



RESEARCH ARTICLE

# Pollen-based screening of coconut (*Cocos nucifera* L.) varieties for tolerance to high temperature and drought stress

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## Abstract

*Cocos nucifera* L. is a vital crop in South Asia, particularly in coastal areas vulnerable to climate change, with heat and drought stress significantly impacting its production. The reproductive stage of coconut is more sensitive to these stresses. Recent studies have employed *in vitro* pollen screening to assess heat tolerance. Osmotic adjustment (OA) has been recognized as a key factor in drought tolerance by maintaining turgor pressure. This study examined the effects of temperature on pollen germination (PG) and tube growth across eight coconut hybrids, with temperatures ranging from 15 to 50 °C. The hybrids showed significant differences in cardinal temperatures ( $T_{min}$ ,  $T_{opt}$ ,  $T_{max}$ ) for PG and tube growth. This work also assessed osmotic stress responses using polyethylene glycol (PEG) solutions in the presence or absence of an osmolyte potassium chloride (KCl), revealing differences in intrinsic OA and osmolyte-induced OA. The Chowghat Orange Dwarf (COD) x Andaman Ordinary Tall (ADOT) hybrid was found to be the most heat tolerant. These findings provide valuable insights into coconut hybrid tolerance to climate change, highlighting the potential for combining intrinsic and osmolyte-induced OA to enhance drought resistance.

**Keywords:** cardinal temperature; coconut hybrids; evaluation; osmotic adjustment; pollen

## Introduction

Global temperatures are projected to rise by 2 - 5 °C by the end of this century, resulting momentous changes in rainfall pattern, crop productivity and associated economic value (1). Coconut is a perennial plantation crop, mainly grown under rainfed conditions (2). Apart from the annual summer stress, prolonged period of dry weather accompanying with high temperatures can be expected with climate change, that adversely affect the coconut productivity (3). The reproductive development of coconut is more sensitive to climate change than vegetative processes. Genotypes which exhibit greater reproductive survivability under high temperature stress conditions during reproductive stage are advantageous (4). Therefore, screening based on reproductive traits and identification of high temperature and drought stress tolerant coconut cultivars are high priority research areas especially considering the changing climatic conditions (5).

Temperature has a significant influence on plant growth and development; mainly reproductive tissues were highly susceptible to temperature changes during the flowering process (6). Several reports have shown that temperature stress

often results in abortion of pollen and asynchronous pollen development (7) especially, it had significant effect on germination of pollen, pollen tube growth, fertilization, abscission of flower and fruit set (8). Thus, additional consideration should be given on the effect of temperature stress on pollen germination (PG). Besides, identification and evaluation of the response of pollen grain to extreme weather conditions is important for sustainable agriculture. Earlier studies highlighted that both high and low temperature stress adversely affect the pollen performance such as reduction of PG rate and pollen tube length (PTL) of different crops viz., almond (9), peanut (10) and coconut (5, 11). A negative impact of high temperature on viability of pollen was reported in crops including rice, wheat, sorghum, common bean, soybean, canola and groundnut (12).

Generally, higher percent of pollen viability was observed in tolerant cultivars under high temperature stress conditions than sensitive genotypes (13). Exploiting pollen viability as a rapid screening tool under high temperature conditions assist to identify the genotypes with more male gametophytic tolerance under high temperature stress (14). Additionally, since pollen is a haploid genetic material, its

genetics is simple compared to other factors used for screening stress tolerance and can be effectively used as a valuable trait to screen heat stress tolerance genotypes (15).

Drought stress is another major abiotic factor that severely affects crop productivity (16). Many potentially valuable mechanisms and characters have been defined for improving the performance of the plants under drought conditions (17, 18). Osmotic adjustment (OA) is one of the important traits, widely recognized as a major mechanism of drought resistance in crop plants. Osmotic adjustment capacity allows maintaining the cell turgor by accumulation of osmolytes even under increasing water stress, which enables in adaptation to water deficit stress (19). Osmotic adjustment expressed in all plant cells since it is a cellular mechanism and hence it is expressed in pollen also. This provides an opportunity to characterize the genotypes based on this trait (20, 21).

Thus, the study was carried out to screen the Dwarf x Tall coconut hybrids namely Chowghat Orange Dwarf (COD) x West Coast Tall (WCT), COD x Andaman Ordinary Tall (ADOT), COD x West African Tall (WAT), COD x Laccadive Ordinary Tall (LCT), Malayan Yellow Dwarf (MYD) x WCT, MYD x ADOT, MYD x WAT and MYD x LCT for drought and high temperature stress tolerance through *in vitro* screening techniques.

## Materials and Methods

### Plant material

Eight Dwarf x Tall (D x T) hybrids were used for the study viz., COD x WCT, COD x ADOT, COD x WAT, COD x LCT, MYD x WCT, MYD x ADOT, MYD x WAT and MYD x LCT. All the palms selected for this study were 25 years old.

### Experimental location

The palms used in the study are being maintained under experimental plot of ICAR-CPCRI (Indian Council of Agricultural Research-Central Plantation Crops Research Institute). The plot is located at 12° 18' N latitude and 75°E longitude. The altitude of the plot is 10.7 m above mean sea level (AMSL). During summer season, this region records a mean maximum temperature of 31.5 °C and minimum of 23.5 °C. The mean relative humidity is 88 % and the location receives approximately 3400 mm rainfall annually. The soil texture is sandy loam with a pH of 4.3–5.5.

