ASSESSMENT OF WATER STRESS TOLERANCE IN SELECTIVELY FERTILIZED COCONUT (*Cocos nucifera* L.) HYBRIDS

By

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(2016-11-118)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

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KERALA, INDIA

2018

DECLARATION

I, hereby declare that this thesis entitled **"Assessment of water stress tolerance in selectively fertilized coconut (*Cocos nucifera* L.) hybrids"** 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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ACKNOWLEDGEMENT

First and foremost I bow my head before the Almighty God who enabled me to successfully complete the thesis work on time.

With immense pleasure I avail this opportunity to express my deep sense of gratitude and indebtedness to my major advisor ***Dr. Roy Stephen****, Professor, Department of Plant Physiology, Chairperson of my Advisory Committee for the meticulous supervision, valuable guidance, constructive suggestions, moral support, unfailing patience, friendly approach, constant inspiration and affection bestowed on me throughout the study period.*

I would like to express my extreme indebtedness and obligation to ***Dr. M. M. Viji,*** *Professor and Head, Department of Plant Physiology and member of my Advisory Committee for the unwavering encouragement, forbearance, well timed support and critical scrutiny of the manuscript which has helped a lot for the improvement and preparation of the thesis.*

I would like to express my extreme indebtedness and obligation to ***Dr. R. V. Manju,*** *Professor, Department of Plant Physiology for the unwavering encouragement, forbearance, well timed support friendly approach, constant inspiration and affection bestowed on me throughout the study period.*

I gratefully acknowledge ***Dr. K. Sreekumar****, Professor of Plant Breeding and Genetics, Instructional Farm, COA, Vellayani and member of my advisory committee for his timely advice and valuable help given to me during the course of investigation.*

I place on record my sincere gratitude to ***Dr. K. N. Satheeshan,*** *Professor of horticulture, RARS Pilicode, Kasaragod for his affectionate encouragement and for the critical scrutiny of the manuscript.*

I express my heartiest gratitude to ***Dr. M. K. Rajesh****, Principal scientist, ICAR-CPCRI, Kasaragod for his ever willing help, valuable guidance and technical support during the course of investigation.*

I am extremely thankful to ADR, staff, labours of RARS, Pilicode for their help extended during collection of leaf samples during course of my study

I am extremely thankful to the staff and labours of RARS, Pilicode for their help extended during collection of leaf samples during course of my study.

I owe my sincere gratitude to my batchmates, Remo, Amritha and saranya for cooperation, support and love rendered during my study period.

Words are inadequate to express my thanks to my dear friends Kingini, Chackochi, Shahi and Naveen whose love, support and affection helped me through many hardships. Without their persistant help and care, this would not have been possible. I wish to place on record the help rendered and support given by my friends Gokul, Fallu, Bernad, Madhu, Reddy, Mathukutty, during my research period.

I take this opportunity to thank my seniors Gayu chechy, Sabana chechy, Anjana chechy, Gritta chechy, Dhanu chechy and manasa chechy who gave me enough mental strength to get through all tedious circumstances. I thank for the support and encouragement of my dear juniors, Akhila pahi, safu, lincy, aura, ammu, arya, lekshmi and for their support and encouragement.

I thankfully remember the services rendered by all the staff members of the Library, COA.

I am obliged beyond words to express my indebtedness to Acha, Amma, vava and unnimol and my dearest family members for their unconditional love, extraordinary patience and support bestowed on me during my hard periods.

Rahul Guptha. K

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
CBB	Coomassive Brilliant Blue
CD (0.05)	Critical Difference at 5% level
CDB	Coconut Development Board
cm	Centimetre
COD	Chowghat Orange Dwarf
CRD	Completely Randomized Design
^0C	Degree Celsius
et al.	And others
Fig.	Figure
g	Gram
g^{-1}	Per gram
ha^{-1}	Per hectare
HSP	Heat Shock Protein
HSPF	Heat Stable Protein Factor
KAU	Kerala Agricultural University
KG	Keraganga
KS	Kerasree
LEA	Late Embryogenic Abundant
LMW	Low Molecular Weight
LO	Laccadive Ordinary
M	Molar
MDA	Malondialdehyde

mg	Milligram
mg g^{-1}	Milligram per gram
mm	Millimetre
MMP	Medium Molecular Weight
No.	Number
RBD	Randomized Block Design
%	Per cent
POD	Peroxidase
REL	Relative Electrolytic Leakage
ROS	Reactive Oxygen Species
RWC	Relative water content
SOD	Superoxide dismutase
S.F	Selectively Fertilized
Via	Through
viz.	Namely
WCT	West Coast Tall

Introduction

1. INTRODUCTION

Cocos nucifera (2n=32), an important plantation crop in the coastal belts of tropical and subtropical areas and it provides food for millions of people in the world. It is often referred as 'tree of life' due to its multifarious uses (Mandal and Mandal, 2011). India is the largest producer of coconut in the world followed by Indonesia and Philippines. India stands first in productivity (10164 nuts/ha), first in production (22167 million nuts) and third in area (2.14 m ha) under coconut. The crop contributes more than Rs. 8000 crores annually to the GDP and earns valuable foreign exchange to the extent of Rs. 2000 crores by way of export of coconut products. The contribution of the crop to the vegetable oil pool in the country is about 5% and the crop sustains 10 million people of the country through cultivation, processing, marketing and trade related activities. Under these circumstances, the crop holds much value in the Indian economy.

According to the survey conducted by the Coconut Development Board (CDB, 2015), four south Indian states particularly Kerala, Tamil Nadu, Karnataka, Andhra Pradesh accounts for 90% of the total coconut production in the country. Kerala has the largest area under cultivation of coconut at 7.9 lakh ha (2015-16) and has the number one position in production (7429 million nuts) among the Indian states. The crop also ranks second in the Gross Value Output (GVO) in Kerala. Around 42 lakh households in the state are engaged in coconut cultivation. Among the districts, Kozhikode has the highest productivity with 11,972 nuts hectare^{-1}, followed by Malappuram (11,840 nuts hectare^{-1}) and Thrissur (11,218 nutshectare^{-1}) and the lowest productivity of 1, 856 nuts hectare^{-1} was reported from Idukki district (CDB, 2017).

The perennial palm belongs to the family of the Arecaceae and subfamily of Cocoideae. It prefers drained loamy or clayey soil and warm humid weather. An ideal climatic condition for the better growth and yield of the palm is an annual

mean temperature of 27^{0}C, evenly distributed rainfall of 1500-2500 mm per annum, and relative humidity above 60% (Carr, 2011).

Tall and dwarf types are two distinct groups of coconut. The tall types start to bear within 6 to 10 years after planting and have a lifespan of about 80- 120 years where as dwarf starts to bear within 3 to 4 years and has a lifespan of about 40 years. Male flowers mature in advance than the female flowers, this leads to cross-pollination and nuts mature in around 365 days after pollination (Gomes *et al.*, 2010).

Drought is the second most important factor reducing the productivity of coconut next to the dreaded root (wilt) disease in Kerala. Drought is a multi-dimensional factor affecting the coconut plants at various levels of organization from cell to organ and to whole plant level (Gomes *et al.*, 2008). Studies showed that about 2/3 of the potential coconut production is generally lost due to either poor nut setting or immature nut fall as a result of water deficit conditions. A coconut palm yielding 80-120 nuts year^{-1} in good rainy season may reduce its yield to 40 nuts year^{-1} or less after a drought period.

Adverse effects of drought in coconuts persist for two and a half years (Rajagopal and Bai, 1999). Though coconut hybrids are high yielding, they are generally susceptible to moisture stress (Rajagopal *et al.*, 2005). Zinn *et al.* (2010) reported that high temperature when coincides with drought in coconut plantation can affect the productivity negatively. A large reduction in the number of male and female flowers, as well as fruit drop, was observed under extreme conditions of temperature and drought (Nainanayake *et al.*, 2008).

Burke *et al.* (2004) reported that temperature stress can be overcome by the exposure of the pollination phase to temperatures (34 – 36^{0}C). According to Ranasinghe *et al.* (2003), low quality of pollen or female flowers produced due to their exposure to water stress during their final stages of development, in turn, reduce the fruit set of coconut.

Selective fertilization is characterized by artificially imposing the desired selective pressure during pollen germination so that the pollen grains which are tolerant to selection pressure only will germinate and fertilize the ovule. It was reported that selectively fertilized hybrids were drought tolerant and had high water use efficiency compared to normal coconut hybrids (Renju *et al.*, 2015).

At this juncture, where the production and productivity of coconut are affected by drought, there is an urgent need to develop drought tolerant coconut hybrids. Selective fertilization being feasible and time saving approach, efforts were made to develop coconut hybrids through selective fertilization. With this back ground the research work has been done with the specific objective to assess the physiological and molecular basis of water stress tolerance in selectively fertilized coconut hybrids and to screen coconut genotypes for water stress tolerance through critical water potential for pollen germination.

Review of Literature

2. REVIEW OF LITERATURE

2.1 COCONUT AND DROUGHT

Coconut is regularly exposed to soil and atmospheric water deficit due to the fact that, it is a perennial palm with an extended effective life span (Rajagopal and Bai, 2002). Drought is one of the important abiotic stresses which can limit the crop growth and yield by altering various physiological and biochemical processes (Vaidya *et al.*, 2015). The research project on "Assessment of water stress tolerance in selectively fertilized coconut (*Cocos nucifera* L.) hybrids" was done with the objective to assess the physiological and molecular basis of water stress tolerance in selectively fertilized coconut hybrids and to screen coconut genotypes for water stress tolerance through critical water potential for pollen germination. The published information available in this area is presented in this chapter.

2.2 DROUGHT RELATED PHYSIOLOGICAL PARAMETER

2.2.1 Stomatal conductance

Gaseous exchange in plants is performed by specialized structures known as stomata, through which transpiration or water loss occurs. Diffusion resistance provided by the stomatal pores and the humidity gradient between the leaves internal air spaces and ambient air are the main factors of transpiration rate. Water potential reduction in leaves leads to closing of stomata (Brodribb and Michele, 2003).

During water stress condition, coconut root produces certain chemical signals based on the water availability. These chemical signals are sent to shoot through the transpiration stream, as a result, decrease in leaf expansion and stomatal closure takes place (Wilkinson and Davies, 2002). Gomes *et al.* (2008) reported that, Abscisic acid (ABA) is the signaling molecule produced by coconut palms when subjected to

drought stress. Root tip produces ABA which is transported to leaf where it reduces stomatal conductance.

According to Repellin *et al.* (1997), leaf gas exchange parameters like stomatal conductance, net photosynthetic rate and leaf inner CO_2 concentration of West Coast Tall' (drought avoiding), 'Malayan Yellow Dwarf' (drought susceptible), and their progeny, the hybrid 'PB 121' (drought resistant) are declined under water stress condition in coconut.

2.2.2 Relative Water Content (RWC)

Voleti *et al.* (1990) reported that when coconut genotypes experience moisture stress, the relative water content gets reduced and also observed that water stress tolerant genotypes have higher relative water content compared to the susceptible ones. Repellin, (1994) reported that when WCT was subjected to water stress, relative water content declined.

In line with Repellin *et al.* (1997), the leaf water potential varies from -0.5 to -0.7 MPa and relative water content (RWC) ranges from 95-97% under moderate stress condition. Leaf water potential varies from -1.3 to -1.5 MPa, leaf relative water content varies from 87 to 91% when plants were subjected to water stress for 10-12 days. Palms when subjected to excessive stress, by withholding water for 20-22 days, leaf water potential ranged from - 1.8 to -2.2 MPa and relative water content reduced up to 72-80%.

An experiment was conducted by Sun *et al.* (2011) in oil palm and observed the adaptation mechanism in growth and physiology. Water stress experienced palms showed less relative water content and less growth rate than well watered ones. This experiment has proved that some hybrids WCT x COD, COD x WCT, LO x GB and LO x COD when exposed to water stress showed reduced relative water content.

2.2.3 Carbon isotope discrimination (‰)

Carbon has generally two naturally occurring stable isotopes, ^{12}C & ^{13}C. ^{12}C occupies 98.9% of the amount of carbon present in the atmosphere and ^{13}C about 1.1%. In plant tissue the relative proportion of ^{12}C to ^{13}C will be less as compared with the atmospheric carbon dioxide, indicating that carbon isotope discrimination occurs during the incorporation of CO_2 into plant biomass (Farquhar, 1989).

In line with Robina *et al.* (2005) water use efficiency is defined as the ability of the crop to produce biomass per unit of water transpired. Carbon isotope discrimination is a tool used for evaluating water use efficiency and carbon discrimination in leaves. Water use efficiency is a plant trait which indicates the drought tolerance of genotype (Kumarasinghe *et al.*, 1996).

Osmond *et al.* (1981) defined intrinsic water use efficiency as the ratio of net assimilation to stomatal conductance, an indicator of long-term tendencies inside the carbon uptake and water loss of plants was Wg derived from isotopes (Seibt *et al.*, 2008). Carbon isotope discrimination ($\Delta^{13}C$) is important for determining the plant's physiological responses and provides information on photosynthesis, water relations (Martinez *et al.*, 2007).

Silvestre *et al.* (2017) conducted an experiment and proved that some physiological parameters like leaf water capacity, net CO_2 assimilation rate, stomatal conductance, transpiration and instantaneous water use efficiency are declined in coconut palms due to the withdrawal of irrigation from the field.

2.3.4 Cell membrane stability index (%)

Gigon *et al.* (2004) cell membranes were the main target under water stress conditions and showed a decrease in membrane lipid content due to inhibition of lipid

biosynthesis. Cell membrane stability (CMS) is one such method for understanding water stress tolerance in plants (Farooqe and Azam, 2006).

Bajji *et al.* (2002) reported that electrolyte leakage from the cells is the index for assessing the degree of cell membrane injury under water stress conditions.

Asemota and Conaire (2010) reported that, when irrigation was withdrawn from oil palm plantations for 9 days, water potential decreased up to -2.4 MPa and this brought about 20 % damage to the cellular membranes and it was measured by electrolyte leakage.

Water stress conditions, drought tolerant plants maintained stability and integrity of their cell membrane (Bajji *et al.*, 2002). Cell membrane damage indicates how much solute leaked from the tissue under water stress (Basyoni *et al.*, 2017). Sensitive plants were more prone to cell damage by degradation of their membranes and damages shown by plants were irreversible (Gigon *et al.*, 2004).

Chaum *et al.* (2013) studied the physiological and biochemical responses of oil palm seedlings under reduced soil moisture of around 20 percent and also studied the responses during the recovery process. They observed that under severe water stress condition relative electrolyte leakage in the leaf tissues increased, in proportion to the decreasing degree of reduced soil moisture.

2.3.5 Transpiration rate

Transpiration rate plays a major role in estimating drought tolerance of plants. Plants which allow less loss of water from leaves through stomata are supposed to be more drought tolerant. Tolerant coconut palms conserve water in their tissues by reducing transpiration rate through the partial closure of stomata for physiological and' metabolic processes and also concluded that tolerant ones have less loss of water through stomata (Rajagopal *et al.*, 1988).

Residual transpiration is the transpiration observed at minimum stomatal opening (Sanchez *et al.*, 2001). Transpiration rate, net photosynthetic rate and stomatal conductance were reduced when coconut plants were imposed by short-term water stress (Gomes *et al.*, 2008).

2.3.6 Leaf temperature

Nainanayake and Bandara (1998) stated that leaf temperature is one of the important drought tolerance related parameter in coconut and also recorded that the high leaf temperature due to lowering of transpirational cooling effect with the onset of stress probably be attributed to the decline in levels of chlorophyll.

Vincent *et al.* (2002) treated leaf temperature as drought tolerant parameter on east coast tall variety of coconut for drought management and recorded a positive correlation among epicuticular wax and leaf temperature.

2.3.7 Epicuticular wax content

Cuticular wax is the outermost thin hydrophobic layer masking the surface of aerial plant components. This offers a number one water-proof barrier and safety to distinct environmental stresses mainly water stress and involved in controlling non-stomatal water loss and fuel exchange (Kerstiens, 2006). Waxes are associated with abiotic stresses like water stress, heat stress and also maintain water balance in the plant cells (Riederer and Schreiber, 2001).

