EFFECT OF HIGH TEMPERATURE ON FEMALE FLOWER RECEPTIVITY AND FERTILIZATION IN COCONUT

By

NEETHU P.

(2014-20-130)

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VELLANIKKARA, THRISSUR-680656

KERALA, INDIA

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

BSc. -MSc. (Integrated) Climate Change Adaptation

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Kerala Agricultural University, Thrissur

ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH

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DECLARATION

I, hereby declare that the thesis entitled **"High Temperature effect on female flower Receptivity and fertilisation in Coconut"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date: **(2014-20-130)**

CERTIFICATE

Certified that this thesis entitled **"High Temperature effect on female flower Receptivity and fertilisation in Coconut"** is a record of research work done independently by **Ms. Neethu P.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Neethu P.

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SYMBOLS AND ABBREVIATIONS

%	- Percent
°C	- degree Celsius
et al	- and others
i.e	- That is
µm	- Micro meter
Cm	- Centimeter
KAU	- Kerala Agricultural University
G	- Gram
CO_2	- Carbon dioxide
IPCC	- Inter-governmental Panel on Climate Change
GDP	- Gross Domestic Product
ICAR	- Indian Council of Agricultural Research
CPCRI	- Central Plantation Crop Research Institute
WCT	- West Coast Tall
pH	- Power of Hydrogen
In-vitro	- Under Laboratory condition
In-vivo	- Under Field condition

CFCs	- Chorofluorocarbon
C_3Plant	- Initial product of the assimilation of carbondioxide through photosynthesis is 3-phosphoglycerate
Mm	- Millimeter
Wm^{-2}	- Watt per square meter
UNFCC	- The United Nations Framework Convention on Climate Change
Ppm	- Parts per million
ET	- Elevated Temperature
$T_{min.}$	- Minimum Temperature
$T_{max.}$	- Maximum Temperature
$T_{opt.}$	- Optimum Temperature
UV	- Ultra Violet Radiation
IR	- Infra-Red Radiation
TTC	- Triphenyl tetrazolium choride
NIS	- Nikon Instrument Software -For image
KH_z	- Kilohertz
FMST	- Federated Malay States Tall
LCT	- Lakshadweep ordinary Tall
COD	- Chowghat Orange Dwarf

MYD	- Malayan Yellow Dwarf
EPP	- Effective Pollination Period
NaoH	- Sodium hydroxide
ANOVA	- Analysis of Variance
LCD	- Liquid Cristal Display
SSD	- Solid State Relay
DHT 22	- Low-cost digital temperature and humidity sensor
DC	- Direct Current
RH	- Relative Humidity
RTD	- Resistance Temperature Detector
LSD	- Least Significant Difference
Pt-100 RTD	- Platinum Resistance thermometer
CTRI	- Cumulative Temperature Response Index

CHAPTER 1

INTRODUCTION

Plant growth and development depends on the temperature limits surrounding the plant and each species have a designated temperature range (a maximum, minimum, and optimum) for their optimal growth. The anticipated changes in the global mean surface temperature over the next three to five decades have been predicted to be in the range of 1.4 to 2.6°C based on the analysis by Representative Concentration Pathways (RCP 8.5) (IPCC, 2014). The future climate scenario envisages more intense and frequent heat waves or extreme temperature events which may last longer. The extreme temperature conditions may prevail for a very short duration of few days with temperature increasing by over 5°C than the normal temperatures. It is believed that those elevated temperatures will have large negative impacts on the overall plant growth, development and economic yield levels. Among the various developmental pathways, sexual reproduction in plants is more sensitive to the elevated temperature conditions than the vegetative stage. Hence, it is anticipated that the plant increase in temperature than vegetative processes, and therefore, plant reproductive organs and their normal developmental processes will be more vulnerable to modulations in the short episodes of high temperature prior to and during early stages of floral bud development. In horticultural crops, field crops and other fruit trees, fruit set is highly sensitive to high temperatures. However, the information about the effect of high temperature on the reproductive phase of plantation crops like coconut is scanty and requires thorough investigation.

Coconut is an important plantation crop grown extensively in 12.3 million ha spread over 92 countries with an annual production of 67 billion nuts (APCC, 2016). Coconut is widely eulogized as *Kalpavriksha* or tree of heaven, since it provides food and livelihood security to 20 million people globally and 10 million people in India involved in cultivation, processing, marketing and trade-related activities. Coconut thus exerts a intense influence on the rural economies around the globe. However, coconut is extremely sensitive to both the abiotic stressors of drought and high temperature. In India coconut is widely cultivated in the western and eastern coasts of Southern states. In the west coast of Karnataka and Kerala states of India the annual rainfall is greater than 2000 mm and the crop is grown at a T_{max} (maximum temperature) of 34 to 36ºC, whereas in the Eastern coast of Andhra Pradesh and Tamil Nadu the quantum of rainfall is low (1000 to 1200 mm) and T_{max} reaches to 40 to 42ºC

(Naresh Kumar *et al*. 2013). The future climate change projections have divulged that temperature would increase in these regions to the tune of 1.1°C to 2.6°C by the finish of the 21st century (IPCC, 2014). Also high temperatures are related with the high vapour pressure deficit and other evaporative driving forces which may cause severe drought in near future.

High temperature conditions are characterized with the shedding of button (female flower of coconut inflorescence) which ultimately leads to great economic loss in coconuts. Therefore, it is expected that the different developmental stages of reproductive (progamic) phase viz. gametophytic and saprophytic stage, pollen germination on receptive stigma, pollen tube growth through style and its kinetics, pollen entry to the ovule and fertilization are highly sensitive to high temperature stress. In coconut Hebbar*et al.,* 2018 and Chaturvedi*et al*. 2017 have already demonstrated the decreased pollen germination and viability at high temperatures. There is a good genotypic variation for high temperature tolerance. However, there are no studies to show the effect of high temperature on female flower receptivity and other progamic phases. This study aims to find the effect of high temperature on female flower receptivity, pollen germination in stigma, pollen tube growth through style etc.

As coconut is a perennial crop and its height and spread is quite large and hence exposing the whole plant to high temperature is a challenge, Therefore a methodology was devised to expose the whole inflorescence on the tree (*in- vivo*) to high temperature few days before and during the receptivity of female flowers and an *in- vitro* protocol was developed to study the progamic phase response to high temperature. Accordingly, the following objectives were fulfilled in the study-

- To study the progamic phase in coconut and its response to high temperature *in- vivo*
- To standardize media for the growth and temperature induction of female flowers *in-vitro* and study the influence of temperature on progamic phase

CHAPTER 2

REVIEW OF LITERATURE

2.1CLIMATE CHANGE

Climate change is not hysteria-it's a fact. Over the past few decades, climate change has become a global issue. "According to IPCC 5thassessment report there is a rise in global mean air temperature. The air temperature is expected to rise by 0.3^{0}C-0.7^{0}C and up to 4.8 degree by the end of the 21th century". It is true in India and elsewhere too as climate change has emerged as the one of the gravest environmental concerns for the existence of human kind. Climate variability and climate change in the recent times have become the most discussed subjects worldwide since the frequency of occurrence of weather-associated disasters such as cold waves, droughts, dust storms floods, heat waves and landslides, have greatly increased. The effect on agriculture of climate change will be one of the main determining factors affecting humanity's potential food security on earth. Agriculture besides being the driver of climate change many times becomes vulnerable to the climate change effects. Moreover there is an element of uncertainity to the sensitivity of agriculture to the climate change due to wide variations of crops and cropping system, rainfall, temperature, soils and other crop husbandry management practices. It all causes a huge threat to the climate of the entire earth system. Because of the increasing concentrations of greenhouse gases such as Carbon dioxide (CO_2), Ozone (O_3), Methane (CH_4), Nitrous oxide (N_2O) and Chlorofluoro-carbons (CFCs), there exists a huge propensity for changes in our climatic conditions and it may exert a mammoth effect on agriculture either directly or indirectly (Bhatia *et al.,* 2004). Furthermore, research evidences including scientific analysis have clearly concluded that climate change is a reality. Among the various sectors of agriculture, plantation-based cropping is one of the high priority sectors that requires an immediate attention to avoid the uncertainties due to climate change as millions of people are dependent on plantation based systems for their livelihoods and contributing to the national GDP through coconut and other crops (Ranasinghe, 2012).

The coconut is perennial palm and it is characterised with a relatively long reproductive phase (extending for a period of 44 months) right from the start of inflorescence primordium until the attainment of fully matured nuts. Hence, coconut cultivation is highly sensitive to the weather extremities such as drought, elevated temperature conditions, floods

etc. Climate change variables like increase in CO_2 concentration will have a positive effect on coconut since being a C_3 plant. But, unfortunately simultaneous increase in temperature and dry spell will drastically reduce the production. Though, coconut is sensitive to high temperature throughout the growth stages, but it is found to be more sensitive during reproductive phases (Hebbar *et al*., 2018). And also Hebbar *et al.* (2018) found that in coconut, "Temperature above 33^0C there is significant decline in pollen germination, however wide variability was found across the genotypes for pollen germination". For the successful fertilization in addition to pollen germination, female flower receptivity and pollen tube growth through the pistil are very important. In this study an attempt is made to study the female flower receptivity and pollen tube growth of coconut at different temperatures in a tall variety WCT (West Coast Tall).

2.2 COCONUT

Coconut is one of the most useful palms in the world, also known as *Cocos nucifera*L. It belongs to Arcaceae family and is the only palm belonging to the *Cocos* genus. It is a tree crop that is monocotyledonous and is a C_3 plant. The palm is typically found on sandy beaches near the sea, where salt spray and brackish soil can be tolerated. It is also well grown in various types of soil. Coconut is broadly divided into two morph forms, the Talls and the Dwarfs. The tall palms can grow up to a height of 15 to 18 m and extend their life span for 60-80 years after flowering. As the name suggests, the dwarf varieties are short in stature and size and starts flowering and yielding economic fruits in 3-4 years after planting, and are also characterised with short span of 40-50 years in comparison to the tall varieties. Coconut generally requires equatorial climate characterised with relatively high humidity and complete humid and warm climate so as to enhance its growth conditions. The optimum temperature requirement for coconut is around 27^0C $\pm 3^0$C and coconut cannot tolerate a prolonged spell of climate or weather extremities. The preferred rain fall is 1300-2300 mm per annum. Coconut is distributed 23^0N & 23^0 S of the equator up to an altitude of about 600m above the mean sea levels. Also coconut growing regions experiencing very high abnormal temperatures, rainfall or RH document huge variations in the yield levels. It can tolerate a wide range of soil acidity and the ideal soil pH for optimal coconut growth conditions warrant the pH of 5 to neutral (Fremond, 1964). Mancot *et al.* (1964) reported that the major soil types that can adequately support the growth of coconut in Indian conditions

are alluvial, laterite, red and coastal sandy loam, coastal sandy soils. The crop can be grown in reclaimed soils too provided the pH varies from 5.2 to 8.0.

India is placed II in the global coconut production and the country produces 15 billion nuts annually. The coconut occupies around 16% of total cultivable land of our country and contributes little over 24% of global coconut production. Interestingly, over 98% of land holdings under coconut cultivation is less than two hectares among the estimated 5 million coconut holdings in the country (Rao and Krishnakumar. 2011). According to Bhaskaran and Leela (1976), low and erratic nature of rainfall greatly influences the yield levels of coconut. Authors have recognised the importance of soil moisture conditions as it influences the development of spadix which eventually determines the yield levels.

The coconut palm is one of the few plants which flowers throughout the year and for this reason may be the only source of nectar and pollen for the floral visitors at certain times of the year. A wider variation exists between ecotypes and geographic regions with regard to flowering phenology in coconut. Even though anemophily obviously occurs in coconut palm, entomophily is the major pollination source and it can be concluded that coconut is predominantly a bee-pollinated palm (Thomas Reji and Josephrajkumar. 2013). So that high temperature may also affect the pollination sources of coconuts.

2.3CLIMATE CHANGE AND COCONUT

Coconut is cultivated and grown in tropical regions between 20^{o}N and 20^{o}S latitude. The optimal weather conditions required for fine growth and economic yield in coconut are annual rainfall between 1300 and 2300 mm that has to reasonably well distributed, mean annual temperature of 27^{o}C, copious sunlight in the range of 250 to 350 Wm^{-2} and a minimum sun shine period of 120 hours per month. Coconut grows well when the humidity is beyond 60% as it is a humid tropical crop (Child 1974; Murray 1977). The recommended dose of watering or irrigation schedule are 200L of water /palm in every 4 days or @ 66% Eo applied in drip irrigation (Rajagopal *et al*. 1989). Any significant alterations or deviations from these suitable conditions generally cause the palm to undergo stressful conditions. It is reported that quantum of rainfall and the duration of dry spells greatly determines the productivity of coconut in under varied agro-climatic zones of the country (Nareshkumar*et al*., 2007). Minimum temperatures above 10°C enhance flowering nevertheless, temperature

greater than 10 °C for one-month cause nut fall (Nareshkumar*et al*., 2008). The coconut simulation models predicted that yield will decline in the east coast region by about 2 percent in 2020, 8 percent in 2050 and 31 percent in 2080. The yield will increase in states such as Kerala, Tamil Nadu and Karnataka and is projected to decline in Andhra Pradesh, Orissa and Gujarat (Nareshkumar*et al.*, 2008). Coconut is generally grown in regions of high rainfall predominantly as a rain fed crop and hence these regions experience 50% less productivity compared to the regions where it is cultivated in irrigated gardens. Coconut encounters a prolonged dry spells during summers besides the occurrence of complete drought years. The phenology of the crop occupies a longer period since it is a perennial. The crop has approximately 44 months of duration between the inflorescence initiation to the maturity of the nuts, has a relatively long pre-fertilization duration of about 32 months, compared to the short (12 months) of post-fertilization period. Hence, the impact of drought occurrence during any of these critical stages of development of the inflorescence results in a yield loss (Rajagopal *et al*., 1996; Rajagopal 2000). This reduction in yield can be observed for a further three more years, making the problem more severe (Naresh Kumar 2002).

Recent research indicated that the climate is changing in areas under plantation crops. Past adverse climate events such as droughts and cyclones severely affected the coconut yields. Despite following the best of crop husbandry practices, yield levels are too low during the years of drought or floods. Rao and Krishnakumar (2011), reported that for the first time in Kerala in the recent past, the coconut production adversely affected due to unprecedented drought in summer. Coconut simulation-based projections as per HAD CM3 scenarios indicate beneficial influence of climate change on coconut in west coast of India provided the current water sources are made available in future as well (Naresh Kumar *et al.* 2008). On the other hand, the plantations in irrigated areas of Tamil Nadu, Karnataka, Andhra Pradesh, Orissa and Gujarat are likely to be affected adversely causing loss of production. Various factors such as elevated temperature conditions, elevated CO_2 concentration, changes in the levels of precipitation, deficiency of nutrient status, frequent flooding will significantly affect coconut plantation. India's 2nd National communication to the UNFCC projected CO_2 increase to 550 to 700 ppm. and temperature rise by 3.5 to 4.3^{o}C by the end of the century (Anonymous 2012). Kasturibai *et al.*(1988) reported that coconut palm experiences stress when exposed to excess radiation above 265 Wm^{-2}, temperature>33 °C and vapor pressure Deficit>26mbar, aggravated by soil water deficit during the period. Thus, coconut in east coast may suffer more due to climate change compared to west coast.

2.4. EFFECT OF CLIMATE CHANGE ON REPRODUCTIVE SYSTEM OF FLOWERING PLANTS

It is the known fact that in flowering plants the reproductive phase or gametophytic stage is generally sensitive to the extreme conditions of temperature (cold or hot stresses). Since a single cold night or hot day could cause severe fatalities to the reproductive phase of the crops. Hence, it is observed that analysis of temperature stress on crop plants suggest that development of pollen and the process of fertilization are the most sensitive reproductive stages.

The coincidence of dry spell in coconut during the sensitive inflorescence stage and the growth of nuts has a significant effect on the productivity of coconut. The vagaries of the dry spell in various agro-climatic zones of India were correlated with the economic nut yield of coconut (Kumar *et al*., 2007). This study unearthed the fact the relatively long duration (44 months) of reproductive phase (starting from the inflorescence initiations to nut maturation), encountering dry spell even for an year would significantly affect the yield levels atleast for 3-4 ensuing years. Thus, it was inferred that the occurrence of dry spells could eventually affect the yield levels for the following four years despite the quantum of rainfall during the same period.

In many plants it was observed that elevated temperature stress affect the reproductive development and yield compared to the photosynthesis (Pettigrew, 2008; Prasad *et al*., 2008). In addition, temperature stress exerts varying effects on the male and female reproductive parts hence causing an asynchrony between them (Herrero, 2003; Hedhly*et al*., 2008). High temperature stress severely reduces the time period of stigma receptivity greatly reducing the chances of successful fertilization. For instance, in peach (*Prunus persica* L.) the stigmas exposed to 30ºC lose their ability to induce pollen germination after 3 days; however the treatment of 20ºC viability lasted for 8 days (Hedhly*et al*., 2005.) Pollination is considered to be one of the most sensitive phenological stages to high temperature conditions. *In-vitro* pollen germination study in coconut genotypes revealed genotypic variation for cardinal temperatures (Tmin.,Topt. and Tmax.) with respect to pollen germination percentage and pollen tube growth. Tall genotypes such as FMST, LCT, WCT, and a dwarf cultivar COD

and hybrids exhibited relatively good adaptability to the high temperature conditions whereas the dwarf variety such as MYD was found to be least adaptable (Hebbar *et al* .2018).

