICAR-Indian Institute of Horticultural Research, Bengaluru for sugar profiling. Authors thank Shiva Kumar for both field and laboratory assistance.

References

Aiyadurai, S.G., Koyamu, K., 1957. Variations in seedling trees of cashew. South Indian Hortic. 5, 153–156.
Aloni, B., Peet, M., Pharr, M., Karni, L., 2001. The effect of high temperature and high atmospheric CO2 on carbohydrate changes in bell pepper (Capsicum annuum) pollen in relation to its germination. Physiol. Plant. 112, 505–512.
Andrew, R. M., 2012. A comparative analysis of the relative water content of the pollen of early diverging angiosperms. Chancellors's Honors Program Projects.
Arzani, K., Nejatian, M.A., Karimzadeh, G., 2005. Apricot (Prunus armeniaca) pollen morphological characterization through scanning electron microscopy, using multivariate analysis. New Zeal. J. Crop Horticult. Sci. 33, 381–388.
Bahadur, S., Ahmad, M., Mir, S., Zafar, M., Sultana, S., Ashfaq, S., Arfan, M., 2018. Identification of monocot flora using pollen features through scanning electron microscopy. Microsc. Res. Tech. 81, 599–613.
Bhattacharya, A., 2004. Flower visitors and fruit set of *Anacardium occidentale*. Ann. Bot. Fenn. 41, 385–392.
Britnie, M., Shu-Mei, C., 2016. Pollen competition in style: effects of pollen size on siring success in the hermaphroditic common morning glory, Ipomoea purpurea. Am. J. Bot. 103 (3), 460–470. http://www.amjbot.org/.
Copeland, H.F., 1961. Observations on the reproductive structures of Anacardium occidentale L. Phyto morphology 11, 314–325.
Damodaran, V.K., Abraham, J., Alexander, K.M., 1965. The morphology and biology of the cashew flower I-flowering habit, flowering season, morphology of the flower and sex ratio. Agric. Res. J. Kerala 3 (1), 78–84.
Dezhong, T., Baoming, W., Gaixiu, D., Xiaofung, F., 1995. Studies on the pollen morphology and ultrastructure of cultivated varieties of apricot (Armeniaca vulgaris Lam). Acta Hortic 403, 140–144.
Dubois, M., Gilles, K.A., Hamilton, G.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Dulberger, R., 1981b. The floral biology of Cassia didymobotyra and C. auriculata (Caesalpiniaceae). Am. J. Bot. 68, 1350–1360.
FAO, 2014. Food and agricultural organization statistical production report on cashew nuts with Shell. Accessed 04/07/2018. Available online http://faostat3.fao.org/fao statgateway.
Feijó, J.A., Malhó, R., Obermeyer, G., 1995. Ion dynamics and its possible role during in vitro pollen germination and tube growth. Protoplasma 187, 155–167.
Franchi, G., 2002. Partially hydrated pollen: taxonomic distribution, ecological and evolutionary significance. Plant Systemat. Evolut. 234, 211–227.
Freitas, B.M., Paxton, R.J., 1996. The role of wind and insects in cashew (*Anacardium occidentale*) pollination in NE Brazil. J. Agric. Sci. 126, 319–326.
Freitas, B.M., Paxton, R.J., 1998. A comparison of two pollinators: the introduced honey bee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew Anacardium occidentale in its native range of NE Brazil. J. Appl. Ecol. 35 (1), 109–121.
Garay, B.R., Barrow, J.R, 1988. Pollen selection for heat tolerance in cotton. Crop Sci 28, 857–859.
Gaurang, P.D., Patel, B.N., Chauhan, D.A., 2015. Influence of temperature on pollen viability and fruit setting in mango. J. Environ. Res. Dev. 10 (01), 47–53.
Gilani, S.A., Qureshi, R.A., Khan, A.M., Potter, D., 2010. Morphological characterization of the pollens of the selected species of genus Prunus Linn. from Northern Pakistan. Afr. J. Biotechnol. 9, 2872–2879.