Epicuticular wax deposition was reported in coconut palms during the period of drought (Kurup *et al.*, 1993). A drought tolerant coconut palm contains more hydrophobic waxes than susceptible ones (Riedel *et al.*, 2009). Escalante *et al.* (2002) reported that Clupeol methyl ester, Isoskimmiwallin, and Skimmiwallin are the major components of epicuticular wax in coconut genotypes.

Plant epidermal cells contribute greater than half of their lipid metabolism to the synthesis of cuticular lipids, which seal and shield the plant shoot. The cuticle is made from cutin polymer and waxes (Debono *et al.*, 2009). Cuticular wax deposition and composition affect drought tolerance and yield in plants. Total leaf cuticular wax load accelerated during water stress stages and overall wax load varies from 1.5 to 2.5 fold (Riedel *et al.*, 2009). In line with Rajagopal *et al.* (1988) transpiration shows a negative relationship with Epicuticular wax content.

2.3.8 Photosynthetic rate

Chaum *et al.* (2013) studied the physiological and biochemical responses of oil palm seedlings under reduced soil moisture (20 percent) and also studied the responses during the recovery process. Water deficit conditions led to the reduction of net photosynthetic rate followed by growth retardation. Cornic (2000) was observed that photosynthetic rate was decreased due to the closure of stomata under water stress as an impact of physiological response.

In line with Galmes *et al.* (2007) reduction in the photosynthetic rate and incomplete recovery was explained in terms of both stomatal and non-stomatal factors. According to Zhou *et al,* (2007) photosynthetic rate was reduced as water stress increased, mainly because of reduction in the rubisco activity, RuBP regeneration, ATP supply; electron transport rate and light capture efficiency.

Gomes *et al.* (2008) studied the effect of photosynthetic rate on two climatic situations like hot-humid climate and semi-arid type of climate area.

2.3 BIOCHEMICAL PARAMETERS RELATED WITH DROUGHT TOLERANCE IN COCONUT

2.3.1 Malondialdehyde (MDA)

According to Kong *et al.* (2016) the ROS generated during water stress, damages the membrane lipid by the process of peroxidation. Usually, membrane lipid peroxidation in plants is detected by measuring Malondialdehyde (MDA) and it is widely used as marker of oxidative lipid injury caused by abiotic stress.

Zhou *et al.* (2015) studied forest trees grown in soil which was exposed with different levels of water stress, and the results indicated that water stress significantly increased superoxide dismutase (SOD) and peroxidase (POD) activities and MDA content.

2.3.2 Proline

Proline is known as a well-known stress indicator as well as a biochemical solute accumulated during the water-deficit period (Yoshiba *et al.*, 1995). Proline accumulation takes place when the plants exposed to water deficit conditions have normally appeared as an osmotic adjustment or osmoregulation defense mechanism in coconut (Gomes *et al.*, 2010).

Gomes *et al.* (2010) reported that accumulation of proline is multiplied approximately 1.5 to 2.1 fold in coconut palms and a tremendous reduction in dry matter production and leaf area throughout the drought in coconut and also observed the accumulation of organic solutes (soluble carbohydrates, soluble amino N, and free proline) in seedlings of coconut during drought.

Proline acts as a solute and performs three essential roles in the stress period *i.e.*, as a metal chelator, an antioxidative protection molecule and a signaling

molecule. Overproduction of proline in the course of the drought in plants imparts stress tolerance through retaining cell turgor or osmotic balance, stabilizing membranes thereby preventing electrolyte leakage. This brings concentrations of reactive oxygen species (ROS) within normal levels, preventing oxidative damage in plants (Hayatab *et al.*, 2012).

Bartels and Sunker, (2005) studied a relationship between the drought tolerance and proline accumulation and also studied the over expression of the D1 -pyrroline- 5-carboxylate synthase gene or by using antisense suppression of the proline dehydrogenase (ProDH) gene in various crops. In coconut, Jayasekara *et al.* (1993) reported excessive ranges of proline in leaves of drought tolerant genotypes during the dry season.

High contents of proline were detected in dwarf coconut palm leaflets exposed to drought (Bai and Rajagopal, 2000). Voleti *et al.* (1990) made conclusion that there occurs an inverse relationship between RWC and proline accumulation in west coast tall, Laccadive ordinary (LO) and a pair of dwarfs Chowghat Orange Dwarf (COD) and Gangabondam (GB) and 4 hybrids *via*, WCT x COD, COD x WCT, LO x GB and LO x COD of coconut subjected to moisture stress.

2.3.4 Total soluble proteins

In C_3 plants protein synthesis is impaired under moisture stress conditions than C_4 plants. New proteins are produced under drought conditions. Heat-shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins - two types of major proteins are expressed during water stress (Taiz and Zeiger, 2010).

Low, medium and high molecular weight proteins were expressed in coconut during drought stress. Kumar *et al.* (2007) also reported that when coconut seedlings of different cultivars and cross combinations were subjected to stresses like drought.

There was an increase in the concentration of heat stable protein fraction (HSPF) in leaf tissue and the total protein concentration reduced under water stress. Leaf water potentials and the percentage of HSPF in leaf tissue are inversely proportional to each other. Asemota and Conaire (2010) observed that protein content of moisture stressed plants were reduced compared to well watered seedlings of oil palm

Particularly under water stress conditions, degradation of protein molecule will occur. For the survival of cells under stress, maintenance of proteins in their functional native conformations by preventing aggregation of non native proteins is important. Heat shock proteins (HSPs) act as molecular chaperones. It regulates functions like protein folding, assembly, translocation, and degradation under water stress conditions (Park and Seo, 2015).

Kumar *et al.* (2007) reported that HSPF, proteins similar to 66 kDa are found in root and leaf tissue. Leaflet tissues have specific proteins in the range similar to 10 kDa and similar to 14 kDa which were not present in the root tissues. Two extra proteins of 66 kDa and 76 kDa appeared in water stressed WCT seedlings. Among the Medium Molecular Weight (MWP)-proteins, a protein of 53 kDa was present in all WCT samples. The Low Molecular Weight (LMW) protein of 20.1 kDa, present in non-stress seedlings disappeared during water stress period.

Cao *et al.* (2011) reported that coconut seedlings adjusted to water stress condition by various changes in morphological, physiological and biochemical mechanisms in the plant body. Adjustment is related with keeping up osmotic homeostasis by metabolic changes mainly by proline incorporating change of stress related protein.

2.3.5 Chlorophyll content

Chlorophyll a and Chlorophyll b are essential pigments of the plant photo systems. Among them chlorophyll a is the primary photosynthetic pigment in plants

which enables to produce energy in the plant. Chlorophyll a concentration is 2-3 times higher than that of chlorophyll b in plants (Kamble *et al.*, 2015).

Plant productivity is a completely unique method that relies significantly upon the quantity of chlorophyll present in the chloroplast. Photosynthetic potential depends on the amount of chlorophyll content in unit leaf region. Therefore, any type of environmental stresses like drought will affect the quantity of chlorophyll within the leaf tissues (Otitoju and Onwurah, 2010).

Photosynthetic activity and stress are associated with chlorophyll content in the leaf and spectral features of plants and water content (Mate and Deshmukh, 2016).

Under water stress, decreasing the amount of chlorophyll content is particularly because of oxidative stress, resulting in photo-oxidation and chlorophyll degradation. Drought stress triggered a large decline in the chlorophyll a content, the chlorophyll b content, and the total chlorophyll content. This decline is leading to photosynthetic inhibition (Anjum *et al.*, 2011). Oil palm seedlings when subjected to PEG-induced water stress, Chlorophyll a, chlorophyll b, and total chlorophyll in the seedlings had been extensively decreased. Ultimately quantum yield of PSII, photon yield of PSII, in addition to net-photosynthetic rate, also showed a decline (Chaum *et al.*, 2012). When palms were subjected to extreme water stress, Chaum *et al.* (2013) diagnosed that chlorophyll a and chlorophyll b content became reduced and severely damaged.

According to Chaum *et al.* (2013) a positive correlation takes place between Relative Electrolytic Leakage (REL) and chlorophyll degradation in water deficit stressed palms. Total chlorophyll content in the coconut palm is substantially decreased under moisture stress and it maintains the chlorophyll content at normal soil moisture (Gomes *et al.*, 2008).

2.4 ENZYMATIC ANTIOXIDANT

2.4.1 Peroxidase (POD)

Peroxidase (POD) activity was increased and then decreased gradually in leaf with the exposure of drought to the plant. Among the observed parameters like relative conductivity, injury index, malondialdehyde (MDA), proline content and peroxidase, only the proline content gets increased during drought. Cao *et al.* (2011) reported that palm showed response mechanisms during drought stress in order to increase plant defense capability.

An experiment conducted by Putra *et al.* in 2016 and explained that, oil palm seedlings when subjected to water stress there was an increase in the activity of enzymatic antioxidants such as superoxide dismutase, catalase, guaiacol peroxidase , glutathione peroxidase, ascorbate peroxidase, Glutathione Reductase, dehydroascorbate reductase and monodehydroascorbate reductase. This rise of antioxidant activity can decrease oxidative damage caused by reactive oxygen species (ROS), there by drought tolerance capacity increases.

Plant drought stress response and tolerance are complex biological processes. Wang (2014) had done an experiment to reveal the drought tolerance mechanism in rubber tree. Peroxidase activity was markedly increased on withdrawing irrigation.

2.4.2 Superoxide Dismutase (SOD)

SODs are divided into four main subtypes and are categorized based upon their cellular locations and metal –ion-binding prosthetic groups. Copper and Zinc SODs (Cu/Zn-SODs) are found primarily in the cytoplasm of eukaryotic cells. Manganese SODs (MnSODs) exist in the mitochondria of eukaryotic cells and the cytoplasm of prokaryotes, iron SODs (Fe-SODs) exists mainly in prokaryotes and the chloroplast of eukaryotic plants, and Nickel SODs (Ni-SODs) exclusively found in prokaryotes (Pradedova *et al.*, 2009).

SOD is the most important antioxidant enzyme, a mettallo-enzyme involved in the detoxification of reactive oxygen species. Kumar *et al.* (2007) reported four to six SOD isoforms in different genotypes of citrus and correlated SOD with abiotic stress tolerance.

The increase or decrease in the activities of the stress sensitive enzymes under desiccation as compared to the pre-stress level indicated the differential adaptability of coconut palms with change in leaf water potential (Bai *et al.*, 2005).

2.5 MOLECULAR PARAMETERS

2.5.1 Protein profiling: SDS- PAGE

Cardena *et al.* (1998) detected the proteins of peroxidase, endopeptidases and coomassie blue stained proteins among the varieties of West African Tall, Malayan Yellow Dwarf and in the hybrid PB121. Low, medium and high molecular-weight proteins were expressed in coconut during drought stress (Kumar *et al.*, 2007).

Kumar *et al.* (2007) reported that, the concentration of Heat Shock Protein Fractions (HSPF) accumulated in maximum during the period of water stress in leaf. A protein of 66 kDa and 76 kDa was expressed in water stressed WCT seedlings.

2.5.2 DNA polymorphism

Manimekhala *et al.* (2004) found the relationship between leaf water potential and molecular markers through RAPD and ISSR analysis. Water stress-related MAPK genes were sequenced in coconut (Bobby *et al.*, 2010).

A cDNA clone of 467 bp encoding MAPK genes were isolated by Bobby *et al.* (2012) from the drought induced coconut seedlings. In coconut seedlings a total of 129 transcripts expressed during the period of water stress. AP2, CBF, MAPK, NAC and 14-3-3, were the candidate gens up regulated during drought.

Xiao *et al.* (2017) observed that, *WRKY* transcription factor that played a major role in the plant stress tolerance. Around 95 genes belonging to the *WRKY* family were identified and characterized in oil palm genome with intron number (0 to 12) and gene length (477 bp to 89,167 bp).

2.6. GENETIC VARIABILITY IN COCONUT- CRITICAL WATER POTENTIAL FOR POLLEN GERMINATION

2.6.1 Floral morphology

In a bearing palm, every leaf axil can produce a spadix or inflorescence which under normal conditions varies from 12 to 15 annually. The inflorescence develops within a strong, tough, pointed, tube like sheath called spathe, which after full development splits from top to bottom and releases the inflorescences

Inflorescence of coconut is known as spadix, is 1-2 m lengthy, and includes a primary axis or rachis, with 30 or more lateral branches called rachillae, each approximately 30-55 cm long and bearing 200-300 male flowers. The coconut inflorescence is monoecious with male and woman flowers in every spadix. Every inflorescence is borne singly, rising from the axil of successive leaves of a bearing palm. Flowering commences at 4-6 years of age, depending on the variety (dwarf plant flower earlier than tall). In ordinary bearers, the quantity of leaves and the spadices remains equal, (i.e., about 12 consistent with yr) (Manthriratna, 1965).

Inflorescences open successively at durations varying from 22 to 30 days, relying on the environmental situation and age of the palm (Ranasinghe *et al.*, 2010). From the second to the 19th days after the opening of the inflorescence, the male flora open, release pollen and fall off. Open male flower remain on an inflorescence for a couple of day, commonly open within the early hours of the day and are shed the same evening. Pollen grains stay best for two days on normal atmospheric conditions (Liyanage, 1954).

2.6.2 Pollen morphology

Coconut pollen grains are monocolpate. The variety typica measured 0.005 to 0.069 mm. in length and 0.028 to 0.030 mm. in diameter. There had been no enormous inter-varietal variations in length or form of pollen grains. The pollen output according to anther in a flower of a healthy tree has been estimated to be between 1, 11,000 to 2, 21,000 grains (Manthriratna, 1965).

2.6.3 Pollen germination medium

Lora *et al.* (2006) claimed that pollen grains shows high variability in different pollen germination medium due to the aggregation of boron, calcium and sucrose. The basic subculture medium used in maximum germination tests of pollen grains is composed of sucrose, and the content of different vitamins may cause variation. Boron and calcium are crucial for the in vitro pollen tube formation (James *et al.*, 1963).

Materials and Methods

3. MATERIALS AND METHOD

The objective of the experiment entitled "Assessment of water stress tolerance in selectively fertilized coconut (*Cocos nucifera* L.) hybrids was to assess the water stress tolerance of selectively fertilized coconut hybrids on physiological and molecular basis and to screen coconut genotypes for water stress tolerance through critical water potential for pollen germination. The experiment was conducted on nine year old field grown coconut palms at RARS Pilicode, Kasaragod (2017-18). Irrigation was withdrawn for a period of three months. Physiological and biochemical analysis were done at monthly intervals.

3.1. EXPERIMENT 1: PHYSIOLOGICAL AND MOLECULAR ASSESSMENT OF WATER STRESS TOLERANCE IN SELECTIVELY FERTILIZED COCONUT HYBRIDS

3.1.1. Location

The study was carried out at RARS Pilicode, Kasaragod, situated at $12^0$11'25" N latitude and 75^0 10' 0" E longitudes at an altitude of 15 m above mean sea level.

3.1.2 Planting material

Nine year old field grown coconut hybrid palms of the following varieties, Kerasree (WCT × MYD) selectively fertilized, Keraganga (WCT × GB) selectively fertilized, Kerasree (WCT × MYD), Keraganga (WCT × GB), West Coast Tall (WCT) were selected for experiment. The hybrids were developed in project funded by the International Foundation for Science IFS, Sweden in the Department of Plant Physiology.

3.1.3 Layout of the experiment and design

The experiment was laid out in RBD (Randomized Block Design) with five treatments and four replications. Five coconut varieties and hybrids were

Plate1. Experimental location

Kerasree

Kerasree S.F

Plate 2. Kerasree and Kerasree S.F after 60 days of water tress

Keraganga

Keraganga S.F

Plate 3. Keraganga and Keraganga S.F after 60 days of water tress

WCT

Plate 4. WCT after 60 days of water tress

selected for the experiment which consists of Kerasree, Keraganga, Kerasree (selectively fertilized), Keraganga (selectively fertilized) and West Coast Tall. 38

3.1.4 Main Items of Observations

3.1.4.1 Physiological parameters

3.1.4.1.1 Stomatal conductance

Using SAI-1 Porometer of company Delta T Devices, the stomatal conductance was recorded from the4th fully opened leaf from the top and was expressed in m moles H_20 m^{-2} s^{-1}.