Some reports show that reproductive growth is sensitive to heat stress, which results in various abnormal effects such as: (a) poor pollen germination (Porch and Jahn 2001, Hebbar*et al.* 2018), (b) loss of pollen viability (Kafizadeh*et al.* 2008), (c) impaired micro-sporogenesis and mega-sporogenesis (Peet*et al.* 1998; Porch and Jahn 2001; Young *et al.* 2004), (d) the absence of pollen on stigma surface and loss of stigma receptivity (Jagadish*et al.* 2007) ,(e) inhibited pollen tube growth (Pressman *et al.* 2006; Kafizadeh*et al.* 2008), (f) loss of ovule function (Gross and Kigel 1994), (g) impaired fertilization (Dupuis and Dumas 1990), (h) limited embryogenesis (Zinn*et al.* 2010), and (i) reduced ovule number and increased ovule abortion (Whittle *et al.* 2009) leading to poor seed set (Young *et al.* 2004). In addition, heat stress may accelerate reproductive growth, thereby reducing the duration of this stage and limiting the yield potential (Boote*et al*. 2005). The relative sensitivity of reproductive stages such as flowering and seed filling to heat stress may vary according to the crop species (Sung *et al.* 2003). Koike *et al.* (1990) conduct a study on rice and he reported that cold stress affects both male and female reproductive development in rice; however, the male organ is more sensitive as compared to female organ. And also, another work by Endo *et al. (*2009), in rice established that heat stress modulates the functions of the tapetum which are important for anther dehiscence and germination of pollen grains on the stigma. Kumar *et al*. (2012) demonstrated that various features such as viability of the pollen, germination of pollen germination, growth of pollen tube, pollen load and receptivity of stigma of chickpea genotypes significantly decreased with an upward movement of temperatures (45/35^{0}C) and the genotypes that are characterised with heat tolerance showed relatively less damage. Erovic *et al*. (2000) , reported that at constant temperature of 20^{0}C, all plum cultivars showed a decline in longevity of ovule viability.

2.5FLORAL BIOLOGY

Coconut palms are monoecious, producing inflorescences that are axillary and interfoliar. The central axis of the inflorescence called the rachis gives rise to the spikelets (rachillae) in a spiral succession. The rachillae or spikelets bear both the staminate and pistillate flowers at their apices and base, respectively. Depending on both genetic and environmental factors, the

number of inflorescence varies from zero through approximately 300. Similarly, for some spicata inflorescence, the range of male flowers goes from several thousand to very few.

The commencement of flowering is an important stage in the life of that in coconut. The coconut inflorescence generally known as spadix. According to the type of palm, climatic and soil conditions the age at which the tree flowers will varies. Dwarf palms in Malaya flower in their third year after planting (Jack and Sands 1929). However, the tall variety was known to flower from fifth year on wards (Patel 1938) whereas the majority of palms flowered only by the end of the twelfth year.

The number of spadix largely depends on the number of leaves produced. It is one of the interesting facts that in a mature palm, the number of leaves and the number of spadix are almost same that is 12 per annum. If each leaf of a palm has an inflorescence in its axial, the tree is considered as regular bearer, otherwise it is known as irregular bearer.

In coconut the period from the development of the flower primordium to harvest of mature nuts is around 44 months. Of this period the opening of spathe to the harvest takes around 12 months.

2.5.1 Male Flower

Within an inflorescence of coconut, male flowers open initially starting at the top of each spikelet and it proceeds towards the base of the spikelet. And they usually open in the morning hours, and then release the pollen and fall of the evening. It is observed that male flowers open between 8:00 am to 10:30 am and most of the male flowers in an inflorescence open between 3 to 10 days of spathe opening (Menon and Pandalai 1958; Helen, 2016). Ranasinghe *et al.* (2010) documented that inflorescences open sequentially at the interval ranging from 22 to 30 days depending on the environmental condition and age of the palm. The male flowers open, liberate the pollen and fall off from the 2^{nd} to 19^{th} days following the inflorescence opening. Male flowers generally remain on the inflorescence just for a day and shed at the same evening. The flowering pattern is an extended type of flowering, since a few flowers open on each day, over a period of weeks. This reduces the chances of geitonogamy (Willmer, 2011). Liyanage (1954) found that in a mature palm, the male phase continues for about 18-25 days. Patel (1938) reported that most of the flowers opened on 15^{th}day after spathe opening. The duration of male phase varies according to the nature of the tree, season,

and locality. The male phase is shorter during the summer months of February to April and longer during rainy and winter seasons. The number of male flowers varies from a very few in Spicata to thousand in some tall varieties. Each male flower contains six alternately arranged perianths. Compared to outer ones the inner perianth is greater than the outer ones. The inner one is about three times greater than the outer ones and the anthers dehisce longitudinally. Within the perianth are six hammer shaped stamen, which on maturity, release pale yellow coloured powdery pollen. There is also a rudimentary pistil with three teeth at the apex bearing nectarines, the nectar of which attracts ants, bees, and other insects. This is suggestive of the predominance of entomophily in pollination (mainly bee pollinated). The phenomenon of absence of this rudimentary pistil in some flowers is a rare occurrence. Also those flowers that are present on the upper most part of the spikes and those present on the sides of the female flowers open first from the apex and proceed downwards to the base.

2.5.2 The Pollen and Pollen tube Growth

The pollen sacs in the fully mature anthers burst along the longitudinal slits coinciding with the partitions of the pollen sacs and release their pollen well before the male flower opening. Coconut pollen grains are spherical (50 µm) when fresh but shrink rapidly after shedding and become ellipsoidal (65-69 µm in length and 28-30 µm in diameter) with a longitudinal suture. When placed in water, the pollen grain immediately gets hydrated regaining its spherical shape and the suture disappears. The pollen has been reported to remain viable for 2-8 days (Whitehead, 1962; Whitehead, 1965b) in laboratory conditions but in nature 75 per cent of shed pollen losses its viability after 12 hours (Furtado, 1924). Aldaba (1921) estimated that around 270 million pollen grains are present in each male flower. The pollen grain comprises a three nucleic one larger and two relatively small. Kidavu and Nambiar (1925) have documented that the viability of the stored pollen grains was only for a period of six days. The pollen tube carries the sperm cells to the ovary as it grows through the extracellular matrix in the pistil. The pistil comprises three different segments namely stigma, style, and ovary, and this physical barrier has to be crossed to effect the fertilization and it offers opportunity for pollen pistil interactions (Bruce McClure, 2007).

Dispersal of pollen is affected by atmospheric variables like temperature and relative humidity since it also influences the time of anther dehiscence and phenology of flower opening. In anemophilous and entomophilous angiosperms, the pollen is hold for a longer

period until the insect pollinator arrives or winds strong enough to dislodge the pollen and carry in air arrives (Lisci and Pacini, 1996). In case of coconut, pollination is attributed by wind, insect or both. By morphology, it is more adapted to insect pollination. Henderson (1986) observed that anthesis and opening of flowers in the early morning with sweet smelling flowers and nectar production emphasize the adaptation for insect pollination. Wasp, bee, fly, beetle, and ant are the principle pollinating agents. In that bees and wasps are the major pollinators of the coconut flower (Sholdt, 1966). Wind also contributes to pollination, mostly within a palm than between palms (Sholdt and Mitchell, 1967). Though anemophily is obviously occurs in coconut palm, the predominant pollination mechanism is entemophilly (Sholdt and Mitchell, 1967; Hedstrom, 1986; Melendez-Ramirez *et al.*, 2004; Conceicao*et al.*, 2004).

In flowering plants the reliable delivery of sperm cells by the pollen tube to the embryo sac within the ovule to effect fertilization determines the rate of reproduction (Lord and Russell, 2002; Boavida *et al*., 2005). This journey is characterised with appearance of the vegetative cell of the pollen grain after adhesion to the stigma. An apical cell growth process maintains the pollen tube cytoplasm and the sperm cells, in the most proximal region of the tube as it elongates and it enters the ovule where it discharges the sperm (Berger F., 2008).

Pollen germination on the stigma is homogeneous and synchronous process. A fluorescent bulge usually emerges from one of pollen grain apertures to form a short pollen tube which grows just beneath the papillae cell wall (Cheung *et al.,* 2010). In the elongation process, the pollen cytoplasm, vegetative nucleus and the sperm cells are carried within the tube growing through intercellular spaces of the pistil tissue (Johnson and Preuss, 2002; Kim *et al.,* 2003; Palanivelu and Preuss, 2000; Ray *et al*., 1997). Thus, this process of pollen germination and pollen tube growth inside the pistil is possible due to many cell–cell interactions, some facilitating while others prohibiting because of incompatibility or inherent mechanism to avert polyspermy (Cheung *et al.,* 2010)

Pollen grains when examined under microscope reveal the presence of shrunken type along with smooth and spherical one. These are infertile or sterile ones. According to the study conducted by Juliano and Quisumbing (1931), this sterile pollen is smaller than the fertile one also it retains longitudinal sutures even 8 under moist condition. These have an inability to attain spherical shape even after contact with water and unlike fertile grains after dehiscence the granular content was absent. On an average, 25% pollen produced in a male

flower is shrunken, suggesting infertility (Aldaba, 1921). This sterile pollen is more in number in diseased palms compared to normal one (Varkey and Davis, 1960). Nampoothiri (1970) suggested that, pollen grains from flowers at the proximal end were found to be most sterile and the ones from the distal end were least sterile. Pollen production in the individual flowers is high during summer or cold months than during the rainy seasons. Earlier studies revealed that pollen can be stored in desiccators over calcium chloride with 50% relative humidity without losing its viability for 10-15days. Viability of pollen can be remained for one year or more, if stored in a combination of low temperature (0 °C) and 50-56% relative humidity (Manthiratna, 1965). For most species to remain its viability low temperature and low relative humidity is required but in case of poaceae, it requires low temperature and high humidity. Freeze drying of pollen is a successful method used for storing pollen and is used in different species like coconut (Whitehead, 1963) and pine (Ching and Ching, 1964). Viability of lettuce pollen decrease rapidly when stored at room temperature (4 °C), whereas viability was still acceptable after 32 days storage in a freezer at -18 °C. Pollen oven dried at 37 °C for 2-8 hrs followed by storage at deep freezer proved to be the best method for storing the pollen without losing viability. Pollen which is stored in this manner for 12 months had retained appreciable level of viability

The role of sugars in pollen germination and pollen tube growth is twofold, for osmotic regulation as well as for nutrition (SreekrishnaBhat, 1990). According to Loguercio (2002), pollen tube ruptures mainly due to a situation known as imbibitions damage.

2.5.3 Female Flower

The globosepistillate flowers (“buttons”) have a diameter of 2-3cm, a light yellow color and a calyx composed of six thick, imbricate (overlapping) perianth lobes in two whorls that,whenyoung,are tightly folded over the pistil. Below the lobes are two bractioles (small bracts) at the juncture of the short stalk (peduncle). The perianth lobes are sub orbicular (almost circular), concave and coriaceous; they enlarge and persist as the ovary develops into a fruit. There are abortive remains of six stamens between the ring of perianth lobes and the large, fleshy, globose ovary. As the pistillate flower develops, the apex of the trilocular ovary emerges between the lobes, with three ridges extending downwards, making it three-sided (triquetrous).Three ridges stretch downwards from the tip of the pistil,making the entire ovary appear globally three-sided ,each side being provided with a groove at its tip. These three

grooves meet in the centre of the apex and form the stigma. After the inflorescence opens, and before fertilisation takes place, the mass of fibrous tissue continues to increase in size and ultimately forces apart the floral leaves disclosing only its rounded upper surface which is surmounted by a white nipple. This nipple is marked by three equidistant grooves which meet at its apex and thus divided it into three triangular sections. When the female flower is ripe, these three segments separated and stand erect as three teeth, exposing the stigmatic surface on which the pollen is to fall in order that the female flower may be fertilized. These three teeth constitute the stigma. Pistillate flowers become receptive early in the morning as indicated by a reflexed and moist stigmatic surface. In addition to the stigmatic appearance, nectar containing 9-12 per cent sucrose is produced from the receptive flowers (Jay, 1974) throughout the day. When receptive, the stigma is expanded as three erect teeth.

The number of female flowers per inflorescence varies, but is usually 20- 40. Young palms have fewer female flowers per spadix. The stigma remains receptive to pollen for 1-4 days (Sholdt and Mitchell, 1967; Henderson, 1988; Ashburner, 1995) before they dry up. The completion of the process of fertilisation is visualized when the stigma turns brown and the tissue collapses to form a small circular region having three shrivelled teeth at the apex of the young fruit. It is accompanied in increase in the size of the six floral leaves and the whorl of perianth leaves remains persistent at the base of the fruit till it matures and dries.

The pollen is deposited on the surface of a receptive stigma during pollination,which has a specialised surface for holding the pollen. The glands on the petals produce exudates at the time of receptivity that reach the stigma tip and act as a necessary medium for germination of pollen. At the base of the ovary, a sticky viscous surface and a direct opening near the top of the fruit coat has nectar secretion. Marechal (1928) found oozing out of sugary fluid through ores situated below or around the stigma. In earlier studies it is observed that there are three orifices, which is a small opening altering with the stigma. The composition of this secreted fluid has different proportions of lipids, carbohydrates, proteins and phenolics (Martin, 1959; Konar and Linkens, 1971; Labarca*et al.*, 1970; Dumas, 1974; Shivanna and Shastri, 1976).

After the deposition of pollen, there exchanges a signal between pollen and stigma cell, in which acceptance leads to pollen germination and tube growth whereas rejection leads to the inhibition of interaction (Heslop-Harrison, 1975). Soon after pollen reaches the stigma, hydration and release of pollen protein (exines and intines) occurs simultaneously (Knox and

Heslops-Harrison, 1975). If the pollen grains are compatible, it emerges and contacts the cuticle of stigmatic papillae.

For the growth of pollen tube, it utilizes nutrients from pistil. From the exudates, lipids which are the chief constituent protect pollen from excessive transpiration and desiccation in case of Petunia hybrids and zeamays (Martin, 1970). In nature water, sugar, amino acids are supplied by the style and nourish the growing pollen tube. Stigmatic and stylar tissue contains high level of boron which further promotes pollen germination and tube growth, boron is also provided by stigma sans styles, facilitates sugar uptake and has a role in pectin production in the pollen tube. Krishnendu*et al*. (2016) found that boron has a vital function in fertilization of flowering crops since it takes part in pollen germination and style tube formation. The time of pollen to germinate vary from species to species, where germination of binucleate pollen occurs faster and easier than that of trinucleate pollen (Vasil, 1960a). *In -vivo* pollen germination duration can vary widely between species. Russel (1982) found that in *Plumbago zeylanica,*, pollen tube initiation began just 15 minutes after pollination where as in Lily pollen tubes grow at a rate of 1000µm per hour (Qin *et al.,*2011) and faster growing pollen tubes of Trandescantia and Hemerocallis show a pollen tube growth rate of 14400µm (Michard*et al* .,2011).

The female flowers production is an important attribute as it determines the final yield of the palms. A female flower remains receptive from 1-3 days. The female phase remains for 3-5 days in tall palms or 8-15 days in dwarf morphoforms depending on the environmental conditions. Shedding of female flower in an inflorescence also occurs which has a bearing on the economic yield of coconut. Button shedding in all the varieties is observed during summer and other during monsoon months (Kasthuribai *et al.* 2003). However as early as 1938 Patel observed button shedding has no correlation with the quantum of rainfall since shedding was high during August, September, and November and slightly less during the other months. Maximum number of female flower was produced during the summer months (march-may) and the minimum during winter (Rao1988; Ratnambal *et al.* 2003).

Prasad Rao and Krishnakumar, K. N. (2011) found that, a gradual decline in female flower production was noticed from summer to post monsoon season and thereafter an increase was noticed during winter in all the cultivars. It is the well-established truth that the effect of weather variables during the two critical stages viz., primordia initiation and ovary development is vital for final female flower production. Female flower or button shedding is

one of the serious issues looked by the coconut ranchers. A coconut inflorescence carries on an average 16-20 female flowers, this is otherwise called potential nuts when the spathe opens. Some female flowers which miss the opportunity of getting fertilized, wither, get detached from the spikelet and shed. However, according to Prasad Rao and Krishnakumar, K.N. (2011), the phenomena of seasonal button shedding is more dependent on agroclimatic features. High temperature (both air and soil), and vapour pressure deficit adversely influenced the button shedding in coconut in absence of soil moisture. It is evident during the summer. The low button shedding from June to November could be attributed to less vapour pressure deficit in the crop environment under no soil moisture stress. 'Rajagopal*et al.,*1989.reported that the nut production under rainfed condition is influenced significantly by the length of dry spells at critical stages and the dry spell during the primordium, ovary development and button size is crucial for the production of nut yield. According to Kumar *et al.,* (2007), longer dry spell affects the nut yield for next four years to follow with stronger impact on fourth year, irrespective of the total rainfall. We know that coconut has a long reproductive phase, so it is difficult to assess the receptive stages which are affected due to high temperature stress, soil moisture stress and the lack of proper humidity under field conditions. But there is lack of literature on interaction between the occurrence of high temperature (dry spell) and its effect on reproductive stages in coconut.

2.5.4 Male and Female Phases

The male flowers are the first to open in an inflorescence, beginning at the top of each spikelet and proceeding towards the base. The pollen is released after the male flower opens and the flower later abscise, the whole process taking one day. In tall palms, the male phase extends for 16-22 days and the female phase usually begins 22 days after the spathe has opened and lasts for 5-7 days. A single female flower remains receptive for 1-4 days (Sholdt and Mitchell, 1967; Henderson, 1988; Ashburner, 1995). Detailed studies in this direction may be helpful in optimizing the frequency of pollination during artificial pollination in coconut. The length of each phase is affected by the season and locality. Usually in tall ecotypes, the two phases do not overlap and hence the chance of intra-spadix pollination is negligible. However, an overlap of male and female phases between subsequent inflorescence is common. Whitehead (1965a) reported that in Jamaican conditions the possibilities of selfing due to overlapping of consecutive inflorescences were substantial in Jamaica Tall and

San Blas varieties. In dwarf varieties there is considerable overlapping of phases in the same inflorescence. Coconut varieties have been classified into four types based on flowering pattern (Rognon, 1976). In Type I flowering pattern (allogamy), there exists no overlap of male and female phases (within the same inflorescence and also between the inflorescences). In Type II flowering pattern (mixed allogamy and indirect autogamy), there is no phase overlap in the same inflorescence but phase overlap occurs between subsequent inflorescence. In Type III flowering pattern (direct autogamy), there is complete overlap of the phases within an inflorescence. In Type IV flowering pattern (mixed mating), flowering is characterized by partial overlap of the female phase within and between inflorescences. Within flowering groups, the degree of phase overlap is highly dependent on environmental conditions.

