SCREENING OF COCONUT VARIETIES FOR TOLERANCE TO HIGH TEMPERATURE STRESS USING POLLEN VIABILITY TEST

By

HELEN MARY ROSE

(2011-20-108)

THESIS

Submitted in partial fulfillment of the requirement

for the degree of

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Faculty of Agriculture

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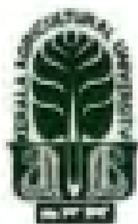

ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2016

DECLARATION

I hereby declare that the thesis entitled **"Screening of coconut varieties for tolerance to high temperature stress using pollen viability test"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
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Helen Mary Rose
(2011-20-108)

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Vellanikkara:

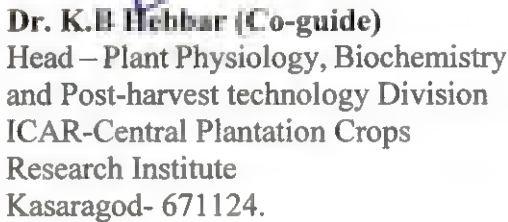
Date: 23.11.2016

Dr. S. Anitha
Associate Professor (Agronomy),
Water Management Research Unit
Thrissur - 680656

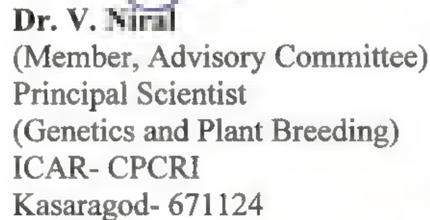
CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Helen Mary Rose, a candidate for the degree of **B.Sc. – M.Sc. (Integrated) Climate Change Adaptation** agree that the thesis entitled "Screening of coconut varieties for tolerance to high temperature stress using pollen viability test" may be submitted by Ms. Helen Mary Rose in partial fulfilment of the requirement for the degree.

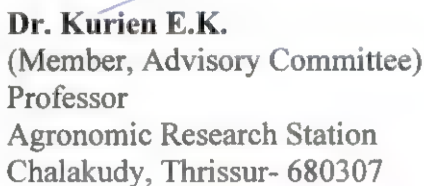
Dr. S. Anitha
(Chairman, Advisory Committee)
Professor (Agronomy)
Water Management Research Unit
Vellanikkara- 680656



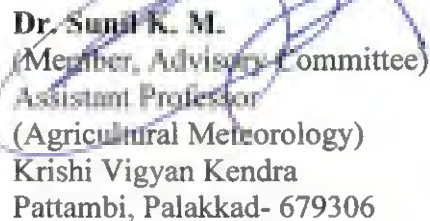
Dr. K.B Hebbar (Co-guide)
Head – Plant Physiology, Biochemistry and Post-harvest technology Division
ICAR-Central Plantation Crops Research Institute
Kasaragod- 671124.



Dr. V. Niral
(Member, Advisory Committee)
Principal Scientist
(Genetics and Plant Breeding)
ICAR- CPCRI
Kasaragod- 671124

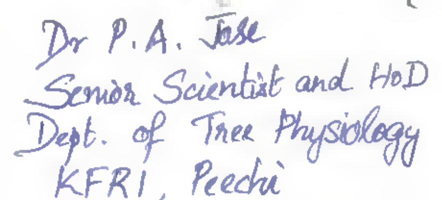


Dr. Kurien E.K.
(Member, Advisory Committee)
Professor
Agronomic Research Station
Chalakudy, Thrissur- 680307



Dr. Sunil K. M.
(Member, Advisory Committee)
Assistant Professor
(Agricultural Meteorology)
Krishi Vigyan Kendra
Pattambi, Palakkad- 679306

EXTERNAL EXAMINER



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5

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Helen Mary Rose

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Dedicated to my parents

8

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	LIST OF TABLES	ix
	LIST OF FIGURES	x
	ABBREVIATIONS	xii
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	3
3	MATERIALS AND METHODS	16
4	RESULTS AND DISCUSSION	44
5	SUMMARY	78
	REFERENCES	79
	ABSTRACT	89
	APPENDIX	90

LIST OF TABLES

Table no.	Title	Page no.
1	Genotypes selected for the study	20
2	Media tested for pollen germination	26
3	Percentage pollen germination of coconut genotypes across different temperatures	54
4	Mean pollen tube length of coconut genotypes across different temperatures	61
5	Optimum temperatures for pollen germination and the corresponding percentage pollen germination of coconut genotypes	65
6	Optimum temperatures for pollen tube length and the corresponding pollen tube length of coconut genotypes	66
7	SOD, POD, MDA total phenol, ECW and chlorophyll content of leaf samples of four genotypes	70

10

LIST OF FIGURES

Figure no.	Title	Page no.
1	Weekly maximum and minimum temperature for the experimental period from October 2015 - July 2016.	17
2	Weekly Rainfall for the experimental period from October 2015 - July 2016	17
3	Weekly sunshine hours for the experimental period from October 2015 – July 2016	18
4	Weekly evaporation for the experimental period from October 2015 - July 2016	18
5	Coconut inflorescence, 2 days after spathe opening	22
6	Counting the number of open flowers	22
7	Dusting the open flowers to collect pollen	23
8	Removal of open flowers	23
9	Pollen collected in a butter paper	27
10	Pollen germination medium	27
11	Pouring the medium on to slides	28
12	Solidified germination medium on the slide	28
13	Dusting the collected pollen on the slide	28
14	Slides dusted with pollen	29
15	Petriplates lined with filter paper	29
16	Moistening the filter paper	30
17	Slides kept in the petriplate	30
18	Petriplates kept in B.O.D. incubator at preset temperature	35
19	Petriplates left to incubate for 2 hours	35

Figure no.	Title	Page no.
20	Number of open flowers collected at 6.30, 8.30 and 10.30am. for a period of 10 days from the day of spathe opening in genotypes (a) MYD (b) COD and (c) COD x WCT	45
21	Pollen germination percentage of flowers collected at 8.30 and 10.30am. from the day of spathe opening to a period of 10 days in (a) MYD, (b) COD and (c) COD x WCT.	46
22	Percentage germination in pollen of WCT and COD collected at 6.30, 8.30 and 10.30am.	49
23	Percentage germination of COD and WCT pollen in various germination media	49
24	Percentage pollen germination of COD and WCT at various temperatures	51
25	Mean percentage pollen germination of various genotypes averaged over the studied temperature range	53
26	Percentage pollen germination at different temperatures averaged over all genotypes	53
27	Percentage pollen germination of different genotypes at (a) 20°C, (b) 25°C (c) 30°C	55
28	Percentage pollen germination of different genotypes at (a) 35°C (b) 40°C	56
29	Mean pollen tube length of various genotypes averaged over the studied temperature range (20°C - 40°C).	59
30	Mean pollen tube length at different temperatures, averaged over genotypes.	59
31	Percentage pollen germination at different temperatures, averaged over (a) tall, (b) dwarf and (c) hybrid genotypes	63

Figure no.	Title	Page no.
32	Percentage reduction in pollen germination of different genotypes from optimum temperature to 20°C and 40°C	68
33	Percentage reduction in pollen tube length of different genotypes from optimum temperature to 20°C and 40°C	68

ABBREVIATIONS

IPCC	Intergovernmental Panel on Climate Change
ppm	Parts per million
μm	Micrometer
μM	Micromolar
mM	Millimolar
ICAR	Indian Council of Agricultural Research
CPCRI	Central Plantation Crops Research Institute
WCT	West Coast Tall
SNRT	San Ramon Tall
FMST	Federated Malay States Tall
LMT	Lakshadweep Micro Tall
SPI	West Coast Tall Spicata
COD	Chowghat Orange Dwarf
MYD	Malayan Yellow Dwarf
CRD	Cameroon Red Dwarf
CGD	Chowghat Green Dwarf
B.O.D.	Biochemical Oxygen Demand
T_{opt}	Optimum Temperature
PVPP	Polyvinyl Pyrrolidone
SOD	Superoxide dismutase
POD	Peroxidase
MDA	Malone Dialdehyde
ECW	Epicuticular Wax
EDTA	Ethylene Diamine Tetraacetate
%PG	Percentage pollen germination
PTL	Pollen Tube Length
Wm^{-2}	Watts per metre square

14

CHAPTER 1

INTRODUCTION

Climate change essentially refers to an unprecedented change in the mean values of the various weather parameters or their variability from what has been observed over the years. The causes of climate change can be natural or anthropogenic in nature. The current accelerated climate change is attributed mainly to anthropogenic emission of greenhouse gases which enhances the natural greenhouse effect. As a consequence of this, temperature and precipitation patterns have already started changing from the normal. Most of the climate models employed to predict future climate agree on the high probability of an increase in atmospheric temperatures in the upcoming years. Needless to say, the phenomenon is going to have a profound impact on all living systems. Plants, being sedentary, and mostly adapted to a narrow range of environmental conditions, will have to experience the repercussions of a changing climate with maximum severity. Since completely mitigating the effects of already emitted greenhouse gases is not feasible in the short term, adaptation to a warmer climate is a necessity.

The threat of climate change is projected to be more in the coastal tract and hilly areas of India where plantation crops like coconut is the predominant crop which provides sustenance to more than 10 million people and contributes Rs.83000 million annually to the Gross Domestic Product (GDP) of the country. Coconut is grown between 20°N and 20°S latitude. It can be grown even at 26°N latitude but the temperature is the main limitation. The optimum weather conditions for good growth and nut yield in coconut are well distributed annual rainfall between 130 and 230 cm, mean annual temperature of 27°C, abundant sunlight ranging from 250 to 350 Wm^{-2} with at least 120 hours per month of sun shine period. Since, it is humid tropical crop it grows well above 60% humidity (Child 1974, Murray 1977). Climate change will affect coconut plantation through higher temperatures, elevated CO_2 concentration, precipitation changes, increased weeds, pests, and disease pressure.

Since coconut is a perennial crop, it has to cope with the impact of climate change during its life cycle. Prolonged periods of temperatures above the maximum tolerable limit can hamper vegetative growth as well as reproductive development and consequently the yield. Pollen in plants play the essential role of transporting the male gamete to the ovary in order to effect fertilization. In cross pollinated plants, pollen have to remain detached from the plant, i.e. in the open environment for a long time. Here, it is exposed to various kinds of stress resulting from fluctuating weather variables. Pollen of plants are sensitive to minor fluctuations in the atmospheric variables, especially temperature and can be used as an effective specimen to study whole plant stress tolerance. Also, pollen find use in breeding programmes and maintenance of gene bank of crops. So it is important to know about its viability under the scenario of global environmental change. Many studies showed that pollen development during various phases of microsporogenesis was sensitive to high temperature stress (Salem *et al.* 2007, Prasad *et al.* 2008, Jain *et al.* 2010). In many field crops high temperature tolerant genotypes were selected through *in-vitro* pollen germination studies (Kakani *et al.*, 2002; 2005). In this study the effect of high temperature on pollen germination and pollen tube length of coconut genotypes was undertaken with the following objectives.

The objectives of the current study were

i) to assess the impact of high temperature stress on the germination and viability of pollen in coconut genotypes

ii) To screen the selected coconut accessions for tolerance to high temperature stress.

CHAPTER 2
REVIEW OF LITERATURE

2.1 CLIMATE CHANGE

According to the IPCC (2013) Fifth Assessment Report, the global mean air temperatures are expected to rise by 0.3-0.7°C by 2035 and up to 4.8°C by the end of the century. This will surely have an impact on the crop production systems worldwide. High maximum and minimum temperatures will lead to a reduction in canopy cover and yield of many crops. Coconut, being a C_3 plant, will be benefited by the increase in the concentration of carbon dioxide in the atmosphere (Naresh Kumar *et al.*, 2008) but the concurrent increase in temperature and the occurrence of frequent dry spells will have a detrimental effect on the production of inflorescences (Peiris and Kularatne, 2008). Minimum temperatures less than 10°C for 1 month caused nut fall and temperatures above 40°C in the summer caused a reduction in leaf area as well as nut yield (Naresh Kumar *et al.*, 2008). Studies using the Infocrop- Coconut Model have suggested that an increase in rainfall along the west coast of India and consequent increase in coconut production, while the production in high productivity areas like Tamil Nadu and Andhra Pradesh are predicted to reduce considerably, by which the economy will be hard – hit (Naresh Kumar *et al.*, 2013).

2.2 COCONUT

The coconut palm (*Cocos nucifera* L.) is a monocotyledonous tree crop. Coconut belongs to the Arecaceae family and is the only species in the genus *Cocos*. Coconut prefers a mean temperature of 27°C with a diurnal variation of 6 to 7°C and a relative humidity more than 60%.

There are mainly two types of varieties based on the stature or height, namely, the talls (Typica) and dwarfs (Nana). Talls grow to a height of 20-30m. They have a longer juvenile phase. The initiation of the reproductive phase or first

flowering usually occurs at 5-12 years of age (Patel, 1938). They have an economic life of 60-70 years.

Dwarfs, as the name suggests, are of short stature and grow up to a height of 8-10m. They flower after 3-5 years of growth and have a lower productive life of 30-40 years. Jack and Sands (1929) studied the flowering of Malaysian Dwarf varieties and reported that Dwarf Yellow started flowering earliest and Dwarf Green last. Dwarf Orange flowered in between the former two.

2.3 FLORAL BIOLOGY

Usually, in a mature palm, one leaf is produced per month. After the initiation of flowering, roughly one inflorescence opens every month. Trees which produce an inflorescence with every successive leaf are called regular bearers and the ones that do not are called alternate bearers. The absence of an inflorescence may indicate abortion of the inflorescence. Environmental factors and moisture availability during the development of the inflorescence are very important factors which determine the rate of abortion of inflorescences. In a study conducted in Kasaragod, Menon and Pandalai (1958) found that maximum abortion of inflorescences occurred in the months from June to October. This was attributed to the drought to which the plant was exposed during the development of inflorescence primordium, 16 months prior to the opening of the inflorescence. The coconut inflorescence is called spadix. It is borne on the posterior side of every leaf axil. Spadix is covered by two sheaths, namely the inner and outer spathe. The inner spathe is softer and yellow or creamy white in colour. The outer spathe is harder, more fibrous and green in colour. The outer spathe stops growing earlier in the life of an inflorescence and is pierced by the inner spathe, which grows outward and becomes green. The spathe, due to the distension of the inflorescence within, ruptures longitudinally on the ventral side. It takes 24 hours to completely rupture, exposing the inflorescence within. Every inflorescence bears spikelets containing both male flowers and female flowers, except the first inflorescence borne on a palm, which may exclusively consist of male flowers.

18

Within an inflorescence, the male flowers mature and open first. They usually open in the morning hours, release pollen and fall off in the evening. Most male flowers have been found to open between 8am. and 10am. and most of the male flowers in an inflorescence open between 3 and 10 days of spathe opening (Menon and Pandalai, 1958). The flowering pattern in coconut can be described as extended flowering, wherein a few flowers open on each day, over a period of weeks. In a mature palm, the male phase continues for about 18-22 days (Liyanage, 1953). The duration of the male phase varies according to the nature of the tree, season and locality. Patel (1938) found that most male flowers open and fall off by the fifteenth day after the opening of the spathe. The number of male flowers in an inflorescence range from a few in Spicata to thousand in some tall varieties. Each male flower contains six perianth arranged in two whorls in alternate succession. The outer whorl is smaller than the inner. Within the perianth six hammer shaped stamen, which on maturity, release yellow powdery pollen. The male flower also contains an abortive pistil with three teeth at the apex, bearing nectar glands. This is suggestive of the predominance of entomophily in pollination.

The female flowers usually mature or become receptive after the male phase. The number of female flowers in an inflorescence is around 15 to 50, except in the case of Spicata, where an average of 130 female flowers are borne on a single inflorescence. In most talls and hybrids, the male and female phases don't overlap, omitting the chances of self - pollination. But in dwarfs, both inter – spadix and intra –spadix overlap of male and female phases is observed.

2.4 POLLEN CHARACTERS

Aldaba (1921) found that about 272 million pollen grains are produced in each inflorescence. The pollen attain maturity and are released from the anthers in a few male flowers even before the opening of the inflorescence. The pollen grains are smooth and spherical when they are released. But on contact with the dry atmosphere, it attains an ellipsoidal shape with a length of 0.063mm and width of 0.02mm. A meridional groove runs along its length, which has been interpreted as an adaptation for easy capture by pollinating insects. Coconut pollen is released in

19

the trinucleate condition, one nucleus being larger than the others. Juliano and Quisumbing (1931) studied the internal structure of coconut pollen in detail and found that not all microspores resulting from the microspore mother cell division are functional. They observed microspores in which just a single one contained a distinct nucleus. Cases of two and three functional nuclei were also observed. Based on their observations, some characteristics of sterile pollen was proposed. They are:

- Absence of granular content after dehiscence
- Inability to attain spherical shape even after contact with water
- Persistence of longitudinal sutures in moist condition.
- Smaller in size than fertile pollen.

Aldaba (1921) proposed that on an average, 25% pollen produced in a male flower is shrunken, suggesting infertility. Pollen from flowers at the distal end were found to be least sterile and the ones from the proximal end were most sterile (Nampoothiri, 1970).

2.5 POLLEN DISPERSAL MECHANISM

Coconut flowers are pollinated both by means of wind as well as insects. By morphology, the flowers are more adapted to insect pollination. Anthesis and opening of flowers in the morning (6am. – 11am.), sweet smelling flowers with nectar production emphasize the adaptation for insect pollination (Henderson, 1986). Sholdt (1966) observed that bees and wasps are the major pollinators of the coconut flower. Wind also contributes to pollination, mostly within a palm than between palms (Sholdt and Mitchell, 1967).

Atmospheric variables like temperature and relative humidity influence the time of anther dehiscence, the phenology of flower opening and the dispersal of pollen. Castor releases pollen only at temperatures above 24°C and relative humidity lower than 70% (Meinders and Jones, 1950). Rainfall is the major factor influencing flowering phenology in *Cocoa* (Adjaloo *et al.*, 2012).

20

2.6 POLLEN VIABILITY

Pollen viability is defined as the ability of the pollen grains to germinate on the stigma and effect fertilization (Vaughton and Ramsey, 1991). It depends on the genetic and physiological makeup of the pollen as well as environmental conditions. Rognon and Nuce de Lamothe (1978) found that collection of pollen 6 to 8 days after spathe opening is most suitable for viability studies. Menon and Pandalai (1958) were of the opinion that pollen should be collected between 3 – 10 days after spathe opening. In breeding programmes, 50% viability of pollen is considered to be the yardstick for using pollen in artificial pollination. Kidavu and Nambiar (1925) studied the viability of coconut pollen and found that in field conditions, the pollen remain viable up to the sixth day after dehiscence. Patel (1938) observed that storage of pollen in tissue paper kept in glass tubes wrapped with blotting paper gave good germination up to third day, but it completely lost viability by the ninth day. He found no difference in viability of pollen from flowers collected at different period from the same inflorescence. On the other hand, Nampoothiri (1970) proved that pollen from flowers at the top of the distal spikelet gave the highest germination while those from the proximal spikelet gave the lowest.

2.7 POLLEN VIABILITY TESTS

The most conclusive test for pollen viability is by using it for artificial pollination of female flowers and checking for the production of seed set. But this process consumes a huge amount of time and effort (Heslop-Harrison *et al.*, 1984), especially in the case of coconut, where seed development takes an entire year to complete. So, alternative methods which are good indicators of actual pollen viability need to be used. Generally, five types of tests are employed to assess pollen viability, namely

- Staining techniques
- *In vitro* and *in vivo* germination

- Measurement of pollen respiration and conductivity of leachates (very rarely used)
- Content of proline
- Artificial pollination to effect seed set (Dafni and Firmage, 2000)

The easiest and most common method used is staining pollen for the detection of cytoplasm using acetocarmine or other cytoplasmic stains. But this method has been found to stain dead pollen. Also, in many cases, it was poorly correlated with actual pollen germination and overestimated the viability (Heslop-Harrison *et al.*, 1984). *In vitro* pollen germination studies are more effective than staining, but they evaluate germination of pollen grains under artificial conditions of germination medium, temperature, humidity etc. This may or may not simulate the actual natural conditions and there is bound to be huge variation between the results when any of the experimental conditions are changed. Variation can also arise from difference in time of sampling, pollen storage conditions etc. But it does show high correlation with natural fruit set in several species (Visser, 1955). *In vivo* germination or the germination on excised or conspecific stigma more or less eliminates the effect of artificial conditions, but adds yet another factor of stigma receptivity and incompatibility issues (Stone *et al.*, 1995). It is more time consuming than *in vitro* studies.

Yet another dye used for pollen viability tests is the tetrazolium dye, which tests for dehydrogenase enzyme activity in the pollen. Dehydrogenase reduces the colourless salt to a coloured substance. Apart from the possibility of overestimating viability (Sedgley and Harbard, 1993), the concentration of the dye needs to be standardized for each species (Shivanna and Johri, 1985) and non – uniform staining may also lead to faulty results (Shivanna and Rangaswamy, 1992).

The Isatin test detects the presence of proline in pollen and is a relatively simple test. But it can only be used if the pollen contain at least 7% proline (Palfi *et al.*, 1988).

The FCR test using fluorescein diacetate tests for two aspects of pollen viability, namely, membrane stability and enzyme activity. It has been found to

correlate well with *in vitro* germination studies (Heslop – Harrison, 1970; 1992) and gives results faster than germination. But in some species, it gives a range of fluorescent shades which makes assessment difficult (Khatum and Flowers, 1996; Sukhvibul and Considine, 1993). The time limit available for counting is very short, almost 15 minutes, and hence is difficult to perform (Kapyla, 1991; Fritz and Lukaszevsky, 1989). Also, it cannot be used on desiccated or dehydrated pollen. In fact, hydration is a key step in preparing the samples for the test (Heslop-Harrison, 1992).

Karapanos *et al.* (2010) studied the respiration of tomato pollen under various temperatures and suggested it as a better test than *in vitro* germination because it gives an indication of the pollen tube growth vigour.

2.8 *IN VITRO* POLLEN GERMINATION

The germination of pollen grains requires a carbon source as the basic requirement. The synthesis of cell wall during pollen tube elongation requires nutrition. In the natural system, the nectar produced on the stigma provides all the necessary chemicals for effecting pollen germination as well as subsequent pollen tube elongation. Jay (1974) reported that nectar collected from coconut flowers contain 9 – 12% sucrose. Hence, sucrose forms an essential component of artificial pollen germination media. The sucrose in germination medium acts as a substrate for the growing pollen tube and also serves to maintain the osmotic potential (Brink, 1924; Visser, 1955). The absorption of exogenously supplied sugar by the growing pollen tube has been demonstrated in many species like *Tecoma radicans* (O'Kelley, 1955), Apple and *Nicotiana alata* (Tupy, 1960) *in vitro* and in *Petunia in vivo* (Linskens, 1954). The effect of various sucrose concentration on the germination of pollen has been studied by many researchers. Patel (1938) found a solution of 5% sugar and 2% gelatin to be suitable for coconut pollen germination, while Aldaba (1921) found maximum germination in 25 – 30% sugar solution. Liyanage (1954) proposed a medium containing 10% cane sugar and 2% gelatin and Furtado proposed 20% sugar solution as the best for germination of coconut pollen. Marechal (1928) obtained better germination on a medium with 25%

23

sucrose and 4.5% gelatin. Sreekrishna Bhat (1990) studied the effect of various concentrations of sucrose, boric acid and a combination of both these components and found that a combination of 10% sucrose and 10 ppm boric acid gave the best germination of 68.8%. Media utilizing a separate osmoticum like polyethylene glycol (PEG) has also been used in some studies (Roy Stephen, 2004).