3.1.4.1.2 Relative water content

To measure the relative water content, fresh weight, dry weight, and turgid weight of known number of leaf discs were taken from the index leaf of the treatment plants. Initially the fresh weight of the sample was taken, the leaf discs were then immersed for three hours in distilled water. After three hours turgid weight was taken. The samples were kept in oven at 80^0C for three days and dry weight was recorded.

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \text{ x } 100$$

3.1.4.1.3 Carbon isotope discrimination

For determining carbon isotope discrimination ratio, the index leaves (4^{th} fully opened leaves) were collected from the experimental palms and oven dried in 80^0C, the fully dried samples were made to fine powder. For carbon stable isotope studies, the samples were send to the National Facility at Department of Crop Physiology, GKVK, UAS, Bangalore and carbon isotope ratio was measured by using isotope ratio mass spectrometer (IRMS).

3.1.4.1.4 Cell membrane stability index

Fully turgid condition was reached by immersing the index leaf (4th fully opened leaf) with their petiole in distilled water for 3 hours and turgid weight was recorded. After the loss of about 40 to 60 % fresh weight, 1cm leaf bites were washed under running water to remove the solutes from the cut ends and 10 uniform sized leaf bites were kept for incubation in test tubes containing 20 ml distilled water and absorbance was recorded at 273 nm (initial leakage of solutes). The test tubes were incubated in water bath at 90^0C and absorbance was taken at 273 nm using spectrophotometer.

$$\% \text{ of leakage} = \frac{\text{initial absorbance of bathing medium}}{\text{final absorbance of bathing medium}} \text{ x } 100$$

3.1.4.1.5 Transpiration rate

Using portable photosynthetic system (CIRAS-3 SW, PP System International, MA, USA) transpiration rate recorded was recorded and expressed in mole H_20 m^{-2} s^{-2}

3.1.4.1.6 Leaf temperature

Using portable photosynthetic system (CIRAS-3 SW, PP System International, MA, USA) leaf temperature was recorded. The equipment is available in the Department of Plant Physiology, College of Agriculture, Vellayani.

3.1.4.1.7 Epicuticular wax content

A sample of 10 cm^2 leaf bites were taken from the 4th fully opened leaf and recorded the initial weight. Leaf bits were immersed for 30 seconds in 10 ml chloroform. The test tubes were kept undisturbed until chloroform gets

evaporated. Final weight of leaf bites was measured. The difference indicated the wax content. ECW was expressed as mg cm^{-2}.

3.1.4.1.8 Photosynthetic rate

Using portable photosynthetic system (CIRAS-3 SW, PP System International, MA, USA) photosynthetic rate was recorded from the 4th fully opened leaf using a ladder. Equipment is available in the Department of Plant Physiology, College of Agriculture, Vellayani.

3.1.5 Biochemical Parameters

3.1.5.1 Estimation of Malondialdehyde (MDA)

MDA content was determined by the Thiobarbituric acid (TBA) reaction as described by Ali *et al.* (2005), with slight modifications. Approximately 0.2 g leaf, coleoptiles and root segments were homogenized with 2 ml of 0.1% Trichloroacetic acid (TCA) and centrifuged at 14,000 rpm for 15 min. After centrifugation, 1 ml of the supernatant was mixed with 2.5 ml 0.5% TBA in 20% TCA and incubated in hot water for 30 min. Thereafter, it was cooled immediately on ice to stop the reaction and centrifuged at 10,000 rpm for 30 min. Absorbance at 532 and 600 nm was determined and MDA concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm.

3.1.5.2 Estimation of Proline

Leaf sample (500 mg) was extracted with 10 ml of 3 % sulphosalicylic acid and centrifuged at 6000 rpm for 15 minutes. Equal volume (2 ml) of acetic acid and ninhydrin was added to the aliquot. Reaction mixture was incubated for 1 hr in water bath and then reaction was arrested by placing on ice bath, 4ml toluene was added. The toluene layer was pipetted out from the extract and the absorbance was read at 520 nm using toluene as blank. The amount of proline was estimated from the standard curve drawn against absorbance versus concentration.

μ moles per gram tissue = (μg proline/ml x ml of toluene) /115.5 X (5 / gram sample)

3.1.5.3 Enzymatic antioxidants

3.1.5.3 a. *Estimation of Peroxidase (POD)*

The method described by Reddy *et al.* (1995) was used to quantify the peroxidase activity in plants under water stress conditions. Phosphate buffer of 0.1M with pH of 6.5 was prepared. The homogenate was centrifuged and supernatant was taken for assay. The enzyme extract of 0.1 ml was added to 3.0 ml of pyrogallol solution and the absorbance was read at 430 nm in spectrophotometer. 0.5 ml H_2O_2 was added to the cuvette containing sample and mixed thoroughly. The change in absorbance was recorded every 30 seconds up to 3 minutes.

3.1.5.3 b. Estimation of Superoxide dismutase (SOD)

The method described by Kakkar *et al.* (1984) was used to estimate the SOD activity in plants. The sample of 0.5 g was ground and homogenized with 3 ml of potassium phosphate buffer and centrifuged at 2000 rpm for 10 minutes. 1.2 ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of prepared enzyme was added to supernatant and total volume was made up to 2.8 ml. The mixture was kept for incubation at 30^0C for 10 minutes by the immediate addition of 0.2 ml of NADH solution, which initiate the reaction and butanol layer was pipetted out and absorbance was recorded at 560 nm.

3.1.5.4 Estimation of total soluble protein

Marion and Bradford (1976) method was used to quantify the total soluble protein present in the leaf sample. In this method bovine serum albumin is used as the standard. Dye solution was prepared by dissolving 100milligram of CBB (Coomassie Brilliant Blue) G250 in 50 ml of 95% ethanol and 100 ml of 85 % (w/v) orthophosphoric acid was added, made up to 200 ml by adding distilled H_20.

0.1 g of index leaf (4th fully opened leaves) was homogenized with 10 ml of phosphate buffer (p^H 7.8). The reaction mixture was centrifuged at 5000 rpm for 10 minutes. From the supernatant, 20 µl was mixed with 5 ml of diluted dye. The solution was mixed thoroughly and kept for 5 to 30 minutes for developing blue colour and absorbance was read at 596 nm. The protein content was expressed as mg g^{-1} FW.

3.1.5.5 Chlorophyll content

Estimation of chlorophyll (DMSO method)

Samples of 0.5 gram was weighed and cut into small leaf discs. Leaf discs were immersed in 10 ml of 80% acetone: DMSO mixture and kept in dark for one overnight. After overnight incubation, absorbance was recorded at 663, 645 nm using spectrophotometer. Chlorophyll content was expressed as mg g^{-1}

$$\text{Chl a} = (12.7 \times A_{663} - 2.65 \times A_{645}) \times \frac{V}{1000} \times \frac{1}{FW}$$

$$\text{Chl b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \frac{V}{1000} \times \frac{1}{FW}$$

$$\text{Total Chl (a+b)} = (8.02 \times A_{663} + 20.2 \times A_{645}) \times \frac{V}{1000} \times \frac{1}{FW}$$

3.1.6 Molecular Studies

3.1.6.1 Protein profiling: SDS – PAGE

Total soluble proteins were separated through electrophoresis as per the method given by Laemelli (1970).

Leaf samples were extracted with 1.5 ml of cold denaturing buffer at 4^0C. The homogenized sample was kept for centrifugation at 5000 rpm for 15 minutes. The supernatant was pipetted out and equal volume of chilled acetone (1:1) was added to precipitate the proteins. The supernatant was discarded and the remaining pellet was kept in 50 µl of denaturing buffer and vortexed. The pellet with buffer mixture was centrifuged at 5000 rpm for 15 minutes. Again the supernatant was

mixed with 10 µl of sample buffer and kept for 3 minutes in boiling water bath. Electrophoresis was done using SDS-PAGE.

Reagents

a) Acrylamide stock (30%)

Acrylamide - 29.2 g

Bis-acrylamide - 0.8 g

Double distilled water - 100 ml

b) Separating (resolving) gel buffer stock (1.5M Tris –HCl, pH 8.8)

Tris base (18.15 g) was dissolved in approximately 50 ml of double distilled water. The pH was adjusted to 8.8 with 6 N HCl and the volume was made up to 100 ml with double distilled water and stored at 4^0C.

c) Stacking gel buffer stock (0.5 M Tris – HCl pH 6.8)

Tris base (6.0 g) was dissolved in approximately 60 ml of double distilled water and adjusted the pH was adjusted to 6.8 with 6 N HCl and the volume was made up to 100 ml with double distilled water and stored at 4^0C.

d) Polymersing agents

AmmoniumperSulphate (APS) 10 percemt prepared freshly before use.

TEMED-Fresh from refrigeration

e) Electrode buffer pH 8.3

Tris base	- 6.0 g
Glycine	- 28.8 g
SDS	- 2.0 g
Double distilled water	- 2 L

f) Sample buffer

Double distilled water	- 2.6 ml
0.5 M Tris HCl pH 6.8	- 1.0 ml
2-mercapto ethanol	- 0.8 ml
Glycerol	- 1.6 ml
SDS 20% (w/v)	- 1.6 ml
0.5% Bromophenol blue	- 0.4 ml

g) Staining solution

Coomassie brilliant blue R 250	- 0.1 g
Methanol	- 40.0 ml
Glacial acetic acid	- 10.0 ml
Double distilled water	- 50.0 ml

Destaining solution is the staining solution without coomassie brilliant blue R 250.

Procedure

Separating gel was first casted followed by stacking gel mixing various solutions as indicated below

a) Preparation of separating gel (12%)

Double distilled water	- 6.7 ml
Tris HCl, pH 8.8	- 5.0 ml
SDS10%	- 0.2 ml
Acrlamide stock	- 8.0 ml

The solution was mixed well and de gassed for 3 minutes and then the following were added immediately.

Freshly prepared 10% Ammonium per Sulphate (APS) - 0.10 ml

Tetra methyl ethylene diamine (TEMED) - 0.01 ml

The separating gel was mixed well and poured immediately between glass plates and a layer of water was added above the polymerizing solution to quicken the polymerizing process

b) Preparation of stacking gel

Double distilled water	- 6.1 ml
Tris HCl, pH 6.8	- 2.5 ml
SDS 10%	- 0.2 ml
Acrlamide stock	- 1.3 ml

The solution was mixed well, degassed and the following were added

APS 10%	- O.5 ml
TEMED	- 0.1 ml

The water layer over the separating gel was removed and washed with a little electrode buffer and then stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

The comb was removed after polymerization; samples were loaded in to the wells. A standard with known molecular weights was also loaded to wells. The electrophoresis was performed at 100 V till the dye reached the separating gel. Then the voltage was increased in 200 V and continued till the dye reached the bottom of the gel. Immediately after electrophoresis the gel was removed from the glass plates and incubated in the staining solution overnight with uniform shaking. The gel was transferred to the destaining solution. The protein appeared as bands and gel was photographed after placing it on GEL DOC (G: BOX, Synegene)

3.1.6.2 Polymorphism **(SSR Marker analysis)**

Isolation of DNA (CTAB method- Maroof *et al.*, 1984). The leaf samples (one gram) were powdered using liquid nitrogen in a mortar and transferred in to 50 ml sterile centrifuge tube by adding 15 ml of CTAB buffer (Extraction buffer- 2% C-TAB, 1.4mM NaCl, 20mM EDTA, and 10mM Tris base (pH 8)). 50 µl of β-mercaptoethanol was added to the mixture and gently shaked. Tubes were kept in water bath for an hour for incubation with constant stirring at successive intervals. After the incubation, 15 ml of chloroform isoamyl alcohol was added to the incubated mixture and kept for centrifugation at 6000 rpm for 20 minutes. The upper layer was transferred to the fresh sterile centrifuge tube was added with cold isopropanol and the tubes were centrifuged at 6000 rpm for 20 minutes. DNA pellet found was washed twice with 1 mL of 70 % ethanol and the tubes were centrifuged at 6000 rpm for 20 minutes. Remaining ethanol was removed gently and pellet was dissolved in 500 µl of TE buffer.

Polymerase chain reaction (PCR)

The PCR is performed as one of the first proofs for the presence of the gene in the transformants.

Constituents of a PCR reaction

DNA : 1µl

dNTPs : 1.5 µl

Forward primers : 1µl

Reverse primers : 1µl

$MgCl_2$: 0.3 µl

Taq polymerase : 2.0 µl

Taq buffer : 2.0 µl

Sterile water : 11.2 µl

Master mix was prepared by mixing forward and reverse primers, dNTPs, Taq buffer, sterile water and Taq polymerase were added in to eppendoff and kept on ice. One µl of each DNA sample was aliquoted in to sterile PCR tubes and 19 µl of master mix was added to each PCR tube and mixed well. The PCR tubes were placed in thermocycler and the program is set as follows.

Primer	Forward sequence	Reverse seequence
CnCir E2	TCGCTGATGAATGCTTGCT	GGGGCTGAGGGATAAACC
CnCir E10	TGGGTTCCATTTCTTCTCTCATC	GCTCTTTAGGGTTCGCTTTCTTAG
CnCir E12	TCACGCAAAAGATAAAACC	ATGGAGATGGAAAGAAAGG
CnCir H40	TTAGATCTCCTCCCAAAG	ATCGAAAGAACAGTCACG

3.2 EXPERIMENT II: EVALUATION OF CRITICAL WATER POTENTIAL FOR POLLEN GERMINATION IN ASSESSING GENETIC VARIABILITY IN COCONUT

3.2.1 Location

The study was carried out at RARS Pilicode, Kasaragod, situated at $12^0 11'$ 25" N latitude and 75^0 10' 0" E longitudes at an altitude of 15 m above mean sea level.

3.2.2 Experimental material

30 genotypes (given in table 1) from the germplasm collection at RARS Pilicode, was selected for study.

3.1.3 Layout of the experiment and design

The experiment was laid out in CRD with 30 treatments and three replications.

3.1.4 Main items of Observation

3.1.4.1 Pollen germination percentage

Male flowers were collected from the selected genotypes in the morning hours, 8.00 am to 10.00 am. Collected pollens were dusted in the standardized pollen germination medium in a Petri plate and kept for incubation for 3 hours. After incubation period Pollen germination was recorded using DLMS Leica microscope.

Table.1 List of coconut genotypes used in the study for assessing genetic variability

Sl. No	Name of the Genotypes
1.	New guinea
2.	Andaman ordinary
3.	Anandaganga
4.	Godavari
5.	Lakshaganga
6.	Keraganga
7.	Navasi
8.	Tanjore
9.	Java
10.	WCT
11.	COD
12.	Philippines
13.	Soubhagya
14.	Thembli
15.	Ayiramkachi
16.	Komadan
17.	Bombay G
18.	Kappadam
19.	Andaman Ranguchen
20.	Lakshadweep micro
21.	Sanramon
22.	MYD
23.	Cochin China
24.	Fiji
25.	Bensahybrid
26.	Kerasree
27.	Lakshadweep ordinary
28.	Bansanda
29.	Jamaica
30.	NCD

Results

4. RESULTS

Kerala the land of coconut occupies first position in area with 6.5 lakh ha and a productivity of 7535 nuts/ha. But the productivity is far behind the national average (CDB, 2015). Drought is the second most important factor reducing the productivity of coconut next to the dreaded root (wilt) disease. Studies showed that about 2/3 of the potential coconut production is generally lost due to either poor nut setting or immature nut fall as a result of water deficit conditions.

Only way towards increasing coconut production in drought affected areas is through development of drought tolerant varieties and hybrids. *In vitro* pollen selection followed by selective fertilization will be the most feasible and cost effective approach in this direction. Hence an experiment entitled "Assessment of water stress tolerance in selectively fertilized coconut (*Cocos nucifera* L.) hybrids" was conducted and results obtained are presented in this chapter after statistical analysis.