2.6. HIGH OR LOW TEMPERATURE EFFECT ON POLLEN TUBE GROWTH, STIGMA RECEPTIVITY, AND FERTILIZATION

Temperature is an important factor controlling plant growth and development. Suitability of a crop to a given location depends on threshold temperature as well as on the length of the growing season (Kakani*et al.*2005; Acar and Kakani, 2010). It is the known fact that temperatures above or below that range will have a negative impact on plant performance. All plant species have an optimum temperature range for efficient physiological functions such as their growth, development and reproduction. In some Plant species there are some reviews available related on the impact of heat stress and cold stress. But these reviews illustrated with limited facts about the consequences of the heat and cold stress on the reproductive process. According to Jagadish *et al*. (2010), heat stress reduces pollen viability and germination due to reduced uptake of iron by pollen tubes or micropores in rice.

The maximum percentage pollen germination and tube length was attained at a temperature between 27^0C and 28^0C in different coconut varieties (Ranasinghe *et al.*, 2010). Sexual reproduction in plants is more sensitive to high temperatures than vegetative processes (Visser 1955). Pollination will be adversely affected by extreme and unexpected variation in climate variables especially temperature. Extreme temperatures not only suppress the germination and pollen tube growth along pistil but also reduce bee and other insect activities, which plays an important role in pollination (Vasilakakis and Porlingis, 1985). Temperature is one of the most important factors that can affect performance of pollen at

programic phase (Hedhly*et al.*2005) and pollen tube kinetics in the style (Jefferies *et al.,* 1982; Elgersma*et al.,* 1989). Temperature above the optimum for the plant may also be detrimental to pollen longevity. It is known fact that temperature stress should severely affect female flower, but not many studies are available to show the effect of high temperature on female flower receptivity and fertilization. High temperature may affect either positively or negatively in plant reproductive mechanism. Hedhly *et al. (2005.)* reported that on peach, high temperature conditions evinced poor response of all the components of the reproductive phase investigated. Eventhough elevated temperature caused acceleration in both the pollen germination and the growths of pollen tube within style it also caused a greater loss in the receptivity of stigma (Hedhly *et al*., 2003). And also, been shown that temperature reduces ovule longevity in cherry (Postweiler *et al*., 1985) and plum (Cerovic *et al.*, 2000). In the grass *Brachypodium distachyon*, temperatures above 32^0C led to a decrease of 97% in pollen germination along with the abnormalities or arresting of pollen development stages and lack of deposition of pollen on the stigma (Harsant *et al.*, 2013); under water stressed condition there was inhibition of starch accumulation during pollen development in rice (Sheoran and Saini, 1996). Several works have been conducted to study the temperature ranges and optimum temperature values for pollen germination and pollen tube growth and fertilisation in different crops. But reports on the effect of heat stress on female reproductive development are limited. Saini*et al.* (1983) reported that high temperature can also limit pollen tube guidance to the ovules by increasing ovule abnormalities and decreasing the proportion of functional ovules. According to Hedhly*et al.* (2009) and Zinn*et al.* (2010), suggested that pollen development and function may be the most thermo sensitive reproductive process to high temperature. For example, chronic high temperature exposure during the meiotic phase of micro gametophyte development result in poor pollen germination and seed set in sorghum bicolour (e.g. Jain *et al.* 2012*).* Karapanos*et al.* (2010) studied the respiration of tomato pollen at temperatures 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. 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.* (2015) studied the response of apricot pollen to three temperatures, namely 10, 15 and 25 °C and obtained high germination at 15 and 25 °C. Gajanayake*et al.* (2011) conducted a 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. Study 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. Considerable variation was observed in the cultivar response to high temperatures. Pinus pollen failed to germinate at temperatures below 16 °C or 22 above 42 °C and showed maximum germination between 30°C and 32 °C (McWilliam, 1958).

In vitro experimental systems have been used by many workers to prove the susceptibility of pollen tube growth to high temperature as an underlying cause for low economic yields in many crops. Snider *et al.* (2009), investigated in vivo fertilization efficiency on high temperature stress; they exposed *Gossypium hirsutum* plants a week prior to flowering, to optimal (30/20◦C) or high day temperature (38/20◦C) conditions during flowering were analysed for fertilization efficiency via UV microscopic observation of pollen tube containing ovules. It was concluded that the high temperature stress caused a considerable decline in the reproductive success either by reducing the number of functional ovules that are available for fertilization and by declining the efficiency of ovule fertilization due to inefficient fertilized growing pollen tubes. Marqural (1992) reported that temperature dramatically influenced early *in-vivo* pollen tube growth rate and he found that, in Pecan under the temperature tested moderate temperature (26^{o}C) was better than relatively high(36^{o}C)or low (22^{o}C) and he also reported that the optimum temperature for the germination and tube growth from *in-vitro* germination of Cape pollen on a solid medium was 27^{o}C.

According to Sanzol and Herrero (2001), Temperature has been identified as an important factor that affects the flower receptivity as it severely dents the various processes such as stigma receptivity, pollen tube growth or ovule development. Sanzol *et al*. (2003), conducted an experiment on apple and pears and reported that a sluggish pollen tube growth is an important hindrance to the EPP (Effective Pollination Period) when the crop is grown in cooler temperate cultivation conditions. Eventhough high temperatures drives the growth of pollen tube, the inclusion of these fruit crops in the high temperature or warm regions frequently causes failures of fruit set

Considering the significance of fertilization in determining the yield levels of crops, a number of studies have been conducted to decipher the most thermo-sensitive stage during the reproductive development. Tomato (*Lycopersicon esculentum*) plants when subjected to

the short-term exposure (3 hours) to excessively high temperatures (40°C) during the meiotic phase (9–7 days prior to anthesis) reveals the greater degeneration of pollen tetrads and of the ovule (Iwahori S., 1965).

Morphological investigations by Endo *et al*. (1997) revealed that the viability of pollen subjected to high temperature treatments were severely affected compared to those that were not. High temperature not only reduced the number of pollen grains on stigma but also substantially reduced their germination. Chakrabarti *et al.* (2009) , deduced that elevated temperature conditions severely impacted the sterility of pollen and affected their germination on stigma in rice and wheat. And these effects are relatively more profound in basmati cultivars of rice and Durum cultivars of wheat. Extreme temperatures not only suppress the germination of pollen grains and or the growth of pollen tube but also reduce activities of the honey bee or other insects thereby reducing the insect pollination (Vasilakakis and Porlingis, 1985). Headhly *et al.*(2005) state that temperature is one of the most important factors that can affect performance of pollen at progamicphase;and pollen tube kinetics in the style (Jefferies *et al.*, 1982; Elgersma*et al.*, 1989). Several works has been conducted to study the temperature ranges and optimum temperature values for pollen germination and pollen tube growth for different species including mango (Mangiferaindica L.) (Sukhvibul*et al*., 2000; Dag *et al*., 2000), almond (Godini*et al*., 1987), sour cherry (Cerovic and Ruzic 1992), sweet cherry (Pirlak 2002), Prunusmume L. (Wolukau*et al*., 2004), apricot (Vachun 1981; Egea*et al*., 1992; Austin *et al.*, 1998; Pirlak 2002), and Pistacia L. spp. (Acar and Kakani, 2010). It is reported that for better pollen germination the optimum temperature required was about 15 °C and 20 °C for apricot, sour cherry and sweet cherry (Vachun 1981; Egea*et al.,* 1992; Cerovic and Ruzic1992). Temperatures above the optimum for the plant may also be detrimental to pollen longevity. Harsant*et al*. (2013) found that in the grass Brachy podium distachyon, temperatures above 32°C led to a decrease of 97% in pollen germination along with abnormalities or arresting of pollen development stages and lack of deposition of pollen on the stigma.

The early stage of pollen germination and pollen tube growth is similar in both *in-vitro* and *in-vivo,* although germination occurs within hours in *in-vitro* but takes days in case of *in-vivo*. Pollen germination *in-vivo* and its pollen tube growth into style or ovary depend on several environment factors. In most of the species, all genotypes performed well during winter compared to offseason. In a study conducted on lablab bean (Uddin*et al*., 2007) suggested that for most of the embryological parameters in winter stigma harvested after 8-12

hours produced better results, but for reaching the maximum level of pollination and fertilization in other seasons a period of 24 hour after anthesis was the safe limit.

2.7. STIGMA RECEPTIVITY

Stigma receptivity refers to the ability of the stigma to support germination of viable, compatible pollen and it has been investigated in very few species (Shivanna, 2003). The optimum stigma receptivity period varies with species as it could be only hours following anthesis as in teak (Tangmitcharoen and Owens, 1997), or it may extent for few days after anthesis as observed in oak and Silene alba (Kalinganire *et al*., 2000,Young and Gravitz, 2002). In tree crop like almond despite the histological investigations delineating the development of ovule, stigma development or its receptivity have not been well explored (Sterling, 1964; Pimienta and Polito, 1983). At the time of coconut stigmatic receptivity, the stigmas are very white and remain so, for one or two days. After this period, the secretion of nectar commences and the stigmas become receptive. The period during which the female flowers remain receptive varies from one to three days (Patel, 1938).

Transfer of the male gamete to the female egg is considered an important process for successful sexual reproduction in organisms which lacks motility. In seed plants this is accomplished by the generation of gametophyte propagules, pollen grains which supplies the sperm nuclei for the fertilization of the egg cell. Pollen grains on reaching the stigma surfaces that are receptive enhance their germination to induce the pollen tube growth to effect fertilization (Steer *et al*., 1988).

Most of the studies stigmatic receptivity was analysed using florescent microscopic studies with aniline blue. On *Moringaoleifera*Lamk (Bhattacharya and Sudhendu 2004,) pollination, pollen germination and stigma receptivity was examined by the method of Martin (1959) and Joshirao&Saoji (1989) first by fixings (with acetic alcohol 1:1), softening (with 4N NaOH) and staining the stigmas (with aniline blue). Utilizing various pollen tube staining techniques the growth of pollen tubes within the pistils was observed at high temperature treatments in plant species such as *Datura stramonium* (Buchholz and Blakeslee, 2006) and *Oenothera organensis* (Lewis, 1942). Following these many number of herbaceous species has been extensively explored for studying the effect of high temperature on their progamic phase (Coast *et al*., 2016; Matsumoto *et al*., 2012; Snider *et al*., 2011a, 2011b; Liu *et al*.,

2006; Kakani *et al*., 2005; Koti*et al*., 2005; McKee and Richards, 1998; Shivanna*et al*., 1991; Elgersma*et al*., 1989). It was studies in many woody species and tree crops too (Acar and Kakani, 2010; Gao*et al*., 2014; Hedhly*et al*., 2005b, 2005a, 2004; Huang *et al*., 2010; Jefferies *et al*., 1982; Jefferies and Brain, 1984; Kakani*et al*., 2002; Koubouris*et al*., 2009; Luza*et al*., 1987; Nygaard, 1969; Pham *et al*., 2015).

Hedhly *et al.* (2003) found that temperature profoundly influences the duration of stigmatic receptivity in sweet cherry since elevated temperature reduced the receptivity of stigma and the low temperature conditions enhanced it. The stigma lost its inability to assist the pollen in penetration, germination of pollen germination followed by proper adhesion of pollen to the stigmatic surface. Among these, the temperature effect was more prominent on the processes of pollen germination and penetration than on the process of pollen adhesion. Even the effect of high temperature on pollen germination was so predominant that germination is severely reduced as early as the first day after anthesis, when the signs of stigma degeneration are not visually apparent.

2.8. COCONUT FEMALE FLOWER NECTAR

Honey-like sugary liquid is secreted from the multiple pores present around and below the stigma (Marechal 1928). Sampson (1923), had documented nectar or honey secretion at the tri-radial orifice at the base of ovary which opens near the apex of fruit coat wherein honey secretion is observed. Thus, the receptive stigma characteristically shows clear, sweet liquid secretion in multiple places namely at the stigmatic base, orificies (three) on the pericarp at the apex of ovary. These pores are the outer manifestation of three intercarpellary channels that are lined with secreting cells and thus there are no separate or dedicated glands that secrete honey at the ovary base (Sampson 1923, Patel, 1938, reviewed by Narayana G.V. 1937). According to Eames and Mac Daniels (1937), that the secreting cells of the stigmatic surfaces are of the same nature as those of nectaries and the nectaries are phylogenetically derived from hydathodes." And he concluded that the secreting cells of the stigma and nectaries, and hydathodes are closely allied. Thus, in the coconut the secretory cells of (1) the septalnectaries, (2) the base of the stigma, and (3) the stigmatic surface, may be essentially of the same origin though of different shape and size. That the secretory cells of the stigma and the hydathodes are of epidermal origin is obvious from their position. As regards the septalnectaries it may be seen that since they arise by partial non-coalescence of the margins of adjacent carpels they also are of epidermal origin.

CHAPTER 3

MATERIALS AND METHODS

The present investigation was conducted to study the high temperature effect on coconut female flower receptivity, germination of pollen on stigma and the growth of pollen tube through pistil under *in vivo* and *in vitro* conditions.

3.1. LOCATION AND GEOGRAPHY

The experiment was conducted at ICAR-Central Plantation Crops Research Institute, Kasargod, Kerala, India. The institute is located at 12˚18' N latitude and 75˚E longitude. It is about 10.7 m above mean sea level. This region is characterised with an average relative humidity of about 88% (sea side) and annual precipitation of ~ 3400 mm rainfall. The soil in this region is predominantly sandy loam type having the reaction of 4.3-5.5pH.

3.2EXPERIMENTAL DETAILS

Plant material: The experiment was conducted on popular local variety West Coast Tall (WCT) grown in ICAR-CPCRI farm, Kasaragod. WCT is a tall popular variety and extensively grown in all the coconut growing belts of India. The cultivar has been in cultivation since time immemorial and has traits such as long living, hardy, multipurpose palm. In an earlier experiment we observed wide adaptability of its pollen to high temperature as it had high germination even at 38^{o}C (Hebbar *et al*., 2018).

3.2.1. Evaluation of in-vivo progamic phase in coconut grown under ambient condition

An experiment was conducted to standardize the histological and staining protocol to elucidate the *in-vivo* coconut pollen germination in stigma, pollen tube growth and its kinetics in style till reaches ovule grown under ambient condition.

3.2.2. Preparation of inflorescence for pollination

Flowering in WCT variety starts after 5 to 6 years of planting. In this experiment 8 to 10-year-old healthy trees were selected, which has a height of nearly 10 to 12 m. As soon as fresh spadix burst open (spathe breaks and rachis and spikelets emerge out), all the male flowers were emasculated leaving the spikelets intact. The male flowers were collected in a bag and disposed of properly. Bagging is done using pollination bags (cloth bags) 6 days before any of the female flowers in an emasculated inflorescence become receptive by experienced pollinator who has very good understanding of coconut floral biology. The receptivity of a female flower is denoted by the splitting of the white stigma and the secretion of nectar (Fig.1&2). Viable pollen from either same plant or adjacent plants was collected and dusted on receptive stigma. Pollinator climbed the tree daily to pollinate the successive receptive flowers, until all the flowers in the inflorescence were pollinated. Each pollination date was recorded. As soon as the stigma of all female flowers necrose or turn brownish-black, the bag was removed, usually, that occurred on the fifth day after the last pollination.

Fig.1.Fully opened Inflorescence bunch just before bagging for *in vivo* treatment

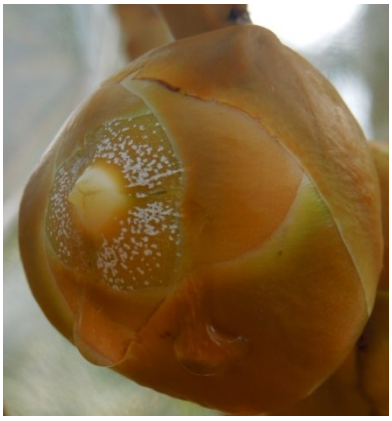


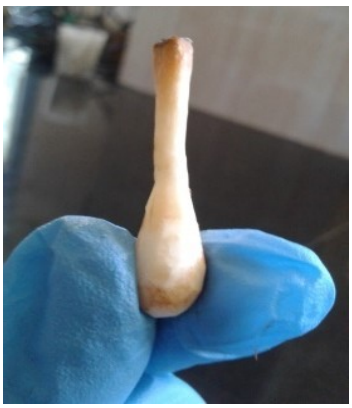
Fig.2. Fully opened stigma with the presence of nectar at the time of receptivity on control condition (ambient)

3.2.3. Sampling of pollinated buttons

Pollinated female flowers were initially collected every two hours to study extent of pollen germination on stigma and the growth of pollen tube through style. Initial observations revealed that *in- vivo* pollen germination and pollen tube expansion occurred only after one day of pollination hence, further observations were restricted to day basis.