Godini, A., de Palma, L., Petruzzella, A., 1987. Interrelationships of almond pollen germination at low temperatures, blooming time and biological behaviour of cultivars. Adv. Hort. Sci. 1, 73–76.

Goode, J.E., Hegarty, F.W., 1965. Measurement of water potential of leaves by the methods involving immersion in sucrose solutions. Nature (London) 276, 109.
Gupta, S., Sen, P., Srinivasan, S., 2014. Impact of climate change on the Indian economy: evidence from food grain yields. Climate Change Econ. 5 (02), 1450001.
Haldankar, P, M., Deshpande, S, B., Chavan, V.G., Rao, E.V.V.B., 2003. Weather associated yield variability in cashewnut. J. Agrometeorol. 5 (2), 73–76.
Handa, A.K., Bressans, R.A., Handa, S., Hasegawa, P.M., 1982. Characteristics of cultured tomato cells after prolonged exposure to medium containing polyethylene glycol. Plant Physiol. 69, 514–521.
Haarer, A.E., 1954. The cashew (*Anacardium occidentale* Linn) nut. World Crops 6, 95–98.
Hebbar, K.B., Helan, M.R., Anusree, R.N., Kannan, S., Niral, V., Arivalagan, M., Alka, G., Samsudeen, K., Chandran, K.P., Chowdappa, P., Vara Prasad, P.V., 2018. Differences in in vitro pollen germination and pollen tube growth of coconut (Cocos nucifera L.) cultivars in response to high temperature stress. Environ. Exp. Bot. 153, 35–44.
Heard, T.A., Vithanage, V., Chacko, E.K., 1990. Pollination biology of cashew in the northern territory of Australia. Aust. J. Agric. Res. 41, 1101–1114.
Huang, J.H., Ma, W.H., Liang, G.L., Zhang, L.Y., Wang, W.X., Cai, Z.J., Wen, S.X., 2010. Effects of low temperatures on sexual reproduction of 'Tainong 1' mango (Mangifera indica). Sci. Hortic. 126, 109–119.
Issarakraisila, M., Considine, J., Turner, D., 1992. Seasonal effects on floral biology and fruit set of mangoes in a warm temperate region of Western Australia. Acta Hortic 321, 626–635.
Janes, B.E., 1974. The effect of molecular size, concentration in nutrient solution and exposure time on the amount and distribution of polyethylene glycol in pepper plants. Plant Physiol. 54, 226–230.
Javady, T., Arzani, K., 2001. Pollen morphology of five Iranian olive (Olea europaea L.) cultivars. J. Agric. Sci. Technol. 3, 37–42.
Kahraman, A., Binzat, O.K., Doğan, M., 2013. Pollen morphology of some taxa of Vicia L. subgenus Vicia (Fabaceae) from Turkey. Plant Systemat. Evolut. 299, 1749–1760.
Kakani, V.G., Prasad, P.V.V., Craufurd, P.Q., Wheeler, T.R., 2002. Response of in vitro pollen germination and pollen tube growth of groundnut (Arachis hypogaea L.) genotypes to temperature. Plant Cell. Environ. 25, 1651–1661.
Kakani, V.G., Reddy, K.R., Koti, S., Wallace, T.P., Prasad, P.V.V., Reddy, V.R., Zhao, D., 2005. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann. Bot. 96, 59–67.
Kevan, P.G., 1975. Pollination and environmental conservation. Environ. Conserv. 2, 293–298.
Khaleghi, E., Karamnezhad, F., Moallemi, N., 2019. Study of pollen morphology and salinity effect on the pollen grains of four olive (Olea europaea) cultivars. S. Afr. J. Bot. 127, 51–57.
Lal, R., 2001. Future climate change: implications for India Summer monsoon and its variability. Current Sci. 81, 1205–1207.