### Pollen collection and growth medium

To test the response of hybrids to high temperature, spikelets (5 to 6 days after inflorescence opening) were collected at 8:30 a.m. from field-grown palms, placed in polythene bags and kept in an ice box to prevent desiccation of pollen. Male flowers were placed on a butter paper and pollen was collected by gently tapping with a nylon brush. Pollen germination medium was prepared which comprised of 8 % sucrose, 0.01 % boric acid and 1 % gelatin in 100 mL deionized water (22) which was solidified with 1 % agar. On a glass slide, 2 mL of germination media was spread and placed in Petri dishes lined with moist filter paper, to maintain the humidity to support germination and to avoid pollen rupture. This setup was kept in the biological oxygen demand (BOD) incubator with different temperatures (between 15 and 50 °C at 5 °C intervals) for 15 min for preconditioning. After the temperature equilibrated, pollens were uniformly sprinkled onto the germination media

and allowed for 2 hr incubation. Five slides per palm at each temperature were considered as replicates.

### Pollen germination (PG) and pollen tube growth under *in vitro*

Pollen germination and pollen tube growth were recorded after 2 hr of incubation in all the treatments. Pollen germination was observed under 10x magnification objective of a Leica compound microscope equipped with a Leica DFC 250 camera. Five microscopic fields per slide were photographed. Pollen grains were considered germinated when the PTL was equal to or exceeded the diameter of the pollen grain (10). After 2 hr of incubation, PTL was measured, as the pollen tubes reached the maximum length in 2 hr and began rupturing thereafter. The PTL was measured using the Leica QWin software and expressed in micrometer (µm). Pollen germination percentage was calculated using the formula:

$$\text{Percentage of pollen germination} = \frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Mean percentage PG and PTL were calculated by averaging the data obtained from all microscopic fields per slide and subsequently, from all slides for each temperature treatment per palm.

### Curve fitting procedures and determination of cardinal temperatures

Response of PG and PTL to the range of temperatures were recorded and using nonlinear curve-fitting procedures, cardinal temperatures—minimum ( $T_{\min}$ —temperature below which there was no PG), optimum ( $T_{\text{opt}}$ —temperature at which maximum PG was observed) and maximum ( $T_{\max}$ —temperature above which there was no PG) were calculated (10). Using a modified bilinear equation, the best-fitting curve was determined, which yielded the highest coefficient of determination ( $R^2$ ) and the lowest root mean square deviation (RMSD). PROC NLIN, a nonlinear regression procedure, was used to estimate the model parameters and determine  $T_{\text{opt}}$  using a modified Newton–Gauss iterative method. Based on  $T_{\text{opt}}$  and the parameters of the bilinear equation,  $T_{\min}$  and  $T_{\max}$  values were calculated using the derivative equations shown below:

$$\text{PG or PTL} = a + [b_1(T - T_{\text{opt}})] + b_2 [\text{ABS}(T_{\text{opt}} - T)] \quad (\text{Eqn. 1})$$

$$T_{\min} = [a + T_{\text{opt}}(b_2 - b_1)] / (b_2 - b_1) \quad (\text{Eqn. 2})$$

$$T_{\max} = [T_{\text{opt}}(b_1 + b_2) - a] / (b_1 + b_2) \quad (\text{Eqn. 3})$$

Where,  $a$ ,  $b_1$  and  $b_2$  are the equation constants,  $T$  is the actual temperature at which percentage PG and PTL were determined and  $T_{\text{opt}}$  is the optimum temperature for PG and PTL (23).

### Cluster analysis

Clustering was done using the parameters viz.,  $T_{\min}$ ,  $T_{\text{opt}}$ ,  $T_{\max}$ , estimated maximum percentage PG, estimated maximum pollen tube growth to group the coconut varieties into smaller number of clusters to study the similarities and dissimilarities among the coconut varieties using the MultiVariate Statistical Package (MVSP) software (24).

### Estimation of OA expressions induced in coconut pollen grains

Spikelets were collected from field in the morning (8.30 am). Male flowers were gently tapped to collect the pollen grains. For

exposing the pollen grains into osmotic stress, 40 % and 50 % PEG-6000 (polyethylene glycol) solutions were added into the cavity slides and pollen grains were sprinkled onto the solutions. To assess the influence of osmolytes, 40 % and 50 % PEG solutions were supplemented with 10 mM and 20 mM KCl. The solutions were placed in cavity slides and pollen grains were gently sprinkled onto them (25). The pollen grains were incubated for 24 hr under room temperature. Four cavity slides were prepared for each variety and each treatment. The projected cytoplasm area of the pollen grains was measured after 24 hr using a compound microscope with a projection screen at a magnification of 400x. The circumference of the pollen grains on the screen was recorded using inbuilt software (Leica QWin). For each treatment 20 pollen grains were randomly selected from each cavity to record observations. For control, pollen grains were sprinkled on the cavity slide containing distilled water and measured the pollen cytoplasm area immediately.

The ratios of projected pollen cytoplasm areas were calculated to quantify OA:

1. The ratios (B/A) of stress induced projected pollen cytoplasm area (mean of both 40 % and 50 % PEG without KCl) to initial cytoplasm area (non-stressed), as a measure of intrinsic OA.
2. The ratios (C/B) of stress induced projected pollen cytoplasm area under external osmolyte supply (mean of 40 % and 50 % PEG with addition of KCl) to stress induced projected pollen cytoplasm area (mean of 40 % and 50 % PEG without KCl), as a measure of induced OA.
3. The ratios (C/A) of stress induced projected pollen cytoplasm area under external osmolyte supply (mean of 40 % and 50 % PEG with addition of KCl) to initial cytoplasm area (non-stressed), as a measure of overall OA.