4.1 EXPERIMENT 1 - PHYSIOLOGICAL AND MOLECULAR ASSESSMENT OF WATER STRESS TOLERANCE IN SELECTIVELY FERTILIZED COCONUT HYBRIDS

4.1.1 Physiological parameters

Stomatal conductance, cell membrane stability index, transpiration rate, leaf temperature, epicuticular wax content, photosynthetic rate, relative water content and carbon isotope discrimination were recorded during the experiment, showed significant variation under water stress condition.

4.1.1.1 Stomatal Conductance (m mol H_20 m^{-2} s^{-1})

Effect of water stress in stomatal conductance is depicted in Table 2. During first month of irrigation withdrawal, Keraganga unveiled lowest mean value of stomatal conductance (19.50 m mol H_20 m^{-2} s^{-1}) which was on par with kerasree

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Table.3 Effect of water stress on stomatal conductance (m mol H_20 m^{-2} s^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	31.75	29.50	28.75
Keraganga SF	26.50	26.00	26.50
Kerasree	21.25	22.25	13.75
Keraganga	19.50	25.25	10.75
WCT	39.50	30.75	42.25
CD(0.05)	5.038	5.141	4.638

Table.4 Effect of water stress on relative water content (%) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	30 days after stress	30 days after stress
Kerasree SF	81.23	80.86	76.465
Keraganga SF	79.55	72.90	71.633
Kerasree	74.65	74.96	69.273
Keraganga	77.99	72.68	68.905
WCT	73.63	72.03	72.360
CD(0.05)	3.089	2.900	2.412

(21.25 m mol H_20 m^{-2} s^{-1}). WCT recorded (39.50 m mol H_20 m^{-2} s^{-1}) which was significantly different from all other treatments and was followed by Kerasree S.F (31.750 m mol H_20 m^{-2} s^{-1}) and Keraganga S.F (21.25 m mol H_20 m^{-2} s^{-1}).

WCT recorded high stomatal conductance during second month of treatment with a mean value of 30.75 m mol H_20 m^{-2} s^{-1} which was on par with both selectively fertilized hybrids, Kerasree S.F (29.50 m mol H_20 m^{-2} s^{-1} and Keraganga S.F (26.00 m mol H_20 m^{-2} s^{-1}). The lowest mean value was recorded for Kerasree (22.25 m mol H_20 m^{-2} s^{-1}) which was on par with Keraganga (25.25 m mol H_20 m^{-2} s^{-1}).

During third month of treatment, WCT recorded high stomatal conductance (42.25 m mol H_20 m^{-2} s^{-1}) which was significantly different from the selectively fertilized coconut hybrids, Kerasree S.F (28.75 m mol H_20 m^{-2} s^{-1}) and Keraganga S.F (26.50 m mol H_20 m^{-2} s^{-i}). The lowest mean was found in normal hybrids, Keraganga (10.75 m mol H_20 m^{-2} s^{-1}) which was on par with Kerasree (13.75 m mol H_20 m^{-2} s^{-1}).

4.1.1.2 Relative water content (%)

Effect of water stress in relative water content is depicted in Table 3. Kerasree S.F had maintained high relative water content (81.23 %) and it was on par with Keraganga S.F (79.55 %) during second month of treatment and significantly different from all other treatments. The lowest mean was detected on WCT (73.63 %) which was found to be on par with normal hybrid, Keraganga (74.65 %)

During the second month, Kerasree S.F recorded high relative water content (80.86 %) which was significantly different from all other treatments and followed by Kerasree (74.960 %) and was found to be on par with Keraganga S.F (72.908 %). The lowest mean value was recorded for WCT (72.03 %) which was on par with Keraganga (72.68 %).

Preeminent relative water content (76.465 %) was recited by Kerasree S.F during the third month of treatment and it was significantly different from other treatments. The lowest value was noticed in Keraganga (69.273 %) and was on par with Keraganga S.F (71.633 %).

4.1.1.3 Carbon isotope discrimination (‰)

Effect of water stress in carbon isotope discrimination is depicted in Table 4. Keraganga recorded highest discrimination (21.49 ‰) followed by Kerasree with a mean value of 20.41 ‰. Kerasree S.F (19.90 ‰) followed by WCT (20.08 ‰) reported minimum discrimination and which were significantly different from all other treatments.

4.1.1.4 Cell membrane stability index (%)

Effect of water stress in cell membrane stability index is depicted in Table 5. Keraganga S.F (72.44 %) registered maximum stability in first month of water stress condition followed by Keraganga (63.11 %) which were significantly different from all other treatments. Membrane stability was lowest for Kerasree (54.60 %) which was significantly different from all other treatments.

During the second month treatment, Keraganga S.F unveiled high (74.27 %) cell membrane stability and it was significantly different from its normal hybrids (61.16 %) and WCT (70.67 %).

The lowest cell membrane stability in third month was detected in kerasree (68.17 %), Kerasree S.F (80.62 %), Keraganga S.F (79.31 %), and WCT (79.16 %).

Table.5 Effect of water stress on Carbon isotope discrimination (‰) of selectively fertilized coconut hybrids

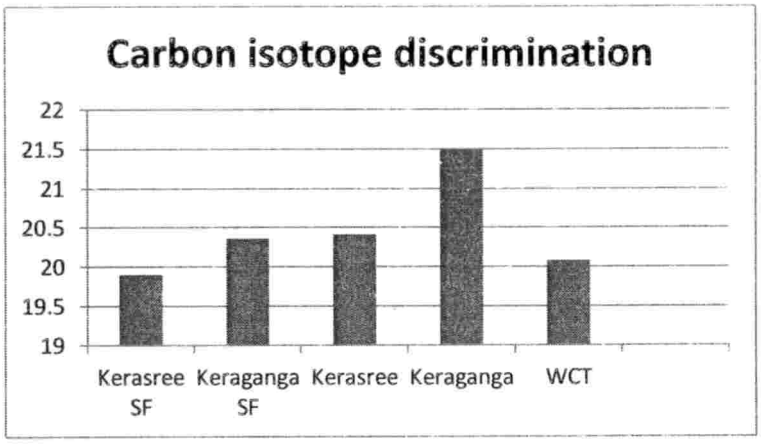

Table.6 Effect of water stress on Cell membrane stability index (%) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	63.14	69.92	80.62
Keraganga SF	72.44	74.27	79.31
Kerasree	54.60	61.16	68.17
Keraganga	68.29	68.67	70.53
WCT	64.91	70.47	79.16
CD(0.05)	2.239	2.409	1.935

4.1.1.5 Transpiration rate (m mol H_20 m^{-2} s^{-1})

Effect of water stress in transpiration rate is depicted in Table 6. WCT (1.45 m mol H_20 m^{-2} s^{-1}) recorded high transpiration rate during first month of water stress, followed by Kerasree S.F (0.85 m mol H_20 m^{-2} s^{-1}) and Keraganga S.F (0.675 m mol H_20 m^{-2} s^{-1}). Kerasree registered least transpiration rate (0.50 m mol H_20 m^{-2} s^{-1}).

Transpiration rate was found to be high in WCT (0.97 m mol H_20 m^{-2} s^{-1}) and was significantly different from all other treatments. Kerasree S.F recorded higher transpiration rate (0.77 m mol H_20 m^{-2} s^{-1}) than its normal hybrid Kerasree (0.47 m mol H_20 m^{-2} s^{-1}). Keraganga S.F recorded higher transpiration rate (0.65 m mol H_20 m^{-2} s^{-1}) as compared to its normal hybrid, Keraganga (0.55 m mol H_20 m^{-2} s^{-1}).

Third month of withdrawal of water from the field unveiled that Kerasree S.F recorded preeminent transpiration rate (0.97 m mol H_20 m^{-2} s^{-1}) which was on par with WCT (0.95 m mol H_20 m^{-2} s^{-1}). Lowest value was recorded for Kerasree (0.50 m mol H_20 m^{-2} s^{-1}).

4.1.1.6 Leaf temperature (°C)

Effect of water stress in leaf temperature is depicted in Table 7. During the period of first month of water stress, WCT recorded a high canopy temperature (33.40°C) which was on par with both normal hybrids, kerasree (31.77 °C) and Keraganga (32.70 °C). Lowest mean value was recorded for kerasree S.F (30.75 °C) which was on par with Keraganga S.F (31.45 °C)

After two months of water stress, Kerasree S.F recorded lowest leaf temperature (30.30 °C) which was on par with Keraganga S.F (31.45 °C) which were significantly different from all other treatments. WCT recorded preeminent leaf temperature (34.37 °C), which was on par with Kerasree (34.27 °C).

Table.7 Effect of water stress on Transpiration rate (m mol H_20 m^{-2} s^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	0.85	0.77	0.97
Keraganga SF	0.67	0.65	0.67
Kerasree	0.50	0.47	0.50
Keraganga	0.62	0.55	0.70
WCT	1.45	0.97	0.95
CD(0.05)	0.227	0.191	0.195

Table.8 Effect of water stress on Leaf temperature (°C) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	30.75	30.30	27.77
Keraganga SF	31.45	31.45	28.77
Kerasree	31.77	34.27	30.60
Keraganga	32.70	32.57	29.77
WCT	33.40	34.37	30.65
CD(0.05)	1.739	1.485	1.005

During the third month of irrigation withdrawal WCT recorded preeminent leaf temperature (30.65 °C) which was on par with normal hybrids, Kerasree (30.60 °C) and Keraganga (29.77 °C). Kerasree S.F recited lowest mean value (27.77 °C) which was on par with Keraganga S.F (28.77 °C).

4.1.1.7 Epicuticular wax content (mg/10cm^2)

Effect of water stress in epicuticular wax content is depicted in Table 8. After the first month of irrigation withdrawal, WCT accumulated high quantity of epicuticular wax (2.89 mg/10cm^2) which was on par with its selectively fertilized hybrid, Kerasree S.F (2.62 mg/10cm^2). Kerasree (1.73 mg/10cm^2) had accumulated wax was minimum among the normal hybrids and selectively fertilized hybrids.

WCT recorded high accumulation of epicuticular wax (3.24 mg/10cm^2) which was on par with Kerasree S.F (2.98 mg/10cm^2), followed by Keraganga S.F (2.39 mg/10cm^2).

In the third month of treatment Kerasree S.F found preeminent epicuticular wax (2.57 mg/10cm^2), which was on par with WCT (2.27 mg/10cm^2) which were significantly different from Keraganga S.F (1.79 mg/10cm^2), normal hybrids, Kerasree (1.17 mg/10cm^2) and Keraganga (1.66 mg/10cm^2).

4.1.1.8 Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1})

Effect of water stress in photosynthetic rate is depicted in Table 9. During the first month of treatment, it was observed that WCT had high photosynthetic rate (5.47 μ mol CO_2 m^{-2} s^{-1}) which was on par with selectively fertilized hybrids, Kerasree S.F (5.20 μ mol CO_2 m^{-2} s^{-1}) and Keraganga S.F (4.92 μ mol CO_2 m^{-2} s^{-1}) and were significantly different from normal hybrids. Keraganga recorded a higher photosynthetic rate (4.07 μ mol CO_2 m^{-2} s^{-1}) than Kerasree (3.225 μ mol CO_2 m^{-2} s^{-1}).

Table.9 Effect of water stress on Epicuticular wax (mg/10cm^2) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	2.62	2.98	2.57
Keraganga SF	2.14	2.39	1.79
Kerasree	1.73	1.92	1.17
Keraganga	2.27	1.91	1.66
WCT	2.89	3.24	2.27
CD(0.05)	0.404	0.278	0.564

Table.10 Effect of water stress on Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	5.20	4.40	4.82
Keraganga SF	4.92	3.05	3.92
Kerasree	3.22	1.25	2.90
Keraganga	4.07	0.80	1.50
WCT	5.47	3.37	1.57
CD(0.05)	0.588	0.685	0.614

Sixty days after stress, Kerasree S.F recorded high photosynthetic rate (4.40 μ mol CO_2 m^{-2} s^{-1}) which was significantly different from normal hybrids and WCT. The photosynthetic rate WCT (3.37 μ mol CO_2 m^{-2} s^{-1}) documented was closely related Kerasree S.F (3.05 μ mol CO_2 m^{-2} s^{-1}). Kerasree (1.25 μ mol CO_2 m^{-2} s^{-1}) followed Keraganga (0.80 μ mol CO_2 m^{-2} s^{-1}).

After the third month of treatment, Kerasree S.F marked higher photosynthetic rate (4.825 μ mol CO_2 m^{-2} s^{-1}), followed by Keraganga S.F (3.92 μ mol CO_2 m^{-2} s^{-1}) and which were significantly different from all other treatment. Kerasree registered photosynthetic rate (2.90 μ mol CO_2 m^{-2} s^{-1}) was higher than WCT (1.57 μ mol CO_2 m^{-2} s^{-1}) and lowest photosynthetic rate was noted in Keraganga (1.50 μ mol CO_2 m^{-2} s^{-1}).

4.1.2 Biochemical parameters

4.1.2.1 Malondialdehyde (μg g^{-1})

Effect of water stress in malondialdehyde is depicted in Table 10. Kerasree (0.74 μg g^{-1}) and Keraganga (0.74 μg g^{-1}) recited high membrane damage in the first month of treatment. Selectively fertilized hybrids, Kerasree (0.58 μg g^{-1}) and Keraganga (0.65 μg g^{-1}) recited a least membrane damage compared with the normal Kerasree and Keraganga. WCT (0.38 μg g^{-1}) recited a minimum Malondialdehyde (MDA) content.

In the second month of treatment, same trend was followed. WCT (0.39 μg g^{-1}) unveiled a lowest minimum of lipid peroxidation as compared with Kerasree (0.75 μg g^{-1}), Keraganga (0.77 μg g^{-1}) and its selectively fertilized hybrids, Kerasree S.F (0.60 μg g^{-1}), Keraganga S.F (0.62 μg g^{-1})

Lipid peroxidation value was highest in Keraganga (0.86 μg g^{-1}) and the lowest value was noticed in WCT (0.47 μg g^{-1}) during the third month of water stress.

Table.11 Effect of water stress on MDA (µg g^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	0.58	0.60	0.59
Keraganga SF	0.65	0.62	0.62
Kerasree	0.74	0.75	0.79
Keraganga	0.74	0.77	0.86
WCT	0.38	0.39	0.47
CD(0.05)	0.079	0.189	0.193

Table.12 Effect of water stress on Proline (µ mol g^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	102.00	254.75	313.53
Keraganga SF	115.25	293.25	293.05
Kerasree	235.50	300.25	266.70
Keraganga	149.25	185.25	214.58
WCT	190.25	222.50	261.29
CD(0.05)	20.172	18.072	8.023

4.1.2.2 Proline (μ mol g^{-1})

Effect of water stress in proline is depicted in Table 11. Preeminent proline accumulation was noted in Kerasree (235.50 μ mol g^{-1}) which was significantly different from all other treatments. Second preeminent proline accumulation was recorded in WCT (190.25 μ mol g^{-1}). Kerasree S.F had a lowest proline accumulation (102.00 μ mol g^{-1}), which was on par Keraganga S.F (115.25 μ mol g^{-1}).

During the second month of treatment, Kerasree maintained high proline accumulation (300.25 μ mol g^{-1}), which was on par with Keraganga S.F (293.25 μ mol g^{-1}) and followed by Kerasree S.F (254.75 μ mol g^{-1}) which were significantly different from Keraganga (185.25 μ mol g^{-1}) and WCT (222.50 μ mol g^{-1}).

After third month of stress Kerasree S.F marked high proline accumulation (313.53 μ mol g^{-1}) which was significantly different from all other treatments. Keraganga S.F (293.05 μ mol g^{-1}) noted second preeminent proline content among the treatments. Kerasree (266.70 μ mol g^{-1}) which was on par with WCT (261.29 μ mol g^{-1}) and the lowest proline accumulation was recorded in Keraganga (214.29 μ mol g^{-1}).