Lanza, B., Marsilio, V., Martinelli, N., 1996. Olive pollen ultrastructure: characterization of exile pattern through image analysis scanning electron microscopy (IA-SEM). Sci. Hortic. 65, 283–294.
Ledesma, N.A., Kawabata, S., 2016. Responses of two strawberry cultivars to severe high temperature stress at different flower development stages. Sci. Hortic. 211, 319–327.
Lee, C.W., Thomas, J.C., Buchmann, S.L., 1985. Factors affecting germination of jojoba pollen. J. Am. Soc. Hort. Sci. 110 (5), 671–676.
Lobell, D.B., Schlenker, W., Costa-Roberts, J., 2011. Climate trends and global crop production since 1980. Science 333 (6042), 616–620.
Maas, J.L., 1977. Pollen ultrastructure of strawberry and other small fruit crops. J. Am. Soc. Horticult. Sci. 102, 560–571.
Maciej, J.E., Dorota, W.S.P., Anna, E., Dominika, D.K., Monika, K.S.K., Piotr, K., Jan, K., 2011. Does climate affect pollen morphology? Optimal size and shape of pollen grains under various desiccation intensity. Ecosphere 2 (10), 117.
Maurizio, I., Aurora, C., Luigi, G.I., Claudio, D.V., Giovanna, A., 2022. High temperature and humidity affect viability and longevity in Olea europaea L. Agronomy 12 (1). https://doi.org/10.3390/agronomy 12010001.
Mazer, S.J., Moghaddasi, A., Bello, A.K., Hove, A.A., 2016. Winning in style: longer styles receive more pollen, but style length does not affect pollen attrition in wild Clarkia populations. Am. J. Bot. 103, 408–422.
Mellenthin, W.M., Wang, C.Y., Wang, S.Y., 1972. Influence of temperature on pollen tube growth and initial fruit development in 'D'Anjou' pear. Hort Science 7, 557–559.
Meng, F., Cui, S., Wang, S., Duan, J., Jiang, L., Zhang, Z., Li, X., 2016. Changes in phenological sequences of alpine communities across a natural elevation gradient. Agric. For. Meteorol. 224, 11–16.
Menon, K.P.V., Pandalai, K.M., 1958. Coconut: A Monograph. Central Coconut Research Committee, New Delhi.
Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Zust, A., 2006. European phenological response to climate change matches the warming pattern. Glob. Change Biol. 12, 1969–1976.
Michael, G., Amir, J., 2019. Report on climate change and heat-induced mortality in India. Insight /Publication. Tata Centre for Development. UCHICAGO. Chicago tcd.uchicago.edu.
Mohammed, A.S., Arfin, K., Carl, B., Juergen, K., Sabrina, B., Sebastian, V., Anke, J., 2018. Phenological sensitivity of early and late flowering species under seasonal warming and altered precipitation in a seminatural temperate grassland ecosystem. Ecosystems. https://doi.org/10.1007/s10021-017-0220-2.
Mortazavi, S.M.H., Torahi, A., Beremeh, L., 2010. A study on the pollen morphology of Khuzestan male date varieties by scanning electron microscopy (SEM). Int. J. Plant Prod. 33, 97–109.
Nayak, M.G., Mohana, G.S., Bhat, P.S., Saroj, P.L., Swamy, K.R.M., 2014. Minimum Descriptors of Cashew Germplasm Accessions. ICAR-Directorate of Cashew research, Puttur Catalogue IV.
Nogue, S., Nascimento, L., Graham, L., Brown, L.A., Gonzalez, L.A.G., Castilla-Beltran, A., Penuelas, J., Fernandez-Palacios, J.M., Willis, K.J., 2022. The spatiotemporal distribution of pollen traits related to dispersal and desiccation tolerance in Canarian laurel forest. J. Veg. Sci. 33, e13147. https://doi.org/10.1111/jvs.13147.
Northwood, P.J., 1966. Some observations on the flowering and fruit setting in the cashew. Trop. Agric. 43, 35–42.