3.2.4 Female flower dissection and staining

The female flowers fertilised under *in- vivo* condition were collected and brought to the laboratory for further observation. Coconuts have large and fleshy styles, and ovary, so carefully dissected the samples using a sharp blade and needle and extracted the stigma, style, ovary of the female flower intact (Fig 3). Then it was cut longitudinally into thin sections and was fixed in a modified Carnoy's fluid (absolute alcohol: chloroform; glacial acetic acid 6:4:1) for a period of 12 hours.



(a)

(b)

(c)

Fig. 3. Dissection of female flower (a) showing intact longitudinal part b) two halves showing stigma, pistil and ovules (c)small longitudinal sections of female flower reproductive parts.

To study the germination of pollen, growth of pollen tube in style and to evaluate the stigma receptivity histological preparations were performed. To stain the pollen grains and pollen tubes various dyes like acetocarmine, acid fuschine, basic fuschine, aniline blue, brilliant blue, Evans blue, methyl orange, iodine potassium iodide, malachite green, methyl red, methyl green, orange G, toluene blue, TTC etc. were tried. In most of these stains coconut pollen either not absorbed or in some of them it was burst. Hence, in coconut to observe the pollen germination and pollen tube growth inside the pistil a method devised by Alexander (1987) was adopted.

Fig4. Experimental attempt to stain the pollen tube with different dyes

It is a quadruple staining procedure, the staining mixture consists of lactic acid 80 ml; aqueous malachite green (1%)-4 ml; aqueous acid fuchsin (1%)- 6 ml; aqueous aniline blue (1%)-4 ml, ; orange G (1 %) in 50% alcohol- 2ml; and chloral hydrate-5 g. Pistils arc fixed for 6 hr in modified Carnoy's fluid (composition of fluid: absolute alcohol: chloroform: glacial acetic acid 6: 4: 1), kept hydrated in the descending alcohols, followed by the transfer to stain and maintained for 24 hr at 45$\pm$2ºC. Then the samples were shifted to a clearing and softening liquid comprising 78 ml lactic acid, 10 g phenol, 10 g chloral hydrate and 2ml 1% orange G. The pistils were maintained for 24 hr at 45$\pm$2ºC, followed by the hydrolysis in the clearing and softening fluid at 58$\pm$2ºC for 30 min. Following hydrolysis, procedure the

tissues were subjected to wash twice in lactic acid and stored in lactic acid for later assessment or mounted directly on a slide for immediate analysis under a Stereomicroscope.

3.3MICROSCOPIC OBSERVATIONS

After the incubation period with clearing and softening solution at 4-5 days the specimens observed under 8x, 3x, 1.5x magnifications objective of a Nikon SMZ800N stereo microscope (Nikon Corporation, Tokyo, Japan) which was paired with an in built Nikon DS-Ril camera. The photographs of the observed microscopic fields were documented at a resolution of 1920×1536 pixels and a scale of 0.61µm/pixel using the NIS (Nikon Instrument Software) Element D version 4.3. Measurements were taken using 'Measurement and Annotations' toolbar of NIS Element. Measurement of pollen tube length was measured in micrometers using the polyline length measurement tool of the same software. The data was exported to an Excel file.

Fig.5.SMZ800N Sterio Microscope (NIKON)

3.4. IMPOSITION OF TEMPERATURE TREATMENT

In-vivo technique: As it was difficult to expose the whole adult palm of coconut to high temperature, in this study a device was developed to expose the individual inflorescence in a tree crown to set temperature.

The device is a transparent plastic cover (heat cover) having 0.40 mm thickness, big enough to accommodate the size of coconut tree inflorescence. It has a front transparent PVC flap (that can be opened and closed through zipper mechanism) and two side pockets for doing the pollination. A frame was also fabricated that acts as a skeletal support for the bag to give proper shape as well as for fixing the heater. The frame is made of MS rods of a Dia. 5mm and size is 40cm×30cm×30cm. A temperature controller with sensor is attached inside the bag. The controller that used in the experiment is a digital PID (Proportional Integral Derivative) controller. A 1 meter Pt-100 RTD sensor (Dia. of 0.5mm and length of 6mm) which is connected to the controller is used to measure the temperature. It continuously monitors the temperature inside the chamber. With a change in the temperature, the resistance of the RTD varies that is detected in terms of voltage drop by the controller and controls the heater to switch on and off to maintain the set temperature. As the PID controller cannot handle the power requirement of the heater (1000 watts), a separate solid-state relay (SSD) was used in the circuit to switch on and off the load.

Fig.6. Experimental set up for *in vivo* growth of female flowers on tree

Fig.7A Fig.7B

Fig.7A. Readymade Panasonic heater attached inside the *in-vivo* chamber, 7.B.Temperature and humidity controller box fixed on the tree

Fig.8.A Fig.8.B

Fig.8.A&B periodic observation of diurnal temperature change and morphological observations during the *in-vivo* treatment

Fig.9 Fig.10

Fig.9.In-vivo high temperature experiment. Fig10. Fully opened inflorescence+ Receptive female flowers+Heat cover to elevate the temperature + Temperature and humidity control devices

3.4.1 The temperature treatments used for *in-vivo* studies were as follows treatments:

T1: Control (Ambient temperature)

T2: Control with heat cover

T3: T2+3^{0}C elevated temperature

T4: T3+70% Humidity

T5:T2+5^{0}C elevated temperature

Replications: 3

Six days before the female flowers were to become receptive the inflorescence was enclosed in heat cover and it was tied at the base of the inflorescence. The temperature treatment to the inflorescence was started as soon as it was enclosed inside the chamber. High temperature and humidity treatment were imposed from 8.00 a.m in the morning till 5.00 p.m in the evening. Every two hour the temperature of the inflorescence with and without chamber was measured using Fluke IR Thermometer and accordingly the elevated

temperature treatments, the heater inside the chamber was set to raise the temperature $+3^0$C. As and when temperature rises above set temperature the heater automatically cuts off and with 1^0C fall in temperature it automatically gets on. Both under control and temperature treatments as and when the female flowers became receptive, they were pollinated through the side openings of the chamber. The temperature treatment was continued, 5 days after the flower was pollinated. The temporal variation in secretion of nectar, stigmatic appearance of button with varying temperatures was observed. The pollinated female flowers were collected after 1, 2, 3, 4 and 5 days to observe the pollen germination on stigmatic surface and pollen tube growth through the style. Further the collected buttons were subjected to histological processes and microscopic observations as described above.

3.5. *IN-VITRO* TECHNIQUE:

A technique was developed to grow the female flower under *in-vitro* condition so as to analyse the germination of pollen on stigma and the growth of pollen tube in response to different temperature and humidity under well controlled condition. It was found that in a growth media of 10% sucrose, 0.01% boric acid and 1% agar freshly collected female flower at the receptive stage could be grown at least for a period of 5 days. In order to maintain temperature and humidity during growth period a setup was developed as shown in Figure below:

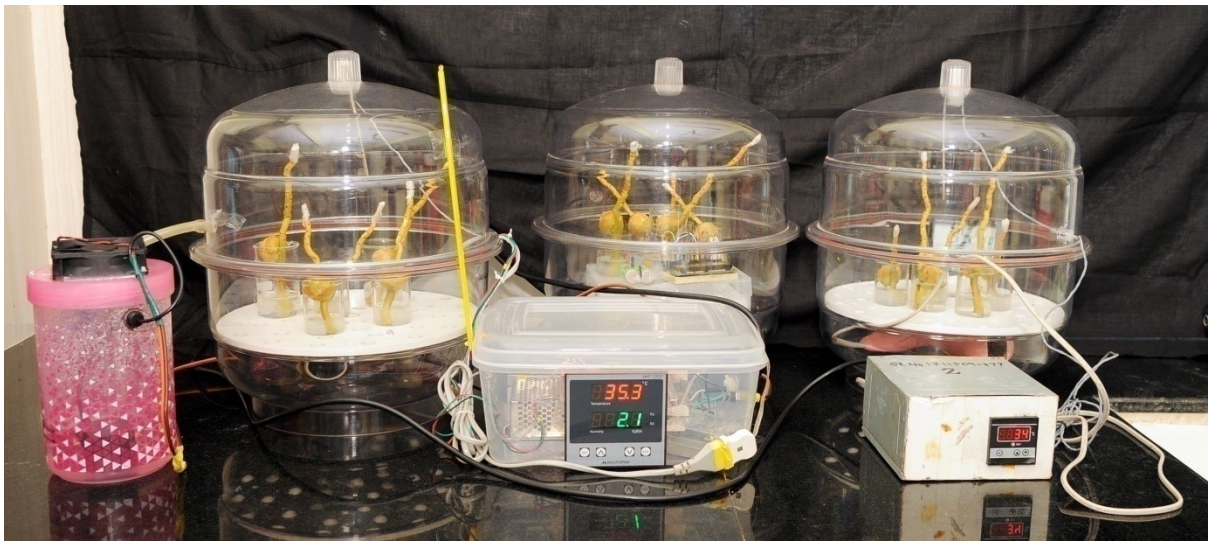


Fig.11. Experimental set up for *in-vitro* growth of female flowers. From left we set up 3 growth chambers 1. Humidity cum temperature control chamber 2. Control chamber, 3.A temperature control chamber,

3.5.1. Control Chamber

Closed desiccators with a small outlet for air flow were considered as a control chamber. A portable Arduino based humidity cum temperature sensor was used to monitor the humidity and temperature in the control chamber

An ardunio was programmed to display the humidity and temperature on a 16×2 LCD module. The sensor used was DHT22 humidity cum temperature sensor. The unit was powered with a 3.7V/3450 mAH Li Iron rechargeable battery. The setup is shown in Fig.12

Fig.12.Control Chamber

3.5.2. Temperature Chamber

The instrumentation components are 1. A temperature controller with sensor, 2. Solid State Relay, 3.A heater with air flow control. 4. Desiccator

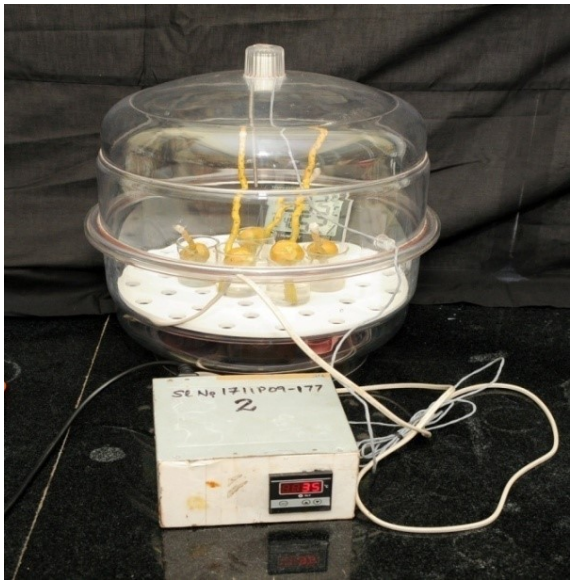


Fig.13.Temperature chamber

A 1meter Pt-100 RTD sensor which is connected to the controller was used to measure the temperature. It continuously monitored the temperature inside the chamber. It is required to increase the temperature inside the chamber. With a change in the temperature, the resistance of the RTD varies which is detected in terms of voltage drop by the controller

A 1000-watt electrical heater with airflow control system (load) was used to rise the temperature. For the convenience, we have employed a readymade Panasonic 1000 watts heater (EH-ND12-P) with a speed control regulation as it has a small footprint and flexible to fix in any direction. The fan fitted in the dryer will create a uniform heat distribution inside chamber so that each part of the inflorescence will be heated uniformly. For our experiment the lowest speed of the fan was used and it was enough for the purpose.

3.5.3. Humidity cum Temperature Chamber

The same temperature controller system with ultrasonic mist generator (wave fr.70 KHz) was employed to create the required humidity in the chamber. A 24V DC fan pushes the fine mist generated to the chamber to keep it humid to the required level. The humidity sensor used is a capacitive type which is connected to the temperature cum humidity controller. The chamber humidity is set at 70%RH ±2% hysteresis and the sensor continuously monitor it. When the humidity level goes below the set value, the ultra sound mist generator switches on and fine mist is sprayed into the chamber. The controller switches off the (mist generator) when the set value is reached.

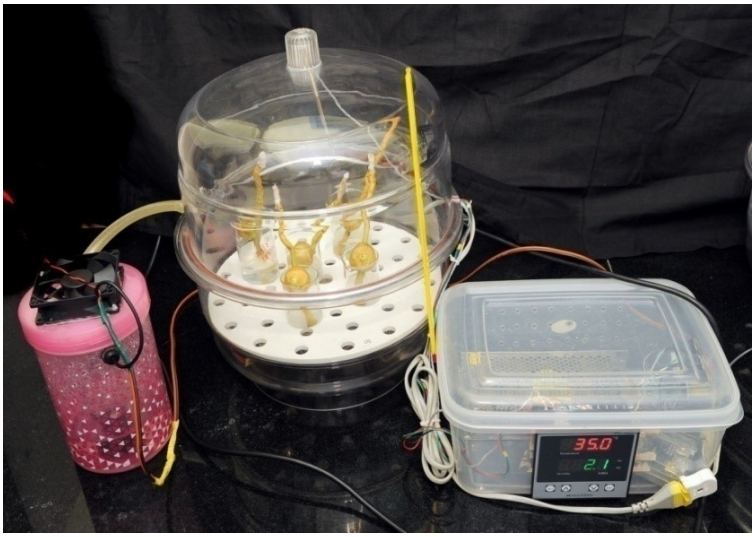


Fig.14.Temperature cum Humidity chamber

Table.1. Treatments under *in-vitro* studies are as follows:

TREATMENT	CHAMBER	TEMPERATURE (^{0}C)	RELATIVE HUMIDITY (%)
T1	Control	29^{0}C	70-75%
T2	Temperature	31^{0}C	<55%
	Temperature + Humidity	31^{0}C	72%
T3	Temperature	33^{0}C	<55%
	Temperature + Humidity	33^{0}C	72%
T4	Temperature	35^{0}C	<55%
	Temperature + Humidity	35^{0}C	72%
T5	Temperature	37^{0}C	<55%
	Temperature + Humidity	37^{0}C	72%
T6	Temperature	40^{0}C	<55%
	Temperature + Humidity	40^{0}C	72%
T7	Temperature	42^{0}C	<55%
	Temperature + Humidity	42^{0}C	72%
T8	Temperature	45^{0}C	<55%
	Temperature + Humidity	45^{0}C	72%

Replications: 3

For this study fresh receptive female flowers were collected from well growing WCT plant and were immediately transferred to already prepared 10% sucrose +.01% boric acid + 1% agar growth medium.

These were placed in controlled chambers with temperature and humidity control. Receptive female flowers were pollinated using stored viable WCT pollen. The response of pollen germination on stigma and the pollen tube growth in the pistil was studied at 29, 31, 33, 35, 37, 40, 42 and 45^{o}C with and without humidity control. Female flowers 1, 2, 3, and 4 days after pollination were dissected for observing pollen germination and pollen tube growth.

3.6. METHOD AND TIME OF POLLEN COLLECTION

In order to study *in- vivo* pollen germination on stigmatic tip of female flower, freshly opened flowers were collected between 8:00 to 8:30 am so as to get maximum germinations (Hebbar*et al*., 2018). For the *in-vitro* study on the other hand dry pollen were used. The pollen was extracted by cutting the spikelet 5cm above the female flower after 5-6 days of the spathe opening. The male flower collected was separated and crushed with a wooden roller. The crushed flower were kept in an incubator at 35°C for 24 hours and the next day it was sieved for removing the debris. The pollen collected was kept in cryogenic vials and stored in -20°C.

3.7. POLLEN GERMINATION

3.7.1. Pollen germination on female flower nectar, stigmatic surface *vs* artificial media

Germination percentage of pollen collected for the study was tested by the method as described by Helen (2016), Anusree (2017) and Hebbar*et al*., (2018) .The medium contains 8% sucrose, 0 .01% boric acid and 1% agar.

The media was prepared separately by mixing all the chemical components in the specified concentrations. Agar (1%) was added to the media and was boiled to dissolve the agar. The media was allowed to cool down a bit and then 1 ml. of 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

In another study female flower nectar was collected on a glass slide from freshly opened (receptive) flowers at 8:30 a.m. and the pollen was dusted on the nectar by means of a

nylon brush. To provide a high humidity during incubation, the Petri plates containing pollen were lined with moist filter paper. This ensured a humidity of about 80% within the Petri plate. And germination assessed after 2 hours, 4 hours, 6 hours, 1 day of incubation.

The germination was observed under 8x magnification objective of a Nikon SMZ800N stereo microscope (Nikon Corporation, Tokyo, Japan) as described elsewhere.

3.8. POLLEN GERMINATION ON STIGMATIP

To calculate the time required for pollen germination on female flower stigma tip, we also conduct an *in vitro* experiment also. For this study we collect freshly receptive flower from field and transferred into the *in vitro* growth medium. Then pollinated with fresh viable WCT pollen, and observed in different time interval under 3x magnification objective of a Nikon SMZ800N stereo microscope (Nikon Corporation, Tokyo, Japan) as described previously.

3.10 CROWN TEMPERATURE MEASUREMENT

The crown temperature of the coconut tree was measured using Fluke 568 IR Thermometer(laser radiation) at the site of *in vivo* experiment.it was measured between 8:30 a.m till 4:30 p.m at 2 hour interval during the pollination period.

3.11. STATISTICAL ANALYSIS

The data was subjected to two factor factorial Analysis of Variance in order to test the significance of difference among pollen tube length using SPSS software. Further, on arriving the fact that there exists significant difference among the temperature and humidity, response curves were developed for the temperature and humidity treatments. The observations were replicated at least three times and the data were subjected to analysis of variance (ANOVA) and least significant difference (LSD; p 5 0.05) with SPSS software.