Besides sucrose, boron is another element that promotes pollen tube growth. The remarkable role of boron in pollen tube growth was first found by Schmucker (1932). The presence of boron in the stigmatic secretion of *Vitis* and styles of *Lilium* has been observed (Gartel, 1952; Thomas, 1952). Vasil (1964) found that a low boric acid concentration of 100 to 150 ppm promoted the pollen tube growth of many plant species. A combination of sucrose and boric acid has a more positive effect on pollen germination than the individual components, because boron forms a sugar borate complex with sucrose and this complex leads to the better translocation of sugar into the pollen tube (Sidhu and Malik, 1986). Overall, the functions of boric acid in pollen germination can be summarized as:

- Promotes sugar absorption by forming sugar – borate complexes.
- Improves oxygen uptake
- Help in the synthesis of pectin for the walls of the growing pollen tube

Besides these elements, calcium also has been found to be essential for pollen tube metabolism. Calcium maintains the integrity of cell membranes by binding pectic carboxyl groups and alters their permeability (Kwack, 1967). Hepler and Winship (2010) established that an external concentration of calcium ion ranging from10μM to 10mM is required to prevent pollen tube bursting without the inhibition of tube elongation itself. In the growing tip of a pollen tube, an internal calcium ion gradient is observed (Michard *et al.*, 2009) and this serves a major role in the directional growth of the pollen tube (Malhó and Trewavas, 1996). Brewbaker and Kwack (1961) demonstrated that pollen populations which were low in number and density had a smaller chance of germinating in the medium used for densely populated samples and that this effect can be overcome by the addition

24

of calcium ion. They also found that other ions like Potassium, Magnesium and Sodium played supporting roles in the uptake and binding of calcium by the pollen tubes. Based on their studies, they found that a medium containing 10% sucrose, 100ppm boric acid, 300ppm calcium nitrate, 200ppm magnesium sulphate and 100ppm potassium nitrate as suitable for the germination of most pollen.

The *in vitro* pollen germination media have been standardized for many crops. For different cultivars of almond and peach, a medium containing 15% sucrose, 0.01% boric acid, 0.01% magnesium sulphate and 1% agar was found to effect maximum pollen germination (Imani *et al.*, 2011). The medium proposed by Taylor (1972) containing 2 g agar, 30 g $C_{12}H_{22}O_{11}$, 5.3 mg KNO_3, 51.7 mg $MnSO_4$, 10.3 mg H_3BO_3, 10.3 mg $MgSO_4.7H_2O$ made up to 100 mL with deionized waterwas used by Kakani *et al.* (2005) for germination of cotton pollen. Liu *et al.*, (2013) studied the influence of various sucrose and boric acid concentrations on the germination of Arecanut pollen and found that a medium containing 5 g/L agar, 0.4 g/L to 0.6 g/L boric acid, 40 g/L sucrose, and water is suitable for the growth of areca pollen. Reddy and Kakani (2007) used a germination medium comprising of 100 g sucrose ($C_{12}H_{22}O_{11}$), 500 mg calcium nitrate[$Ca(NO_3)_2.4H_2O$], 120 mg magnesium sulphate ($MgSO_4$),100 mg potassium nitrate (KNO_3) and 120 mg boric acid (H_3BO_3) dissolved in 1000 ml of deionized water for Capsicum cultivars. Mortazavi *et al.* (2010) optimized the germination medium for Date Palm pollen, which consisted of 50 mg L^{-1} boric acid, 200 mg L^{-1} calcium nitrate and 15% (w/v) sucrose. Kavand *et al.* (2014) demonstrated the cultivar variation in requirement of boric acid and calcium nitrate for the germination of date pollen. A modification of the Brewbaker and Kwack medium with 4% sucrose, suggested by Lora *et al.* (2006) was found to be the best for germination of coconut pollen belonging to two Brazilian accessions by Moura *et al.* (2015). Karun *et al.* (2014) used a medium containing 8% sucrose and 0.01% boric acid for studying the *in vitro* germination of cryopreserved coconut pollen.

The pollen also requires contact with the atmosphere for effective respiration to occur. When liquid media is used for *in vitro* pollen germination, the

omission of a coverslip or the use of a cavity slide and employing the 'hanging drop' (Righter, 1939) method has been found to be effective for unhindered respiration. The hanging drop method prevents the evaporation of the germination medium and prevents the pollen tube growth from being retarded by the flat surface of a culture slide. But solid or semi- solid media provide the advantages of providing ample surface area for contact with the atmosphere and of the pollen not sinking, as may occur with liquid media. Solid media are easier to handle (Beck and Joley, 1941) as well as observe under the microscope since the pollen grains fall on a single plane of view whereas in the case of liquid media, the pollen germinate at various depths in the media, making focusing on one plane and counting difficult. Growing pollen on an agar medium provides it with moisture at a constant relative humidity and favourable aerobic conditions too (Kubo, 1955; 1960).

Besides the media, the results of *in vitro* pollen germination also depend on how the pollen is dispersed on the medium. In the case of liquid media, the pollen is dusted on the media and mixed well within it using a laboratory needle. But it can be observed that the pollen move towards the periphery of the medium and lay clustered there so that they have to be spread out again during the time of observation. In the case of solid media, the pollen is either directly transferred from the male flower to the slide by lightly tapping it, or is brushed on the medium by means of a nylon brush. In any case, uniform distribution has to be ensured to eliminate the population effect, as found by Brewbaker and Kwack (1961).

2.9 DURATION OF GERMINATION

In vitro germination of binucleate pollen occurs faster and easier than that of trinucleate pollen (Vasil, 1960; Johri and Shivanna, 1977). Trinucleate pollen may require specific chemical components, a special mode of hydration or a high osmotic potential for successful germination *in vitro* (Mulcahy and Mulcahy 1988, BarShalom and Mattson 1977; Shivanna and Sawhney, 1995). Because trinucleate pollen are released after a second mitotic division, they may be deprived of sufficient reserves for longevity and germination (Frankel and Galun, 2012). *In*

vivo pollen germination duration can vary widely between species. Russel (1982) found that in *Plumbago zeylanica*, pollen tube initiation began 15 minutes after pollination. Lily pollen tubes grow at a rate of 1000μm per hour (Qin *et al*., 2011) and faster growing pollen tubes of *Tradescantia* and *Hemerocallis* show a pollen tube growth rate of 14400μm (Michard *et al*., 2009). Coconut pollen is released in the trinucleate stage. Pollen germination *in vivo* occurred six hours after pollination in coconut (Sreekrishna Bhat, 1990).

2.10 THERMO-TOLERANCE OF POLLEN

Pollen are exposed to diverse environmental conditions in the period between dispersal and pollination. The longevity of pollen during this period is affected by air temperature, relative humidity, air composition and pressure. Intrinsic factors like pollen vigor also decide longevity (Visser 1955, Johri and Vasil, 1961; Linskens, 1964). Since weather variables keep on changing diurnally and seasonally, it becomes important to find the optimum temperature for pollen longevity and germination. After studying the effect of a range of temperatures on Pine pollen, McWilliam (1958) found that 16 to 42°C was suitable for their germination. Temperatures above the optimum growth temperature for the plant may also be detrimental to pollen longevity. In the grass *Brachypodium distachyon*, Harsant *et al*. (2013) found that temperature above 32°C led to a decrease of 97% in pollen germination, compounded by abnormalities or arresting of pollen development stages and lack of deposition on the stigma. Gajanayake *et al*. (2011) studied the effect of high temperature on different cultivars of ornamental peppers and concluded that a temperature range of 11.8°C to 41.2°C was suitable for pollen germination with an optimum temperature of 26.8°C. Considerable variation was observed in the cultivar response to high temperatures. *Pinus* pollen failed to germinate at temperatures below 16°C or above 42°C and showed maximum germination between 30°C and 32°C (McWilliam, 1958). In sweet cherry, *Prunus avium*, temperature above 20°C accelerated the growth of pollen tube in the style, although the number of pollen tubes reaching the ovary remained constant, being affected both by temperature and genotype (Hedhly *et al*., 2004). Karapanos *et al*.

(2010) studied the respiration of tomato pollen at temperature ranging from 15°C to 35°C and found that respiration decreased at 30°C and 35°C after the initial hour due to the effect of temperature. They observed maximum pollen germination at 15°C and maximum pollen tube length at 25°C.

Agar *et al.* (2015) studied the genetic variation that exists in the tolerance of wheat pollen to heat stress during meiosis and anthesis using kernel weight per spike, kernel number per spike and 1000 kernel weight as indicators of pollen fertility. Sorkheh *et al.* (2011) exposed various Iranian wild almond species to temperatures ranging from 10°C to 50°C and obtained a variation in maximum germination from 68% to 90% between various genotypes. The response of various cotton cultivars to a range of temperatures was studied by Kakani *et al.* (2005) and found that cotton pollen can tolerate temperature ranging from 15°C to 43°C. The maximum, minimum and optimum temperature for pollen germination were calculated by fitting a modified bilinear model. Singh *et al.* (2008) used a similar method in *Brassica napus* and calculated the minimum, optimum and maximum temperature for pollen germination to be 6.4, 24.3 and 33.7°C. He also identified the cultivar which was most tolerant to high temperature stress.

Reddy and Kakani (2007) used the concept of a Cumulative Temperature Response Index (CTRI) to screen various *Capsicum* varieties for high temperature stress tolerance and classified the varieties as tolerant, intermediate and sensitive to high temperature stress. A Mexican variety was identified as the sole tolerant one. Gajanayake *et al.* (2011) added a few more parameters like cell membrane thermostability, canopy temperature depression and chlorophyll stability index to the CTRI calculation in addition to the pollen parameters in order to find the tolerance level of ornamental pepper cultivars. Kakani *et al.* (2002) studied the heat stress tolerance of groundnut pollen and found that it can tolerate temperatures from 14°C to 43°C with an optimum temperature of 30°C. Milatovic *et al.* (2016) studied the response of apricot pollen to three temperatures, namely 10, 15 and 25°C and obtained high germination at 15 and 25°C. Ranasinghe *et al.* (2010) conducted an experiment to screen six coconut cultivars for their tolerance to high

28

temperature stress and found the mean cardinal temperatures (T_{min}, T_{opt}, T_{max}) averaged over cultivars to be 20.0 °C, 28.0 °C, and 38.8 °C, respectively, for pollen germination. Based on the results, the variety Dwarf Green was identified as showing the widest temperature adaptability. Till date, no studies of the kind have been reported in Indian coconut varieties.

CHAPTER 3

MATERIALS AND METHODS

An experiment was conducted to study the effect of high temperature on the coconut pollen germination and its viability as well as to identify the contrasts in coconut genotypes based on the thermotolerance of pollen. The procedure involved in performing the experiment is outlined in the following sections.

3.1 EXPERIMENT LOCATION AND GEOGRAPHY

The experiment was conducted at ICAR- Central Plantation Crops Research Institute Farm, Kasaragod. Kasaragod Farm is located at 12° 30'N latitude and 75°E longitude at an altitude of 10.7m above mean sea level. On an average, the area receives 3400mm annual rainfall. The soil type is sandy loam.

3.2 WEATHER

The experiment was conducted from December 2015 to July 2016. The weather data for the experimental period was continuously recorded. The weekly averaged values of maximum and minimum temperature, rainfall, sunshine hours and evaporation are presented in the following sections.

3.2.1 Temperature

The average maximum temperature (T_{max}) was 32.2°C while the minimum temperature (T_{min}) was 22.8°C. T_{max} was low during December (28.8^{0}C) and increased during April and May (34.4°C) and T_{max} showed an increasing trend from the month December to May (Fig. 1). Similarly, T_{min} ranged from18.4°C (January) to 27.3°C (April).

3.2.2 Rainfall

During the experimental period, 332mm total rain fall was recorded, in which, the first 10 weeks got 71mm rainfall and remaining was recorded from 31st to 43rd weeks (Fig.. 2).

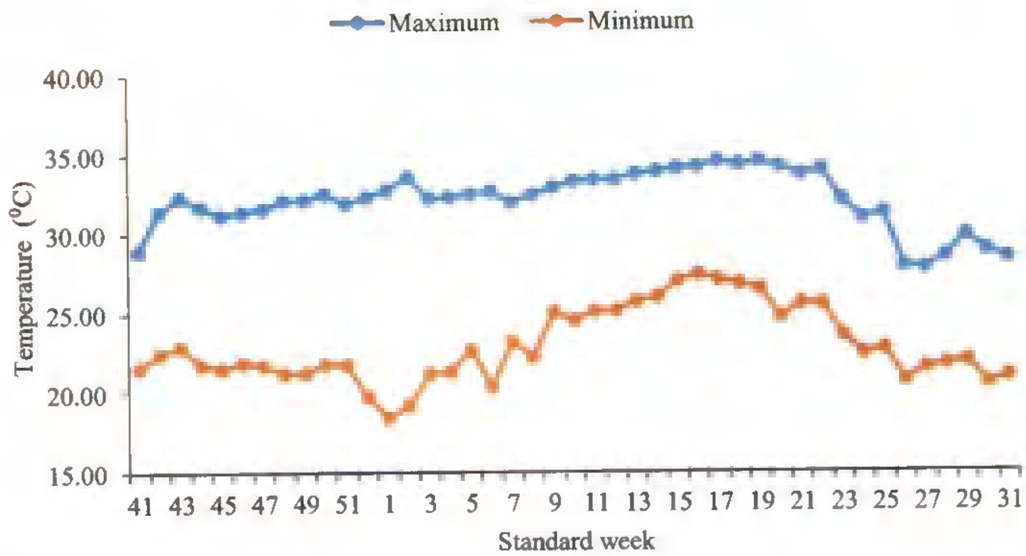

Fig. 1 Weekly maximum and minimum temperature for the experimental period from October 2015 - July 2016.

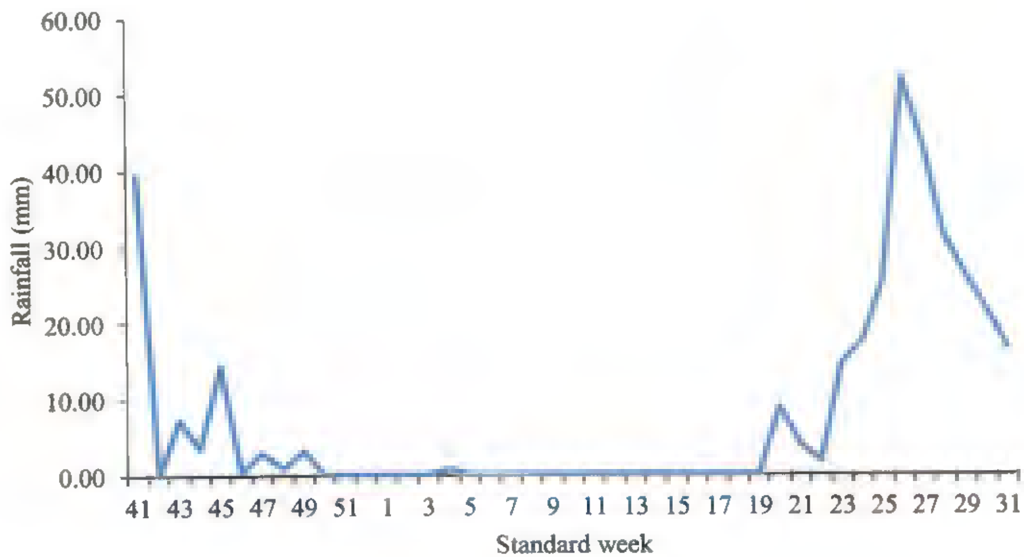

Fig. 2 Weekly Rainfall for the experimental period from October 2015 - July 2016

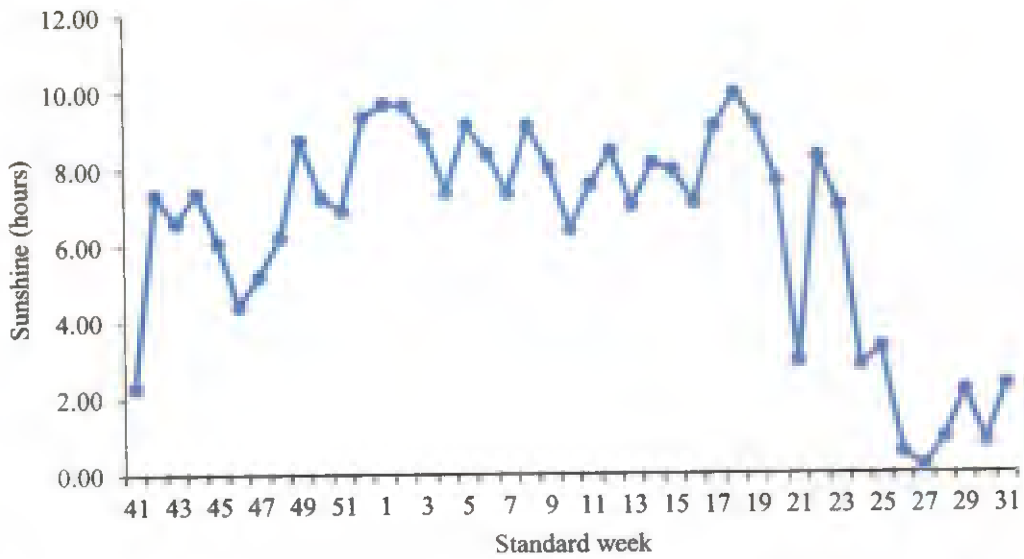

Fig. 3 Weekly sunshine hours for the experimental period from October 2015 – July 2016.

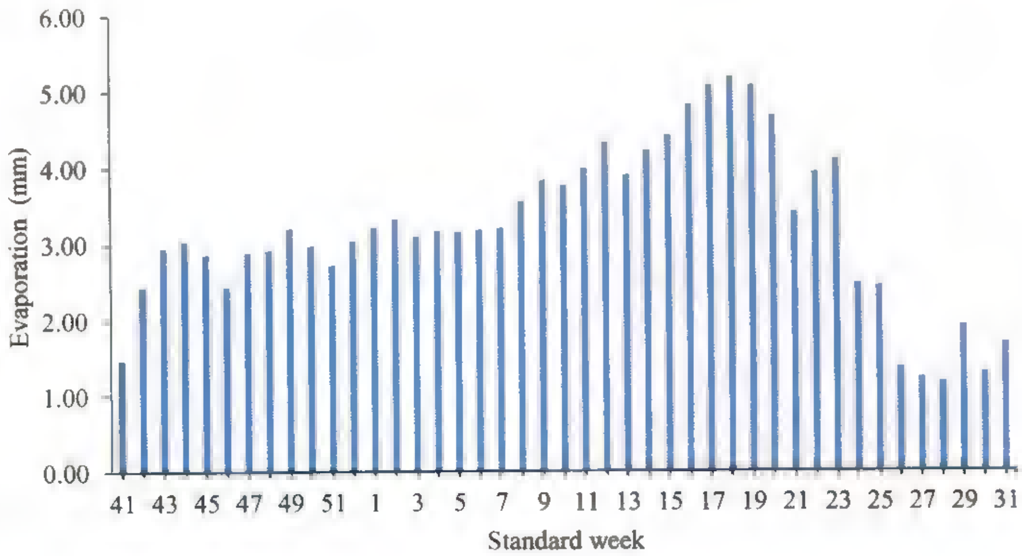

Fig. 4 Weekly evaporation for the experimental period from October 2015 - July 2016.

3.2.3 Sunshine hours

On an average, 8.03 hours of sunshine was recorded during the experimental period. The maximum sunshine hour was 9.8 hrs, which was recorded from 29th to 31st weeks (Fig. 3).

3.2.4 Evaporation

The maximum evaporation of 5.17mm was recorded during the 30th week and the minimum evaporation of 1.17mm was recorded during the 40th week. Throughout the period, about 3.18 mm of average evaporation was recorded and the rate of evaporation increased during a period extending from 15th to 30th weeks (Fig. 4).

3.3 GENOTYPE SELECTION

For this experiment, 11 genotypes belonging to talls, dwarfs and hybrids have been selected for *in vitro* pollen germination studies to screen for high temperature tolerance. The list of the selected genotypes is given in Table 1.

Genotypes of different origin were selected for the study in the expectation that the variation exhibited in the morphological characters may also be applicable in the tolerance to heat stress.

3.4 PALM SELECTION

Six palms aged between 20-25 years belonging to each of the selected genotypes were chosen for the study. The selection was done based on the expression of typical morphological characters of the genotype as described in the "Coconut Descriptors Part – 1 and 2" (Ratnambal *et al.*, 1995; 2000). All the palms were grown under recommended package of practices.

Table 1 Genotypes selected for the study

Talls	**Dwarfs**	**Hybrids**
West Coast Tall (WCT)	Chowghat Orange Dwarf (COD)	Chandra sankara (COD X WCT)
San Ramon Tall (SNRT)	Malayan Yellow Dwarf (MYD)	Kalpa samrudhi (MYD X WCT)
Federated Malay States Tall (FMST)	Cameroon Red Dwarf (CRD)	
Lakshadweep Micro Tall (LMT)	Chowghat Green Dwarf (CGD)	
West Coast Tall Spicata (SPI)		

3.5 POLLEN COLLECTION

3.5.1 Collection time

For studying the time of maximum flower opening, three palms, two dwarfs and one hybrid, were selected. Fresh and completely opened spadix was used for the pollen collection. A day before the actual date of collection, at 4.30pm., all the opened male flowers were removed and discarded. On the day of collection at 6.30am.all the dehisced male flowers from the inflorescence were collected and counted. At 8.30am. and again at 10.30am., the male flowers opened were counted and collected separately in a petridish. The open flowers collected at 8.30am. and 10.30am. were lightly tapped with a nylon brush so that they shed pollen and the same was collected on a butter paper. The collected pollen was used for studying %PG. Since the flowers collected at 6.30am. also included the flowers opened from previous day, they were not included for the pollen germination study.