4.1.2.3 SOD (activity g^{-1} min^{-1})

Effect of water stress in SOD is depicted in Table 12. Selectively fertilized Kerasree hybrid recorded a higher amount SOD (4.67 activity g^{-1} min^{-1}) followed by Keraganga S.F (4.05 activity g^{-1} min^{-1}), which were significantly different from normal hybrids and WCT. Kerasree (2.95 activity g^{-1} min^{-1}) examined higher SOD content than Keraganga (2.68 activity g^{-1} min^{-1}) during first month of treatments.

In the second month of treatments, higher SOD activity was detected in selectively fertilized Kerasree hybrid (5.72 activity g^{-1} min^{-1}), followed by Keraganga

Table.13 Effect of water stress on SOD activity (activity g^{-1} min^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	4.67	5.72	6.22
Keraganga SF	4.05	4.70	4.95
Kerasree	2.95	3.70	3.95
Keraganga	2.68	3.18	3.05
WCT	2.25	3.03	4.12
CD(0.05)	0.458	0.780	0.815

Table.14 Effect of water stress on Peroxidase activity (activity g^{-1} min^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	0.92	1.75	3.20
Keraganga SF	0.77	1.35	1.85
Kerasree	0.67	2.07	2.32
Keraganga	0.52	1.50	2.02
WCT	0.55	1.05	1.70
CD(0.05)	0.281	0.550	0.865

S.F (4.70 activity g^{-1} min^{-1}), which were significantly different from normal hybrids and WCT. Among the normal hybrid Kerasree (3.70 activity g^{-1} min^{-1}) recorded preeminent SOD content than Keraganga (3.18 activity g^{-1} min^{-1}).

Selectively fertilized Kerasree hybrid was recorded a high amount of SOD (6.22 activity g^{-1} min^{-1}) and followed by Keraganga S.F (4.95 activity g^{-1} min^{-1}) in the last month of treatment and found that both the selectively fertilized hybrids were significantly different from normal hybrids and WCT. WCT recorded preeminent accumulation of SOD (4.12 activity g^{-1} min^{-1}) than kerasree (3.95 activity g^{-1} min^{-1}) and Keraganga (3.05 activity g^{-1} min^{-1})

4.1.2.3. Peroxidase (activity g^{-1} min^{-1})

Effect of water stress on peroxidase activity is depicted in Table 13. Preeminent amount of peroxidase activity was marked in Kerasree S.F (0.92 activity g^{-1} min^{-1}) under first month of treatment, which was on par with Keraganga S.F (0.77 activity g^{-1} min^{-1}) and Kerasree (0.67 activity g^{-1} min^{-1}) which was significantly different from WCT

Kerasree recorded preeminent peroxidase activity (2.07 activity g^{-1} min^{-1}), which was on par with Kerasree S.F (1.75 activity g^{-1} min^{-1}). Kerasree and its selectively fertilized hybrid were significantly different form WCT (1.05 activity g^{-1} min^{-1}) in the second month of treatment.

In the case of third month, Kerasree S.F (3.20 activity g^{-1} min^{-1}) recorded maximum peroxidase activity which was significantly different from all other treatments. Among the normal hybrids Kerasree (2.32 activity g^{-1} min^{-1}) had maximum peroxidase activity than Keraganga (2.02 activity g^{-1} min^{-1}). WCT noted slightly higher accumulation of peroxidase than kerasree and Keraganga in third month of treatments.

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4.1.2.4 Total soluble proteins (mg g^{-1})

Effect of water stress in total soluble proteins is depicted in Table 14. Higher content of total soluble proteins was recorded in WCT (6.43 mg g^{-1}) which was on par with its selectively fertilized hybrid, Kerasree S.F (6.10 mg g^{-1}). The Keraganga S.F had high soluble protein content (5.38 mg g^{-1}) than its normal hybrid. Normal Kerasree hybrids recorded least total soluble proteins (4.85 mg g^{-1}).

During the second month of treatment, Kerasree S.F recorded maximum accumulation of soluble proteins (6.87 mg g^{-1}), followed by Keraganga S.F (6.09 mg g^{-1}). Kerasree S.F and Keraganga S.F were significantly different from normal hybrids. Keraganga (5.073 mg g^{-1}) recorded lowest quantity of soluble proteins than WCT (5.18 mg g^{-1}) as well as Kerasree (5.38 mg g^{-1}).

Principal aggregation of soluble protein was marked in Kerasree S.F (7.70 mg g^{-1}), which was on par with Keraganga S.F (7.09 mg g^{-1}) and followed by WCT (5.81 mg g^{-1}). The Kerasree (4.38 mg g^{-1}) reported with lowest amount of soluble protein as compared to Keraganga (4.67 mg g^{-1}) during third month of water stress.

4.1.2.5 Chlorophyll content (mg g^{-1})

Effect of water stress in chlorophyll content is depicted in Table 15. Chlorophyll content was retained by kerasree S.F (1.39 mg g^{-1}) after one month of water stress, which was on par with Keraganga S.F (1.24 mg g^{-1}) and which were significantly different from normal hybrid, Kerasree and Keraganga. WCT (1.04 mg g^{-1}) retained highest chlorophyll content than Kerasree and Keraganga.

Kerasree S.F detected with higher chlorophyll content (1.18 mg g^{-1}) during second month of stress treatment, which was significantly different from all other treatments. Second elevated chlorophyll content was retained by Keraganga S.F

Table.15 Effect of water stress on Total soluble protein (mg g^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	6.10	6.87	7.70
Keraganga SF	5.38	6.09	7.09
Kerasree	4.85	5.38	4.38
Keraganga	4.94	5.07	4.67
WCT	6.43	5.18	5.81
CD(0.05)	0.749	0.603	1.055

Table.16 Effect of water stress on Chlorophyll content (mg g^{-1}) of selectively fertilized coconut hybrids

Hybrids and Varieties	30 days after stress	60 days after stress	90 days after stress
Kerasree SF	1.39	1.18	1.26
Keraganga SF	1.24	0.98	0.95
Kerasree	0.99	0.66	0.55
Keraganga	0.91	0.85	0.46
WCT	1.04	0.72	0.62
CD(0.05)	0.188	0.153	0.126

(0.98 mg g^{-1}). Least chlorophyll content was found in kerasree (0.66 mg g^{-1}) as compared with Keraganga (0.85 mg g^{-1}) followed by WCT (0.72 mg g^{-1}).

During third month of treatment, a consistent increase in chlorophyll content was registered in Kerasree S.F (1.26 mg g^{-1}), which was significantly different from all other treatments. The minimal quantity of chlorophyll was listed in Keraganga (0.46 mg g^{-1}) when compared with WCT (0.62 mg g^{-1}) followed by kerasree (0.55 mg g^{-1}).

4.1.3 Molecular parameters

4.1.3.1 Polymorphism: SSR markers

Drought related SSR primers CnCirE2, CnCirE10, CnCirE12 and CnCirH4 were evaluated. CnCirE2 marker was observed as polymorphic and can distinguish normal hybrid of Keraganga. CnCirE10 marker was also ploymorphic with WCT and Keraganga S.F which also linked with drought tolerance of WCT. CnCirE12 were used to distinguish selectively fertilized hybrids from normal hybrid and WCT.

4.1.3.2 Protein polymorphism: SDS-PAGE

A protein of 66-70 kDa was expressed in WCT which was reported to be associated with drought tolerance.

Plate 6. Polymorphism: SSR marker- CnCirE2

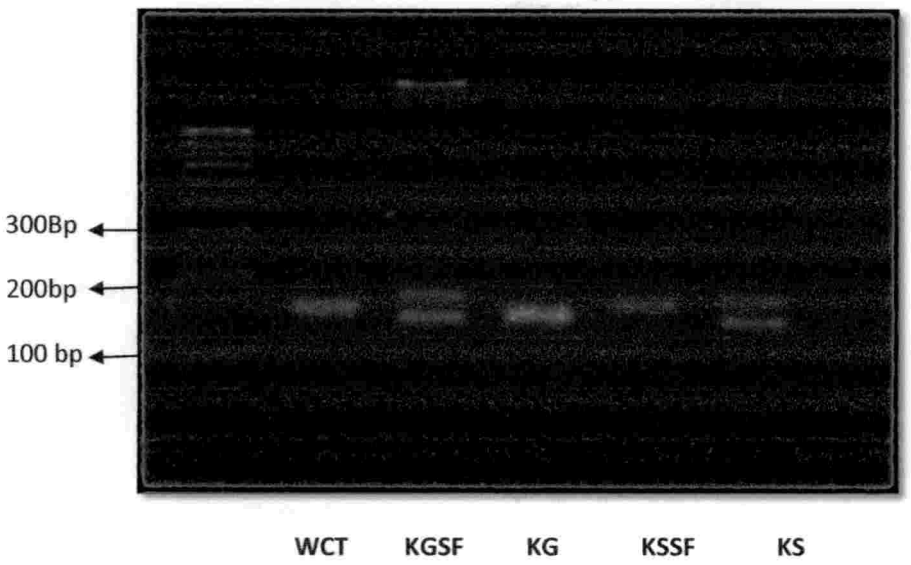


Plate 7. Polymorphism: SSR marker- CnCirE10

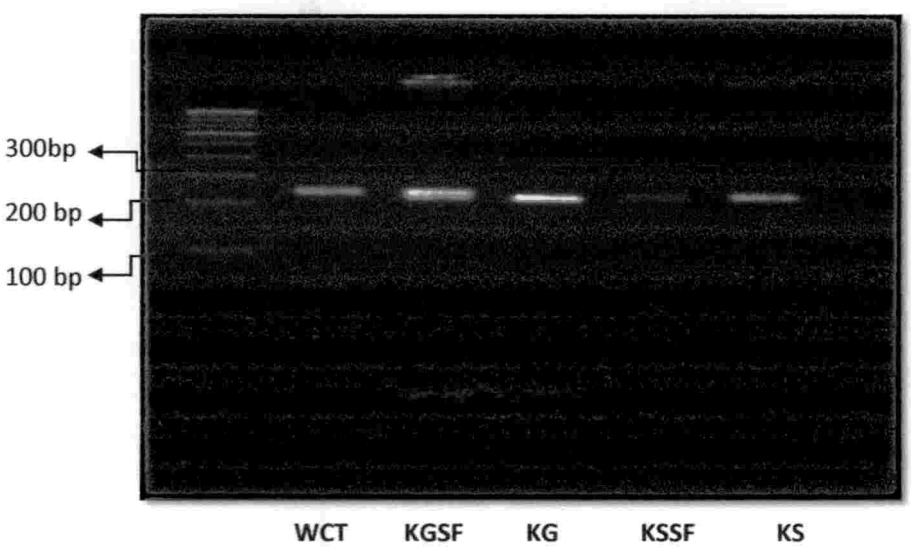
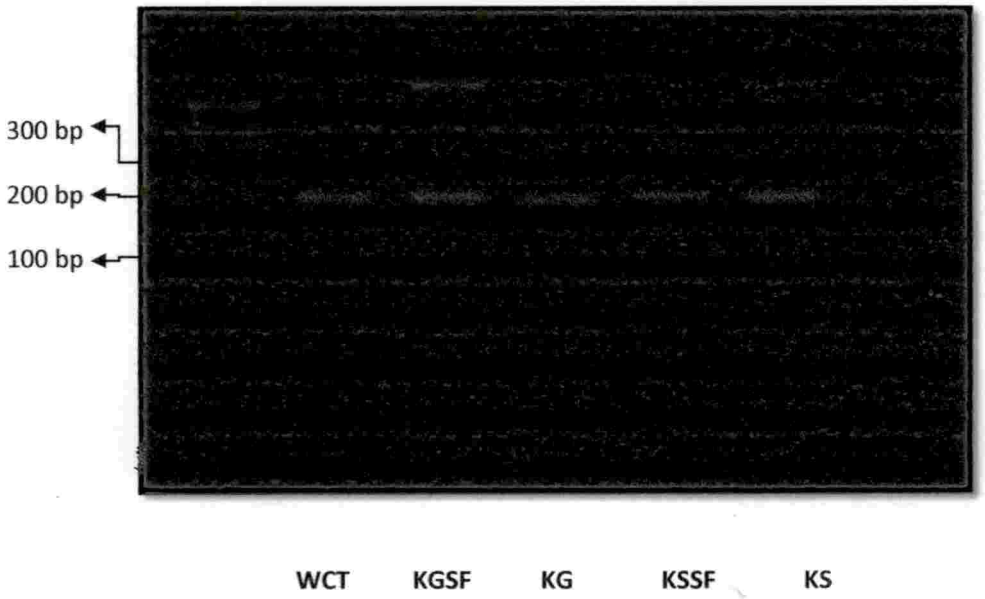
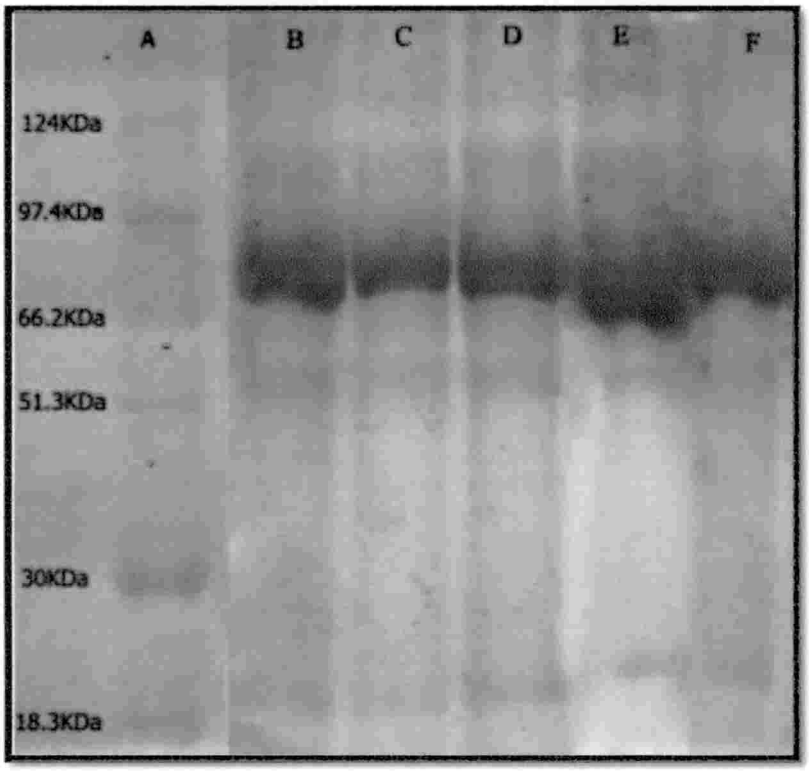


Plate 8. Polymorphism: SSR marker- CnCirE12



Protein Profiling: SDS PAGE

Marker KS KG KGSF WCT KSSF



4.2 EXPERIMENT 2 - EVALUATION OF CRITICAL WATER POTENTIAL FOR POLLEN GERMINATION IN ASSESSING GENETIC VARIABILITY IN COCONUT

4.2.1 Pollen germination percentage (%)

Pollen germination percentage is depicted in Table 16. Andaman ordinary, Lakshaganga, Keraganga, Soubhagya, Jamaica, Bansanda and WCT genotypes reported highest critical water potential for pollen germination was -0.5 MPa. WCT (50.29%) recorded highest pollen germination followed by Tanjore (31.70 %), Andaman ordinary (29.82 %). Lowest critical water potential for pollen germination was observed in Sanramon (-0.2 MPa) with 27 % of pollen germination at that water potential.