Where, A is the normal size of the pollen grains, B is the effect of osmotic stress on pollen grains and C is the response to osmolyte (25).

### Statistical analysis

For the screening of coconut varieties for temperature stress tolerance, PG and PTL were assessed *in vitro* using six plants per variety, with five slides prepared for each temperature treatment. Therefore, observations from 30 slides for each temperature of a variety were used for curve fitting to determine the cardinal temperatures. SAS software version 9.3 (26) was

used for curve fitting analysis and clustering. Comparison of coconut hybrids and temperature response for PG and pollen tube growth were analysed using two-way analysis of variance (ANOVA).

## Results and Discussion

### The effect of controlled temperatures in PG

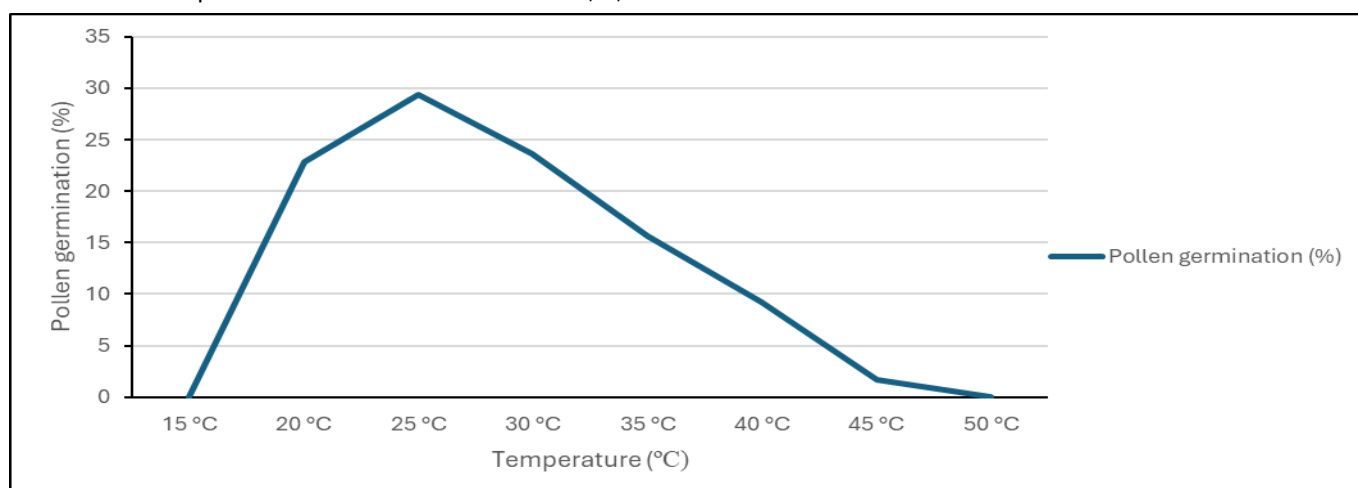
The coconut hybrids showed differences in PG and pollen tube growth at different temperature levels and among the different temperature ranges, the hybrids performed well at 20 to 30 °C. Temperature significantly influenced PG and PTL. Over the hybrids, maximum PG and PTL were observed at 25 °C and minimum at 50 °C on an average. A linear decrease in PG and PTL was observed for every above and below temperature change from this optimum temperature (25 °C) (Fig. 1 & 2).

A significant difference was observed between different coconut hybrids for PG and cardinal temperature (Table 1). Percentage of PG ranged from 21.8 % (MYD × ADOT) to 35.4 % (COD × ADOT) (Table 1, Fig. 3). Cardinal temperatures differed greatly among cultivars. Values of  $T_{min}$  ranged from 14.4 °C (COD × WAT and COD × LCT) to 16.6 °C (MYD × ADOT) with an average of 15 °C. Optimum temperature ( $T_{opt}$ ) ranged from 19.81 °C for MYD × LCT to 28.02 °C for COD × WAT with an average  $T_{opt}$  of 23.07 °C. The  $T_{max}$  values ranged from 46.11 °C for MYD × ADOT to 50.56 °C for COD × ADOT with an average  $T_{max}$  of 48.28 °C.