CHAPTER 4

RESULTS

The present investigation was conducted to study the high temperature effect on coconut female flower receptivity, pollen germination on stigma and the pollen tube growth through pistil under *in vivo* and *in-vitro* conditions. The response of pollen tube growth of WCT genotype to different temperature and temperature with humidity was determined *in-vitro* laboratory condition as well as in an *in-vivo* study involving controlled pollination in field grown plants during the summer months. For this study West Cost Tall (WCT) genotype belonging to tall family with wide adaptability to climatic variability was selected. For *in-vitro* study a growth medium sufficient enough to sustain the viability of female flowers for 5 days was standardized. For the *in-vivo* studies to expose the whole inflorescence to set temperature a heat cover was devised. The results obtained from the study are described below.

4.1WEATHER

The experiment was conducted from 23 September 2018 to 15 October 2019. 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.

4.1.1 Temperature (°C)

The average maximum temperature (T_{max}) was 34.3°C while the minimum temperature (T_{min}) was 24.78°C. T_{max} was low during December (25.91ºC) and increased during April and May (34.3°C) and T_{max} showed an increasing trend from the month December to May. Similarly, T_{min}ranged from18.4°C (January) to 27.3°C (April) (Fig.15.)

4.1.2 Rainfall

During the experimental period, 365.07 mm total rain fall was recorded, of which the first 10 weeks got 71mm rainfall and remaining was recorded from 31st to 43rd standard weeks. There was no rainfall recorded during 11 to 31weeks (Fig .15.) which coincided with the imposition of drought treatments.

4.1.3 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 31stweeks (Fig.16.).

4.1.4. Evaporation

The maximum evaporation of 5.32mm was recorded during the 26th week and the minimum evaporation of 2.15mm was recorded during the 47th 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

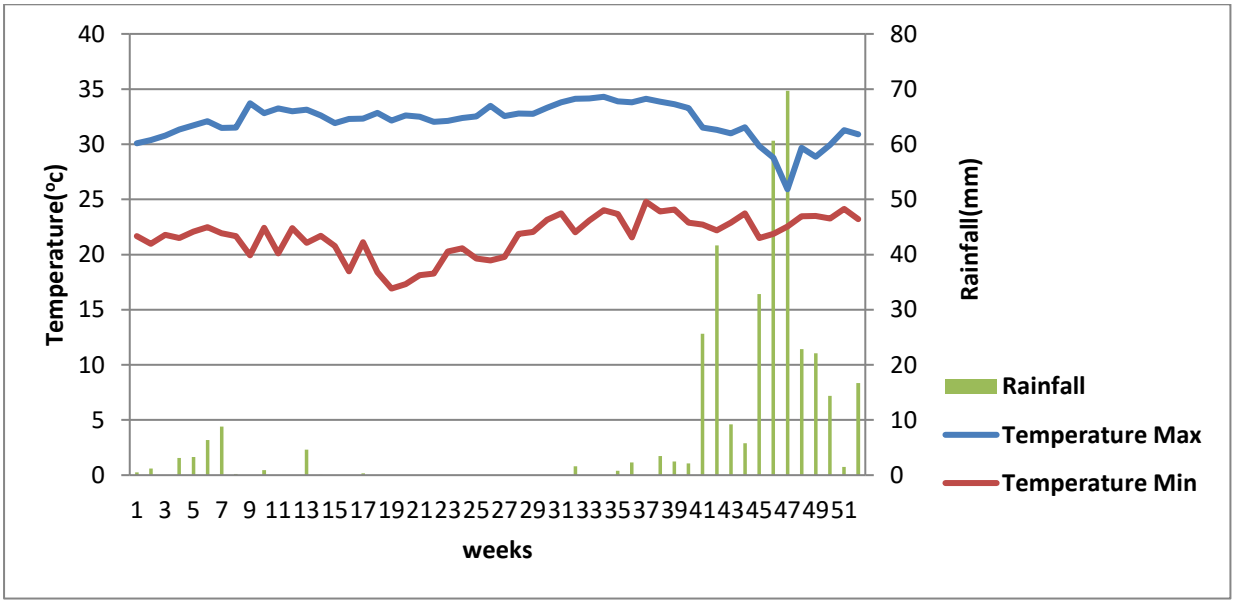


Fig.15.Weekly maximum and minimum temperature and rainfall for the experimental period from September 2018 to October 2019

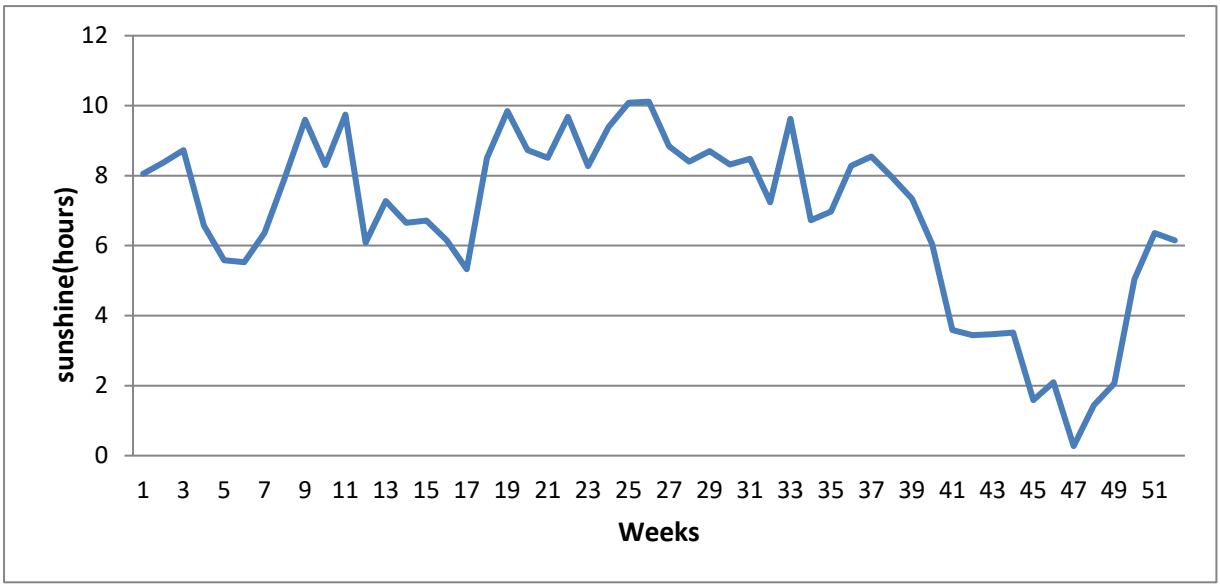


Fig 16.Weekly sunshine hours for the experimental period from September 2018 to October 2019.

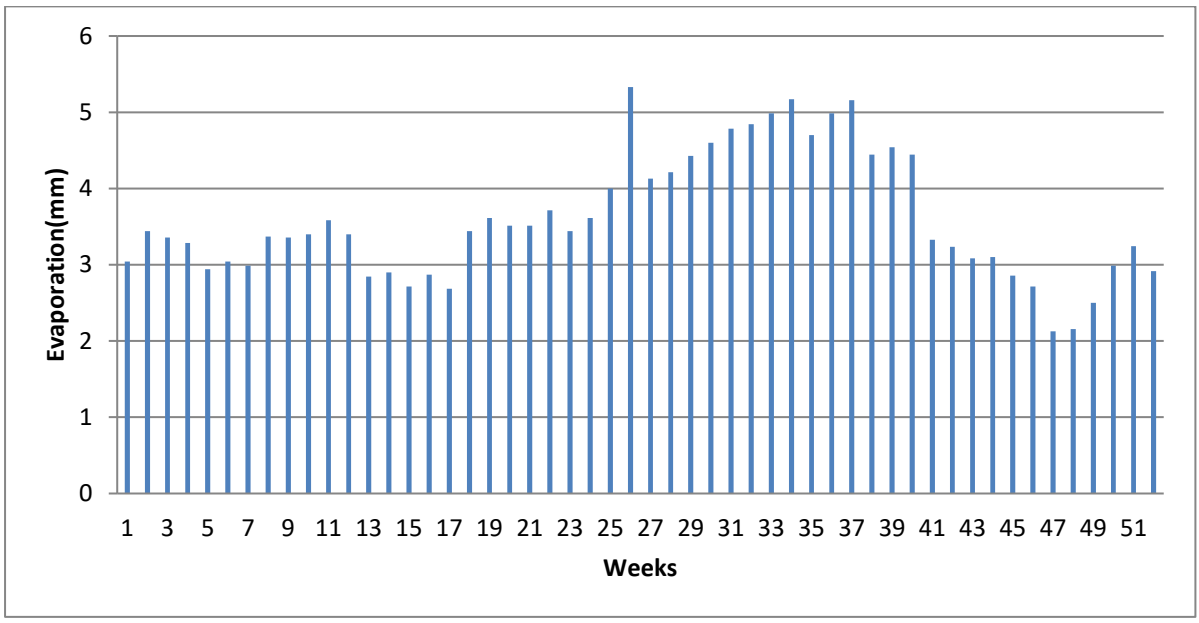


Fig.17. Weekly evaporation for the experimental period from September 2018 to October 2019.

4.2. PROGAMIC PHASE OF COCONUT UNDER NATURAL CONDITION

In coconut no information was available regarding the time elapsed for the pollen germination on stigma, pollen tube growth in pistil to reach the ovule. In order to study the above aspects artificial pollination of receptive female flowers was done under *in-vivo* conditions. Bagged female flowers, hand pollinated under ambient condition (28 to 29ºC) were collected at hourly intervals for histological observations. As it was observed that pollen germination happened only after one day (Fig.18.), the sample collection and its observation was restricted to daily basis.

The pollinated female flowers collected at regular intervals were dissected in laboratory and stained to observe under stereo microscope.

Dissection of pollinated female flowers

(a) (b) (c)

Fig.18. Dissection of female flower (a) showing intact longitudinal part b) two halves showing stigma, pistil and ovules (c)small longitudinal sections of female flower reproductive parts.

Coconuts have large and fleshy styles, and ovary, so carefully dissected the samples with sharp blade into 3-dimension section including stigma, style, ovary of the female flower. The samples were stained following splitting up into longitudinal sections. Compared with the styles of *Cucumis melo* and Luffa *cylindrica* which are large and fleshy, the styles of , coconut is too hard. Therefore, the fixing, staining, clearing and softening procedures are time consuming. Pollen tube fixation in modified Carnoy's fluid had taken 24 hours to fix the stain. The sample with staining solutions which was kept in hot air oven at 50^0C for 24 hr,

took 2 days at 45^0C for clearing and softening. Again, it required fresh clearing and softening medium for 4 days at 45^0C for observing under microscope.

4.3. IDEAL STAIN FOR OBSERVATION

We made an attempt to staining the pollen grains and pollen tube with different vital and non-vital dyes of the compositions presented in the Table. 3. but were not suitable to observe the specimen under stereo microscope.

Table:2A & 2B. List of stains used to observe pollen germination on stigma and pollen tube growth in pistil.

Sl. No.	**Stain**	**Stain concentration**	**Percentage pollen germination**	**Remarks**
1	Acetocarmine	1%, 0.1%, 0.01%	0	Pollen shrinks, no germination
2	Acid fuchsine	1%	35	Both pollen tube and pollen head takes the dye
3	Aniline blue	1% 0.1% 0.01% 0.001%	0 5 24 54	Pollen germinates at low concentration but bursting is seen in most cases. Only pollen head absorbs the dye
4	Basic fuchsine	1%	0	No germination
5	Beetroot juice		0	No germination
6	Brilliant blue	0.1% 0.01%	13 5	Pollen tube does not take up the color
7	Evans Blue	0.1 %	0	No germination
8	IKI(iodine potassium iodide)	0.1%	0	No germination

Table:3B. List of stains used to observe pollen germination on stigma and pollen tube growth in pistil.

Sl. No.	Stain	Stain concentration	Percentage pollen germination	Remarks
9	Methyl orange	1% 0.1% 0.01%	0 14 25	Pollen tube does not take up the color
10	Malachite green	1% 0.1% 0.001%	0 15% 30%	Pollen germinates at low concentration but pollen tube doesn‘t take up the dye
11	Methyl red	1%	0	No germination
12	Methyl green	1%	0	No germination
14	Orange G	1%	0	No germination
15	Toludine blue	0.1% 0.01%	40 80	Pollen head absorb colour but not tube
16	TTC	0.1%	20%	Pollen head and tube doesn‘t takes up the dye

In most of the earlier pollen tube staining studies they adopted fluorescent microscopic study using aniline blue staining method however it requires a fluorescence microscope (Linskens and Esser 1957). Here we tried with 16 different dyes and concluded that pollen tubes only take vital dyes, but pollen heads could absorb normal dyes but not suitable to observe under stereo microscope. Since, staining of pollen tube is a difficult task we followed quadruple staining procedure for staining pollen tubes in pistil for light microscopy developed by Alexander (1987) with little modification. It was a mixture mad by

the combination of malachite green, aniline blue, acid fuchsin, orange G and Chloral hydrate. We chose the same combination without Chloralhydrate in different concentration of stock solutions and standardized a quadruple staining mixture for stereomicroscopic study. Accordingly, acid fuchsin and aniline blue stains were used to colour the nuclear material whereas the Orange G stains both the nuclear material and the cellulose of cell walls, and the Malachite green stains only the cellulose of the cell walls. In an acid medium both the acid fuchsin and aniline blue helped in staining the pollen grains, pollen tubes and stylar tissue dark blue, however the clearing the softening procedures ensured that the colour absorbed by the stylar tissue was removed. The pollen and pollen tubes thus showed dark blue against a light or dark purple background. This procedure that was followed was simple and efficient using dilute solutions of NaOH however it consumes lot of time.

4.9 POLLEN GERMINATION ON FEMALE FLOWER NECTAR, STIGMATIC SURFACE *VS* ARTIFICIAL MEDIA

The response of pollen germination of WCT genotype to temperature was studied in a media as standardized earlier (Helen (2016), Anusree (2017) and Hebbar*et al*. (2018), female flower nectar of WCT collected during its receptivity, and on stigmatic surface of the buttons during receptivity.

The following observations were made:

In the germination media freshly collected pollens required just two hours for germination as observed in earlier studies (Hebbar*et al*.2018). Pollens were started to germinate after 30 minutes incubation at 27^0C room temperature, and reach maximum germination and elongated tube length at 2 hours incubation. But in female flower nectar, pollen germination requires more time and just to emerge it required 24 hours at 75-80% humidity. Pollen tube growth was not observed when they are incubated in nectar. Similarly, on the stigmatic surface it required on an average one day $\pm$6 hours for germination. Pollen tube growth is slow and requires 4 to 5 days for complete elongation.

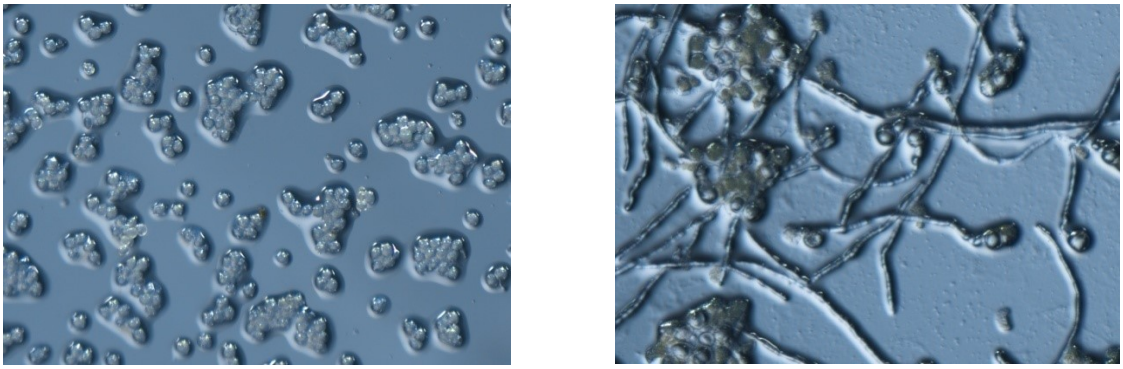
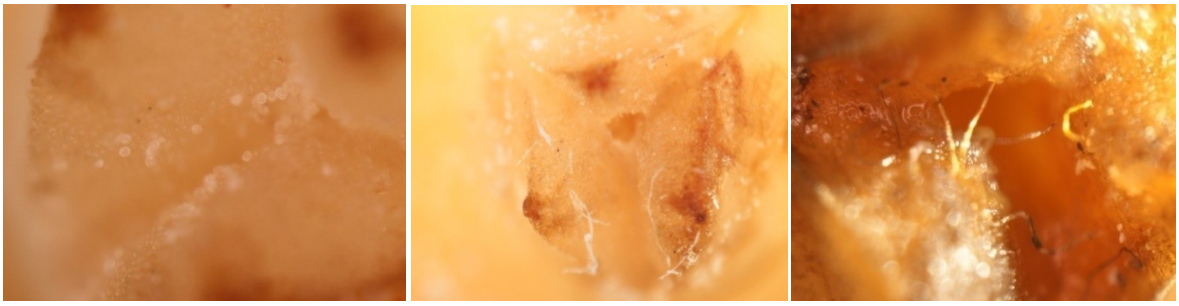


Fig.19.A Ungerminated pollens on nectar after 4 hours incubation. Fig.19.B Germinated pollens in artificial germinating media.