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Table.17 Pollen germination percentage (%) of coconut genotypes at different water potential

Genotypes	-0.2 MPa	-0.3 MPa	-0.4 MPa	-0.5 MPa	-0.6 MPa
New guinea	81.70	49.60	18.37	0.28	0.70
Andaman ordinary	91.33	63.28	47.12	29.82	3.66
Anandaganga	79.16	56.83	34.22	16.02	0.70
Godavari	61.66	20.34	9.08	0.28	0.70
Lakshaganga	81.66	56.62	40.36	22.10	0.70
Keraganga	91.33	69.86	46.149	25.51	2.52
Navasi	61.66	23.51	0.28	0.28	0.70
Tanjore	90.66	59.88	43.44	31.70	2.72
Java	75.33	35.64	0.28	0.28	0.70
WCT	90.66	70.26	60.13	50.29	4.92
COD	57.66	22.13	15.65	0.28	0.70
Philippines	86.00	36.81	24.50	0.28	0.70
Soubhagya	83.66	59.69	39.21	23.47	2.59
Thembli	47.66	33.73	19.03	0.28	0.70
Ayiramkachi	73.00	45.63	25.26	16.58	0.70
Komadan	87.00	40.36	16.29	0.28	0.70
Bombay G	76.00	57.04	43.46	19.27	1.55
Kappadam	84.66	40.00	17.97	0.28	0.70
Andaman Ranguchen	74.00	33.19	21.07	0.28	0.70
Lakshadweep micro	88.33	66.20	23.77	0.28	0.70
Sanramon	27.00	0.28	0.28	0.28	0.70
MYD	45.00	36.70	30.53	24.76	3.42

Cochin China	65.33	29.02	11.38	0.28	0.70
Fiji	65.00	31.07	0.28	0.28	0.70
Bensahybrid	89.00	43.27	27.02	14.43	1.83
Kerasree	91.33	47.88	31.51	14.14	0.70
Lakshadweep ordinary	77.33	43.27	26.45	0.28	0.70
Bansanda	72.66	55.66	44.29	27.87	0.70
Jamaica	80.00	52.26	49.99	23.51	2.20
NCD	86.66	20.86	5.33	0.28	0.70
CD	10.551	6.699	5.055	7.550	0.395

Discussion

5. DISCUSSION

Coconut is regularly exposed to soil and atmospheric water deficit due to the fact that, it is a perennial palm with an extended effective life span (Rajagopal and Bai, 2002). Drought is one of the important abiotic stresses which can limit the crop growth and yield by altering various physiological and biochemical processes (Vaidya *et al.*, 2015). The research project on "Assessment of water stress tolerance in selectively fertilized coconut (*Cocos nucifera* L.) hybrids" was done with the objective to assess the physiological and molecular basis of water stress tolerance in selectively fertilized coconut hybrids and to screen coconut genotypes for water stress tolerance through critical water potential for pollen germination.

5.1 EXPERIMENT 1- PHYSIOLOGICAL AND MOLECULAR ASSESSMENT OF WATER STRESS TOLERANCE IN SELECTIVELY FERTILIZED COCONUT HYBRIDS

Morphological and biochemical changes in plants under water deficit lead to acclimation, subsequent functional damage and the loss of plant parts as water stress becomes more severe (Silva *et al.*, 2016). In addition to the complexity of drought itself (Passioura, 2007), plant responses to drought are complex and different mechanisms are adopted by plants when they encounter drought (Jones, 2004). Short-term responses of coconut to water stress such as low biomass accumulation and partitioning, reduced stomatal conductance to water vapor and leaf water potential which often impair photosynthesis and transpiration have been extensively documented (Gomes and Prado, 2010).

Coconut is frequently exposed to soil and atmospheric drought because it is a perennial palm with long productive life span (Rajagopal and Bai, 2002; Gomes *et al.*, 2002). The effects of water deficit on the physiology, growth and productivity of coconut have been widely reported (Gomes and Prado, 2010). Due to long maturation

and generation period of coconut the adverse effects of drought persist for two and a half years (Rajagopal and Bai, 1999).

5.1.1 PHYSIOLOGICAL PARAMETERS

Coconut hybrids are high yielding; but are generally susceptible to moisture stress. Drought susceptibility of COD x WCT and drought tolerant nature of LCT x GB and LCT x COD were reported (Rajagopal *et al.*, 2005) based on water relation. Carr (2011) suggested that in plantation crops, stomatal conductance can be used as an index for water stress tolerance and also correlated the same with transpiration rate and photosynthetic rate. Increased stomatal conductance was reported in the present experiment for selectively fertilized dwarf genotypes under water deficit treatment, it may be due to high stomatal stomatal frequency. These results are identical to the findings of Jayasekara *et al.* (1996), where they reported lower level of stomatal conductance in fifteen year old coconut palms under soil water deficit situations. WCT registered highest stomatal conductance than all other treatment, may be due to the presence of high stomatal frequency under water deficit condition.

The data on stomatal conductance, photosynthetic rate and transpiration rate in the current study showed a decrease for all the treatments under water stress condition. Same trend was reported by with Passos *et al.* (2005), who found that stomatal conductance and photosynthetic rate decreased in dwarf coconut under water stress condition. It may be due to the closure of stomata by the inception of drought in coconut (Repellin *et al.*, 1997).

In our experiment, tall genotype (WCT) showed highest stomatal conductance than dwarf genotypes (Kerasree and Keraganga). Our findings validated with Rajagopal and Bai, (2002) who did an experiment in tall and dwarf genotypes of coconut, reported that tall genotypes showed superiority over dwarf genotypes under water stress conditions and showed a slow recovery in stomatal conductance after rewatering.

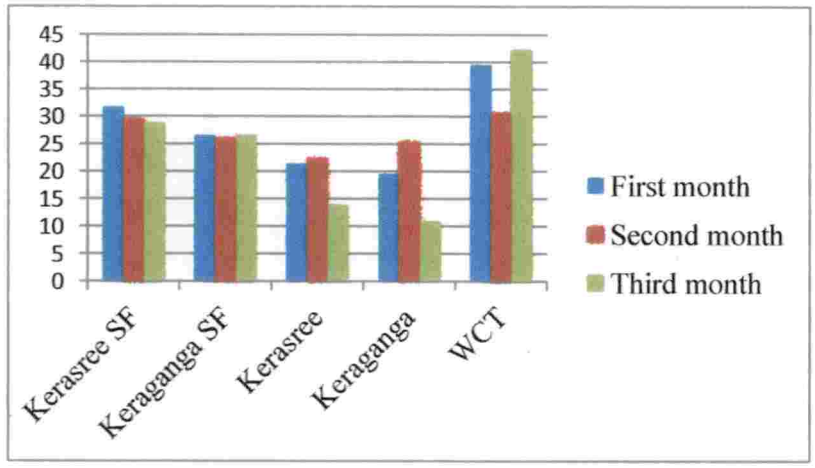

Fig. 1 Effect of water stress on Stomatal conductance (m mol H_2O m^{-2} s^{-1}) of selectively fertilized coconut hybrids

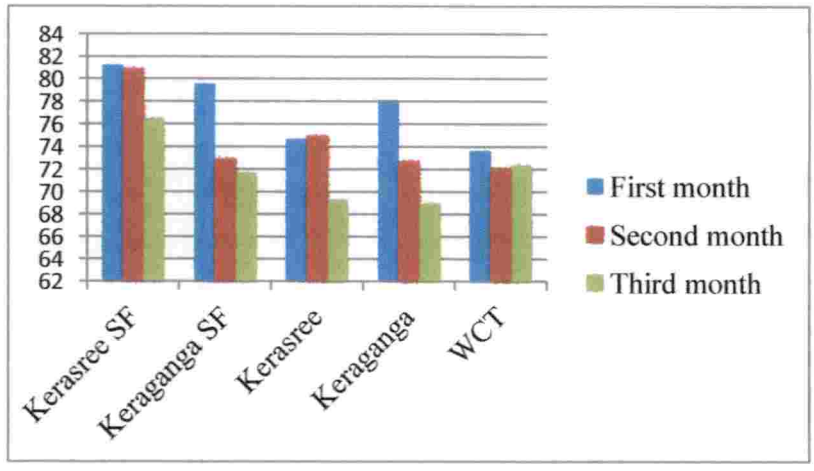

Fig. 2 Effect of water stress on Relative water content (%) of selectively fertilized coconut hybrids

The trend in transpiration data was corroborated with the study of Sucre and Suárez (2011), where a marked reduction in leaf transpiration rate was noticed in plants under drought by closing stomata for retaining water in the tissues. Goncalves *et al.* (2010) disclosed a positive correlation between stomatal conductance and transpiration rate. In the present study, selectively fertilized hybrids showed higher transpiration rate than normal hybrids and WCT. The same trend was registered by Voleti *et al.* (1993) and reported that dwarf genotypes expressed high transpiration rate due to high consumption of water than tall varieties.

Photosynthetic rate was found to be higher in case of Kerasree S.F and Keraganga S.F than WCT. This consonant finding is disclosed by Rajagopal *et al.* (2007) in coconut that, slight reduction in photosynthetic rate was noticed in dwarf coconut than tall ones in the course of water stress. Gomes *et al.* (2008) reported that photosynthetic efficiency in dwarf coconut showed positive adaptation to frequent drying and recovery cycles. The decreased trend in photosynthetic rate may be due to abscisic acid accumulation in leaves of drought stressed coconut palms and its involvement with stomatal regulation of gas exchange during and after stress were investigated (Gomes *et al.*, 2010).

Normal hybrids, Kerasree and Keraganga reported minimum stomatal conductance and photosynthetic rate in the present investigation, corroborate with the findings of Rajagopal and Bai (2002) who claimed that, lowest stomatal conductance played a major role in the reduced photosynthetic rate. Photosynthetic rate was found to be inversely proportional to water stress agreeing with the findings of Cornic (2000), who reported that photosynthetic rate decreased due to the closure of stomata under water stress as an impact of physiological response and also due to the reduction in the rubisco activity, RuBP regeneration, ATP supply, electron transport rate and light capture efficiency (Zhou *et al.*, 2007).

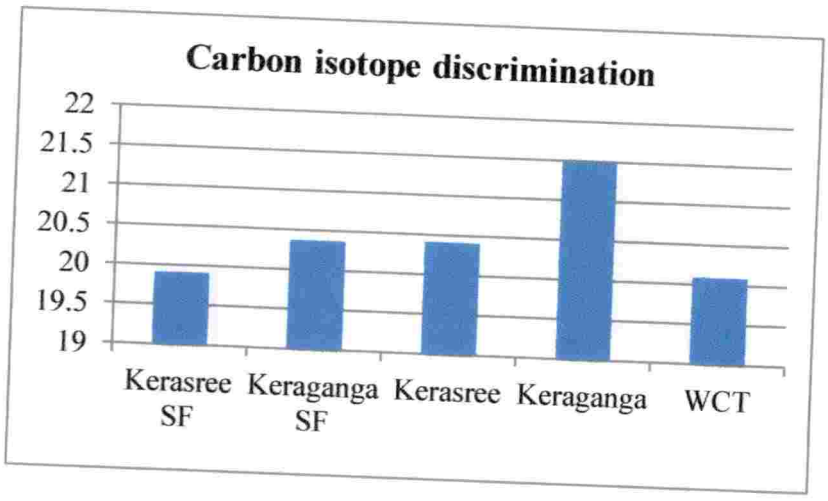

Fig. 3 Effect water stress on carbon isotope discrimination (‰) of selectively fertilized coconut hybrids

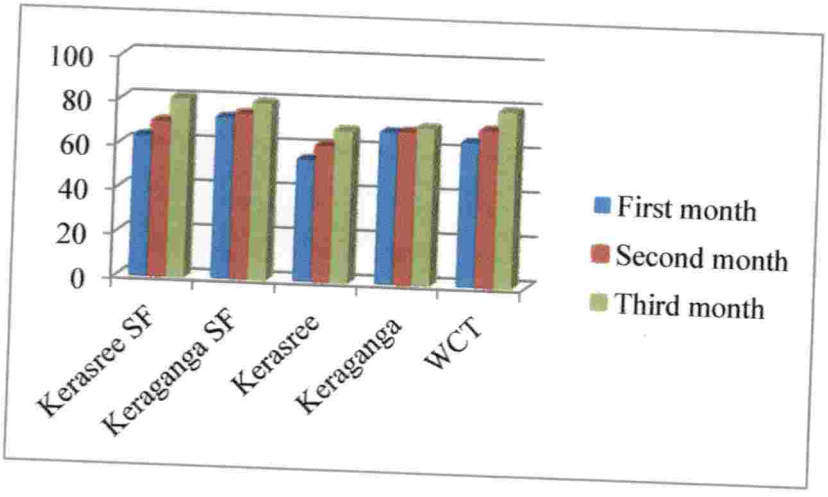

Fig. 4 Effect of water stress on Cell membrane stability (%) of selectively fertilized coconut hybrids

Sun *et al.* (2011) in oil palm revealed that relative water content reduced gradually by the withdrawal of water for 90 days. Repellin *et al.* (1997) made a conclusion that, reduction of stomatal conductance was not related with the value shown by the treatment. Same conclusion was obtained in this study. Kerasree S.F when compared with WCT, showed highest relative water content in its leaves in water stress situation and lowest volume of water transpired was noted. The results validated with the findings of Rajagopal and Bai (2002) who reported that maintenance of high leaf water status is the basis of drought tolerance mechanisms in coconut.

Normal hybrids recorded lowest photosynthetic rate, relative water content and higher leaf temperature than selectively fertilized hybrids. Our results corroborate with the findings of Lawlor and Cornic (2002). The foliar photosynthetic rate in higher plants is known to decrease as the relative water content decreases and it may be due to the decrease in the water potential which results in reduced cell growth, root growth and shoot growth and also causes inhibition of cell expansion and reduction in cell wall synthesis (Chaitanya *et al.*, 2003).

Gomes and Prado (2010) listed out the leaf anatomical characters that confer the water stress tolerance in coconut, *i.e.* stomatal frequency, stomatal resistance and epicuticular wax content. WCT showed preeminent deposition of epicuticular wax and corresponding reduction in the transpiration rate in present investigation. Kurup *et al.* (1993) reported the negative correlation between wax and transpiration rate. Riedel *et al.* (2009) noted that tolerant genotypes load up more quantity of epicuticular wax than susceptible one and It was reported that selectively fertilized hybrids were drought tolerant and had high water use efficiency compared to normal coconut hybrids (Renju *et al.*, 2015).

On the basis of epicuticular wax content, Kerasree S.F was the drought tolerant genotype. Rajagopal *et al.* (1990) discloses the criteria for drought tolerance

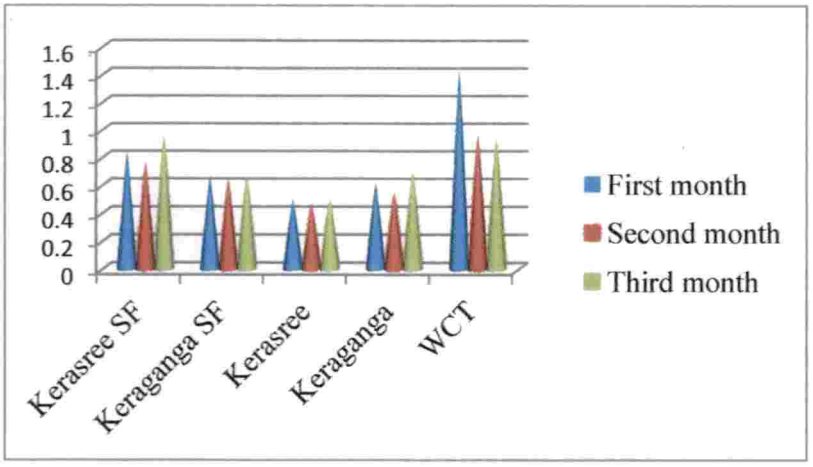

Fig. 5 Effect of water stress on Transpiration rate (m mol H_2O m^{-2} s^{-1}) of selectively fertilized coconut hybrids

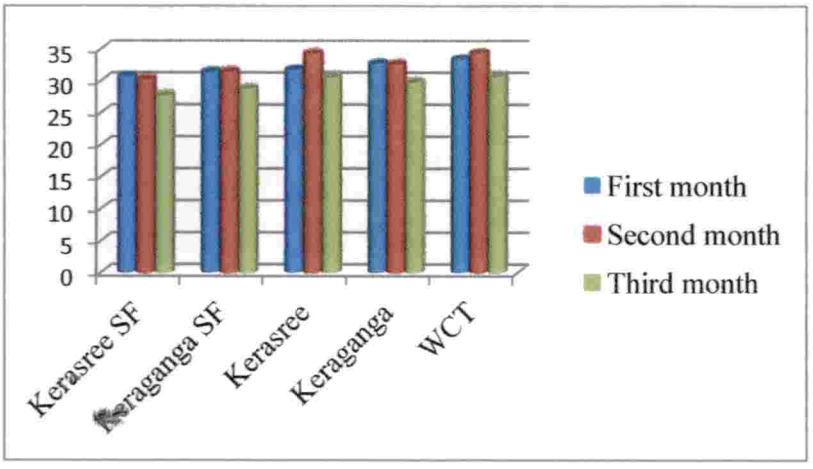

Fig. 6 Effect of water stress on leaf temperature (°C) of selectively fertilized coconut hybrids

as, tolerant genotype will have high deposition of epicuticular wax. According to Jenks (2001), epicuticular wax played an important role in drought tolerance in crops by water retention by limiting transpiration.