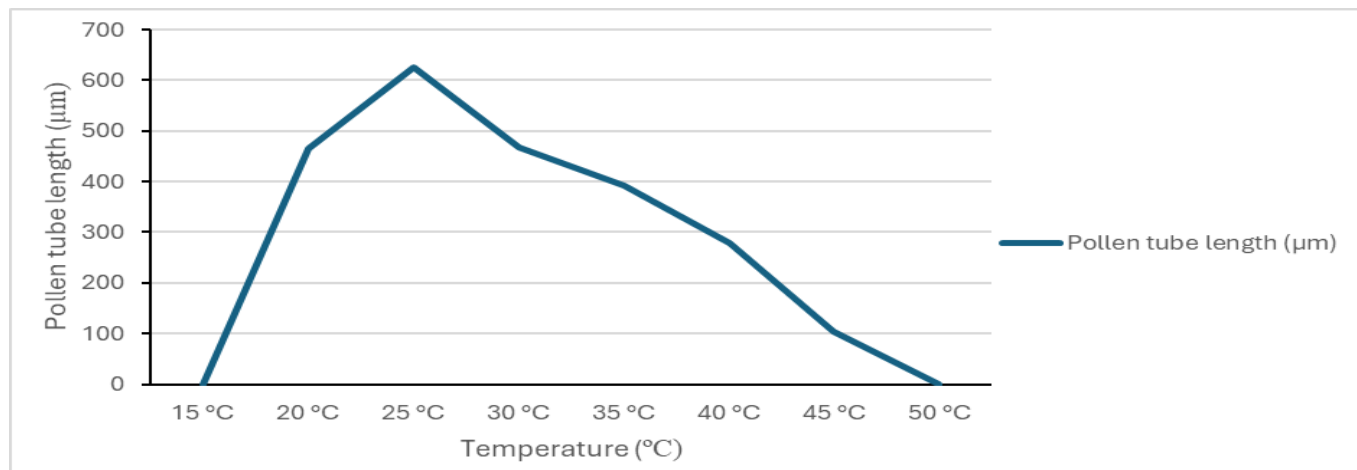
The eight coconut hybrids differed significantly with regard to pollen tube growth. The PTL ranged from 465.3 µm (MYD × ADOT) to 701.8 µm (COD × WCT) with a mean of 618.4 µm (Table 2). The average  $T_{min}$ ,  $T_{opt}$ ,  $T_{max}$  for pollen tube growth were 15.2 °C, 22.9 °C and 50.09 °C respectively.

### Clustering of genotypes

A dendrogram obtained based on variables like  $T_{min}$ ,  $T_{opt}$  and  $T_{max}$  for PG, estimated maximum PG and estimated maximum pollen tube growth showed similarities among the genotypes and accordingly genotypes under study were grouped into three clusters. The first cluster includes, MYD × LCT and MYD × WAT. The second cluster includes COD × ADOT, MYD × WCT and COD × LCT and the third cluster consisted of WCT, MYD × ADOT, COD × WCT and COD × WAT (Fig. 4). The optimum temperature for PG was highest for the hybrids under cluster II. The estimated maximum value for PG was highest (35.4 %) for cluster II.



**Fig. 1.** Mean percent of PG at different temperatures, averaged over hybrids.

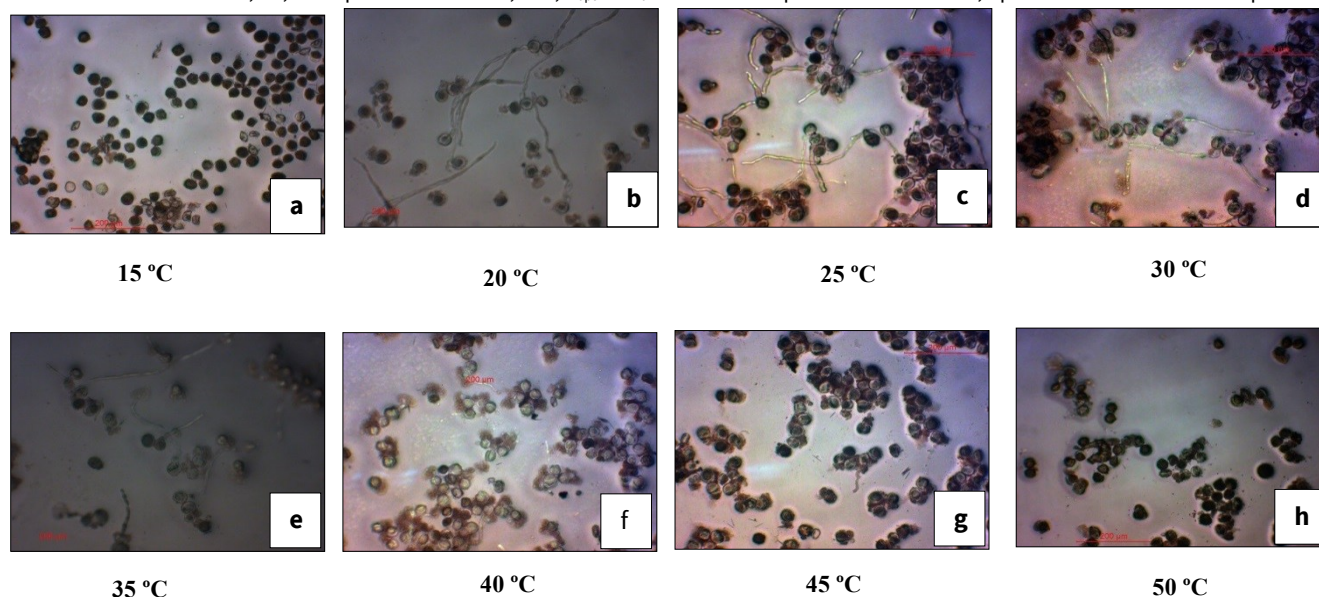


**Fig. 2.** Mean PTL at different temperatures, averaged over hybrids.

**Table 1.** Maximum PG percentage, modified bilinear equation constants and cardinal temperatures for PG of coconut hybrids in response to temperature