3.5.2 Method of pollen collection

Most of the experiments conducted on coconut pollen utilizes dried pollen due to the ease with which relatively large quantities of pollen can be obtained and the convenience of storing the pollen. But it is a known fact that freshly dehisced pollen has maximum germination capacity. But the major constraint in coconut is the availability of freshly collected pollen as it loses viability immediately after collection, depending on the prevailing environmental conditions before, during and after anther dehiscence. In order to get maximum germination, the freshly opened flowers collected at 8.30am. were used in this study.

Fig. 5 coconut inflorescence, 2 days after spathe opening

Fig. 6 Counting the number of open flowers

Fig. 7 Dusting the open flowers to collect pollen

Fig. 8 Removal of open flowers

3.6 STANDARDIZATION OF POLLEN GERMINATION MEDIUM

An experiment was conducted to find out the optimum medium for coconut pollen germination and pollen tube elongation. The details of the same are presented below:

Design - Factorial CRD

Replications - 3

Varieties - 2

V_1 – Chowghat Orange Dwarf

V_2- West Coast Tall

Treatments - 5

T_1- 6% sucrose + 0.01% boric acid

T_2 - 8% sucrose + 0.01% boric acid

T_3 - 10% sucrose + 0.01% boric acid

T_4 -10% sucrose + 0.01% boric acid + 0.05% $MnSO_4$ + 0.005% KNO_3 + 0.01% $MgSO_4.7H_2O$

T_5 -10% sucrose + 0.01% boric acid + 0.025% $Ca(NO_3)_2$ + 0.02% $MgSO_4$ + 0.01% KNO_3

Treatment combinations – 2 x 5 = 10

Duration – 2 hours

The selected media were prepared separately by mixing all the chemical components in the specified concentrations. Finally, 1% agar was added to the media and they were boiled to dissolve the agar. The media were allowed to cool down a bit and then 1- 2ml of each media was poured on to clean glass slides and allowed to solidify. The pollen collected was dusted on the solid medium by means of a nylon brush and germination assessed after two hours of incubation.

3.7 OPTIMIZATION OF CONDITIONS FOR POLLEN GERMINATION

The pollen is to be uniformly distributed over the medium to eliminate the population effects on pollen germination (Brewbaker and Kwack, 1961). For this,

38

dipping the nylon brush in pollen, tapping off the excess, positioning the brush above the slide at about 2 – 3 cm from the medium and blowing on the brush from above with an even force, moving it from end to end of the slide had been found to be an effective method and hence adopted for the study.

Pollen germinates better in a high humidity atmosphere. To provide an almost saturated atmosphere during incubation, the petriplates in which the pollen is incubated were lined with moist filter paper. This ensured a humidity above 80% within the petriplate.

Table 2 Media tested for pollen germination

Serial no.	Composition	Medium
M1	6% Sucrose; 0.01% Boric acid; 1% Agar	Kumari *et al.*, 2009
M2	8% Sucrose; 0.01% Boric acid; 1% Agar	Karun *et al.*, 2014
M3	10% Sucrose; 0.01% Boric acid; 1% Agar	Sreekrishna Bhat, 1990
M4	10% Sucrose; 0.01% Boric acid; 0.05% Manganese sulphate; 0.005% Potassium nitrate; 0.01% Magnesium sulphate; 1% Agar	Taylor (1972), modified by author
M5	10% Sucrose; 0.01% Boric acid; 0.025% Calcium nitrate; 0.02% Magnesium sulphate; 0.01% Potassium nitrate; 1% Agar	Niles and Quesenberry, 1992

Fig.9 Pollen collected in a butter paper

Fig.10 Pollen germination medium

41

Fig.11 Pouring the medium on to slides

Fig.12 Solidified germination medium on the slide

Fig.13 Dusting the collected pollen on the slide

Fig.14 Slides dusted with pollen

Fig.15 Petriplates lined with filter paper

Fig.16 Moistening the filter paper

Fig.17 Slides kept in the petriplate

44

3.8 OPTIMIZATION OF POLLEN GERMINATION DURATION

The pollen from two accessions, namely, WCT and COD were dusted on the medium by means of a nylon brush. Three slides per medium per accession were used as replicates. The slides dusted with pollen were incubated at room temperature within petriplates lined with moist filter paper for a duration of two hours. After the incubation period, the slides were observed under the microscope and scored for finding the %PG.

At room temperature and in a medium containing 8% sucrose and 0.01% boric acid, coconut pollen germinated after about 45 minutes of incubation. Since the main aim of the present study was to evaluate pollen germination and tube length at higher temperatures where these processes would be slower, a duration of two hours was set for incubation of pollen before observation. At room temperature, it was found that at durations longer than two hours, the PTL exceeded the span of the microscopic field and made measurement of the PTL impossible. Hence, all observations were made at two hour incubation period.

3.9 TEMPERATURE TREATMENTS

A preliminary investigation was conducted by exposing the pollen on germination medium to temperatures in the range of 15°C - 50°C at an interval of 5°C for two hours. Pollen from WCT and COD were used for this experiment. %PG was assessed after the incubation period. Based on the results from this experiment, the temperature range to be tested in the main experiment was set as 20°C - 40°C at an interval of 5°C. The experiment details are as follows:

Design - Factorial CRD

Replications - 6

Varieties - 11

Talls

V1 – West Coast Tall

V2 – San Ramon Tall

V3 – Federated Malay States Tall

45

V4 – Lakshadweep Micro Tall

V5 – West Coast Tall Spicata

Dwarfs

V6 – Chowghat Orange Dwarf

V7 – Malayan Yellow Dwarf

V8 – Cameroon Red Dwarf

V9 – Chowghat Green Dwarf

Hybrids

V10 – Chowghat Orange Dwarf x West Coast Tall

V11 – Malayan Yellow Dwarf x West Coast Tall

Treatments - 5

T1 - 20°C

T2 - 25°C

T3 - 30°C

T4 - 35°C

T5 - 40°C

Treatment combinations – 11 x 5 = 55

Duration – 2 hours

The set temperatures were maintained in five B.O.D. incubators. After the preparation of slides with the germination media, the slides were kept in covered petriplates and placed in the incubators with preset temperatures for 15 minutes in order to bring the germination medium to the set temperature of the incubator. After this period, the slides were taken out and dusted with the pollen from the selected palms of the 11 genotypes chosen for the study. Six palms per treatment per genotype were taken as replicates. Four slides per temperature treatment per palm were used. The slides were then kept back in the incubator.

3.10 OBSERVATIONS

After an incubation period of 2 hours, the petriplates containing the pollen germinated slides were taken out of the incubator and observed under 10x magnification objective of a Nikon Eclipse NI- U microscope (Nikon Corporation,

46

Tokyo, Japan) paired with a Nikon DS - Ri1 camera. The photographs of the observed microscopic fields were taken at a resolution of 1920 x 1536 pixels and a scale of 0.61µm/pixel with the help of the software, NIS Elements D version 4.3. Five microscopic fields per slide were photographed. Measurements of germination count were made using the 'counts' tool in the 'Measurement and Annotations' toolbar of NIS Elements D. A pollen grain was considered to be germinated when its tube length exceeded the diameter of the pollen. PTL was measured in micrometers using the Polyline length measurement tool of the same software. The data was exported to an Excel file. %PG was calculated using the formula:

$$\% PG = \frac{\text{Number of germinated pollen x 100}}{\text{Total number of pollen}}$$

The %PG and PTL data was averaged over all the microscopic fields per slide and all the slides per temperature treatment per palm.

3.11 STATISTICAL ANALYSIS

The data was subjected to two factorial Analysis of Variance using the SAS 9.3 Software. Genotype was set as factor 1 with 11 levels and temperature was set as factor 2 with 5 levels. There were 6 replications per genotype. Duncan's Multiple Range Test was employed to group the factor means according to their significant difference.

For calculating the optimum temperature for pollen germination for each genotype, the mean values of %PG of all the palms belonging to the genotype at different temperatures was taken. The equation for finding out the optimum temperature is:

$$T_{opt} = \frac{T(l) + (PG_{max} - PG(l)) \times 10}{(PG_{max} - PG(u)) + (PG_{max} - PG(l))]}$$

where, T(l) is $(T_{max} - 5)$ and T(u) is $T_{max} + 5$.

PG_{max}- pollen germination at T_{max}

PG(u) - pollen germination at T(u)

47

PG(l) - pollen germination at T(l).

T_{max} – temperature at which maximum pollen germination was observed

The assumption used in computing T_{opt} is: rate of increase in pollen germination from T(l) to T_{opt} is same as the rate of decrease in pollen germination from T_{opt} to T(u) and the change is linear.

In a similar way, optimum temperature for pollen tube length was also calculated. The equation used for the same is:

$$T_{opt} = \frac{T(l) + (PTL_{max} - PTL(l)) \times 10}{(PTL_{max} - PTL(u)) + (PTL_{max} - PTL(l))]}$$

where T(l) is (T_{max} - 5) and T(u) is T_{max} +5.

PTL_{max}- pollen tube length at T_{max}

PTL(u) - pollen tube length at T(u)

PTL(l) - pollen tube length at T(l).

T_{max} – temperature at which maximum pollen tube length was observed

45

Fig.18 Petriplates kept in B.O.D. incubator at preset temperature

Fig.19 Petriplates left to incubate for 2 hours

3.12 BIOCHEMICAL MEASUREMENTS IN LEAF TISSUE

To check whether there is any correlation between the thermo tolerance of pollen and that of vegetative tissue, estimation of stress related biochemical parameters were conducted for the leaf tissue. For this, leaflets were sampled from the third fully opened leaf of palms belonging to four genotypes, namely, MYD x WCT, COD x WCT, CGD and MYD.

3.12.1 Leaf tissue extraction for the assay of scavenging enzymes

Enzyme extraction from leaf tissue

Enzyme extract was prepared by following the method of Chempakam *et al.*, (1993) as standardized for coconut leaf tissue.

Reagents

1. Sodium phosphate buffer (0.1 M; pH 7.6): Disodium hydrogen phosphate heptahydrate (Na_2HPO_4. $7H_2O$; 26.805g) was dissolved in distilled water (500ml) to get solution A. Sodium dihydrogen phosphate dehydrate (NaH_2PO_4. $2H_2O$: 15.605g) was dissolved in distilled water (500ml) to get solution B. Solution A (435ml) and solution B (65ml) were mixed and volume was made to 1000 ml with distilled water to prepare sodium phosphate buffer (0.1M: pH 7.6). The pH of solution was adjusted.
2. Insoluble poly vinyl polypyrrolidone (PVPP)

Procedure

Plant tissue sample (2 g) was homogenized in prechilled mortar and pestle with 0.1M sodium phosphate buffer (20 ml) and insoluble PVPP (1g). This homogenate was centrifuged at 12000 rpm at 4°C for 15 minutes. Supernatant was collected and again centrifuged at 12000 rpm for 15 minutes at 4°C. This enzyme extract was used for the assay of scavenging enzymes viz., super oxide dismutase and peroxidase.

3.12.1.1 *Assay of Superoxide dismutase (SOD)*

Super oxide dismutase (SOD) specific activity was assayed by following the method of Beauchamp and Fridovich (1971).

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Reagents

1. Potassium phosphate buffer 0.1M (PH 7.8): Potassium hydroxide (1.122g) was dissolved in distilled water (100ml) to get solution A and potassium dihydrogen phosphate (2.722g) was dissolved in distilled water (100ml) to get solution B. Solution A (90ml) and Solution B (100ml) were mixed and made to 200ml using distilled water to prepare potassium phosphate buffer (0.1M; PH 7.8).
2. Sodium carbonate solution1.5M (pH 10.2).
3. Nitro blue tetrazolium (NBT) solution: NBT (5.5mg) was dissolved in distilled water (10mL).
4. Methionine solution: Methionine (194mg) was dissolved in distilled water (10mL).
5. EDTA disodium salt solution: EDTA (37.2mg) was dissolved in distilled water (10mL).
6. Riboflavin solution: Riboflavin (4.89mg) was dissolved in distilled water (10mL) and 1mL from the solution was diluted to 100mL using distilled water.

Procedure

Reaction mixture

Potassium phosphate buffer	1.6mL
NBT solution	0.3mL
Methionine solution	0.3mL
EDTA disodium salt solution	0.3mL
Enzyme extracts	0.1mL

Light control

Except enzyme extract all other reagents in reaction mixture were added and mixed in a test tube and then kept in light.

Dark control

Reaction mixture with enzyme extract was kept in dark condition.

Sample

Reaction mixture with enzyme extract was kept in light condition.

Reagent blank

Potassium phosphate buffer served as reagent blank.

51

Enzyme assay

Reagents in reaction mixture were added one after another and the reaction was started by adding riboflavin solution (0.3ml). After the addition of riboflavin solution, test tubes were incubated at 30°C for 30 minutes in dark chamber to serve as dark control. Another one test tube was incubated at 30°C for 30 minutes in florescent light as light control (reaction mixture without enzyme extract) and samples were incubated at 30°C for 30 minutes in florescent light as light control (reaction mixture with enzyme extract). After 30 minutes, absorbance was taken at 560nm by using UV visible spectrophotometer (Shimadzu UV 160A, Japan) against reagent blank.

Calculation of specific activity

(100 (ODS/ODLC) 100)/50 = X

Where, X/mg protein in enzyme extract = specific activity in units.

1unit is defined as the 50% reduction of the blue color formed by NBT 30 minutes^{-1} mg protein^{-1}.

$OD\ S = OD_T - OD_C$

OD_T = Absorbance of sample

OD_C = Absorbance of dark control

OD_{LS} = Absorbance of light control

3.12.1.2 ***Assay of peroxidase (POD)***

Specific activity of peroxidase enzyme was assayed by following the method Kar and Mishra (1976).

Principle

The enzyme activity was assayed using *O*-dianisidine as hydrogen donor and H_2O_2 as electron acceptor. The rate of formation of yellow range colored dianisidine dehydrogenation product is a measure of the peroxidase activity and can be assayed spectrophotometrically at 430nm.

Reagents

1. Sodium phosphate buffer (0.1 M; pH 7.6): Disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 . 7H_2O$; 26.805g) was dissolved in distilled water

52

(500ml) to get solution A. Sodium dihydrogen phosphate dehydrate ($NaH_2PO_4.2H_2O$: 15.605g) was dissolved in distilled water (500ml) to get solution B. Solution A (435ml) and solution B (65ml) were mixed and volume was made to 1000ml with distilled water.

2. Hydrogen peroxide (H_2O_2): Hydrogen peroxide (0.5%) was diluted up to 10ml using sodium phosphate buffer.
3. 0.2% O-dianisidine: O-dianisidine (20mg) was dissolved in methanol (10ml)

Procedure

Sodium phosphate buffer (pH 7.6)	2.7ml
0.2% O-dianisidine	0.1ml
0.5% H_2O_2	0.1ml
Enzyme extract	0.1ml

Read the change in OD at 430nm continuously for 4 minutes at 30 seconds.

Reagent blank

Reaction mixture except enzyme extract served as reagent blank.

1 unit is: change in OD to 1 per minute per gram tissue.

3.12.1.3 *Lipid peroxidation (MDA) assay*

Leaf lipid peroxidation was estimated by following method of Heath and Packer (1968).

Reagents

1. Tri chloro acetic acid (0.1%): TCA (1g) weighted and dissolved in distilled water and made up to 1000 mL using distilled water.
2. TCA (20%): TCA (100g) was dissolved in distilled water and volume was made up to 500 mL using distilled water.
3. Thio barbituric acid (TBA) (0.5%) in TCA (20%): TBA prepared freshly by dissolving 500 mg in TCA (100 mL; 20%) solution.

Enzyme extraction from leaf tissue

Plant tissue sample (0.250g) was homogenized in a pestle and mortar with extraction buffer 0.1% tri chloro acetic acid (10 mL). Centrifuge the sample at 10000 rpm in 4^0C for 10 minutes. Supernatant was taken for the assay.

53

Procedure

Pipette out 0.5 mL of enzyme extract and 4.5 mL of TBA solution (0.5%) in TCA (20%) was added and kept in boiling water bath for 30 minutes. After water bath test tubes were quickly cooled using ice bath to stop the reaction and immediately OD was taken at 532 and 600 nm using UV- Visible spectrophotometer (ShimadzuUV160A, Japan).

Reagent blank

Blank was prepared with 5 mL of 20% TCA with 0.5% TBA

Calculations

$$\text{MDA equivalent} = \frac{(OD_{532} - OD_{600}) \times V \times 1000}{\varepsilon \times M}$$

Where,

V = Grinding volume

ε = Molar extinction coefficient of MDA = 155 Mm/cm

M = Weight of tissue taken.

3.12.2 Estimation of chlorophyll Content

The chlorophylls are the essential components for photosynthesis and occur in Chloroplasts as green pigments in all photosynthetic plant tissues. They are bound loosely to Proteins but are readily extracted in organic solvents such as acetone or ether. There are at least five types of chlorophylls in plants. Chlorophylls a and b occur in higher plants, ferns and mosses. Chlorophylls c and d are only found in algae and in certain bacteria.

Principle

Chlorophyll is extracted in 80% acetone and absorbance at 663nm and 645nm are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

Reagents

1. Dilute analytical grade acetone to 80% (prechilled).

Procedure

500mg finely chopped leaf tissue extracted with 10ml 80% acetone. Keep it overnight decants the supernatant and extracted the residue with 10ml 80%

acetone. Decant the supernatant and pooled. Make up the volume to 25ml. Take the OD at 663 and 645nm against 80% acetone.

Calculation

$$\text{Total Chlorophyll} \left(\frac{\text{mg}}{\text{g}}\right) = \frac{[20.2(\text{A}645) + 8.02(\text{A}663)] \text{ x V}]}{1000 \text{ x W}}$$

Where,

A = Absorbance at specific wavelengths.

V = Final volume of chlorophyll extract in 80% acetone.

W = Fresh weight of the tissue extracted.

3.12.3 Estimation of epicuticular wax (ECW)

The epicuticular wax content was estimated using the method of Ebereon *et al.*, (1977) as standardized for coconut leaf samples by Rajagopal *et al.*, (1989).

Reagents

1. Dichromate reagent: powdered potassium dichromate (20g) was dissolved in distilled water (40 mL) and to this concentrated sulphuric acid (1000 mL) was added and then heated below boiling point in a preheating water bath to prepare dichromate reagent.
2. Standard ECW: The ECW (40 mg) extracted from coconut leaf lets was dissolved in potassium dichromate reagent (20mL) to obtain stock standard. Standard solutions of 0.4 mg to 2 mg concentration were prepared from the stock standard.

Procedure

Twenty leaflets segment 3*2 cm^2 area from the third fully opened leaf from the top were immersed in chloroform (15 mL) and then vigorously shaken for 20 seconds and chloroform was completely collected in test tubes. After complete evaporation of chloroform added 5 mL potassium dichromate reagent and kept it in a boiling water bath for 30 minutes. After that the volume was made upto 17 mL using distilled and OD readings was taken at 590 nm using UV- Visible spectrophotometer (Shimadzu UV 160A Japan).

3.12.4 Leaf tissue extraction for total phenol estimation

Leaf tissue (0.250g) was homogenized in a pestle and mortar using 80% ethanol (10 ml) and kept in preheated water bath for 30 minutes at 70 °C. Filter the homogenate through Whatman No.1 filter paper and similarly repeated the extraction using 80% ethanol (10 ml) and filtered. Thereafter ethanol was evaporated completely by heating it at preheated water bath (70°C), and the remaining portion was dissolved in 10 ml distilled water and vortexed for 2 minutes. This extract was used for the estimation of the total phenol.

Estimation of Total Phenol

Total Phenols were estimated by using Folin ciocalteus method (Bray and Thorpe 1954).

Principle

Phenols react with phosphomolybdic acid in FC reagent in alkaline medium and produce blue colored complex (Molybdenum Blue) which gives the absorption maxima at 650 nm.

Reagents

1. Sodium Carbonate Solution (20%), Prepare fresh each time
2. Folins Ciocalteus Reagent (Commercially available, Merck India)
3. Standard: Catechol (100 mg) was dissolved in distilled water and made up the volume to 100 ml with distilled water. Stock standard (10 mL) was diluted to 100 mL with distilled water to get working standard. A series of standard solutions were prepared by taking 0.1 to 1ml of working standard and made up to 1ml in each tubes using distilled water.

Procedure

Pipette out the sample extract (0.1 mL) and working standard solution (0.1mL to 1 mL) into seperate series of test tubes and made up the volume to 1 ml with distilled water. To this added FCR (0.5 mL) and vortexed for 15 seconds and added 20% Sodium carbonate solution (2 ml). Vortexed for 15 seconds and kept at room temperature for 1 hour incubation in dark condition. After that sample was diluted to 25 mL using distilled water.

Reagent blank

Distilled water (1 mL) + FCR (0.5 mL) + sodium carbonate (5 mL,20%). Blank is also run simultaneously.OD was taken at 650 nm by using UV- VIS spectrophotometer (Shimadzu UV 160A, Japan) and from the graph drawn using standards, calculated the amount of total phenol by using catechol standard and expressed the results mg catechol equ.g FW^{-1}.

CHAPTER 4
RESULTS AND DISCUSSION

An investigation entitled "Screening of coconut varieties for tolerance to high temperature stress using pollen viability test" was conducted at ICAR-CPCRI Farm, Kasaragod to understand the response of coconut pollen germination and its viability at different temperatures so as to select the genotypes for high temperature tolerance. For this study, 11 genotypes belonging to talls, hybrids and dwarfs were selected. The ideal time for pollen collection, medium of germination, optimum temperature for germination were standardized. The results obtained from the study are described below.

4.1 POLLEN COLLECTION TIME

Pollen viability is related to characteristics such as environmental humidity and temperature (Nepi and Pacini, 1993). It varies from season to season which has not been clearly studied in coconut. In order to find the time of flower opening and pollen viability during the relatively dry months of January and February, open flowers and their pollen were collected from three contrasting genotypes of dwarfs and hybrids.

Observations on the number of flowers opened at various time intervals, i.e. from 4.30pm. on the previous day to 6.30am. on the day of observation, from 6.30am. to 8.30am. and 8.30am. to 10.30am. were taken from the day of spathe opening to 10 days in two dwarf (MYD and COD) and one hybrid (COD x WCT) palms and the following results were obtained.