Data on wax and transpiration rate disclosed a correlation in line with the Jenks (2002). Kerasree S.F showed highest epicuticular wax content as well as less transpiration. In the present experiment, under water deficit condition all treatments reported a hike in the deposition of wax up to second month. This result was corroborative with the findings of Rajagopal *et al.* (1988), who proposed that in coconut palms genotypes showed 32% increase in deposition of epicuticular wax under severe water stressed condition.

Reduction in the epicuticular wax deposition was observed in the third month of water deficit period. It may be due to the rain fall experienced in the field. This observation can be justified by the reports of Sutter & Langhans (1979), mentioned that low light intensity and high relative humidity can result in reduced wax deposition.

According to Xu *et al.* (2008), cell membrane stability was considered as one of the best physiological components for drought tolerance in plants. Cell membrane stability could be considered as an index for drought tolerance (Sayar *et al.*, 2008). Gomes *et al.* (2008) noted that, cell membrane stability was used to the tolerant and susceptible genotypes of coconut and a slighter elevation of leakage was also disclosed by the author in water stressed coconut palms. At the cellular level, the cell membrane as well as the endomembrane system change dramatically their disposition and limit organelle function as well as cell integrity in response to the stress (Gigon *et al.*, 2004)

Kerasree S.F noted highest percentage of membrane stability in relation to the normal hybrids. The result obtained in the present investigation was ratified with Gomez *et al.* (2010), where they reported higher membrane stability in drought

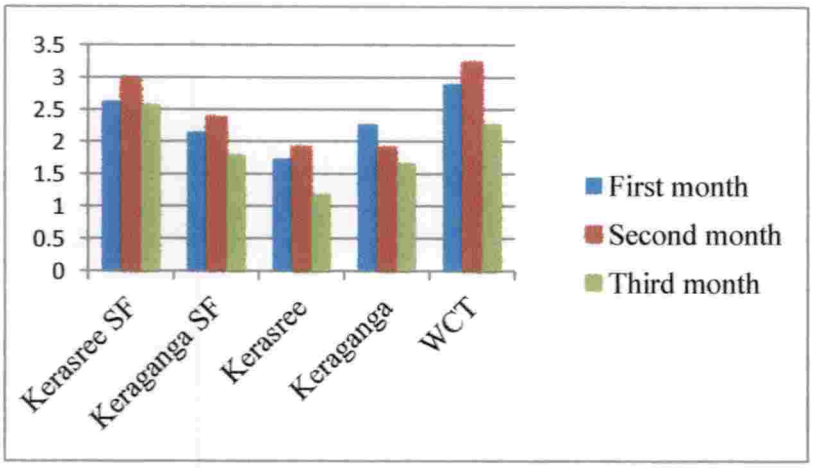

Fig. 7 Effect of water stress on Epicuticular wax (mg cm^{-2}) of selectively fertilized coconut hybrids

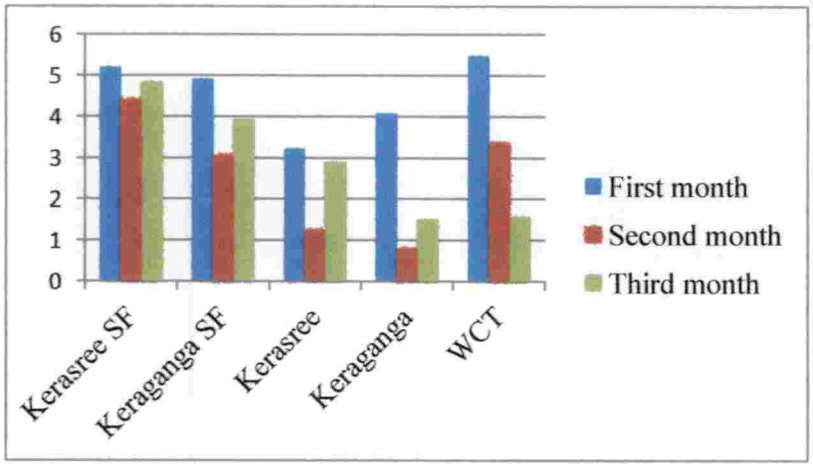

Fig. 8 Effect of water stress on Photosynthetic rate (µ mol CO_2 m^{-2} s^{-1}) of selectively fertilized coconut hybrids

tolerant Brazilian green dwarf coconut genotypes subjected to drought stress. Selectively fertilized hybrids reported higher membrane stability than WCT in the present experiment. The result was justified by the findings of Repellin *et al.* (1994), that water deficit induced a reduction in total leaf lipid content, mainly that of the chloroplast membranes, an effect particularly expressive in the less drought-tolerant genotypes.

Leaf temperature increased significantly in the absence of transpirational cooling and consequently enhanced the breakdown of chlorophyll leading to reduction in the rate of photosynthesis (Nainanayake and Bandara, 1998). Highest leaf temperature was recorded for WCT (showed high transpiration rate) and findings negatively correlated with the Nainanayake and Bandara (1998).

During third month of water stress, WCT reported highest wax content and also showed highest leaf temperature. The results corroborate with Vincent *et al.* (2002) who treated leaf temperature as drought tolerant parameter on east coast tall variety of coconut for drought management and recorded a positive correlation among epicuticular wax and leaf temperature.

In line with Robina *et al.* (2005) Water use efficiency (WUE) is defined as the ability of the crop to produce biomass per unit of water transpired. Carbon isotope discrimination is a tool used for evaluating water use efficiency. Water use efficiency is a plant trait which indicates the drought tolerance of genotype (Kumarasinghe *et al.*, 1996).

Water use efficient genotypes will exhibit least discrimination towards stable isotopes in water deficient situation and hence can be used to assess genotypic variation in WUE and physiological responses to environmental factors (Johnson *et al.*, 1990). In current experiment for evaluating water use efficiency through stable isotope

discrimination, Kerasree S.F which reported lowest discrimination value for stable carbon isotope can be considered as the most water use efficient.

5.1.2 BIOCHEMICAL PARAMETERS

Superoxide dismutase, peroxidase and malondialdihide content, increased with the extension of drought stress (Cheng Xu *et al.*, 2010). Membrane lipid peroxidation in plants was detected by measuring MDA and it is widely used marker of oxidative lipid injury caused by abiotic stress. Lowest membrane damage was noticed from the value of MDA in WCT followed by selectively fertilized Kerasree. The results of our investigation was agreeing with the study conducted by Zhou *et al.* (2015) in forest trees, high damage were observed and it may be due to the process of peroxidation (Kong *et al.*,2016).

Proline has many roles in the course of water stress, mainly reducing the effect caused by the free radical and stabilization of membranes in period of drought experienced under field condition. A preeminent inflation of proline in leaf during water stress was detailed by Pagter *et al.* (2005). Drought tolerant genotypes developed maximum accumulation of proline for osmotic adjustment was occurred during water stress (Heuer, 1999). Jayasekara *et al.* (1993) indicated that, tolerant genotypes in coconut were noticed with high amount of proline under water stress condition. Bai and Rajagopal, (2004) disclosed that, dwarf genotypes experienced a hike in the proline accumulation under water stress condition in coconut.

In our experiment a hike in the proline content was recorded in dwarf genotypes, Kerasree S.F, Keraganga S.F than WCT. The experimental results were corroborative with the results of Bai and Rajagopal, (2004). In line with Jayasekara *et al.* (1993), Kerasree S.F developed highest proline content than normal hybrids and WCT and also indicated the drought tolerance ability under water stress conditions.

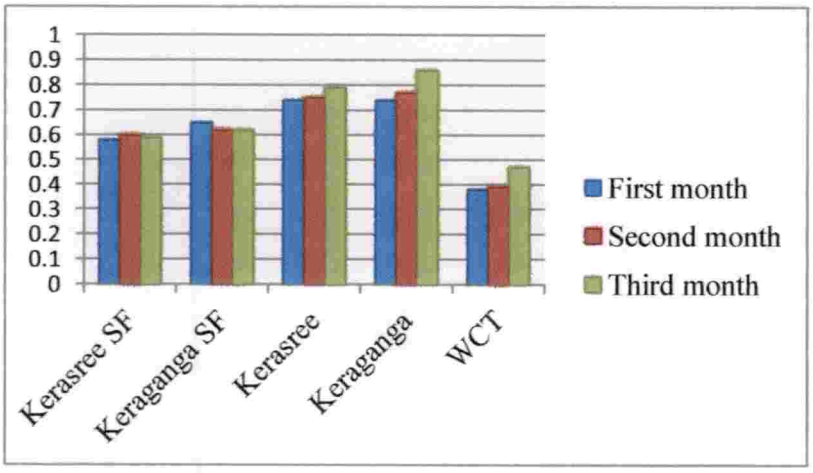

Fig. 9 Effect of water stress on MDA (µg g^{-1}) of selectively fertilized coconut hybrids

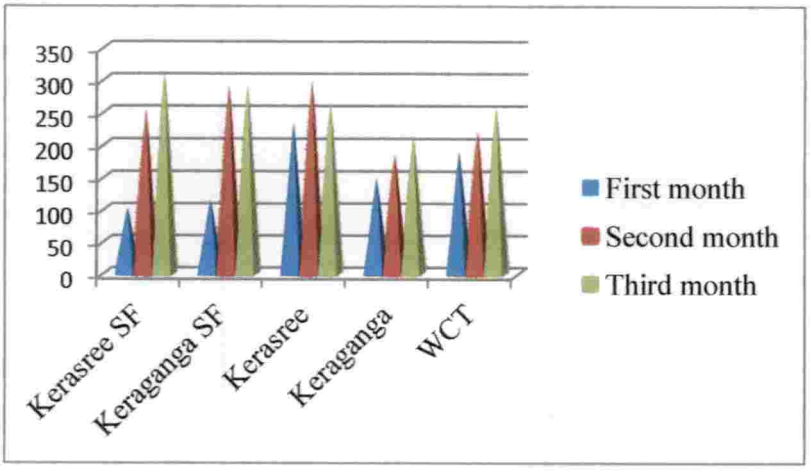

Fig. 10 Effect of water stress on Proline (µ mol g^{-1}) of selectively fertilized coconut hybrids

According to Sudhakar *et al.* (2001), peroxidase activity was found to be a drought tolerant trait to reduce the damages caused by the reactive oxygen species produced during cell metabolism and protection against oxidative stress. In the present investigation, SOD and Peroxidase activity in Kerasree S.F showed a hike during drought conditions and Malondialdehyde noted a decreasing trend during water stress condition. Our findings corroborate with the results of Chempakam *et al.* (1993) who reported a negative correlation between anti oxidant enzyme activities and lipid peroxidation in coconut.

Drazkiewicz (1994) noted that, increased chlorophyllase activity during the period of water deficit situation caused the reduction of chlorophyll content. Vyas (2001) found that chlorophylls were closely associated with water stress tolerance and suggested these parameters as biochemical markers for the identification of drought tolerant genotype in cluster bean. Selectively fertilized Kerasree reported highest chlorophyll content than normal hybrids and WCT under water stress condition. Reduction in the chlorophyll content indicates the clear damage of chloroplast membrane (Sunoj *et al.*, 2016).

The palm which reported higher leaf temperature also retained lesser chlorophyll content it may be due to leaf temperature increased significantly in the absence of transpirational cooling and consequently enhanced the breakdown of chlorophyll leading to reduction in the rate of photosynthesis (Nainanayake and Bandara, 1998).

Normal hybrids recorded lowest photosynthetic rate along with higher reduction of chlorophyll content in the leaves than Kerasree S.F and Keraganga S.F. It may be due to Retardation in the content of photosynthetic pigment in response to drought stress was attributed to the ultra-structural deformation of plastids including the protein membranes forming the thylakoids which in turn causes untying of photosystem II which captures photons, so its efficiency declined, thus causing

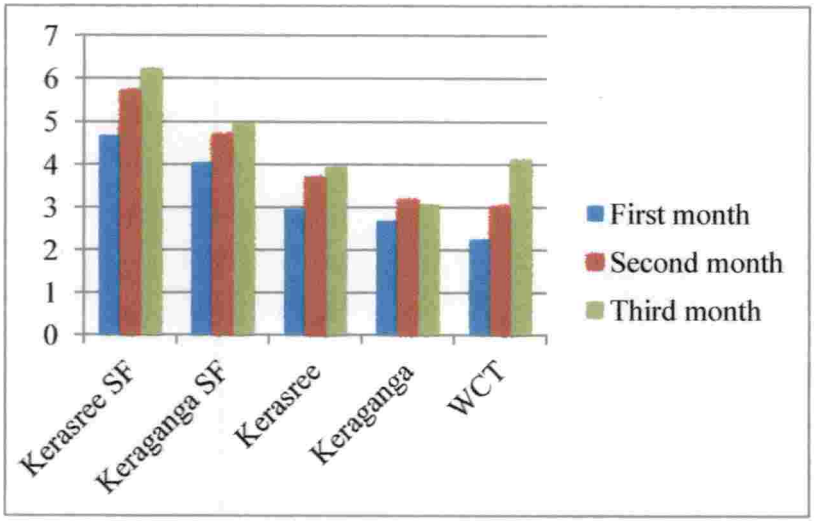

Fig. 11 Effect of water stress on SOD activity (activity g^{-1} min^{-1}) of selectively fertilized coconut hybrids

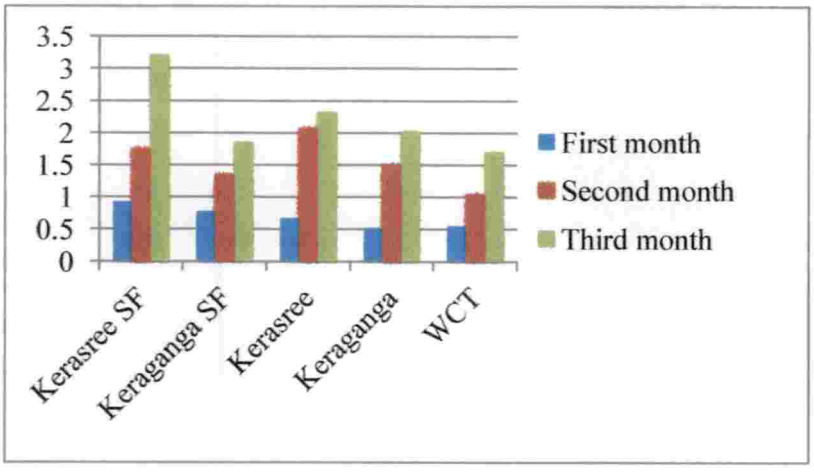

Fig. 12 Effect of water stress on Peroxidase activity (activity g^{-1} min^{-1}) of selectively fertilized coconut hybrids