Hybrids	Maximum PG percentage	Equation constants				Cardinal temperatures (°C)		
		a	b1	b2	R <sup>2</sup>	T <sub>min</sub>	T <sub>opt</sub>	T <sub>max</sub>
COD × WCT	27.7±4.4	32.28	1.99	-3.21	0.91	15.0	21.21	47.66
COD × WAT	31.86±4.8	35.51	0.38	-2.22	0.94	14.4	28.02	47.33
COD × LCT	27.8±4.2	32.55	0.54	-2.06	0.92	14.4	26.88	48.29
COD × ADOT	35.4±4.6	37.69	2.36	-3.64	0.99	15.0	21.28	50.56
MYD × WAT	26±4.2	29.03	1.83	-2.97	0.87	15.0	21.05	46.33
MYD × WCT	32±5.4	39.39	2.90	-4.30	0.92	15.0	20.47	48.45
MYD × LCT	28±5.0	34.64	2.96	-4.24	0.97	15.0	19.81	46.90
MYD × ADOT	21.8±3.9	24.50	0.79	-1.82	0.75	16.6	25.00	46.11
WCT	27±3.7	28.53	1.47	-2.50	0.91	15.0	22.00	49.03
<b>Mean</b>	<b>28.6</b>					<b>15.0</b>	<b>23.07</b>	<b>48.28</b>

a: estimated maximum PG; b1, b2: equation constants; T<sub>min</sub>, T<sub>opt</sub> & T<sub>max</sub>: cardinal temperatures: minimum, optimum and maximum respectively.



**Fig. 3.** Response of pollen grains under *in vitro* conditions.

**Table 2.** Maximum PTL, modified bilinear equation constants and cardinal temperatures for PTL of coconut hybrids in response to temperature

Hybrids	Maximum PTL (µm)	Equation constants				Cardinal temperatures (°C)		
		a	b1	b2	R <sup>2</sup>	T <sub>min</sub>	T <sub>opt</sub>	T <sub>max</sub>
COD × WCT	701.8±89.7	772.11	40.25	-65.99	0.88	15.0	22.3	52.26
COD × WAT	601.5±84.7	683.79	32.80	-58.56	0.94	15.0	22.5	49.02
COD × LCT	594.7±81.7	659.37	32.21	-56.99	0.91	15.0	22.4	49.00
COD × ADOT	681.0±90.3	745.62	31.41	-58.84	0.96	15.0	23.3	50.44
MYD × WAT	646.02±90.6	699.91	52.61	-76.49	0.80	15.0	20.4	49.73
MYD × WCT	606.9±82.6	685.40	32.55	-68.56	0.98	15.0	20.9	51.88
MYD × LCT	651.2±92.9	756.32	39.00	-66.76	0.93	15.0	22.2	49.40
MYD × ADOT	465.3±62.1	589.1	13.28	-33.24	0.66	16.7	26.9	51.06
WCT	617.1±91.2	713.02	41.54	-68.37	0.96	15.0	21.5	48.06
<b>Mean</b>	<b>618.4</b>					<b>15.2</b>	<b>22.9</b>	<b>50.09</b>

a: estimated maximum PTL; b1, b2: equation constant; T<sub>min</sub>, T<sub>opt</sub> & T<sub>max</sub>: cardinal temperatures: minimum, optimum and maximum respectively.

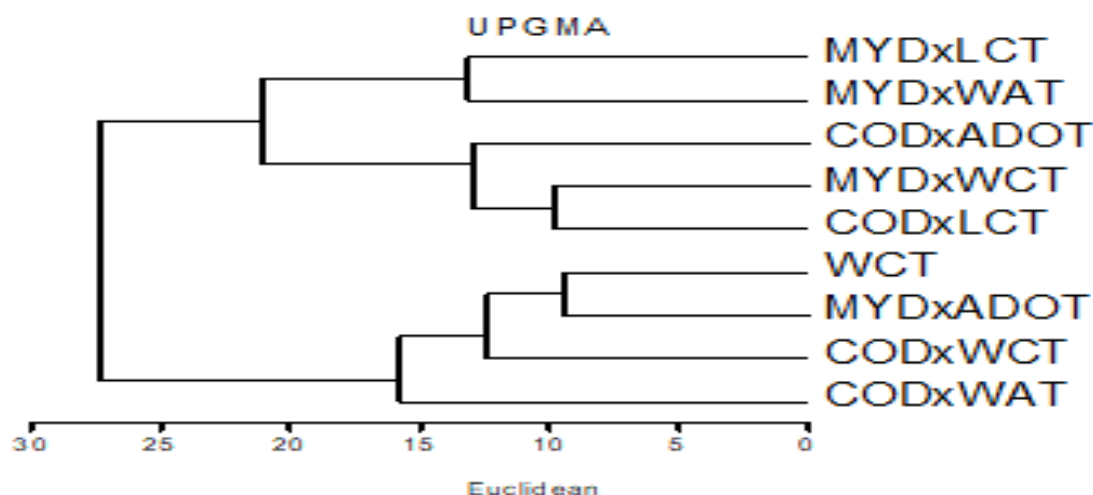


### Osmotic adjustment in pollen grains

In the present study coconut hybrids had different responses to the applied osmotic stress treatments. Each pollen grain cell was measured on the slide. The pollen grain responses to different osmotic stresses were different among coconut hybrids. Projected area of pollen cytoplasm of coconut hybrids decreased after immersion in PEG solutions and greatly increased after addition of KCl in the PEG solutions (Fig. 5 & 6). The intrinsic OA value (B/A) ranged from 0.957 to 1.177 with a

Coconut hybrids had clear temperature optima for PG and PTL, above and below which there is a linear decrease in PG and PTL in every change in temperature. This response of pollen was well defined by a bilinear regression model. Similar responses of pollen to different temperature ranges have been observed in coconut (5, 11), groundnut and cotton (10, 27), capsicum (28), pepper (29) and sorghum (30).