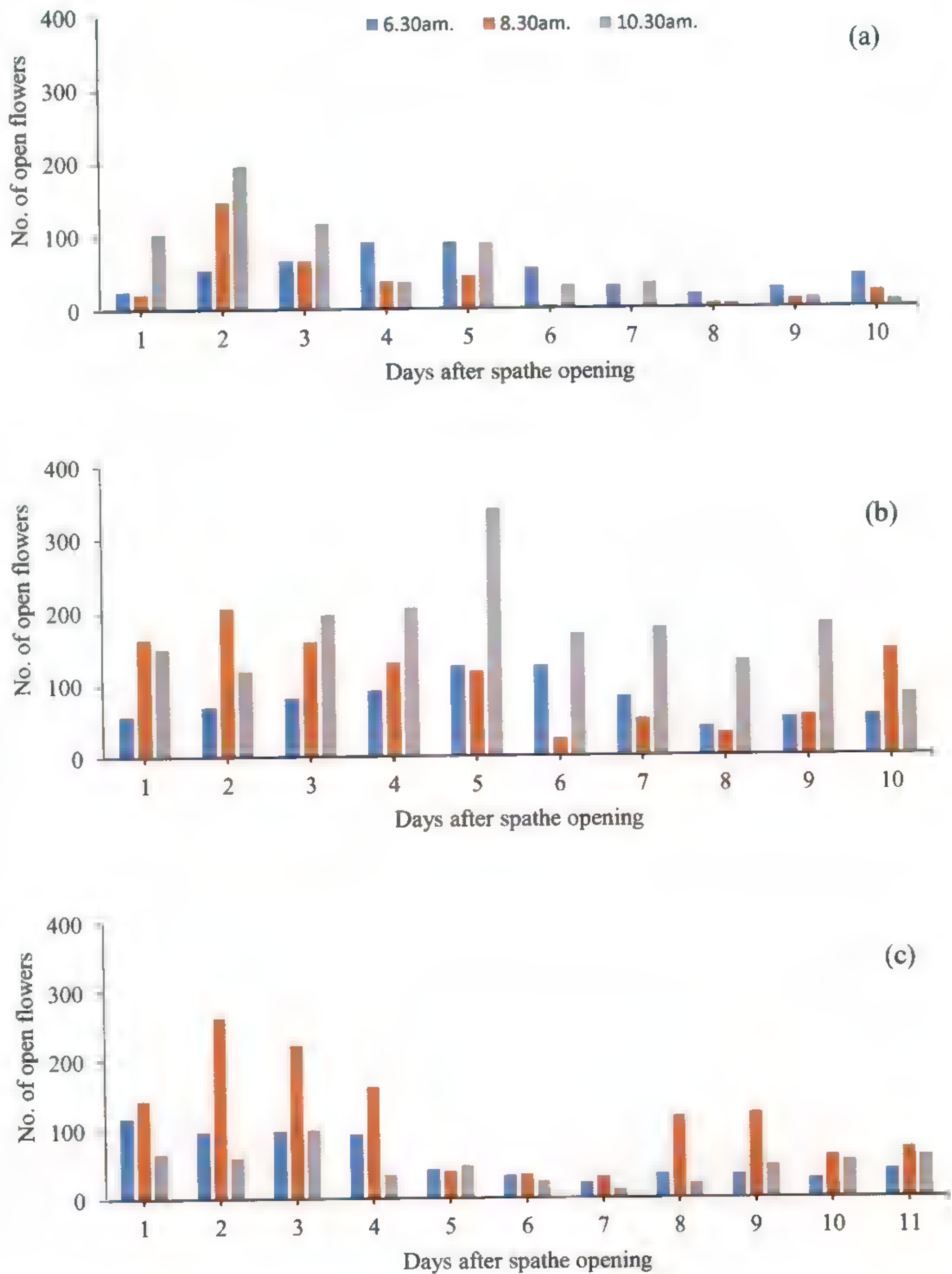


Fig. 20. Number of open flowers collected at 6.30, 8.30 and 10.30am. for a period of 10 days from the day of spathe opening in genotypes (a) MYD (b) COD and (c) COD x WCT

59

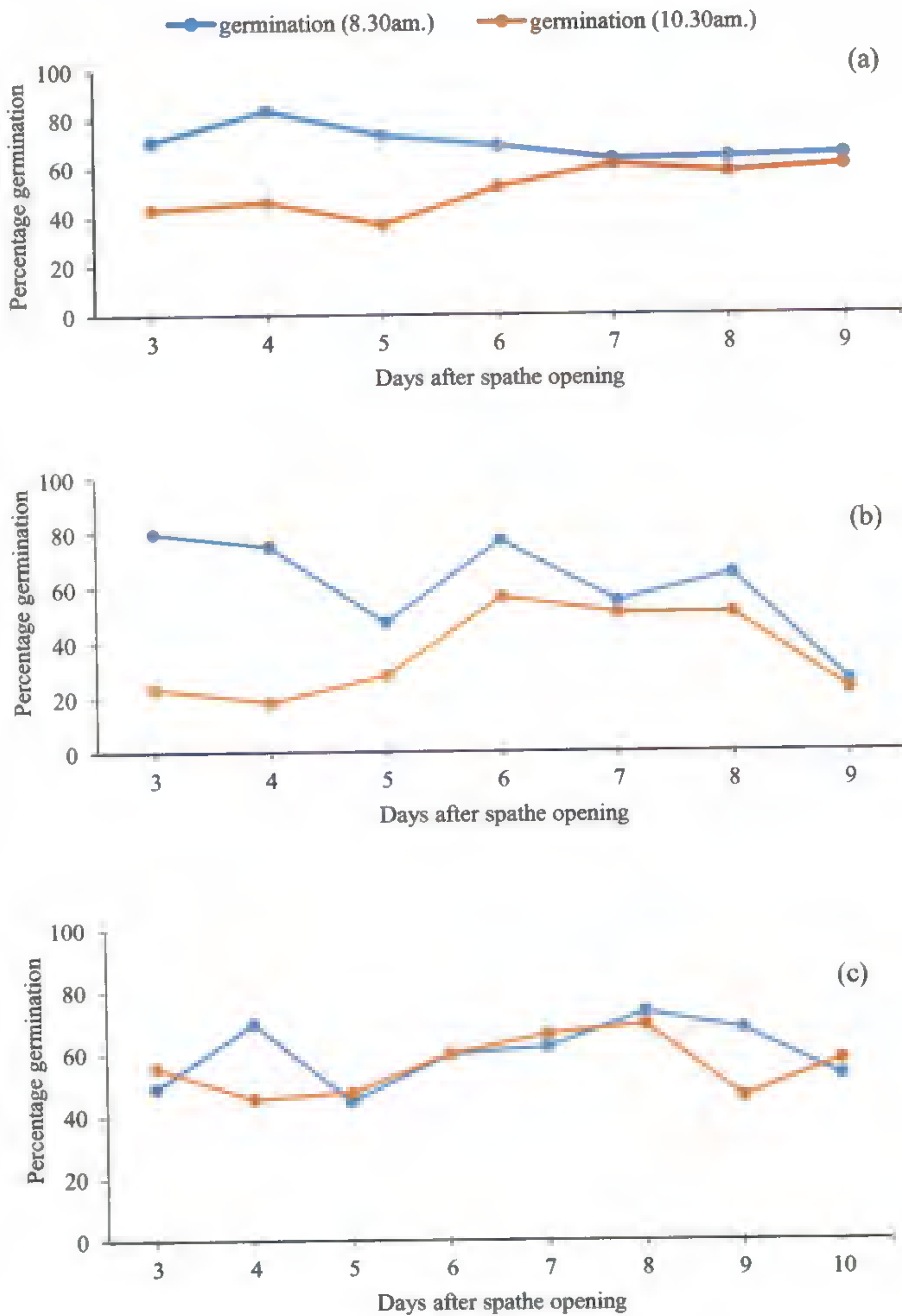


Fig. 21 Pollen germination percentage of flowers collected at 8.30 and 10.30am. from the day of spathe opening to a period of 10 days in (a) MYD, (b) COD and (c) COD x WCT.

60

From the Fig. 20(a), 20(b) and 20(c), it is clear that the flower opening pattern varies quite distinctly with genotypes. High number of open flowers were seen around 4 to 6 day in MYD, 2 to 5 day in COD and 1 to 4 day in COD x WCT after opening of the spathe. In dwarfs, being mostly self – pollinated, the male flowers begin opening even before the spathe is completely open and the inflorescence is exposed. Hence, it may be concluded that more flowers mature during the initial stages during the male phase of an inflorescence.

Aldaba (1921) has observed that the male flowers mostly open early in the morning, shed pollen throughout the morning hours and fall off by midday. In the present study, it was observed that flower opening was late in dwarfs MYD and COD, between 8.30am. and 10.30am. This agrees with the findings of Menon and Pandalai (1958), who observed that most of the male flowers open between 8am. and 10am. But in hybrids, the time of maximum flower opening was early and extended from 6.30 to 8.30am. There have been no reports of genotypic variation in the time of flower opening. The variation observed in the case of hybrids may be a consequence of reduction in sensitivity to temperature. However, in our future studies, we selected flowers opened between 6.30am. and 8.30am.

Pollen obtained from the flowers collected at 8.30am. and 10.30am. were used for the germination studies. Similar to the variation observed in open flowers collected across the genotypes, there was a quite good variation in germination of pollen (Fig. 21(a),(b),(c)). In general, germination was high between 6 - 8 days after spathe opening. In the dwarfs MYD and COD, germination was high in the flowers which opened between 6.30am. and 8.30am. compared to those that opened between 8.30am. and 10.30am. However, 6 to 7 days after spathe opening, this difference was reduced. In hybrids, on the other hand, there was not much difference between these two timings. But hybrids too showed a trend of low germination during early days after inflorescence opening. Earlier, Nampoothiri (1970) also showed similar finding in coconut. Female flowers generally remain receptive for 3- 5 days. Based on the above findings, in rest of the studies, the pollen collected at 6 – 8 days of flower opening were used.

61

4.2 POLLEN GERMINATION

The pollen collected from the genotypes COD and WCT were subjected to germination studies and the results are presented in Fig. 22.

As can be observed from the Fig.ure, the germination of pollen collected from fresh flowers at 8.30am. was found to be higher than that of pollen collected at 6.30am. and 10.30am. in both the genotypes tested. Hence, pollen collected from fresh flowers collected at 8.30am. were used for further studies.

4.3 IDEAL GERMINATION MEDIUM

The percentage pollen germination of WCT and COD were evaluated in five different germination media of the following compositions:

M1 – 6% Sucrose; 0.01% Boric acid; 1% Agar

M2 - 8% Sucrose; 0.01% Boric acid; 1% Agar

M3 - 10% Sucrose; 0.01% Boric acid; 1% Agar

M4 - 10% Sucrose; 0.01% Boric acid; 0.05% Manganese sulphate; 0.005% Potassium nitrate; 0.01% Magnesium sulphate; 1% Agar

M5 - 10% Sucrose; 0.01% Boric acid; 0.025% Calcium nitrate; 0.02% Magnesium sulphate; 0.01% Potassium nitrate; 1% Agar

The following observations were made:

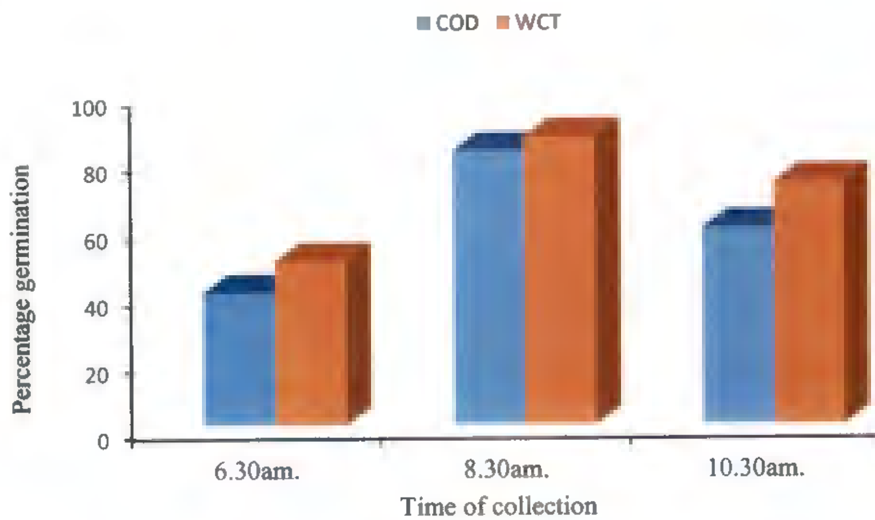


Fig. 22 Percentage germination in pollen of WCT and COD collected at 6.30, 8.30 and 10.30am.

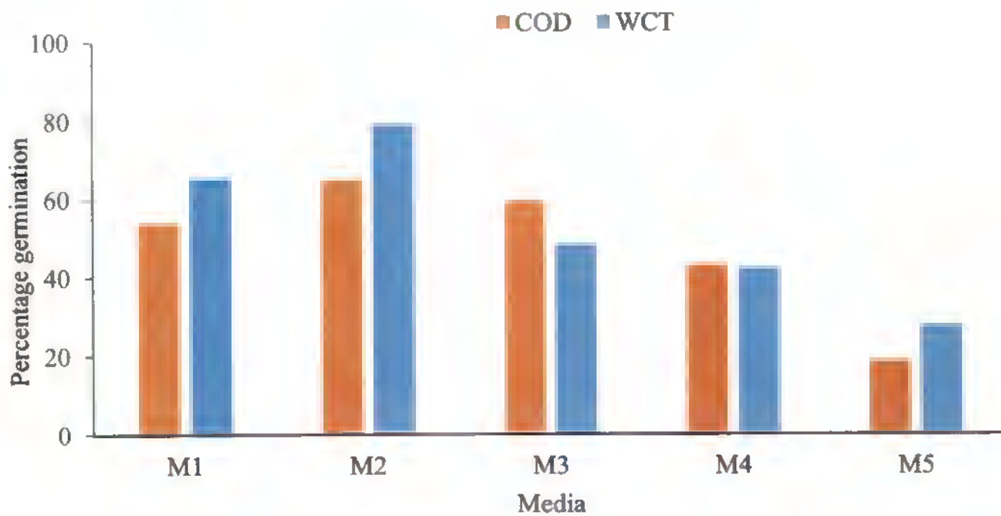


Fig. 23.Percentage germination of COD and WCT pollen in various germination media

174033

The germination percentage was the highest in M2 containing 8% sucrose, 0.01% boric acid and 1% agar for both COD and WCT (Fig. 23). Hence, further studies were conducted using the above medium.

At higher concentrations of sucrose and the addition of calcium, magnesium, manganese and potassium salts, pollen and pollen tube bursting was observed. This could be because of the imbalance in osmotic potential. Sucrose in the germination medium has the dual purpose of acting as a substrate and an osmoticum, as proposed by Shivanna and Johri (1989) and Johri and Vasil (1961). But it can enter pollen and increase the concentration of the intracellular material. It may also lead to the leaching of metabolites and ions into the germination medium by increasing the permeability of the pollen tube (Taylor and Hepler, 1997).

4.4 POLLEN RESPONSE TO TEMPERATURE RANGES

Percentage pollen germination of WCT and COD incubated on germination medium for two hours at temperatures ranging from 15°C - 50°C was observed and is presented in Fig. 24. In both the genotypes, pollen germination was negligible at 15°C. It increased to 40% in COD and 57% in WCT at 20°C. Maximum germination of 50% and 67% was observed at 25°C and 30°C for COD and WCT respectively. Beyond this temperature, there was a steep decline in germination in both the varieties. At 40°C, germination declined to 8 and 16% in COD and WCT respectively.

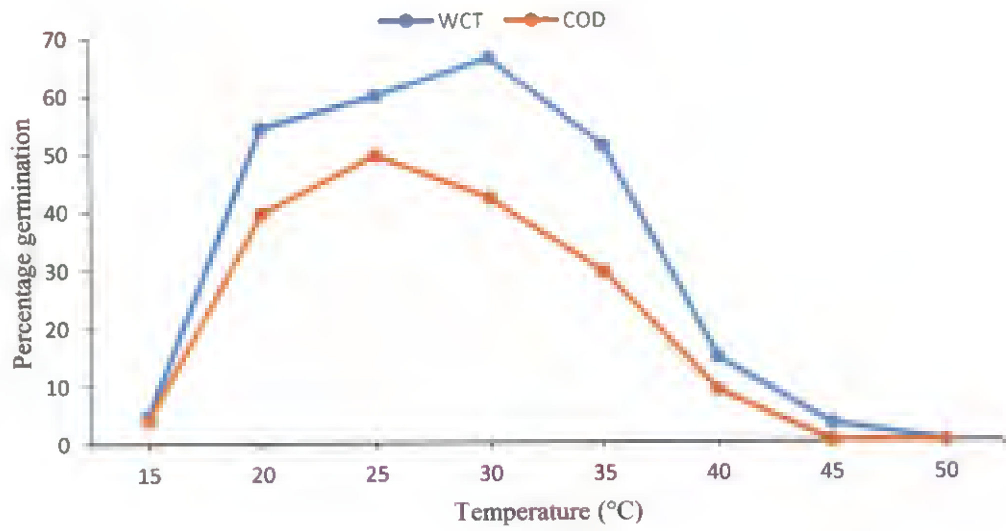


Fig. 24 Percentage pollen germination of COD and WCT at various temperatures

Hence, it can be concluded that coconut pollen have a base temperature of 15°C, below which germination is hardly possible. It is similar to most other species of Cotton (Reddy and Kakani, 2007) and Groundnut (Kakani *et al*., 2002). Ranasinghe *et al.* (2010) obtained a germination of around 12% for pollen incubated at 20°C for 24 hours, collected in October. In the present study, germination rates were in the range of 30% to 50% when fresh pollen collected in March were exposed to the same temperature. However, the experimental details of the above study is not available. Based on the above understanding in order to screen genotypes, five temperatures, 20°C, 25°C, 30°C, 35°C and 40°C were selected.

4.5 GENOTYPE AND TEMPERATURE EFFECT ON POLLEN GERMINATION AND VIABILITY

4.5.1. Percentage pollen germination

Percentage pollen germination of 11 genotypes was tested at temperatures ranging from 20°C - 40°C.

Percentage germination of genotypes across the temperatures studied showed significant difference (Fig..25). Germination was high in the hybrid COD x WCT (47%), and talls WCT (50%) and was the least in dwarfs MYD (13%). The mean value was 34.5%; all the talls and hybrids had higher percentage germination while dwarfs except COD had lower germination percentage from the mean.

Similarly, germination at different temperatures averaged over all genotypes showed a significant difference (Fig. 26). Germination was on par at 25°C and 30°C. It decreased to 37% and 36% at 20°C and 35°C respectively. It was the least at 40°C.

Though genotype x temperature effect was non – significant, still wide variation was observed in genotypic response to different temperatures for pollen germination (Table 3).

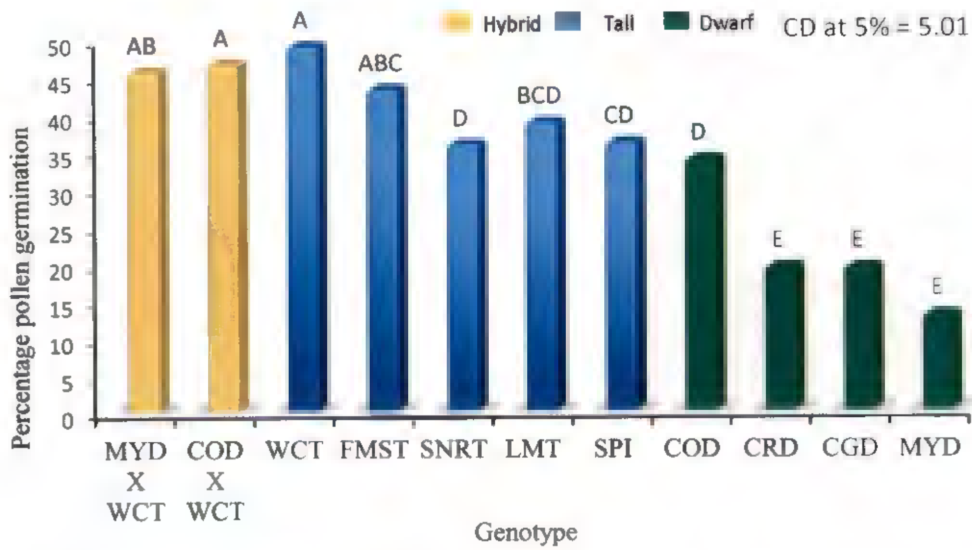


Fig. 25. Mean percentage pollen germination of various genotypes averaged over the studied temperature range

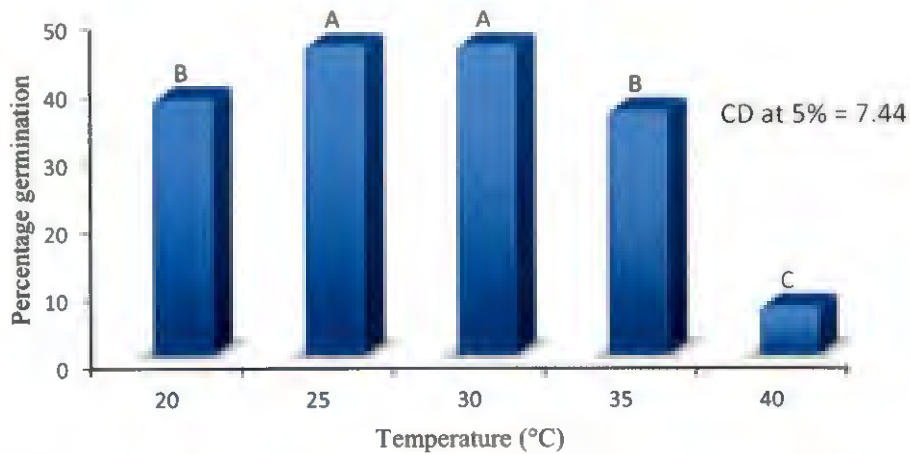


Fig. 26. Percentage pollen germination at different temperatures averaged over all genotypes

Table 3 Percentage pollen germination of coconut genotypes across different temperatures

Genotypes	Temperature (^{0}C)					Mean
	20	25	30	35	40	
CGD	18.08	28.44	29.82	18.95	0.89	19.24^{E}
COD	39.37	49.34	41.83	29.16	8.76	33.69^{D}
CRD	11.25	27.99	28.34	27.68	1.23	19.29^{E}
COD x WCT	58.3	64.32	57.11	45.23	7.87	46.57^{A}
FMST	50.06	56.64	52.69	47.08	9.24	43.15^{ABC}
LMT	44.22	46.07	51.57	44.31	7.19	38.67^{BCD}
MYD x WCT	49.31	61.73	60.38	44.27	12.35	45.61^{AB}
MYD	9.94	17.01	22.74	14.52	0	12.85^{E}
SNRT	42.19	46.23	43.7	35.83	10.54	35.69^{D}
SPI	40.09	45.72	48.54	39.41	6.11	35.98^{CD}
WCT	54.01	59.53	66.08	50.73	14.47	48.97^{A}
Mean	37.89^{B}	45.73^{A}	45.71^{A}	36.11^{B}	7.1517^{C}	34.52
CD at 5%						
Genotype	7.44					
Temperature	5.01					
GxT	NS					