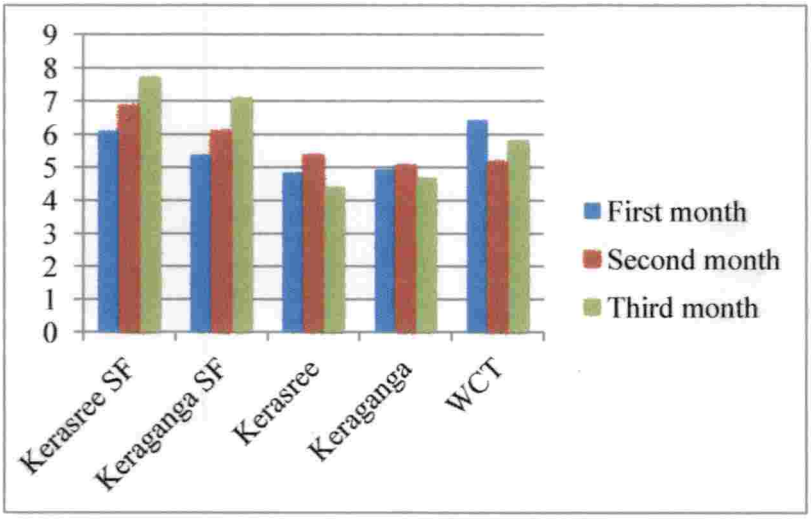

Fig.13 Effect of water stress on Total soluble protein (mg g^{-1}) of selectively fertilized coconut hybrids

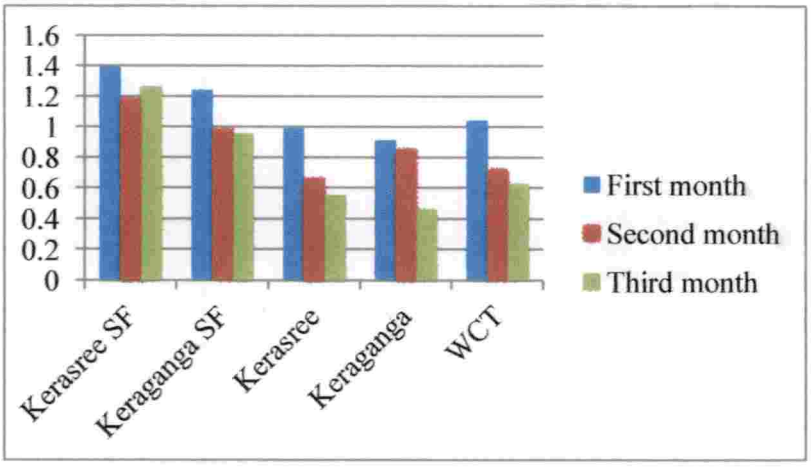

Fig. 14 Effect of water stress on Chlorophyll content (mg g^{-1}) of selectively fertilized coconut hybrids

declines in electron transfer, ATP and NADPH production and eventually CO2 fixation process (Zhang *et al.*, 2006).

In coconut palm, total chlorophyll (Chla + Chlb) and total carotenoids in water stressed plants dropped significantly, restored when plants were subject to full irrigation (Gomes *et al.*, 2008). The photosynthetic pigments in oil palm subjected to mannitol induced and PEG-induced water deficit stress decreased drastically, depending on the osmotic potential in the culture medium (Chaum *et al.*, 2012).

In Selectively fertilized Kerasree, highest concentration of total soluble protein was recorded and it indicated the drought tolerant nature of the genotypes. The result of our investigation ratify the statement of Asemota and Conaire, 2010 observed that Protein content of moisture stressed plants were reduced compared to well watered seedlings in oil palm seedling. Kerasree S.F recorded highest soluble protein among all treatments under water deficit condition. It may be due to the highest photosynthetic rate of kerasree S.F. Findings of the present study contradicted with the results observed by Kala and Godara (2011) in citrus, Rodriguez *et al.* (2002) in sunflower and Mafakheri *et al.* (2011) in chickpea under drought stress condition

5.1.3 MOLECULAR PARAMETERS

Low, medium and high molecular-weight proteins were expressed in coconut during drought stress (Kumar *et al.*, 2007).

In our experiment, WCT showed a band on protein profiling under water stress condition which corroborate with the result of Kumar *et al.* (2007) who reported that, the concentration of Heat Shock Protein Fractions (HSPF) accumulated in maximum during the period of water stress in leaf. A protein of 66 kDa and 76 kDa was expressed in water stressed WCT seedlings.

Drought related SSR primers CnCirE2, CnCirE10, CnCirE12 and CnCirH4 were evaluated and polymorphism was observed in three markers. This marker can be used to distinguish the selectively fertilized hybrids from normal hybrids based on the drought tolerance.

5.2 EVALUATION OF CRITICAL WATER POTENTIAL FOR POLLEN GERMINATION IN ASSESSING GENETIC VARIABILITY IN COCONUT

Male gametophytic selection can be described as a change in gene frequencies resulting from selection pressures applied to the stages of the male gametophytic generation. Selection can occur on both the female and male gametophytic generations. Because of the peculiar features of the male gametophytic generation, such as the large numbers of individuals competing with each other during development in the anther and during pollen germination and tube growth in the style (Ottaviano and Gorla, 1993),

Transcriptome profile of the total number of expressed genes during male gametophyte development shows the number of genes expressed is reduced during the late phase of development. There is a quantitative overlap between the genes expressed in Arabidopsis seedlings (14 464), mature pollen (7177), and sperm cells (5829) (Borges *et al.*, 2009). A large overlap between the genes expressed in sperm cells and seedlings (4757 or 82% of genes expressed in sperm). Approximately 11% of sperm cell expressed genes (642) appear to be unique to sperm (Honys and Twell, 2004).

Pollen fittness is controlled by several factors, (1) the amount of viable pollen produced by a single plant (2) pollination ability (3) postpollination ability (4) fertilization ability (Pfahler, 1975). Pollen quality (viability and function) in maize shows a large amount of genetic variability such as temperature stress tolerance (Schoper *et al.*, 1986). High Pollen tube growth rates are directly correlated with plant vigour (Ottaviano *et al.*, 1980).

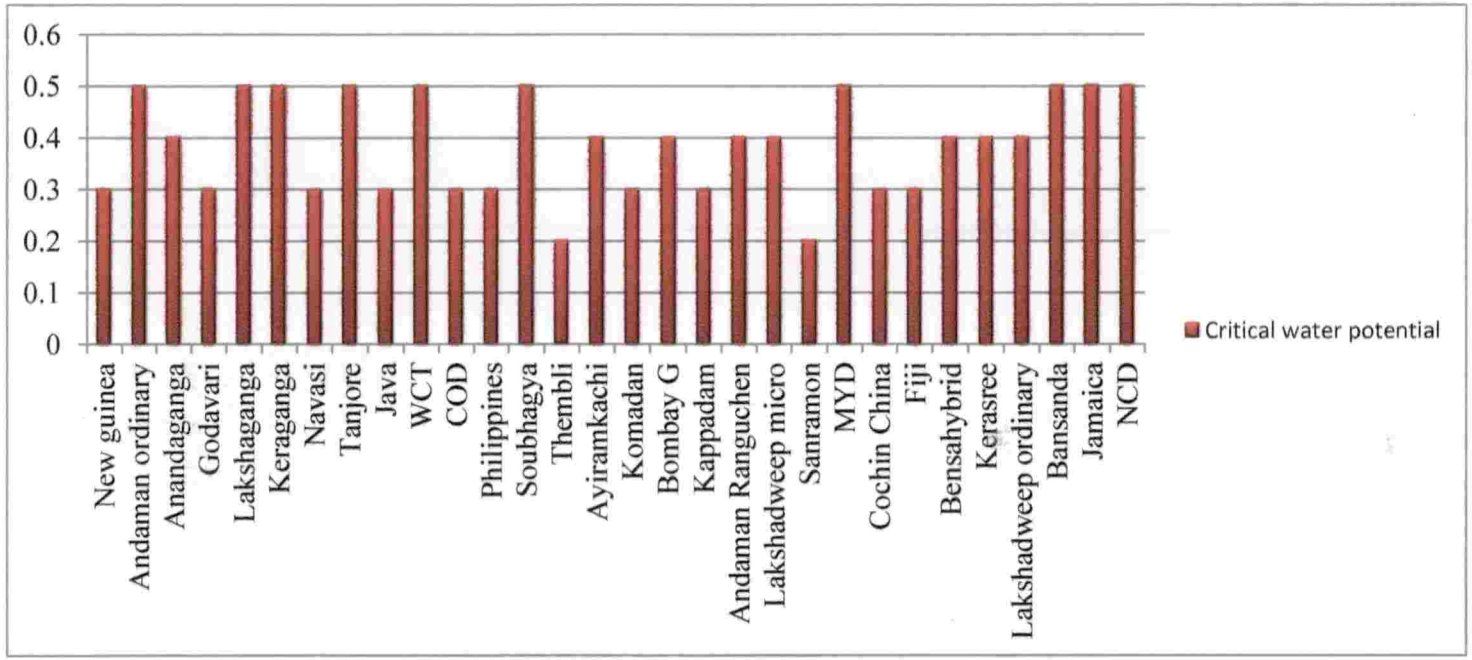

Fig.15 Critical water potential for pollen germination of coconut genotypes

Summary

6. SUMMARY

Coconut known as the 'tree of life' or 'Kalpavriksha' is a benevolent provider of the basic needs of millions of people. It is one of the most important tropical plant species which can adapt to different local environments. Kerala the land of coconut occupies first position in area with 6.5 lakh ha and a productivity of 7535 nuts/ha. But the productivity is far behind the national average

Drought is the second most important factor reducing the productivity of coconut next to the dreaded root (wilt) disease in the state of Kerala. Drought is a multi-dimensional factor of stress affecting the coconut plants at various levels of organization from cell to organ and to whole plant level. A coconut palm yielding 80-120 nuts/year in good rainy years may reduce its yield to 40 nuts/year or less after a drought period. Adverse effects of drought in coconuts persist for two and a half years.

Though coconut hybrids are high yielding, they are generally susceptible to moisture stress. Only way towards increasing coconut production in drought affected areas is through development of drought tolerant varieties and hybrids. *In vitro* pollen selection followed by selective fertilization will be the most feasible and cost effective approach in this direction. This study is an attempt to evaluate physiological and molecular basis of water stress tolerance in selectively fertilized coconut hybrids and to screen coconut genotypes for water stress tolerance through critical water potential for pollen germination. The salient findings of the study are summarized below.

A significant fall in leaf water status was observed in all coconut genotypes under water deficit condition. Proportions of relative water content were highest in Kerasree S.F (6 %) compared to WCT. Keraganga S.F (3 %) recorded higher relative water content than its normal hybrid. Lowest discrimination value was recorded for Kerasree S.F (3 %) and Keraganga S.F (1 %) compared to its normal hybrids.

This showed that selectively fertilized hybrids have high water use efficiency. Selectively fertilized hybrids had highest membrane stability and percentage of leakage was lower as compared to normal hybrids. Reduction in the chlorophyll content was lower in selectively fertilized hybrids as compared to normal hybrids under water stress. Three fold of increase in photosynthetic rate was observed in Kerasree S.F under water stress condition compared with WCT.

An increase of 119 % of proline concentration under stress was observed in selectively fertilized hybrid of kerasree. Activities of antioxidants like peroxidase and superoxide dismutase were high in S.F hybrids as compared to normal hybrids in under water deficit situation. Lesser membrane damage was observed in Kerasree S.F (20 %) and Keraganga S.F (24 %) compared with its normal hybrids.

Genetic variation of critical water potential for pollen germination was observed in 30 coconut genotypes that include Talls, dwarf and hybrids. In WCT, there was expression of a heat shock protein with a molecular weight of 66-70 KDa. Drought related SSR primers CnCirE2, CnCirE10, CnCirE12 and CnCirH4 were evaluated and polymorphism was observed in three markers.

Selective fertilization is characterized by artificially imposing the desired selective pressure during pollen germination, so that the pollen grains which are tolerant to selection pressure only will germinate and fertilize the ovule. The study revealed that selectively fertilized hybrids were drought tolerant compared to normal coconut hybrid and water stress tolerance trait can be added along with high yield in coconut hybrids through selective fertilization.

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Appendices

Table 1. Weather parameters of RARS, Pilicode during the experimental period (Jan 2018-May 2018)

Date	Temperature(°C)	Humidity (%)
Jan 1, 2018	31.2	93
Jan 8, 2018	31.08	92.83
Jan 15, 2018	31.39	93.86
Jan 22, 2018	30.64	92.76
Jan 29, 2014	31.07	92.71
Feb 5, 2018	31.21	92.29
Feb 12, 2018	31.74	91.72
Feb 19, 2018	31.77	89.71
Feb 26, 2018	32.29	88.57
Mar 5, 2018	32.29	91.86
Mar 12, 2018	32.36	90.29
Mar 19, 2018	33.31	86.00
Mar 26, 2018	33.91	85.00
Apr 5, 2018	33.33	87.29
Apr 12, 2018	32.29	87.29

Apr 19, 2018	30.60	93.00
Apr 26, 2018	31.00	92.83
May 5, 2018	30.81	92.76
May 12, 2018	31.19	92.71
May 19, 2018	31.93	92.74
May 26, 2018	32.24	89.12

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Assessment of water stress tolerance in selectively fertilized coconut (*Cocos nucifera* L.) hybrids

By

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(2016-11-118)

Abstract of the thesis

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

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KERALA, INDIA

2018

ABSTRACT

The present study entitled "Assessment of water stress tolerance in selectively fertilized coconut (*Cocos nucifera* L.) hybrids" was carried out in the Department of Plant Physiology, College of Agriculture, Vellayani during 2016-2018. The main objective was to assess the physiological and molecular basis of water stress tolerance in selectively fertilized coconut hybrids and to screen coconut genotypes for water stress tolerance through critical water potential for pollen germination.

The study was conducted as two experiments. In the first experiment, Nine year old field grown coconut palms of the following varieties, Kerasree (WCT × MYD) selectively fertilized, Keraganga (WCT × GB) selectively fertilized, Kerasree (WCT × MYD), Keraganga (WCT × GB) and West Coast Tall (WCT) planted at Regional Agricultural Research Station, Pilicode, Kasargod were used as the experimental material for this study. The experiment was laid out in Randomized Block Design (RBD) with four replications during February 2018- April 2018. Water stress was imposed by withdrawing irrigation for three months and the physiological and biochemical observations were recorded at monthly interval.

The selective fertilization technique is characterized by artificially imposing the desired selective pressure during pollen germination, so that the pollen grains which are tolerant to selection pressure only will germinate and fertilize the ovule.

On physiological analysis the selectively fertilized Kerasree and Keraganga showed higher stomatal conductance, transpiration rate, photosynthetic rate, relative water content and cell membrane stability index than normal Kerasree and Keraganga hybrids. Epicuticular wax deposition was highest in Kerasree S.F (2.57 mg/10cm^2) which was on par with WCT (2.27 mg/10cm^2). Among all genotypes lowest carbon

isotope discrimination value was observed in Kerasree S.F (19.90 ‰) followed by WCT (20.08 ‰) which is an indication of high water use efficiency. Leaf temperature was also lowest in Kerasree S.F (27.77°C).

The biochemical parameters like total soluble proteins, proline content and anti oxidant enzymes *viz* SOD and peroxidase activities were maximum in selectively fertilized Kerasree. The selectively fertilized coconut hybrids recorded the lowest membrane damage (lipid peroxidation value) under water stress condition compared to normal hybrids. Kerasree S.F retained maximum chlorophyll content (1.26 mgg^{-1}) followed by Keraganga S.F (0.95 mgg^{-1}).

In SDS- PAGE analysis a specific protein of around 66-70 kDa was expressed in WCT. Molecular analysis was done using drought related four SSR primers *viz* CnCirE2, CnCirE10, CnCirE12 and CnCirH4 were evaluated and polymorphism was observed for three markers.

The second experiment was conducted to screen 30 coconut genotypes for water stress tolerance through critical water potential for pollen germination. Significant genetic variation in critical water potential for pollen germination was observed. Critical water potential varied from -0.2MPa to 0.5MPa.

The study revealed that selectively fertilized hybrids were more drought tolerant compared to normal coconut hybrids. By selective fertilization technique it may be possible to add water stress tolerance trait to high yielding coconut hybrids.