The mean PG observed was 28.6 %, which is comparable to earlier reports of 23 % (5), 40 % (31) and 48 % (11). Pollen tube



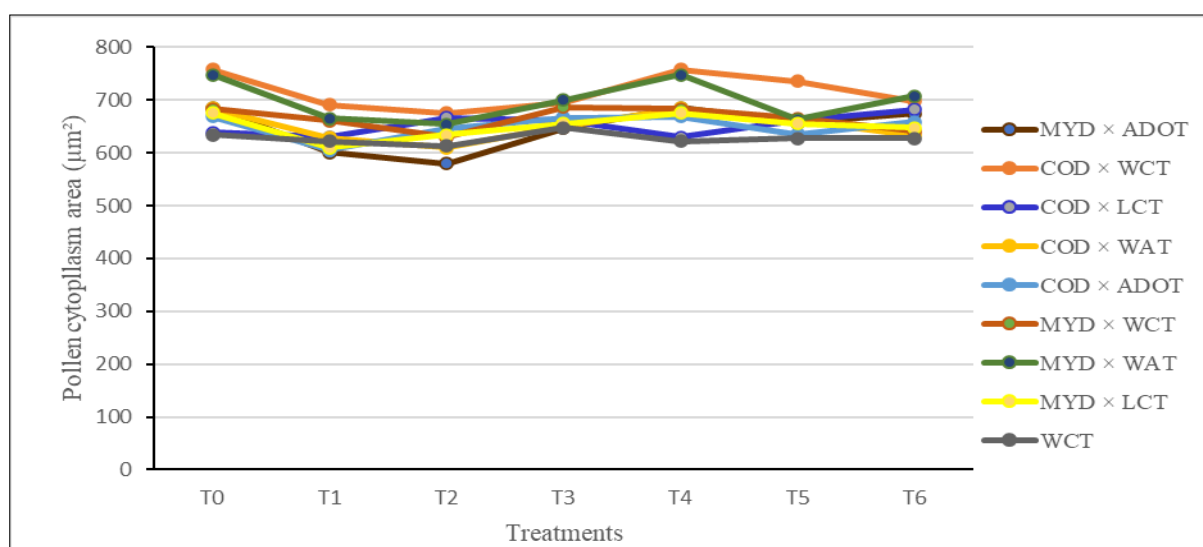
**Fig. 4.** Clustering of coconut genotypes studied for PG at different temperatures.

mean value of 1.067. Higher intrinsic OA value recorded with MYD x LCT. The same hybrid recorded higher total OA value. The induced OA value ranged from 1.010 (COD x LCT) to 1.127 (MYD x ADOT) with an average of 1.053 (Table 3).

Temperature stress is one of the key abiotic stresses which disturb the plant reproduction process and thereby affecting the fruit set (13). In the present study, response of pollen grains of different coconut varieties to a range of temperature from 15 to 50 °C at an interval of 5 °C was observed and the variations among the varieties for cardinal temperatures were evaluated.

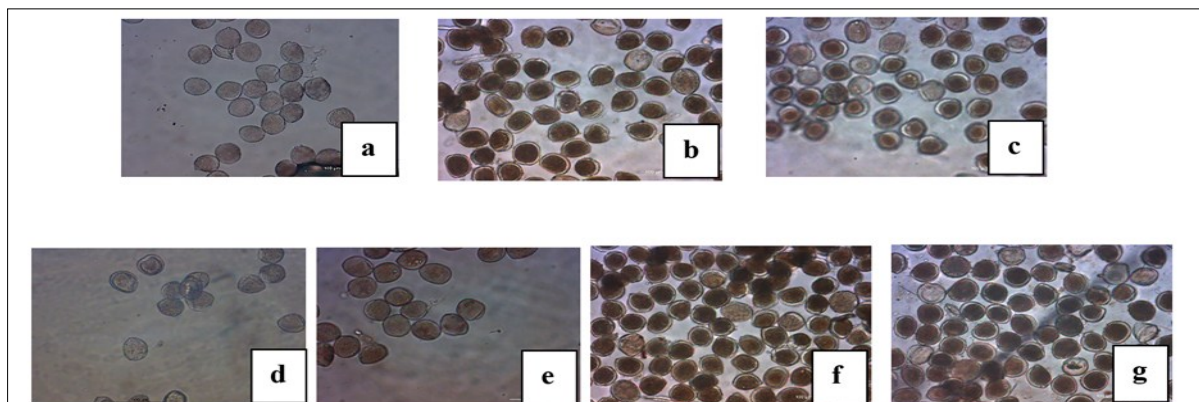
started to grow within a few min after germination and maximum growth was observed after two hr of incubation. After that it started rupturing. The mean PTL observed in this study was 618.4 µm which is similar to previously reported values (5, 30, 31) when pollen was grown on artificial media. It indicated that the recorded differences in PG and PTL in the current study reflected variability among the hybrids.

COD x ADOT and MYD x WCT performed better in terms of PG under  $T_{max}$  than  $T_{opt}$ . Thus, among the evaluated varieties, COD x ADOT and MYD x WCT are more adaptable to high temperature stress.