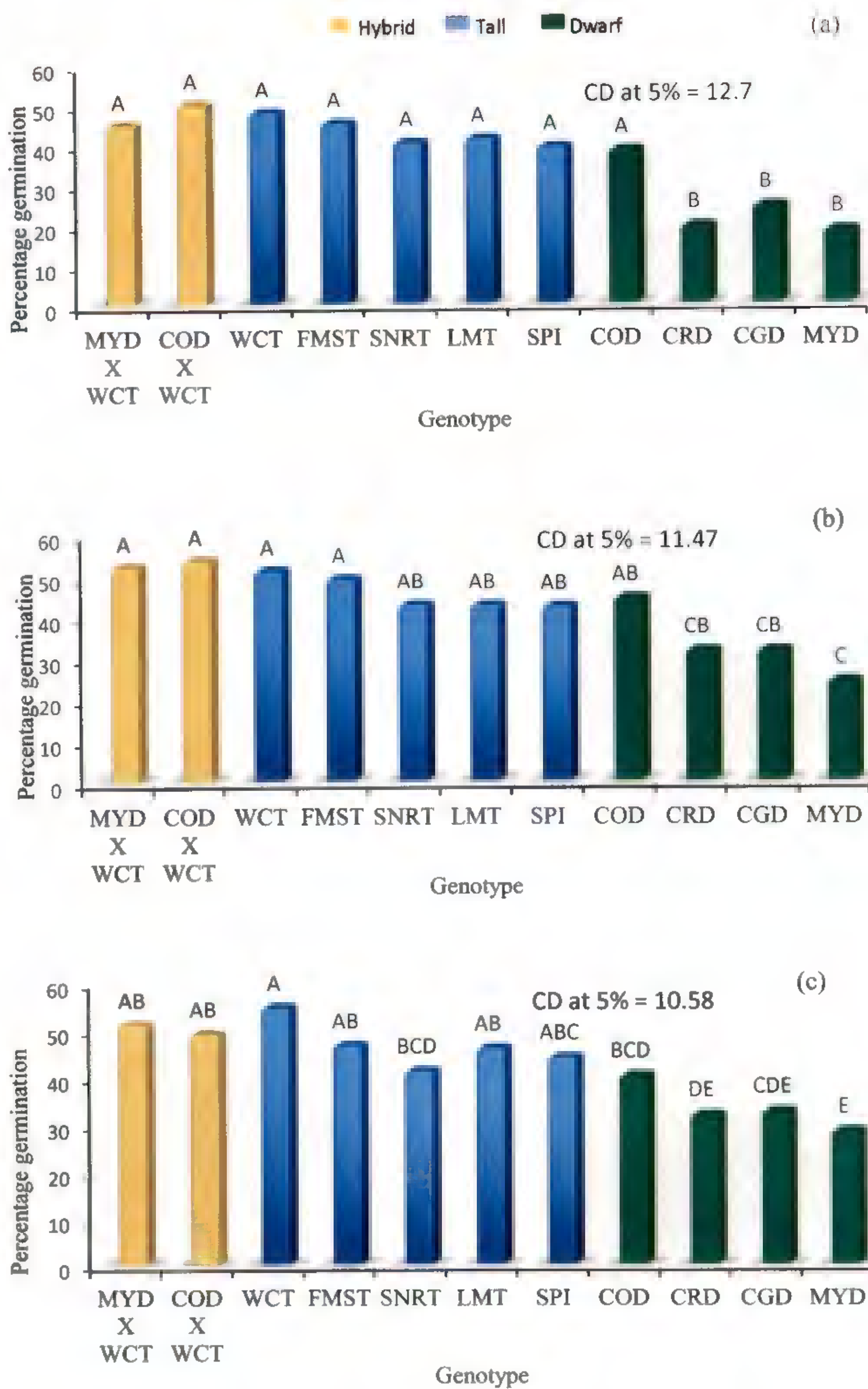


Fig. 27. Percentage pollen germination of different genotypes at (a) 20°C, (b) 25°C (c) 30°C

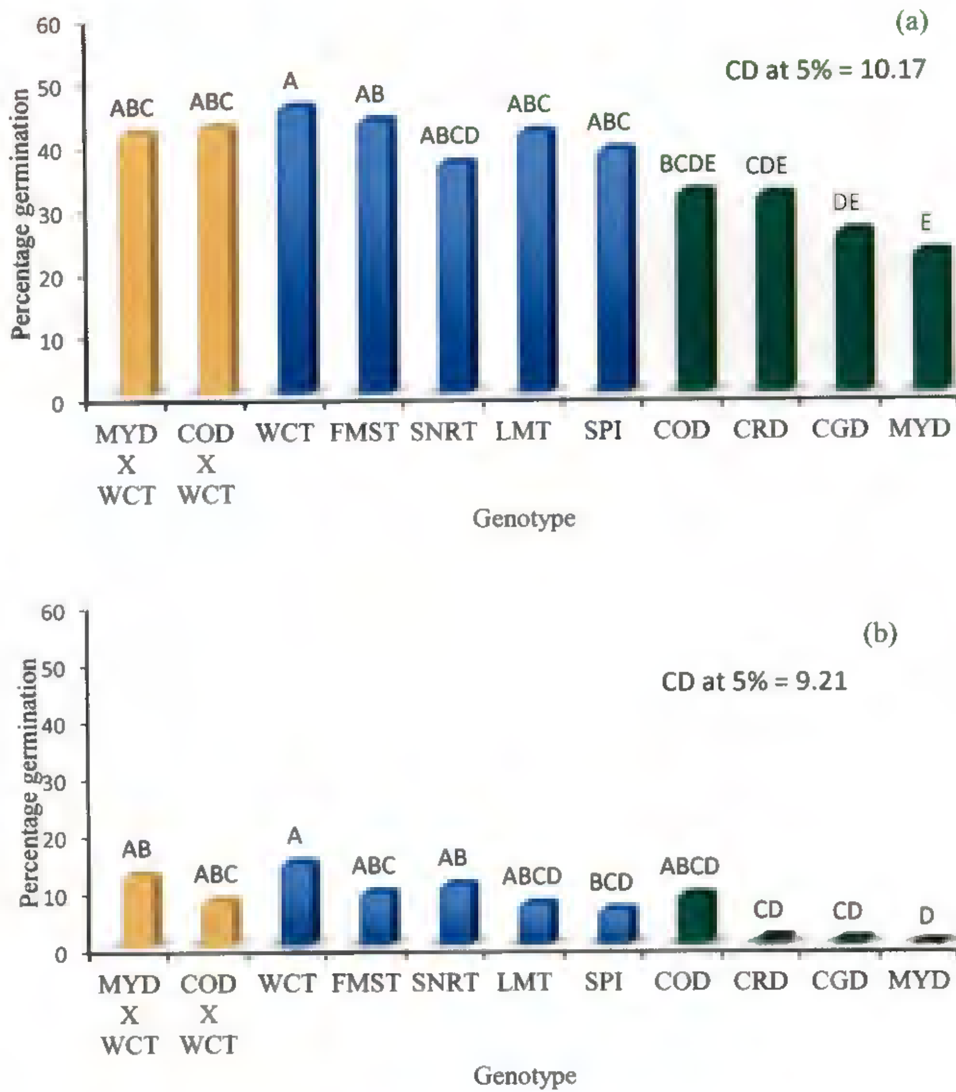


Fig. 28. Percentage pollen germination of different genotypes at (a) 35°C (b) 40°C.

Both lower and higher temperatures from optimum, the difference in %PG between dwarfs and rest of the cultivars (talls and hybrids) widened. The decrease was more in dwarfs.

At 20°C, dwarf varieties CRD, CGD and MYD showed significantly reduced germination than the other varieties, while it was non – significant for talls and hybrids (Fig. 27(a)). The mean %PG of dwarfs except COD reduced by 23% from the rest of the genotypes. The mean germination was 38%.

At 25°C, hybrids and the tall varieties WCT and FMST had significantly high germination compared to the rest of the talls and dwarfs (Fig. 27(b)). It was the least in MYD. The mean of %PG of dwarfs except COD reduced by 17.97% from other genotypes. At 25°C, the mean germination increased to 46% from 38% of 20°C.

At 30°C, the difference between the genotypes for %PG narrowed. WCT had the highest germination (55%) followed by hybrids and the talls FMST and LMT. MYD had the lowest germination of 28.34% (Fig. 27(c)).

At 35°C, the mean %PG reduced to 36% from 46% of 30°C. Almost similar response was seen between temperatures 20 and 25°C. WCT recorded the highest germination followed by FMST (Fig. 28(a)). It was low in dwarfs and was the least in MYD (22.3%).

Distinct difference in %PG was seen at 40°C. WCT showed the highest germination followed by MYD x WCT and SNRT. It was too low in dwarfs except COD and was zero in MYD (Fig. 28(b)). The mean germination of all genotypes reduced to 7% at 40°C from 35°C.

4.5.2 Pollen tube length

PTL of 11 genotypes was evaluated across temperatures ranging from 20°C - 40°C.

Mean PTL showed significant variation among the genotypes studied across the temperature range (Fig. 29). Highest mean PTL was recorded for the hybrid COD x WCT (408.9μm). All talls and hybrids showed significantly higher

PTL compared to dwarfs. The mean PTL of all genotypes across all temperatures was found to be 343.97μm. Dwarfs showed values much lower than the mean with MYD having the lowest at 180.82μm.

This could be explained by the fact that dwarfs are predominantly self - pollinated and the pollen need to travel a shorter distance to effect pollination and take relatively less time than the pollen of talls and hybrids. Thus, the lesser viability might be a consequence of evolution for shorter periods of exposure to the environmental conditions. Observations by Pacini *et al.* (1997), in two anemophilous species, *Festuca arundinaceae* and *Mercurialis annua,* where the pollen flight distance is very less, confirm that the pollen are short lived.

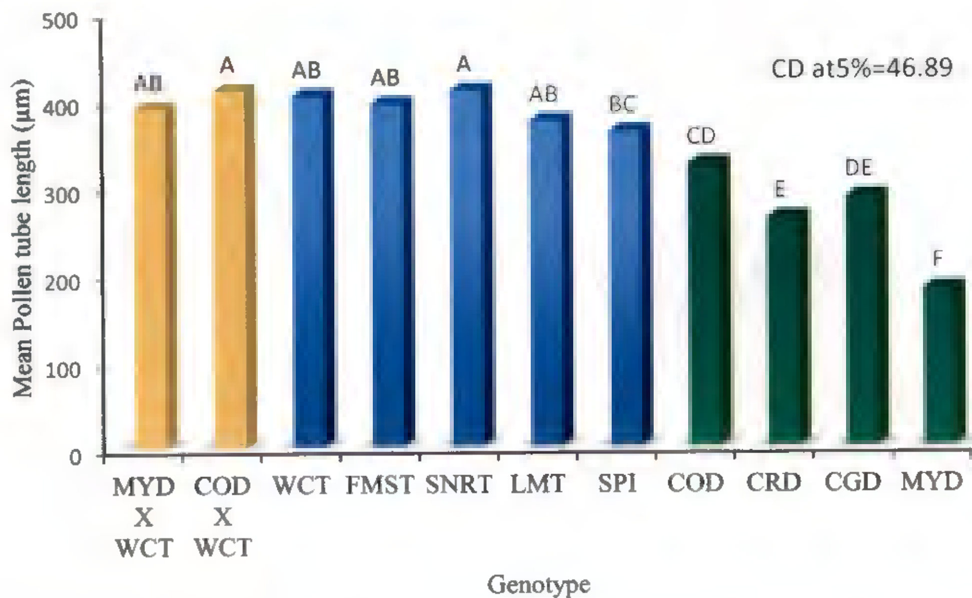


Fig. 29. Mean pollen tube length of various genotypes averaged over the studied temperature range (20°C - 40°C).

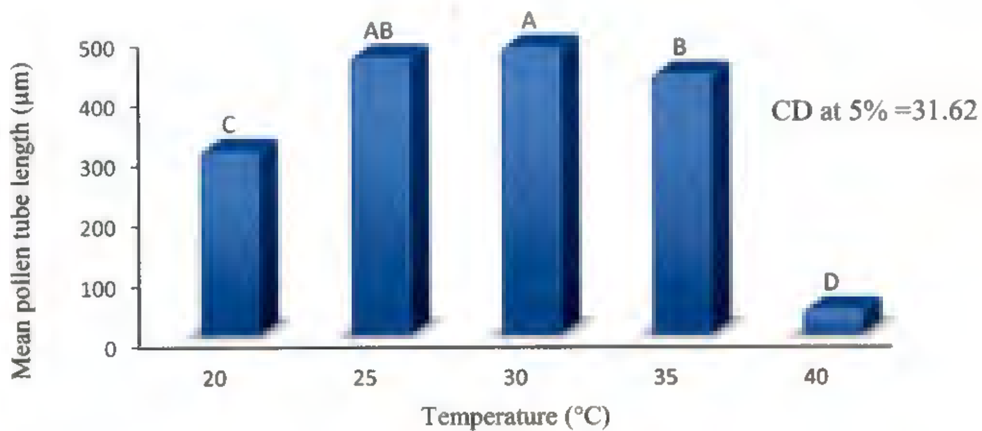


Fig. 30. Mean pollen tube length at different temperatures, averaged over genotypes.

Similar to the trend observed with %PG, mean PTL at different temperatures averaged for all genotypes showed significantly higher values at 25 and 30°C, 462 and 478µm respectively (Fig. 30). Both at 20°C and 35°C, there was a significant decline in length by 35.92% and 9.5% from 30°C. Beyond this, the mean tube length underwent a steep decline to 41.5µm at 40°C. This suggests that generally for coconut, %PG as well as PTL are equally affected by sub optimum temperatures. It can also be concluded that while low temperatures arrest or slow down the growth of the pollen tube, high temperatures don't allow the pollen to produce pollen tubes. There is a possibility of the breakdown of cellular metabolism within the pollen grain when exposed to high temperatures and the leaching of cytoplasmic content due to rupture of the cell membrane.

Table 4 Mean pollen tube length of coconut genotypes across different temperatures.

	Temperature ^{0}(C)					
Genotypes	**20**	**25**	**30**	**35**	**40**	**Mean**
CGD	130.17	420.71	498.88	362.29	14.77	285.36^{DE}
COD	319.93	471.69	424.63	352.22	48.58	323.41^{CD}
CRD	118.93	334.35	391.96	441.77	22.1	261.82^{E}
COD x WCT	455.18	545.21	535.83	413.84	94.56	408.92^{A}
FMST	360.15	513.6	538.11	513.12	38.42	392.68^{AB}
LMT	330.04	503.77	509.78	503.47	15.88	372.59^{AB}
MYD x WCT	422.3	505.65	518.94	390.58	102.95	388.08^{AB}
MYD	131.64	218.72	281.9	271.84	0	180.82^{F}
SNRT	394.26	522.55	520.95	572.6	27.7	407.62^{A}
SPI	330.27	505.33	466.89	448.3	51.6	360.48^{BC}
WCT	374.19	541.54	567.01	485.9	40.56	401.84^{AB}
Mean	306.09^{C}	462.11^{AB}	477.72^{A}	432.36^{B}	41.56^{D}	343.97
CD at 5%						
Genotype	46.89					
Temperature	31.62					
GxT	104.86					

The interaction effect of genotype x temperature for pollen tube length was significant (Table 4). At low temperature of 20°C and high temperatures of 35°Cand 40°C, dwarfs except COD showed a steep decline in their tube length. In all the temperatures, it was the least for MYD.

4.6 RESPONSE OF TALLS, DWARFS AND HYBRIDS

The pollen germination of genotypes were analyzed for individual groups of talls, dwarfs and hybrids, as shown in Fig. 31.

In talls, at 20°C, 25°C, 30°C and 35°C, there was no significant difference in germination. However, at 40°C it was significantly reduced.

Dwarfs, on the other hand, had significantly low germination at 20°C and 35°C compared to the germination of 25°C and 30°C. At 40°C, it was drastically reduced.

Hybrids had high germination at 25°C and it decreased significantly at 30°C. It was significantly low at 20°C and 35°C and steep decline was seen at 40°C.

So a clear contrast is seen between talls and dwarfs in terms of pollen germination at high temperatures. Dwarfs are found to be more susceptible to high and low temperature stress. Hybrids showed a temperature tolerance in between talls and dwarfs. Although the steep decline at 40°C continued for hybrids also, it was higher than that of dwarfs, showing that the maximum temperature of pollen germination for hybrids will be above 40°C. The genotypes chosen for the study can be ranked according to their increasing tolerance to high temperature stress as Dwarfs < Hybrids < Talls.

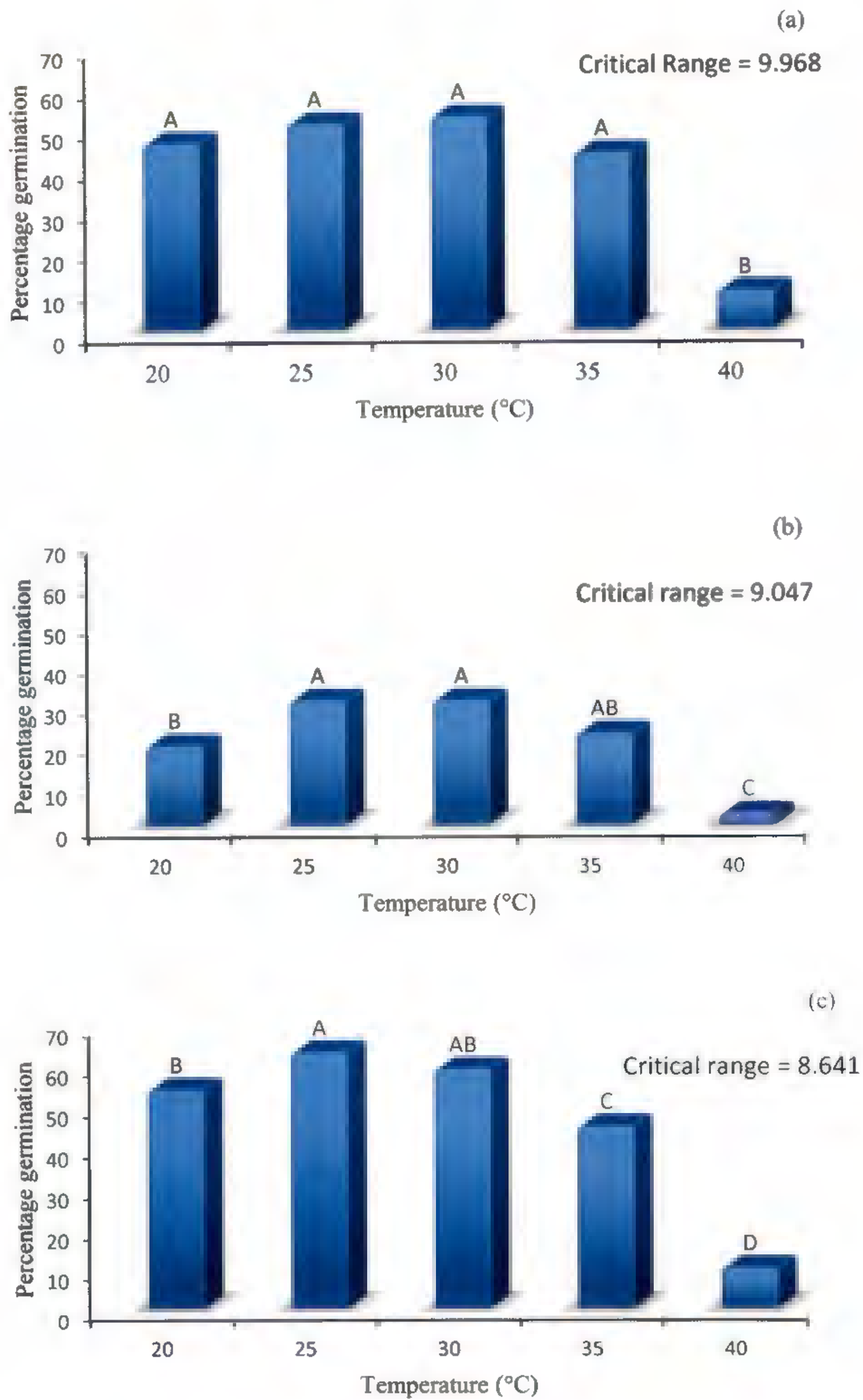


Fig. 31 Percentage pollen germination at different temperatures, averaged over (a) talls, (b) dwarfs and (c) hybrid genotypes

Genotypes among these groups viz. talls, dwarfs and hybrids too showed wide variability for %PG across temperatures. Tall variety WCT had high pollen germination at all the temperatures, followed by FMST. Hybrid MYD x WCT performed better than COD x WCT at high temperature. The dwarf COD was the best at all temperatures. Similar kind of genotypic variation was seen in other crops like cotton (Kakani *et al.*, 2005) and Pistacia (Acar and Kakani, 2010) under field condition.

4.7 OPTIMUM TEMPERATURES FOR POLLEN GERMINATION AND POLLEN TUBE LENGTH

The optimum temperature required for pollen germination across the genotypes ranged from 24.55°C to 29.31°C with a mean of 27.28°C (Table 5). This is similar to the optimum temperature (27°C) required for the growth of coconut (Child, 1964). However, the interesting fact is that there exists an optimum temperature variability across the genotypes for pollen germination. This data is useful while evolving varieties for those areas where the temperature is expected to rise under climate change. Tall variety LMT (29.31°C), hybrid MYD x WCT (29.02°C) and dwarf MYD (29.11) had high optimum temperature for germination. COD, COD x WCT and CGD have relatively low temperature requirement.

Table 5 Optimum temperature for percentage pollen germination and the corresponding percentage pollen germination of coconut genotypes

Genotype	Optimum temperature for %PG	%PG at optimum temperature
MYD x WCT	29.02	62.81
COD x WCT	24.55	64.85
WCT	27.99	68.71
FMST	26.25	57.63
SNRT	26.15	46.81
LMT	29.31	52.33
SPI	27.36	50.02
COD	25.70	50.39
CRD	28.47	28.54
CGD	26.12	38.25
MYD	29.11	24.21
Mean	27.28	49.5

Table 6 Optimum temperature for pollen tube length and the corresponding pollen tube length of coconut genotypes

Genotype	Optimum temperature for PTL	PTL at optimum temperature
MYD x WCT	25.94	529.73
COD x WCT	29.06	552.83
WCT	27.39	580.30
FMST	29.95	538.34
SNRT	30.87	615.30
LMT	29.87	509.93
SPI	28.20	529.93
COD	27.63	496.47
CRD	31.06	481.01
CGD	28.64	520.14
MYD	33.63	289.19
Mean	29.29	513.02

Interestingly, the optimum temperature of the hybrid MYD x WCT was high (29.02°C) while it was low for COD x WCT. It was similar to the temperature requirement of MYD and COD. The optimum temperature of coconut hybrids MYD x WCT and COD x WCT was derived from maternal parents MYD and COD respectively, suggesting it as an inheritable trait and could be used in evolving tolerant varieties.

The optimum temperature for PTL ranged from 25.94°C to 33.63°C with a mean of 29.29°C (Table 6). It is 2°C more than the mean temperature required for pollen germination. MYD, CRD, SNRT and LMT required high temperature while MYD x WCT, COD and WCT required relatively low temperature.