Pacini, E., Guarnieri, M., Nepi, M., 2006. Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. Protoplasma 228, 73–77.
Pavithran, K., Ravindranathan, P.P., 1974. Studies on the floral biology in cashew (Anacardium occidentale L. J. Plantat. Crops 2, 32–33.
Péreza, V., Herrerob, M., Hormazaa, J.I., 2019. Pollen performance in mango (Mangifera indica L., Anacardiaceae): Andromonoecy and effect of temperature. Sci. Hortic. 253, 439–446.
Pham, V.T., Herrero, M., Hormaza, J.I., 2015. Effect of temperature on pollen germination and pollen tube growth in longan (Dimocarpus longan Lour.). Sci. Hortic 197, 470–475.
Pirlak, L., 2002. The effects of temperature on pollen germination and pollen tube growth of apricot and sweet cherry. Gartenbauwissenschaft 67 (2), 61–64.
Prasad, P.V.V., Craufurd, P.Q., Summerfield, R.J., 1999. Sensitivity of peanut to timing of heat stress during reproductive development. Crop. Sci. 39, 1352–1357.
Prasada Rao, GS.L.H.V., Giridharan, M.P., Jayaprakash Naik, B., Gopakumar, C.S., Krishnakumar, K.N., Xavier, T., 2001. Influence of weather factors on bud break, flowering and nut quality of cashew (Anacardium occidentale L.). Indian J. Agric. Sci. 71 (6), 399–402.
Pressman, E., Peet, M.M., Pharr, D.M., 2002. The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. Ann. Bot. 90, 631–636.
Purseglove, J.W., 1968. Tropical Crops Dicotyledons. Longmans, London, p. 332.
Quamar, M.F., Ali, S.N., Pandita, S.K., Singh, Y., 2017. Modern pollen rain from Udhampur (Jammu and Kashmir), India: insights into pollen production, dispersal, transport and preservation. Palynology 42, 1–11.
Rao, V.N.M., Hassan, M.V., 1957. Preliminary studies on the floral biology of cashew (*Anacardium occidentale* L.). Indian J. Agric. Sci. 27, 277–288.
Reddi, E.U.B., 1991. Pollinating agent of cashew-wind or insects? Indian Cashew J. 20, 13–18.
Reddi, E.U.B., 1993. Pollination studies of cashew in India: an overview. In: Veeresh, GK, Umashaankar, R, Ganeshaiah, KN (Eds.), Proceedings of the international symposium on pollen tropical. UAS, Bangalore, pp. 321–324.
Rejani, R., Rupa, T.R., Nayak, M.G., 2013. Suitability of cashew growing areas in India – an appraisal using GIS. J. Agrometeorol. 15 (Special Issue-II), 123–128.
Richardson, A.D., Keenan, T.F., Migliavacca, M., Ryu, Y., Sonnentag, O., Toomey, M., 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agric. For. Meteorol. 169, 156–173.
Roman, G., Tim, T., Patrick, L., Isabel, J., 2022. Expected global suitability of coffee, cashew and avocado due to climate change. PLOS ONE 17 (1), e0261976. https://doi.org/10.1371/journal.
Roubik, D.W., 1995. Pollination of Cultivated Plants in the Tropics, 118. FAO, Rome.
Rui, D., Zhongjie, L., Zhengyu, Y., Yanrong, W., Chao, C., 2020. Scanning electron microscopy observations of pollen morphology in common vetch (Vicia sativa) subspecies. J. Agric. Sci. 158, 646–659. https://doi.org/10.1017/S0021859620001033.
Rupa, T.R., Rejani, R., Bhat, M.G., 2010. Climate change effects on cashew and adaptation strategies. In: Proceedings of the National Dialogue on Climate Resilient Horticulture held on 28-29 January at IIHR. Bangalore, pp. 200–211.