**Fig. 5.** Cytoplasmic area of pollen grains subjected to osmotic stress, with or without the addition of KCl.

T0 - Control; T1 - Pollen grains 40 % PEG; T2 - Pollen grains 50 % PEG; T3 - Pollen grains 40 % PEG with 10 mM KCl; T4 - Pollen grains 40 % PEG with 20 mM KCl; T5 - Pollen grains 50 % PEG with 10 mM KCl; T6 - Pollen grains 50 % PEG with 20 mM KCl).



**Fig. 6.** Effect of osmotic stress and osmolytes on the size and shape of pollen grains (scale bar = 100  $\mu$ m).

a- Control; b- Pollen grains 40 % PEG; c- Pollen grains 50 % PEG; d- Pollen grains 40 % PEG with 10 mM KCl; e- Pollen grains 40 % PEG with 20 mM KCl; f- Pollen grains 50 % PEG with 10 mM KCl; g- Pollen grains 50 % PEG with 20 mM KCl .

**Table 3.** Variation among varieties in intrinsic, osmolyte induced and total OA as expressed in pollen grains

Hybrids	Intrinsic OA (B/A)			Induced OA (C/B)			Total OA (C/A)		
COD $\times$ WCT	1.051			1.055			1.109		
COD $\times$ WAT	1.013			1.061			1.074		
COD $\times$ LCT	1.061			1.010			1.072		
COD $\times$ ADOT	0.957			1.052			1.006		
MYD $\times$ WAT	1.122			1.068			1.198		
MYD $\times$ WCT	1.056			1.037			1.095		
MYD $\times$ LCT	1.177			1.058			1.245		
MYD $\times$ ADOT	1.073			1.127			1.210		
WCT	1.098			1.013			1.112		
Mean	1.067			1.053			1.125		
CD (0.05 %)	V	T	V $\times$ T	V	T	V $\times$ T	V	T	V $\times$ T
	0.010	0.011	0.032	0.008	0.009	0.026	0.01	0.11	0.33

A: normal size of the pollen grains; B: effect of osmotic stress on pollen grains; C: response to osmolyte

COD  $\times$  ADOT recorded higher  $T_{max}$  value (50.56  $^{\circ}$ C) for PG.  $T_{max}$  or lethal temperature for PG was chief parameter which describes the genotypic tolerance against high temperature stress. It was reported in many crops that, under high temperature stress conditions, the genotype which showed higher  $T_{max}$  value for PG recorded maximum fruit and seed set percentage (26, 30). The  $T_{max}$  value for PG and the capability of pollen to germinate and grow well at supra optimal temperatures were the key parameters for identifying high temperature-tolerant coconut varieties (5). Different coconut genotypes and hybrids viz., WCT, LCT, COD, Gangabondam Green Dwarf (GBGD), Federated Malay States Tall (FMST), COD  $\times$  WCT and MYD  $\times$  WCT recorded high  $T_{max}$  both for PG and viability and all these genotypes except COD are considered to be abiotic stress tolerant genotypes (32). In the present study, COD  $\times$  ADOT had a higher PG of about 35.4 % and higher lethal temperature ( $T_{max}$ ) of 50.56 %.

MYD  $\times$  ADOT recorded higher  $T_{opt}$  for PG followed by COD  $\times$  ADOT. The widest temperature range was calculated for COD  $\times$  WAT ( $T_{max} - T_{min} = 37.26$   $^{\circ}$ C) followed by MYD  $\times$  WCT (36.88  $^{\circ}$ C) and COD  $\times$  ADOT (35.44  $^{\circ}$ C), indicating wider tolerance to temperature changes for pollen tube development.

In the present study, among the hybrids, COD  $\times$  ADOT had an average PG of about 35.4 %, higher  $T_{max}$  for PG of 50.56  $^{\circ}$ C, widest temperature range ( $T_{max} - T_{min}$ ) for PG of 35.56  $^{\circ}$ C and the second highest pollen tube growth rate.

In general, high temperature is more sensitive to PG than pollen tube growth. If the matured pollen grains shed on the receptive stigma, it starts to germinate within 30 min (5). If the environmental temperatures are not conducive for PG

(temperature is higher than  $T_{max}$  or lower than  $T_{min}$ ), the pollen grains may not germinate. However, pollen tube growth is happening inside the female flower. In coconut, female flower is covered with a thick outer skin and inside there is a fibrous layer, hence pollen tube growth which is happening inside the style, is less sensitive to high temperature as compared to the PG (33). But there is an indirect effect of high temperature such as there is a lack of carbohydrate content in the style of the female flower that may affect the pollen tube growth (34). Hence the lethal temperature or  $T_{max}$  for PG and  $T_{opt}$  for pollen tube growth had a greater role in describing the genotypic tolerance against high temperature stress.

The estimated  $T_{opt}$ ,  $T_{min}$  and  $T_{max}$  values for PG and PTL were used for cluster analysis, which grouped the genotypes into three distinct clusters. The varieties under Cluster II had high  $T_{max}$ , it indicates that those varieties have wider adaptability (COD  $\times$  ADOT and MYD  $\times$  WCT) and these hybrids also recorded high PG. Varieties under Cluster III is relatively tolerant to  $T_{max}$  (COD  $\times$  LCT, COD  $\times$  WCT, COD  $\times$  WAT and MYD  $\times$  LCT) and these varieties were moderately adaptable to high temperature. On the other hand, varieties under Cluster I recorded low  $T_{max}$  and low germination, indicated that those varieties exhibited low performance under high temperature compared to the other hybrids.