4.8 PERCENTAGE REDUCTION IN GERMINATION AND POLLEN TUBE LENGTH

The percentage reduction in %PG and PTL from the optimum temperature to the lowest (20°C) and highest (40°C) temperature was calculated.

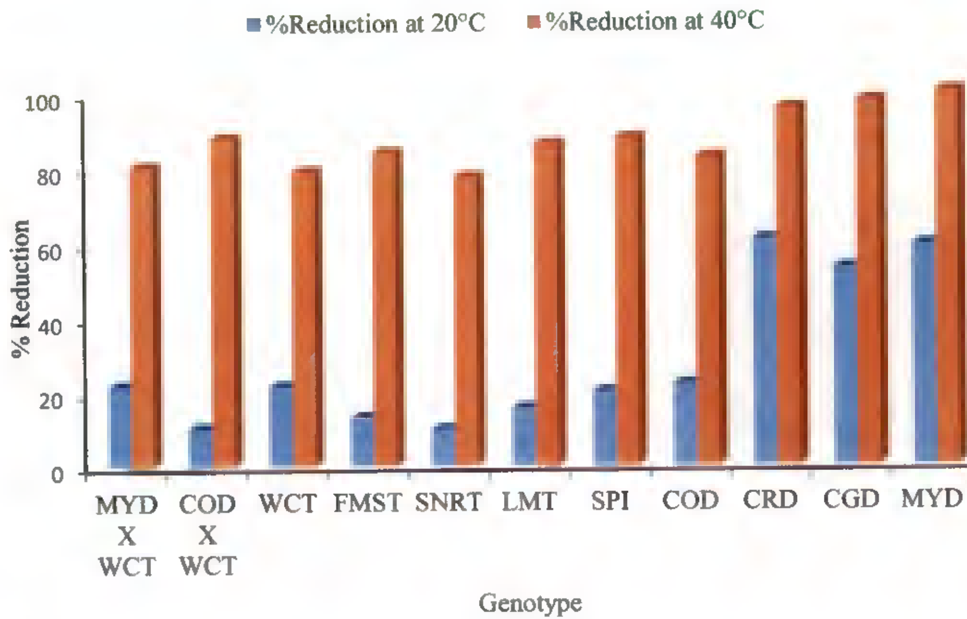


Fig. 32. Percentage reduction in pollen germination of different genotypes from optimum temperature to 20°C and 40°C

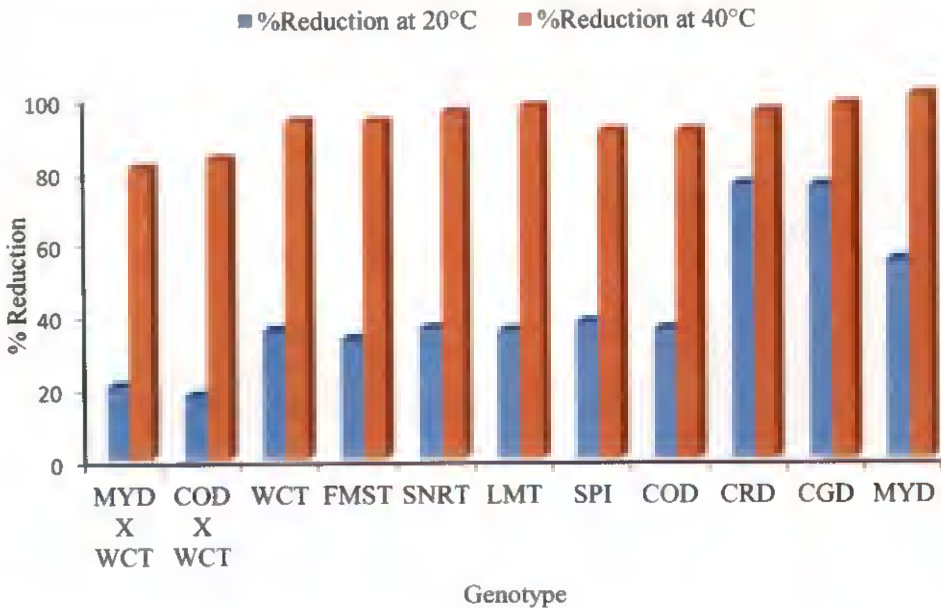


Fig. 33.Percentage reduction in pollen tube length of different genotypes from optimum temperature to 20°C and 40°C

From Fig. 32, it is clear that dwarfs except COD are more sensitive to low temperatures from optimum compared to hybrids and talls. At 20°C, germination of dwarfs reduced by 58 to 60%, hybrids 10 to 22% and talls 8 to 24%. Similar response was seen at high temperature, i.e. at 40°C. In dwarfs, the reduction ranged from 83% to 100%; talls 48% to 88% and hybrids 80% to 88%. On an average, the reduction was less in talls.

PTL response was almost similar to %PG (Fig. 33). At 20°C, reduction was high in dwarfs (36% to 75%) followed by talls (33% to 38%) and the least for hybrids (18% to 20%). At high temperature, percentage reduction was less for hybrids and high for talls and dwarfs.

4.9 BIOCHEMICAL PARAMETERS

The biochemical parameters related to stress response were estimated from the leaf tissue of two hybrids (MYD x WCT and COD x WCT) as well as two dwarfs (CGD and MYD). The data obtained was analyzed using SAS 9.3 software and the results obtained are summarized in Table 7.

The hybrids COD x WCT and MYD x WCT have higher content of superoxide dismutase and peroxidase compared to dwarfs. The increase in the amount of SOD and POD in heat stress induced leaves of fingered citron has been studied earlier by Chen *et al.* (2012). So the current observation indicates that the vegetative tissue of these varieties are also tolerant to oxidative stress. Of the four varieties studied, MYD had the lowest superoxide dismutase, peroxidase as well as epicuticular wax content. MYD also has the highest MDA content, which has been linked to the level of free radical damage to the cell membranes in earlier studies (Tommasino *et al.*, 2012). This agrees with the observations of low pollen germination under stressed conditions for MYD. The bursting of pollen grains at high temperature may be attributed to free radical damage. But cell membrane thermostability studies are required to confirm this. However, it can be inferred that though MYD has higher optimum temperature for pollen germination, its tolerance to high temperature stress both in terms of vegetative as well as

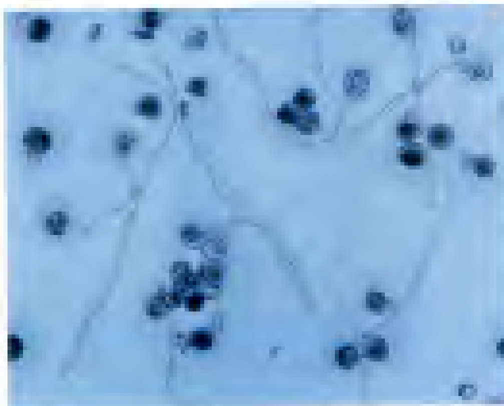
reproductive tissue is less compared to the other varieties studied. Hence, it is unlikely to thrive well in warmer future climates.

Fig. 19- 24 show the pollen germinating at various temperatures in the genotypes studied. From these, it can be clearly observed that low temperatures do not affect the integrity of the pollen tube membrane. It only affects the rate of pollen tube growth. But at temperatures higher than optimum for the respective genotype, bursting of pollen tube or pollen as such is observed in as less a duration as two hours. Since coconut pollen, under natural conditions, take four to six hours to germinate (Sreekrishna Bhat, 1990), prolonged conditions of high temperature during the pollination period will be detrimental to pollen germination and subsequent seed setting. The production of an effective seed set also depends on other factors like the presence of insect pollinators as well as the condition of the female flower when exposed to heat stress. These can be addressed in future studies to actually pin point the specific effects of high temperature stress on the reproduction of coconut. But pollen germination is an easier and faster screening method for heat tolerance in plants.

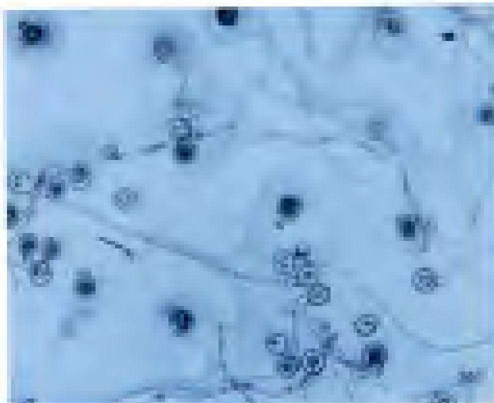
Table 7. SOD, POD, MDA, total phenol, ECW and chlorophyll content of leaf samples of four genotypes

Variety	SOD (mg /protein)	POD (s.act./ unit/g f.wt.)	MDA (nmol/ g.f.wt)	Phenol (mg/g)	ECW ($\mu g/cm^2$)	Chlorophyll (mg/g)
MYD×WCT	10.69^{A}	79.25^{A}	62.81^{A}	16.31^{A}	116.99^{B}	1.47^{A}
COD×WCT	8.98^{AB}	55.2^{B}	56.43^{B}	13.86^{B}	110.57^{B}	1.66^{A}
CGD	7.69^{BC}	27.72^{C}	38.28^{C}	13.73^{B}	132.45^{A}	1.66^{A}
MYD	6.42^{C}	21.4^{D}	63.12^{A}	14.18^{AB}	109.32^{B}	1.38^{A}
CD at 5%	1.98	4.07	6.51	2.42	9.85	0.42

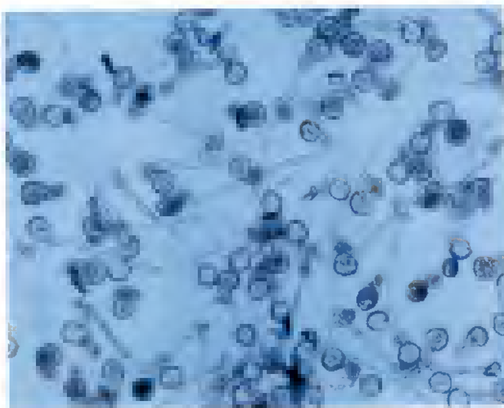
20°C



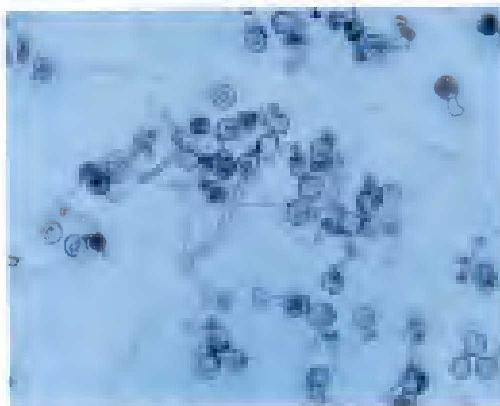
25°C



30°C



35°C



40°C

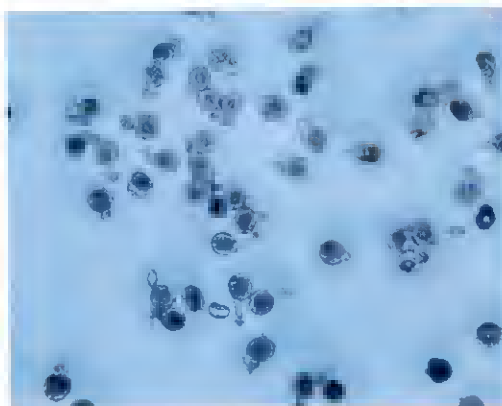
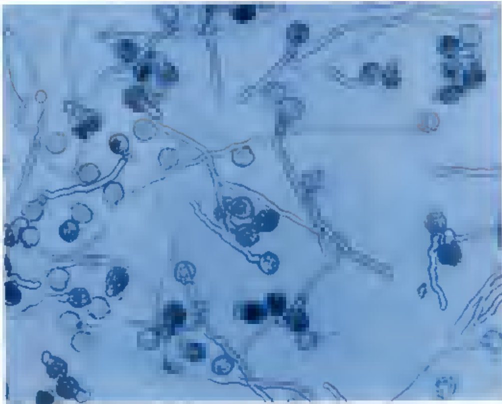
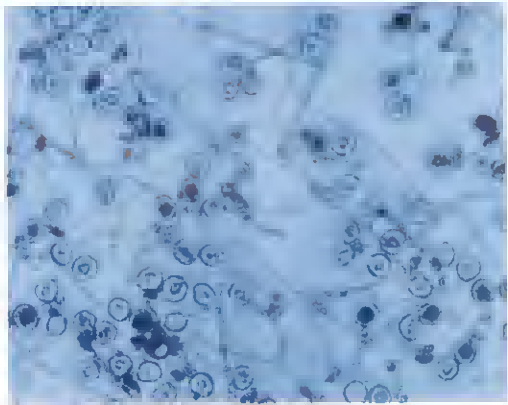


Fig.19 Bright field optical microscopic view (10X) of germinating pollen grains of MYD x WCT at the temperatures 20°C to 40°C

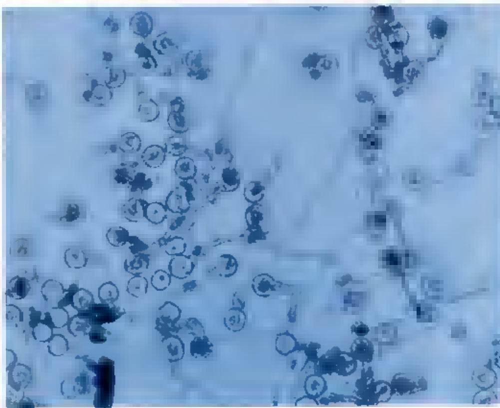
20°C



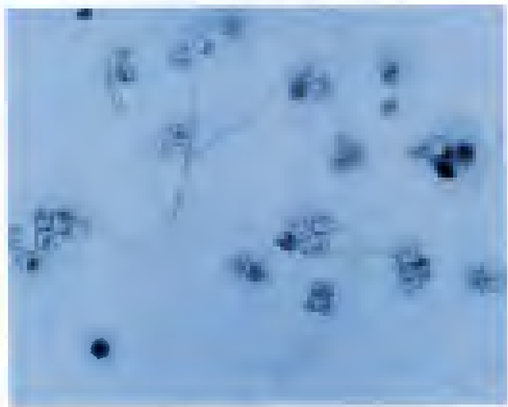
25°C



30°C



35°C



40°C

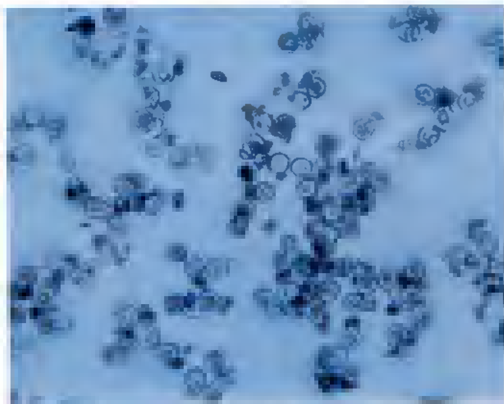
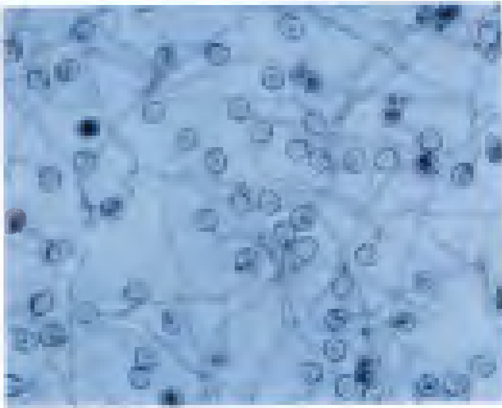
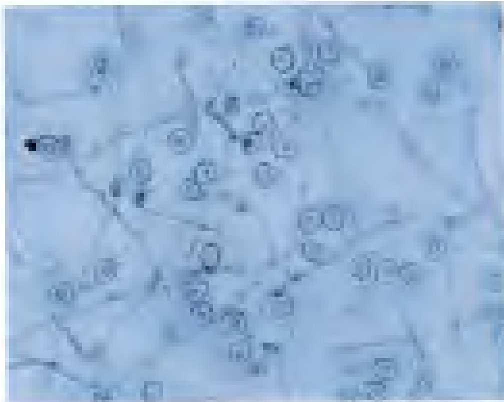


Fig.20 Bright field optical microscopic view (10X) of germinating pollen grains of COD x WCT at the temperatures 20°C to 40°C

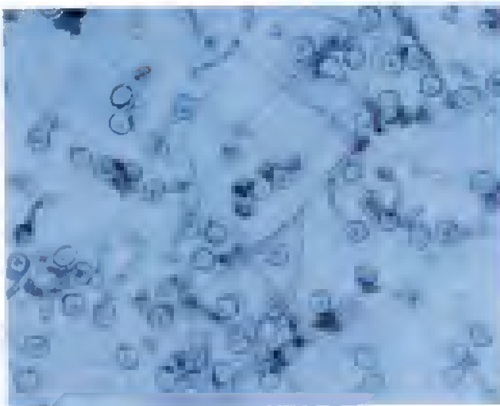
20°C



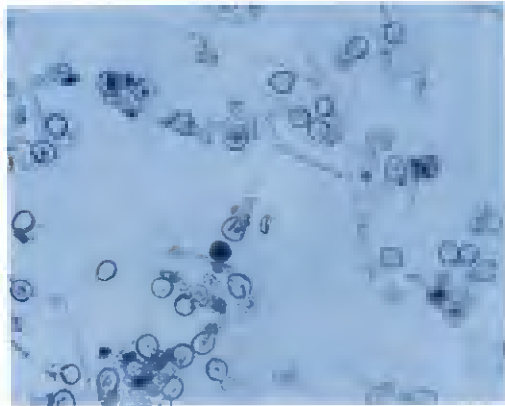
25°C



30°C



35°C



40°C

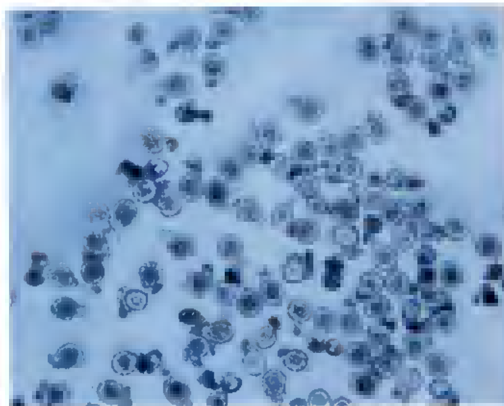
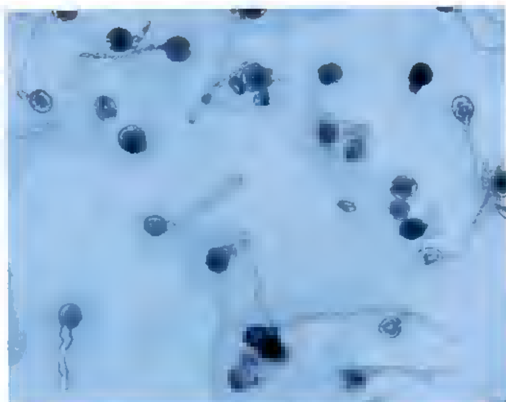
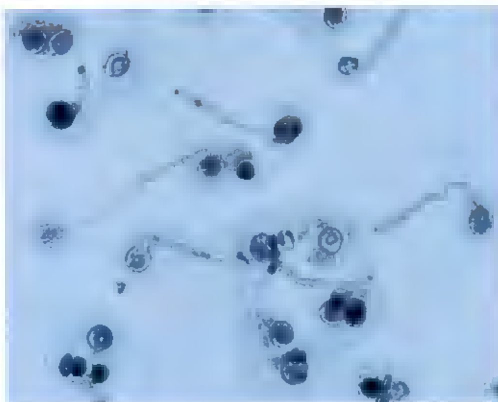


Fig.21 Bright field optical microscopic view (10X) of germinating pollen grains of WCT at the temperatures 20°C to 40°C

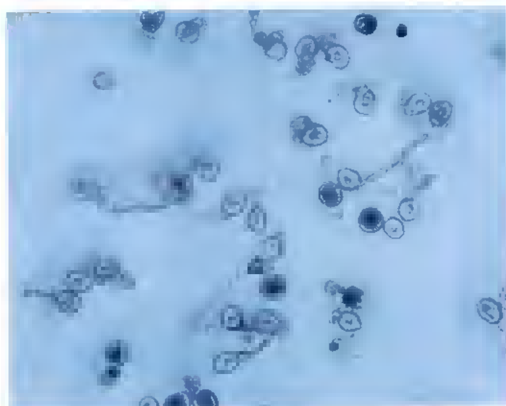
20°C



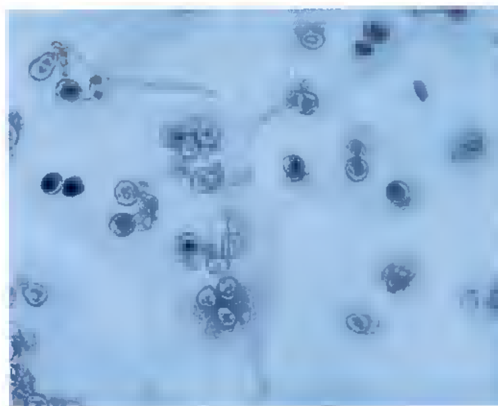
25°C



30°C



35°C



40°C

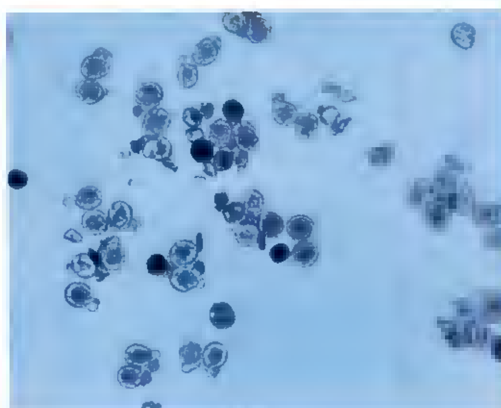
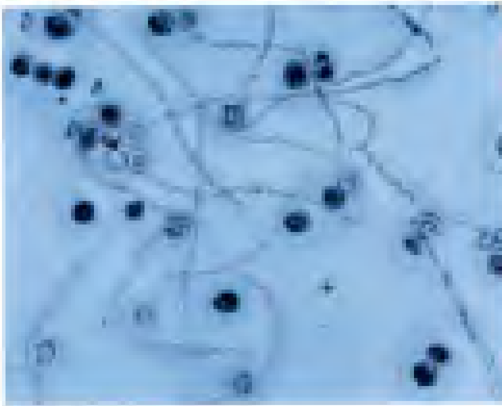
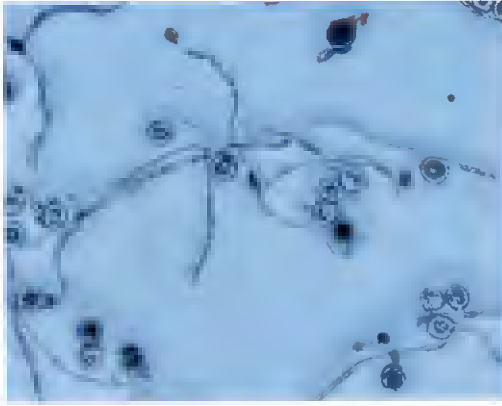