(A) (B) (C)

Fig.20. Pollen grains on the receptive stigma. (A). Ungerminated pollens 2 hours after pollination (B).Germinated pollen grains on stigma at 1 day after pollination (C).shows geminated pollens on stigma tip at 2 day after pollination

Fig21.Pollen tubereachedmicrophylar region four days after pollination in control plant

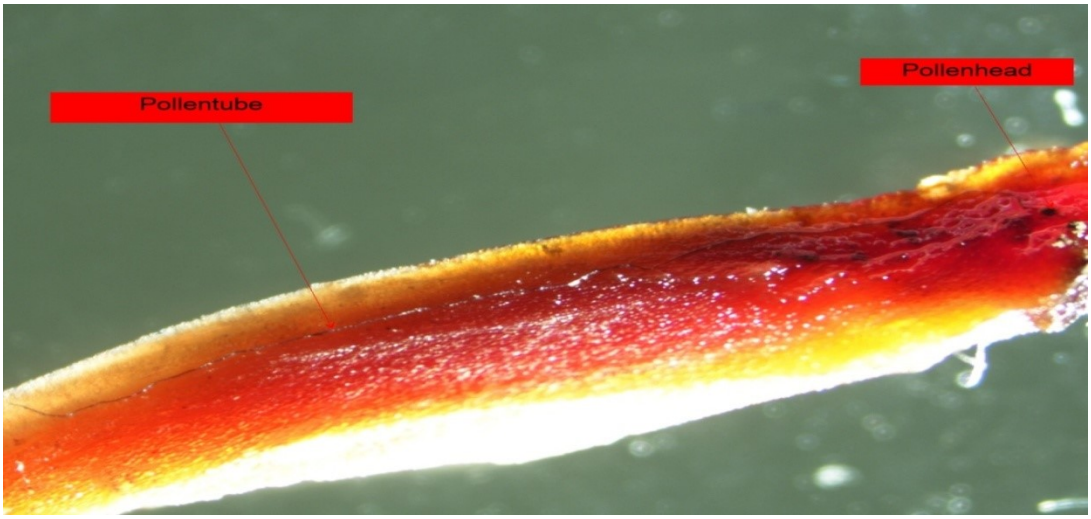


Fig 22.Pollen tube growth in style

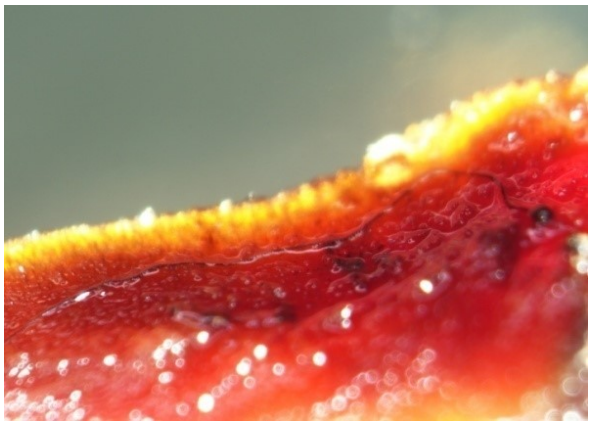


Fig.23. Germinated pollen on the stigma tip under 3x magnification

In coconut the distance from stigma to ovule is around 1.9 cm and pollen tube required approximately four days to reach this distance at a temperature of around 28 to 29ºC at the time of pollination.

4.2 HIGH TEMPERATURE EFFECT UNDER *IN- VIVO*

4.2.1Diurnal temperature

The temperature and humidity of the treatments were maintained from 8.30 in the morning to 5.30 in the evening and were monitored at regular intervals. A representative diurnal variation of temperature in control and various treatments is depicted in Fig.18. The experiment was conducted in the month of April. In the control plants the temperature near the inflorescence in the tree crown was around 28^0 C. As the day progressed ambient

temperature increased and peaked around 2.30 pm (34 ^{0}C) and later declined to 27^{0}C at 4.30 pm. When the inflorescence was enclosed in a heat cover the internal temperature increased by around 1 to 1.5^{0}C throughout the day from ambient. In the elevated temperature treatment of 3^{0}C and 5^{0}C from ambient, from the figure it is clear that there was a consistent increase in set temperature throughout the day. In the treatment with humidity too the set temperature was maintained consistently.

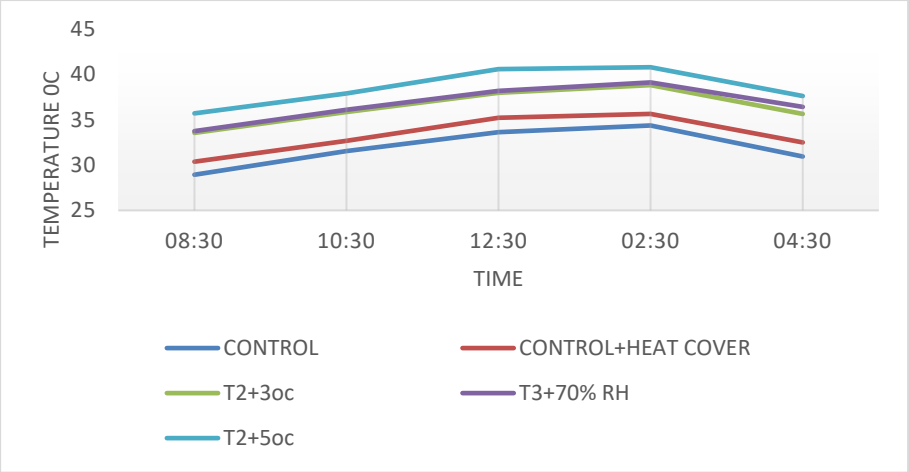


Fig.24. A representative diurnal record of the temperature around the inflorescence in the chamber control and chamber control+3ºC in comparison to ambient control during April 2018.

4.4.2. Temperature during the experimental period

The response of female flower receptivity, morphological changes, pollen germination on stigma and pollen tube growth was studied in an inflorescence enclosed in a heat cover (Fig. 18) with set temperature and humidity controls for a period of 10 days. Heat covers and temperatures were set 5 days before the receptivity of female flower. Female flowers were exposed to ambient (T1) temperature and humidity (Temperature 29-34^{0}C, Relative humidity around 70-75%), T2 (TI+ heat cover), T3 (T2+3ºC), T4 (T3+70% RH), and T5 (T2+5ºC) with and without humidity control. The device used to regulate the temperature was quite effective and could maintain the set temperature during the experimental period as depicted in Fig.18A. Control temperature was around 31-32ºC, T2 around 33.5ºC, T3 and T4 around 36ºC, T5 around 39ºC. Thus, the female flowers were exposed to temperature between 32 to 39ºC.

Fig.25

Fig.26

Fig.25 An *in-vivo* experimental set up with heat cover on the tree. Fig.26 Diurnal variations in temperature on control plant monitored at regular intervals of time

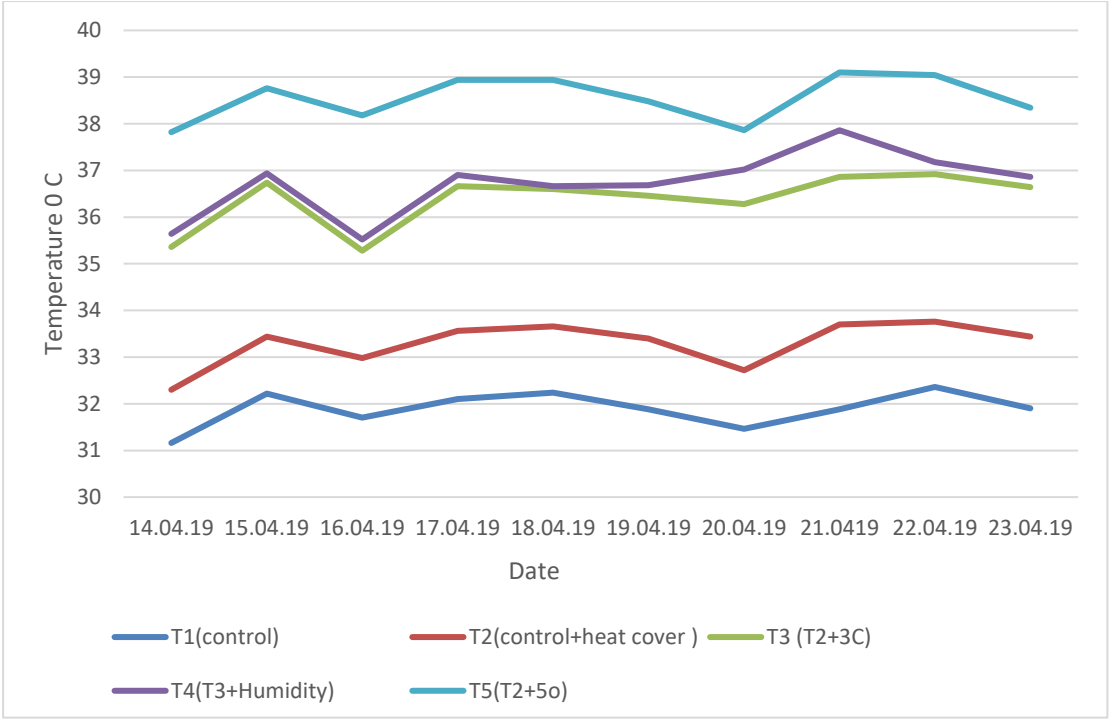


Fig.27. Temperature of control and high temperature treatments during the experimental period

4.4.5 High temperature effect on female flower receptivity and Female Flower Morphological Change

As the female flowers were to become receptive some of the following changes were noticed.

- The pistillate flowers present at the top of the spadix opened initially
- The size of the pistillate or female flower is double the size of what it was while opening of the inflorescence
- The stigmatic region of the pistillate flower was observed protruding from the perianth lobes
- Small drops of nectar started to ooze out from the three orifices alternating with the stigma. The nectar secretion just starts just 2-3 days before the stigma tip opening
- At the time of receptivity three equidistant grooves on the apex divided into three triangular sections, and stand erect as three teeth. It reveals the stigma region so that pollen falls on it to effect germination and fertilization The stigmas remain white in colour and they maintain this colour for a day to two

(A) (B) (C)

Fig.28. various stages of female flower receptivity under ambient condition. A show the stigmatic region emerged out from the perianth lobes, B. Secretion of nectar from the orifices. C. stigma opened and white in colour with nectar

Temperature had effect on emergence of stigmatic regions, nectar secretion and stigmatic appearance of the growing female flower. Some of the changes observed at flower receptivity and some of the morphological changes observed.

Temperature	No.of female flowers in a bunch	No.of female flower became receptive	Pre-receptive nectar secretion and amount	Stigma characters
T1(control)	22 20 15	22 19 13	Tiny drops of nectar at the orifice was observed from 2-3 days pre-receptivity to 2-day post receptivity	Clear white stigma. Browning of stigma occurred 1-2 hours post pollination
T2 (control + heat cover)	16 23 18	13 22 17	Nectar secretion was similar to control but the amount was slightly higher.	Similar to control, except that few buttons(2to3) stigmatic surface became brown
T3 (T2+3^{0}C)	18 21 16	13 19 12	Nectar secretion started within 3-4 hour after exposure to temperature, the amount of secretion was higher and continued till one day before stigma opening. At the stage of stigma receptivity, it was dry.	Almost all flowers stigma turned into brown colour at the time of receptivity.

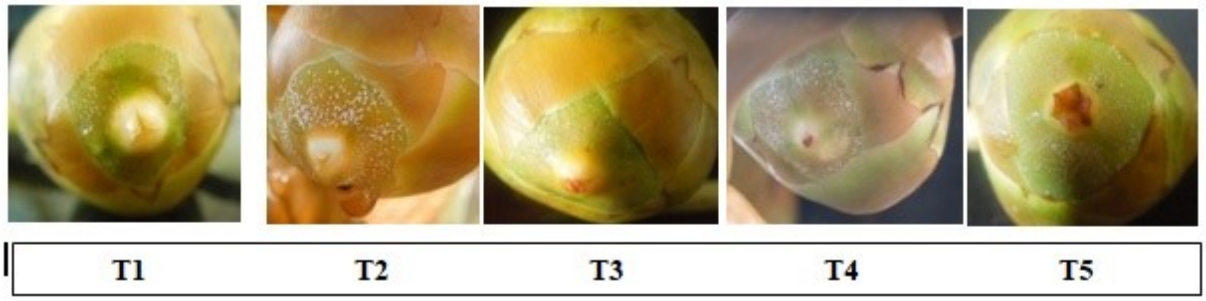
Table 3A

Temperature	No:of female flowers in a bunch	No.of female flower became receptive	Pre-receptive nectar secretion and amount	Stigma characters
T4(T3+70% RH)	25 22 17	24 20 14	Nectar secretion started within 3-4 hours after exposure to temperature as in T3 and had high amount of secretion. Secretion was there on the receptive day	Almost half of the flower stigmas were normal while rest turned into brown colour as in T3.
T5 (T2+5^{0}C)	23 21 18	12 16 7	Nectarsecretion started15-30 hrs after exposure to temperature but it fully stopped 2 days prior to stigma receptivity	Almost all the stigmas turned into dark brown and became dry at the time of receptivity

Table 3B

From the Table.3A & 3B.it is clear that exposure to high temperature leads to early nectar secretion in female flower orifices i.e., 5 to 6 days prior to receptivity. However, at the time of stigma receptivity stigmatic surface becomes dry and observed brownish discoloration. On the other hand, in control plants at a temperature around 29^{0}C, there was ample nectar at stigma receptivity; stigma is wet and white in color. Here in these plants' nectar secretion was started just 2 days prior to stigma receptivity. When the inflorescence temperature was elevated either to 36^{0}C or 39^{0}C, the relative humidity inside the heat cover had reduced to around 50% from control 75%. Rising humidity with elevated temperature treatment to a certain extent alleviated the high temperature effect. There was reasonably extended nectar production till the receptivity of stigmas and to a large extent prevented the browning of stigma. At high temperature of 39ºC (T5) more buttons were dry at receptive stage while only few buttons produced nectar.

Stigma tip observation



Figures.29. Appearance of stigmatic surface and nectar secretion observation in response to temperature and humidity

This pictures clearly shows how high temperatures influence the appearance of stigma. T1 (control), T2 (control +heat cover) shows clear white stigma at the time of receptivity. High temperature T3 (T2+3^{0}C), T4 (T3+70%RH), T5 (T2+5^{0}C) stigma tip appeared as brownish in color and there was hardly any nectar. In T4 with humidity the tip was relatively white and little nectar was seen.

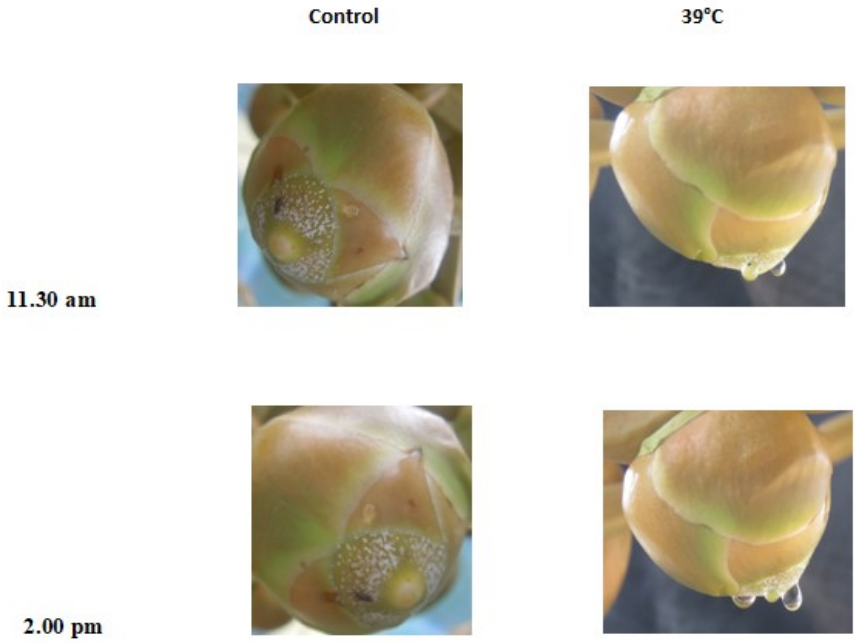


Fig .30. The nectar secretion of control and temperature induced buttons 2 hour after exposure to 39^{o}C.

From this experiment it is clear that high temperature had an impact on the physical appearance of the buttons which in turn affected the stigmatic receptivity and fertilization period

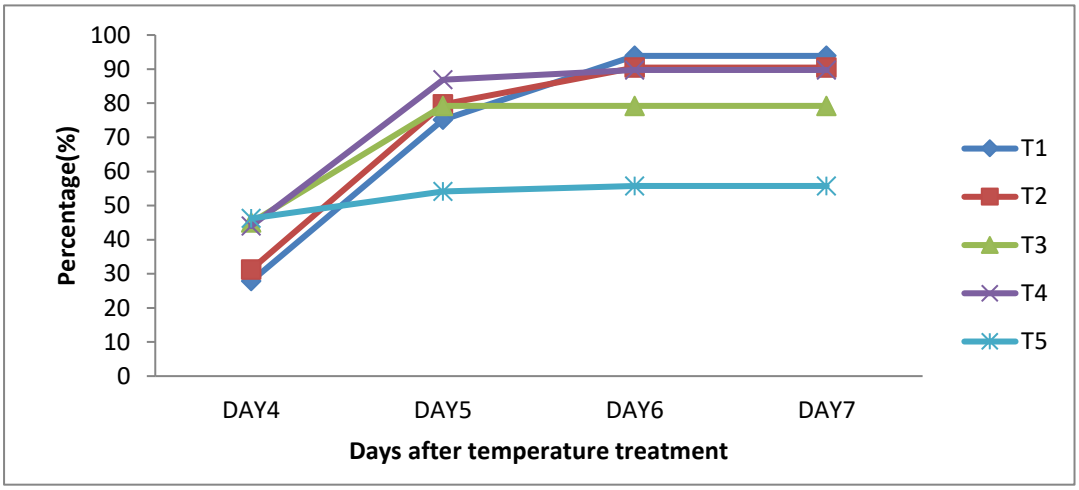


Fig.31. Percentage progression of female flower receptivity from 4th day of high temperature and humidity treatment

At the time of observation i.e., four days after temperature and humidity treatment ambient grown control plants and heat cover enclosed inflorescences had 31% of the female flowers were receptive. During the same period in temperature treatment 46% of the female flowers became receptive. It continued to increase 79% on 5th day and 89% on 7th day in control and heat cover enclosed inflorescences. High temperature +3ºC increased the receptivity to 79% and +5ºC to 54% with no further increase on 6th and 7th day. However, maintaining humidity in +3ºC temperature treatment reduced the heat effect and receptivity was on par with control plants.