The improvement of high temperature stress tolerance is difficult due to its low heritability (35), as it is strongly influenced by environmental factors such as humidity and other associated variables (36). Different parameters can be studied to determine the high temperature tolerance of a plant. However, fruit set is the decisive factor for high temperature tolerance trait

of a genotype and highly correlated with pollen viability trait in plants (37). Under high temperature conditions, tolerant genotypes showed greater pollen viability compared to sensitive genotypes (14). Thus, male gametophytic stress tolerant genotypes can be identified by screening the genotypes based on its pollen viability under high temperature. Focusing on one of the key aspects of the heat tolerance mechanism-pollen screening-can provide valuable insights, as pollen is a haploid structure and its genetic analysis is relatively simple compared to complex traits such as fruit production and yield, which are influenced by multiple factors including flower formation, tolerance of male and female reproductive phases, fruit set and development (38).

Selection can be made at male gametophytic level and this can be used as an effective screening tool in plant breeding programmes by studying the association between sporophytic and gametophytic responses to biotic and abiotic stress tolerance (39). Drought tolerance is a highly complex trait. The results of earlier studies aimed at developing selection criteria for drought tolerance were not promising; consequently, progress in breeding for drought tolerance has been limited (40). Male gametophytic selection (MGS), or pollen selection, offers a promising alternative approach under such complex conditions.

The present study indicated that PEG, an osmotic agent, influenced PG under *in vitro* conditions. Osmotic adjustment is widely documented as one of the key mechanisms of drought tolerance in crop plants. Osmotic adjustment is realized under water stress conditions by decreasing the osmotic potential as a result of accumulation of organic and inorganic osmolytes (41). The term OA is used when new solutes accumulate, rather than when the concentration of existing solutes increases under water stress conditions (42). To maintain cell turgor and protect cellular functions, plants accumulate solutes within their cells and this process is referred to as OA (43). Sugars, sugar alcohols, amino acids and other low molecular weight substances are the compatible solutes that reduce the cytosol osmotic potential (44).

In the present study, the pollen grain size of all the studied coconut varieties was reduced when treated with 40 % and 50 % PEG solutions under *in vitro* conditions. The higher intrinsic OA was observed in pollen grains of MYD x LCT. The intrinsic OA maintain the turgor pressure of the cell and hence the size of the pollen grain. It leads to sustain the biochemical and physiological activities of the cell under water stress conditions. In earlier studies, the correlation between the intrinsic OA in pollen and response of the plant to tolerate water stress has reported in wheat (45) and sorghum (46).

The addition of the osmolyte KCl to the PEG medium resulted in an increase in pollen size compared to the treatment with PEG alone. The pollen grain size increased with the supplementation of 10 mM and 20 mM KCl over PEG alone. This might be because of the upregulation of related genes and increase of osmolyte under water stress condition to protect the pollen grains through supplemented osmolytes. The pollen grains responded to osmolyte K<sup>+</sup> and coconut hybrids regained their original size indicating operation of induced OA in these hybrids. Higher induced OA was observed with MYD x ADOT.

Genetic diversity for OA has been reported in several crops, including sunflower (47), chickpea (48), barley (49), wheat

(50), sorghum (25) and maize (51). In wheat, the OA capacity was reported as inherited trait and controlled by alternative alleles at a single locus (52). Osmotic adjustment is recognized in all plant cells, including pollen grains and up to 70 % of sporophyte genes are expressed in gametophyte level. Hence valuable genotypes can be selected based on gametophyte level. The selection based on gametophyte level are reported in breeding programs for many crop species (53). In the present study, we assessed the diversity of coconut hybrids for tolerance to drought, based on the response of pollen grains to induced osmotic stress under *in vitro* condition and observed the hybrids MYD x LCT had higher intrinsic OA and total OA whereas MYD x ADOT had higher induced OA. Earlier studies supported that there is association between pollen responses in the contrasting genotypes to moisture stress with sporophyte or plant response (24, 42, 45).

## Conclusion

Pollen germination and pollen tube growth of eight hybrids under *in vitro* condition were significantly varied with different temperature ranges. The response of pollen to temperature was best described by modified bilinear model. Among the cardinal temperatures,  $T_{max}$  for PG and  $T_{opt}$  for PTL are important parameters to decide the reproductive success during the high temperature stress condition. Hybrids exhibiting higher  $T_{max}$  values for PG and higher  $T_{opt}$  values for PTL were considered tolerant to high temperature stress. Based on cardinal temperatures for PG and PTL, COD x ADOT and MYD x WCT were tolerant to high temperature stress. The level of drought tolerance in coconut varieties could be identified by a rapid screening method originally developed for wheat. There are significant differences among the coconut hybrids in both intrinsic and induced OA. No correlation was observed between intrinsic and osmolyte induced OA indicating that these are governed by different genes. Thus, combining both mechanisms in a single background might help to improve degree of drought resistance in coconut varieties. Future work could focus on identifying the specific genes controlling intrinsic and osmolyte-induced OA to better understand their separate contributions to drought tolerance in coconut. Additionally, validating the performance of the tolerant hybrids under field conditions will help confirm their adaptability and stability under real heat and drought stress.

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## Authors' contributions

RS, VN and KS conceptualized and designed the study. RS, MN and AV conducted the experiments. MRA, VS and RS were responsible for collecting the scanning electron microscopy data. KPC performed the data analysis. RS drafted the original manuscript and MN and VN critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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