Fig.22 Bright field optical microscopic view (10X) of germinating pollen grains of LMT at the temperatures 20°C to 40°C

20°C

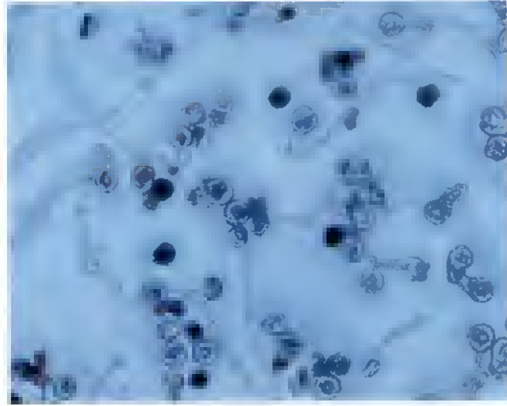


25°C



30°C

35°C



40°C

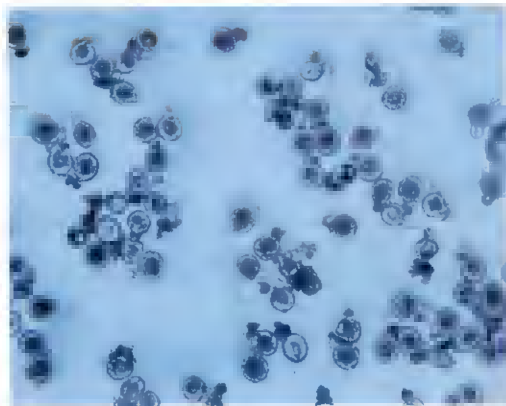
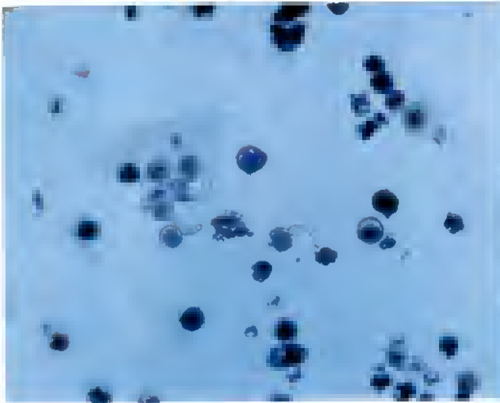
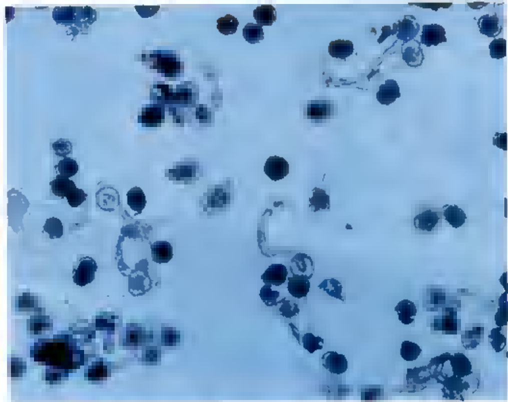


Fig.23 Bright field optical microscopic view (10X) of germinating pollen grains of COD at the temperatures 20°C to 40°C

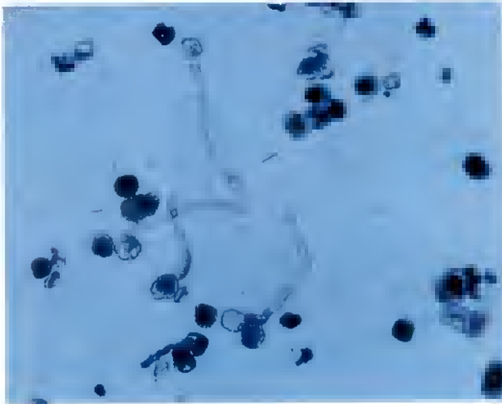
20°C



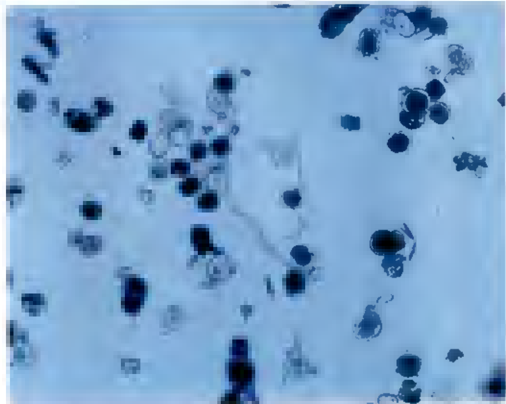
25°C



30°C



35°C



40°C

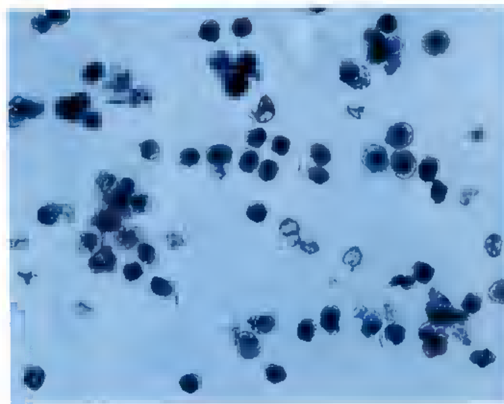


Fig.24 Bright field optical microscopic view (10X) of germinating pollen grains of MYD at the temperatures 20°C to 40°C

From the study it is clear that in coconut there is wide genotypic variability for pollen germination. Tall variety like WCT, FMST, hybrid MYD x WCT and to certain extent the dwarf variety COD showed comparatively high pollen germination at 40°C. Whereas most of the dwarfs had negligible germination at this temperature. In other field crops like Cotton (Kakani *et al*., 2005), Ground nut (Kakani *et al*., 2002) etc. the existing genetic variability is made use of to select or breed genotypes suitable for warmer areas or where there is the threat of high temperature event to happen under climate change. Hybrids have higher antioxidant potential like superoxide dismutase and peroxidase as compared to dwarfs and are found to better tolerate high temperature stress.

SUMMARY

An experiment was conducted at ICAR – CPCRI Farm, Kasaragod to study the effect of high temperature stress on the *in vitro* pollen germination of 11 coconut genotypes and to find the genotypes that are tolerant to high temperature stress. The selected genotypes consisted of both indigenous (WCT, SPI, LMT, COD, CGD) as well as exotic (SNRT, FMST, MYD, CRD) ones. Biochemical measurements in leaf tissue were also conducted. The major findings from the study are summarized below:

- High temperatures affected pollen germination and pollen tube growth negatively.
- There exists a genotypic variation in the response of pollen germination to high temperature.
- The tall variety WCT was found to have higher pollen germination at high temperatures compared to the other genotypes studied.
- Tall as well as hybrid varieties performed well under high temperature when compared to dwarfs.
- Temperatures lower than optimum slows down the germination of pollen and higher temperatures cause bursting of pollen as well as pollen tubes in the initial stages of its growth.
- The temperature requirement for maximum pollen tube length was found to be higher than that required for pollen germination, as has been observed in other crops.
- The optimum temperature for pollen germination of hybrids was found to be derived from their maternal parents.
- WCT among talls and COD among dwarfs, both indigenous genotypes and MYD x WCT among the studied hybrids were found to be superior in their performance at high temperatures under the experimental conditions. These may be used in future for evolving climate change tolerant genotypes for the region studied.

- Hybrids have higher antioxidant potential than dwarfs, suggesting that the vegetative tissue of hybrids is also tolerant to high temperature stress.

REFERENCES

Acar, I. and Kakani, V.G. 2010. The effects of temperature on *in vitro* pollen germination and pollen tube growth of *Pistacia* spp. *Scientia Horticulturae* 125: 569-572.

Adjaloo, M.K., Oduro, W. and Banful, B.K. 2012. Floral phenology of upper Amazon Cocoa trees: Implications for reproduction and productivity of Cocoa. *ISRN Agron.* 2012: 1-8.

Agar, O., Tan, D.K.Y., Barton, D.A. and Trethovan, R.M. 2015. Genetic variation in wheat pollen heat tolerance. "Building Productive, Diverse and Sustainable Landscapes", Proceedings of the 17th ASA Conference, Hobart, Australia.

Aldaba, V.C. 1921. The pollination of the coconut. *Phil. Agric.* 10:195-207.

Bar-Shalom, D. and Mattson, O. 1977. Mode of hydration, an important factor in the germination of trinucleate pollen grains. *Botanisk Tidsskrift bd.* 71: 245-251.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 44(1):276-87.

Bray, H. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. *Methods of Biochem. Anal.* 52: 1-27.

Brewbaker, J.L. and Kwack, B.H. 1963. The essential role of Calcium ion in pollen germination and pollen tube growth. *Am. J. Bot.*, 50(9):859-865.

Brink, R. A. 1924. The physiology of pollen. I. The requirements for growth. *Am. J. Bot.* 11: 218-228.

Burke, J. J., Velten, J and Oliver, M. J. 2004. *In vitro* analysis of cotton pollen germination. *Agron. J.* 96: 359-368.

Chempakam, B., Kasturi Bai, K.V. and Rajagopal, V. 1993. Lipid peroxidation and associated enzyme activities in relation to screening for drought tolerance in coconut (*Cocos nucifera* L.). *Plant Physiol. Biochem.* 20:5-10.

Chen, W.R., Zheng, J.S. and Li, Y.Q. 2012. Effects of high temperature on photosynthesis, chlorophyll fluorescence, chloroplast ultrastructure, and antioxidant activities in fingered citron. *Russ. J. Plant Physiol.* 59: 732.

Child, R. 1964. *Coconuts*. Longmans Green and Co., London.335p.

Dafni, A. and Firmage, D. 2000. Pollen viability and longevity: Practical, ecological and evolutionary implications. *Plant Syst. Evol.* 222: 113 – 132.

Ebercon, A., Blum, A. and Jordan, W. R. 1977. A rapid colorimetric method for epicuticular wax content of sorghum leaves. *Crop Sci.* 17: 179- 180.

Frankel, R. and Galun, E. 2012. *Pollination Mechanisms, Reproduction and Plant Breeding.* Springer Science & Business Media. 284p.

Fritz, S.E. and Lukaszewski, A.J. 1989. Pollen longevity in wheat rye and Triticale. *Plant Breed.* 102: 31-34.

Furtado, C.X. 1924. A study of the coconut flower and its relation to fruit production. *Gardens Bull.* Singapore. 3: 261-273.

Gajanayake, B., Trader, B.W., Reddy, K.R. and Harkess, R.L., 2011. Screening ornamental pepper cultivars for temperature tolerance using pollen and physiological parameters. *HortScience* 46, 878–884.

Harsant, J., Pavlovic, L., Chiu, G., Sultmanis, S. and Sage, T.L. 2013. High temperature stress and its effect on pollen development and morphological components of harvest index in the C3 model grass *Brachypodium distachyon. J. Exp. Bot.* 64(10): 2971–2983.

Heath, R.L. and Packer, L.1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*125:189–198.

Hedhly, A., Hormaza, J.I. and Herrero, M. 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). *Am. J. Bot.* 91:558-64.

Henderson, A. 1986. A review of pollination studies in the Palmae. *Bot. Rev.* 52:221-259

Hepler, P.K. and Winship, L.J. 2010. Calcium at the cell wall-cytoplast interface, *J. Integr. Plant Biol.* 52:147–160.

Heslop-Harrison, J. and Heslop-Harrison, Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Tech.* 45: 115-120.

Heslop-Harrison, J. and Heslop-Harrison, Y. 1992. Intracellular motility, the actin cytoskeleton and germinability in the pollen of wheat (*Triticum aestivum* L.) *Sex Plant Reprod.* 5: 247-255.

Heslop-Harrison, J., Heslop-Harrison, Y. and Shivanna, K. R. 1984. The evaluation of pollen quality and further appraisal of the fluorochromatic (FCR) test procedure. *Theor. Appl. Genet.* 67:367-379.

Imani, A., Barzegar, K., Piripireivatlou, S. and Masomi, S.H. 2011. Storage of apple pollen and *in vitro* germination. *Afr. J. Agric. Res.* 6(3): 624-629.

IPCC [Intergovernmental Panel on Climate Change]. 2007. *Climate Change 2007: Synthesis report. Fourth Assessment Report*. http;//www.ipcc.ch/pub.

Jack, H.W. and Sands, W.N. 1929 Observations of the dwarf coconut palm in Malaya. *Malay. Agric. J.* 17:140-170

Jay, S.C. 1974. Nectar and honey collection by honeybees from coconut flowers. *Bee World* 55:105-111

Johri, B. M. and Shivanna, K. R. 1977. Differential behavior of 2and 3-celled pollen. *Phytomorphology* (March): 98-106.

Johri, B. M. and Vasil, I. K. 1961.The Physiology of pollen. *Bot. Rev.* 27(3): 325-381

Juliano, J.B. and Quisumbung, E. 1931. Morphology of the male flower of *Cocos nucifera* L. *Phil. J. Sci.* 45: 449-445.

Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, P. V. V., Reddy, V. R. and 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.

Kakani, V.G., P.V.V. Prasad, P.Q. Craufurd, and T.R. Wheeler. 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.

Kapayla, M.I. 1991. Testing the age and viability of airborne pollen. *Grana* B 29: 430-433.

Kar, M. and Mishra, D. 1976. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiol.* 57: 315-319.

Karapanos, I. C., Akoumianakis, K. A., Olympios, C. M. and Passam, H.C. 2010. Tomato pollen respiration in relation to in vitro germination and pollen tube growth under favourable and stress-inducing temperatures. *Sex. Plant Reprod.* 23(3):219-224.

Karun, A., Sajini, K.K., Niral, V., Amarnath, C.H., Remya, P., Rajesh, M.K., Samsudeen, K., Jerard, B.A. and Engelmann, F. 2014. Coconut (*Cocos nucifera* L.) Pollen Cryopreservation. *CryoLetters.* 35(5): 407-417.

Kavand, A., Ebadi, A., Shuraki, Y.D. and Abdosi, V. 2014. Effect of calcium nitrate and boric acid on pollen germination of some date palm male cultivars. *Eur. J. Exp. Biol.* 4(3): 10- 14.

Khatum, S., Flowers, T.J. 1995. The estimation of pollen viability in rice. *J. Exp. Bot.* 46: 151-154.

Kidavu, M.G. and Nambiyar, E.K. 1925. *Pollination in coconut*. Yearbook, Dept. Agric., Madras.

98

Kumari, A., Komal, R., Rajesh, R. and Pandey, A. K. 2009. *In vitro* pollen germination, pollen tube growth and pollen viability in *Trichosanthes dioica* Roxb. (Cucurbitaceae). *Int. J. Plant Rep. Biol.* 1(2): 147- 151.

Kwack, B.H. 1967. Studies on cellular site of Calcium action in promoting pollen growth. *Plant Physiol.*, 20: 825-833.

Linskens, H.F. 1954. Biochemical studies about the incompatibility reaction in the style of *Petunia. Congr. Int. Bot. Paris.* 9: 146-147.

Linskens, H.F. 1964. Pollen physiology. *Annu. Rev.Plant Physiol.*, 15: 255-270.

Liu, L., Huang, L. and Li, Y. 2013. Influence of Boric Acid and Sucrose on the germination and growth of *Areca* pollen. *Am. J. Plant Sci.*4: 1669-1674.

Liyanage, D.V. 1950. Sex-life of the coconut palm. *Ceylon Coconut Quarterly* 1:33-35.

Liyanage, D.V. 1954. Controlled pollination of coconut palms. *Ceylon Coconut Quarterly*. 5(3): 135-139.

Lora, M. A. J. 2006. Low temperature storage and in vitro germination of cherimoya (*Annona cherimola* Mill) pollen. *Scientia Horticulturae*, 1(1):91-94.

Malhó, R. and Trewavas, A.J.1996. Localized apical increases of cytosolic free calcium control pollen tube orientation, *Plant Cell* 8:1935–1949.

Marechal, H. 1928. Observations and preliminary experiments on the coconut with a view to developing improved seednuts for Fiji. *Agric. J. Fiji* 1:16-45.

McWilliam, J.R. 1958. The role of the micropyle in the pollination of *Pinus. Botanical Gazette*.120:109–117.

Meinders, H.C. and Jones, M.D. 1950. Pollen shedding and dispersal in the castor plant *Ricinus communis. L. Agron. J.* 42:206-209.

Menon, K.P.V. and Pandalai, K.M. 1958. *Coconut: A Monograph*, Central Coconut Research Committee, New Delhi.

99

Michard, E., Alves, F. and Feijó, J.A.2009. The role of ion fluxes in polarized cell growth and morphogenesis: the pollen tube as an experimental paradigm, *Int. J. Dev. Biol.* 53: 1609–1622

Milatović, D., Nikolić, D. and Radović, A. 2016. The effect of temperature on pollen germination and pollen tube growth of apricot cultivars. *Acta Horticulturae*. 1139: 359-362.

Mortazavi, S.M.H., Arzani, A. and Moieni, A.J. 2010. Optimizing storage and *in vitro* germination of date palm (*Phoenix dactylifera*) Pollen. *Agric. Sci. Tech*. 12(2):181-189.

Moura, C. R. F., Machado, C. A. and Lédo, A. S. 2015. *In vitro* germination and viability of pollen grain of coconut accessions. *Revista Ciência Agronômica*. 46(2): 421-427.

Mulcahy, G. and Mulcahy, D. 1988. The effect of supplemented media on the growth in vitro for bi- and trinucleate pollen. *Plant Sci.* 55: 213-216.

Murray, D.B. 1977. Coconut palm. In: Alvim, P and Kozlowski, T.T.(eds.) *Ecophysiology of tropical crops*. Academic Press, New York. pp 387- 407.

Nampoothiri, K.U.K. 1970. Pollen studies in coconut with special reference to sampling procedure. *Indian J. Agri. Sci*. 40: 457-460.

Naresh Kumar, S. and Aggarwal, P.K. 2013. Climate change and coconut plantations in India: Impacts and potential adaptation gains. *Agric. Syst* 117: 45-54.

Naresh Kumar, S., Kasturi, B.K.V., Rajagopal, V. and Aggarwal, P.K. 2008. Simulating coconut growth, development and yield using info crop-coconut model. *Tree Physiol*. 28: 1049-1058.

Nepi, M. and Pacini, E. 1993. Pollination, pollen viability and pistil receptivity in *Cucurbita pepo*. *Ann. Bot.* 72: 527-536.

Niles, W. L. and Quesenberry, K. H. 1992. Pollen Germination of Rhizoma Peanut cv. florigraze. *Peanut Sci.* 19(2):105-107.

O'Kelley, J.C. 1955. External carbohydrates in growth and respiration of pollen tubes *in vitro*. *Am. J. Bot.* 42:322-327.

Pacini, E., Franchi, G.G., Lisci, M. and Nepi, M. 1997. Pollen viability related to type of pollination in six angiosperm species. *Ann. Bot.* 80: 83-87.

Palfi, G., Gulyas, S., Rajki, E.S. and Cseuz, L. 1988. The Proline test - a method to the demonstration of the tolerance of water deficiency and of frost - and to the qualification of pollens. *Acta Univ. Szeged. Acta Biol.*34: 11-25.

Patel, J.S. 1938. *The Coconut: A Monograph*. Madras, Government Press.

Peiris, T.S.G. and Kularatne, J.D.J.S. 2008. Assessment of climate variability for Coconut and other crops: A Statistical approach. *Cord* 24(1):35-53.

Qin, Y., Wysocki, R.J., Somoqyi, A., Feinstein, Y., Franco, J.Y., Tsukamoto, T., Dunatunga, D., Levy, C., Smith, S. and Simpson R. 2011. Sulfinylated azadecalins act as functional mimics of a pollen germination stimulant in Arabidopsis pistils. *Plant J* .68: 800–815.

Rajagopal, V., Rama Dasan, A., Kasturibai, K.V, and Balakrishna, D. 1989. Influence of irrigation on leaf water relations and dry matter production in coconut palms. *Irrig. Sci.*10: 73–81.

Ranasinghe, C. S., Waidyarathna, K. P., Pradeep, A. P. C. and Meneripitiya, M. S. K. 2010. Approach to Screen Coconut Varieties for High Temperature Tolerance by *in-vitro* Pollen Germination. *COCOS*. 19: 01-11.

Ratnambal, M. J., Nair, M.K., Muralidharan, K., Bhaskara Rao, E.V.V. and Pillai, R.V. 1995. *Coconut Descriptors Part I.* C.P.C.R.I., Kasaragod, Kerala, India.

Ratnambal, M.J., Niral, V., Krishnan, M. and Ravikumar, N. 2000. *Coconut Descriptors Part II,* CD- ROM, C.P.C.R.I., Kasaragod, Kerala, India.

Reddy, K.R. and V.G. Kakani. 2007. Screening Capsicum species of different origins for high temperature tolerance by *in vitro* pollen germination and pollen tube length. *Sci. Hort.* 112:130-135.

Rognon, F. and de Nuce de Lamothe, M. 1978. Harvesting and conditioning of pollen for the pollination of coconut seed gardens. *Oleagineux* 33:17-23.

Russell, S.D. 1982. Fertilization in *Plumbago zeylanica*: entry and discharge of the pollen tube in the embryo sac. *Can. J. Bot.* 60(11): 2219-2230

Salem, M. A., Kakani, V. G., Koti, S. and Reddy, K. R. 2007. Pollen - based screening of Soybean genotypes for high temperatures. *Crop Sci.* 47: 219 – 231.

Schmucker, T. 1932. Zur Blutenbiologie tropscher *Nymphaea* – Arten II. Bor alsentscheidender Faktor, *Planta* 18: 641- 650.

Sedgley, M. and Harbard, J. 1993. Pollen storage and breeding system in relation to controlled pollination of four species of *Acacia* (Leguminosae: Mimosoideae). *Aust. J. Bot.* 41(5):601 – 609.

Shivanna, K. R. and Johri, B. M. 1989. *The angiosperm pollen structure and function*. Willey Eastern Ltd. New Delhi.390p.

Shivanna, K.R and Rangaswamy, N.S. 1992. *Pollen Biology: A Laboratory Manual*. Berlin, Heidelberg: Springer-Verlag.

Shivanna, K.R. and Johri, B.M. 1985. *The Angiosperm Pollen structure and Function*. New Delhi: Wiley Eastern Ltd.