4.5. HIGH TEMPERATURE TREATMENT UNDER *IN VITRO*

A technique was developed to grow the female flower under *in-vitro* condition so as to study the pollen germination on stigma and pollen tube growth in response to different temperature and humidity under well controlled condition. Fresh receptive female flowers collected at 8:30 am from well growing WCT plant which were immediately transferred to already prepared 10% sucrose +.01% boric acid + 1% agar growth medium could sustain the viability of the flower for at least five days. These were placed in controlled chambers with temperature and humidity control after pollination using stored viable WCT pollen. The

results of pollen germination on stigma and the pollen tube growth in the pistil studied at 29, 31, 33, 35, 37, 40, 42 and 45ºC with and without humidity control is presented below.

4.5.1. Diurnal temperature

The temperature and humidity of the *in- vitro* treatments in the chambers were maintained from 8.30 in the morning to 5.30 in the evening and were monitored at regular intervals and a representative diurnal value depicted in Fig.30

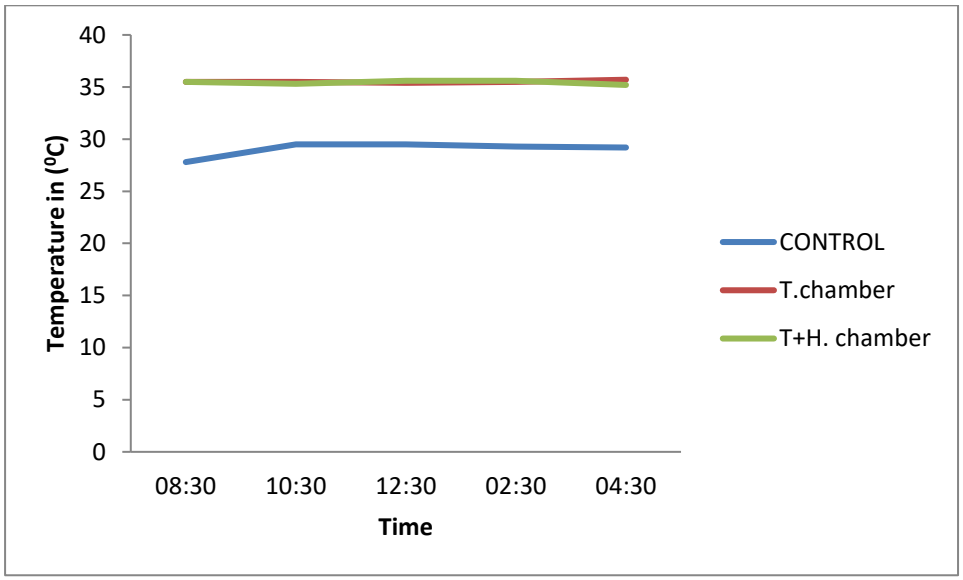


Fig.32. A representative diurnal temperature of control (28ºC) and elevated temperature (35ºC) chambers during the period of temperature treatment.

In control chamber the flowers were always at 28 to 29ºC with 70% RH. When the temperature of the chamber was elevated the RH declined to around 50%. From the Fig.30 it is clear that the flowers in the chamber were perfectly exposed to the set temperature. In one set of the flowers the RH was maintained at 70% with respective set temperature.

4.10 EFFECT OF TEMPERATURE ON POLLEN TUBE GROWTH

4.10.1in *vivo* experiment

The results of a time course measurement of pollen tube length in pollinated flowers under *in-vivo* are presented below. Pollinated flowers of after Ist day, 2nd day, 3rd day and 4th day were collected from ambient condition, and 3^{0}C and 5oC increase in temperature with and without humidity control.

The results showed that though pollen germination started on the first day, but the pollen tube length was negligible. There was a significant increase in pollen tube growth from 2nd to 4th day and it reached from 3248 µm to 19737 µm. With heat cover the tube length reduced to 12893 µm. There was no tube expansion with 30C elevated temperature to the chamber without humidity. And a small-scale germination with low pollen tube penetration occurred at the same temperature treatment with the presence of 70% of humidity. From this evidence it is clear that relative humidity has a major role in the pollen germination. The highest mean pollen tube length was 19736.82 µm (1.9cm) observed in control treatment on 4th day after pollination.

Table.4. Mean pollen tube length of pollen across different in vivo temperature treatments

Treatment	**day1**	**day2**	**day3**	**day4**
T1	0.00 ^{A}	3248.73^{A}	13968.94^{A}	19736.82^{A}
T2	0.00 ^{A}	2654.47 ^{A}	9432.36 ^{B}	12893.82 ^{B}
T3	0.00 ^{A}	0.00 ^{B}	0.0000 ^{C}	0.00 ^{D}
T4	0.00 ^{A}	321.46 ^{B}	856.01 ^{C}	1882.55 ^{C}
CD at 5%	0	555.28	40.60	10.98
p value	0	<.0001	<.0001	<.0001
significant		*	*	*

CD at 5% indicates critical difference at 5% level of significance. Different letter in each column after mean value are significantly different at 5% level according to Duncan's multiple range test.

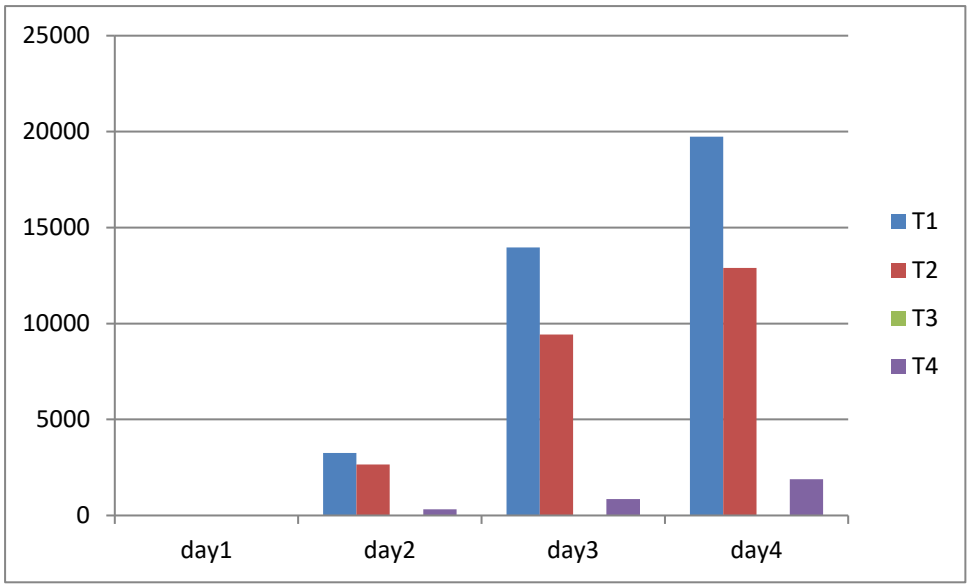


Fig.33. Deviation in mean pollen tube length along with different temperature treatment

4.10.*IN-VITRO* EXPERIMENT

Pollen tube length of treatment samples were evaluated across temperatures ranging from 29 ^{0}C - 45 ^{0}C with and without relative humidity control.

Mean pollen tube length along stylar region show significant variation across the temperature range 29 ^{0}C - 45 ^{0} C. Even under *in-vitro*, pollen tube from stigma to reach ovule took 4 days as in *in-vivo* at 28^{o}C. With increase in temperature pollen tube length traversing in style is significantly reduced (Table5). Even at a temperature of 31^{o}C it traversed only 19214.01µm and with further increase in temperature more drastic reduction was seen. There was no pollen tube expansion at 40^{o}C. As seen with *in-vivo* condition, maintenance of humidity with ET to certain extent minimised the effect of temperature (Fig .31). At 31^{o}C the decline was not significant and across all temperature the decline was minimal. Even at 40^{o}C with humidity there was pollen tube expansion of 1419.5µm. significant and across all temperature the decline was minimal.

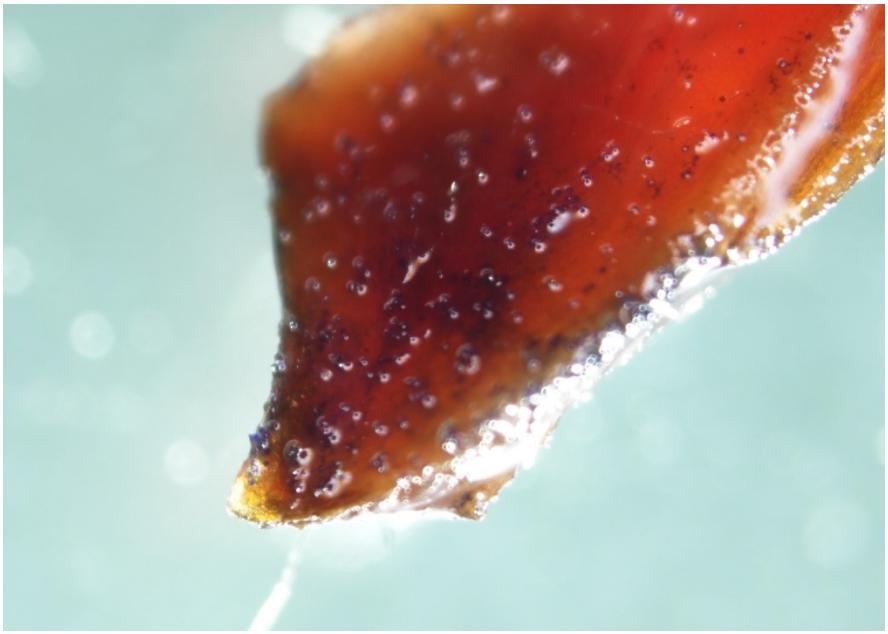


Fig. 34.Ungerminated pollens on stigmatic tip at 40^0C temperature treatment at 3x magnification

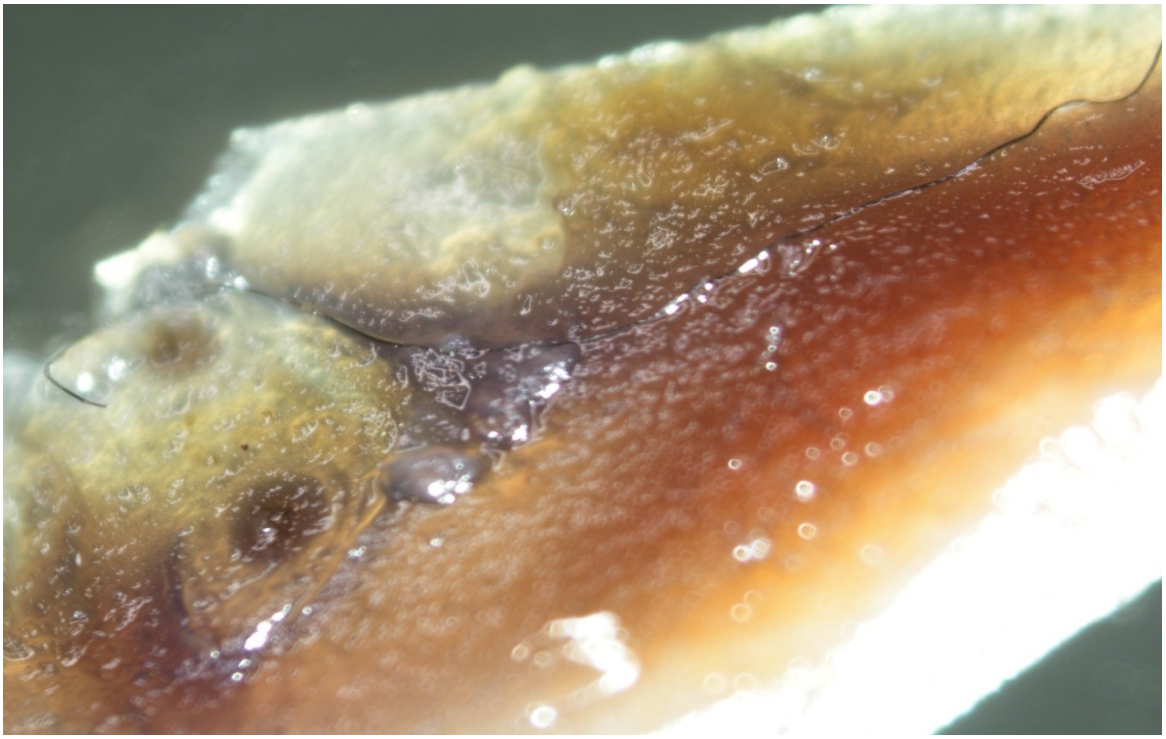


Fig.35. Pollen tube entry into the ovule at 4^{th}day after pollination under 3x magnifications

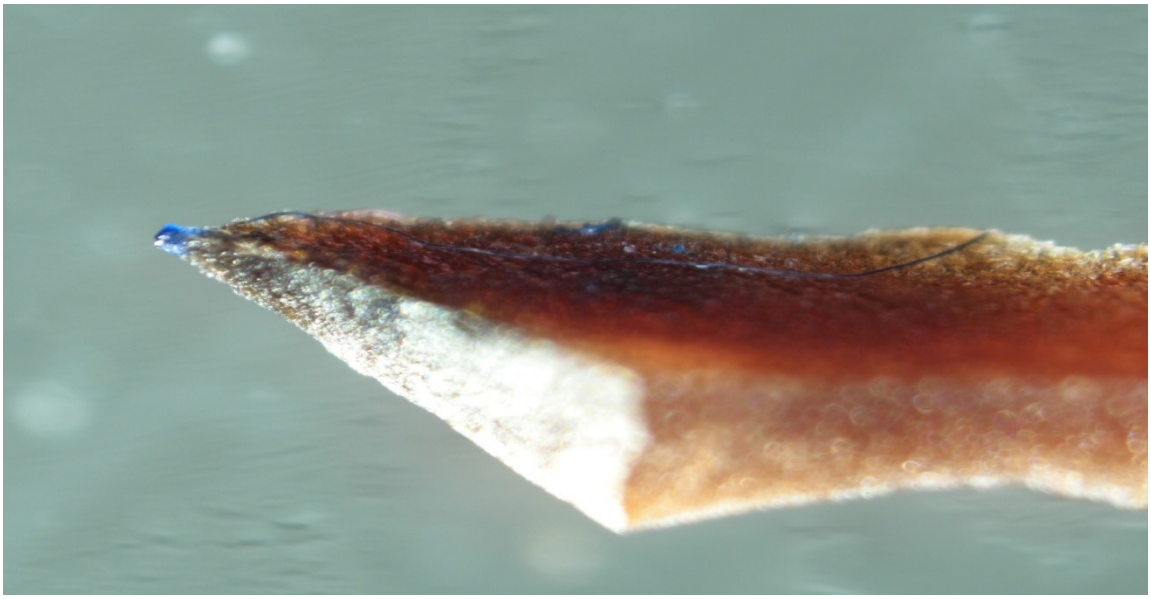


Fig.36. Retard pollen tube growth on stigma tip at 37^0C temperature after 4^{th} day of the pollination. (3x) magnification

Temperature	DAY 2		DAY 3		DAY 4	
	without RH	With RH	without RH	With RH	without RH	With RH
Ambient (28^0C+70 RH)		3972.41^{A}		14362.9^{A}		19909.51^{A}
31^0C	3494.60^{ABC}	3775.70^{AB}	5000.29^{D}	12008.60^{B}	8157.80^{CD}	19214.01^{A}
33^0C	3455.94^{BC}	3471.29^{BC}	4627.44^{D}	7980.00^{C}	6186.81^{DE}	14688.71^{B}
35^0C	1107.27^{D}	3205.70^{C}	1148.80^{EF}	6195.32^{CD}	1215.75^{FG}	9882.43^{C}
37^0C	908.66^{D}	1224.03^{D}	1167.13^{EF}	2641.22^{E}	1205.72^{FG}	3816.66^{EF}
40^0C	0.00^{E}	337.82^{E}	0.00^{F}	641.66^{F}	0.00^{G}	1419.53^{FG}
CD VALUE		**p-Value**		**p-Value**		**p-Value**
Temperature:	239.87	0.000	936.05	0.000	1819.10	0.000
Humidity:	151.70	0.000	592.01	0.000	1150.50	0.000
Tem* Humidity	339.22	0.000	1323.77	0.000	2572.60	0.000

CD at 5% indicates critical difference at 5% level of significance. Different letter in each column after mean value are significantly different at 5% level according to Duncan's multiple range test.