Shivanna, K., Sawhney, V., 1995. Polyethylene glycol improves the in vitro growth of Brassica pollen tubes without loss in germination. J. Exp. Bot. 46, 1771–1774.
Shivashankara, K.S., Geetha, G.A., Roy, T.K., 2019. Metabolite profiling in Mango (Mangifera indica L.) pollen grains in relation to viability. J. Hortic. Sci. 14 (1), 33–42.
Sreekumar, K.M., Vasavan, N., Madhu, S;, Sijila, J., 2011. Managing tea mosquito bug (Helopeltis antonii Sign.) in cashew by augumenting red ants Oecophylla smaragdina (F.). J. Plant. Crops 1, 110–113.
Stern, R.A., Gazit, S., 1998. Pollen viability in lychee. J. Am. Soc. Hortic. Sci. 123, 41–46.
Subbaiah, C.C., 1984. A polyethylene glycol based medium for in vitro germination of cashew pollen. Can. J. Bot. 62, 2473–2475.
Sundararaju, D., 2011. Studies on extent of pollination and fruit set in cashew. J. Plant Crops 39 (1), 157–160.
Sukhvibul, N., Whiley, A.W., Vithanage, V., Smith, M.K., Doogan, V.J., Hetherington, S. E., 2000. Effect of temperature on pollen germination and pollen tube growth of four cultivars of mango (Mangifera indica L.). J. Hort. Sci. Biotechnol. 75 (2), 64–68.
Suonan, J., Aimee, T.C., Zhenhua, Z., Jin Sheng, H., 2017. Asymmetric winter warming advanced plant phenology to a greater extent than symmetric warming in an alpine meadow. Funct. Ecol. 31, 2147–2156.
Takkis, K., Tscheulin, T., Petanidou, T., 2018. Differential effects of climate warming on the nectar secretion of early- and late-flowering mediterranean plants. Front. Plant Sci. 9, 874.
Thakur, D.R., Thakar, S.S., 1970. Pollen morphology and germination in some temperate drupe plants. J. Palynology 6, 96–100.
Tweddle, J., Dickie, J., Baskin, C., Baskin, J, 2003. Ecological aspects of seed desiccation sensitivity. J. Ecol. 91, 294–304.
Vanitha, K., Raviprasad, T.N., 2019. Diversity, species richness and foraging behaviour of pollinators in cashew. Agric. Res. 8 (2), 197–206. https://doi.org/10.1007/s40003-018-0370-2.
Veeraraghavan, P.G., Pushpalatha, P.B., 1990. Rainfall and crop production in cashew. The Cashew 8–10.

Venkatesh Hubballi, N., 2021. Cashew development in India- challenges and opportunities. In: National Conference on Cashew held on 26-27 February at Panjim. Goa, pp. 9–14. http://dccd.gov.in.
Venkateswarlu, B., Rama Rao, C.A., 2010. Rainfed agriculture: challenges of climate change. Agric. Today Yearbook 43–45.
Westwood, M.N., Challice, J.S., 1978. Morphology and surface topography of pollen and anthers of Pyrus species. J. Am. Soc. Horticult. Sci. 103, 8–37.
Wunnachit, W., 1992. Floral structure and phenology of cashew in relation to yield. J. Hortic. Sci. 67 (6), 769–777.
Wunnaehit, W., Jenner, C., Sedgley, M., 1992. Pollen vigour and composition in relation to andromonoecy in cashew (Anacardium occidentale L.: Anacardiaceae). Sex Plant Reprod 5, 264–269.
Yadukumar, N., Raviprasad, T. N., Bhat, M.G., 2010. Effect of Climate Change on Yield and Insect Pests Incidence on Cashew. Challenges of Climate Change - Indian Horticulture. (Eds.) Singh, H.P., Singh, J.P. and Lal, S.S. Westville Publishing House, New Delhi pp. 224.
Zhangh, Q., Croes, A.F., 1982. A new medium for pollen germination in vitro. Acta Bot. Neerl. 31, 19.