Shivanna, K.R. and Sawhney, V.K. 1995. Polyethylene glycol improves the *in vitro* growth of *Brassica* pollen tubes without loss in germination. *J. Exp. Bot.* 46(292): 1771-1774.

Sholdt, L. L .1966. Insects associated with the flowers of the coconut palm, *Cocos nucifera* L., in Hawaii. *Proc. Hawaii Entomol. Soc.* 19:293-296

Sholdt, L. L. and Mitchell, W.A. 1967. The pollination of *Cocos nucifera* L. in Hawaii. *Trop. Agric.* 44:133-142

Shukhvibul, N., Considine, J.A. 1993. Medium and long storage of *Anigozanthos manglesii* (D. Don) pollen. *N.Z. J. Crop. Hort. Sci.*

Sidhu, R.J.K., Malik, C.P. 1986. Metabolic role of boron in germinating pollen and growing pollen tubes. In: Mulcahy, D.L., Mulcahy, G.B., Ottavio, E. (Eds.) *Biotechnology and Ecology of Pollen*, Springer, New York. pp. 373-378.

Singh, S.K., V.G. Kakani, D. Brand, B. Baldwin, and Reddy, K.R. 2008. Assessment of cold and heat-tolerance of winter-grown canola cultivars by pollen-based parameters. *J. Agron. Crop Sci*.194:225–236.

Sorkheh, K., Shiram, B., Rouhi, V. and Khodambashi, M. 2011.Influence of temperature on the *in vitro* pollen germination and pollen tube growth of various native Iranian almonds (*Prunus L.* spp.) species. *Trees* 25:809–822.

Sreekrishna Bhat, S. 1990. Cyto- embryological investigations in dwarf coconuts (*Cocos nucifera L.)* M.Phil. thesis, Mangalore University.

Stephen, R., Naik, B. J., Haridass. 2004. *In vitro* pollen selection for water stress tolerance in coconut (*Cocos nucifera* L.). *J. Plant. Crops* 32(Supplement): 46-49.

Stone, J.L., Thomson, J.D., Dent-Acosta, S.J. 1995. Assessment of pollen viability in hand-pollination experiments: A review. *Am. J. Bot.* 82: 1186-1197.

Taylor, L.P. and P.K. Hepler. 1997. Pollen germination and tube growth. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:461–491.

Taylor, R.M. 1972. Germination of Cotton (*Gossypium hirsutum* L.) Pollen on an Artificial Medium. *Crop Sci.* 12(2):243- 244.

Tommasino, E., Griffa, S., Grunberg, K., Ribotta, A,. Lopez, C. E., Carloni, E., Quiroga, M., Luna, C.M. 2012. Malondialdehyde content as a potential biochemical indicator of tolerant *Cenchrus ciliaris* L. genotypes under heat stress treatment. *Grass and Forage Sci*. 1365-2494.

Tupy, J. 1960. Sugar absorption, callose formation and the growth rate of pollen tubes. *Biol. Plant* 2: 169-180.

Vasil, I.K. 1960. Studies on Pollen germination of certain Cucurbitaceae. *Am. J. Bot.* 47(4):239-247.

Vasil, I.K. 1964. Effect of boron on pollen germination and pollen tube growth. In: *Pollen Physiology and Fertilization*, Linskens HF (Ed.) North-Hall Publishing Company, Amsterdam.107- 119.

Vaughton, G., Ramsey, M. 1991. Floral biology and inefficient pollen removal in *Banksiana spinulosa* var. *neoangelica* (Proteaceae). *Aust. J. Bot.* **39**: 167-77.

Visser, T. 1955. Germination and storage of pollen. *Meded. Landb. Wageningen* 55: 1-68.

SCREENING OF COCONUT VARIETIES FOR TOLERANCE TO HIGH TEMPERATURE STRESS USING POLLEN VIABILITY TEST

By

HELEN MARY ROSE

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ABSTRACT OF THE THESIS

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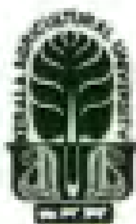

ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

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105

ABSTRACT

Temperature is the major factor that limits the growth and production of coconut in the humid tropics. Due to the current accelerated climate change, temperature is expected to rise at the rate of 0.2°C per decade. Coconut pollen, since they have to stay viable under field conditions from anthesis to pollination, are more vulnerable to the effects of high temperature. The present study evaluates the impact of high temperature stress on coconut through *in vitro* pollen germination technique and attempts to screen the selected genotypes for tolerance to high temperature stress. Pollen samples collected from palms belonging to 11 genotypes were allowed to germinate on an *in vitro* germination medium containing 8% sucrose and 0.01% boric acid at the temperatures 20°C, 25°C, 30°C, 35°C and 40°C for a duration of two hours. Percentage pollen germination and pollen tube length were assessed after this period. The tall variety WCT had highest percentage germination at all temperatures, followed by the hybrid COD x WCT. Generally for all genotypes, germination decreased drastically at 40°C. On an average, COD had much higher germination than the rest of the dwarf varieties studied, while MYD had the lowest germination of all the genotypes. Mean pollen tube length also showed a significantly low value (41.55 μm) at 40°C. Highest mean pollen tube length was recorded for the hybrid COD x WCT (408.9μm). All talls and hybrids showed significantly higher pollen tube lengths compared to dwarfs. Optimum temperature for pollen germination and pollen tube length for all genotypes was calculated. Tall variety LMT (29.31°C), hybrid MYD x WCT (29.02°C) and dwarf MYD (29.11) had high optimum temperature for germination. The mean optimum temperature for pollen germination was 2°C greater than that for pollen tube length. WCT among talls and COD among dwarfs were found to be tolerant to high temperature stress.

APPENDIX

Weather data for the experimental period (October 2015 – July 2016)

Standard weeks	Temperature (°C)		Sunshine Hours	Rainfall (mm)	Evaporation USA (mm)
	Maximum	Minimum			
41	28.84	21.56	2.26	39.29	1.47
42	31.36	22.43	7.30	0.00	2.43
43	32.29	22.86	6.59	7.11	2.94
44	31.64	21.71	7.36	3.49	3.03
45	31.13	21.49	6.06	14.23	2.86
46	31.33	21.86	4.39	0.33	2.43
47	31.53	21.69	5.17	2.83	2.89
48	32.04	21.17	6.19	0.80	2.91
49	32.09	21.20	8.70	3.14	3.20
50	32.47	21.79	7.23	0.00	2.97
51	31.86	21.71	6.89	0.03	2.71
52	32.26	19.73	9.31	0.00	3.03
1	32.70	18.39	9.66	0.00	3.20
2	33.50	19.16	9.61	0.00	3.31
3	32.14	21.14	8.87	0.00	3.09
4	32.24	21.26	7.36	0.81	3.16
5	32.41	22.61	9.10	0.00	3.14
6	32.59	20.33	8.34	0.00	3.17
7	31.90	23.10	7.33	0.00	3.20
8	32.37	22.17	9.07	0.00	3.54
9	32.83	24.96	7.99	0.00	3.83
10	33.21	24.44	6.37	0.00	3.74
11	33.31	25.03	7.54	0.00	3.97
12	33.30	25.03	8.41	0.00	4.31
13	33.66	25.63	7.00	0.00	3.89
14	33.83	25.90	8.10	0.03	4.20
15	34.03	26.94	7.91	0.00	4.40
16	34.11	27.33	7.07	0.00	4.80
17	34.44	27.00	9.04	0.00	5.06
18	34.24	26.73	9.89	0.00	5.17
19	34.43	26.40	9.13	0.00	5.06
20	34.11	24.64	7.63	8.80	4.66
21	33.61	25.53	2.90	3.80	3.40
22	33.90	25.46	8.24	1.66	3.91
23	32.00	23.47	6.97	14.40	4.09

24	30.84	22.40	2.80	17.34	2.46
25	31.19	22.66	3.24	25.20	2.43
26	27.79	20.70	0.53	51.69	1.37
27	27.66	21.47	0.14	42.74	1.23
28	28.40	21.70	0.89	31.00	1.17
29	29.71	21.89	2.11	26.03	1.91
30	28.77	20.50	0.80	21.49	1.29
31	28.29	20.94	2.26	16.54	1.69

Number of open flowers collected at 6.30, 8.30 and 10.30am. for a period of 10 days from the day of spathe opening in genotypes (a) MYD (b) COD and (c) COD x WCT

(a) MYD

Days after spathe opening	**Number of flowers open at**		
	6.30am.	**8.30am.**	**10.30am.**
1	56	163	150
2	69	164	119
3	81	165	196
4	91	166	205
5	125	167	340
6	125	168	169
7	82	169	177
8	40	170	132
9	52	171	183
10	55	172	85

(b) COD

Days after spathe opening	Number of flowers open and at		
	6.30am.	8.30am.	10.30am.
1	24	21	102
2	53	22	195
3	65	23	116
4	90	24	36
5	89	25	89
6	54	26	31
7	30	27	34
8	18	28	5
9	26	29	13
10	44	30	9

(c) COD x WCT

Days after spathe opening	Number of flowers open at		
	6.30am.	8.30am.	10.30am.
1	117	143	66
2	97	144	60
3	98	145	100
4	93	146	35
5	42	147	48
6	33	148	26
7	22	149	13
8	35	150	22
9	34	151	48
10	28	152	54
11	40	153	61

Pollen germination percentage of flowers collected at 8.30 and 10.30am. from the day of spathe opening to a period of 10 days in (a) MYD, (b) COD and (c) COD x WCT

(a) MYD

Days after spathe opening	Percentage germination of pollen collected at							
	8.30am.				10.30a.m			
	R1	R2	R3	Mean	R1	R2	R3	Mean
3	76.13	82.78	79.44	79.45	22	26.5	21.1	23.2
4	68.41	80.7	74.54	74.55	23.86	12.39	18.11	18.12
5	37.24	56.78	46.99	47	15.85	39.87	27.86	27.86
6	74.16	79.09	76.62	76.62	47.53	65.04	56.27	56.28
7	59.69	49.55	54.62	54.62	61.54	57.01	32.62	50.39
8	73.06	61.34	59.7	64.7	46.33	54.74	50.52	50.53
9	29.44	21.6	25.52	25.52	21.08	21.17	25.22	22.49

(b) COD

Days after spathe opening	Percentage germination of pollen collected at							
	8.30am.				10.30am.			
	R1	R2	R3	Mean	R1	R2	R3	Mean
3	63.64	72.01	75.31	70.32	40.5	33	54.3	42.6
4	80.88	86.52	82.2	83.2	46.71	48.7	41.69	45.7
5	68.74	77.06	72.87	72.89	43.5	39.6	25.8	36.3
6	78.32	66.7	60.48	68.5	45.68	67.74	41.74	51.72
7	57.19	62.74	68.98	62.97	58.59	67.52	57.07	61.06
8	60.73	63.81	66.77	63.77	56.09	69.14	47.63	57.62
9	63.25	67.49	63.84	64.86	55.38	62.53	63.47	60.46

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(c) COD x WCT

Days after spathe opening	Percentage germination of pollen collected at							
	8.30am.				10.30am.			
	R1	R2	R3	Mean	R1	R2	R3	Mean
3	45.62	51.53	48.59	48.58	52.58	58.03	55.29	55.3
4	67.82	73.65	66.28	69.25	52.14	68.38	45.26	55.26
5	42.14	38.38	55.26	45.26	58.11	56.55	27.33	47.33
6	65.54	58.34	54.44	59.44	55.73	59.42	63.68	59.61
7	63.19	61.14	60.68	61.67	62.39	68.55	66.73	65.89
8	71.92	75.85	70.96	72.91	79.62	55.21	71.93	68.92
9	63.91	61.04	77.46	67.47	52.16	43.21	42.15	45.84

Percentage pollen germination of COD and WCT under different media

Media	Percentage Pollen Germination (COD)				Percentage Pollen Germination (WCT)			
	R1	R2	R3	Mean	R1	R2	R3	Mean
M1	52.96	54.24	54.66	53.95	65.12	66.54	63.88	65.18
M2	66.35	65.08	62.89	64.77	85.29	73.39	77.72	78.8
M3	58.74	55.64	63.63	59.34	45.68	48.96	49.63	48.09
M4	43.98	46.9	38.09	42.99	40.34	43.42	42.11	41.96
M5	16.27	19.51	20.1	18.63	24.63	30.45	27.18	27.42

Percentage pollen germination of WCT and COD at the temperature range of 15°C - 50°C

Temperature(°C)	Percentage Pollen Germination	
	WCT	COD
15	4.66	3.85
20	54	39.37
25	59.53	49.34
30	66.08	41.83
35	50.73	29.16
40	14.47	8.76
45	3.01	0
50	0	0

Percentage pollen germination of different genotypes at temperatures 20 - 40°C

Genotype	Replication	Percentage pollen germination at temperature				
		20°C	25°C	30°C	35°C	40°C
MYD x WCT	1	46.80	63.96	55.03	47.71	18.90
	2	67.60	69.44	64.44	38.85	14.93
	3	43.62	41.25	52.09	15.44	8.16
	4	35.46	68.44	57.50	51.51	14.50
	5	65.53	67.78	76.76	62.41	17.60
	6	36.87	59.50	56.49	49.69	0.00
COD x WCT	1	58.48	63.48	51.31	43.79	9.69
	2	61.45	66.73	67.92	48.77	5.34
	3	61.29	66.73	48.66	43.34	12.71
	4	44.05	48.94	57.65	52.49	0.00
	5	58.29	64.97	55.94	45.25	8.72

	6	66.23	75.05	61.21	37.73	10.77
WCT	1	34.14	50.68	47.82	32.79	5.59
	2	47.34	56.89	71.39	56.93	11.47
	3	50.05	50.85	49.68	37.08	14.87
	4	79.40	79.18	76.88	64.58	15.81
	5	80.32	84.13	79.16	69.74	28.66
	6	32.82	35.44	71.53	43.28	10.40
FMST	1	72.45	77.25	75.03	69.26	19.01
	2	21.77	27.40	25.39	30.02	6.05
	3	71.52	76.46	72.58	42.04	21.01
	4	52.49	65.42	57.12	62.54	0.00
	5	49.85	61.47	54.44	49.17	5.60
	6	32.25	31.84	31.59	29.42	3.77
SNRT	1	45.79	49.10	47.56	30.40	21.27
	2	72.35	71.17	61.42	41.74	8.15
	3	16.76	33.67	37.52	31.60	6.41
	4	20.20	24.01	30.93	29.27	6.73
	5	22.57	27.78	25.54	21.91	7.32
	6	75.46	71.65	59.24	60.03	13.37
LMT	1	69.54	60.84	77.51	69.25	22.84
	2	55.77	48.46	56.89	47.89	0.00
	3	16.84	25.44	27.06	19.12	0.00
	4	37.37	42.93	48.16	39.89	6.17
	5	12.21	27.89	24.61	20.70	0.00
	6	73.59	70.85	75.17	68.98	14.16

SPI	1	34.89	36.75	38.29	33.46	0.00
	2	20.64	24.56	26.87	20.88	0.00
	3	59.59	64.28	65.38	50.76	0.00
	4	46.59	57.72	65.14	52.09	0.00
	5	24.17	28.09	31.52	19.50	7.52
	6	54.65	62.91	64.01	59.78	29.15
COD	1	45.33	70.85	27.29	13.27	9.66
	2	48.11	59.14	48.50	31.78	0.00
	3	69.96	74.59	77.44	61.28	9.39
	4	7.94	13.90	20.41	10.64	0.00
	5	32.27	33.08	32.90	19.38	0.00
	6	32.64	44.46	44.43	38.63	33.54
CRD	1	5.62	43.60	38.88	33.40	4.00
	2	20.29	33.84	33.86	28.20	0.00
	3	11.46	17.55	14.08	12.28	3.40
	4	7.00	22.65	26.47	35.73	0.00
	5	12.75	31.36	34.25	36.24	0.00
	6	10.38	18.93	22.49	20.25	0.00
CGD	1	36.87	44.03	49.70	25.66	0.00
	2	31.81	39.47	37.58	26.53	1.89
	3	6.97	36.99	36.23	19.21	3.46
	4	13.54	17.73	16.37	13.97	0.00
	5	8.36	14.18	22.91	13.43	0.00
	6	10.93	18.26	16.14	14.87	0.00
MYD	1	4.14	19.57	16.66	9.89	0.00
	2	9.72	12.18	27.00	13.47	0.00

	3	11.53	16.03	15.83	13.36	0.00
	4	8.15	13.55	22.89	17.69	0.00
	5	10.35	20.45	28.78	19.02	0.00
	6	15.76	20.28	25.27	13.66	0.00

Pollen tube length of different genotypes at temperatures 20 - 40°C

Genotype	**Replication**	**Pollen tube length(µm) at temperature**				
		20°C	**25°C**	**30°C**	**35°C**	**40°C**
MYD x WCT	1	572.03	578.60	510.99	467.66	101.05
	2	362.60	386.65	532.71	237.81	42.87
	3	423.91	495.85	458.89	358.98	301.59
	4	311.67	484.31	614.98	414.99	100.01
	5	472.52	560.13	408.97	379.31	72.14
	6	391.07	528.36	587.07	484.72	0.00
COD x WCT	1	437.47	506.59	469.88	438.73	239.04
	2	497.44	579.15	600.84	347.10	76.83
	3	494.75	572.11	503.39	483.72	48.48
	4	459.53	536.59	727.23	463.31	0.00
	5	452.93	558.35	511.67	398.64	95.70
	6	388.93	518.50	401.99	351.56	107.29
WCT	1	163.94	542.82	543.50	391.21	63.93
	2	304.29	509.97	555.76	515.31	42.20
	3	416.61	558.28	684.14	512.31	40.80
	4	401.34	578.48	493.72	432.86	38.67
	5	529.39	567.06	620.28	500.27	30.44
	6	429.55	492.64	504.63	563.41	27.31

FMST	1	464.59	699.94	698.23	540.84	29.11
	2	176.24	498.98	452.75	410.87	24.64
	3	446.26	493.58	556.87	487.51	28.57
	4	460.23	474.37	542.84	586.72	0.00
	5	320.33	439.70	408.62	530.66	65.30
	6	293.28	475.03	569.32	522.15	82.89
SNRT	1	549.49	550.04	468.66	341.31	45.55
	2	675.16	588.65	656.14	602.49	23.17
	3	216.10	461.54	509.07	644.40	22.73
	4	215.59	512.52	455.62	625.99	27.59
	5	144.69	476.35	535.93	637.39	24.71
	6	564.51	546.22	500.31	584.03	22.47
LMT	1	292.55	458.96	404.02	579.82	37.93
	2	388.80	405.08	446.36	291.33	0.00
	3	121.01	570.86	676.03	565.21	0.00
	4	446.53	455.58	515.08	552.80	27.45
	5	165.33	546.49	501.27	522.29	0.00
	6	566.03	585.68	515.94	509.35	29.88
SPI	1	423.38	540.72	466.08	371.39	0.00
	2	263.46	517.01	538.20	478.76	0.00
	3	346.36	568.80	472.12	445.74	0.00
	4	398.71	588.73	391.11	398.94	0.00
	5	205.81	423.44	447.15	553.25	142.27
	6	343.91	393.29	486.69	441.75	167.34
COD	1	512.15	536.43	427.68	398.30	96.16
	2	501.29	643.84	515.26	419.22	0.00

	3	570.32	500.07	441.93	439.05	136.62
	4	54.25	414.55	383.81	317.87	0.00
	5	123.29	385.76	340.42	285.56	0.00
	6	158.32	349.49	438.66	253.30	58.72
CRD	1	87.17	415.67	422.62	480.36	79.33
	2	158.24	343.07	501.04	489.49	0.00
	3	72.93	182.18	217.78	202.56	53.25
	4	67.19	251.12	314.13	346.36	0.00
	5	175.09	421.78	480.48	566.62	0.00
	6	153.00	392.25	415.70	565.24	0.00
CGD	1	236.73	506.43	678.72	451.14	0.00
	2	258.29	406.50	425.66	290.76	49.39
	3	67.21	444.43	606.23	341.77	39.21
	4	60.18	549.80	516.86	454.54	0.00
	5	65.53	400.56	509.88	345.62	0.00
	6	93.06	216.55	255.89	289.92	0.00
MYD	1	138.78	260.32	182.31	287.54	0.00
	2	180.23	115.76	267.51	187.61	0.00
	3	111.87	200.76	303.19	261.72	0.00
	4	57.89	180.52	324.51	205.43	0.00
	5	149.54	231.48	358.65	373.46	0.00
	6	151.54	323.50	255.23	315.27	0.00

Pollen germination at different temperatures, averaged over (a) talls, (b) dwarfs and (c) hybrids

(a) Talls

Temperature	Mean %PG	Duncan grouping
20	46.113	A
25	50.836	A
30	52.514	A
35	43.471	A
40	9.512	B

(b) Dwarfs

Temperature	Mean %PG	Duncan grouping
20	19.661	B
25	30.695	A
30	30.682	A
35	22.577	AB
40	2.722	C

(c) Hybrids

Temperature	Mean %PG	Duncan grouping
20	53.805	B
25	63.021	A
30	58.749	AB
35	44.748	C
40	10.11	D

Percentage reduction in pollen germination of different genotypes from optimum temperature to 20°C and 40°C

Genotype	%PG at T_{opt}	%PG at 20°C	%PG at 40°C	%Reduction at 20°C	%Reduction at 40°C
MYD x WCT	62.81	49.31	12.35	21.48	80.34
COD x WCT	64.85	58.3	7.87	10.11	87.86
WCT	68.71	54.01	14.47	21.39	78.94
FMST	57.63	50.06	9.24	13.14	83.96
SNRT	46.81	42.19	10.54	9.87	77.48
LMT	52.33	44.22	7.19	15.49	86.25
SPI	50.02	40.09	6.11	19.86	87.78
COD	50.39	39.37	8.76	21.86	82.61
CRD	28.54	11.25	1.23	60.58	95.68
CGD	38.25	18.08	0.89	52.73	97.67
MYD	24.21	9.94	0	58.94	100.00

174033

Percentage reduction in pollen tube length of different genotypes from optimum temperature to 20°C and 40°C

Genotype	PTL at T_{opt}	PTL at 20°C	PTL at 40°C	%Reduction at 20°C	%Reduction at 40°C
MYD x WCT	529.73	422.30	102.95	20.28	80.57
COD x WCT	552.83	455.18	94.56	17.66	82.90
WCT	580.30	374.19	40.56	35.52	93.01
FMST	538.34	360.15	38.42	33.10	92.86
SNRT	615.30	394.26	27.70	35.92	95.50
LMT	509.93	330.04	15.88	35.28	96.89
SPI	529.93	330.27	51.60	37.68	90.26
COD	496.47	319.93	48.58	35.56	90.21
CRD	481.01	118.93	22.10	75.27	95.41
CGD	520.14	130.17	14.77	74.97	97.16
MYD	289.19	131.64	0.00	54.48	100.00