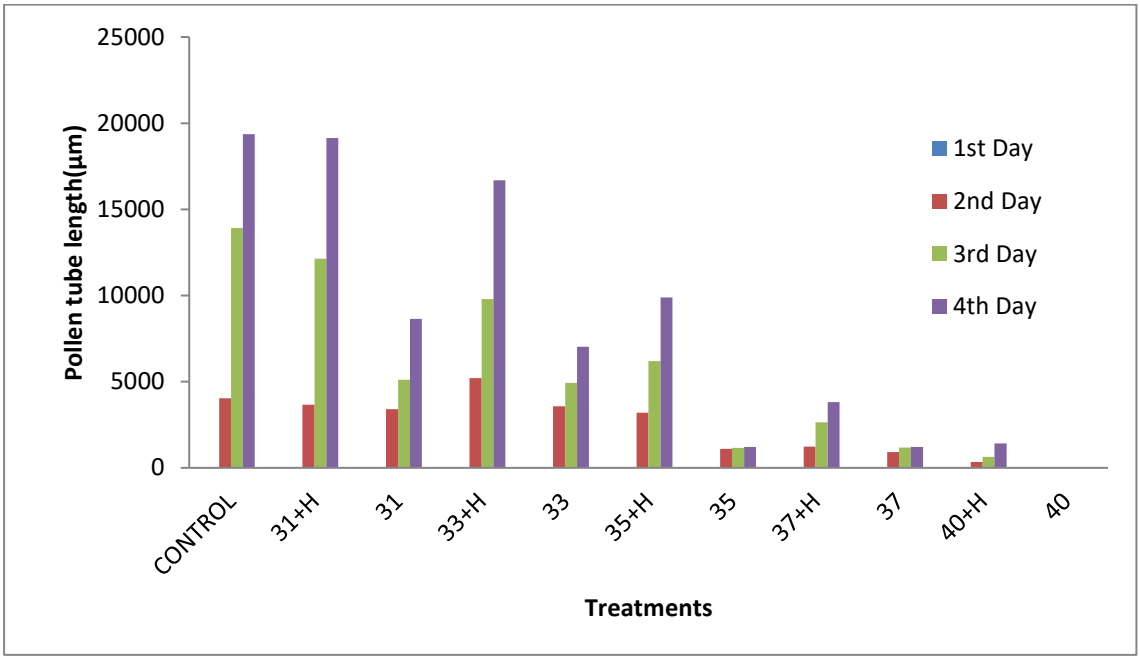


Figure.37. Shows deviation in mean pollen tube length along with different temperature treatment under laboratory condition.

CHAPTER 5

DISCUSSION

Climate change studies have projected that heat stress and very high temperatures are going to be very frequent, with greater intensity and would extend for longer period than what is being anticipated (Meehl*et al*., 2007). Also, the frequency of occurrence of these extreme heat events, albeit for a very short period of few days characterised with temperature conditions of >5°C, may become a new normal. It has already been demonstrated that any temperature deviations beyond the normal range suited for the particular species have serious repercussions for fruit and seed set (Prasad *et al*., 2017). Furthermore among the various stages of plant life, sexual reproduction stage is more vulnerable to increased temperature compared to vegetative stages. Hence, the reproductive organs of the plants are susceptible to any modulations in the temperatures, though for a short period, before or during the initial stages of floral bud development (Prasad *et al*., 2017). This phenomenon has been observed in many of the cultivated and undomesticated plant species that demonstrate poor growth or erratic fruit and seed setting properties when subjected to extreme temperature conditions (both low and high) during the flowering phase.

Coconut has a very narrow temperature range (optimum 27+3ºC) (Child. 1974), however, its cultivation is mostly restricted to semi-arid tropical/tropical climate of the countries where the temperature fluctuation is minimal. Thus, the temperature is the major driving force for its distribution and expansion. In India it is mostly restricted to west coast and east coast of south India where the difference in minimum and maximum temperature is minimal while in north with extreme cold and hot temperature coconut is highly vulnerable and even if it thrives nut production is severely dented (IPCC.,2014). Hebbar *et al*. (2016) reported that in the west coastal regions of Southern Indian states of Karnataka and Kerala that are characterised with an annual rainfall more than 2000 mm coconut is grown until the T_{max} (maximum temperature) of 34 to 36ºC. On the other hand in the Eastern coast of Indian states of Andhra Pradesh and Tamil Nadu the rainfall quantum is low around (1000 to 1200 mm) and the T_{max} could reach as high as 40 to 42ºC in some of the growing regions. Coconut flowers almost an inflorescence per month throughout the year and therefore its probability of getting exposed to high temperature episodes is very high.

After identifying the sensitivity of reproductive development of a crop to variations in the temperature conditions it is indispensable to investigate the sensitive stages of reproduction viz. gamete development (pollen development), progamic phase (pollen viability, pollen germination, pollen tube growth rate, pollen dynamics, stigma receptivity and ovule viability) and embryo development. Most of the published studies have focussed on the effect of temperature on the development of male gametes, their morphology, biochemical composition and functionality of the pollen grains (Distefano *et al.*, 2018; Lora *et al.*, 2009; Koti*et al*., 2005; Prasad *et al*., 2002; Sato *et al.*, 2002; Aloni*et al*., 2001;). On the other hand few investigations have studied the sporophytic generation of the crop starting from the postzygotic stage to the reproductive phase (Hedhly, 2011; Hedhly*et al.*, 2009; Sage *et al.*, 2015; Zinn*et al.*, 2010). The progamic phase or it is called as receptivity period, elapses from the process of pollination to fertilisation. It is one of the most critical phases of the plant reproduction process. Also, this is the critical phase of sexual reproduction where in specific interactions between the male gametophyte and the female counterpart occurs. Thus, the progamic phase is susceptible to various environmental conditions. Among all the environmental factors, the prevailing temperature conditions severely affect each of the processes in the progamic phase starting from the receptivity of stigma, germination of pollen grain, growth of pollen tubes and degeneration of ovule all are affected (Montalt *et al.* 2019;Hedhly, 2011). In coconut, in an earlier in-vitro pollen germination study involving 12 coconut genotypes it was showed that the mean cardinal temperatures ranged from 23.5 °C to 29.5 °C, 9.7 °C to 16.5 °C and 40.1 °C to 43.9 °C for Topt, Tmin. andTmax., respectively (Hebbar*et al.*, 2018). However, there was no study on the progamic phase. Therefore, the major objective of the study was to understand the progamic phase i.e., female flower receptivity, pollen germination in stigma, pollen tube expansion through pistil till it reaches ovule and to see these stages respond to elevated temperatures under *in-vivo* and *in-vitro* conditions.

5.1. PROGAMIC PHASE OF COCONUT

In coconut there was no information available on time elapsed for the pollen germination on stigma, pollen tube growth in pistil and the time required to reach the ovule. In order to study the above aspects artificial pollinated female flowers were collected at hourly intervals for histological observations. As it was observed that pollen germination happened only after one day (Fig.20. B.), the sample collection and its observation were restricted to daily basis. Generally, the dissected flowers were stained using an aniline blue dye and microscopic

observation was performed using a fluorescent microscope at different time interval (Linskens and Esser,1957). However, since we were only having steriomicroscope we adopted a quadruple staining technique (Alexander,1957) which is relatively time consuming and cumbersome. Still we could observe the pollen germination and pollen tube growth as it had taken dark blue color to pollen grains and pollen tube which is distinct from the background colour. From our observations we could see that pollen germination occurred only after one day of pollination and the pollen tube required reaching from stigma to ovule a distance of 1.9 cm nearly four days. It is a relatively long period compared to most of the other species (Russel., 1928, Marquard.,1992, Sanzol*et al*.,2003, Mc Williams.1958.,Michard *et.al*.,2011) .But in species like Pecan. Pear, Apple they also require 3 to 4 days for the pollen tube to traverse through the style (Marquard.1992, Mc Williams.1958., Sanzol *et al*.,2003). In coconut it is the first report showing the pollen tube movement through the style and time required to reach the ovule is four days. With this understanding we analysed the effect of elevated temperature treatment on pollen germination and pollen tube expansion in style.

5.2. HIGH TEMPERATURE TREATMENT

It is difficult to expose the whole coconut palm to high temperature. In this study, for in-vivo experiment, we enclosed whole inflorescence instead of whole tree with a heat cover made of transparent plastic which is fitted with a heat blower to rise the temperature and a misting device to regulate the humidity inside the chamber. With the help of heater, the internal temperature near the inflorescence was 3 and 5^{o}C higher than the control chamber and the data suggest that the temperature increase was quite consistent. However, it is quite challenging to climb the tree every now and then measure the control chamber and set the elevated temperature in accordance with control chamber. Alternatively, we attempted an *in-vitro* technique of exposing the pollinated flowers to high temperature under laboratory conditions. For this study, female flowers just before the receptive stage excised along with their peduncle form the bunch and placed in a nutrient medium containing 10% sucrose+.01% Boric acid+1%Agar. As soon as they become receptive, they were pollinated with viable WCT pollens and placed in desiccators with controlled temperature from 27 to 45^{o}C with and without humidity control at 70%. In the nutrient medium the flower was viable up to 5 days. Hedhly *et al.,* (2005), used florist's foam to culture emasculated flowers of Peach. The flowers were sampled at regular intervals for histological study and microscopic observation. This is a simple technique compared to the one adopted by Marquard R. D.

(1992), who used hot plate to maintain temperature gradient by warming one end by hot plate and cooling the other with compressed freon, similar to the system described by Hensley *et al.* (1982). Aluminium blocks containing tubes and flowers were placed on the plate at temperatures between 20 and 30^0C and remained in place for an l-h equilibration period for the *in-vivo* study.

5.3 HIGH TEMPERATURE AND FEMALE FLOWER RECEPTIVITY

The results had shown that high temperature had clear effect on emergence of stigmatic regions, nectar secretion and the appearance of stigma. The nectar secretion was advanced in female flower orifices i.e., 5 to 6 days prior to receptivity as against 2 days in control plants. However, by the time of stigma receptivity stigmatic surface becomes dry and observed brownish discoloration. On the other hand, in control plants at a temperature around 29^0C, there was ample nectar during stigma receptivity; stigma is wet and it was white in colour. Coconut is predominantly a bee-pollinated palm, and hence lack of nectar during receptivity would not attract the insects for pollination thus affecting the fertilization at high temperature (Thomas and Josephrajkumar, 2013).The early nectar secretion and the dry appearance of stigmatic surface, and brown discoloration were the indication stigma has lost the capacity to offer to support the pollen germination, pollen tube penetration etc. In similar studies on the crops viz., sweet cherry (Hedhly *et al.,*2003,), pecan (*Caryaillinoinensis)* (Marquard,1992) and Peach (Hedhly *et al*.,2005) high temperature had reduced stigmatic receptivity, low temperature enhanced it.

However, it is interesting to see that the high temperature effect was to a certain extent negated by maintaining the flowers under high humidity. There was reasonably extended nectar production till the receptivity of stigmas and to a large extent prevented the browning of stigma. From this result it is clear that atmospheric humidity level has an important role during pollination.

5.4. POLLEN TUBE GROWTH WITH HIGH TEMPERATURE

5.4.1 Under*in-vivo* condition

Pollinated flowers after 1^{st} day, 2^{nd} day, 3^{rd} day and 4^{th} day were collected from ambient condition, and 3^0C and 5^0C increase in temperature with and without humidity

control. And measurements (length) of pollen tube were made with the help of NIS-imaging software under stereomicroscopy.

The data from the experiment suggest that the rising elevated temperature in atmosphere will have negative effect on the pollen germination on the stigma, pollen tube growth towards ovule. At high temperature of 3 and 5ºC above ambient, it not only reduced pollen germination but also reduced the pollen tube expansion through the style. Marquard (1992) claimed that pollen tube growth was strongly influenced by temperature Pollen tube growth was more sensitive to reduced humidity along with high temperature. High humidity maintenance with high temperature allowed the tube to grow though at a slower pace compared to controls, requiring more time to reach the ovules thus may ensure fertilization. Therefore, in coconut growing regions of east coast where the humidity is very low the expected temperature rise may affect the fertilization period and the coconut productivity.

5.4.2 Under *in-vitro* condition

Mean pollen tube length along stylar region show significant variation across the temperature range 29ºC - 45ºC at 2-3º C interval. At a higher temperature above 33º C drastic decrease in pollen tube length was observed. The average length of WCT female flower reproductive part (stigma to ovary) was 1.82 cm. The highest mean pollen tube length was 19364.59 µm (1.9cm) observed in control treatment on 4^{th} day after pollination. High temperature at 40ºC there was no pollen germination in stigma. Thus, our finding is in conformity with the observations of Hedhly*et al*., (2011) who reported that temperature strongly affects each process in programic phase, i.e, stigmatic receptivity, pollen grain germination, Pollen tube growth and ovule degeneration.

CHAPTER 6

SUMMARY AND CONCLUSION

Climate change studies have projected that heat stress and very high temperatures are going to be very frequent, with greater intensity and would extend for longer period than what is being anticipated in some of the coconut growing regions. In most of the crops it was deduced that among the various phase of plant's life cycle it is the reproductive phase that is more vulnerable to the elevated temperature conditions. In coconut too it was reported that during the episodes of high temperature there is high frequency of button shedding but the reason is not well understood. In an earlier study we had seen a stage of reproductive phase i.e., pollen germination is highly sensitive to high temperature and we had shown wide variability in pollen germination across genotypes and most of the genotypes have optimum temperature of 29^{o}C (Hebbar*et al*. 2018). However, of late it was shown in other field crops that even if the pollen is germinated yet times the fertilization are failed because of poor female flower receptivity, pollen germination on stigma, pollen tube growth through style and its interaction with ovule. These stages are not studied in coconut and therefore our objective was to decipher the progamic phase of coconut and how it is affected by temperature under *in-vivo* and *in-vitro* condition.

In order to decipher the progamic phase we collected the pollinated flowers at regular intervals from a local tall variety 'WCT' dissected and stained suitable to be observed under stereo microscope. From the study we could see that in coconut pollen germination in stigma occur after one day and pollen tube traverses through style and reaches ovule four days after pollination. This is the first time we are reporting the time required for pollen tube to reach ovule in coconut.

High temperature both under *in- vivo* and *in-vitro* affected the pollen germination and pollen tube expansion through style in addition to the changes associated with female flower receptivity. We noticed early secretion of nectar from orifice in high temperature treatments well before receptivity making it devoid of nectar to attract the insects at receptivity might result in poor pollination and nut set. Similarly, early drying of stigma tip is seen with high temperature. High temperature not only reduced pollen germination but also delayed or devoid of pollen tube growth through style resulting in either poor or no fertilization. However, we noted that the high temperature effect to certain extent was minimised by

maintaining the flowers at high relative humidity suggesting those coconut growing regions where the RH is low increasing temperature might have deleterious effect on fertilization and nut set. Thus, the understanding from this study will not only enable us to breed genotypes tolerant to high temperature at reproductive stage but also evolve management practice like creating environment with high humidity whenever there are expected episodes of high temperature to ensure higher fertilization. It has already been demonstrated that any temperature deviations beyond the normal range suited for the particular species have serious repercussions for fruit and seed set (Prasad *et al*., 2017). Furthermore among the various stages of plant life, sexual reproduction stage is more vulnerable to increased temperature compared to vegetative stages. Hence, the reproductive organs of the plants are susceptible to any modulations in the temperatures, though for a short period, before or during the initial stages of floral bud development (Prasad *et al*., 2017). Earlier reports shows that many cultivated and wild plant species within different climatic regions worldwide perform poorly and display erratic fruit and seed set when subjected during their flowering phase to high and low temperatures beyond their optimum needs.

Coconut has a very narrow temperature range (optimum 27+3ºC) (Child 1974), however, its production is primarily confined to the semi-arid tropical/tropical climate of countries where the fluctuation in temperature is minimal. Thus, the main driving force for its distribution and expansion is temperature . In India it is mostly restricted to west coast and east coast of south India where the difference in minimum and maximum temperature is minimal while in north with extreme cold and hot temperature coconut is highly vulnerable and even if it thrives nut production is severely dented (IPCC.,2014). In the west coastal regions of Southern Indian states of Karnataka and Kerala that are characterised with an annual rainfall more than 2000 mm coconut is grown until the T_{max} (maximum temperature) of 34 to 36ºC. On the other hand in the Eastern coast of Indian states of Andhra Pradesh and Tamil Nadu the rainfall quantum is low around (1000 to 1200 mm) and the T_{max} could reach as high as 40 to 42ºC in some of the growing regions (Hebbar *et al.* 2016). Coconut flowers throughout the year almost an inflorescence per month and therefore its likelihood of getting exposed to episodes of high temperature are very high.

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EFFECT OF HIGH TEMPERATURE ON FEMALE FLOWER RECEPTIVITY AND FERTILIZATION OF COCONUT

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ABSTRACT OF THE THESIS

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ABSTRACT

Coconut is a most economically important perennial crop; it has to cope with the impact of climate change during its life cycle. Prolonged periods of temperatures above the maximum the maximum tolerable limit can be detrimental to the plant production. Temperature effect in the reproductive phase is critical for the success of plant sexual reproduction, and new knowledge is needed to optimize breeding programmes to obtain new varieties that adapt to a climate change scenario. Using the popular local variety West Coast Tall (WCT), we evaluated the effect of different temperature regimes on female flower receptivity, the response of pollen germination on stigma and the pollen tube growth in the pistil was studied under both in vivo and *in vitro* .In this study a device was developed to expose temperature to individual inflorescence in a tree crown. The experimental device set up included plastic cover (heat cover) having 0.40 mm thickness+ readymade Panasonic heater+ temperature controller with sensor+ Humidifier. For the treatments with elevated temperature, the heater inside the heat cover was switched on to raise the temperature $+3^0$C and $+5^0$C from the ambient control. And also, for a growth chambers technique was developed to grow the female flower under in-vitro condition to study the pollen germination on stigma and pollen tube growth in response to different temperature and humidity under well controlled condition. The WCT genotypes showed maximum pollen germination and tube growth at 29^0C. Beyond 31^0C both pollen germination and tube growth significantly reduced. From this study it is clear that high temperature effected loss of female flower receptivity through morphological changes as well. The result showed that as the temperature increases, the female flower fertilization is affected by both reduced pollen germination on stigma and poor and pollen tube growth through pistil. Further, high temperature exposure will lead to the stigmatic surface become dry and observed brownish discoloration. The result showed that as the temperature increases, the female flower fertilization is affected by both reduced pollen germination on stigma and poor and pollen tube growth through pistil. Further work is being attempted to screen genotypes for their pollen germination on stigma and growth of pollen tube at different temperature to identify temperature tolerant genotypes